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Multi-matrix approach to microplastic pollution in the bivalve *Donax trunculus*, sediment and water along the Mediterranean coasts☆

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ABSTRACT

Plastic pollution is a widespread issue in marine ecosystems worldwide, and at the basin level, the Mediterranean represents one of the main hotspots for plastic debris. Here, we present MPs pollution levels in the bivalve *Donax trunculus*, commonly known as wedge clam, considering both young and adult individuals, as well as sediment and water matrices across a national scale, covering the Tyrrhenian, Ionian, and Adriatic coasts of Italy. The aim is to provide an overview of MPs pollution in coastal ecosystems and assess whether wedge clams can act as an early warning sentinel for sandy habitats. Results highlighted that the Adriatic and Ionian coasts exhibited higher MPs levels than the Tyrrhenian coast across all matrices, with MPs pollution predominantly consisting of blue acrylic fibers. *D. trunculus* proved to be an excellent sentinel for MPs pollution in sediment. The findings also revealed that young individuals had more MPs than adults, highlighting potentially severe harm to the growth of marine organisms. As a commercially valuable species, this study underscores the urgent need to further investigate this issue, especially given its critical implications for both marine ecosystem health and human well-being.

1. Introduction

Plastic pollution is one of the most pressing challenges of our time (Rochman and Hoellein, 2020; Yu and Singh, 2023) since global marine systems are estimated to contain 82–358 trillion plastic particles weighing 1.1–4.9 million tons (Eriksen et al., 2023). Low recycling rates and improper disposal practices have led to the widespread accumulation of plastic waste entering marine environments (Kibria et al., 2023). Once introduced, plastics persist in these ecosystems, floating on the sea surface, in the water column, or accumulating on the seafloor and along shorelines, impacting regions globally, from polar areas to the equator (Eriksen et al., 2023; Suaria and Aliani, 2014). At a regional level, the Mediterranean Sea, being a semi-enclosed basin with a high population density, is one of the world's most polluted areas (Cincinelli et al., 2019). Referred to as the 'Mediterranean plastic soup' (Suaria et al., 2016), it has been suggested as the sixth major garbage patch, potentially containing up to 10 % of the global plastic mass (Zambianchi et al., 2017). In terms of size classes, microplastics (1 μm < MPs < 5 mm) (Frias and Nash, 2019), originating from both primary (intentionally produced in small sizes) and secondary (resulting from the fragmentation of larger plastics) sources, are among the most prevalent and have garnered significant global attention (Hu et al., 2022). Compared to larger sizes, MPs are considered particularly hazardous, as many organisms can ingest particles of this dimension, whether in the form of fibers, fragments, or films (Nardi et al., 2024). Additionally, due to their hydrophobic nature, rough surfaces, and high surface-to-volume ratio, MPs can adsorb contaminants (namely metals, POPs, PAHs, pharmaceuticals, pesticides, and additives) and microorganisms (pathogens, alien species) from the environment (Hu et al., 2022), acting as "Trojan horses" that may increase toxicity (Bhagat et al., 2021; Cesarini et al.,

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2022). Human health is also imperiled since MPs in the environment can enter the human body through the food chain, e.g. through the ingestion of contaminated seafood, livestock and agricultural products (Hu et al., 2022).

The rising concern about the spread of this contaminant and the linked toxicity has led to the development of monitoring studies using marine species, including commonly used early warning sentinels (Savoca et al., 2024). Bivalve mollusks have been proposed as sentinels for monitoring MPs pollution in marine environments (OSPAR, 2011). As sedentary filter feeders, bivalves actively adsorb pollutants from their environment, including those suspended in the water column or settling on the seafloor (Porter et al., 2023). This property makes them highly responsive to indicators of environmental pollution (Livingstone et al., 2000). In addition to their ecological importance in marine food webs, they hold substantial socio-economic value as bivalves are widely farmed and consumed as seafood worldwide (Olivieri et al., 2022). An additional property that makes them suitable as an early warning system is representing abiotic matrices, namely water and sediment (Porter et al., 2023). In the literature, there is a wide range of studies examining the accumulation of MPs in the biota, water, and sediment analyzed separately (Martellini et al., 2018; Olivieri et al., 2022). In contrast, a few studies have investigated the bioaccumulation of MPs across both biotic and abiotic matrices in an integrated manner worldwide, and even fewer studies have focused on the Mediterranean Sea. In this regard, Kazour et al. (2019), using the sentinel bivalve *Spondylus spinosus*, and Fagiano et al. (2023), employing *Mytilus galloprovincialis* and *Arca noae*, conducted sampling on the eastern and western Mediterranean coasts, respectively, observing distinct concentrations of MPs across their matrices, although no correlation was found between the sentinel species and the surrounding environment.

Given the gap in the existing literature on this topic, it is fundamental to identify species that can serve as reliable sentinels, effectively reflecting MPs pollution in the surrounding environment (Savoca et al., 2024). The present study aims to investigate MPs pollution along the Italian coastline, using the bivalve *Donax trunculus* (*Linnaeus, 1758*), commonly known as wedge clams, with sediment and water matrices. Indeed, it could be hypothesized that this species, which inhabits the first meters from the shore and beneath the sand, could represent the pollution present in both matrices. To further refine the analysis, *D. trunculus* individuals were classified into two size categories (young and adult) to evaluate potential differences in MPs levels between these groups.

2. Material and methods

2.1. Study area

Samples of intertidal water, sediment and wedge clams were collected from 11 sampling sites along the Mediterranean coast, including Tyrrhenian, Ionian and Adriatic sides (Fig. 1). Sampling activities were carried out once per site between March to September 2022, according to the geographical distance and weather conditions.

