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Research Article
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Microplastic Ingestion in the Rocky Intertidal Zone: An Assessment of the Grazing Gastropod *Phorcus turbinatus*

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Abstract: Microplastic (MP) pollution has become a ubiquitous threat to marine ecosystems, yet studies on grazing gastropods in the rocky intertidal zone remain limited. This study investigates the microplastic contamination in the top-shell snail (n=36), *Phorcus turbinatus*, collected from the highly industrialized İskenderun Bay (Northeastern Mediterranean). The soft tissue of the collected samples underwent chemical digestion, filtration, and extraction processes, after which all suspected microplastic particles were documented. Microplastics were detected in 36% of the individuals, with a mean abundance of 0.42±0.55 MP items/individual and 0.28±0.40 MP items/g (wet weight). Morphological characterization revealed an overwhelming dominance of fibers (92.3%) and black-colored particles (76.9%), with the majority of MPs (72%) being smaller than 1 mm. A moderate positive correlation was observed between shell size and MP burden, suggesting size-dependent accumulation. These results indicate that *P. turbinatus* is susceptible to microplastic ingestion via grazing which reveal their potential canditance as bioindicators for monitoring benthic pollution in rocky coastal habitats. However, the role of *P. turbinatus* as a reliable bioindicator remains to be validated through more extensive spatio-temporal monitoring.

Keywords
- Microplastics
- *Phorcus turbinatus*
- Bioindicator
- İskenderun Bay

1. INTRODUCTION

The exponential increase in global plastic production has led to the ubiquitous presence of plastic waste in aquatic ecosystems, where it fragments into smaller particles known as microplastics (MPs). These persistent pollutants, have size range between 1 µm to 5 mm, have permeated every trophic level of the marine food web (Marmara et al., 2023), posing severe risks to biota ranging from physiological stress to physical blockages in digestive systems (Raju et al., 2022). While initial research primarily focused on microplastic accumulation in commercial fish species (Aydin et al., 2023; Marmara et al., 2023), research on invertebrates has been disproportionately centered on bivalves due to their high filtration rates (Kibria et al., 2022). Consequently, there is still a significant lack of data regarding other invertebrates inhabiting the littoral zone (de Sá et al., 2018; Curren et al., 2024), particularly gastropods that occupy distinct ecological niches in benthic rocky habitats (Kögel et al., 2020; Weber et al., 2021; Wootton et al., 2022).

Grazing gastropods are crucial components of rocky ecosystems. Their special feeding strategy involves scraping algae from the substrate which makes them more vulnerable to MP ingestion (Doyle et al., 2020). Together with grazing behavior, their close interaction with the substrate, this species exhibits a stronger correlation with sediment-associated pollution loads rather than those suspended in the water column. For that reason, these organisms have been successfully applied to monitor pollutants in the benthic environment (Aranda et al., 2022, Duysak & Ersoy, 2014). Similar to other contaminants, Curren et al. (2024) proposed the usage of gastropods as microplastic bioindicators due to their global distribution across intertidal and marine sediments, their remarkable tolerance to environmental stressors, their feeding behavior and vital role in material circulation.

The Northeastern Mediterranean Sea, particularly İskenderun Bay, is characterized as a significant "hotspot" for microplastic contamination due to its semi-enclosed basin dynamics and intense anthropogenic pressure (Gündoğdu, 2017; Liubartseva et al., 2018; Gündoğdu & Çevik, 2019; Çevik et al., 2022; Kılıç, 2024a). As highlighted in recent regional studies, coastal regions receive a substantial plastic load from not only terrestrial environments (i.e., surface runoff, wastewater disposal, urbanized and industrial coastal area) (Çevik et al., 2022; Kılıç, 2024a), but also freshwater environments (Yılmaz et al., 2022), atmospheric inputs (Yücel et al., 2023).

