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To achieve open access to scholarly journal literature, we recommend two complementary strategies.

I. Self-Archiving: First, scholars need the tools and assistance to deposit their refereed journal articles in open electronic archives, a practice commonly called, self-archiving. When these archives conform to standards created by the Open Archives Initiative, then search engines and other tools can treat the separate archives as one. Users then need not know which archives exist or where they are located in order to find and make use of their contents.

II. Open-access Journals: Second, scholars need the means to launch a new generation of journals committed to open access, and to help existing journals that elect to make the transition to open access. Because journal articles should be disseminated as widely as possible, these new journals will no longer invoke copyright to restrict access to and use of the material they publish. Instead, they will use copyright and other tools to ensure permanent open access to all the articles they publish. Because price is a barrier to access, these new journals will not charge subscription or access fees, and will turn to other methods for covering their expenses. There are many alternative sources of funds for this purpose, including the foundations and governments that fund research, the universities and laboratories that employ researchers, endowments set up by discipline or institution, friends of the cause of open access, profits from the sale of add-ons to the basic texts, funds freed up by the demise or cancellation of journals charging traditional subscription or access fees, or even contributions from the researchers themselves. There is no need to favor one of these solutions over the others for all disciplines or nations, and no need to stop looking for other, creative alternatives.

Open access to peer-reviewed journal literature is the goal. Self-archiving (I.) and a new generation of open-access journals (II.) are the ways to attain this goal. They are not only direct and effective means to this end, they are within the reach of scholars themselves, immediately, and need not wait on changes brought about by markets or legislation. While we endorse the two strategies just outlined, we also encourage experimentation with further ways to make the transition from the present methods of dissemination to open access. Flexibility, experimentation, and adaptation to local circumstances are the best ways to assure that progress in diverse settings will be rapid, secure, and long-lived.

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RESEARCH ARTICLE

The impact of lunar phases and illegal light use on purse seine fishery in Türkiye's Mediterranean waters

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ABSTRACT

This study examines the purse seine fishing activities along the Mediterranean coast of Türkiye between 2013 and 2020, with a particular focus on the apparent fishing hours in regions such as Iskenderun, Mersin, Anamur, Antalya, and Fethiye. Data obtained through the Global Fishing Watch platform have been used to assess the impact of lunar phases and illegal light usage on fishing practices. Analyses indicate intensified fishing activities in regions like Fethiye, where light usage is permitted, and Iskenderun, where it is prohibited. This intensity is especially evident during the dark lunar phases, highlighting increased fishing efforts associated with illegal light usage. The research observes how fishing activities differ in regions with varying legal regulations, such as Iskenderun and Fethiye. Despite the prohibition, an increase in fishing activities due to illegal light usage has been observed in Iskenderun, raising questions about the effectiveness and enforceability of current bans. In Fethiye, where light usage is allowed, there has been a significant increase in fishing hours. Findings and analyses reveal the adverse effects of illegal light usage on sustainable fishing practices and the health of marine ecosystems. The results offer significant insights for developing policies and management strategies to ensure the ecological and economic sustainability of fishing activities along Türkiye's Mediterranean coast.

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Introduction

Purse seine fishing is a pivotal method for capturing pelagic species globally, with significant implications for Türkiye's

fishery sector. It accounts for 30% of the world's and 70% of Türkiye's fishery production, predominantly targeting anchovy in the Black Sea and small pelagic species in the Marmara and Aegean seas (Demirci & Demirci, 2006; Watson et al., 2006;

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Aydın, 2012; TurkStat, 2019; Tunca et al., 2021). Its utilization in the Mediterranean is restricted to specific months due to economic considerations, leading to a smaller fleet and fewer operational periods (Koyun et al., 2022).

Using light at night can enhance fishing efficiency by attracting a diverse range of fish species. However, this technique's effectiveness is moderated by the lunar cycle, with natural illumination during full and quarter phases reducing fish attraction to artificial light sources. Thus, fishing during darker lunar periods is preferred (Tosunoğlu et al., 2021).

Among various fishing methods in the Mediterranean, the purse seine method is noted for its relatively limited impact on the ecosystem. Research indicates that this method is highly selective, targeting small pelagic fish species and resulting in a low discard rate of about 2-3% (Tsagarakis et al., 2012). In the Eastern Mediterranean, particularly during the summer months, there is a large fishing fleet targeting large pelagic fish, while capturing small pelagics is more economically sustainable during spring and summer (Lucchetti et al., 2008). Generally, in the Mediterranean and especially in the Aegean Sea, night fishing involves the use of skiffs equipped with powerful lights to attract various schools of fish. However, this type of fishing in shallow waters, such as in the Eastern Mediterranean and the Bay of Iskenderun, not only attracts pelagic fish but also many demersal fish species, potentially leading to overfishing pressure. Consequently, Türkiye has implemented sustainable management practices for the use of light in purse seine fishing, allowing it in the Aegean and the Black Sea while imposing a complete ban in the Mediterranean. These measures are designed to reduce impacts on demersal stocks and avoid conflicts between fishing fleets (MAF, 2020).

Despite these regulations, illegal fishing activities, particularly those involving the use of light at night, pose a significant challenge to fisheries management (Göktürk & Deniz, 2017; Demirci et al., 2019). This study aims to examine purse seine fishing practices between 2013 and 2020 across five regions in the Mediterranean, with a particular focus on the relationship between lunar phases and illegal light use in fishing activities. By analyzing the patterns of fishing hours and the prevalence of illegal practices during darker nights, the study seeks to highlight the ongoing challenges in enforcing the ban on light usage in purse seine fishing in the Eastern Mediterranean. The ultimate goal is to provide data-driven insights to support more effective regulatory measures and reduce overfishing pressure in the region.

Material and Methods

Data Collection

Fishing activity

This study collected data on the time Turkish purse seine fishing vessels spent at sea along five Mediterranean coastal regions from 2013 to 2020 (Figure 1). The data were sourced from the Global Fishing Watch website, which provides daily totals of operating hours for purse seiners within specific coordinates. Turkish purse seine vessels have been trackable on this system since 2013. However, satellite signals are sometimes unreachable or deliberately disrupted for security purposes. Additionally, data collection is challenged by vessels turning off their tracking devices, which contravenes regulations against such actions during fishing operations.

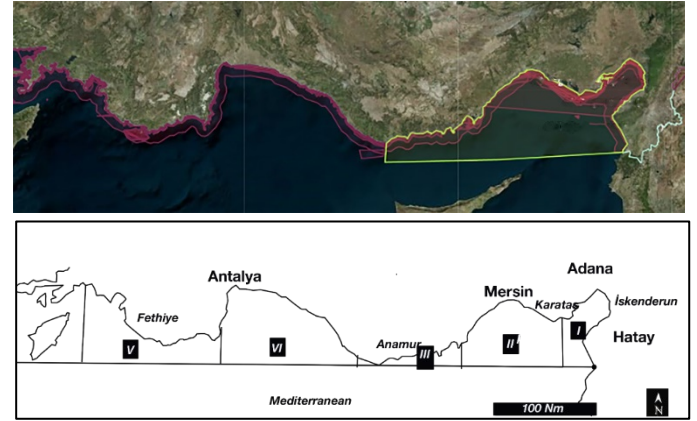


Figure 1. Regulated areas for Mediterranean purse seine fishing according to Turkish fisheries management: complete prohibition in red zones, light use banned in yellow zones

Meteorological data

The meteorological data utilized in this study were sourced daily from five marine stations (İskenderun, Karataş, Mersin, Anamur, Antalya, and Fethiye) situated along the Mediterranean coast of Türkiye, spanning the same period as the purse seine vessel data, provided by the Turkish State Meteorological Service. The collected parameters included (1) cloud cover, measured on a scale from 0 to 8, (2) wind direction, categorized into eight cardinal directions, and (3) wind speed, recorded in kilometers per hour.

Lunar phases

Lunar Phases were calculated by using the Hijri calendar and the Gregorian calendar excel converter to cover all the days of the research. The verification of this calculation was carried out on the website of the Turkish Historical Society. In the

assessment process, the first quarter of the lunar month corresponds to the transitional period at the end and the beginning of each month. This period has been designated as five days in the study. It is defined as the first three days of the new lunar month according to the Hijri calendar, and the last two days of the previous month. This 5-day span is considered the period of lunar darkness. Similarly, the days from the 12th to the 16th of the lunar month, when the lunar is at its brightest, are considered the period of lunar illumination. The remaining 20-day span is regarded as a normal period in terms of the lunar's condition.

Data Analyses

Statistical analyses were designed to match the seasonal patterns of the fishing industry. Turkish fishing regulations permit activity in the Mediterranean from September 15 to April 15 in areas deeper than 25 meters within a 12-mile limit. Occasionally, this period has been extended by 15 days at both the start and end in certain years. After April 15, purse seine fishing moves beyond the 12-mile limit for two months (MAF, 2020). In the Eastern Mediterranean, certain species from the Scombridae family and sardines, are observed and have the primary target of fishermen. However, it is not feasible to catch these species throughout the entire season. Therefore, purse seine fishermen in the area resort to using light to target demersal and semi-demersal species.

Fishing seasons were segmented as follows: September to December marked the season's start, January to April the season's end, and April to August the off-season. Analysis focused on mean daily Apparent Fishing Hours (AFH) during these intervals. Data were organized using Microsoft Excel, with further analyses conducted through Past v14 (Øyvind et al., 2001) and R (R Core Team, 2020).

Specific hypotheses were tested at İskenderun and Fethiye: (1) Fishing activity does not correlate with lunar phases, and (2) High cloudiness occurs at night when lunarlight's effect diminishes. One-sample chi-square tests compared categories within each variable group. Pearson's chi-squared test analyzed the independence between variables and AFH.

Upon rejecting the null hypothesis, the contingency coefficient (C) quantified the relationship between two categorical variables. A value of C close to zero indicates independence. The contingency coefficient's value increases with table size, approaching 1.0 (or 100%) for a perfect association.

A Box-Cox transformation was applied to AFH data before the one-way ANOVA test to satisfy the normal distribution

requirement. Levene's test assessed variance homogeneity. The one-way ANOVA tested the hypotheses.

Kendall's tau test examined the relationships between AFH and wind speed, cloudiness, and their combined effect. The Polyserial rho test assessed the correlation between the frequency of fishing activity and wind speed, and cloudiness.

Results

The distribution and size of purse seine fishing vessels along Türkiye's Mediterranean coastline show significant variability. Adana has one vessel, 28.5 meters long. Antalya has six vessels, averaging 35.4 meters with a standard deviation of 13.1, indicating a wide range in sizes. Mersin also has six vessels, averaging 31.2 meters. Hatay stands out with 10 vessels, averaging 26.4 meters.

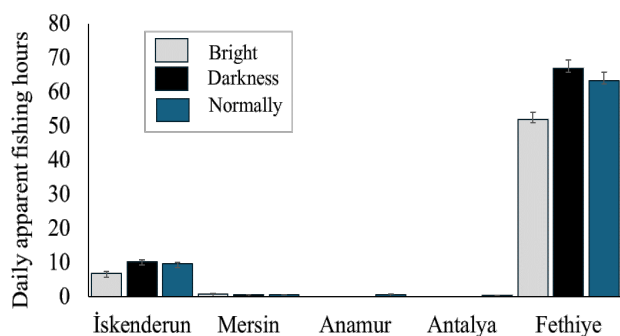
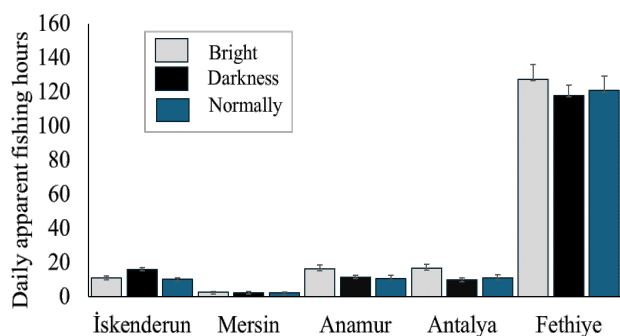
It's important to note that vessel registration in specific cities does not confine their activities to Türkiye's Mediterranean region. Purse seine vessels are highly mobile, often moving between the Mediterranean and Aegean seas. This mobility can range from short stints within a fishing season to prolonged periods spanning several years. The dynamic nature of purse seine fishing reflects the sector's response to factors such as fish stock distribution, weather conditions, market demands, and fishing regulations.

Therefore, while registration data provides a snapshot of vessel presence and size in Mediterranean Turkish ports, it is crucial to consider the broader dynamics of the fishing industry, including vessel mobility and the complex interplay of environmental and economic factors influencing fishing practices.

The data, differentiated by lunar phase (bright, darkness, or normal), reveals a notable disparity in average daily fishing hours. The bulk of purse seine fishing activity is concentrated in Iskenderun and Fethiye. In Iskenderun, significant yearly fluctuations in daily fishing hours are influenced by the lunar phase. During bright lunar phases, fishing hours range from 0.15 hours in 2016 to 14.01 hours in 2018. In periods of darkness, hours range from 4.83 in 2015 to 16.28 in 2013. Under normal lunar conditions, hours span from 4.26 in 2015 to 14.87 in 2018. In Fethiye, the longest fishing durations are recorded. Bright lunar phase hours range from 37.85 in 2016 to 72.48 in 2015. During dark phases, hours vary from 37.00 in 2016 to 89.09 in 2015. Normal lunar phases show hours between 35.65 in 2016 and 88.76 in 2015. In contrast, Mersin, Anamur, and Antalya show considerably lower figures, indicating less intense purse seine fishing activities in these areas (Figure 2; Table 1).

Table 1. The daily average apparent fishing times (hours) with purse seine nets in the Eastern Mediterranean, between the years 2013 and 2020, during the fishing season (October-April)

Area	Lunar Phase	2013	2014	2015	2016	2017	2018	2019	2020
İskenderun	Bright	9.21	9.61	2.03	0.15	4.99	14.01	9.70	4.02
	Darkness	16.28	9.94	4.83	5.48	12.68	10.52	10.53	11.05
	Normal	9.72	12.33	4.26	5.98	8.59	14.87	8.22	12.55
Mersin	Bright	0.07	1.66	0.83	3.14	0.07	0.05	0.01	0.27
	Darkness	0.05	1.24	0.43			0.27		0.52
	Normal	0.31	0.47	1.27	0.02		0.04	0.53	0.87
Anamur	Bright								
	Darkness								
	Normal		0.27	0.28			1.31		
Antalya	Bright								
	Darkness								
	Normal		0.27	0.28			0.78		0.25
Fethiye	Bright	42.90	71.34	72.48	37.85	45.30	47.53	58.07	40.08
	Darkness	55.25	60.82	89.09	37.00	54.30	72.40	78.26	87.10
	Normal	53.96	70.98	88.76	35.65	46.70	61.80	77.98	70.65
Total		187.76	238.91	264.54	125.26	172.62	223.58	243.29	227.36

**Figure 2.** The daily average apparent fishing times (hours) with purse seine nets in the Eastern Mediterranean, between the years 2013 and 2020, during the fishing season (October-April)**Figure 3.** The daily average apparent fishing times (hours) with purse seine nets in the Eastern Mediterranean, between the years 2013 and 2020, off-season (May-July)

The data on average daily purse seine fishing hours during the off-season (May-July) from 2013 to 2020 highlights an intriguing dynamic in the Mediterranean region. Despite the off-season being considerably shorter than the main fishing season, this period is characterized by intense fishing activity. Numerous purse seine fishing vessels converge on the region for tuna fishing, and despite the off-season's shorter duration, fishing hours are significantly higher. Moreover, fishing activities spread more broadly across the Mediterranean, indicating an expansion in operational areas for tuna fishing. In Iskenderun, average daily fishing times during bright lunar phases vary from 2.32 hours (2014) to 33.18 hours (2020). In darkness, the hours range from a minimal 0.31 hours (2018) to 24.05 hours (2014). Under normal lunar phases, fishing hours fluctuate between 3.66 hours (2018) and 17.69 hours (2020). Mersin, Anamur, Antalya, and Fethiye show varied fishing times, with Fethiye exhibiting the highest fishing times, a trend consistent with the main fishing season. Under bright lunar phases in Fethiye, fishing hours range from 49.80 hours (2014) to an astounding 223.13 hours (2016). In darkness, fishing times vary from 76.84 hours (2016) to 205.07 hours (2019). Normal lunar phases see fishing hours between 56.83 hours (2013) and 239.99 hours (2019). The overall average daily fishing times for all mentioned locations under all conditions from 2013 to 2020 range from a minimum of 251.05 hours in

Table 2. The daily average apparent fishing times (hours) with purse seine nets in the Eastern Mediterranean, between the years 2013 and 2020, off-season (May-July)

Area	Lunar Phase	2013	2014	2015	2016	2017	2018	2019	2020
İskenderun	Bright	11.34	2.32	5.11	7.17	7.60	3.69	16.04	33.18
	Darkness	14.04	24.05	18.20	12.43	15.32	0.31	19.20	24.63
	Normal	11.51	8.18	9.44	6.72	9.47	3.66	14.28	17.69
Mersin	Bright		6.68	0.39	0.07	0.01	0.43	9.43	0.01
	Darkness		9.24	0.03	0.19	0.01	0.17	6.64	0.08
	Normal	0.03	8.33	0.84	0.11	0.61	0.23	6.51	0.04
Anamur	Bright			2.22	16.56	4.44	40.73	27.92	4.85
	Darkness						11.92	19.41	3.00
	Normal	1.15	0.71	4.14	4.63	3.47	31.90	29.35	10.03
Antalya	Bright			3.19	16.56	4.44	42.14	27.92	4.85
	Darkness					1.72	13.12	19.41	4.24
	Normal	0.31	1.02	4.42	4.16	3.63	33.20	29.93	10.85
Fethiye	Bright	68.03	49.80	69.22	223.13	150.33	125.65	176.35	156.59
	Darkness	87.80	103.08	87.59	76.84	135.42	132.55	205.07	114.90
	Normal	56.83	71.49	82.27	109.21	101.61	163.51	239.99	141.07
Total		251.05	284.90	287.05	477.77	438.09	603.20	847.47	526.01

2013 to a maximum of 847.47 hours in 2019. Interestingly, from 2013 to 2020 range from a minimum of 251.05 hours in 2013 to a maximum of 847.47 hours in 2019. Interestingly, variations in fishing times do not appear to be significantly influenced by the lunar phase during the off-season. The integration of the specific names of the locations (İskenderun, Mersin, Anamur, Antalya, and Fethiye) provides a clearer understanding of the fishing dynamics in these regions (Figure 3; Table 2).

While exploring the impact of the lunar's phase on fishing efforts, the study concentrated on purse seine fishing activities during the peak fishing season, particularly in high-activity regions: İskenderun and Fethiye. The data presents the daily average apparent fishing hours for these regions over several years. A closer look at the figures indicates that fishing efforts in İskenderun Bay tend to increase during the dark lunar phase. This trend was statistically confirmed using the Kendall method, which considered the daily activity status of purse seine fishing vessels. The heightened fishing activity during the dark lunar phase was statistically significant, with a p-value less than 0.05.

Additionally, the study investigated the influence of wind speed and cloud cover on fishing efforts. It was determined that cloud cover could diminish the effect of lunar light on fishing activities. For this analysis, days characterized by dark lunar phases were excluded. A concise summary of the

findings is offered by emphasizing the regions of İskenderun and Fethiye, without referring to specific table citations or area codes.

As wind speed increases, fishing effort predictably declines, possibly due to the difficulties of operating in turbulent sea conditions. However, the influence of cloudiness on fishing effort presents a more intricate picture. While conventional wisdom would suggest that an increase in cloudiness - reducing natural lunar light - would lead to a decrease in fishing effort, our findings indicate this is not uniformly the case, suggesting other factors may be at play that offset the impact of reduced natural illumination. The research further underscores the intricate interplay of various environmental factors, including the lunar phase, wind speed, and cloud cover, on purse seine fishing effort in the Mediterranean's regions of heightened activity, specifically İskenderun and Fethiye. In İskenderun, data indicate a significant increase in fishing activities during the lunar's dark phase (Figure 4). This pattern strongly suggests that lunar illumination (or the lack of it) plays a critical role in these operations. However, in Fethiye, does not observe such a trend, indicating that the relationship between lunar phases and fishing efforts might be specific to certain regions and potentially dependent on local factors (Figure 5). The observed inconsistency in the impact of cloudiness on fishing effort in both regions could potentially be attributed to the use of artificial lighting by the fishing vessels. Despite restrictions, the

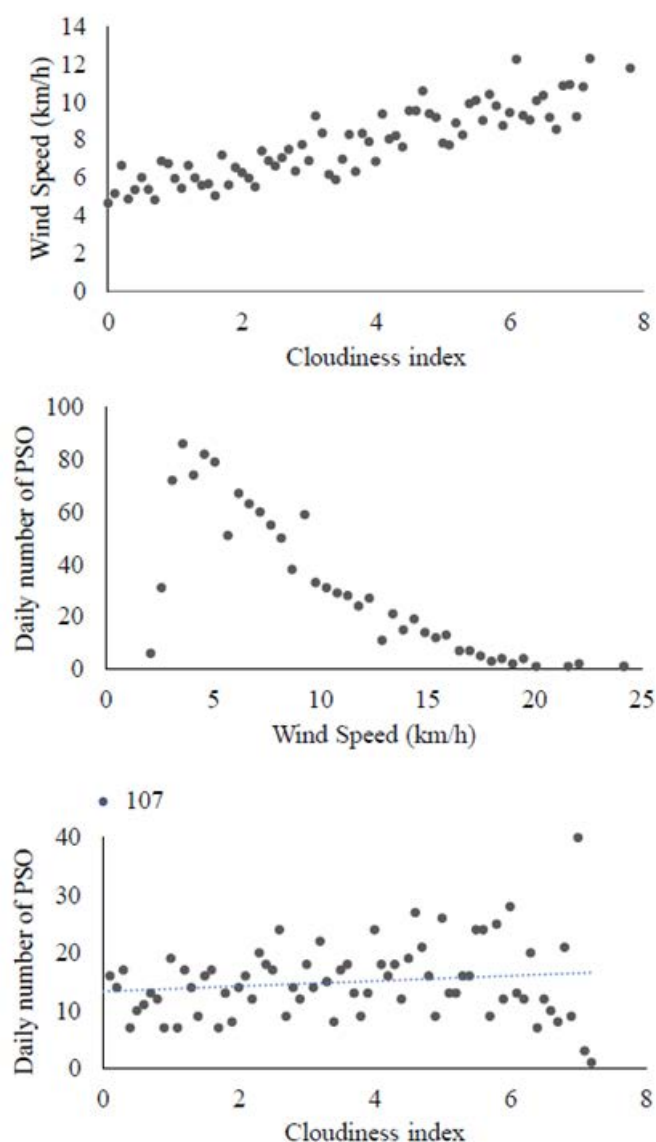


Figure 4. The effect of the first region wind speed and cloudiness index on daily purse seine operations. 2013-2020

use of light in purse seine fishing is known to improve catch yield. In conditions of increased cloud cover and consequent reduction in lunar light, vessels could be relying on artificial light sources, hence maintaining or even increasing their fishing activities.

Discussion

To evaluate the results, a general assessment of the Turkish purse seine fleet is necessary. Türkiye is renowned among Mediterranean countries for its highly effective purse seine fishing fleet, largely thanks to the abundant anchovy stocks in the Black Sea (Ceyhan & Tosunoğlu, 2021). These anchovy stocks play a crucial role in the national fishery, contributing to nearly half of the total yield. In the Marmara Sea, the focus of purse seine fishing shifts to Mediterranean and Aegean

sardines, as well as sizable tuna species (Karakulak & Alıçlı, 2002; Koyun et al., 2022). However, the availability of these species is seasonal, leading to intermittent fishing activities that lack year-round consistency. This results in regional fishing mobility, with fishing efforts relocating in response to the seasonal presence of target species.