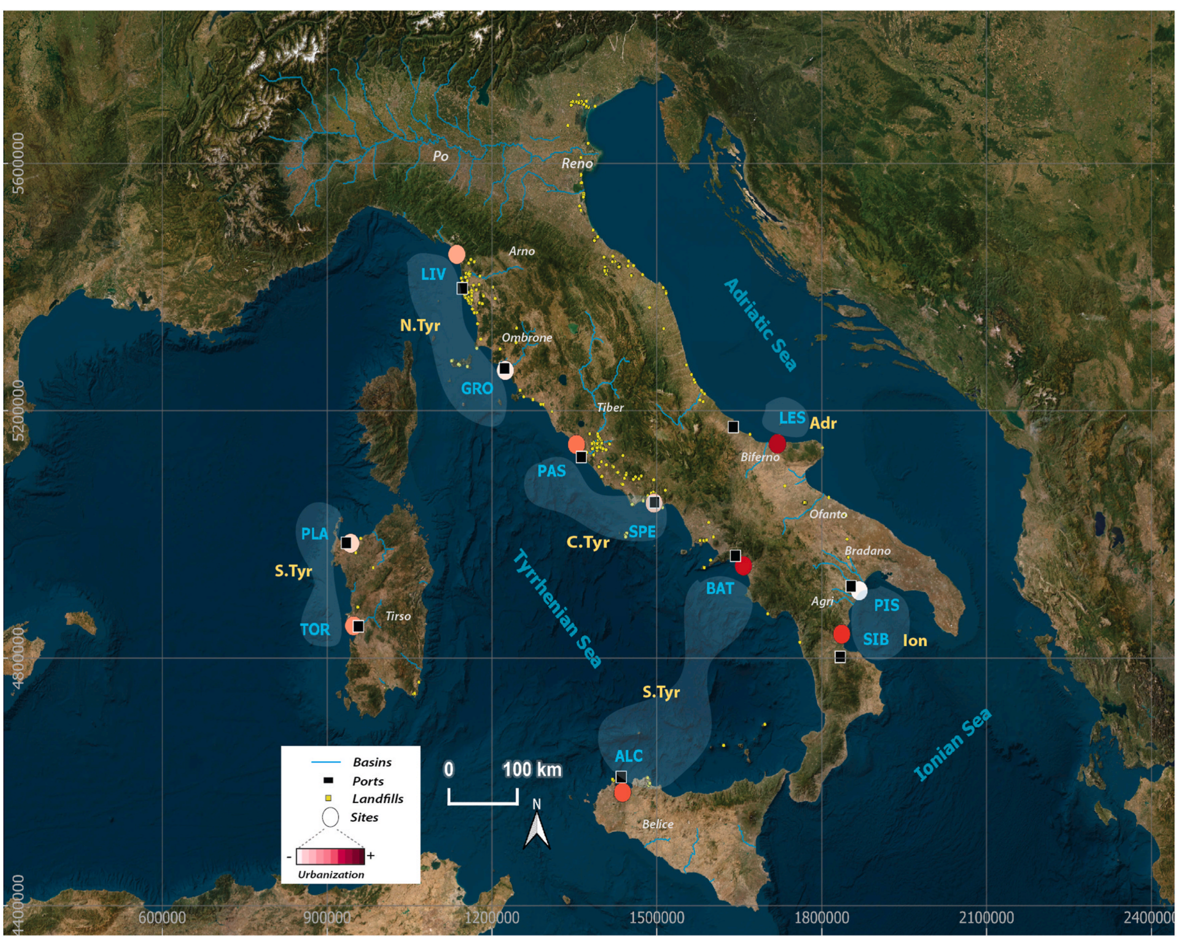

Fig. 1. Map of the sampling sites along the Italian coast. Liv (Livorno) and Gro (Grosseto) are included in the N. Tyr (North Tyrrhenian side), Pas (Passoscuro) and Spe (Sperlonga) in the C. Tyr (Central Tyrrhenian side), Bat (Battipaglia), Alc (Alcamo), Pla (Platamona), and Tor (Torregrande) in the S. Tyr (South Tyrrhenian side), Pis (Pisticci) and Sib (Sibari) in the Ion (Ionian side), and Les (Lesina) in the Adr (Adriatic side). Black squares indicate the nearest ports to sampled sites, blue lines represent main basins, and yellow dots mark landfills. White-to-red gradient dots show sampled stations, with color intensity reflecting site urbanization by using QGIS 3.16.8 software.

The sampling sites were selected from both urban and rural areas, representing diverse sources of pollution, including riverine discharges, industrial effluents, different types of ports, and their designation as high-tourism locations (Table 1). Detailed information on the population density, types of ports, blue flag presence and tourism intensity is provided in Table 1.

2.2. Sample collection and biological parameters

Samples from the three different matrices, *D. trunculus*, sediment and water, were collected along the same transects, in the coastal zone at an approximate depth of 1–1.20 m.

The bivalve *D. trunculus* was selected for this study due to its representativeness and widespread distribution along Mediterranean and Atlantic sandy coasts (Bayed, 1990). As an edible bivalve, it also represents an important resource in various countries along these coastlines. *D. trunculus* specimens were obtained using a hand dredge along a transect of c.a. 10 m. At each site, 30 individuals of *D. trunculus* were collected and categorized based on shell length into two groups: 15 adults (shell length > 1.5 cm) and 15 young individuals (shell length < 1.5 cm) (Manca Zeichen et al., 2002). The samples were wrapped in aluminum foil and kept refrigerated during transport to the laboratory, where the samples were stored at −20 °C until the subsequent analysis. The soft tissues were extracted from the shells, and six pools, each consisting of five individuals, were prepared for each sampling site, according to Narmatha Sathish et al. (2020) and Ben-Haddad et al. (2022). The wet weight of each pool was then measured.

Sediment samples were collected in triplicate using a grab sampler along the same transect where the wedge clam samples were collected (Prata et al., 2019). The sediment samples were placed in aluminum containers for transport to the laboratory.

For water samples, three replicates of 5 L each were collected manually at each site along the same transect of *D. trunculus* and sediment, considering the water column, precisely from 15 cm below the surface, and were preserved in glass bottles (Narmatha Sathish et al., 2020). Both water and sediment samples were stored at +4 °C until the following analysis.

2.3. Experimental procedures

The *D. trunculus* tissue samples were digested following the protocol of Corami et al. (2020) and Suebpala et al. (2018) and adapted according to the requirements of the analyzed matrix. Briefly, the pools of wedge clams' tissues were treated with H_2O_2 (30 % w/v, 3 times the tissue volume) in an incubator at 37 °C for 48 h. Thereafter, if fragments of tissues were still visible by naked eye, the samples were kept at c.a. 24 °C (room temperature) until a clear solution was obtained (for a maximum of two further days). Daily monitoring was performed to mix the solutions until the complete digestion of tissues. Sediment samples were digested following the protocols of Coppock et al. (2017) and adapted based on the samples treated in the present study. Samples were heated at 40 °C until completely dried. After that, 100 g d.w. of sediment for each replicate was placed in 200 ml $ZnCl_2$ hypersaline solution (density 1.8 g cm^{-3}), stirred for 15 min using a magnetic stirrer, and decanted overnight to separate the plastics from the matrix due to a different density gradient. After the overnight separation, the supernatant was digested with H_2O_2 30 % for 24 h. The following steps were repeated until the sample appeared free from sediment and organic matter, allowing for filtration. Samples of water were analyzed by the method of Masura et al. (2015), with the addition of 30 % H_2O_2 solution to digest the biological material in a ratio of 1:5. The solution was then kept at room temperature for a period of 72 h until the reaction stopped.