To assess the impact of this pollution, the selection of appropriate bioindicator organisms is essential. In this context, *Phorcus turbinatus*, a widespread and sedentary gastropod in the Mediterranean intertidal zone, presents itself as an ideal sentinel species.

Despite the growing body of literature on microplastic accumulation in the region's commercial species, data regarding microplastic ingestion in *P. turbinatus* remain relatively fragmented. Although some studies have investigated microplastic and nanoplastic ingestion levels in the same family (Ehlers et al., 2022; Janssens & Garcia-Vazquez, 2021; Li & Han, 2022), there is still a need for region-specific baseline data to understand the contamination profile in the Northeastern Mediterranean Sea. This study aims to meet this need by investigating the presence, abundance of microplastics in *P. turbinatus* specimens collected from the Northeastern Mediterranean coast.

2. MATERIAL and METHODS

2.1. Study Area and Sampling Strategy

This study was conducted along the coast of İskenderun Bay, located in the Northeastern Mediterranean Sea. This region is characterized as a pollution hotspot due to limited water circulation and significant anthropogenic pressures (Özhan et al., 2016; Gündoğdu & Çevik, 2019), including urbanized coastal area, industrial activities, maritime traffic, and riverine inputs coming from Seyhan, Ceyhan (Gündoğdu & Çevik, 2017), and Asi Rivers (Kılıç et al., 2024; Yılmaz et al., 2022).

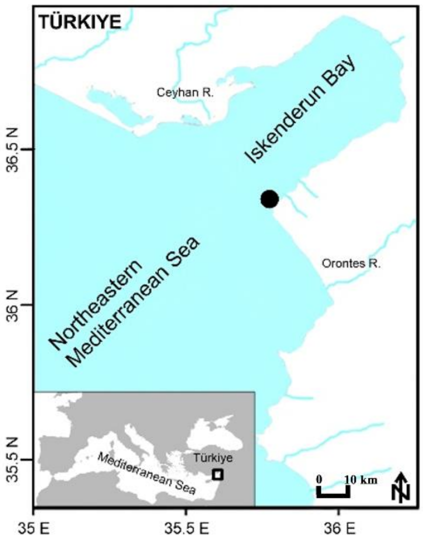

Figure 1. Representative sampling area is indicated by a filled circle on the map.

A total of 36 specimens of *Phorcus turbinatus* were collected randomly from the rocky intertidal zone along the coastline between Kale and Arsuz during low tide in May 2025 (Fig 1). Because of the restricted distribution of the organism in the region, a pooled sampling approach was adopted rather than utilizing distinct sampling stations. To avoid cross-contamination, all samples were rinsed with ambient seawater, wrapped in aluminum foil, and transported to the laboratory in ice-filled cooler boxes. Upon arrival, samples were stored at -20°C until analysis.

2.2. Sample Preparation and Biometric Measurements

In the laboratory, samples were thawed at room temperature and rinsed with distilled water to remove external sediments and debris. Biometric parameters, including shell length, width, and height, were measured using a digital caliper (0.01 mm precision), and total wet weights were recorded (0.01 g precision). The soft tissues were carefully dissected from the shells using stainless steel forceps and weighed separately to determine the wet tissue weight.

2.3. Digestion and Microplastic Extraction

To isolate microplastics (MPs) from the organic tissue, an oxidative digestion protocol was employed following established methodologies for gastropods. In this study, the entire soft tissue of each gastropod was processed as a whole for chemical digestion. No individual tissue separation (e.g., muscle or digestive tract) was performed, ensuring that the total microplastic burden within the organism's body was captured. The dissected soft tissues were placed in clean glass beakers, and approximately 100 mL of 30% hydrogen peroxide H_2O_2 was added to each sample. The beakers were covered with aluminum foil and incubated on a hot plate at 50°C for 24 hours until the organic matter was completely digested and the solution became clear (Yücel & Kılıç, 2023). The chemical digestion process was conducted using H_2O_2, selected for its high efficiency and relatively low environmental impact, as it primarily decomposes into water and oxygen. To ensure human health and safety, all procedures were performed in a ventilated fume hood by personnel wearing appropriate personal protective equipment (PPE), including lab coats, nitrile gloves, and safety goggles. Following the completion of the digestion, all chemical residues were neutralized and disposed of according to the institutional environmental safety guidelines.