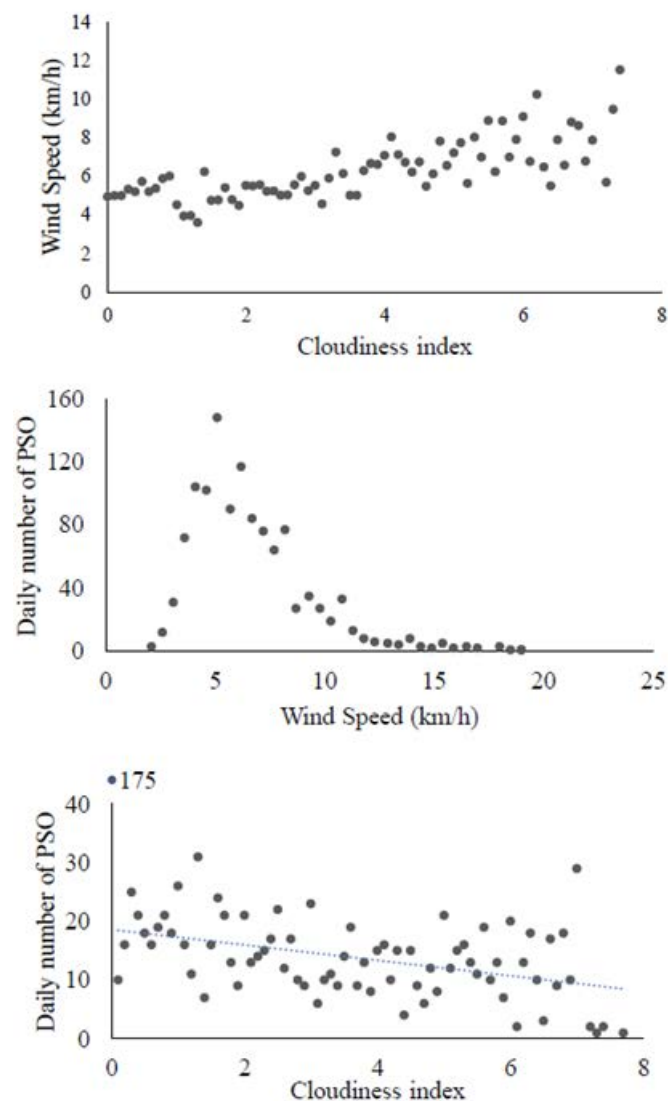


Figure 5. The effect of the five-region wind speed and cloudiness index on daily purse seine operations. 2013-2020

The use of artificial light in purse seine fishing, particularly in the Aegean Sea, has been a long-standing practice, although it remains prohibited in the Mediterranean Sea (Göktürk & Deniz, 2017). Despite this prohibition, evidence suggests that Mediterranean purse seine fishers might still use light during the fishing season, inferred from the increased apparent fishing hours during the lunar's dark phase. This indicates a possible reliance on artificial light, as natural lunar cycles wouldn't typically impact fishing activities to this extent.

Cloud cover also appears to play a significant role in purse seine fishing activities (Cirim et al., 2023). Increased fishing efforts during overcast conditions, irrespective of lunar illumination, suggest that both natural and artificial light sources are pivotal in these operations. Fishing vessels often utilize powerful lights to attract fish by night, a technique also employed by various other fishing methods and even some non-fishing marine vessels.

The use of light in purse seine fishing incorporates an array of techniques. Typically, fishing vessels equipped with a high light source illuminate the fishing grounds after dark and wait for an adequate amount of fish to congregate. Once the desired volume of fish is amassed, the purse seine fishing vessel undertakes its operation. This practice of nocturnal light emission is also employed by trawlers, longline fishing vessels, and extending net boats. Interestingly, this practice is occasionally executed by commercial marine vessels not actively engaged in fishing operations.

Despite the effectiveness of light usage in attracting significant fish stocks, its use, particularly in the Mediterranean, is considered illicit and subject to rigorous inspection by the Ministry of Agriculture and the Coast Guard Command. Infringements of this prohibition are met with punitive measures aimed at curbing such practices. Nonetheless, the exact nature of these sanctions, their enforcement, and their effectiveness in deterring such practices require further exploration.

In the Mediterranean region, particularly in the Eastern Mediterranean and Iskenderun Bay, the illegal practice of using lights in purse seine fishing has been prevalent despite being banned for a considerable period (Göktürk & Deniz, 2017). This illicit fishing activity poses significant disruptions, primarily to purse seine fishing vessels. The main objective of purse seine fishing in this region is to capture limited seasonal aggregating species. Long-term and continuous visible fishing operations are not feasible in this area. Consequently, during periods when purse seine fishermen are unable to catch pelagic species, especially during lunarlit nights, they resort to using lights to conduct night fishing. However, this practice is illegal, and as a result, these fishermen occasionally face severe penalties and sanctions.

Compared to the overall Mediterranean region, this specific area exhibits a shallower coastal structure. When lights are employed, they not only attract pelagic fish but also economically valuable demersal species, leading to a diverse composition of the catch in purse seine fishing (Can et al., 2006; Can & Demirci, 2012). This situation creates unfair

competition among different fishing fleets, particularly those engaged in bottom trawling, which targets demersal fish. These fleets express their concerns regarding the disturbance caused by purse seine fishing activities. Despite the prohibition on using lights in purse seine fishing in the region, the practice persists, exerting additional pressure on demersal fish populations that are already subjected to excessive fishing.

In purse seine fishing, fishermen in this region use a variety of methods. As shown in Figure 6, lights powered by batteries and generators, placed on the sea surface with a simple buoy system, create challenges for enforcement because they appear ownerless. Additionally, other fishing vessels in the area, especially trawlers, support purse seine vessels in illegal light usage by turning on lights at night. In the current situation, preventing the use of lights and ensuring adequate enforcement seem difficult.

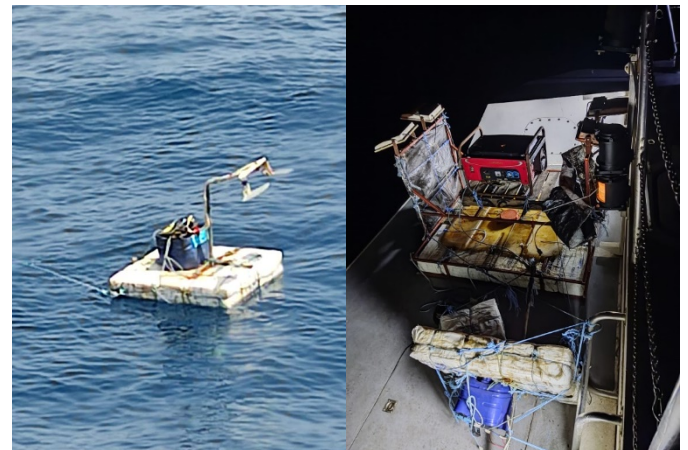


Figure 6. Illegal light usage devices in Iskenderun Bay used by purse seine fishermen, with lights on the left powered by batteries and those on the right by generators, placed using a simple buoy system

To mitigate the effects of using lights in purse seine fishing in this region, additional legal regulations are necessary. Granting permission for the use of lights in purse seine fishing would escalate fishing pressure significantly in the area, as purse seine vessels from various locations in Türkiye would converge on the region for both the utilization of lights and fishing purposes. It is evident that additional measures need to be implemented to ensure the economic and biological sustainability of the fishing industry. Monitoring the landed catch from the limited number of purse seine fishing vessels in this region and implementing additional measures to address economic and biological concerns are crucial. Consequently, it would be beneficial to impose restrictions on purse seine fishing vessels from venturing out to sea during periods of darkness, if deemed necessary. Such regulations would effectively regulate

the use of lights in purse seine fishing, eliminate unfair competition among fishing fleets, and alleviate the impact of current fishing pressure on fish stocks.

Conclusion

The Turkish Mediterranean coasts are important fishing areas for Türkiye, but these regions face challenges such as overfishing, intense maritime activities, industrialization, and high population density along the coast. To address these issues and mitigate their impacts, targeted solutions must be implemented. Monitoring and regulation of purse seine fishing vessels, which are few in number but have a significant impact on the ecosystem, should be increased. An action plan should be promoted by fisheries inspectors and managers. These measures will contribute to the ecological and economic sustainability of fishing activities on the Turkish Mediterranean coasts.

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Compliance With Ethical Standards

Authors' Contributions

SD: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing

EŞ: Methodology, Data curation, Writing – original draft, Writing – review & editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data Availability

The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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RESEARCH ARTICLE

Microplastic contamination of *Holothuria (Thymiosycia) arenicola* Semper, 1868, *Holothuria pardalis* Selenka, 1867, sediments and seawater from Karachi Coast, Northern Arabian Sea, Pakistan

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ABSTRACT

Microplastics (MPs) are posing an increasing threat to the marine environment, affecting marine ecosystems and posing potential risks to human health through the food chain. This study aims to investigate the MP contamination of *Holothuria (Thymiosycia) arenicola* Semper, 1868, *Holothuria pardalis* Selenka, 1867, sediments and seawater from Buleji coasts of Karachi, Pakistan. The MP contamination was determined in the different body parts of sea cucumber species and both seawater and sediment samples were contaminated with microplastics. The results show that *H. arenicola* contains more microplastics than *H. pardalis*. In terms of shape and colour, fibres were the most common form of MPs (>99%), with black being the predominant colour. The highest to the lowest amount of MPs was determined as gut (52±26 pieces/individual in *H. arenicola* and 31±14 pieces/individual in *H. pardalis*), coelomic fluid (18±10 pieces/individual in *H. arenicola* and 26±15 pieces/individual in *H. pardalis*), respiratory tree (22±11 pieces/individual in *H. arenicola* and 14±9 pieces/individual in *H. pardalis* and tentacles (13±8 pieces/individual in *H. arenicola* and 10±5 pieces/individual in *H. pardalis*), respectively. Determination of MP pollution in these sea cucumbers and their surrounding environment is very important in terms of the importance of these organisms in the marine ecosystems. The biological impacts of MPs on sea cucumbers and other marine organisms can eventually affect humans through the food chain. Therefore, the paper advocates for the development of policies to monitor and reduce MP pollution in marine ecosystems.

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Introduction

Anthropogenic pollutants, especially microplastics, which have become a major part of the world's marine litter, are posing an increasing threat to the marine environment. MPs, defined as plastic particles measuring less than 5 mm, originate from various sources, including the breakdown of larger plastic items and the shedding of synthetic fibres (Mathalon & Hill, 2014; Isobe et al., 2017; Concoli et al., 2019; Cincinelli et al., 2019).

Their widespread presence in marine ecosystems not only increases the risk to marine organisms but also raises concerns about their eventual entry into the human food chain. The Arabian Sea, a vital marine ecosystem (Zafar et al., 2018), is no exception, and the coastal waters of Karachi have been reported to harbour significant amounts of plastic pollution, which is likely to have detrimental effects on local marine biota (Ahmed et al., 2023; Tariq et al., 2024).

Among the numerous species affected by MPs, sea cucumbers, especially *Holothuria (Thymiosycia) arenicola* Semper, 1868 and *Holothuria pardalis*, are of significant ecological importance (Bat et al., 2020; Bhuyan et al., 2024). Sea cucumbers play an important role in maintaining benthic health through their feeding activities, which facilitate nutrient cycling, sediment bioturbation, and organic matter recycling (Sezgin et al., 2008; Ahmed & Bat, 2015, 2020; Ahmed et al., 2017, 2023). Additionally, these echinoderms act as biological indicators of coastal ecosystem health due to their sensitivity to environmental changes and pollutants (Ahmed et al., 2015, 2017, 2018, 2019). Their placement at the lower trophic levels of the marine food web also raises implications for larger marine and human consumers, making them critical to studying the effects of MPs in marine environments (Ahmed et al., 2023).

Despite their ecological importance, there is a scarcity of research specifically assessing the accumulation and potential health effects of MPs in sea cucumbers, particularly in the context of the Arabian Sea. Understanding the concentration of MPs in species *H. arenicola* and *H. pardalis* is essential, not only for assessing the overall health of coastal ecosystems but also for evaluating the risks posed to human health through seafood consumption. Given the rising trend of MP pollution globally, coupled with the increasing demand for seafood, there is an urgent need to investigate and characterize the level of contamination within these economically and ecologically significant organisms.

The accumulation of MPs in sea cucumbers, particularly in regions such as the Arabian Sea, poses significant ecological concerns. Sea cucumbers, as deposit feeders, play a crucial role in marine ecosystems by recycling nutrients and enhancing sediment health. However, their feeding habits make them particularly vulnerable to MP ingestion. This study aims to fill a critical gap in the current understanding of MP pollution in marine food webs by analysing samples of *H. arenicola* and *H. pardalis* collected from the intertidal zone of the Buleji coast in Karachi. Through this investigation, we seek to elucidate the extent of MP contamination in these sea cucumbers and discuss the broader implications for marine ecosystems and food safety. The results of this research will contribute to the growing body of literature on MP pollution, inform conservation strategies, and raise awareness about the urgent need for regulatory frameworks to mitigate plastic waste in the Arabian Sea.

Material and Methods

Sampling and Analysing

Sea water, sediment and sea cucumbers (*H. arenicola* and *H. pardalis*) samples were collected from Buleji (Lat: 24°50'20.41" N Long: 66°49'24.15" E), Karachi coast in 2022 (Figure 1). Sea cucumbers were collected on seasonally as North-east monsoon (Nov-Feb), inter-monsoon_1 (Mar-Apr), South-west monsoon (May-Sept), Inter-monsoon_2 (Oct). Sea water and sediments for also collected for analysis of MP monthly from January to December 2022.

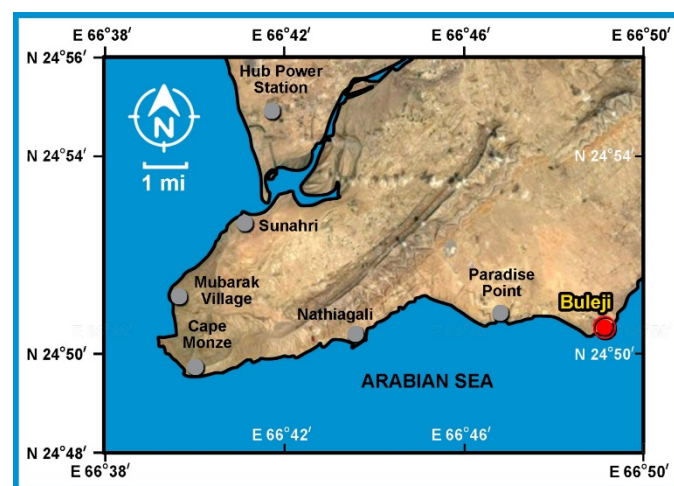


Figure 1. Study area map

The sea cucumbers (total 80 specimens) were randomly collected from intertidal zone. The collection took place during low tide to minimize disturbance to the natural habitat and to ensure that the sea cucumbers were accessible for measurement. Length (cm) and weight (g) of sea cucumbers

were measured. The body parts of sea cucumbers were removed separately and microplastic analysis in sea cucumbers was carried out in gut, respiratory tree, tentacles, and coelomic fluid. Different body parts placed in separate beakers and digestion process were applied with 30% H₂O₂. After all organic matter was digested, ZnCl was added to solutions, for the MPs to float on the surface. The particles on the surface of the solution were taken and filtered on filter paper (Whatman glass microfiber GF/C filter discs 1.2 µm pore size), and the filters were dried and stored for future analysis (Ahmed et al., 2021).

Sea water and sediment samples were collected from the Buleji coasts. 1 litre surface water samples were collected in glass bottles, which were submerged in water with floats attached to their exterior to maintain the top at a depth of 5 cm. Sediment samples were collected with a metal spoon from the top 5-10 cm of the sediment during low tide. Sediment samples were dried for three days at 65°C to reach a stable weight. A density separation process was then applied to the sediment samples. In a glass beaker, 200 g of dried sediment was mixed with 500 mL of saturated NaCl solution (density 1.2 g/cm³) and manually agitated for 2 minutes before being allowed to settle for 2 hours and floating solids were taken clean beakers. Digestion processes were applied to seawater and sediment samples. The digestion process was conducted using 20 mL of 30% H₂O₂ at 65°C for 24 hours for remove organic materials in the solutions. The final solutions were then filtered (Whatman glass microfiber GF/C filter discs 1.2 µm pore size), and the filters were dried and stored for future analysis (Ahmed et al., 2021).

The filter papers were investigated under a stereo microscope to determine the presence of MPs as well as group them according to MP groups based on shape and colour (Idris et al., 2022).

Quality Control and Quality assurance

Precautions were taken to detect and prevent the potential risk of contamination. To avoid contamination, cotton lab coats and gloves were worn during each stage of research from sampling to analysing. All tools and surfaces were washed with distilled water. Additionally, during every step, aluminium foil was used to cover the filters, beakers and solutions in order to minimize airborne contamination. Contamination control filters were used for detect airborne contamination.

Data Analysis

The number (pieces/individual for sea cucumbers, pieces/kg for sediment, pieces/L for seawater), shape (fibre,

fragment, film, and bead), and colour of MPs were recorded after each filter was examined under a stereo microscope (50X magnification).

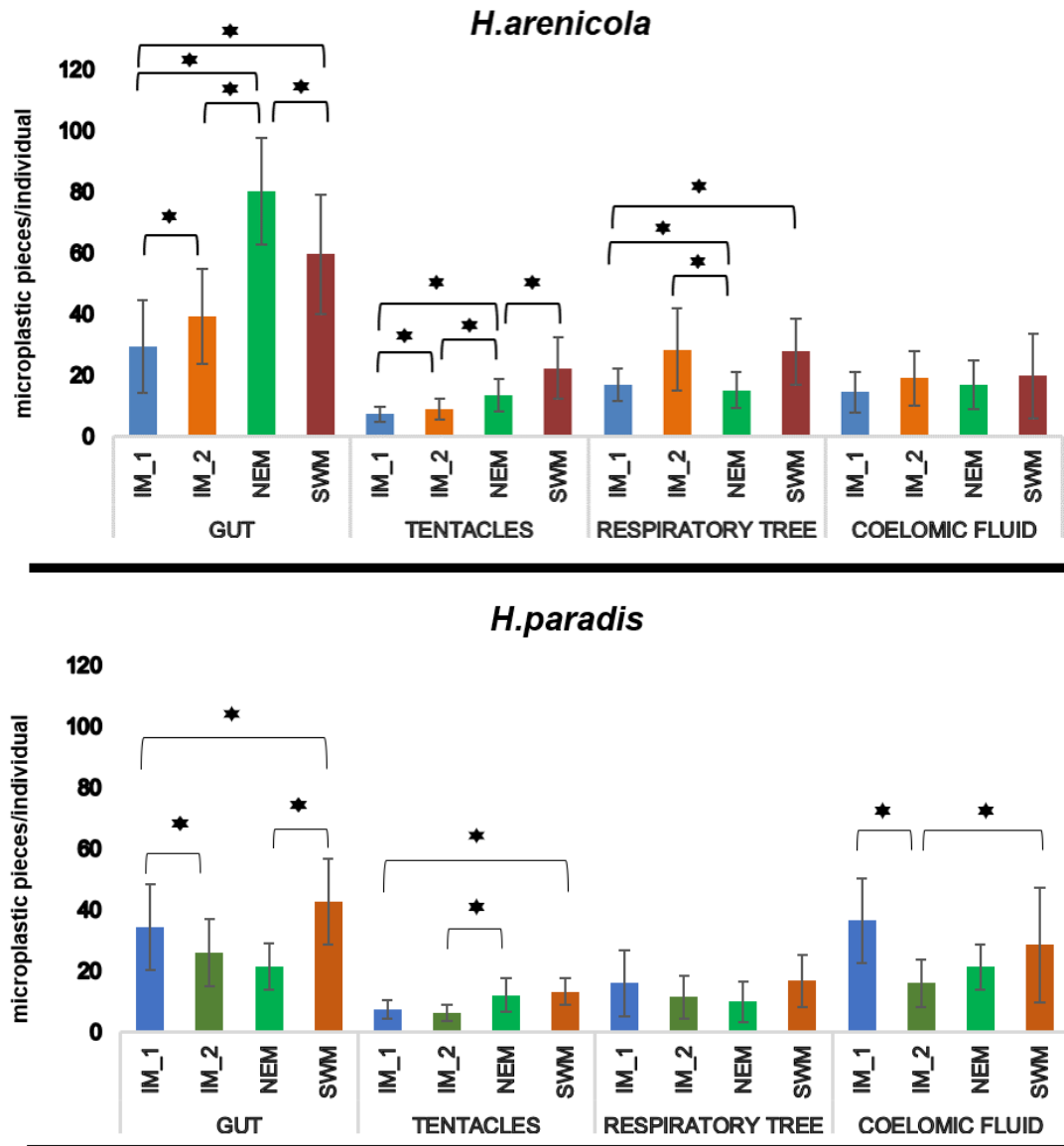
All obtained data were processed and maintained using MS Excel (Microsoft 365) and presented as mean and standard deviation (SD). The Kruskal-Wallis test was used to evaluate MP amounts among the groups (seasons in different body parts) and Mann Whitney U test was used to evaluate MP amounts between the species because the data did not have a normal distribution. The relationship between MP amounts in sediment and seawater samples and additionally sediment/seawater and different organs of sea cucumbers were investigated with Pearson correlation. The p-value was accepted as 0.05.

Results

The mean length and weight of the sea cucumber species were 23±5.30 cm and 190.18±36.41 g in *H. arenicola* and 11.30±1.87 cm and 37.58±11.14 g in *H. paradis*. The MP amounts in different parts of the *H. arenicola* and *H. paradis* and also statistical differences were shown in Figure 2 (p<0.05).

MP abundance in the gut of sea cucumber species was 52±26 pieces/individual in *H. arenicola* and 31±14 pieces/individual in *H. paradis*. The MP abundance of gut in *H. arenicola* was higher than in *H. paradis* and this was statistically significant (p<0.05). MP abundance in the tentacles of sea cucumber species was 13±8pieces/individual in *H. arenicola* and 10±5 pieces/individual in *H. paradis*. The respiratory tree of sea cucumber species contained 22±11 pieces of MP in *H. arenicola* and 14±9 pieces of MP in *H. paradis*. The MP abundance of respiratory tree in *H. arenicola* was higher than in *H. paradis* and this was statistically significant (p<0.05). Finally, MP abundance in the coelomic fluid of sea cucumber species were 18±10 pieces/individual in *H. arenicola* and 26±15 pieces/individual in *H. paradis*. The MP abundance of coelomic fluid in *H. paradis* was higher than in *H. arenicola* and this was statistically significant (p<0.05).

MPs were found in all seawater and sediment samples obtained from Buleji (Figure 3). MP abundance in sediment samples was a mean of 293±111 pieces/kg in sediment and 78±28 pieces/L in seawater. MP concentration was found as a maximum in August in both seawater and sediment samples. The lowest MP abundance was found in November in seawater and February in sediment samples. There was no relationship between microplastic abundance in seawater and in sediment samples (p>0.05).



NEM: North-east monsoon (Nov-Feb), IM_1: Inter-monsoon_1 (Mar-Apr),
SWM: Southwest-monsoon (May-Sept), IM_2: Inter-monsoon_2 (Oct)

Figure 2. The MP concentrations in the different body parts of *H. arenicola* and *H. paradisi*. Asterisk upper the vertical bars in each graph indicate the values are significantly different ($p < 0.05$) between seasons

There was a correlation between the amount of MP in the gut ($R: 0.78$), respiratory tree ($R: 0.73$), coelomic fluid ($R: 0.65$), and the number of MPs in the sediment ($p < 0.05$) and there was no correlation between the amount of MP in the seawater and the different organs of the sea cucumber species.

Four different shapes of MPs were found in samples (fibre, fragment, film and bead). Fibre was found as dominant MP shape in all samples. Fibre ratio was found over 99% in sea cucumber species and seawater samples and over 97% in sediment samples (Figure 4). Bead was found in only seawater.

Black was the dominant colour of MP detected in sea cucumber species followed by red, blue, and green (Figure 5).

