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Comparative assessment of microplastic ingestion among deep sea decapods: Distribution analysis in Sardinian and Catalan waters

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ABSTRACT

Microplastic (MP) pollution is a widespread and concerning environmental issue. The benthic layer is known as one of the major accumulation sinks, yet knowledge gaps still remain in describing the interactions of its biota with MPs. This work represents a comprehensive comparative analysis of MP ingestion in the four deep-sea crustacean decapods *Aristeus antennatus* (Risso, 1816), *Aristaeomorpha foliacea* (Risso, 1827), *Nephrops norvegicus* (Linnaeus, 1758) and *Parapenaeus longirostris* (Lucas, 1846) sampled from two distinct regions of the Mediterranean Sea in order to underscore the species-specific characteristics driving their MP ingestion variations.

Results show that 72.5% of the individuals had ingested MPs, with fibers being the most common shape (91.36%), followed by tangles (8.64%). Catalan specimens had significantly higher fiber loads (mean; 3.69 fibers ind^{-1}) than Sardinian ones (mean; 1.62 fibers ind^{-1}), and distinct patterns among species were observed within the two regions. *A. antennatus* showed the highest MP load in the Catalan area whereas *N. norvegicus* was the most contaminated decapod in Sardinia. The observed variations are likely the result of a combination of factors, ranging from potentially different environmental exposure levels among regions to differences in the uptake and retention of MPs due to each species feeding ecology and morphological features. Overall, the results underscore the relevance of comprehensive assessments to properly interpret the trends in MP ingestion in crustacean decapods.

1. Introduction

Plastic pollution is one of the most concerning contemporary threats for marine environments. About 250,000 Tons of plastic are globally floating at sea and no decrease is foreseen for the next future (Andrady, 2011; Eriksen et al., 2014). Among plastics, microplastics (MPs) have raised widespread interest as environmental pollutants. Defined as plastic pieces smaller than 5 mm, their origin may be both by intentional manufacture and by fragmentation of larger debris due to weathering and biological fragmentation processes, making them ubiquitous contaminants (Arthur et al., 2009; Boucher and Friot, 2017). The first evidences of MPs as marine pollutants date back to the early 1970s (Carpenter et al., 1972; Carpenter and Smith, 1972) but after twenty years of research, our understanding on the effects of this pollutant is far from conclusive, with important gaps not only on their effects but also on their pathways and sinks (Frias and Nash, 2019; GESAMP, 2021; Thompson et al., 2024).

Once dispersed in the marine environment, the combined action of both biological and physical factors change the physicochemical features of plastic items, eventually triggering their sinking to the seafloor (Andrady, 2011; Kaiser et al., 2017; Pinto et al., 2019; Latva et al., 2022; Wallbank et al., 2022; Li et al., 2016). This, together with the persistence of plastic, particularly in the deep-sea where weathering processes are slow, results in the progressive accumulation of MPs in these environments (Chamas et al., 2020; Zhang and Peng, 2022).

As a result, benthic ecosystems are considered the largest sink for plastic and MP contamination (Van Cauwenberghe et al., 2013; Haegerbaeumer et al., 2019; Pitacco et al., 2022) which inevitably leads to their potential interaction with the biota present. Numerous potential effects across all levels of the biological hierarchy could arise (Cau et al., 2024), ranging from the impairment of bacterial activities to plastic accidental ingestion, which has been widely demonstrated in species

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with different trophic levels (Savoca et al., 2021; Pitacco et al., 2022).

Within deep-sea benthic communities, decapod crustaceans have gathered great attention due to their great gastronomic value and ecological interest (Cau et al., 2023). In the Mediterranean in particular, *Aristeus antennatus* (Cau et al., 2018; Carreras-Colom et al., 2018, 2020) and *Nephrops norvegicus* (Carreras-Colom et al., 2022a, 2022b; Cau et al., 2020, 2023) have been thoroughly studied in the context of MP contamination, with more recent attention given to *Aristaeomorpha foliacea* and *Parapenaeus longirostris* (Bono et al., 2020; D'Iglio et al., 2022; Yücel, 2022; Leila et al., 2023; Ciaralli et al., 2024). Regardless of the methodological approach or sampling area, these studies have revealed significantly high levels of plastic ingestion in these species compared to other faunistic groups, such as fish (Marmara et al., 2023), underscoring the need for further research to fully understand the extent of this impact and identify which species may be most at risk.