Following digestion, the solution was filtered through 50 µm mesh filters using a vacuum filtration system. The filters were then placed in sterile glass petri dishes and dried at room temperature for microscopic examination.

In this study, the detection limit for microplastics was set at 50 µm, corresponding to the mesh size of the filters used during the extraction process. Consequently, microplastics smaller than 50 µm were not accounted for, and the reported concentrations reflect the abundance of particles within the 50 µm to 5 mm size range.

2.4. Microscopic Examination and Characterization

The dried filters were visually inspected under a stereo microscope (e.g., Olympus SZX7) to identify potential microplastics. Particles were categorized according to their physical characteristics. Shape (fiber, fragment, pellet, film), color (black, blue, red, transparent) and size (which was measured using image analysis software (e.g., Olympus Stream Enterprise)) were noted. The minimum detection threshold for visual inspection was set at 50 µm, corresponding to the mesh size used during filtration. Any particles appearing smaller than this in initial drafts were excluded to ensure physical plausibility and methodological consistency. To reduce the risk of false positives from organic materials (e.g., chitin or cellulose), strictly defined morphological criteria were applied. Only particles that was absent of cellular or organic structures, uniform color throughout the particle, and consistent thickness were noted as suspected particles (Hidalgo-Ruz et al., 2012). To enhance the reliability of our visual identification, we performed the hot needle test as a secondary validation step. According to previous validation studies, the hot needle test shows a high correlation (over 90%) with spectroscopic techniques such as Raman microscopy for confirming the synthetic nature of suspected microplastics (Beckingham et al., 2023). By applying these rigorous physical and morphological criteria, we minimized the potential for false positives in our counts.

2.5. Quality Assurance and Contamination Control

To ensure data integrity and minimize the risk of airborne contamination, all analytical procedures were performed in a restricted-access laboratory equipped with High-Efficiency Particulate Air (HEPA) filtration. Strict cleaning protocols were implemented; all laboratory surfaces and glassware were rinsed thoroughly with filtered distilled water prior to and after each dissection. To prevent atmospheric deposition, glassware was immediately covered with aluminum foil following the introduction of samples (Bessa et al., 2019). Additionally, all reagent solutions, including hydrogen peroxide were filtered prior to use. Laboratory personnel adhered to strict contamination control measures, including the use of cotton lab coats and non-synthetic gloves. Procedural blanks, consisting of two wet filters placed in open Petri dishes, were employed during each analytical batch (dissection, digestion, and microscopic inspection) to monitor background contamination. No particle was detected in blank filters.

To validate the efficiency of the extraction method, a recovery assessment was conducted using polyethylene (PE), a widely accepted reference material for spiking experiments (Way et al., 2022). PE particles were ground and sieved to isolate the 51-505 µm size fraction. A known quantity of these particles was spiked into approximately 0.1 g of mantle tissue from a randomly selected specimen and subjected to the identical digestion and filtration protocol used for the samples. The method yielded a recovery efficiency of 90% for PE, demonstrating the high extraction capability of the procedure. It should be noted that reported abundance values are conservative estimates, as is standard practice in many MP studies when the primary purpose of the recovery test is quality assurance rather than correction.