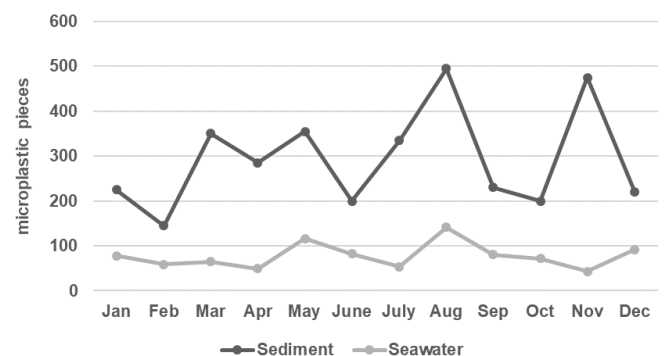


Figure 3. MP abundance in seawater (pieces/L) and sediment (pieces/kg) samples in Buleji coasts

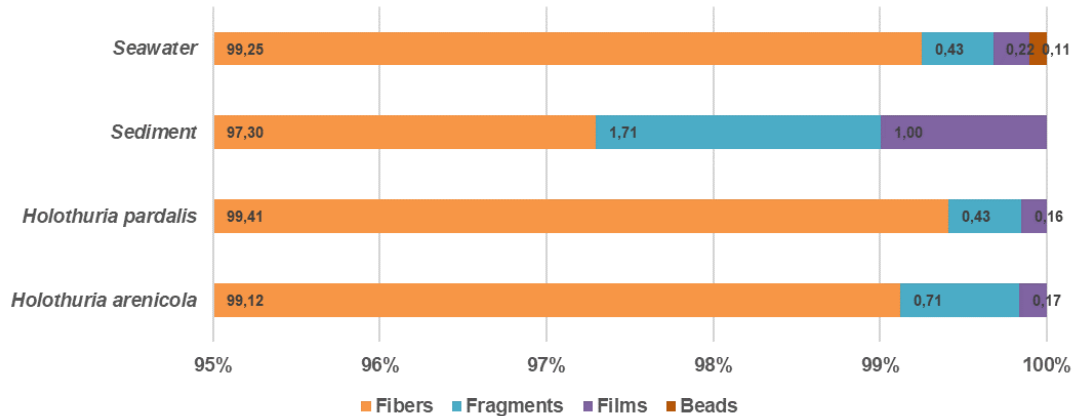


Figure 4. The shapes of MP in seawater, sediment and sea cucumber samples

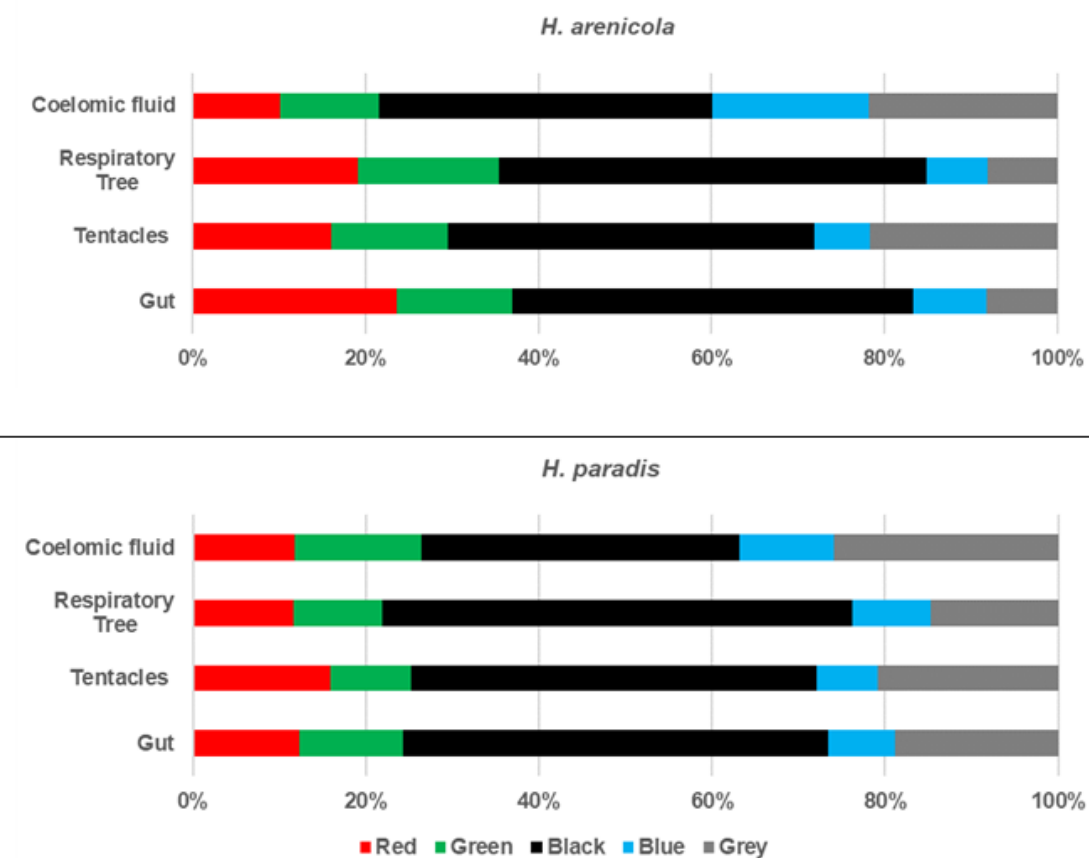


Figure 5. The colours of MPs in sea cucumber species

Discussion

Despite the growing body of literature on marine litter and MP pollution globally (Aydın et al., 2023), there remains a significant knowledge gap regarding its impact on marine life within the Arabian Sea, particularly along the coasts of Karachi (Ahmed et al., 2023; Tariq et al., 2024). Currently, there are very few studies focused on the accumulation of MPs in local marine organisms, especially keystone species like the sea cucumbers. This lack of targeted research emphasizes not only the novelty

of our results but also the urgency to understand how MPs influence the health of marine ecosystems in Karachi coasts.

The results show that *H. arenicola* contains more microplastics than *H. pardalis*. This situation could be related to the size of the organisms. Generally, larger individuals can accumulate more microplastic particles because their bodies are bigger, allowing them to ingest more food, and in this process, environmental pollutants, including microplastics, have a greater accumulation potential (Welden et al., 2018). Additionally, larger individuals typically spend more time and

cover larger areas, which could result in more exposure to microplastics (Porter et al., 2023). Furthermore, larger sea cucumber species may forage over wider areas for food, which increases the likelihood of ingesting microplastic particles. However, to fully understand this, more data and research are needed. Besides size, factors such as age, differences between species can also influence microplastic accumulation.

MP accumulation in sea cucumbers, particularly in the Arabian Sea, is an emerging area of concern due to the ecological implications of MPs on marine life. Sea cucumbers, as deposit feeders, are particularly susceptible to MP ingestion, which can occur during their feeding processes as they sift through sediments. Studies have shown that MPs can be found in the digestive tracts of various sea cucumber species, indicating a significant level of exposure and potential bioaccumulation (Mohsen et al., 2019; Idris et al., 2022; Ahmed et al., 2023; Sulardiono et al., 2023). The presence of MPs in *H. arenicola* and *H. pardalis* raises immediate concerns regarding the bioavailability of MPs within the marine food web and their potential transfer up the trophic levels, impacting larger marine fauna and human consumers alike. The results obtained from our study reveal the extent of MP contamination in these vital sea cucumber species, thereby serving as a critical indicator of the broader health of the marine environment in which they reside. Such results underscore the interconnectedness of marine ecosystems, where MPs in sea cucumbers may cascade through food webs, ultimately affecting biodiversity, and human health.

Research conducted in different marine environments has documented the presence and types of MPs ingested by sea cucumbers. For instance, a study on *H. atra* in Indonesia with fibres being the dominant type (Sulardiono et al., 2023). Similarly, another study reported a high abundance of MPs in sea cucumbers from the seagrass ecosystem of Bintan Island, Indonesia, again with fibres being the dominant type (Idris et al., 2022) and also similarly fibre was dominant and all sea cucumber individuals contaminated with MPs in *H. leucospilota* in the Karachi coasts of Pakistan. These results showed the similarity with our results.

In the context of the Arabian Sea and Karachi, the accumulation of MPs can be attributed to various anthropogenic activities, including coastal development and tourism, which contribute to marine pollution (Ahmed et al., 2023). The ingestion of MPs by sea cucumbers can lead to adverse health effects, including potential toxicity and disruption of physiological processes. For example, MP fibres

can breach the tissues of the respiratory tree during respiration, leading to their presence in the coelomic fluid, which may affect the overall health of the organism (Mohsen et al., 2020, 2023). Furthermore, the ingestion of MPs has been linked to the transfer of harmful additives and pollutants, posing additional risks to marine ecosystems (Sharma et al., 2021).

The implications of MP accumulation in sea cucumbers extend beyond individual health, as these organisms play a crucial role in marine ecosystems. They contribute to nutrient cycling and sediment turnover, and their consumption of MPs raises concerns about the potential transfer of these contaminants through the food web (Courteney-Jones et al., 2017; Mohsen et al., 2019). As such, monitoring MP levels in sea cucumbers can serve as an effective method for assessing the health of marine environments and the extent of plastic pollution (Courteney-Jones et al., 2017; Mohsen et al., 2019).

The sources of MPs in the Arabian Sea and Karachi are multifaceted, including urban runoff, sewage discharge, and the degradation of fishing gear (Ahmed et al., 2023; Jawad Al-Shaikh Ali et al., 2023). In this study, synthetic fibres are a major contributor to MP pollution, in previous studies conducted in the region, it was generally assumed that they were released into the marine environment through wastewater discharge (Ahmed et al., 2023). Additionally, physical processes such as UV degradation can fragment larger plastics into MPs. This is particularly concerning given the high levels of MP contamination reported in sediments along the Arabian coast, which directly correlates with the levels found in local marine fauna, including sea cucumbers (Idris et al., 2022; Ahmed et al., 2023).

Research has demonstrated that MPs can accumulate in the digestive systems of sea cucumbers, with studies revealing a positive correlation between MP concentrations in sediments and those found in the organisms themselves (Ahmed et al., 2023). This suggests that sea cucumbers could serve as bioindicators for monitoring MP pollution in marine sediments. The ingestion of MPs can lead to various physiological impacts, including potential toxicity and disruption of normal feeding, digestive processes and growth (Mohsen et al., 2020, 2023). Furthermore, the presence of MPs in sea cucumbers raises concerns about the transfer of these pollutants through the food web, ultimately affecting higher trophic levels, including humans who consume seafood (De-la-Torre et al., 2019).

The presence of MPs in various anatomical parts of sea cucumbers, including the gut, respiratory tree, tentacles, and coelomic fluid, has become a critical area of research due to its

implications for marine ecosystems and food safety. The highest to the lowest amount of MPs was determined as gut (52 ± 26 pieces/individual in *H. arenicola* and 31 ± 14 pieces/individual in *H. paradis*), coelomic fluid (18 ± 10 pieces/individual in *H. arenicola* and 26 ± 15 pieces/individual in *H. paradis*), respiratory tree (22 ± 11 pieces/individual in *H. arenicola* and 14 ± 9 pieces/individual in *H. paradis* and tentacles (13 ± 8 pieces/individual in *H. arenicola* and 10 ± 5 pieces/individual in *H. paradis*), respectively in this study. Results showed statistical differences.

In the gastrointestinal tract, studies have shown that sea cucumbers can accumulate different types of MPs, including fibres, fragments, and pellets (Mohsen et al., 2019; Idris et al., 2022; Ahmed et al., 2023; Sulardiono et al., 2023). For instance, research on MP abundance in the gut content of sea cucumber showed that MP content ranged from 0-30 pieces per individual in *Apostichopus japonicus* in China (Mohsen et al., 2019), 72.3 pieces per individual in *Stichopus horrens* in Malaysia (Husin et al., 2021), 15.3 to 40.45 pieces per individual in *H. leucospilota* in Pakistan (Ahmed et al., 2023). The present study is similar to the studies mentioned above and MP abundance in the gut were found 52 ± 26 pieces/individual in *H. arenicola* and 31 ± 14 pieces/individual in *H. paradis*.

The respiratory tree of sea cucumbers also serves as a site for MP accumulation. MPs can enter the respiratory system during the process of respiration, where water is drawn in through the anus and filtered through the respiratory tree (Mohsen et al., 2023). Studies have reported MP concentrations in the respiratory trees of sea cucumbers, with findings indicating that these organisms can ingest between 8.9 to 9.55 MP pieces per individual in Karachi (Ahmed et al., 2023), MP abundance in the tentacles of sea cucumber species was 13 ± 8 pieces/individual in *H. arenicola* and 10 ± 5 pieces/individual and consequently, similar results were obtained in nearby regions. The inhalation of MPs can lead to potential physiological impacts, including respiratory distress and impaired gas exchange, which are critical for the survival of these organisms (Mohsen et al., 2020, 2023).

Moreover, MPs have been detected in the tentacles of sea cucumbers, which are essential for feeding and sensory perception. The tentacles can inadvertently capture MPs while the sea cucumber feeds, further contributing to the overall burden of MPs within the organism (Ahmed et al., 2023). Studies have reported MP concentrations in the respiratory trees of sea cucumbers (*H. leucospilota*) were 8.9 to 9.55 MP pieces per individual in Karachi (Ahmed et al., 2023), MP

abundance in the tentacles of sea cucumber species was 13 ± 8 pieces/individual in *H. arenicola* and 10 ± 5 pieces/individual in *H. paradis*, similar results were obtained in nearby regions. The presence of MPs in the tentacles may also affect the organism's ability to detect food and interact with its environment, potentially leading to reduced feeding efficiency and altered behaviour (Sulardiono et al., 2023; Ahmed et al., 2023).

The coelomic fluid, which serves as a medium for gas exchange and nutrient transport in sea cucumbers, has also been found to contain MPs. Research indicates that MPs can transfer from the digestive tract into the coelomic fluid, with studies reporting among the 0-19 MP particles per individual in this fluid (Mohsen et al., 2019, 2020). MP abundance in the coelomic fluid of sea cucumber species were 18 ± 10 pieces/individual in *H. arenicola* and 26 ± 15 pieces/individual in *H. paradis* in this study. This transfer raises concerns about the systemic effects of MPs on the health of sea cucumbers, as they may interfere with physiological processes and lead to toxicological effects (Mohsen et al., 2019, 2023).

The composition of microplastics were evaluated in the current study. The size and shape of MPs ingested can vary significantly, with fibres often being the most prevalent due to their widespread presence in marine environments (Mohsen et al., 2023; Ahmed et al., 2023; Sulardiono et al., 2023). The feeding behaviour of sea cucumbers, which involves the selective ingestion of sediment particles, contributes to the accumulation of MPs in their digestive systems (Widianingsih et al., 2023). Black was the dominant colour of MP detected in sea cucumber species. Microplastics primarily arise from the degradation of plastics and specifically, tire wear particles, which are predominantly black, contribute significantly to aquatic and terrestrial microplastic pollution (Han et al., 2024). The runoff from the road can be a source of microplastics as the sampling location is on the roadside. Additionally, wastewater effluents containing black microplastics from textile industries further augment their environmental concentrations. Wastewater infrastructure is inadequate in Pakistan's major cities, especially in metropolitan areas such as Lahore, Karachi, and Islamabad. So, these plastic particles may be present in the research areas from wastewater effluents.

There are seasonal fluctuations in the microplastic abundance of sea cucumbers, and they found statistically significant. However, no clear seasonal increase or decrease in each body part was observed. Changes in environmental conditions during different seasons can impact the sources and transport mechanisms of microplastics (Anastasiou, 2020). For

instance, increased rainfall during certain seasons may lead to higher runoff from urban and agricultural areas, consequently enhancing the availability of microplastics in adjacent marine environments. Despite the observed seasonal fluctuations in the surrounding environment, studies have indicated that there is no clear, uniform pattern regarding microplastic abundance in various body parts of sea cucumbers.

A positive relationship was found between in the different organs and the amount of MPs in the sediment in this study. Similar results were found by Ahmed et al. (2023) and Mohsen et al. (2019). Determination of MP pollution in these sea cucumbers and their surrounding environment is very important in terms of the importance of these organisms in the marine ecosystems. This research can be used as an indicator for the sea cucumber might be used as a bioindicator of MP pollution in the sediment (Mohsen et al., 2019; Ahmed et al., 2023). Sea cucumbers are one of the indicator biotas that play an important role in ecology, contribute to sediment turnover and nutrient cycling, and help fertilize the substratum by stirring the bottom (Husin et al., 2021; Idris et al., 2022). The implications of MP accumulation extend beyond individual species; they threaten the overall health of marine ecosystems.

Conclusion

In conclusion, the accumulation of MPs in seawater, sediment, and sea cucumbers in the Arabian Sea is a significant environmental issue that warrants further investigation. Understanding the extent of MP ingestion and its ecological consequences is essential for developing effective management strategies to mitigate marine pollution and protect marine biodiversity.

MPs in the different parts of sea cucumbers highlight the pervasive nature of plastic pollution in marine ecosystems. The implications of such accumulation are profound, affecting not only the health of sea cucumbers but also the broader marine food web and human health through seafood consumption. Continued research is essential to understand the full extent of MP impacts on marine organisms and to develop strategies for mitigating plastic pollution in marine environments.

Our research highlights the pressing need for continued monitoring and investigation of MP pollution along the Karachi coast and beyond. Future studies should aim to include a broader range of marine organisms, evaluating not only their MP loads but also the biological effects on their physiology, behaviour, and reproductive success. Longitudinal studies could provide critical insights into seasonal and spatial

variations in MP accumulation, enhancing our understanding of source and sink dynamics within this marine environment. Additionally, findings of this study can inform policymaking and conservation efforts aimed at mitigating plastic pollution. By raising awareness of the ramifications of MP contamination on marine ecosystems and food safety, stakeholders—including policymakers, conservationists, and the fishing community—can be encouraged to implement strategies aimed at reducing plastic waste and enhancing ecosystem resilience. While our study lays a foundational understanding of MP presence in local sea cucumber *H. arenicola* and *H. pardalis* populations, it also serves as a clarion call for increased scientific inquiry into the implications of MPs on marine biodiversity and ecosystem health. By fostering a greater understanding of these issues, we hope to contribute to the development of effective strategies to protect our marine environments for future generations.

Compliance With Ethical Standards

Authors' Contributions

QA: Conceptualization, Investigation, Writing – review & editing;

AÖ: Writing – original draft, Formal Analysis, Data visualization;

QMA: Conceptualization, Writing – review & editing;

LB: Writing – review & editing, Supervision

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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RESEARCH ARTICLE

Diversity, distribution and checklist of brachyuran crabs inhabiting along the Mumbai and Konkan coast of Maharashtra, India

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ABSTRACT

The present paper includes a diversity of brachyuran crabs inhabiting along intertidal and shallow subtidal regions of Mumbai and Konkan coasts of Maharashtra, India. During a survey from 2014 to 2016, a total of 55 species of crabs, belonging to 45 genera and 22 families, were recorded from these areas. According to the Shannon Weiner index (H'), the maximum diversity was recorded around Mumbai rocky shores, having mixed types of microhabitats. The values of Shannon-Weiner index (H') varied between 2.3 and 3.0 for Mumbai as well as Jaitapur, whereas, evenness varied between 0.86 and 0.97, while richness fluctuated between 3.2 and 5.9. Seasonal observations revealed maximum diversity in pre monsoon and post monsoon at Marine Drive shore. The present study also reports total 10 species with 4 new records from intertidal regions of west coast of India (*Xenophthalmus wolfii*, *Ozius rugulosus*, *Achaeus cf spinosus*, *Leptodius cf sanguines*); 5 new distributional records for Maharashtra state (*Ozius tuberculosus*, *Ocypode brevicornis*, *Atergatis laevigatus*, *Pilumnopus convexus*, *Scylla olivacea*); and 1 first record of genus from Indian Ocean (*Anomalifrons garthii*). The results of present study can be used as baseline data for conservation and management of these ecologically sensitive fauna.

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Introduction

The intertidal areas are the boundary between marine and terrestrial habitats, which are subjected to variable degrees of

physical factors and biological processes (Nybakken, 1993; Bertness, 1999), and their interactions determine the spatial and temporal patterns of biological resources on rocky shores (Terlizzi et al., 2002). Even though occupied almost entirely by

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marine organisms, they are regularly visited by shorebirds and even passerines that feed on intertidal animals. The crabs are common and ecologically important predators in benthic marine ecosystems. Most of the crabs feed on detritus or organic matter and play role in marine food web as prey or predator thus maintaining the ecosystem functions, in addition; they serve as an important food source livelihood for coastal communities. They are abundant in the rocky intertidal area which provides a unique habitat for them to hide under the rocks and burry in the sand and mud holes. The rocky intertidal zone serves as a natural laboratory for elucidating the role of physical and biological factors in determining the abundance and distribution of organisms in nature (Tomanek & Helmuth, 2002).

The brachyuran species assemblage in intertidal habitats of the west coast of India have been poorly studied until Fabricius (1775) reported first brachyuran crab from west coast of India. After a gap of 60 years, Milne Edwards (1852) reported 6 species from Bombay coast. Thus, studies on brachyuran fauna of Indian seas were initiated by Milne Edwards (1834) and de Man (1887) but confined to the investigations of species inhabiting the deep seas. However, the first consolidated work on brachyuran crabs from west coast of India was published by Pillai (1951), who described 51 marine and estuarine species from Travancore coast (now Kerala coast). Along Maharashtra coast, the first work on shallow water intertidal regions was published by Chhapgar (1957) who described 81 species. Thereafter, Sankolli & Shenoy (1975) reported one majid crab from Ratnagiri district of Maharashtra, followed report of three new records of portunid crabs from Maharashtra coast by Aravindakshan & Karbhari (1985). Subsequently, Khot et al. (2016) reported a brachyuran crab for first time from Maharashtra coast and established the relationship between carapace length and weight (Khot & Jaiswar, 2018). A perusal of literature on crabs from intertidal regions of west coast of Maharashtra is confined to Chhapgar (1957). In recent times, the aquatic biodiversity, including crustaceans, is affected. The brachyuran crabs, being benthic feeders, are most affected. Thus, there is need to assess the status of this group of animals. Since, Maharashtra is most industrialized state, it has become essential to assess the present status of marine crabs from the state. Further, there is lack of record on brachyuran crab diversity after Chhapgar who reported crabs from Maharashtra in 1991. Thus, the understanding of the status of crab diversity in intertidal regions is important where anthropogenic pressure is ever increasing in the sea. Thereafter, no data are available on documentation or taxonomy of brachyuran crabs from

Maharashtra. Therefore, the understanding of the status of crab diversity in intertidal regions is important where anthropogenic pressure is ever increasing in the sea.

Material and Methods

Study Area

The intertidal areas from Mumbai were Marine Drive (MD), Girgaon Chowpatty (GR), Bandstand (BA), and Uttan (UT) whereas Ganeshgule (GG) and Ambolgad (AM) from Ratnagiri; and Girye (GI) and Devgad (DV) from Sindhudurg districts were selected for the study (Figure 1). Ratnagiri and Sindhudurg district fall in Konkan region and separated by 250 km from Mumbai region. From Mumbai region, four locations were selected, whereas two locations each from Ratnagiri and Sindhudurg district were chosen to compare the variation in fauna due to anthropogenic extent.

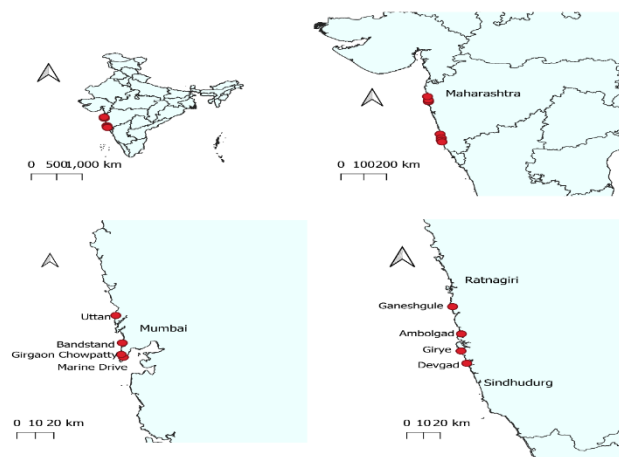


Figure 1. Study area

Habitat: Intertidal areas of Mumbai

Marine Drive

The Marine Drive (18.94°N, 72.82°E), a part of Queen's Necklace area, is three km long rocky shore which ends at Girgaon Chowpatty. The substratum of the shore is rocky with a few sandy patches and tidal pools. The upper intertidal area is artificially reclaimed with large size cement blocks and a road wall is constructed to avoid erosion of the shore, where gastropods are abundant due to lush growth of algae. The middle part of the shore is comprised of coral boulders, stones with sponges, debris like bricks and concrete structures and can be called as Zoanthus with algae & sponge zone. The lower zone, with flat rocks and boulders, is covered with Zoanthus, patches of corals, algae, gorgoniids etc. Originally the shore was

sandy, but today, it is entirely changed, much similar to the rocky shore. The rocks are covered with silt-mud discharged from municipality pipelines. The area harbours different types of algae, sponges, molluscs, crabs, corals, zooanthids, brittlestar, etc. The area is attraction for tourists for its scenic beauty. Thus, the upper intertidal area is highly disturbed due to tourist activities while mid and low tidal zones are less affected and harbour rich biological diversity.

Girgaon Chowpatty

The shore, popularly known as Girgaon Chowpatty (18.95°N, 72.81°E), is part of the Queen's Necklace area 3km from Marine Drive. The upper intertidal area is covered with coarse sand and packed with food stalls. The middle intertidal area is covered with silty-muddy, while lower intertidal area is covered with mixed substratum of sandy-muddy and silt nature. Large number of people visit here every day, which is the cause for poor ecological conditions of the shore. The shore area is gently sloping and more than 500 m is exposed during spring low tide (at 0.06 m).

Bandstand

It is an open type of natural rocky shore (19.04°N, 72.81°E), composed of rock beds and stones of various dimensions, about 15km away from Marine Drive. It is also subjected to direct wave action throughout the year. Maximum exposure was recorded to be 198 m during spring low tide (0.03 m). Shore profile is very uneven. The upper intertidal zone is covered with conglomerate rocks harbouring barnacles and oyster. Middle zone is rich in gastropod and patchy coral zone, while lower zone is represented by gastropod-algal zone. The shallow tidal pools covered mostly with algae and gorgoniids, rocky crevices, under rocks, coral boulders, cobbles and boulders with algae is the characteristics of the area.

Uttan

It is situated towards the northern end of Mumbai (19.26°N, 72.81°E), approx. 54 km from Marine Drive. Many small and large drainage channels discharge effluents into it. The upper intertidal zone is covered with coarse sand, mid intertidal zone is covered with silt and mud, while the lower intertidal zone is covered with big flat conglomerate rocks and small pebbles. The habitat recorded here are rocks, rocky crevices, boulders with algae, shallow tidal pool with algae, along with sandy, sandy-muddy and silty-muddy patches. Maximum exposure was recorded to be 0.12 m during low tide.

Habitat: Intertidal areas from Ratnagiri district

Ganeshgule

It is a small and cleaner beach (16.87°N, 73.29°E) with a small village, situated 25 kms away from Ratnagiri town. The total beach area is 1.5 km, covered with white sand in upper, middle and lower intertidal zone. One end the beach is covered with rock patch while on other end a channel discharges freshwater during monsoon. The upper intertidal zone is occupied by medium and larger laterite and conglomerate rocks covered with barnacles, algae, anemones & gastropods; middle intertidal zone with *Sabellaria* reefs and lower zone with zoanthids. The habitat is comprised of under rocks, rocky crevices, shallow tidal pools with zooanthids, cobbles & boulders with algae, shallow tidal pools with algae. Local women collect bivalves for food during low tides.