Filtration of all analyzed matrices was performed using a vacuum pump and 0.7 μm mesh glass microfiber filters (Whatman GF/F 47 mm), ensuring that all particles, including the smallest ones within the MPs range (≥ 1 μm), if present, were considered across the matrices. The filters were stored in sterile glass petri dishes and screened with a stereoscope for MPs detection. Suspected MPs were marked on the filter, counted and classified according to shape (i.e., beads, fibers or fragments) and colors (i.e., red, green, blue, white, transparent, yellow or black). The colour of MPs was assigned according to commonly used color categories following Lusher et al. (2020). Suspected MPs were categorized into five size classes, defined based on the smallest and largest MPs identified in our samples: 0.1–0.5, 0.5–1, 1–1.5, 1.5–2, > 2 mm. The first two size classes (< 1 mm) fall under small MPs and were selected accordingly to Ben-Haddad et al. (2022) and Narmatha Sathish et al. (2020), while the last three, typically grouped as large MPs (1–5 mm), were further subdivided here for more detailed categorization. For

Table 1
Information on the location of sampling sites (latitude and longitude), population size, types of harbors present at the sampling localities, the presence of Blue Flag certification, and the intensity of tourism activities. *Population information is available in: https://demo.istat.it/; **Ports information is available at: https://earth.google.com/web/; ***Tourism information is available at https://www.istat.it/tag/turismo/.

Sites	Abbreviation	Latitude	Longitude	Population*	Nearest ports**	Beach Blue Flag	Tourism***
Livorno	Liv	43.91791	10.203868	325 978	**Livorno Port (41 km)** Industrial, touristic, fishing	no	very high
Grosseto	Gro	42.69263	10.995728	216 290	**Marina di Grosseto Port (2.5 km)** Touristic	yes	very high
Passoscuro	Pas	41.89554	12.16051	4 173	**Fiumicino Port (14 km)** Commercial, Industrial, touristic, fishing	no	very high
Sperlonga	Spe	41.25987	13.427813	3 041	**Sperlonga Port (1 km)** Touristic	yes	very high
Battipaglia	Bat	40.57129	14.889908	49 635	**Salerno Port (16 km)** Commercial, touristic, fishing	no	high
Sibari	Sib	39.81282	16.494332	4 549	**Corigliano Calabro Port (9.56 km)** Touristic	yes	very high
Platamona	Pla	40.81907	8.468655	23	**Porto Torres Port (7.95 km)** Commercial, Industrial, touristic, fishing	yes	moderate
Torregrande	Tor	39.90603	8.516522	388	**Oristano Port (5.67 km)** Industrial, touristic	yes	moderate
Pisticci	Pis	40.29603	16.782003	16 911	**Metaponto Port (5.34 km)** Touristic	yes	very high
Lesina	Les	41.90267	15.445955	6 231	**Capojale Port (18.71 km)** Fishing	no	high
Alcamo	Alc	38.025088	12.914047	44 655	**Castellamare del Golfo Port (2.99 km)** Commercial, fishing, touristic	no	high

fibers, the maximum length was considered, while for fragments and films, the largest dimension of the MPs was used to categorize them into the five described size classes. After stereoscopic identification, we randomly selected and analyzed 20 % of the total MPs (Malloggi et al., 2024; Olivieri et al., 2022). The selection included all the study sites and the three investigated matrices, ensuring a homogeneous representation of suspected MPs from all resulting samples.

The subsamples were chemically characterized using Raman spectroscopy with a Renishaw In-Via micro-Raman spectrometer, and the suspected MPs were directly analyzed by positioning the filters under the Raman apparatus.

Raman spectra were processed with Spectragryph software (Menges, 2023) and OpenSpecy (Cowger et al., 2021), refining baselines, smoothing with Savitzky–Golay filtering, and verifying library matches above 70 %. To reduce the risk of external contamination, a quality assurance/quality control (QA/QC) protocol was implemented (Cesarini et al., 2023; Gallitelli et al., 2020). Detailed information on Raman spectroscopy technique and QA/QC procedures employed is provided in the supplementary material.

2.4. Data analysis

The Shapiro-Wilk test was performed to assess the normality of the datasets. Kruskal-Wallis analysis was conducted to evaluate how the number of MPs varies across sampling sites in all matrices and among the different groupings of sites belonging to the Tyrrhenian, Ionian, and Adriatic sides. Differences among the sampled sites and between the Tyrrhenian, Ionian, and Adriatic sides were assessed using Dunn's post-hoc test. Additionally, MPs data from *D. trunculus*, sediment, and water were classified into three pollution levels (low, medium, high). The high level concentration was obtained from the site most polluted, and the lowest was set to 0 (considered indicative of no contamination). To define the three classes, the high level was divided into three equal ranges (Figs. 2 and 3). To assess the distribution of MPs in wedge clams in relation to their environment, and specifically between the sediment and water matrices, a regression correlation analysis was performed. The Mann-Whitney *U* test was conducted to assess significant differences in MPs levels between young and adult clams. Additionally, a linear regression analysis was performed to examine the relationship between MPs abundance in clams (items/g and items/ind) and the mean tissue weight (g) in *D. trunculus* individuals. A significance level of $p < 0.05$