Research in laboratory-controlled conditions has shown that MP ingestion may have several noxious repercussions on crustaceans, including loss of appetite, increased oxidative stress and gut microbiota alterations, among others (Timilsina et al., 2023; Welden and Cowie, 2016a). Naturally occurring strategies might be exploited to prevent total blockage: highly exposed species like decapods crustaceans have been suggested to be capable of getting rid of ingested MPs through ecdysis and regurgitation, in addition to egestion through feces (Welden and Cowie, 2016b; Carreras-Colom et al., 2018; Saborowski et al., 2019). In particular, ecdysis was suggested as one of the main potential drivers of the spatial patterns observed in *A. antennatus* in the NW Mediterranean Sea (Carreras-Colom et al., 2020). However, to our best knowledge, species-specific moulting rates throughout the year represent a relevant gap in knowledge that prevents a proper assessment of whether moulting could be a successful strategy among decapod crustaceans to cope with MPs ingestion.

Despite the progress made in understanding the topic of plastic ingestion in Mediterranean benthic decapods, most studies have focused on one or two species at a time hence limiting comparisons across species once the spatial and temporal differences are accounted. A further challenge in this field is the variety of methodologies employed by different authors, which hinders the comparison of studies (Monteiro and da Costa, 2022). Overall, while these studies provide valuable insights, they do not offer a comprehensive basis for analysing the biological and ecological factors that determine MP ingestion across decapod species.

The aim of the present study is to conduct a novel and comprehensive comparative assessment of MP ingestion in four ecologically and commercially significant decapod crustaceans (*Aristeus antennatus*, *Nephrops norvegicus*, *Aristaeomorpha foliacea*, and *Parapenaeus longirostris*) from two distinct areas in the western Mediterranean—the Sardinian and Catalan coasts. By employing a standardized and consistent methodology, this study seeks to uncover species-specific and spatial variations while identifying previously unrecognized drivers of MP ingestion, thereby advancing our understanding of the factors influencing microplastic accumulation in marine organisms.

2. Materials and methods

2.1. Sampling and data collection

Four species of decapod crustaceans were selected in the present study: the red shrimp *Aristeus antennatus* (Risso, 1816), the giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827), the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758), and the deep-water rose shrimp *Parapenaeus longirostris* (Lucas, 1846). Specimens were collected across different seasons in 2021 and 2022 by commercial fishing vessels in