Ambolgad

The small beach (16.64° N, 73.33° E), spread in a length of 1km, is separated by a distance of 5 km from Jaitapur and 55 km from Ratnagiri. The sandy beach is divided in to three zones viz. upper intertidal zone with white sand, middle zone with mixture of brown sand and clay with black layer of organic deposits providing suitable habitat for dotillids, and lower intertidal zone with brown fine sand with suitable habitat for starfishes. The rocky patch on the shore is further divided in to upper intertidal zone with large conglomerate and laterite rocks covered with oysters and can be called as oyster zone, middle zone is covered with medium size rocks covered with small gastropods, a gastropod zone; while lower zone is covered with shallow tidal pools with zoanthids.

Habitat: Intertidal areas from Sindhudurg district

Girye

The shore is situated near a small town Girye (16.56°N, 73.22°E), at a distance of 10 km from Jaitapur under Sindhudurg district. The beach covers an area of 2.5 km. At one end, the sandy zone area of about 2 km in length, having white sand in upper, middle and lower intertidal zone and the rocky patch covers an area of about approximately 800 m, where upper intertidal zone is occupied by white sand inhabited by *Ocypode* spp, the middle zone possesses few cobbles & grapsid crabs, while the lower intertidal zone is occupied by *Zoanthus* and coral reef zone. Fishing is carried out by locals for xanthid crabs, cowries, and bivalves for food.

Devgad

It is a town (16.37°N, 73.38°E) situated at 30 km distance from Jaitapur under Sindhudurg district. The sandy area covers about 1.5 km, upper intertidal zone having white sand, middle zone with ocypodid crabs and lower zone with fine brown sand. At other end the rocky patch is spread in an area of about approximately 500 m. The habitat in these zones are conglomerate rocks with barnacles, gastropods, algae & anemones, shallow tidal pools with algae, cobbles & boulders with algae, rocky crevices, under rocks, tidal pools with coral boulders and under rocks.

Sampling and Identification

Monthly sampling of crabs was carried out from intertidal areas around Mumbai and Jaitapur during 2014-2016 by following quadrature method, (NaGISA, 2006) protocol version II and handpicking using traps and forceps. Burrowing crabs were collected by digging the burrow. Random sampling was done by using the quadrature method. Three quadrates, covering an area of 1m² each, were fixed in high, mid and low tide level during spring low tide. The number of each species of crabs present in 1m² quadrates area was counted. At each quadrate, crabs were recorded by turning over the stones and small boulders and subsequently by putting back to their original position. The density of each species was expressed as average no/m². The abundance was calculated as total number of individuals of species present in given area and categorised as Abundant, Common, Frequent, Occasional and Rare. Specimens were also collected from local fishermen operating nets in the certain areas. At each site, local fishermen used hand net, gill net. Field photographs, habitat and human pressure were also recorded during collection. Representative specimens were stored in icebox immediately to avoid shedding of legs or chelae. The specimens were brought to laboratory and washed with tap water to remove mud, adhering sand and silt.

Taxonomic surveys, for assessing the crustacean assemblages, were performed at eight selected sites, representing different type of habitats. The collected crabs were identified by using standard identification key (Ng, 1998) "The living marine resources of the Western Central Pacific" Vol. 2; Chhapgar (1957); Sethuramalingam & Ajmal Khan (1991); NIO database on Marine life of India, Volume 2 "Marine crabs of India"; Jeyabaskaran et al. (2002); CD on Brachyuran Crabs of West Coast, India, at <http://www.nio.org/Biology/brachyuran/index.html>; Marine species identification portal (www.speciesidentification.org);

research papers and monographs. The latest classification and scientific names of the species were adopted from Ng et al. (2008); website of www.sealifebase.com and [WORMS \(http://www.marinespecies.org/\)](http://www.marinespecies.org/). Identification of crabs up to species level was done by observing the general characters such as carapace shape, chelipeds, ambulatory legs, anterolateral margins, frontal margins, maxillipeds, and pair of two pleopods. The first male pleopod was used as main character to distinguish between closely related species (Sankarankutty, 1962).

Identified specimens were photographed using Canon SX520HS for bigger size body parts, while for smaller parts and gonopods, Stereo microscope (Olympus, SZX16) was used. Morphometric details such as carapace length, carapace width, weight and sex were noted for each specimen. Representative specimens were then preserved in 70% alcohol and deposited in ICAR-Central Institute of Fisheries Education and Bombay Natural History Society (BNHS) Mumbai, India with appropriate labelling. Water parameters, such as pH, temperature and salinity, of the collection areas were analysed as per APHA (1998). The analysis of Pearson's correlation between different environmental variables was performed using software STATISTICA, ver.7.

Diversity

Univariate and multivariate analyses of brachyuran abundance data were carried out using the statistical software, PRIMER v6, developed by Plymouth laboratory U.K. The univariate techniques computed were Shannon-Wiener diversity index ($H'_{\log 2}$); Margalef's richness index (d), and Pielou's evenness index (J'). Multivariate analyses included the ordination of fourth root transformed data using Bray-Curtis similarities by non-metric multi-dimensional scaling (MDS). Graphical representations like k-dominance curve, was figured based on fourth root of transformed data, and cluster analysis was performed to discern similarities between sampling stations. The value of Shannon and Weiner diversity index increased with increase in richness and evenness. It will be zero if the sample in consideration has only one species and would be maximal when all species of the sample, in consideration, have even abundances (Sagar & Singh, 1999). K-dominance plot is also known as 'ranked species abundance plot' which measures the quantity of each taxon computed for abundance, biomass, % cover or other biotic measures. The Abundance and Biomass curve (ABC) indicates undisturbed community, if biomass curve is above the abundance curve; gross disturbance if abundance curve lies above biomass curve and moderate

disturbance if the two lines intersect (Clarke & Warwick, 2001). A 'W' value measures the extent to which the biomass curve lies above the abundance curve (Clarke & Gorley, 2006). The taxonomic diversity index indicates average taxonomic distance between any two individuals chosen at random, provided they belong to two different species (Warwick & Clarke, 1995). The taxonomic distinctness index is the average path length between any two individuals chosen at random, provided they belong to two different species.

Results

Physicochemical Parameters

The values of salinity varied from 33.7 ppt (BA) to 35.1 ppt (UT) at the sites around Mumbai, and 30.1 ppt (AM) to 35.5 ppt (DV) around Jaitapur with maximum in pre monsoon. pH was found to vary from 7.9 (MD, GR, BA) to 8.2 (UT) around sites of Mumbai and 7.9 (AM) to 8.3 (DV) around sites of Jaitapur, with maximum in post monsoon and minimum in monsoon. The water temperature varied from 27.5°C to 28.9°C around Mumbai and 25.7°C to 30.5°C around Jaitapur.

Distribution and Species Composition

During the present study, a total of 55 species of crabs, belonging to 45 genera under 22 families, were recorded around Mumbai and Jaitapur (Table 1). The study also revealed a significant correlation between density of crabs and salinity (Table 2). Out of 55 species, 48 species were recorded from Mumbai area and 24 species from Jaitapur, where 17 species belonging to 9 families were common in both areas. The

average density of crabs ranged from 2 to 5 ind/m² and 3 to 4 ind/m² around Mumbai and Jaitapur coasts, whereas biomass ranged from 28.6 to 180 g/m² and 78.69 to 152.66 g/m², respectively. The population density was high during pre-monsoon and post-monsoon; wherein, maximum density was recorded at Girgaon Chowpatty followed by Uttan beach, Bandstand and Marine Drive around Mumbai locations, and Devgad and Ganeshgule around Jaitapur coast. Maximum number of species was recorded from Marine Drive (26) followed by Girgaon Chowpatty (19), Uttan (17), Devgad (16), Ganeshgule (14) Bandstand, Ambolga and Girye (13) site. Family wise percentage composition revealed maximum species under Portunidae followed by Xanthidae and Pilumnidae around Mumbai, whereas around Jaitapur, maximum species were recorded under Portunidae followed by Epialtidae and Calappidae (Figure 2). More number of families and species were recorded from Mumbai (48 species belonging to 21 families) than Jaitapur (24 species belonging to 11 families). Habitat wise analysis indicated, maximum number of species from rocky shores mixed with coral patches (33) followed by sandy-muddy (15), weedy and silty-mud (13), sandy (9) and muddy (6). Based on their abundance, the total number of individual of species present in given area were categorised as Abundant, Common, Frequent, Occasional and Rare (Table 1). Common species recorded in all seasons throughout the study area were *Charybdis lucifera*, *C. annulata*, *Thalamita crenata*, *Portunus pelagicus*, *Leptodius exaratus*, *Menippe rumphii*, *Grapsus albolineatus*, *Metopograpsus messor* and *Ocypode ceratophthalmus*.

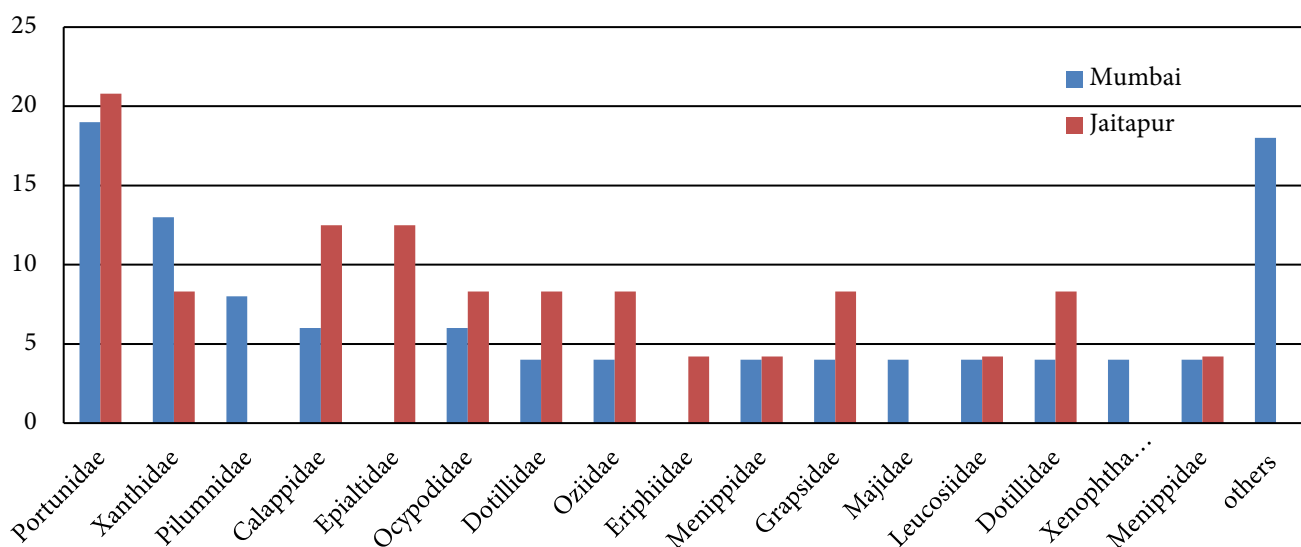


Figure 2. Family wise percentage of species around Mumbai and Jaitapur

Table 1. Abundance and checklist of brachyurans around coastal areas of Mumbai and Jaitapur, Maharashtra

Sr. no.	Species checklist	MD	GR	BA	UT	DV	GI	AM	GG
Family: Portunidae Rafinesque, 1815									
1.	<i>Scylla serrata</i> (Forskål, 1775)	-	-	C	F	-	-	-	-
2.	<i>S. tranqueberica</i> (Fabricius, 1798)	-	C	-	-	-	-	-	-
3.	<i>S. olivacea</i> (Herbst, 1796)*	-	-	-	O	-	-	-	-
4.	<i>Portunus segnis</i> (Forskål, 1775)	-	F	F	F	F	F	-	F
5.	<i>P. sanguinolentus</i> (Herbst, 1783)	C	-	C	C	C	-	-	C
6.	<i>Charybdis annulata</i> (Fabricius, 1798)	F	-	A	O	F	F	F	A
7.	<i>C. lucifera</i> (Fabricius, 1798)	A	-	F	F	F	F	F	F
8.	<i>Charybdis feriatus</i> (Linnaeus, 1758)	O	-	O	O	-	-	-	-
9.	<i>Thalamita crenata</i> Ruppell, 1830	-	-	A	F	-	F	F	F
Family: Leucosiidae Samouelle, 1819									
10.	<i>Philyra globus</i> (Fabricius, 1775)	-	R	-	-	-	-	-	-
11.	<i>Ryphila cancellus</i> (Herbst, 1783)	-	-	-	-	C	C	C	-
12.	<i>Seulocia pubescens</i> (Miers, 1877)	-	O	-	-	-	-	-	-
Family: Calappidae De Haan, 1833									
13.	<i>Ashtoret lunaris</i> (Forskål, 1775)	-	O	-	-	C	C	C	C
14.	<i>Matuta planipes</i> Fabricius, 1798	-	O	-	-	-	-	-	O
15.	<i>Calappa lophos</i> (Herbst, 1782)	-	O	-	-	O	-	-	-
Family: Euryplacidae Stimpson, 1871									
16.	<i>Trissoplax dentata</i> (Stimpson, 1858)	-	F	-	-	-	-	-	-
Family: Oziidae Dana, 1851									
17.	<i>Ozius rugulosus</i> Stimpson, 1858**	-	-	C	-	-	-	-	-
18.	<i>O. tuberculosus</i> H. Milne Edwards, 1834*	-	-	-	-	-	-	F	-
19.	<i>Epixanthus frontalis</i> (Milne Edwards, 1834)	-	C	-	C	C	-	-	C
Family: Menippidae Ortmann, 1893									
20.	<i>Myomenniepe hardwicki</i> (Gray, 1831)	O	-	C	-	-	-	-	-
21.	<i>Menippe rumphii</i> (Fabricius, 1798)	A	-	F	-	-	A	C	C
Family: Eriphiidae MacLeay, 1838									
22.	<i>Eriphia smithii</i> MacLeay, 1838	-	-	-	-	-	O	-	-
Family: Dotillidae Stimpson, 1858									
23.	<i>Dotilla myctiroides</i> (H. Milne Edwards, 1852)	-	C	-	C	-	-	A	-
24.	<i>D. blanfordi</i> Alcock, 1900	-	C	-	A	-	-	A	-
Family: Majidae Samouelle, 1819									
25.	<i>Schizophrys aspera</i> (H. Milne Edwards, 1834)	F	-	-	-	-	-	-	-
26.	<i>Prismatopus aculeatus</i> (H. Milne Edwards, 1834)	-	-	-	-	-	-	-	-
Family: Inachidae MacLeay, 1838									
27.	<i>Achaeus cf spinosus</i> **	O	-	-	-	-	-	-	-

Table 1. (continued)

Sr. no.	Species checklist	MD	GR	BA	UT	DV	GI	AM	GG
	Family: Parthenopidae MacLeay, 1838								
28.	<i>Enoplolambrus pransor</i> (Herbst, 1796)	-	R	-	-	-	-	-	-
	Family: Ocypodidae Rafinesque, 1815								
29.	<i>Ocypode ceratophthalmus</i> (Pallas, 1772)	-	C	-	C	C	F	C	A
30.	<i>O. brevicornis</i> H. Milne Edwards, 1837*	-	-	-	-	R	-	-	-
31.	<i>Austruca annulipes</i> (H.Milne Edwards, 1837)	-	C	-	-	-	-	-	-
32.	<i>Gelasimus vocans</i> (Linnaeus, 1758)	-	C	-	-	-	-	-	-
	Family: Grapsidae MacLeay, 1838								
33.	<i>Metopograpsus messor</i> (Forskål, 1775)	-	-	F	C	F	F	F	F
34.	<i>Grapsus albolineatus</i> Latreille in Milbert, 1812	A	-	-	F	F	C	F	F
	Family: Macrophthalmidae Dana, 1851								
35.	<i>Macrophthalmus sulcatus</i> (H.Milne Edwards, 1852)	-	C	-	C	-	-	-	-
	Family: Plagusiidae Dana, 1851								
36.	<i>Plagusia squamosa</i> (Herbst, 1790)	O	-	-	-	-	-	-	-
	Family: Varunidae H. Milne Edwards, 1853								
37.	<i>Varuna litterata</i> (Fabricius, 1798)	-	O	-	-	-	-	-	-
	Family: Xanthidae MacLeay, 1838								
38.	<i>Leptodius exaratus</i> (H. Milne Edwards, 1834)	C	-	A	C	C	C	C	C
39.	<i>L. cf sanguineus</i> (H. Milne Edwards, 1834)**	R	-	-	-	-	-	-	-
40.	<i>Macromedaeus crassimanus</i> (Milne Edwards, 1867)	-	-	-	-	R	-	-	-
41.	<i>Atergatis laevigatus</i> *	R	-	-	-	-	-	-	-
42.	<i>A. integerrimus</i> (Lamarck, 1818)	O	-	-	-	-	-	-	-
43.	<i>Atergatopsis amoyensis</i>	R	-	-	-	-	-	-	-
44.	<i>Demania baccalipes</i> (Alcock, 1898)	R	-	-	-	-	-	-	-
	Family: Epialtidae MacLeay, 1838								
45.	<i>Menaethius monoceros</i> (Latreille, 1825)	-	-	-	-	O	O	-	-
46.	<i>Doclea rissoni</i> Leach, 1815	-	-	-	-	-	-	R	-
47.	<i>Hyastenus planasius</i> (Adams & White, 1848)	O	-	-	-	O	-	-	-
	Family: Dorippidae MacLeay, 1838								
48.	<i>Dorippoides facchino</i> (Herbst, 1785)	-	R	-	-	-	-	-	-
	Family: Pilumnidae Samouelle, 1819								
49.	<i>Pilumnus longicornis</i> Hilgendorf, 1878	O	-	-	-	-	-	-	-
50.	<i>Heteropilumnus angustifrons</i> (Alcock, 1900)	C	-	C	C	-	-	-	-

Table 1. (continued)

Sr. no.	Species checklist	MD	GR	BA	UT	DV	GI	AM	GG
51.	<i>Pilumnopus convexus</i> (Maccagno, 1936)*	O	-	-	-	-	-	-	-
52.	<i>Glabropilumnus laevis</i> (Dana, 1852) Family: Hymenosomatidae MacLeay, 1838	O	-	-	-	-	-	-	-
53.	<i>Elamena cristatipes</i> Gravely, 1927 Family: Xenophthalmidae Stimpson, 1858	R	-	-	-	-	-	-	-
54.	<i>Anomalifrons garthii</i> (Sankarankutty, 1969)***	R	-	-	-	-	-	-	-
55.	<i>Xenophthalmus wolfii</i> Takeda & Miyake, 1970**	O	A	-	-	-	-	-	-

Note: A: Abundant; C: Common; F: Frequent; O: Occasional; R: Rare. *New record from Maharashtra. **New record from west coast of India. ***First record of genus from Indian Ocean.

Table 2. Spearman rank order correlations

Relations	Valid	Spearman	t(N-2)	p-level
pH & Density	24	0.369104	1.862789	0.075899
pH & Biomass	24	0.339889	1.695142	0.104158
Temp & Density	24	0.195686	0.935944	0.359463
Temp & Biomass	24	0.347183	1.736444	0.096470
Salinity & Density	24	0.473565	2.521937	0.019413
Salinity & Biomass	24	0.097317	0.458633	0.650996

Note: Spearman Rank Order Correlations MD pairwise deleted. Marked correlations are significant at $p < .05000$.

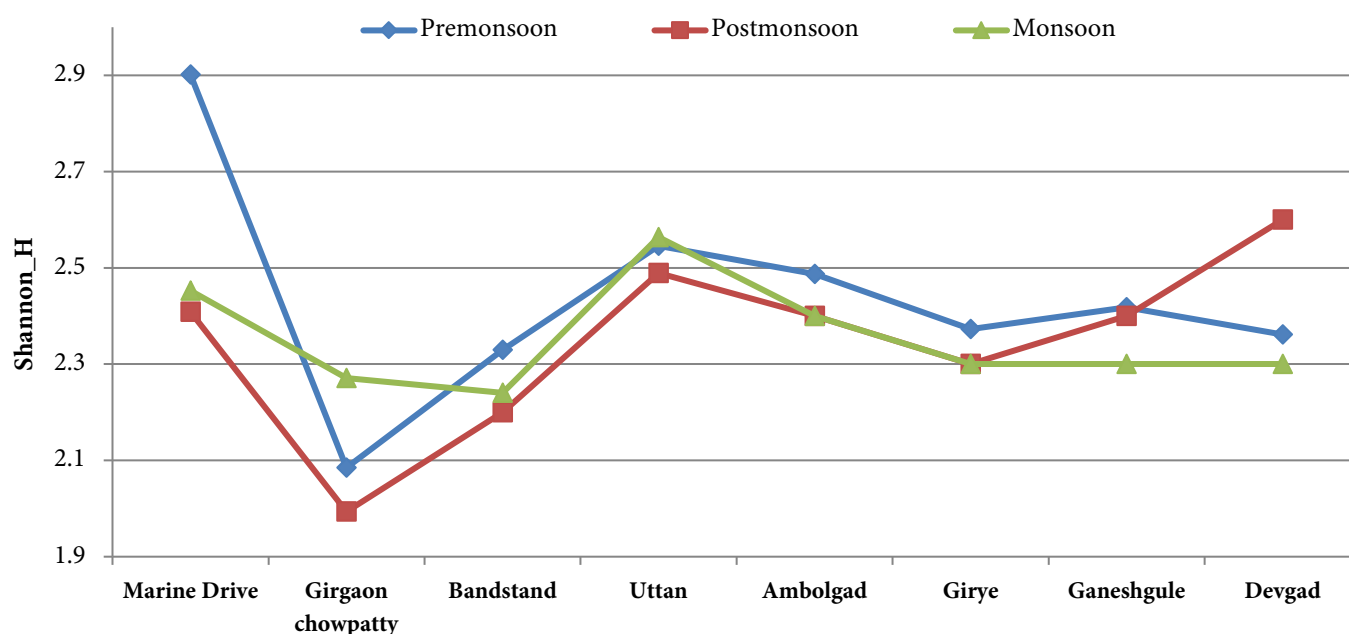
Figure 3. Seasonal and spatial variations in diversity index H' at different sites

Table 3. Diversity indices at different sites at Mumbai and Jaitapur locations

Diversity indices	Total species (S)	Total individuals (N)	Species richness (d)	Pielou's evenness (J')	Shannon diversity [H'(loge)]	Taxonomic diversity (Delta)	Taxonomic distinctness (Delta*)	Average Taxonomic distinctness (Delta+)	Variation in Taxonomic distinctness (Lambda+)	Average Phylogenetic diversity (Phi+)	Total Phylogenetic diversity (sPhi+)
Marine Drive	25	58	5.911	0.955	3.073	55.753	57.853	57.933	42.396	48.800	1220
Girgaon Chowpatty	18	41	4.578	0.869	2.511	53.951	59.542	59.216	15.071	56.667	1020
Bandstand	13	36	3.349	0.921	2.361	51.492	56.125	53.846	105.720	49.231	640
Uttan	16	40	4.066	0.942	2.611	53.564	57.077	54.667	98.222	48.750	780
Ambolgad	13	41	3.231	0.976	2.505	55.488	59.168	58.974	19.461	58.462	760
Girye	13	37	3.323	0.956	2.452	54.745	58.712	58.205	32.676	56.923	740
Ganeshgule	14	49	3.340	0.935	2.466	53.554	58.153	57.143	57.771	52.857	740
Devgad	15	40	3.795	0.957	2.591	55.051	58.422	58.095	42.086	53.333	800

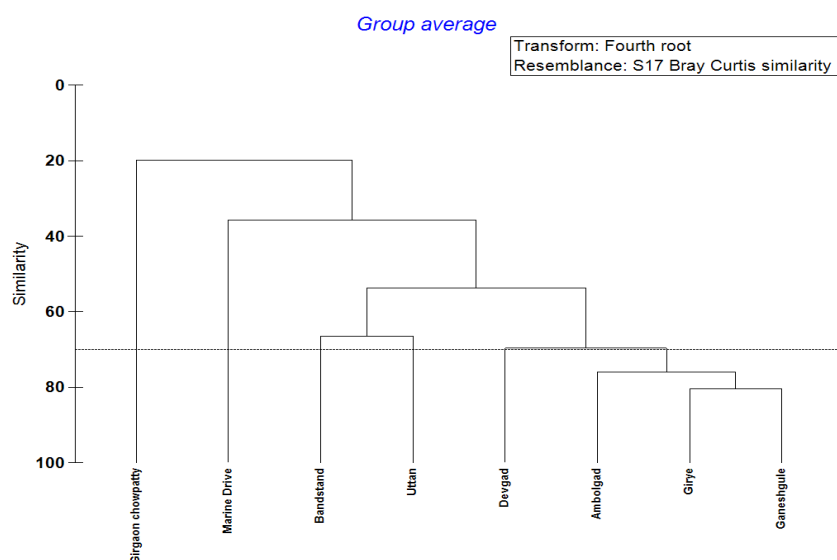


Figure 4. Cluster analysis among all locations of Mumbai and Jaitapur shows 80% of similarity among Jaitapur locations

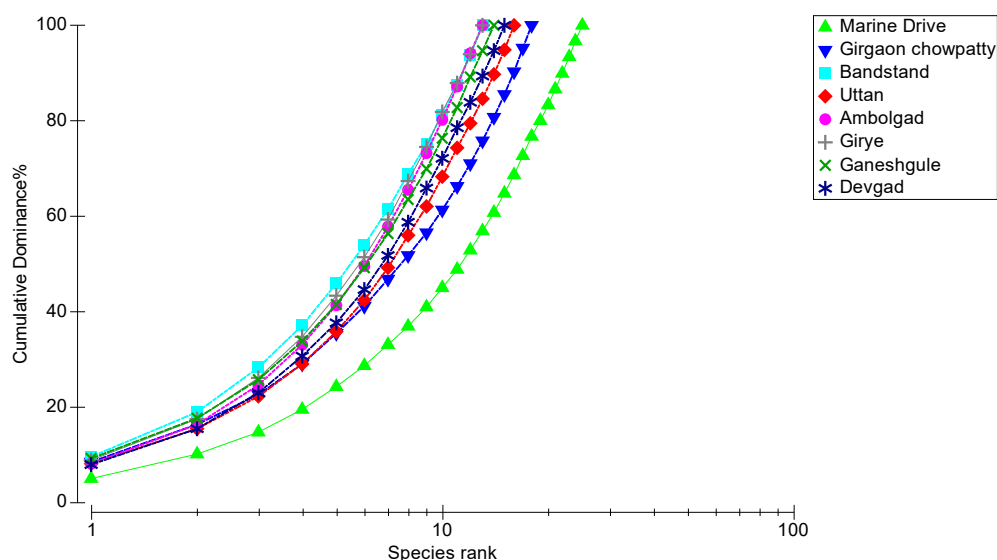


Figure 5. K- dominance plot for all sites (Mumbai locations-Marine Drive, Girgaon Chowpatty, Bandstand, Uttan; Jaitapur locations-Ambolgad, Girye, Ganeshgule, Devgad)

Diversity Indices Estimated for Location Around Mumbai and Jaitapur

The diversity indices are presented in Table 3. The Shannon-Weiner index (H') values varied between 2.3 and 3.0 for Mumbai as well as Jaitapur. Maximum value (3.0) was recorded at Marine Drive site. Season wise analysis revealed maximum diversity in pre monsoon and post monsoon (Figure 3). The evenness varied between 0.86 and 0.97, whereas the richness fluctuated between 3.2 and 5.9. Maximum richness was recorded at Marine Drive as the species were evenly distributed while maximum evenness was recorded at Ambolgad. The minimum (51.49) and maximum (55.75) taxonomic diversity was recorded at Bandstand and Marine Drive, respectively. The taxonomic distinctness was more in

Bandstand (59.54) and Ambolgad (59.16). The average taxonomic distinctness was high in Girgaon Chowpatty (59.21) and Ambolgad (58.97) than Bandstand (53.84). The variation in taxonomic distinctness was maximum at Bandstand (105.72) and Uttan (98.2). The average phylogenetic diversity was maximum at Ambolgad (58.46) and minimum at Uttan and Marine Drive (48.8). Total phylogenetic diversity was maximum at Marine Drive (1220) and minimum at Bandstand (640).