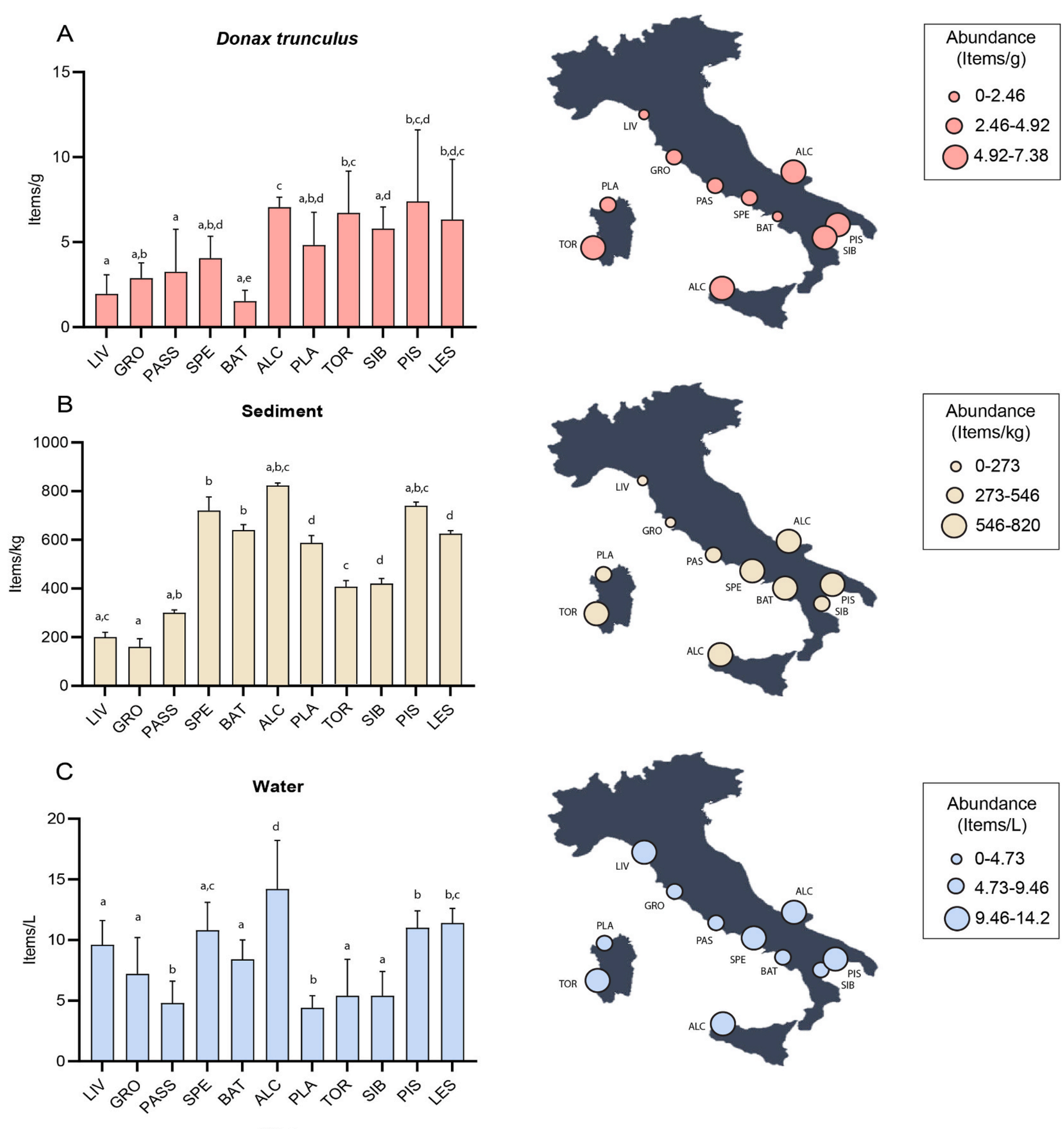

Fig. 2. Distribution of microplastics in *Donax trunculus*, sediment and water matrices in the sampling sites. Differences between conditions are marked with lowercase letters ($p < 0.05$). The size of the dots indicates the level of contamination, classified into three overall levels in the three matrices.

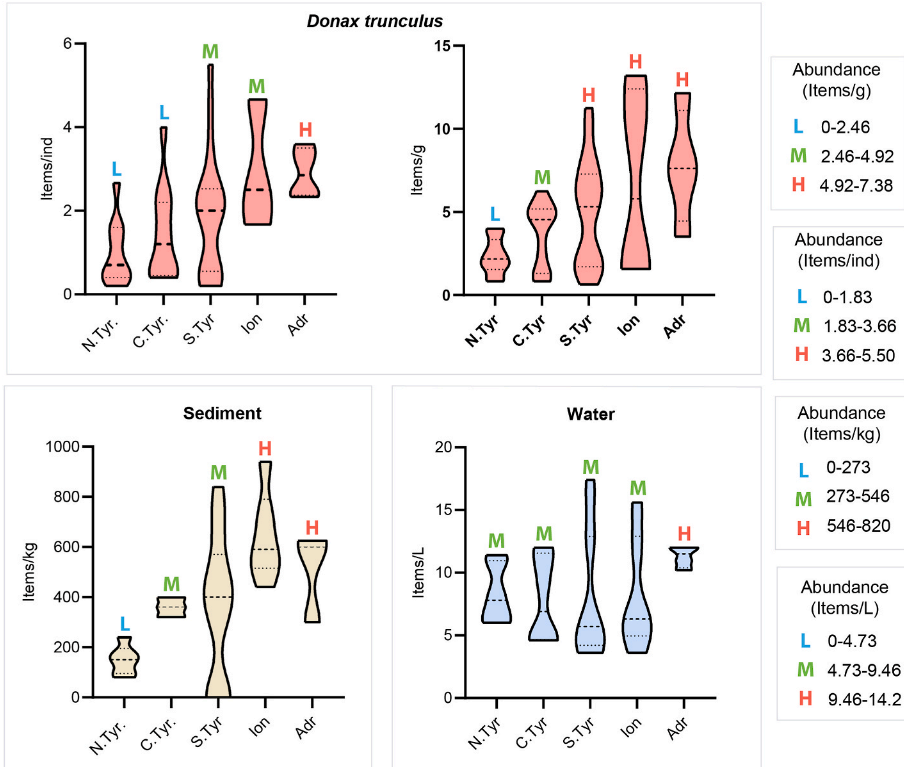

Fig. 3. Abundance of microplastics in *Donax trunculus*, sediment and water matrices in the North Tyrrhenian (N.Tyr), Central Tyrrhenian (C.Tyr), South Tyrrhenian (S.Tyr), Ionian (Ion), Adriatic (Adr) sides. Abundance of microplastics in *Donax trunculus* is expressed in items/ind and items/g, in sediment in items/kg and in water as items/L. The degree of contamination in the three matrices was classified as low (L), medium (M), and high (H). Dotted lines in bold and normal font represent the data's median and quartiles, respectively.

was applied to all statistical tests performed in the analysis. Statistical analyses were performed using GraphPad 8.0.1 and Past 4.03.

3. Results

3.1. Microplastics pollution along the Mediterranean coast

Pollution by MPs was detected in all 11 sampled sites, and across all the analyzed matrices (Fig. 2). In general, the mean abundance of MPs in *D. trunculus* was 4.66 ± 1.3 items/g (corresponding to 4660 ± 1300 items/kg) and 2.67 ± 0.64 items/ind, with values ranging from 0.64 to 13.2 items/g (corresponding to 640–13200 items/kg) and 0.47 to 2.77 items/ind. In sediment, the mean MPs abundance was 470.91 ± 240.22 items/kg, ranging from 160 to 820 items/kg. Regarding the water matrix, the mean MPs concentration was 8.42 ± 3.24 items/L, with a range of 4.40–14.20 items/L.

The abundance of MPs in clams correlated significantly with MPs pollution in intertidal sediment ($R^2 = 0.52$, $p < 0.001$), but not with MPs pollution in water ($p > 0.05$). The distribution and concentration of MPs across different size classes of clams in this study are presented as mean ± SD in Table 1S, both in terms of items/g and items/ind. Results from the Mann-Whitney *U* test indicate that the levels of MPs in *D. trunculus* individuals differed significantly between the two size classes, with a higher quantity of items observed in young compared to adult wedge clams. This difference is present both when considering items/g ($U = 28$, $Z = 10$, $p = 0.035$) and items/ind ($U = 23.5$, $Z = 2.4$, $p = 0.016$). Additionally, a slight negative correlation ($R^2 = 0.12$, $p = 0.019$, $F = 5.88$) was found between the weight of clams (g) and the number of MPs detected (items/g) (Fig. 1A_S), whereas the correlation was also negative but not significant when considering items/ind (Fig. 1B_S).