2.6. Statistical Analyzes

Data normality was assessed using the Shapiro-Wilk test. Since microplastic abundance data typically do not follow a normal distribution, spearman's rank correlation analysis was conducted to evaluate the relationship between the biometric size of the gastropods (shell length, weight) and the number of ingested microplastics. Statistical significance was accepted at $p < 0.05$

3. RESULTS and DISCUSSION

3.1. Biometric Measurements and Microplastic Abundance

A total of 36 *Phorcus turbinatus* specimens were collected and examined for microplastic (MP) presence. The specimens had a mean shell height of 3.43±0.47 mm, mean shell width of 3.02±0.30 mm and a mean total weight of 14.42±3.72 g. The mean soft tissue weight (on wet weight basis), which is critical for density-based MP assessment, was recorded as 1.46±0.51g.

Microplastics were detected in specimens, corresponding to a frequency of occurrence (FO%) of 36%. A total of 13 MP particles were extracted, with a maximum of 2 MP particles were found per individual. The mean MP abundance was calculated as 0.42±0.55 MP items/individual and 0.28±0.40 MP items/g (wet weight). The detection of microplastics in the soft tissues of *P. turbinatus* confirms that littoral grazers are actively interacting with these pollutants in their natural habitat. This field observation is particularly concerning when considered alongside laboratory findings by Li & Han (2022), who demonstrated that plastic exposure induces oxidative stress and immune suppression in the related species *Monodonta labio*, suggesting that wild populations may be experiencing similar physiological impairments.

Microplastic ingestion rates in sea snails varied significant variability in previously published studies globally. Exceptionally high abundance rates have been reported in South Asian waters; for instance, *Littoraria scabra* from Indonesia contained an average of 86.88 particles/individual (Patria et al., 2020), and *Babylonia spirata* from India contained 32 MPs/individual (Abisha et al., 2024). In contrast, relatively lower abundances were observed in other regions, such as 19.6 MPs/ind in *P. lineatus* and *Steromphala umbilicaris* topshells from Spain (Janssens and Garcia-Vazquez, 2021), 10.10±6.24 MP items/ind in *Rapana venosa* from China (Liu et al., 2021) and 0.20-1.80 MP/ind in *Littorina littorea* from the Bay of Bengal (Rahman et al., 2025). The results of the present study (0.42 MP/ind in *P. turbinatus* situate the Northeastern Mediterranean at the lower end of this global spectrum. This finding is in strong agreement with Ehlers et al. (2022), who reported consistently low microplastic loads (~0.38 MP/ind) in grazing snails (*Phorcus* spp. and *Steromphala* spp.) across the North Sea, Mediterranean Sea, and Atlantic Sea. Similarly, reported values in this study are comparable to those reported for other grazing gastropods in the Mediterranean basin. For instance, Yücel & Kılıç (2023) reported MP abundances ranging from 0.20 to 0.45 items/ind in *Patella caerulea* from the same region (İskenderun Bay). These comparisons highlight a distinct regional disparity: while intertidal gastropods in European and Mediterranean waters appear to maintain a low and stable contamination baseline, populations in South Asian regions face significantly higher exposure pressures, likely driven by differences in local pollution loads.

Since these organisms feed by grazing on algae within the littoral rocky zone that could be considered as ultimate sink for microplastics (Ehlers & Ellrich, 2020), they play an important role in the transport and distribution kinetics of microplastics. Firstly, a recent study demonstrated that gastropod pedal mucus acts as a trap for microplastics, thereby facilitating their ingestion by marine snails (Gutow et al., 2019). Secondly, feeding activity through grazing may lead to the mechanical fragmentation of microplastics during feeding, contributing to the formation of secondary microplastics (Porter et al., 2019). From an ecological perspective, this indicates that these organisms are not only susceptible to microplastic contamination but also act as active agents in the sink and transport processes of these pollutants.

3.2. Characterization of Microplastics (Shape, Color, and Size)

The morphological characteristics of the extracted MPs were analyzed in terms of shape, color, and size (Fig. 2). In terms of shape, fibers were the overwhelmingly dominant type, constituting 93% of all identified particles, while fragments accounted for only 7%.