The dendrogram revealed four separate clusters for all seven locations (Figure 4). Maximum similarity was recorded between stations Girye and Ganeshgule (80.41%) at Jaitapur, and between Bandstand and Uttan (66.5%) in Mumbai region. Girgaon Chowpatty is totally different from other locations in terms of substratum (sandy, muddy and silty) and hence species

variation was also observed. The average dissimilarity in species composition of crabs from Marine Drive and Girgaon Chowpatty was 97.9%, where *Dotilla* spp was responsible for 10.3% of dissimilarity between two stations. The maximum species dominance was recorded at Girgaon Chowpatty and Bandstand (Figure 5) on account of dominance of *Dotilla* spp and *Xenophthalmus wolffi*. According to W statistics, the ABC curve seems quite healthy, indicating undisturbed community at all locations except at Bandstand and Ganeshgule where

moderate disturbance is seen (Figure 6 a-h). Funnel plot and ellipse plot revealed clear separation of Bandstand and Uttan, as these are more diverse from other locations (Figures 7-9). The species (*Ozium rugulosus*, *S. serrata* and *S. olivacea*) were recorded only once from Bandstand and Uttan, unlike other locations. Overall results indicate Jaitapur locations as more diverse than Mumbai, with respect to average taxonomic distinctness.

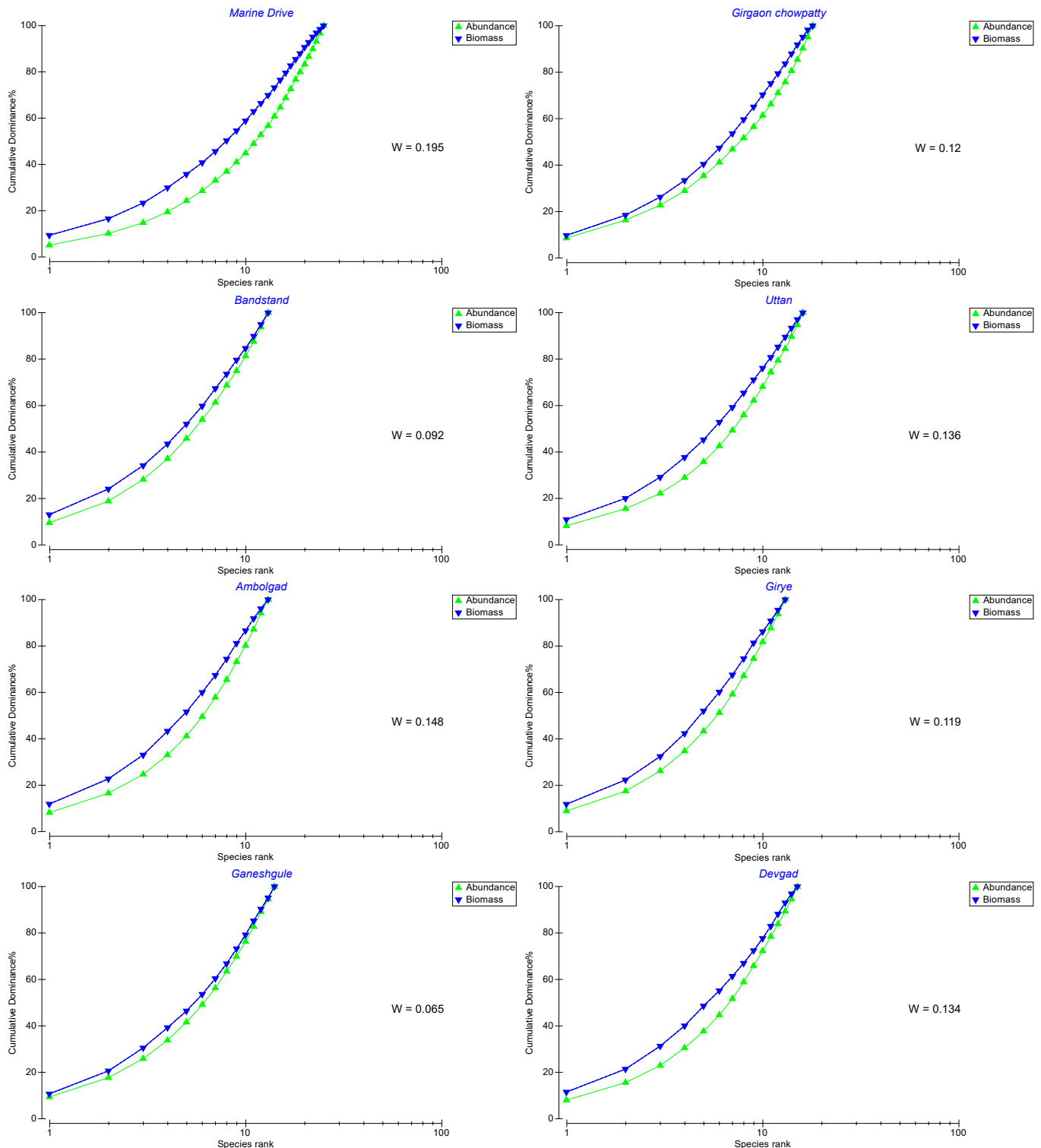


Figure 6. ABC curve for all locations (a-Marine Drive, b-Girgaon Chowpatty, c-Bandstand, d-Uttan, e-Ambolgad, f-Girye, g-Ganeshgule, h-Devgad)

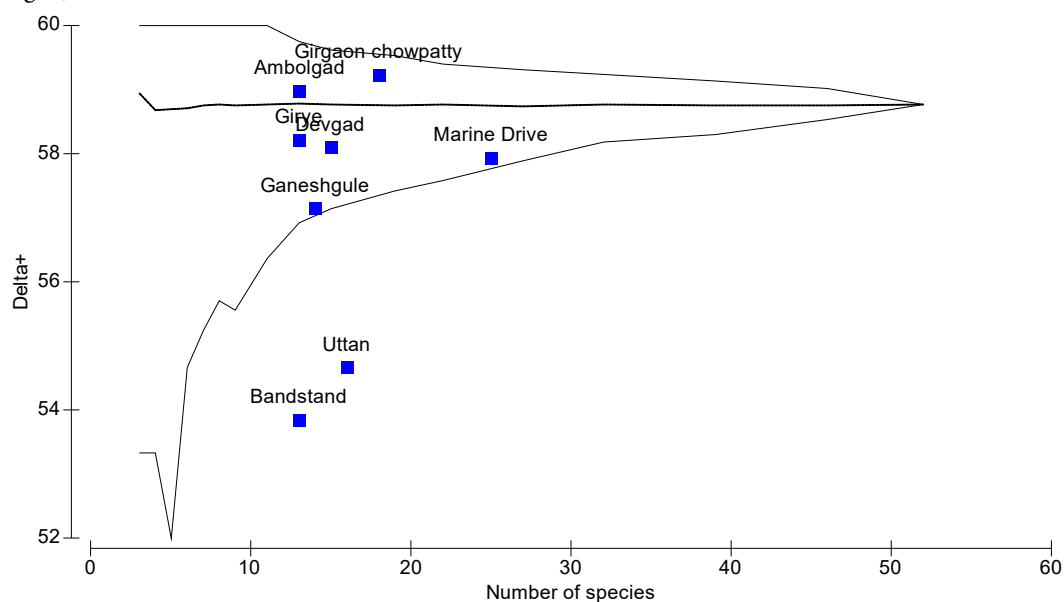


Figure 7. Funnel plot of average taxonomic distinctness for all locations

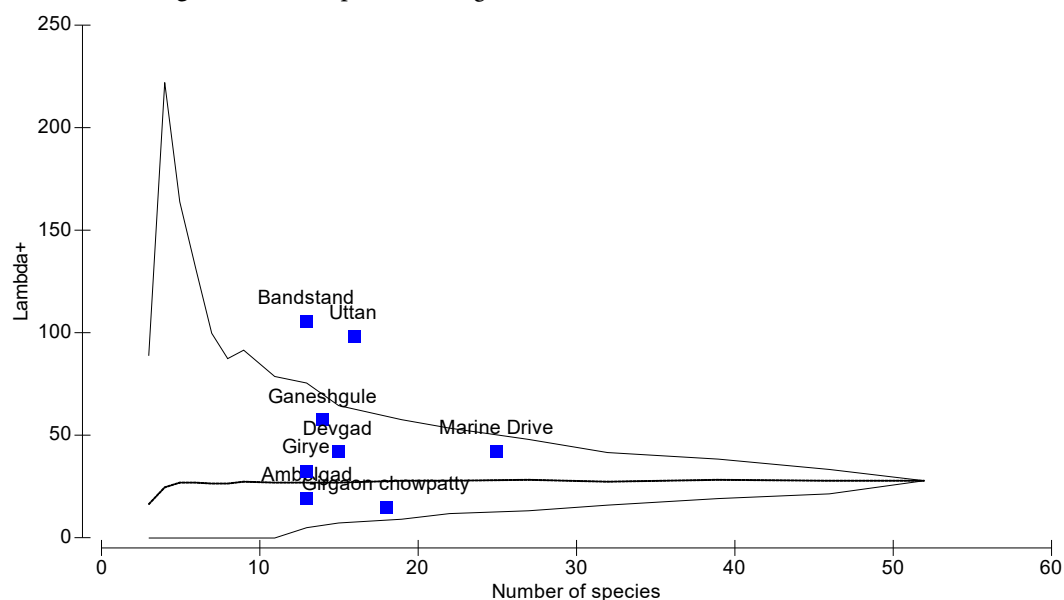


Figure 8. Funnel plot of variation in taxonomic distinctness for all locations

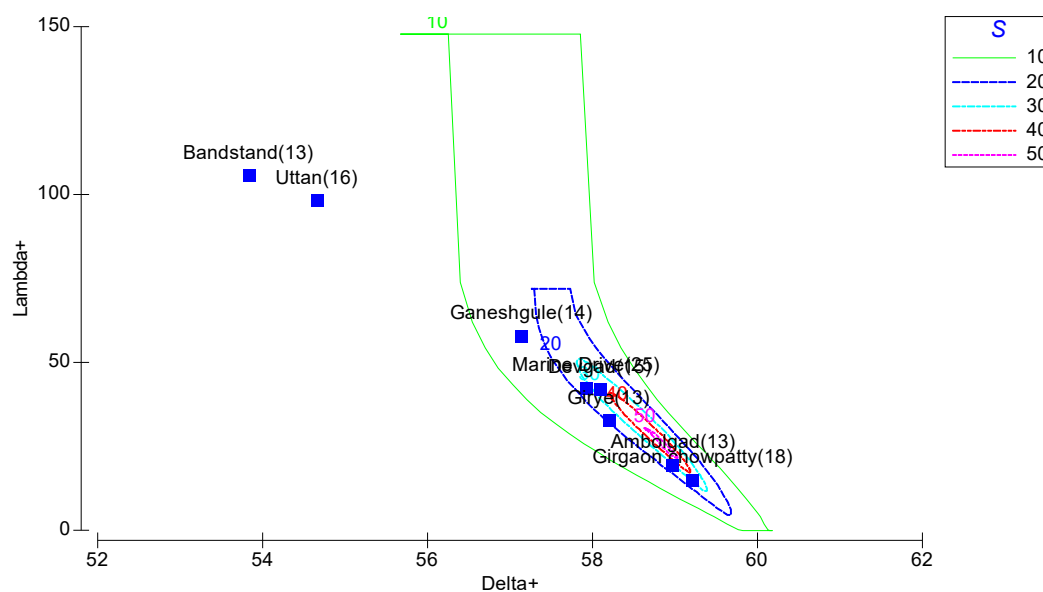


Figure 9. Ellipse plot of average taxonomic distinctness (delta+) and variation in taxonomic distinctness (lambda+) at all locations

The present study also reports total 10 species with 4 new records from intertidal regions of west coast of India (*Xenophthalmus wolfii*, *Ozius rugulosus*, *Achaeus cf spinosus*, *Leptodius cf sanguines*); 5 new distributional records for Maharashtra state (*Ozius tuberculosus*, *Ocypode brevicornis*, *Atergatis laevigatus*, *Pilumnopus convexusus*, *Scylla olivacea*); and 1 first record of genus from Indian Ocean (*Anomalifrons garthii*) (Figures 10-18).



Figure 10. *Atergatis laevigatus*



Figure 11. *Pilumnopus convexusus*



Figure 12. *Ocypode brevicornis*



Figure 13. *Achaeus cf spinosus*



Figure 14. *Leptodius cf sanguineus*



Figure 15. *Ozius rugulosus*



Figure 16. *Ozius tuberculosus*



Figure 17. *Anomalifrons garthi*



Figure 18. *Xenophthalmus wolfii*

Discussion

The data gathered during the present study suggests that the diversity of crabs is profoundly influenced by the nature of the habitat. As Jaitapur is approximately 300km away from Mumbai, differences were observed in climate; habitat and nature of soil that could be responsible for species variation in Mumbai and Jaitapur locations. Observations recorded around Mumbai locations indicated Girgaon and Uttan areas to be more polluted as compared to Marine Drive and Bandstand. Uttan beach is polluted by oil spills, chemical, plastics and domestic sewage brought by Manori and Vasai creeks. Girgaon

Chowpatty and Bandstand are famous recreational spots, hence maximum quantity of waste (plastic & litter) was observed here, whereas Bandstand is polluted mostly due to domestic sewage. Marine Drive shore is covered by cement tripods, restricting anthropogenic activity but two outlets of effluent discharges from townships are responsible for degradation of this area. Around Jaitapur, Girye area is less polluted; Ambolgad is slightly affected by domestic sewage, whereas Ganeshgule and Devgad is moderately affected by tourist activity.

The variations in the density, biomass and dominance of species in different ecological zones like reef lagoon, reef flat, reef flat with crest was reported by Iglesias & Raso (1999). Al-Wazzan et al. (2020) reported that gleaning activity removes the crab and damages the physical structure by breaking and turning upside down the rocks. Khot et al. (2019a) also supported this view and reported the effect of gleaning on the density and biomass of crabs from coastal areas of Mumbai. In the present study, variation in species as per habitat and zonation was recorded similar to reports from different geographical areas.

The present study reports 33 species from rocky coral reef mixed habitat alone. De Szechy et al. (2001) recorded 12 species of brachyuran crabs from the rocky shores of Rio de Janeiro and Sao Paulo of Brazil; whereas, Flores & Paula (2001) recorded 7 species from rocky shore of central Portugal. Trivedi et al. (2012) reported 19 species of brachyuran crabs from different habitats of Gulf of Kutch, India, while Dev Roy (2013) reported 49 species of brachyuran crabs from coral reef habitat from west coast of India.

Family wise dominance was reported from Kanyakumari area (Sruthi et al., 2014), mangrove ecosystem of Uran, Maharashtra (Pawar, 2017), Gujarat (Beleem, 2018). They also reported maximum number of species in Portunidae followed by Xanthidae, Pilmunidae, Calappidae and Epialtidae, similar to the findings of the present study. Based on abundance and spatiotemporal collection, Vignoli et al. (2004) separated decapod crustaceans into Common, Rare, Abundant categories. Kumar et al. (2007) classified *Portunus* spp, *Charybdis* spp, *Matuta* spp, *Scylla* spp, as abundant species. Pawar (2012) categorized species distribution as common (*Scylla serrata* and *Leptodius exaratus*) and occasional (*Portunus pelagicus*, *P. sanguinolentus* and *Charybdis cruciata*), which is in agreement with the findings of present study.

With respect to species and location, species reported by Bi-Shaikh (2002) and Lokhande et al. (2015) from oyster zone at Colaba (*Uca annulipes*, *U. vocans*, *Menippe rumphi*, *Leptodius crassimanus*, *Heteropilumnus angustifrons*) and Mirya Bay,

Ratnagiri district (*Ocypode ceratophthalmus*, *Ashtoret lunaris* and *Philrya corallicola*), respectively, are similar to the findings of the present study. The present study reports 3 new records from Maharashtra state (Khot et al., 2021), 1 new record from west coast of Maharashtra and 1 new record of genus from Indian Ocean (Khot et al., 2019b).

Rocky shores are the most dynamic habitats subjected to varying degrees of environmental factors such as winds, tidal waves, salinity, slope, temperature, desiccation etc. which may have direct impact on diversity and distribution of flora and fauna inhabiting the rocky shore. The salinity and substratum characteristics are the most important key factors that can influence the spatial distribution of brachyuran crabs (Macintosh, 1988; Balasubramanian & Kannan 2005; Sridhar et al., 2006). It also plays vital role in regulating physiology of marine organisms (Lesser, 2006). Thus, it is also responsible for the reduction in number of species from particular areas (Varadharajan et al., 2009, 2013; Pandya, 2011; Pandya & Vachhrajani, 2011). During the present study, a significant correlation ($R=0.47$, $P=0.01$) was found between salinity and density of crabs. This could be related to temperature which is responsible for rates evaporation. However, lower salinity recorded during was the result of influx of rain water into the sea.

Temperature is the most important factor of all the physical environmental factors, which controls the life history of marine organisms. Water temperature is relatively less ($24-26^{\circ}\text{C}$) in the Arabian Sea and Bay of Bengal during the time of the northeast monsoon than other seasons. During the summer months, the temperature gradient runs approximately north-south on both the sides of the Indian coasts, with highest values of 32°C (Venkatraman, 2007). The results of present study are in agreement with many other workers (Subrahmanyam, 1959; Nair et al., 1980; Achuthankutty et al., 1981; Joydos, 2002; Bindhu, 2006; Sawant et al., 2007; Kadam & Tiwari, 2011; Paralkar, 2012; Gadhavi, 2015; Lokhande et al., 2015). The non-significant relation between temperature and abundance of crab indicates that these organisms are adapted to take shelter under rocks, crevices or other hideouts in extreme temperatures. Paralkar (2012) opined that the influx of fresh water during monsoon largely affects the buffering in nearshore and estuarine systems and bringing pH below 8.0. However, a non-significant relationship was seen with pH and density.

Species diversity is a measure of community structure and most important parameter to understand the health status and productivity of an ecosystem. In present study, Shannon-Weiner index ranged from 2.3 to 3.0, indicating very moderate

pollution and Margalef's richness ranged of 3.2 to 5.9, indicating rich diversity. Thus, evaluation of the species diversity, richness, evenness and dominance based on the biological components of the ecosystems are essential to know detrimental changes in the environment. The Shannon-Weiner diversity index (H') is widely used for comparison of diversity between different habitats (Clark & Warwick, 2001). In healthy environment, Shannon-Weiner index range between 2.5 and 3.5 (Ajmal Khan et al., 2005; Magurran, 1988). Wilhm & Dorris (1996) stated diversity values <1.0 , in estuarine waters with heavy pollution. Values between 1.0 and 3.0 indicated moderate pollution where values exceeding 3.0 characterised unpolluted waters.

Maximum diversity and richness was recorded from Marine Drive, as it favoured ecological microhabitat for crabs, comprising of coral reefs, seaweed cover, silty-muddy bottom, coral boulders with sponges, rocky crevices, rocks covered with zoanthids and gorgoniids. Different authors have reported similar results from different areas and habitats. Beleem et al. (2014) reported values of H' , J' and d as 0.84 to 2.4, 0.76 to 0.91 and 3.33 to 0.65, respectively, from Diu coast of Gujarat. Hebling et al. (1994) reported range of H' (0 to 1.99) and J' values (0 to 0.71) based on the majoid crab of Ubatuba. Similar results were obtained on the brachyuran communities from the same Ubatuba area by Fransozo et al. (1993) and Carmona-Suarez (2000). In present study, maximum evenness was recorded from Devgad and Uttan locations, while lower value of evenness was recorded from Girgaon Chowpatty (0.86). The reason for lower evenness is dominance of two species *Dotilla* spp and *Xenophthalmus* sp. Both species were dominant as the habitat is comprised of sand-silt and muddy bottom, with food availability from surrounding areas and tidal flow favoured them to reproduce and grow both species. They contributed 16.5% of the total fauna, resulting in lower H' values (Kumar & Wesley, 2010). Richardson (2004) noted that the dominance of single foraminifera community in Twin Cays, Belize resulted in reduction of evenness. In the present study, Mumbai and Jaitapur locations showed high similarity. On the other hand, the contribution to dissimilarity, between the two locations, was attributed to conditions at Marine Drive and Girgaon Chowpatty of Mumbai. Kumar & Wesley (2010) reported dissimilarity between sites from Maldives. Jitpukdee et al. (2015) showed clear separation of benthic macrofauna at different stations based on cluster analysis. In the present study, the MDS plot displayed clear separation of Girgaon Chowpatty from other locations due to difference in habitats. Clarke & Warwick (2001) stated a stress value of $< 0.10 - 0.20$ to provide

a good representation. In the present study, the 2D stress value was found to be 0.01, displaying significant separation between sampling locations. In present study, dominance of *Dotillids* and *Xenophthalmids* was recorded from Girgaon Chowpatty. According to W statistics, in the present study, the ABC curve seems quite healthy, except at Bandstand and Ganeshgule which indicated moderate disturbance. The findings are in agreement with report of Jaiswar et al. (2007a, 2007b). However, the moderate disturbance could be attributed to the inclusion of small non-commercial species in analysis whose weight was almost negligible and effect of tourism at both sites. Ajmal Khan et al. (2005) used the conventional and the new indices to compare the diversity of brachyuran crabs in two mangrove areas (natural and artificial). Shan et al. (2010) showed positive correlation with species richness and Shannon diversity with average taxonomic distinctness (AvTD) and negative correlation with variation in taxonomic distinctness (varTD). Ellingsen et al. (2005) applied taxonomic distinctness as a diversity measure on benthos of Norwegian continental shelf and found that annelids and crustaceans were positively correlated to latitude and depth whereas mollusc were not related to latitude and depth. In present study, no relationship was seen between new diversity indices and traditional diversity indices as well as with other diverse groups, though found by others (macrobenthos: Warwick & Clarke, 1995; Clarke & Warwick, 1998; asteroids: Price et al., 1999; copepods: Woodd-Walker et al., 2002; fish: Hall & Greenstreet, 1998; Rogers et al., 1999; corals: Brown et al., 2002). Species diversity index was reported to be maximum (2.98) in monsoon in Kerala coast (Kumar et al., 2007), maximum diversity (0.492) at Wilson Island (Kumaralingam et al., 2012), from Pondicherry and Pichavaram mangrove ecosystem east coast of India (Ravichandran et al., 2001, 2007; Ajmal Khan et al., 2005; Soundarapandian et al., 2008; Satheeshkumar, 2012; Kamalakkannan, 2015; Prasanna et al., 2017). In India, very limited investigators have reported brachyurans from mangroves of west coast (Haragi et al., 2010; Bandekar & Kakati, 2011; Shukla et al., 2013; Pawar, 2012, 2015, 2017). The present study reports significant spatial variation between sites ($p=0.0001$, $P<0.05$) and non-significant temporal variation between seasons ($p=0.55$, $P>0.05$) with Shannon diversity and evenness values which supported with the findings of Kumar & Wesley (2010); Jeyabaskaran (1997) and Flores & Paula (2001). The values of richness index showed both non-significant temporal and spatial variation between seasons and sites.