Considering the Tyrrhenian, Ionian, and Adriatic coasts (Fig. 3), as regards MPs in wedge clam tissue, N.Tyr and C. Tyr side (with a mean of 0.98 ± 0.77 items/ind, 2.32 ± 1.09 items/g and 1.42 ± 0.47 items/ind, 3.65 ± 1.94 items/g respectively) exhibited lower MPs levels compared to the S.Tyr (1.90 ± 1.41 items/ind, 5.03 ± 3.15 items/g), Ion (2.58 ± 0.82 items/ind, 6.59 ± 5.93 items/g) with the highest value found in the Adr (3.05 ± 0.59 items/ind, 7.72 ± 3.54 items/g). Regarding sediments, the sampling sites in the N.Tyr had the lowest mean MPs concentration of 150 ± 57.61 items/kg than C.Tyr (380 ± 40 items/kg), S.Tyr (356 ± 290 items/kg), Ion (640 ± 178.88 items/kg) and Adr (508 ± 180 items/kg) (Fig. 3). Lastly, for water samples, Adr had the highest mean MPs concentration with 11.4 ± 1.03 items/L while the lowest value was recorded in S.Tyr (8.1 ± 5.01 items/L).

A more detailed analysis for each sampled site reveals that for the *D. trunculus* matrix, Les, Tor, and Alc sites were significantly more contaminated than Liv, Pas and Bat ($p < 0.05$). For the sediment matrix, Liv and Gro were significantly less polluted compared to Spe, Bat, Alc, and Pis ($p < 0.05$). Regarding the water matrix, Pas and Bat were the least contaminated sites, while Spe and Alc were the most contaminated ($p < 0.05$).

3.2. Qualitative characterization of microplastics pollution

In all three matrices (*D. trunculus* tissue, sediment and water), fibers were the predominant form of MPs detected (81.8 %, 73.5 %, 89.9 % respectively), followed by fragments (11.2 %, 22.9 %, 8.8 %) and films (7.0 %, 3.7 %, 1.3 %) (Fig. 4A). In detail, fibers prevalence in *D. trunculus* tissues ranged from a minimum of 41 % in Adr to a maximum of 100 % in N.Tyr, in sediment matrix the percentage of fibers ranged from a minimum of 63 % in S.Tyr, while reached the maximum of 100 % in S. Tyr, and in water from 83 % in C.Tyr and Adr to 97 % in S.Tyr. Films observed in the tissues of wedge clams ranged from 0 % in N.Tyr to 14 % in Adr, in sediment from 0 % in N.Tyr to 13 % in C.Tyr, and in water from 0 % in N.Tyr and S.Tyr to 3 % in C.Tyr and Adr. Fragments were

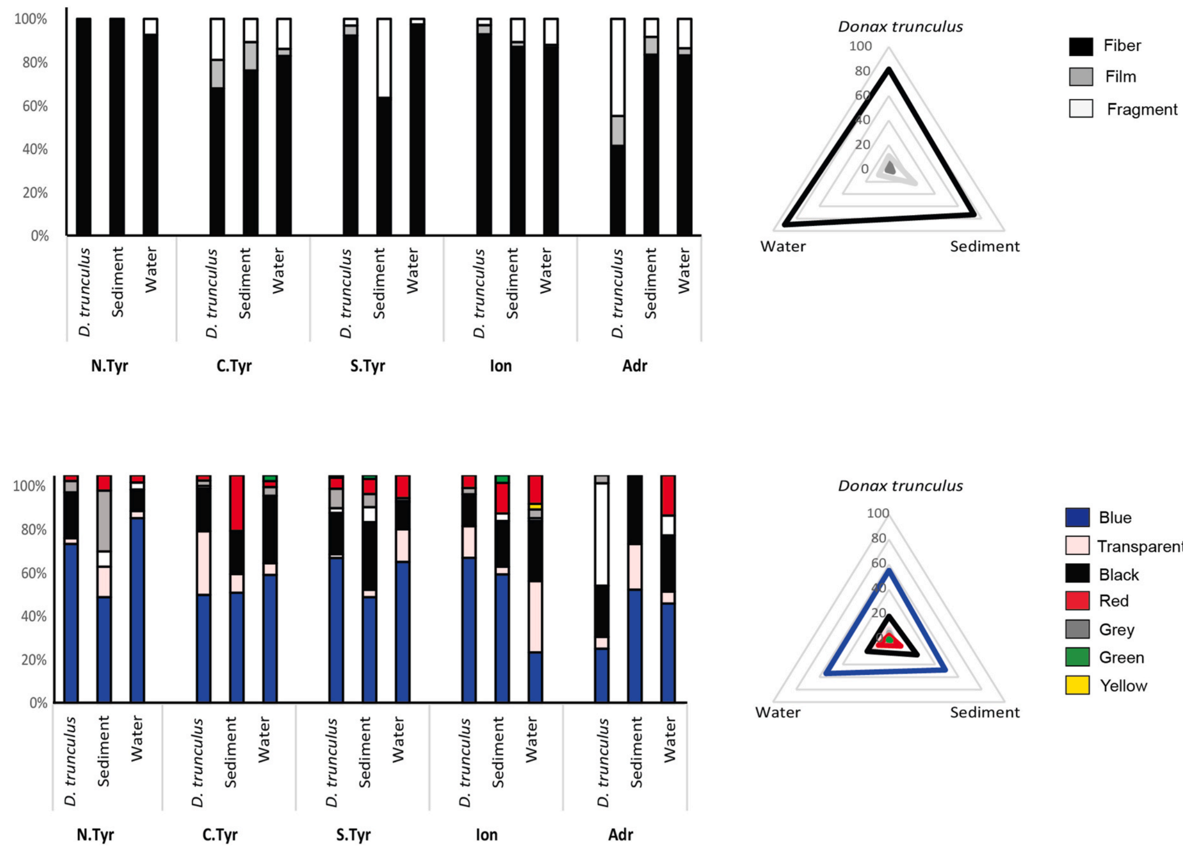

Fig. 4. A. Percentage of microplastics shape found in *Donax trunculus*, sediment, water in the North Tyrrhenian (N.Tyr), Central Tyrrhenian (C.Tyr), South Tyrrhenian (S.Tyr), Ionian (Ion), Adriatic (Adr) sides and considering all the sampling sites collectively, **B.** Percentage of microplastics colour found in *Donax trunculus*, sediment and water in the North Tyrrhenian (N.Tyr), Central Tyrrhenian (C.Tyr), South Tyrrhenian (S.Tyr), Ionian (Ion), Adriatic (Adr) sides and considering all the sampling sites collectively.

detected in *D. trunculus* tissues from 0 % in N.Tyr to 45 % in Adr, in sediment from 0 % in N.Tyr to 37 % in S.Tyr, and in water from 3 % to 14 % in C.Tyr and Adr.