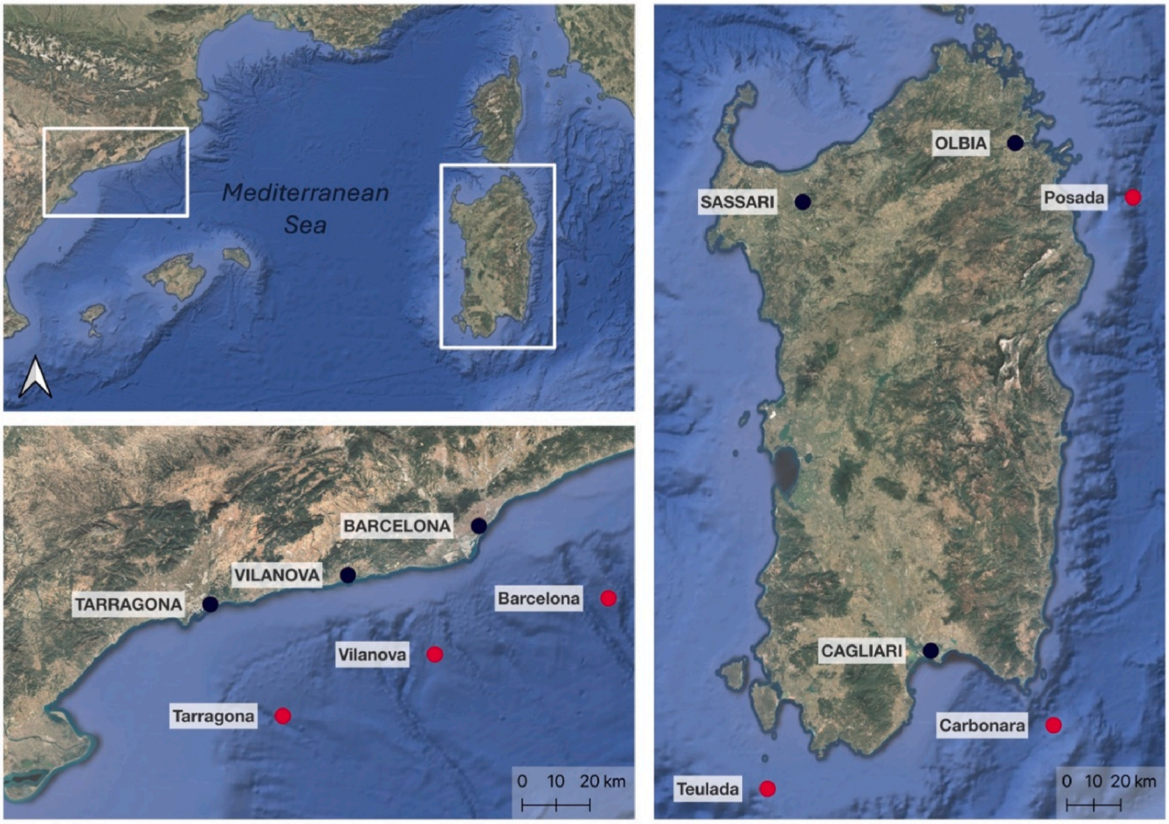

Fig. 1. Map of the study areas showing the whole area of interest (top left) and the specific sampling locations (in red) along the Sardinian (right) and Catalan (bottom left) coasts. Black pinpoints show the three most inhabited cities in the two regions. Basemap provided by Map data ©2015 Google and EMODnet Bathymetry Consortium (2022). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

proximity to the Carbonara, Teulada and Posada submarine canyons in Sardinia (FAO Geographical Sub-Area GSA 11.2), and along the lower slope of the Catalan coast and close to the Vilanova submarine canyon in Catalonia (FAO Geographical Sub-Area GSA 6) (Fig. 1).

After collection, specimens were dissected, biological data were recorded, and then the gastrointestinal tracts (GITs) were stored at −20 °C. Cephalothorax length (CL, in mm), total weight (TW, in g), and sex were recorded. Maturity stages were also determined following Carbonell et al. (2006) and Follesa and Carbonara (2019) criteria.

Moulting stage was assessed relying on the carapace hardness, as proposed by Milligan et al. (2009) for *N. norvegicus*. The consistency of the carapace (hard, soft or jelly) was employed to estimate the time-frame since the individuals underwent moulting (Carreras-Colom et al., 2022a).

GITs' contents were visually inspected under a stereomicroscope, and suspected plastic items were classified by shape as isolated or tangled "balls" of fibers. All isolated fibers were carefully inspected through optical microscopy to further classify the item as synthetic, cellulosic but of potential anthropogenic origin and organic (Rodríguez-Romeu et al., 2020; Carreras-Colom et al., 2022a, 2022b). Tangled balls of fibers were categorized according to their looseness and number of constituent fibers, defining classes from 1 (loose with few fibers) to 4 (highest number of tightly tangled fibers) as proposed by Carreras-Colom et al. (2018). Further characterization was done by means of Fourier Transformed Infrared Spectroscopy (FTIR) for a subsample (11%) of the items previously identified as artificial (Carreras-Colom et al., 2020, 2022b).

2.2. Data analysis

The prevalence of MPs and tangled fibers was determined by counting the number of individuals with at least one MP or tangle, respectively, among the total of individuals analyzed. For each individual, the total fiber load (nMPs), calculated as the total abundance of MPs found, and the total fiber length per individual (iTL), measured as the sum of lengths of each fiber recovered were calculated.

All variables were tested for interspecific and spatial differences following assessment of normality and homoscedasticity using the Shapiro-Wilk and Bartlett's tests, respectively. Since neither normality nor homoscedasticity conditions were met, generalized linear models (GLMs) were used to evaluate differences among species, sampling sites, and regions for each response variable. The models were fitted using Poisson families to best match the observed distributions.

The overall significance of each independent variable was tested using a likelihood ratio (Chi-squared) test. When significant effects were found, post hoc pairwise comparisons were performed using estimated marginal means ("emmeans" package) (Lenth R, 2024), with p-values adjusted using the Tukey method to limit the influence of Type I errors.

To provide a complete interregional comparison, differences in the prevalence of MPs and tangles were tested by employing the Pearson's Chi-squared test and differences in the stomach repletion rates were assessed using the Mann-Whitney *U* test.

Graphical representations were carried out through the "ggplot2" (Wickham, 2016), "wesanderson" (Ram and Wickham, 2023), and "colorspace" packages (Zeileis et al., 2020). All analyses were conducted in RStudio (R version 4.4.0) (R Core Team (2024); RStudio Team (2020), with a significance level set at $\alpha = 0.05$.