Conclusion

In the present study, a checklist and diversity of brachyuran crabs inhabiting along intertidal and shallow subtidal regions of Mumbai and Jaitapur coasts of Maharashtra were surveyed with findings of total 55 species of brachyuran crabs. The study showed rich diversity among both the coasts with different types of habitats where rocky habitat mixed with coral patches showed maximum diversity. Furthermore, new records were also reported from west coast of India and genera from Indian Ocean. The data obtained from present study can be used as baseline data and reference datasets for identification of brachyuran crabs for research studies on marine crab's taxonomy along Maharashtra coast in future. In order to conserve and sustainability use the biodiversity, continuous monitoring and surveys are required for future research.

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Compliance With Ethical Standards

Authors' Contributions

MK: Conceptualization, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing

AKJ: Conceptualization, Formal analysis, Writing – review & editing

AKP: Writing – review & editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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All data generated or analysed during this study are included in this published article.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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REVIEW ARTICLE

Health benefits of fish oil and the application of encapsulated fish oil in meat and dairy products

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ABSTRACT

Fish oil is widely recognized for its health benefits, which are attributed to its high content of unsaturated fatty acids, notably docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Numerous clinical studies have demonstrated the positive impact of n-3 fatty acids on various health conditions, including cardiovascular disease and diabetes. However, despite benefits, dietary intake of n-3 fatty acids often falls below recommended levels. Given the widespread consumption of meat and dairy products, enriching these foods with fish oil presents a promising strategy for increasing dietary PUFA intake. The incorporation of fish oil into food products can be achieved through direct addition, emulsion, or encapsulation techniques. Encapsulation technology offers a promising solution by enhancing the oxidative stability of fish oil and mitigating undesirable fishy flavors & odours in the final product. This article will explore the health benefits of fish oil, discuss various methods for incorporating fish oil into foods and evaluate the potential of encapsulated fish oil in meat and dairy products.

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Introduction

Fish oil is the main source of *n*-3 long chain polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (20:5*n*-3, EPA) and docosahexaenoic acid (22:6*n*-3, DHA), in the human diet. Fatty acids are essential components that facilitate the structural integrity of cells, tissues, and organs. In addition to their structural role, they serve as the

fundamental building blocks for various bioactive ingredients. Studies have shown that *n*-3 PUFAs not only decrease the risk of heart diseases but also exhibit anti-inflammatory properties and may reduce the risk of certain cancers and diabetes (Damerau et al., 2022; Patel et al., 2022). However, dietary intake of *n*-3 PUFAs is below recommended levels, despite its health benefits. A primary contributing factor to this situation is the prevalence of saturated fat in modern diets. The primary

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sources of saturated fatty acids (SFAs) are red meat and high-fat dairy products. The preponderance of saturated fat in the diet is largely attributable to cheese, milk and milk products, meat, fats (hard/solid), and butter (Perna & Hewlings, 2023). SFAs typically comprise nearly half of the total fat content in meat, with meat representing approximately half of the maximum recommended intake of SFAs (Geiker et al., 2021). The World Health Organization (WHO) has expressed support for research initiatives aimed at replacing SFAs in meat products (López-Pedrouso et al., 2021). In order to meet the daily requirements of essential PUFAs through dietary intake, there is a necessity for increased food fortification strategies. A viable option to avoid losing the benefits provided by PUFA is to protect their integrity by using appropriate encapsulating technologies. The encapsulation process is defined as the coating technique of small capsules of solids, gases, and liquids. This process offers several advantages, including protection of the encapsulated materials from oxidation, improvement of the stability of the oil, and extension of its shelf life. Additionally, it enables the controlled release of the encapsulated materials at specific rates (Badr et al., 2020; Selim et al., 2021; Rahmani-Manglano et al., 2024). Given their widespread global consumption, meat and dairy products appear to be promising candidates for such fortification efforts. This study elucidates the effects of fish oil on health and the efficacy of the technique of utilizing it in encapsulated form in meat and dairy products, as well as its effects on product properties.

Fish Oil

The Food and Agriculture Organization of the United Nations (FAO) has reported a consistent increase in the total production of fisheries over the past several decades. From 1950 to 2022, the production of live weight equivalent fisheries increased from 19 million tons to over 185 million tons, exhibiting an average annual growth rate of 3.2% (FAO, 2024). Only 40% of the catching fisheries is utilized for human consumption, and the remaining 60% is wasted (Rohim et al., 2024). Fish oil can be produced from whole fish or by-products from fish processing. The primary products derived from the valorization of fish manufacturing by-products are fish meal and fish oil (Rohim et al., 2024). Fish by-products consist of oil ranging from 1.40% to 40.10%, which depends on the species and tissue type. The economic value of fish oil has exhibited a marked increase day by day (Kalkan et al., 2025). Fish oil is main source of long-chain n-3 PUFA and contain docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3). The majority of commercially available fish oils have a 2:1 ratio of EPA to DHA.

In consideration of the abundant fatty acids it contains, fish oil is recommended by numerous health authorities around the world. More specifically, they recommended intake for n-3 fatty acids particularly EPA and DHA. The following Table 1 illustrates a selection of global n-3 PUFA intake recommendations.

Table 1. Global n-3 PUFA intake recommendations

Organization / Impact Area	Organization Type	Consumption Advice	Fatty Acid Type	Publication Date
Ministry of Health of The Republic of Türkiye General Directorate of Public Health - Healthy Nutrition and Active Life Department / Türkiye	Governmental Health Ministry	1g /day	EPA+ DHA	2019
GOED n-3	Global n-3 association	Adult: 500 mg/day Pregnant or lactating woman: 700 mg/day	EPA+DHA	2014
European Food Safety Authority (EFSA) / Europe	Authoritative body	Adult: between 250 and 500 mg/day	EPA+DHA	2014
The French Agency for the Safety of Health Products (AFFSA) / France	Authoritative body	500 mg / day 250 mg/ day 250 mg/day	EPA +DHA EPA DHA	2010
International Society for the Study of Fatty Acids and Lipids (ISSFAL) / Global	Expert scientific organization	Adult: at least 500 mg/day	EPA+DHA	2004
Scientific Advisory Committee on Nutrition (SACN) / United Kingdom	Authoritative body	0.45 g / day	n-3 PUFA	2004
World Health Organization (WHO) / Global	Authoritative body	1-2% energy of day	n-3 PUFAs	2003

It is noted that the high presence of these long-chain PUFAs makes n-3 oils susceptible to oxidation (Kolanowski, 2024; Kalkan et al., 2025). Due to its susceptibility to oxidation, the use or ingestion of fish oil for the purpose of obtaining nutritional benefits presents a significant difficulty. This challenge can be addressed through various approaches, including the addition of antioxidants such as, vitamin C, vitamin E, and polyphenols. Moreover, the presence of undesirable flavors and odors in fish oil presents a significant challenge for its utilization in the food processing industry. Because the structure of ω -3 PUFAs contains a large number of carbon-carbon double bonds, it is easily affected by oxygen, light, and heat during processing and storage, as a result of oxidative composition into a large number of unstable and easily decomposed undesirable volatiles (Sun et al., 2022). Presently, using nano-, micro-, and encapsulation methods has made it possible to keep fish oil's nutritional value while concurrently impeding its oxidation and the concomitant release of undesirable odors and flavors (Jamshidi et al., 2020). Apart from that, the addition of flavorings can be an effective solution to overcome the fishy taste and odours, thereby increasing the consumer acceptance of products include fish-oil.

Importance of n-3 PUFAs for Human Health

Fatty acids (FA) are carboxylic acids composed primarily of a long, unbranched hydrocarbon chain with an even number of carbon atoms. The majority of fats in foods are triglycerides, defined as a single glycerol unit combined with three fatty acid molecules. The classification of fatty acids based on the degree of unsaturation (presence of double bonds) leads to the identification of three classes: Saturated fatty acids or briefly SFA, Monounsaturated fatty acids or briefly MUFAS, and Polyunsaturated fatty acids or briefly PUFAS (Lima et al., 2022). According to the guidelines established by the World Health Organization (WHO), the proportion of total energy derived from total fat intake should not exceed 30%, while the intake of saturated fatty acids (SFA) should not surpass 10%. Additionally, the recommendation is to prioritize the consumption of polyunsaturated fatty acids (PUFA), with an intake that constitutes more than 6% of total energy. Limiting total fat intake and replacing SFA intake with polyunsaturated fatty acids (PUFA) could be a solution to reducing various diseases (Badar et al., 2021). The nutritionally important PUFAs for human health are n-3 and n-6 groups. Both omega families include different forms of fatty acids (Mariamenatu &

Abdu, 2021). PUFAs are vital to the human body; however, an excessive consumption of n-6 over n-3 has been demonstrated to have detrimental consequences on human health, contributing to the development of numerous contemporary chronic inflammatory diseases, cardiovascular diseases, and some types of cancer. The optimal n-6/n-3 fatty acid ratio to achieve beneficial health effects is a matter of variation among institutions and countries. The optimal n-6/n-3 ratio values proposed by Canada, the Food and Agriculture Organization of the United Nations, and the Chinese Nutrition Society (2000) were 4–10, 5–10, and 4–6, respectively. In general, the n-6/n-3 ratio should not exceed 5 (Mariamenatu & Abdu, 2021; Cao et al., 2024). n-3 and n-6 both cannot be producing by human body and recognized as essential fatty acid n-3 and n-6 fatty acids have been approved as a therapeutic tool for the prevention of several health hazards globally (Kapoor et al., 2021). n-3 PUFA intake has beneficial effects on pregnant woman's mental health. It is recommended that women obtain a sufficient amount of n-3 PUFA during pregnancy, whether through dietary choices such as fish and seafood or through nutritional supplements (Tung et al., 2023). n-3 PUFAs have been demonstrated to enhance the proliferation of beneficial bacteria, including *Bifidobacterium*, in the gastrointestinal tract (Fu et al., 2021). n-3 polyunsaturated fatty acids (PUFAs) can play a pivotal role in the host's defense against infections. This function is attributed to the ability of n-3 PUFAs to limit excessive inflammation and to enhance immune responses (Husson et al., 2016). The presence of high levels of n-3 fatty acids in foodstuffs has been demonstrated to exert a positive influence on the human condition, particularly with regard to cardiac, cerebral, and nervous system function (Jamshidi et al., 2020). Table 2 presents some research findings investigated the relationship between the intake of n-3/PUFAs and the various diseases.

In the context of fish oil, the primary product that comes to mind is fish oil supplements. Globally, fish oils are among the most prevalent dietary supplements. In the United States, 17.7% of adults take dietary supplements, and of those, over one-third are fish oil supplements (Jairoun et al., 2020). Nutritional supplements contain EPA and DHA from fish oils, derived from anchovy, tuna, and cod liver (Sprague et al., 2018). Nonetheless, a considerable proportion of dietary supplements containing EPA and DHA available on the market contain lower amounts of these components than indicated on the labeling (Bannenberget al., 2020). Study from Türkiye investigated that most of the 15 commercial fish oil on the Turkish market. Result showed that label claims for EPA were

reasonably accurate for the products studied, but that DHA levels in some supplements differed significantly from the labels (Karsli, 2021). Another study conducted in France found that the total n-3 content of 2.5% of the samples examined did not

correspond to the content indicated on the labeling of the products (Pasini et al., 2022). Given the potential for misleading content, this calls for further investigation into the competence of these fish oil supplements.

Table 2. Summary of some research findings of relationship between n-3/ PUFAs intake and various diseases

Findings	Number of Participants	Number of Reported Cases After Experiment	Reference
Insulin sensitivity was enhanced in type 2 diabetic patients who received supplementation of n-3 polyunsaturated fatty acids (PUFA)	44	No follow-up	Farsi et al. (2014)
The ingestion of tuna and dark fish, as well as α -linolenic acid (ALA) and marine/fish n-3 acids (PUFAs), was associated with a reduced risk of major cardiovascular disease (CVD) in women without a history of CVD.	713,559 (women)	A total of 1,941 cases of major cardiovascular disease (CVD) were documented, including instances of myocardial infarction (MI), stroke, and cardiovascular death.	Rhee et al. (2017)
Fish intake and n-3 PUFA associated with reduced risk of psychological distress in women during and after pregnancy.	<ul style="list-style-type: none"> • 75,139 pregnancies in the first trimester • 79,346 pregnancies in the second or third trimester • 77,661 pregnancies in the postpartum period. 	No follow-up	Hamazaki et al. (2018)
Patients with high intake of fish oil n-3 PUFAs demonstrated prolonged disease-free survival. It was observed that patients who ingested marine-source n-3 on a weekly basis exhibited a 35% reduced risk of cancer recurrence or mortality in comparison with those who did not consume.	1011 colon cancer patients	343 patient colon cancer recurrences and 305 patients died.	Van Blarigan et al. (2018)
Higher concentrations of EPA were associated with a lower risk of incident Alzheimer Disease. This study results supports a beneficial role of n-3 PUFAs for cognitive health in old age.	1264 healthy participants aged 84 ± 3 years	For 7 years follow-up time 233 participants developed dementia	Van Lent et al. (2021)
n-3 PUFA consumption augmented the reduction of abdominal fat mass and percentage in overweight or obese individuals on a weight loss diet.	40 Adult	No follow-up	Salman et al. (2022)
Dietary intake of n-3 fatty acids could lower risk of all-cause dementia or cognitive decline by ~20%, especially for docosahexaenoic acid (DHA) intake	1135 participants without dementia	No follow-up	Wei et al. (2023)

Food Product Enrichment Techniques With Fish Oil

Notwithstanding the substantial health benefits associated with fish oil, its utilization within the food industry is constrained by several factors. Such limitations include its low solubility, susceptibility to oxidation, the presence of an undesired fishy flavor, and suboptimal handling properties (Jamshidi et al., 2020). The direct addition of fish oil, the emulsification of fish oil, and the encapsulation of fish oil techniques are the most commonly used methods for adding fish oil to fortified foods.

Direct oil addition is basic method for enriched food products. However, it is not suitable for food products in many ways. The direct addition of oil to food products has been revealed to possess a number of significant drawbacks, including an increased susceptibility to oxidation reactions, a reduced solubility, and an elevated hardness, which is attributed to the formation of smaller fat globules (Badar et al., 2021). It was suggested that bulk fish oil should be used with antioxidants in food products to prevent fish oil oxidation and reduce lipid hydroperoxides, which influence the profile of volatiles and develop unpleasant off-odors and off-flavors (Drusch et al., 2008). For instance, regarding enriching meat products with bulk/direct fish oil, EPA and DHA are significantly increased. However, the increase in lipid oxidation, accompanied by the resulting changes, such as undesirable taste and odor, led to the limited use of this technique in industry (Drusch et al., 2008; Serfert et al., 2010; Pérez-Palacios et al., 2019).

Emulsification constitutes another technique for enriching food products with fish oil. An emulsion is defined as a mixture of two immiscible liquids, typically water and oil, wherein one liquid is dispersed in the other (Kim et al., 2021). Conventional emulsions can be classified into two primary categories: oil-in-water (O/W) and water-in-oil (W/O) emulsions. Common fish oil emulsions are of the oil-in-water (O/W) type. Fats are crucial for maintaining water-holding capacity, forming stable emulsions, and enhancing emulsion stability (Shin et al., 2019). The fortification of food products can be achieved through the direct incorporation of fish oil emulsions, for instance, in meat systems or beverages. However, several challenges are associated with substituting animal fats with emulsion oils in meat products. Primarily, the hydrophilic nature of meat presents a challenge in the incorporation of hydrophobic oils. Secondly, increasing the unsaturated fatty acid (UFA) content can heighten susceptibility to lipid oxidation, thereby reducing

shelf-life (Yıldız Turp & Serdaroglu, 2012). To ensure product stability, oils must be effectively emulsified within the myofibrillar protein matrix or with the aid of exogenous emulsifiers (Bolger et al., 2017). The addition of fish oil emulsion to dairy products can increase oxidation, adversely affect sensory quality and alter microstructure. The type of emulsion used can influence oxidative stability, with combinations of milk proteins and phospholipids. Conflicting results observed in different dairy products limit the ability to draw definitive conclusions about the effects of emulsified fish oil. Therefore, in industry when manufacturing dairy products with added fish oil, a multi-faceted approach to minimizing oxidation should be considered, including the use of different emulsion types, the incorporation of antioxidants, and the evaluation of different forms of fish oil (Horn et al., 2012). There are still relatively few types of fish oil emulsion systems. Existing delivery systems for fish oil emulsions have become obsolete due to their inability to meet the evolving demands for enhanced nutritional and health benefits. Therefore, new emulsion systems need to be developed (Chen et al., 2024). Alternatively, the emulsions can undergo processing into microcapsules. Encapsulated fish oil is a promising technique prior to its incorporation into food products. The food industry utilizes encapsulation for a variety of purposes, including the concealment of undesirable odors, flavor, or taste, the preservation of bioactive components, the addition of functional and nutritional elements, and the controlled release of encapsulated components at a designated location, time, and rate (Kumar et al., 2024). Encapsulation of fish oils prevents oxidation of polyunsaturated fatty acids, thus preventing loss of nutritional value and deterioration of taste (Soyuçok et al., 2019).

Fish Oil Encapsulation Methods

Macroencapsulation

Macroencapsulation and encapsulation are related but distinct techniques that differ primarily in their scope and application. Macroencapsulation is a sub-technique of encapsulation, the broader category of which encompasses a variety of encapsulation techniques. Macroencapsulation involves the creation of capsules of a larger size than microencapsulation and nanoencapsulation. Generally, the size of macrocapsules is 2 mm or more (Korkmaz & Tunçtürk, 2024). The primary application of macroencapsulation pertains to the domain of food ingredients and supplements. In comparison to nano and microencapsulation techniques,

macroencapsulation typically elicits a lower degree of toxicity concerns due to its larger capsule size. This method often employs natural and edible coatings, such as gelatin, waxes, or gum coatings, which are relatively safe (Rezagholizade-Shirvan et al., 2024). The larger size of macrocapsules enhances visual appeal and consumer attraction, particularly when the capsule itself is a key aspect of the product experience. Moreover, the shell can be designed to rupture easily, facilitating rapid and significant release of the active ingredient, which is advantageous in applications requiring a sudden and intense effect, such as flavor release in chewing gum (Ngamnikom et al., 2017). A review of the extant literature reveals an absence of studies addressing the macroencapsulation of fish oil. This paucity of research may be attributed to the prevailing perception that alternative encapsulation methods are more appropriate, given its distinct taste and odor.

Microencapsulation

Microencapsulation is another specific technique within the broader category of encapsulation. It involves the encapsulation of ingredients into microspheres, microcapsules or microparticles, with a focus on protecting these substances and enhancing their delivery (Subasri et al., 2024; Thakur et al., 2024). A microcapsule is defined as a minute, spherical particle with a diameter ranging from 50 nanometers to 2 millimeters. These particles can assume diverse forms, including hard or soft gelatin or liquid suspension. Microcapsules are characterized by their dispersion within a solid matrix devoid of a discernible outer wall phase, in addition to intermediate forms, as well as particles or droplets encased within a membrane. The composition of a microcapsule is divided into two fundamental components: the core material and the coating/wall material. The core material is defined as the material to be coated. The coating/wall material is the material that is placed on the core material, thereby imparting thickness to it. The coating material should permit the release of the core material under specific circumstances without reacting with the core material (Bower et al., 2024). The process of microencapsulation has several notable benefits to fish oil. Firstly, it has been shown to enhance the oxidation stability of the oil. Secondly, microencapsulation has been demonstrated to reduce the intensity of the fishy odor characteristic of fish oil. Moreover, microencapsulation has been shown to render the oil suitable for incorporation into various food products (Yeşilsu, 2023). A multitude of methodology exists for the preparation of microcapsules. Spray drying is still the most widely used microencapsulation technique. It is still one of the most preferred method of

microencapsulation in various food industries due to lower cost of production and requirement of less sophisticated set up. It is highly automated, cost effective and produces a good quality product. Other microencapsulation techniques including fluidized bed coating, extrusion, cocrystallization and liposome-entrapment impart great stability to food ingredients in the dry state but release their content readily only the encapsulated product is exposed to high water activity environment (Timilsena et al., 2020). Liposome-entrapment is successful to deliver their content in special conditions. However, the primary challenge associated with this method pertains to the expansion of the microencapsulation process to a commercial scale (Desai & Park, 2005). Other encapsulation methods include interfacial polymerization, organic phase separation, molecular inclusion, coacervation, freeze drying, spray drying, spray chilling, microfluidic jet, electro-spraying, and electrospinning (Noore et al., 2021; Gültekin Subaşı et al., 2021; Yan & Kim, 2024).

Study by Yang et al. (2024a) investigated the effect of spray drying (SD), spray freeze-drying (SFD), freeze-drying (FD), and microwave freeze-drying (MFD) on the characteristics of fish oil microcapsules. According to their results, the microencapsulate fish oil prepared with SD yielded the highest encapsulation efficiency (86.98%), followed by SFD (77.79%), FD (63.29%), and MFD (57.89%). The higher efficiency of spray drying can be attributed to the liquid film it generates during the drying process. In regard to the fatty acid composition of the samples, the spray drying technique yielded the highest levels of total PUFA. The percentages of EPA were as follows: Fish oil (16.86%), SD (12.83%), SFD (12.52%), FD (10.54%), and MFD (11.73%). The DHA percentages were as follows: Fish oil (11.87%), SD (7.28%), SFD (6.63%), FD (5.55%), and MFD (7.52%). A comparison of uniaxial or coaxial electro-spraying and spray drying of fish oil showed that spray drying produced larger capsules with higher encapsulation efficiency ($EE > 84\%$), while uniaxial electro-spraying produced submicron capsules with EE 69-72%. Coaxial electro-spraying had the lowest EE (53-59%). For spray drying and monoaxial electro-spraying, the EE is closely related to its physical stability. As a result, coaxial electro-spraying had the lowest oxidative stability among the techniques studied (Rahmani-Manglano et al., 2023).

Study conducted by Jokar et al. (2024) investigated fish oil microencapsulated by using Arabic gum (AG) and Persian gum (PG) as wall materials. Optimal microencapsulation of fish oil was achieved using a 26:4 gum Arabic-gum Persian blend, a 4:1 wall-to-oil ratio, 210°C drying temperature, and a high feed flow rate. Microencapsulation efficiency (79.49%), low

moisture content (3.39%), low peroxide value (10.98 meq O₂/kg oil), and a relatively small particle size (39.05 µm), indicating good oxidative stability and potential for increased storage life. The Microencapsulation Efficiency (MEE) of 79.49% achieved in this study is reported notably high compared to values reported in previous literature. The low moisture content (MC) of 3.39% is mentioned as advantageous for long-term storage, as lower moisture levels generally reduce the risk of microbial growth and spoilage.

Nanoencapsulation

Nanotechnology has emerged as a highly promising technological approach with the potential to transform conventional food science and the food industry. The process of nanoencapsulation entails the encapsulation of small particles of core materials (in this case, fish oil) within a wall material or encapsulant, with a nanometer size (smaller than 1 µm/0.001 mm) (Silva Sales et al., 2023). The selection of nanoencapsulation method is contingent upon the type of core material to be encapsulated and the polymer utilized as the encapsulant or wall material. The available techniques encompass nanoprecipitation, gelling *via* ionic emulsification, emulsion–diffusion, and emulsification–evaporation of the solvent (Ferreira & Nunes, 2019). Important sign of success of nanoencapsulation is encapsulation efficiency (EE). It refers to the amount of oil contained in the nanoparticles, which is related to the nanoparticle's stability and protection against oxidation. EE values ranging from 60% to 80% were identified in fish oil nanoparticles produced by homogenization, while values exceeding 80% were observed in fish oil nanoparticles derived from ultra-sound, nanoprecipitation, and low-energy self-emulsification methodologies (Ilyasoglu & El, 2014). The choice of emulsifier can also determine the efficiency of nanoencapsulation. Tween 80 was the main emulsifier used for both edible and essential oils nanoparticles, followed by Tween 20 (Silva Sales et al., 2023). The wall material is another important parameter. In their study, Raeisi et al. (2019) nanoencapsulated fish oil and garlic essential oil using various percentages of chitosan and Persian gum-chitosan. The results showed that the 2:1 w/w ratio of Persian gum and chitosan will offer the best performance of nanoencapsulated fish oil-garlic oil for use in the food industry. Current studies highlight that nanoencapsulation may be a solution to overcome the limitations of fish oil in food applications. Nanoencapsulation has demonstrated efficacy in preserving the integrity of ingredients by mitigating the effects of evaporation, oxidation,

light-induced reactions, and degradation due to exposure to heat and moisture. Nanoencapsulation technique can mask undesirable flavors and enhance solubility and sensory characteristics (Sun et al., 2021).