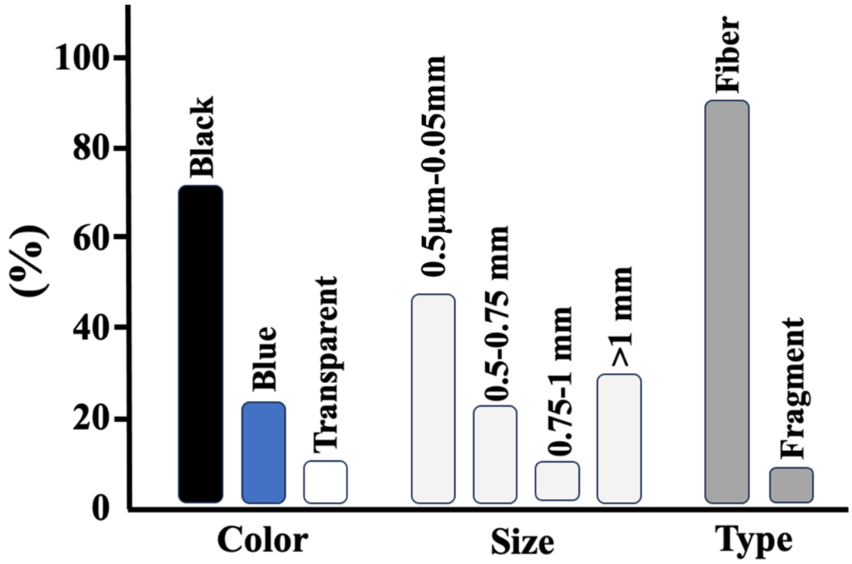

Figure 2. Distribution of microplastic characteristics showing their composition according to color, size range and type.

This dominance of fibers is aligned with the findings of Rahman et al. (2025) from the Bay of Bengal and Patria et al. (2020) from Indonesia. However, the morphological profile of the recovered microplastics notably diverges from the findings of Ehlers et al. (2022), who reported a dominance of paint fragments in *P. turbinatus* populations from the Western Mediterranean, attributed to maritime traffic and harbor activities. In contrast, the overwhelming predominance of fibers in the present study reflects the specific land-based pollution characteristics of İskenderun Bay (Kılıç, 2024a). This pattern is likely driven by substantial terrestrial inputs, particularly from the Asi River (Kılıç et al., 2024) and municipal wastewater discharges (Kılıç et al., 2023), which are well-documented vectors for synthetic fibers originating from domestic laundry and agricultural runoff. Furthermore, these findings align with previous regional studies that have consistently identified fibers as the dominant shape in both surface waters (Güven et al., 2017; Gündoğdu & Çevik, 2017; Çevik et al., 2022; Kılıç, 2024a) and various taxonomic groups within the Bay, including gastropod (Yücel & Kılıç, 2023), cephalopod (Kılıç et al., 2026), crustacean (Yücel & Kılıç, 2025), and commercial fish species (Güven et al., 2017; Kılıç et al., 2024b). These comparison highlights that while the quantity of MP ingestion (abundance) may be consistent across the Mediterranean, the quality (type) of ingested plastics reflects local environmental pressures.

Regarding color distribution, black was the most prevalent color, representing 73% of the particles, followed by blue (20%). Dominance of blue and black particles with coherent with previous studies in Northeastern Mediterranean Sea (Güven et al., 2017; Yücel & Kılıç, 2023).

The size distribution of the recovered microplastics was heavily skewed towards smaller particles, with 72% of the identified items measuring less than 1 mm. This result is coherent to the previous reports in grazing gastropods (Ehlers et al., 2022; Yücel & Kılıç, 2023; Kılıç & Uğurlu, 2024; Rahman et al., 2025). This prevalence of smaller fractions is likely driven by the anatomical constraints of the species, specifically its small mouth aperture (Jâms et al., 2020), or the mechanical fragmentation of larger plastic debris into smaller pieces during the radular scraping process. Despite the dominance of small-sized particles, the isolated microplastics exhibited a broad size range (108-3515 µm). The ingestion of larger particles, which exceeds typical grazing targets, suggests that high-energy hydrodynamic forces-particularly wave action in the intertidal zone-may force the accidental intake of larger particles (Brate et al., 2018).