The most common MPs colors considering all the three matrices, were blue (55.4 %, 48.6 %, and 54.3 % in *D. trunculus*, sediment, and water, respectively) and black (18.6 %, 24.3 %, and 18.9 % in *D. trunculus*, sediment, and water, respectively), followed by red, gray, transparent, green, and yellow in lower percentages (Fig. 4B). In *D. trunculus*, blue was ranging from 4 % in Adr to 70 % in N.Tyr, from 47 % in N.Tyr to 57 % in Ion in sediment, and from 23 % in Ion to 81 % in N. Tyr in the water matrix. Black was the second most frequent color, ranging from 14 % to 22 % in *D. trunculus*, from 0 % in S.Tyr to 30 % in Adr in sediment, and from 9 % in N.Tyr to 29 % in C.Tyr in water. Other colors were observed at lower percentages.

The range of MPs > 2 mm were the most frequent particles detected in sediment and water matrices (46 %) (Fig. 5A). The 0.1–0.5 mm and 0.5–1 mm size range were predominantly found in *D. trunculus* at 14 % and 28 %, respectively. The 1–1.5 mm size range was primarily detected in sediment (32 %). Despite the observed trend of smaller MPs size ranges in wedge clams compared to abiotic matrices, the results were not significantly different. Regarding both the sampling sites and matrices, fibers > 2 mm were the most identified, followed by the other size classes, which did not exhibit any clear or statistically significant trend.

The polymers identified by Raman measurements were grouped into nine macro-categories (Fig. 5B), seven synthetics: namely acrylic, PA (polyamide), PPP (polyphenylenes), PO (polyolefins), PB (polybutylene), PUR (polyurethane), PET (polyethylene terephthalate), and two cellulosics, namely cotton and regenerated cellulose (e.g. rayon, viscose). Acrilyc was the most abundant polymer, representing 71.79 %, 72.73 %, and 68 % of the polymers found in wedge clams, sediment, and water, respectively (Fig. 4B).

4. Discussion

Marine compartments have frequently been studied as separate environments in plastic pollution research (Muhammad Ubaid et al., 2021). Consequently, there is limited knowledge about the MPs distribution across physically and biologically interconnected compartments (Fagiano et al., 2023). This study adopts a comprehensive approach to concurrently assess the abundance and composition of MPs across multiple matrices along an extensive stretch of the Italian coastline within the Mediterranean context, employing comparable measurement approaches between the studied matrices of *D. trunculus* tissues, sediment and water samples.

4.1. Microplastics pollution along the Italian coasts

Considering both biotic and abiotic matrices, the Adriatic Sea exhibited higher levels of MPs (7.72 ± 3.54 items/g), with mean values classified as high pollution, while the Tyrrhenian coast showed reduced pollution levels (2.32 ± 1.09 items/g, 3.65 ± 1.94 items/g, 5.03 ± 3.15 items/g in N.Tyr, C.Tyr, S.Tyr respectively), ranging from low to medium pollution. This is evident in Fig. 2, where MPs levels are reported for each sampling area, and in Fig. 3, where they are grouped according to the three coastal sectors of Tyrrhenian (N. Tyr, C. Tyr, S. Tyr), Ionian (Ion), and Adriatic (Adr) sides.

Studies on MPs in *D. trunculus* and other bivalves from the same regions along the Mediterranean coasts have reported generally similar or lower values than those found in this study when considering both

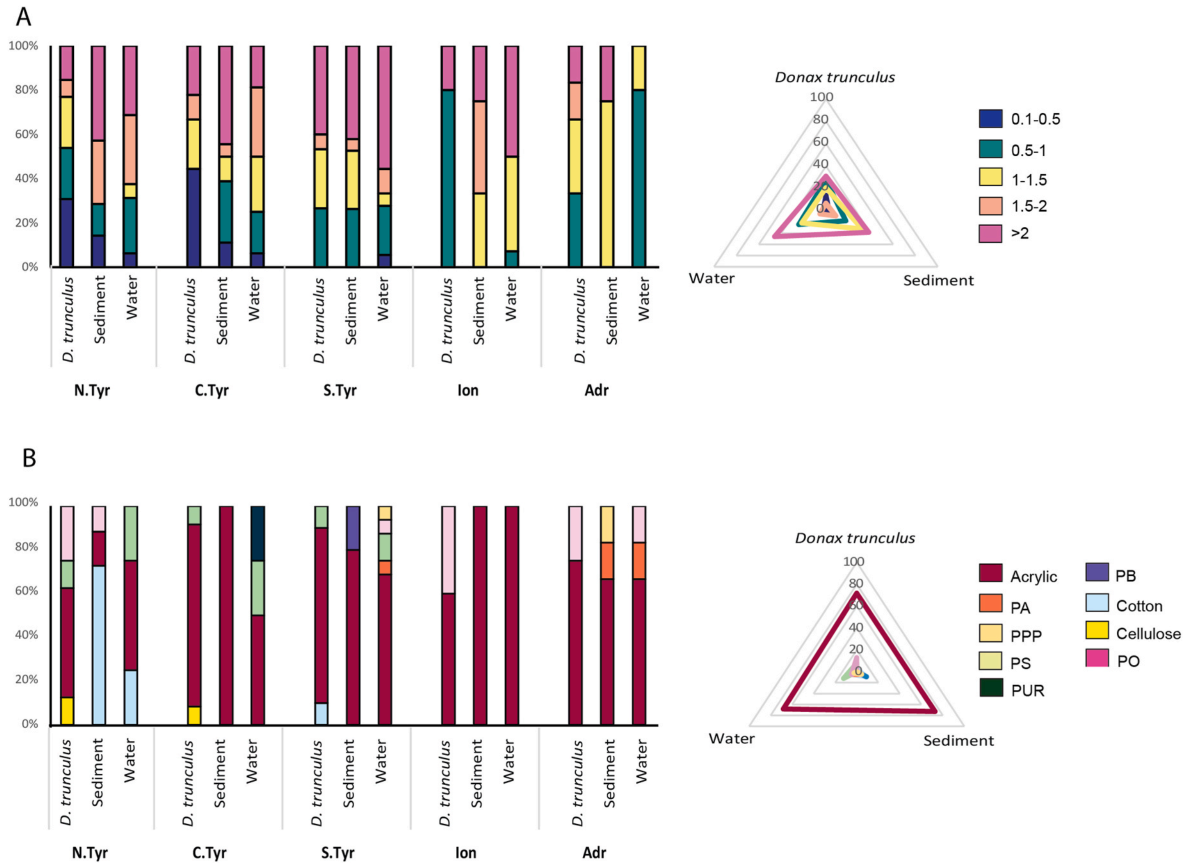

Fig. 5. **A.** Percentage of microplastics sizes found in *Donax trunculus*, sediment, water in the North Tyrrhenian (N.Tyr), Central Tyrrhenian (C.Tyr), South Tyrrhenian (S.Tyr), Ionian (Ion), Adriatic (Adr) sides and considering all the sampling sites collectively, **B.** Percentage of microplastics polymer type in *Donax trunculus*, sediment, water in the North Tyrrhenian (N.Tyr), Central Tyrrhenian (C.Tyr), South Tyrrhenian (S.Tyr), Ionian (Ion), Adriatic (Adr) sides and considering all the sampling sites collectively.

items/ind and items/g as units of measurement (Gomiero et al., 2019; Kazour et al., 2019; Malloggi et al., 2024; Olivieri et al., 2022). This confirms the trend of lower MPs levels in the Tyrrhenian compared to the Adriatic side over time. Overall, the range of MPs detected in wedge clams in this study aligns with global concentrations reported for bivalves. The worldwide mean MPs levels in bivalves range from 0.04 to 20.0 items/g (Ding et al., 2022), with Indian Ocean countries showing the highest mean levels (above 11 ± 8.2 items/g), while mean values reported in other countries are at least half of these. However, it must be considered that clams generally tend to accumulate higher MPs levels than mussels, oysters, and scallops (Ding et al., 2022), highlighting the need for caution when comparing MPs levels in bivalves with different lifestyles (e.g., rocky mussels vs. sandy clams).