The Impact of Encapsulated Fish Oil on Meat Products

Meat and meat products are essential part of the human diet. Meat is a primer source of essential amino acids and supplies amino acid derived metabolites and peptides that have important bioactive properties (Geiker et al., 2021). Meat and meat products, such as sausages, meatballs, meat cakes, and various other local delicacies, consistently enjoy high market demand. These products are highly valuable for consumers, mainly due to their sensory properties (Liu et al., 2024). Despite their importance as a source of high-quality proteins and certain vitamins (most notably vitamin B6 and B12) and minerals (including iron, selenium, and zinc), the lipid profile of meat and meat products is nutritionally deficient. Meat and meat products contain high amount of SFAs and low PUFA contents and to the higher content in n-6 PUFA than in n-3 (Pérez-Palacios et al., 2019). High consumption of (SFAs) is associated with various diseases and health problems, especially cardiovascular disease (CVD). A reduction in the consumption of SFAs has been indicated as a factor associated with a considerable decrease in the risk of CVD (Te Morenga & Montez, 2017). Different strategies focused on enhancing the lipid profile of meat and meat products have been evaluated. It has been stated that the use of different encapsulated unsaturated fatty acids in place of animal fat in fermented meat products does not pose a technological challenge and contributes to the development of health-promoting foods (Soyuçok et al., 2019). Although different alternatives are being investigated to improve the fat profile, one of the strongest candidates is encapsulated fish oil due to its rich n-3 PUFA content and minimum effect on sensory attributes. The addition of encapsulated fish oil to meat products has the potential to improve their structural attributes and sensory characteristics. In the studies carried out, it was observed that the incorporation of encapsulated fish oil into meat products with different characteristics did not generally have a negative effect on the sensory properties of the product. This situation demonstrates the importance of the encapsulation technique in preventing the negative sensory properties that occur when fish oil, which has a uniquely strong flavor and odour, is used directly in products.

Table 3. Advantages and disadvantages of encapsulation techniques

Advantages	Disadvantages
Encapsulation techniques allow for sustained and targeted material delivery	The development of stable and effective encapsulated products can be complex and is a matter of extensive optimization
Encapsulation technology protects sensitive compounds from the effects of external factors	Other newly developed technologies, with the exception of spray drying, may be difficult to adapt to the industry
Encapsulation can mask unpleasant flavors and odors	Advanced encapsulation methods can be expensive
Maintains stability of bioactive compounds during processing and storage	Some encapsulation techniques require complex equipment's
Advance encapsulation techniques have strong control over particle size and morphology, allowing different formulations to be achieved with particles of desired size	Some encapsulation techniques can involve complex post-processing purification steps
Encapsulation has been demonstrated to extend the shelf life of components	Encapsulation include freeze drying, hot air fluidized bed, and flash drying etc. require high energy consumption

Note: References are Abdul Mudalip et al. (2019); Ozkan et al. (2019); Abdul-Al et al. (2023); Thakur et al. (2024).

Study revealed by Stangierski et al. (2020) investigated the effect of adding microencapsulated fish oil powder to chicken sausages on mechanical, structural and sensory properties. Microencapsulated fish oil powder (ME) improved the textural properties of chicken sausages, such as hardness, gumminess, chewiness and water activity. The sensory evaluation results indicated that there were equal ratings for the external color and the color of the cross section of all samples. The sausages products with microencapsulated fish oil powder (ME) were rated highest for their consistency (the thickest), especially when heated. As a result of storage sensory analysis, the most attractive sausages on the first and 21st day of storage were the sample with microencapsulated fish oil powder. Another study investigated the fatty acid profile poultry sausages with ME and liquid fish oil. Interactive effects were observed between the type of fish oil and storage time, on the EPA and DHA content. Researchers have indicated that it is quite challenging to specifically determine EPA and DHA in microencapsulated fish oil, and that their distribution in the product cannot be tracked. As a result, this study proposed liquid fish oil than microencapsulated fish oil for poultry sausages (Kawecki et al., 2021). The composition of wall material is one of the important parameters in the encapsulation. Pourashouri et al. (2021) investigated the impact of wall material (tragacanth (TRG) and carrageenan (CGN) on fish oil microencapsulation and its subsequent incorporation into chicken nuggets. Samples prepared respectively: Control -chicken nuggets with no fish oil, FOL-chicken nuggets with added free fish oil (1%), TRG-chicken nuggets containing fish oil encapsulated with tragacanth, CGN-chicken nuggets with fish oil encapsulated

with carrageenan. TRG microcapsules provide a slightly higher level of protection for EPA and DHA during storage. EPA+DHA mg/g samples values are respectively: Control 0.05 mg/g, FO 2.01 mg/g, TRG 2.04 mg/g, CGN 2.02 mg/g. Also, TRG were found to be closer to the control group in terms of sensory characteristics (texture, crustiness, oiliness, juiciness, hardness, taste, flavor, and fish odor). TRG was found to be a more effective wall material than CGN in encapsulating fish oil and preserving nugget quality.

The microencapsulation of fish oil can be accomplished through the creation of monolayered (Mo) or multilayered (Mu) emulsions, each of which offering different properties and influencing the application of these encapsulated oils in meat products differently. Study of Solomando et al. (2020) evaluated the use of (Mo) and (Mu) fish oil microcapsules in cooked and dry-cured sausages. Monolayered and multilayered emulsions of fish oil were spray-dried to obtain their corresponding microcapsules (designated as Mo and Mu, respectively). Two distinct sausage products were prepared: cooked (C-SAU) and dry-cured sausages (D-SAU). In both sausages evaluated, the amount of EPA and DHA increased from the control (mentioned as not detected) to the fortified batches. Two sample group showed no significant differences between Mo and Mu enriched products in C-SAU (0.16 and 0.15 mg EPA/g sample and 0.31 and 0.32 mg DHA/g sample, respectively) and D-SAU (0.17 and 0.19 mg EPA/g sample and 0.39 and 0.42 mg DHA/g sample, respectively). In conclusion, the study demonstrated that both monolayer and multilayer fish oil microcapsules can be successfully incorporated into meat products to enhance their n-3 fatty acid contents. However,

further sensory analysis is recommended, particularly to investigate the effects of storage duration and microcapsule type on sensory quality. A study of chicken nuggets containing encapsulated fish and garlic oil revealed that the use of 8% (w/w) encapsulated fish oil-garlic oil provided the optimal antioxidant and antimicrobial properties during storage. However, sensory analyses indicated that 4% (w/w) encapsulated fish oil added sample was more acceptable, in terms of overall acceptability, taste, and odor, during storage (Raeisi et al., 2021). Given the considerable variance in the optimal ratio of encapsulated fish oil to meat product, which is dependent on the specific product and its desired properties, it is not feasible to offer a generalized recommendation for all products.

The Impact of Encapsulated Fish Oil on Dairy Products

Dairy products are significant dietary sources of essential nutrients, including protein, fat, vitamins, and minerals, contributing to their widespread global consumption. Cow milk is a rich source of fat, lactose, protein with a high biological value, minerals relevant for skeletal growth like calcium, phosphorous, magnesium, and several trace elements and vitamins like zinc, iodine, vitamins B2, B12, D, and A. Given dairy-based products extensive consumption across all age groups and their critical role in human nutrition, dairy products are important categories for enhancing fatty acid content through the incorporation of fish oil. Dairy-based foods can be categorized into three groups: liquid (milk and fermented milk products), semi-solid (yogurt & certain soft cheeses and ice-cream), and solid (primarily cheeses) (Scholz-Ahrens et al., 2020; Qazi et al., 2024). A variety of encapsulation methods such as emulsification, coacervation, spray/freeze-drying, and liposomes are available for encapsulation of oil in dairy products. Nevertheless, it must be acknowledged that a single method is not generally applicable to all purposes and products. The optimal encapsulation technique must be selected with consideration for the bioactive characteristics, such as molecular structure, polarity, molecular weight, and solubility, as well as the physicochemical properties of the dairy matrix. Suitable wall materials should be selected for the encapsulation of bio actives towards their incorporation into milk and dairy products (Adinepour et al., 2022). Nanoencapsulated fish oil, comprising n-3 fatty acids, was incorporated into a probiotic fermented milk product. The results of study demonstrated that the incorporation of nanoencapsulated fish oil led to an increase in probiotic

bacterial counts decreased the oxidation of EPA and DHA without any adverse effects on the sensory attributes of the product (Moghadam et al., 2019). Another study on milk investigated the encapsulation of fish oil in hollow solid lipid micro and nanoparticles (HoSoLiP) followed by its incorporation into skim milk. Milk fortified with fish oil encapsulated with (HoSoLiPs) remained stable for 3 weeks, whereas milk enriched with direct fish oil exhibited a shorter shelf life, remaining stable for only 1 week. These findings demonstrate that the incorporation of fish oil-loaded HoSoLiPs into milk represents a novel approach for enriching dairy products with n-3 fatty acids (Yang et al., 2024b). The effects of fish oil and alternative microcapsule wall materials are being investigated in some studies. Mahfoudhi et al. (2022) tested the efficacy of almond gum as a new wall material for microencapsulating fish oil and used it to produce fortified yogurt. The results showed that the almond gum/gelatin mixture protected the fish oil from oxidation and masked the undesirable fishy odor and taste of the fortified yogurt. At the end of storage, yogurts containing microencapsulated fish oil retained more n-3 fatty acids (ME 82%, CO 67%) The effect of nanoencapsulated fish oil on the physicochemical properties and sensory quality of yogurt was investigated by researchers. Nanocapsule fish oil were incorporated into yogurt at 15mL/100 g. The results demonstrated an increase in the stability of n-3 fatty acids, such as DHA and EPA, with high sensory acceptability (Ghorbanzade et al., 2017). Another study investigated to develop functional yoghurt with encapsulated fish oil using the nanoemulsion technique as n-3 source. The sample containing nanoemulsion encapsulated fish oil was found to retain 71.75% of its fatty acid content during storage. The overall results show that the developed yoghurt showed significant improvements in both sensory properties and oxidative quality (Shanuke et al., 2025). The fortification of frequently consumed daily foods, such as yogurt, is importance in supporting the recommended amounts for health. In their study, Murage et al. (2021) stated that n-3-fortified yogurt is a source of n-3 fatty acids, providing a sufficient amount of heart-healthy nutrients to reach the recommended daily intake of 150 mg/day with a single serving (150 g) of yogurt.

Conclusion

Consumer's interest in enriched food products is increasing because they offer health benefits besides than food. Meat and dairy products are considered staple foods that are widely consumed across diverse geographical regions and cultural

contexts. Fish oil is main source of PUFA and n-3 fatty acids. Many health authorities make recommendation regular intake for n-3 fatty acids particularly EPA and DHA. The presence of high levels of n-3 fatty acids in foods has positive influence on the human health condition to especially, cardiac, cerebral, and nervous system function. Consequently, fish oil is a substantial source of polyunsaturated fatty acids (PUFAs) and n-3 acids, which have been demonstrated to offer substantial health benefits. However, the daily intake of fish oil remains below the recommended levels. Enrichment food products with fish oil is important field that is the focus of significant research. The inherent susceptibility of fish oil to oxidation and its potential to negatively impact product sensory qualities, such as taste and odor, has necessitated the exploration of encapsulation technologies. While encapsulation offers significant potential for enhancing the functionality and quality of food products, several challenges remain. First, it should be noted that not all products or applications are suitable for encapsulation. Some products may not be able to withstand the encapsulation process, while others may not be compatible with the materials used. Second, the high cost of nano-/microencapsulation technology limits its industrial application. In addition, ensuring the uniform distribution of active ingredients and the effectiveness of microcapsules in protecting them from environmental factors during production and storage is a significant challenge. While a number of clinical trials have evaluated the impact of fish oil intake on human health, more research is needed to assess the specific health effects of consuming fish oil-enriched meat and dairy products. Further research endeavors should concentrate on enhancing encapsulation methodologies, contemplating cost-effectiveness, and meticulously appraising the health benefits of consuming fish oil-enriched food products in human populations.

Compliance With Ethical Standards

Authors' Contributions

GYT: Conceptualization, Supervision, Writing – review & editing

SÖ: Conceptualization, Investigation, Writing – original draft

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data availability is not applicable to this article as no new data were created or analyzed in this study.

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REVIEW ARTICLE

Microplastic distribution and composition in various ecosystems of the Marmara Region: Current gaps and research needs

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ABSTRACT

Microplastics are ubiquitous worldwide and are increasingly recognized as a significant environmental problem. In Türkiye, research on microplastics has expanded over the years, providing valuable insights into the issue. However, studies focusing on different ecosystems are still limited and critical knowledge gaps have not been filled. The aim of this study, therefore, is to compare the composition of microplastics in five different ecosystems—marine water, freshwater, marine sediment, freshwater sediment and soil—across Istanbul and the Marmara region, while also identifying similarities and potential sources of pollution. Furthermore, the study emphasizes the need for more comprehensive research on microplastic pollution and its prevention within the different ecosystems of the region. For this purpose, a total of 26 studies were reviewed, in which 312 samples were collected from 304 stations, 222 of which were located in Istanbul. These stations were categorized by ecosystem type and the predominant composition of microplastics. Results revealed that there is a lack of studies on microplastics in surface waters and soil samples at various locations in Istanbul. While studies in the Marmara region have primarily focused on surface water samples from different locations in the Sea of Marmara, research on lentic and lotic systems, sediments and soils is still insufficient. The results also show that various environmental and anthropogenic factors, including water currents and direction, meteorological conditions, maritime traffic, human and industrial activities, proximity to residential areas and wastewater treatment plants, and atmospheric transport influence the concentration and composition of microplastics. In addition, seasonal and annual variations and the effect of station depth on the accumulation of microplastics were observed. Given that, the analyzed stations represent only a small fraction of the region's ecosystems, this study underlines the urgent need for further research to address existing

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knowledge gaps. The lack of comprehensive studies hinders the effective management of plastic and microplastic litter in the Marmara region. To enable meaningful comparisons at both local and international scales, adapting standardized methodologies in microplastic research is essential.

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Introduction

The increasing production of plastics and the inadequate disposal of plastic waste have led to the widespread distribution of microplastics in the environment (Mortula et al., 2021). These new pollutants persist in both aquatic and terrestrial ecosystems and pose a significant threat to global biodiversity (Horton et al., 2017; Zhang et al., 2023). Given their potential negative impacts on biota, addressing microplastic pollution is essential from both environmental and human health perspectives.

Microplastics, typically defined as particles with a size of 5 mm to 1 µm, can enter ecosystems via different pathways (Bhatt & Chauhan, 2023). Plastics can break down into smaller fragments and microplastics (<5 mm) through physical, chemical and biological processes in the environment, which, in conjunction with the slow degradation process of plastics, leads to an accumulation of plastic fragments of different sizes (Devereux et al., 2023). Due to their physical properties, such as size and specific gravity, they can be transported horizontally and vertically through air, soil and water. In surface waters, low-density microplastics can easily enter coastal areas through surface currents (Ivar do Sul & Costa, 2014). Polymers such as polyethylene (PE), polypropylene (PP), expanded polystyrene (EPS) and ethylene vinyl acetate (EVA), which have a lower density than seawater, tend to float in marine environments and are more likely to be found in surface waters (Woodall et al., 2013). In contrast, denser polymers such as polyamide (PA), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), polycarbonates (PC), acrylonitrile butadiene styrene (ABS), polyurethane (PU) and acrylic more likely to sink and accumulate in sediments (Engler, 2012; Uddin et al., 2020).

The physical properties of microplastics, including shape, color, and polymer structure, provide valuable insights into their origins within ecosystems (Kroon et al., 2018; Schwarz et al., 2019; Zhu et al., 2021). Studies have identified laundry

washing as a major source of microplastic fibers, with wastewater treatment plants serving as potential entry points for these pollutants into surface waters (Napper & Thompson, 2016; Wentworth & Stafford, 2016). In addition to wastewater discharge, microplastics can be transported over long distances through atmospheric deposition, allowing them to reach terrestrial ecosystems far from water bodies and densely populated areas (Zhang et al., 2020; Surendran et al., 2023).

The first study on the distribution and composition of microplastics in the surface waters of the Sea of Marmara was conducted in 2018 and provided initial data on microplastic distributions (Tunçer et al., 2018). However, pollution in the region has continued to increase and poses an urgent ecological challenge. Addressing this problem requires a comprehensive study of the composition and sources of microplastics. The current lack of comprehensive data limits the development of a solid framework for understanding the distribution and impacts of microplastic pollution. Without accurate identification of the sources of pollution, formulating effective waste prevention and reduction strategies remains difficult (Zhou et al., 2024). Therefore, an expansion of research on microplastics in both aquatic (marine, brackish and freshwater) and terrestrial ecosystems in the Marmara region is essential.

The aim of this study, therefore, is to compare the composition of microplastics in five different ecosystems — seawater, freshwater, marine sediment, freshwater sediment and soil — in the entire Marmara region and to identify commonalities and potential sources of pollution. By analyzing the relationships between the sampling stations, this study attempts to determine the possible sources of the detected microplastics. In addition, areas where microplastic pollution has not yet been sufficiently investigated will be identified to support future research and contribute to the development of a comprehensive map of microplastic pollution in the Marmara region.

Material and Methods

Study Area

The Marmara region (40°63'N, 28°12'E), covering around 67,000 square kilometers and accounting for 8 % of Türkiye's total land area, is home to 26,650,405 people as of the 2022 census (Aydın et al., 2023). Istanbul, the most populous city in Türkiye with 15,907,951 inhabitants, is located in this region, which also includes Bursa, the fourth most populous city with 3,194,720 inhabitants (TÜİK, 2022). Istanbul (41.013611°N, 28.955°E) covers an area of 5,421 square kilometers, connects the Sea of Marmara with the Black Sea via the Bosphorus. The city of Istanbul has four natural lakes, five reservoirs and a total of 106 rivers, 67 of which are on the European side and 39 on the Asian side (Dinç & Bölen, 2014). In the Marmara region, 40 Organized Industrial Zones operate wastewater treatment plants with a total treatment capacity of 942,515 m³/day, while the amount of wastewater actually treated is around 545,000 m³/day (Ersoy & Özbay, 2023). Similarly, according to the report published by the Marmara Municipalities Union (2021), the total amount of wastewater discharged from wastewater treatment plants in seven provinces of the region amounted to 4,658,098 m³, of which Istanbul accounted for 3,564,835 m³. Given the significant pollution that these discharges, along with other anthropogenic factors, introduce into the Sea of Marmara, a detailed assessment of their environmental impact is essential.

Review Protocol and Data Analysis

In this study, research papers on microplastics conducted in the Marmara region and Istanbul province were searched in the

“Scopus” and “Science Direct” databases, regardless of the year of publication. The keywords used for the search were “microplastics” AND “Marmara” and “microplastics” AND “Istanbul”. The studies were analyzed using data that included at least one of the following: frequency of microplastics, color, shape and polymer structures of the samples collected from the stations identified within the study areas. These data were categorized into five main groups: Seawater, Freshwater, Sediment and Soil, corresponding to each type of aquatic and terrestrial ecosystem. The abundance of microplastics at surface water stations was expressed as MP/m³, while the abundance of microplastics in sediment and soil stations was expressed as MP/kg. The stations from the study areas identified in the articles obtained from the literature review were mapped using Google Earth based, and the data was visualized using JPM 18.0 and Flourish.

Results and Discussion

Distribution of Microplastic Studies in Marmara Region

This study, which compiles data on the abundance and characterization of microplastics in different ecosystems in the Marmara region, examines a total of 26 research articles. These studies were conducted at a total of 304 stations, 222 of which were in Istanbul. A total of 312 samples were collected from these stations. Most of the samples were sediment samples, including 104 from seawater sediments and 40 from freshwater sediment. Data were collected for the surface water of the Sea of Marmara (87 seawater), the rivers flowing into the Sea of Marmara (17 freshwater) and the wastewater discharged from wastewater treatment plants into the Sea of Marmara (4 wastewater treatment plant effluents) (Figure 1).



Figure 1. Sampling points in the Marmara Region within the scope of relevant studies (Each color on the markers represents a different sample type taken from the stations. Seawater and freshwater samples are given in blue, seawater and freshwater sediment samples in orange, soil samples in purple, other samples (e.g., organisms) in grey, and wastewater treatment plants in green)

A review of the studies revealed that the number of stations where studies on microplastics were conducted in the Marmara Region was not sufficient to make the data fully representative. While surface water studies have been conducted in the Bosphorus and southern coastal waters of Istanbul, research in the northern coastal waters is limited. In addition, there are few studies on the abundance and characterization of microplastics in rivers and lakes (Mülayim et al., 2022; Akdogan et al., 2023). It is also noteworthy that sediment studies were mainly conducted at stations where water samples were also collected (Çullu et al., 2021; Sari Erkan, 2021a; 2021b). Soil studies are limited to two research studies in which samples were taken from urban and industrial areas at eight stations on the European side of Istanbul (Tunali et al., 2022).

General Distribution and Characterization of Microplastics in Marmara Region

Distribution and characterization of microplastics in seawater and sediments

An analysis of the Marmara region shows that the densest stations are to be found in Istanbul. A study in Istanbul found that the stations on the piers near the coast had the highest microplastic frequency, while the stations on the open sea had the lowest values (Sari Erkan et al., 2021a). Microplastic levels were significantly higher at stations with heavy shipping traffic, near sewage treatment plants and industrial activities. Similarly, in a study conducted by Sönmez et al. (2023), it was found that microplastic concentration increased with increasing spatial proximity to wastewater treatment plants. Stations where wastewater treatment plant discharges reached the Sea of Marmara via the rivers showed high microplastic concentration across all sampling periods, highlighting wastewater treatment plants as a major source of microplastics. In another study by Tunçer et al. (2018), it was found that the regional differences in microplastic abundance in the Sea of Marmara could be due to the different treatment capacities of wastewater treatment plants. In a study conducted by Gürkan & Yüksek (2022), surface water and water column samples from various stations in the Sea of Marmara were analyzed and found that microplastic abundance in surface water was significantly lower than in the water column at the same stations. It is hypothesized that this difference is due to the tendency of polymers to sink or float depending on their density and shape, as well as water currents. On the other hand, Terzi et al. (2022) found the highest microplastic abundance on the northern coasts of the

Marmara region, and it was stated that this situation is because the region is one of the most populated and industrialized areas of Türkiye. In addition, various studies show that microplastic concentration is higher in the Marmara region compared to similar studies in other parts of the world (Çullu et al., 2021; Sari Erkan et al., 2021a; Gürkan & Yüksek, 2022; Sönmez et al., 2023). This situation is directly related to the dense population, land and sea transport, industrial activities and inadequate waste management.

Various studies show that the composition of microplastic changes over time (seasonally and monthly) (Çullu et al., 2021; Sönmez et al., 2023). The main reasons for this variability include seasonal weather events, current direction and speed, human activities and shipping traffic. It has been observed that the abundance of microplastic increases especially during rainy periods. This increase can result from the transport of road litter to receiving areas and sewage systems due to the effect of rain and the sedimentation of microplastic in the atmosphere at the surface. The most common types of microplastic in the surface waters of the Marmara region are white, blue and black and consist of PE, PP, PET and EVA in the form of particles and fibers. When examining the distribution of microplastic concentrations (MP/m³) in surface waters according to color, shape and polymer types (Figure 2), it was found that blue and black EVA fibers dominated.