3.3. Correlation Analysis

A Spearman correlation analysis revealed a moderate positive correlation between total weight and microplastic count (r=0.44), as well as between shell height and microplastic count (r=0.30). This suggests that larger and older individuals, which presumably possess higher grazing rates and longer exposure histories, tend to accumulate more microplastics. This phenomenon of 'size-dependent' accumulation is consistent with metabolic scaling; as gastropods grow, their grazing activity on algal biofilms increases to meet elevated energy demands, thereby raising the probability of encountering adhered microplastics. Our findings strongly corroborate the results of Patria et al. (2020), who similarly reported a positive correlation between body weight of snail and microplastic abundance in the *Littoraria scabra*. Both studies collectively suggest that larger gastropods act as greater sinks for microplastics due to their intensified interaction with contaminated substrates.

3.4 Study Limitations

Despite the significant baseline data provided by this research, several limitations must be acknowledged to provide a balanced context for the findings. First, the study was conducted during a single sampling season in May 2025. This temporal snapshot limits our ability to account for seasonal variations in microplastic concentrations, which are often influenced by fluctuating hydrodynamic conditions and precipitation-driven land-based inputs. Second, due to the specific population distribution and habitat accessibility during the study period, sampling was focused on a representative area rather than the entire coastline of İskenderun Bay, which may not fully capture the spatial heterogeneity of the gulf. The absence of polymer characterization using spectroscopic techniques such as μ-FTIR or Raman spectroscopy remains a primary limitation. While this is a common challenge in resource-limited settings, extensive measures were taken to mitigate the risk of misidentification and false positives. These included the application of strictly defined morphological criteria, such as verifying the absence of organic structures and ensuring uniform color and thickness, alongside the performance of the hot needle test. Literature indicates that these physical and morphological assessments can achieve over 90% accuracy in confirming synthetic polymers. Future research incorporating multi-seasonal sampling and chemical polymer identification will be essential to further validate and expand upon these preliminary findings.

4. CONCLUSION

This study provides one of the first assessments of microplastic ingestion by the grazing gastropod *Phorcus turbinatus* in the Northeastern Mediterranean Sea. The presence of microplastics in over one-third of the examined specimens confirms that rocky intertidal grazers are directly interacting with plastic pollution. Unlike filter feeders that capture particles from the water column, *P. turbinatus* reflects the contamination status of the benthic boundary layer, ingesting particles trapped in epilithic algal communities. The distinctive dominance of fibers in the samples serves as a pollution fingerprint for İskenderun Bay, indicating intense pressure from land-based sources such as domestic wastewater and riverine inputs, rather than maritime-based sources (e.g., paint chips) observed in other parts of the Mediterranean. Furthermore, the correlation between body size and microplastic load suggests that bioaccumulation risk increases with the age and grazing activity of the organism. From an ecological perspective, the ingestion of microplastics by *P. turbinatus* raises concerns regarding the transfer of pollutants to higher trophic levels, as this species is a prey item for various crustaceans and fish. Consequently, *P. turbinatus* proves to be a valuable complementary bioindicator for assessing the specific impact of microfibers in rocky coastal ecosystems. Future research should focus on the temporal dynamics of this contamination and its potential physiological effects on gastropod health.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Data curation: EK, EU; Conceptualization: EK; Methodology; EK, EU; Formal analysis: EK; Investigation: EK, EU, NY; Visualization: EU; Writing – original draft: EK; Writing – review & editing: EU, NY. All authors approved the final draft.

ETHICAL STATEMENTS

No ethics committee permissions is required for this study.

DATA AVAILABILITY STATEMENT

Data will be made available on request.

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