Likewise, sediment samples exhibited values consistent with those reported in the literature from the same regions, reinforcing the concept of a distribution pattern of MPs along the Mediterranean coast even in abiotic matrices. In this context Blašković et al. (2018) and Cannas et al. (2017) reported ranges of 62–466 items/kg for the Tyrrhenian side (Tuscany), conversely more than double the MPs concentration for the Adriatic coast, precisely in Venice Lagoon (700 items/kg) and in the Ravenna Riviera (1512 ± 187 items/kg) (Martellini et al., 2018). Similarly, the range of MPs detected in sediment in this study aligns with literature data for the Mediterranean, where reported values do not exceed 2000 items/kg. On a global scale, however, the highest MPs concentrations in sediment have been recorded in Asia, reaching up to 9187.5 items/kg (Tran Nguyen et al., 2020).

Regarding water samples, the obtained results are not easily comparable with those reported in the literature, as many studies investigating MPs in water, whether focusing exclusively on this matrix or including species from offshore areas, have conducted sampling at significantly greater distances from the coastline. These studies have employed specific sampling techniques, such as manta trawls, and presented MPs pollution data using different units of measurement, notably MPs/km^2. On the other hand, Narmatha Sathish et al. (2020) that, similarly to this study extended their samplings including the bivalve *Donax cuneatus*, sediment and water, applied an analogous sampling method for seawater describing a mean of 30.98 ± 7.87 items/L in water samples in the Gulf of Mannar (India). This step facilitated direct comparisons in MPs pollution across the three investigated matrices.

Land-based sources (e.g. high tourism activity, presence of discharges into the sea, ports) are identified as the primary sources of MPs pollution both in the Tyrrhenian and Adriatic coasts (Malloggi et al., 2024; Martellini et al., 2018; Olivieri et al., 2022). In addition, Ombrone, Albegna, Osa, and Tevere rivers (on the Tyrrhenian side) and Adige and Po rivers (on the Adriatic side) further exacerbate MPs pollution in these regions (Cannas et al., 2017). In general, MPs pollution across different coasts appears to be partially influenced by the interaction of multiple factors originating from inland areas as reported in Table 1.

Although our samples were collected in coastal zones, disparities in pollution levels, lower on the Tyrrhenian coast compared to higher levels on the Ionian and Adriatic coasts, seem to be influenced by factors extending beyond the coastal zone, such as hydrodynamic processes, including currents, upwelling and downwelling, gyres, and fronts, which could promote plastic accumulation in enclosed areas like the Adriatic Sea (Kazour et al., 2019). Notably, the Eastern Mediterranean is recognized as a region with significant MPs pollution levels (Cózar et al., 2015). Differences between the two parts of the Mediterranean Sea can be explained by the different circulation patterns: while western currents facilitate dispersion, eastern currents act as attractors, accumulating plastic debris (Mansui et al., 2015) with the progressive formation of smaller plastics within coastal zones, that persist in the water column or settle into the sediment (Kazour et al., 2019). Suaria and Aliani (2014) observed a similar trend in their studies on floating macro-litter in the Mediterranean Sea, reporting high plastic densities in the Adriatic Sea and along the Northwestern African coast. According to UNEP/MAP (2012), these regions also feature some of the highest coastal population densities in the entire Mediterranean basin. In addition, MPs pollution in the Southern Tyrrhenian, Ionian, and Adriatic seas is partly attributed to anticyclonic eddies, which create retention zones for plastic debris (Howell et al., 2012). It should be remarked that MPs concentrations could be also influenced by other factors, such as sampling season, proximity to the coast, variability in measurement units, bivalve species analyzed and methodology (sampling methods, MPs analysis protocols applied in different matrices), thus the comparison of results across different studies require caution and need standardization (Malloggi et al., 2024). In this context, in the digestion step, wedge clams are often processed in pooled samples (as in this study) to facilitate sample handling and recovery. While this approach may reduce analytical precision, it is widely employed due to the small size of wedge clams relative to other bivalves (e.g. mussels and oysters). Another aspect is that reagent choice is a key variable for standardizing digestion protocols. The most commonly used, 30 % H_2O_2, 10 % KOH, and 15 % H_2O_2, are typically applied between 40 and 60 °C (Prata et al., 2019). Despite evidence of potential polymer alterations at these reagents (Karami et al., 2017), temperature plays a more critical role. Below 40 °C, MPs show no significant damage (or minimal ones) to MPs' integrity, although some residual organic matter may persist in the samples (Karami et al., 2017). Thus, these protocols remain effective and reliable when applied under mild thermal conditions (T ≤ 40 °C).

4.2. Qualitative characterization of microplastics pollution

Blue, black and red fibers were the most common colors of MPs found in all the detected matrices and sampled sites followed by fragments and films (Fig. 4). This trend is ubiquitous not only in the Mediterranean context but worldwide in different organisms (bivalves, fishes, polychaetes) and matrices (Olivieri et al., 2022; Suaria, 2024). Considering the small dimensions of wedge clams, fiber shapes are easier to ingest and expel from the digestive tract, instead of other tridimensional MPs (i.e. films and fragments) (Taylor et al., 2016; Woods et al., 2018). The limited clam body sizes also explain the predominance of smaller fibers observed within the clam tissues (0.5–1 mm, 1–1.5 mm), whereas fibers >2 mm were most common in water and sediment. Similar size ranges, namely 1–1.5 mm, have been reported for wedge clams in Morocco (Ben-Haddad et al., 2022), on the Latium and Tuscany coasts (Malloggi et al., 2024; Olivieri et al., 2022), and by Expósito et al. (2022) in Spain. Regarding water samples, analogous MPs dimensions to those observed in the present study were reported by Suaria (2024) with 1.79 ± 1.82 mm in length and 22.6 ± 18.4 μm in diameter, in sampling activities across the Western Mediterranean Sea.