The highest microplastic concentration in sediment samples was found at the coastal and shallow water stations (Baysal et al., 2020; Sari Erkan et al., 2021b; Mülayim et al., 2022). The predominant polymers in the sediments of the Marmara region were PP, PE, PS, polyphenylene sulphide (PPS) and ABS polymers, which are commonly found in blue and black colors in the form of fragments and fibers. Çullu et al. (2021) detected blue PE particles in the surface water of the Küçükçekmece Lagoon. Similarly, in the study conducted by Baysal et al. (2020) in the southeast of the Sea of Marmara (Pendik-Tuzla region), PE was found to be the dominant polymer. Sediment studies conducted at 15 sites in Istanbul revealed that MP concentration was higher during the rainy season, similar to surface water (İşlek et al., 2023). These seasonal variations were associated with the increased human activities, traffic and changes in current speed and direction. Olguner et al. (2023) investigated the influence of shipping traffic, tourism, and fishing and population concentration on microplastic pollution in the Bosphorus, where about 45,000 ships pass annually. High concentrations of microplastics were found in relatively less urbanized areas such as Anadolu Hisarı and Rumeli Feneri, while the lowest microplastic

Distribution and characterization of microplastics in freshwater and sediments

Akdogan et al. (2023) conducted fresh water and sediment investigations at six stations in the Ergene Basin. While black PET fibers were the predominant type of microplastic in the surface waters, black PS particles predominated in the sediments. The study revealed significant temporal variations in the amount of microplastics in the sediments and a correlation between the microplastic concentrations in the river morphology. These findings highlight the possible influence of upstream industrial areas and wastewater treatment plants on the amount of microplastics at the sampled river stations. Similarly, in a study conducted by Mülâyim et al. (2022) in Lake Durusu in Istanbul, transparent PP particles were identified as

the predominant microplastic type. This was attributed to the degradation of improperly disposed plastic waste used in packaging and other commercial applications. In another study, Almas et al. (2022) investigated the Susurluk Basin and found that fibers were the predominant microplastic type in sediment samples. Despite these findings, research on microplastic pollution in freshwater environments within the Marmara region remains limited. Given the critical role of rivers and lakes as transport pathways for microplastics into marine ecosystems, further studies are needed to comprehensively assess sources, distribution patterns and seasonal variations in these areas. Expanding research efforts in freshwater systems will enable a more comprehensive understanding of regional microplastic pollution and its wider environmental impacts.

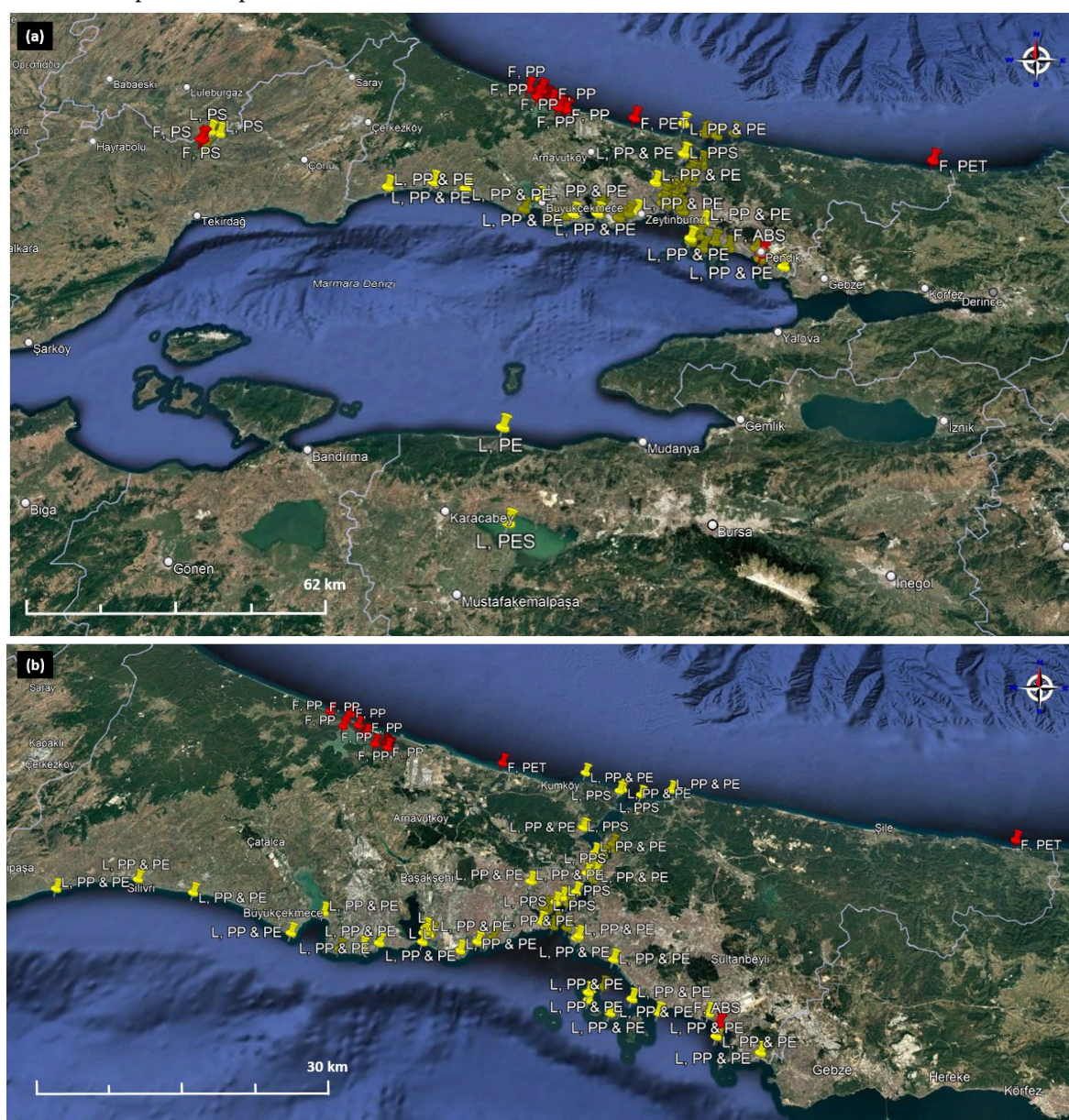


Figure 4. Dominant physical and chemical characteristics of microplastics detected in sediments a) Marmara Region and b) Istanbul (Fragment = F (Red Pin), Fiber = L (Yellow Pin), Film = M (Green Pin), Filament = T (Purple Pin), Pellet = P (Orange Pin))

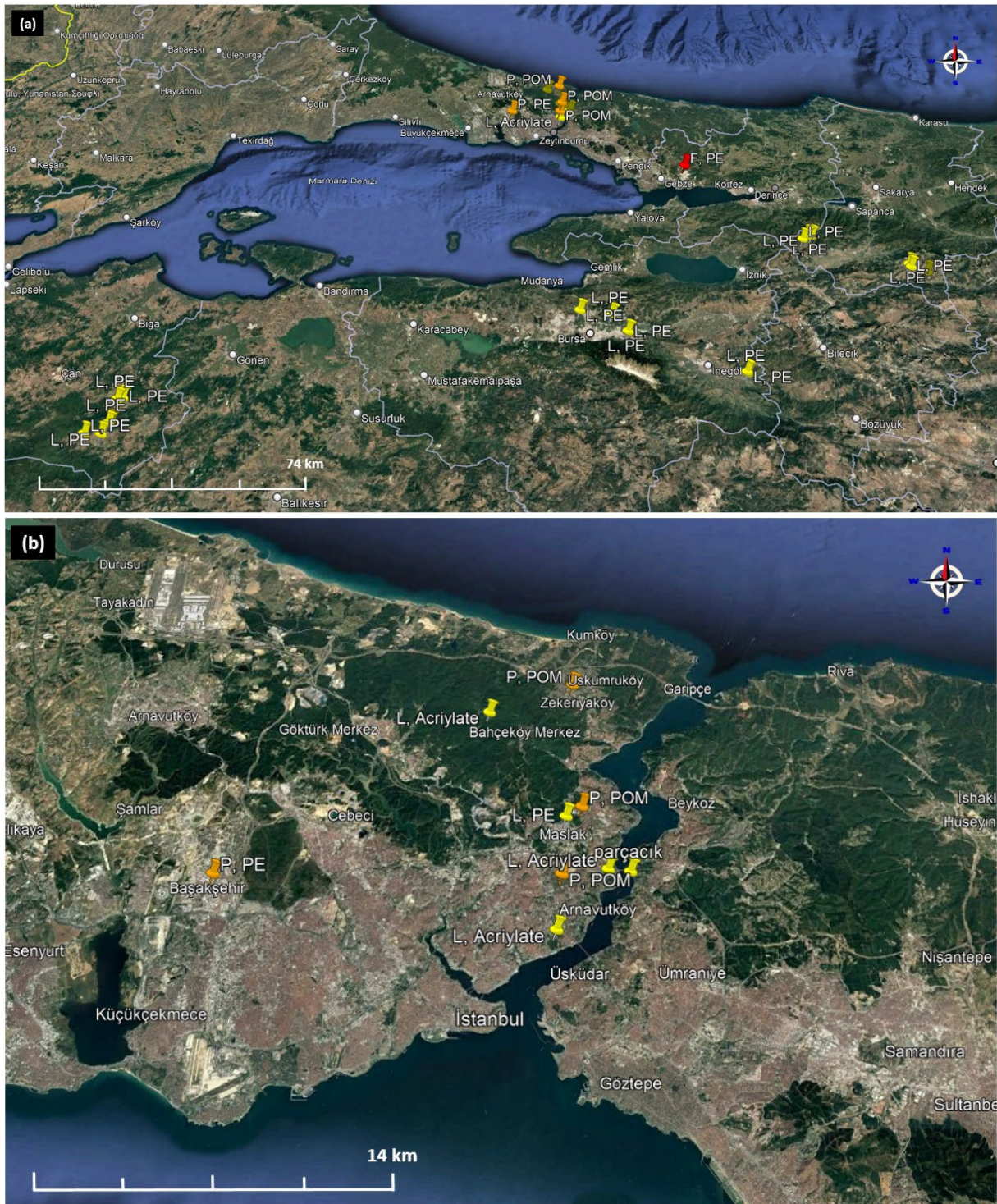


Figure 5. Dominant physical and chemical characteristics of microplastics detected in soils **a)** Marmara Region and **b)** Istanbul (Fragment = F (Red Pin), Fiber = L (Yellow Pin), Film = M (Green Pin), Filament = T (Purple Pin), Pellet = P (Orange Pin))

Distribution and characterization of in soils

Studies on soil microplastic pollution in the Marmara region remain limited, with research to date focusing primarily on microplastic concentrations in recreational, agricultural and industrial soils. In a study conducted in Sakarya, Bursa and Çanakkale, five agricultural and one urban soil samples were analyzed. The results showed that microplastic concentration

was higher in agricultural soils than in urban soils (Akca et al., 2024). In particular, blue PE fibers were the predominant microplastic type in urban soils (parks, roadsides and green areas), reflecting the composition observed in the agricultural soils of the same cities, although they are geographically separated. This pattern suggests that inefficiently treated irrigation water or atmospheric transport may play a role in the accumulation of microplastics. Tunalı et al. (2022) also

investigated the distribution and morphology of microplastics in soil samples from three recreational, three urban and three industrial areas. The highest microplastic concentration was found in a soil sample from the Belgrade Forest, while urban soil samples from a park and a college campus also showed higher concentrations than industrial areas. These results confirm the hypothesis that microplastics tend to accumulate in soils over time. The predominant MP types varied depending on land use: recreational areas showed red PO and acrylate fibers, urban areas were dominated by red POM and polyether pellets, while industrial soils were dominated by yellow PE

pellets. Despite these findings, soil microplastic pollution in the Marmara region is still poorly understood. Given the critical role of soil as both a sink and a potential source of microplastics, further research is needed to assess the long-term environmental behavior, transport mechanisms and potential risks to terrestrial ecosystems. As plastics can be transported, they can accumulate both in the water column and in sediments, e.g., in the river bed, which can serve as temporary sinks depending on the specific characteristics of the river or river section (Horton et al., 2017).

Table 1. Current studies on microplastic pollution in various environmental compartments in Marmara Region

Sampling Area	Number of Stations	Predominant Microplastic					Reference
		Concentration	Size (μm)	Shape	Color	Type	
Seawater	5	37.37 particles/L	>50	fragment	blue	-	Çullu et al. (2021)
Seawater	13	0.071 particles/L	300 - 2000	fiber	black	-	Gürkan & Yüksek (2022)
Seawater	43	1284.74 particles/km ²	>1000	fragment	white	-	Sari Erkan et al. (2021b)
Seawater	9	146.63 particles/L	100 – 249	fiber	transparent	EVA	Sönmez et al. (2023)
Seawater	16	0.019 particles/ L	2070	fiber	-	PET	Terzi et al. (2022)
Seawater	14	1.263 particles/m ²	-	fragment	white	-	Tunçer et al. (2018)
Seawater Sediment	14	0.3 – 85.6 g/kg	-	fragment	black	ABS	Baysal et al. (2020)
Seawater Sediment	1	1960 particles/kg	20–200	fragment	-	-	Belivermiş et al. (2021)
Seawater Sediment	3	3332 particles/kg	<100	fiber	black	-	İşlek et al. (2023)
Seawater Sediment	15	326.62 particles/kg	<1000	fiber	black	PPS	Olguner et al. (2023)
Seawater Sediment	43	1957.37 particles/kg	>300	filaments	blue	-	Sari Erkan et al. (2021a)
Seawater Sediment	43	4337.5 particles/kg	>1000	fiber	blue	PE	Sari Erkan et al. (2023)
Freshwater	1	6.90 particles/L	1000 - 2000	fiber	black	PET	Akdogan et al. (2023)
Freshwater	5	28.78 particles/L	>50	fragment	blue	-	Çullu et al. (2021)
Freshwater Sediment	1	277.76 particles/kg	45 - 1000	fiber	black	PS	Akdogan et al. (2023)
Freshwater Sediment	1	-	1380	fiber	transparent	PE	Almas et al. (2022)
Freshwater Sediment	1	-	1700	fiber	black	PE	Almas et al. (2022)
Freshwater Sediment	2	2648.3 particles/kg	<100	fiber	black	-	İşlek et al. (2023)
Freshwater Sediment	11	134.76 particles/kg	-	fragment	blue	PP	Mülayim et al. (2022)
Soil	55	160 particles/kg	<1000	fiber	blue	-	Akca et al. (2024)
Soil	27	3556.4 particles/kg	<1000	fiber	red	Acrylate	Tunali et al. (2022)

General Evaluation and Future Perspectives

Table 1 contains a comprehensive dataset on microplastic pollution in different environmental compartments in the Marmara region, showing the differences in the number of stations, concentration values and relevant references. Several important observations can be derived from these data, which take into account both the spatial distribution and the methodological consistency of the studies. Studies on marine waters have the largest spatial coverage, with the number of stations ranging from 5 to 43. In particular, the study conducted by Sari Erkan et al. (2021b) at 43 stations provides a large-scale assessment of microplastic pollution in the marine environment and highlights the importance of extensive sampling for understanding spatial variability. Marine sediments show a similar pattern, with the number of stations reaching 43 in some cases (Sari Erkan et al., 2023). This parallel trend between water and sediment studies indicates that research is strongly focused on the deposition of microplastics from the water column to the seafloor. However, the variations in concentration values, ranging from 0.019 to 146.63 particles/L in seawater (Figure 6a) and from 326.62 to 4337.5 particles/kg in sediment (Figure 7a), indicate significant differences in pollution levels, possibly influenced by hydrodynamic conditions, sedimentation rates and proximity to pollution sources. The high accumulation in sediments is consistent with global findings that sediments serve as a long-term reservoir for microplastics.

Freshwater environments appear to be less extensively studied, with the number of stations ranging from 1 to 5 (Çullu et al., 2021; Akdoğan et al., 2023). This limited research coverage raises concerns about data gaps, especially when considering the role of rivers in the transport of microplastics from the terrestrial to the marine environment. Reported concentrations vary considerably, with the highest value being 28.78 particles/L (Çullu et al., 2021). Considering that freshwater systems act as transition pathways for microplastics before they reach the marine environment, the relatively small number of stations highlights the need for further research to capture the dynamics of microplastic transport and retention in freshwater ecosystems. Studies of freshwater sediments show even greater variability, with concentrations ranging from 0.3 to 2648.3 particles/kg (İşlek et al., 2023) (Figure 7a). The highest concentration found in sediments indicates that depositional environments in freshwater systems can act as sinks for microplastics. However, due to the limited number of sampling stations, it is difficult to generalize trends, especially in relation to land-based pollution sources and hydrological processes that influence sedimentation rates. Figure 6b shows a chord diagram illustrating the relationships between the composition of microplastics in different ecosystems based on polymer types, colors and physical forms. The identified polymers include common plastic types such as PE, PP, PET and EVA. Notably, PE and PP are more prevalent in seawater, while freshwater ecosystems have a greater diversity of microplastic types. In terms of color distribution, blue, black and opaque microplastics are predominant.

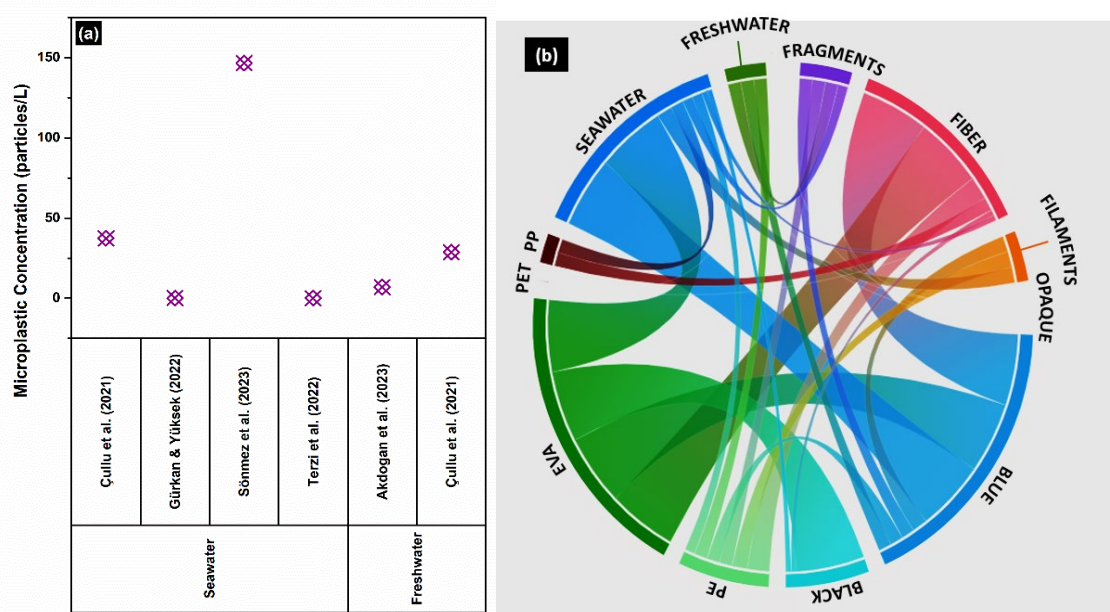


Figure 6. Current reports on (a) microplastic abundance across surface water stations in the Marmara Region and (b) dominant compositions of microplastics

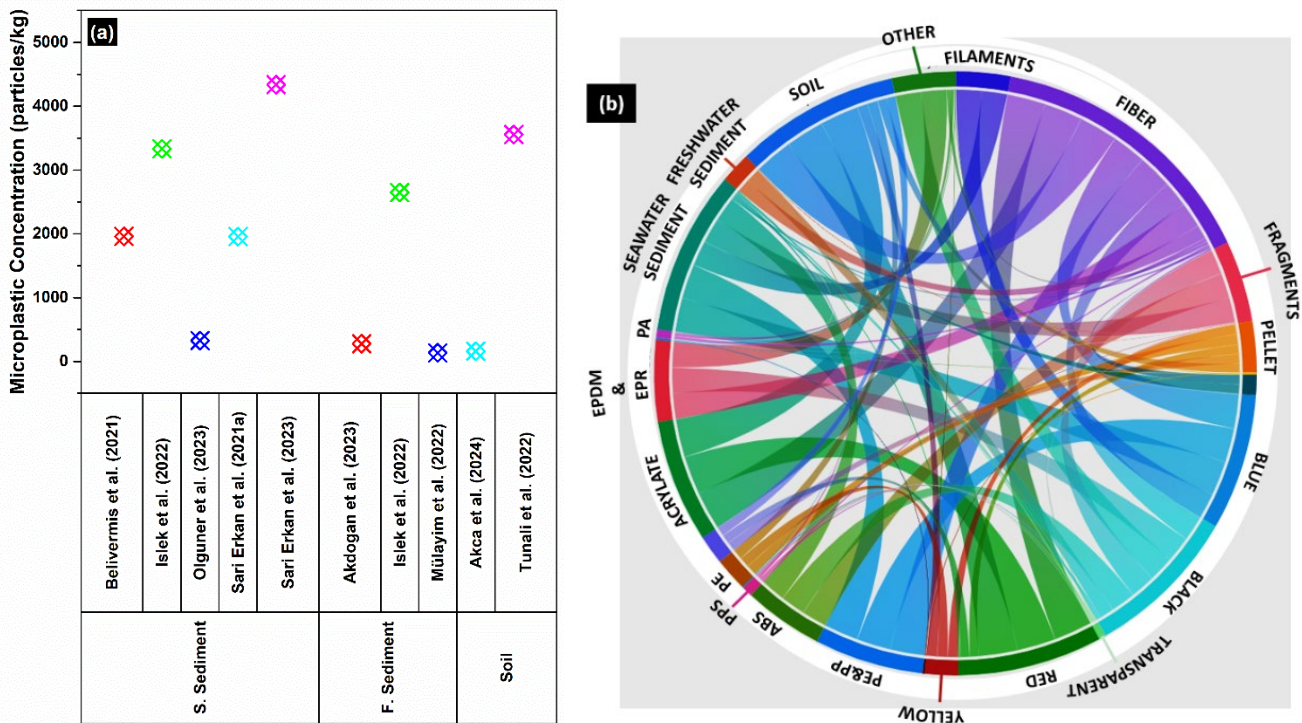


Figure 7. Current reports on (a) microplastic abundance across sediments and soils in the Marmara Region and (b) dominant compositions of microplastics



Figure 8. Hotspot stations for microplastic studies (Blue markers indicate stations with microplastic studies, while red markers represent key stations)

Soil studies demonstrate a wider geographical range, with the number of stations reaching 55 (Akca et al., 2024) and 27 (Tunali et al., 2022). These studies show some of the highest measured microplastic concentrations with values of 3556.4 particles/kg (Tunali et al., 2022) (Figure 7a). The significant accumulation of microplastics in soils highlights the potential role of agricultural activities, urban runoff and atmospheric deposition in shaping contamination patterns. As soils can act as both sources and sinks for microplastics, the high variability

of reported concentrations suggests that local land use patterns and anthropogenic activities strongly influence contamination levels. As given in Figure 7b, the soil samples contain predominantly fiber-based microplastics, with acrylate and PP which is consistent with the use of plastics in agriculture, geotextiles and the degradation of plastic mulch films. The presence of red-colored acrylate fibers also indicates that synthetic textiles and coatings contribute significantly to microplastic contamination in the terrestrial environment. The

results suggest that soil serves as a long-term sink for microplastics, with retention mechanisms differing from those in aquatic environments.

A comprehensive review of all data shows that the shape, color, and polymer type of microplastics found predominantly in an ecosystem can provide valuable insights to its origin. However, it has been shown that the natural conditions of the ecosystem and human activities play a crucial role in the distribution of microplastics. In short-term studies conducted in closely located environments, a varying microplastic distribution pattern was detected. These differences underline the dynamic nature of microplastic communities over different time periods. It was found that the concentration and composition of microplastics can change seasonally and even monthly. It was also found that factors such as water depth and proximity to the coast influence the distribution of microplastics in water and sediment samples.

The lack of standardization of reporting units and the disproportionate focus on the marine environment make it difficult to compare different environmental areas. These inconsistencies in reporting not only make it difficult to combine results, but also limit our ability to draw reliable conclusions about the distribution of microplastics in different ecosystems. A more standardized research approach is needed to better assess the extent of microplastic pollution in different environments. There are significant gaps in microplastics research in several areas, including Istanbul, particularly in surface water and soil sampling (Figure 8). These gaps highlight the need for more comprehensive monitoring of microplastic pollution beyond the marine environment. Surface waters, sediments and soils, which can contain large amounts of microplastics, have not been adequately studied.

Conclusion

The Marmara region, with its dense population, industrial activity, transportation networks and tourism, is facing an increasing threat from microplastics. However, the lack of adequate data on this important pollutant prevents effective action from being taken. This study aims to provide a valuable reference for future research on microplastics and its sources in the Marmara region, especially in Istanbul.

To the best of the author's knowledge, this is the first baseline study on the abundance of microplastics in the Marmara region, providing valuable insights for policy makers and efforts to reduce plastic pollution. The findings can serve as a reference for future monitoring of microplastics in the Sea of

Marmara, particularly in assessing the effectiveness of measures aimed at reducing land-based microplastic inputs into surface waters.

Given the complex industrial and urban development of the region, it is crucial to extend monitoring measures to different environmental areas. While most studies have focused on the marine environment, which is an important aspect of the problem, pollution of freshwater ecosystems, soils and air deposition is equally important. Since freshwater bodies eventually flow into the sea, pollution from non-marine sources has a direct impact on marine ecosystems. Therefore, research should also focus on key areas such as river basins, wetlands and urban soils to track the movement of microplastics from land and freshwater to the sea. Expanding research in these areas is essential to fully understand the extent of microplastic pollution and develop effective solutions. A more balanced approach to research will improve our understanding of the behavior of microplastics and their long-term impact on human health and biodiversity.

Compliance With Ethical Standards

Authors' Contributions

CA: Conceptualization, Writing – original draft, Formal analysis, Writing – review & editing

VZS: Writing – original draft, Formal analysis

AI: Formal analysis

NS: Conceptualization, Writing – original draft,

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data Availability

All data generated or analyzed during this study are included in this published article.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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