Acrylic resins occurred with the highest frequencies in *D. trunculus* and surrounding environments (sediment and water), confirming the wedge clam's value as a sentinel of environmental MPs pollution (Fig. 5). PO and PS are the other materials that followed in prevalence across the matrices. All these polymers were already detected in the Mediterranean Sea with high frequencies in marine organisms and abiotic matrices (Expósito et al., 2022; Fagiano et al., 2023; Rios-Fuster et al., 2022). Synthetic polymers are mainly used in clothing and garment applications, home and office furnishings, transportation, and various other industrial sectors (Suaria, 2024). In addition these polymers are often combined with additives that enhance their resistance and durability over time (Olivieri et al., 2022). Although the origin of these synthetic fibers is difficult to determine, laundering and atmospheric deposition are identified as the main sources (Carr, 2017; Napper et al., 2023), and are favored by the spread of anthropogenic activities (industries presence, surrounding WWTPs) and debris (i.e. torn fishing nets and broken ropes used in coastal areas) (Napper and Thompson, 2016) (Table 1). Furthermore, their ubiquitous presence in clams, sediment and water samples suggests that, despite the polymers having different densities (but still higher than seawater), they are continuously resuspended by the water circulation along the coast, before they permanently sink on the seafloor (which lastly explains the higher quantity of MPs in biota and sediment compared to water). This concept was already explained by Paluselli et al. (2024) for greater seawater depth samplings, where MPs fibers could be advected and resuspended by Mediterranean deep or intermediate currents.

In our samples, regenerated cellulosic and cotton fibers were also observed at varying concentrations across the matrices. Recent literature has also reported an increasing detection of natural fibers in seawater (Suaria et al., 2020), sediment (Sanchez-Vidal et al., 2018), and biotic samples (Taylor et al., 2016) on a global scale. A possible explanation is that natural textiles like wool and cotton shed and release more fibers than synthetic polymers during laundering (De Falco et al., 2019). Moreover, cellulosic fibers are often treated with additives (retardants and dyes), contributing to their persistence and role as potential vectors for hazardous substances in the environment (Suaria et al., 2020). However, further research on the degradation rate and effects on the biota of textile fibers is necessary, since their prevalence, fate, and ecological impacts are still understudied. It is also important to consider that the 20 % subsampling strategy used for polymer characterization, although generally regarded as a reasonable percentage in such studies (Ben-Haddad et al., 2022), may have introduced a bias both in the reported percentages and in the potential presence of additional polymers that were not investigated due to their exclusion from the subsample selection.

4.3. *Donax trunculus* as early warning system for microplastic pollution in sandy habitats

MPs in wedge clams correlated positively with sediments but not with water, revealing that MPs concentration in biota was one order of magnitude higher than in sediment and two orders higher than in seawater. This highlights its effectiveness as a sentinel for monitoring MPs pollution in coastal sandy seabeds. Wedge clams not only reflected the quantitative levels of MPs pollution in the surrounding environments but also provided insight into its qualitative composition. The most abundant fibers, identified as blue, black, and red in color and acrylic polymer, were found both in the sediment and water samples and within the bivalve tissues. This demonstrates that *D. trunculus* did not exhibit selective filtering of specific plastic types, but instead represented the broader MPs pollution present in sediment and water. *D. trunculus* has been previously used as a sentinel for plastic levels in both natural environments and indoor experiments (Ben-Haddad et al., 2022; Expósito et al., 2022; Malloggi et al., 2024; Olivieri et al., 2022), and has proven to be a useful early warning system also for other contaminants (Botelho et al., 2018; Lamine et al., 2023; Secco et al., 2024), reinforcing the hypothesis that this species could serve as an effective sentinel for monitoring emerging contaminants in the marine environment.

4.4. Microplastics accumulation in different size classes of *Donax trunculus*

A negative correlation was observed between the mean tissue weight (g) and the number of MPs (items/g) in *D. trunculus*, indicating an allometric relationship between body size and MPs concentration. This pattern is likely due to the larger surface-area-to-volume ratio in smaller individuals (Narmatha Sathish et al., 2020). Allometric variation is well-documented in influencing metal concentrations relative to body size (Saavedra et al., 2004), and in this study, we identified a similar relationship between MPs concentration and clams body size. In addition, young individuals accumulated significantly more MPs than adults (Table S1). This result aligns with Narmatha Sathish et al. (2020), who observed higher MPs accumulation in young *D. cuneatus* individuals on the Tuticorin coast (India). This pattern may be attributed to the increased filtration rate observed in smaller bivalves compared to larger ones (Winter, 1973), which likely leads to higher MPs uptake in small-sized clams and lower uptake in large-sized clams. These results suggest that smaller individuals are particularly at risk from MPs pollution. Further studies, including indoor experiments, are necessary to better understand the potential risks posed to young individuals by MPs ingestion and its long-term effects on their health and development.

5. Conclusion

The present study focused on MPs pollution on the Mediterranean coasts considering a multi-matrix approach including bivalve *D. trunculus*, sediment and water samples. The study revealed a consistent trend of MPs pollution across all three matrices at the sampled sites in the Mediterranean, with higher MPs levels observed along the Adriatic, Ionian, and Southern Tyrrhenian coasts compared to the Northern and Central Tyrrhenian coasts. According to other studies, this gradient can be attributed to coastal factors, as well as the influence of currents and marine circulation.

While this study aimed to provide a comprehensive snapshot of MPs pollution along the Italian coastline to highlight potential spatial trends in distribution, future studies with repeated samplings across time would be valuable to explore temporal variability.

D. trunculus proved to be an effective early-warning sentinel for sediment MPs pollution in the surrounding environment. Although the species, due to its small size, can ingest MPs up to certain sizes (excluding the larger plastic debris), it could serve as a valuable sentinel organism for monitoring MPs and other emerging contaminants. Indeed, both from a quantitative (MPs abundance) and qualitative (shape, color, size, and polymer type) perspective, it provided key insights into the pollution of the surrounding environment.

Studies on *D. trunculus* and other bivalves inhabiting sandy environments worldwide should be expanded, as these species play a key ecological role, acting as a link between primary producers and higher levels of food chains. This study also underscores the potential risk of this emerging contaminant, as young individuals accumulated higher quantities of MPs, potentially leading to subcellular physiological harm. Lastly, we highlight the critical importance of these studies, not only for aquatic ecosystems but also for human health, as many of the species investigated are also used for human consumption.

CRediT authorship contribution statement

Silvia Secco: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giulia Cesarini:** Writing – review & editing, Methodology, Formal analysis. **Luca Gallitelli:** Writing – review & editing, Methodology, Formal analysis. **Giuseppe Suaria:** Writing – review & editing, Methodology. **Andrea Paluselli:** Writing – review & editing, Methodology. **Michael Di Gioacchino:** Writing – review & editing, Formal analysis. **Armida Sodo:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Massimiliano Scalici:** Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126318.

Data availability

Data will be made available on request.

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