2.3. Quality assurance and quality control

Due to their ubiquity in indoor environments, monitoring airborne MPs within the working area and setting precautions to prevent the contamination of samples is crucial in MP studies (Hidalgo-Ruz et al., 2012; Torre et al., 2016). Dissections were performed in a laminar flow cabinet and all the employed equipment was thoroughly rinsed with filtered deionized water (200 μm) prior to use. The working area was isolated by means of a cabinet built adapted from the design proposed by Torre et al. (2016). Involved personnel wore bright green cotton lab coats for the duration of the whole laboratory protocol to easily track contamination coming from the loss of fibers from the clothing. Monitoring of the potential contamination from airborne MPs was conducted using Petri dishes placed inside the cabinet during the screening process. Any MPs-like items found on the exterior of organs before emptying their contents were considered airborne contamination and discarded. During the visual characterization, organic materials (e.g., plant or algae fragments, antennae) misidentified as plastics during the screening of GITs, as well as not weathered fibers matching the color of the lab coat or the cabinet sleeves (i.e. bright green or orange), were also discarded. No further corrections were made.

3. Results

A total of 212 adult individuals were included in the study; 70 *A. antennatus* (AA), 30 *A. foliacea* (AF), 49 *N. norvegicus* (NN) and 63 *P. longirostris* (PL), with CL ranging from 22.8 to 64.8 mm. Sex ratios and maturity stages varied among locations and regions, with Sardinian samples showing a ~1:2 males:females ratio (0.52) whereas Catalan samples had a much more prominent presence of females (~1:4; 0.28). Higher stomach repletion was observed in Catalan samples (57% on average) compared to the Sardinian ones (38%) when considering all species together. *Nephrops norvegicus* was the only species with a lower stomach repletion in Catalonia than Sardinia (Mann-Whitney; $p < 0.001$). Overall, 4% of the Sardinian individuals and 39% of the Catalan individuals presented a soft carapace, with the higher prevalences being observed in AA and PL (36 and 32%, respectively, both locations considered altogether). Detailed data on individual's size, sex, stomach and intestine repletion, and hardness of the carapace clustered by region can be found in Supplementary Table S1. Similarly, mean fiber load and total fiber length data is provided in Supplementary Table S2.

In total, 509 MPs including isolated fibers, ranging in length from 0.37 mm to 32.72 mm, and tangles of fibers were found across 211 individuals from the four species. Overall, 72.5% of the specimens had at least one isolated or tangle of fibers within the GIT, with the number of MPs per individual ranging from 1 to 15.

No other shaped-particles other than fibers, wether isolated or tangled, were found. Isolated fibers accounted for most of the items found (465 items; 91.36%) while the rest were classified as tangles of fibers (44 items; 8.64%). The majority of items were located in the stomach, accounting for 76.3% of the isolated fibers and 93.2% of the tangled ones.

Regarding the size of isolated fibers, the majority measured between 1 mm and 5 mm (288 items; 61.9%), followed by the ones longer than 5 mm (94 items; 20.2%) and shorter than 1 mm (43 mm; 9.2%).

As for tangles, which were present in 28 individuals (13% of the total), class 2 tangles exhibited the highest incidence rate (16 items; 38.1%), while class 1 (11 items, 26,2%) and class 3 (10 items, 23.8%) were slightly less prevalent, and the lowest abundance was recorded for class 4 (five items; 11.9%). Among species, AA and PL were the ones exhibiting a higher prevalence of tangles (11.6 and 19.4%, respectively), followed by NN (8.2%) and AF (8.2%).

Of the total of isolated fibers, 349 were visually identified as synthetic and 116 as cellulosic, and FTIR analysis from a subsample confirmed this classification with a success rate of 81.8%. Overall the polymer composition was dominated by polyethylene terephthalate (PET; 40.5%), followed by acrylonitrile (ACR; 21.6%), polyamide (PA; 16.2%), cellulose (CEL; 16.2%), and polypropylene (PP; 5.4%) (Suppl. Fig. S1).

Monitoring controls revealed an average deposition rate of 0.0011 fibers $\cdot$ min^{-1} $\cdot$ cm^{-2} which represented an average of 0.28 items per individual screened. All fibers recovered from said controls were visually identified as of potential cellulosic composition.

3.1. Interspecific differences

Significant differences were observed among species in both the total abundance (nMPs) and length of fibers (iTL) in both the Sardinia ($p < 0.0001$ for both assumptions) and Catalonia regions ($p = 0.011$; $p < 0.0001$).

Pairwise tests on Sardinian samples revealed lower nMPs in AA compared to AF (GLM; $z = -2.793$, $p = 0.027$) and NN (GLM; $z = -3.572$, $p = 0.002$). The differences observed for nMPs were further confirmed by iTL as well, as AA showed lower iTL than AF (GLM; $z = -3.379$, $p = 0.0041$) and NN (GLM; $z = -2.654$, $p = 0.0397$). In addition, iTL analysis showed that PL ingested a similar number of fibers, but these were shorter than those retrieved from AA (GLM; $z = -0.6979$, $p < 0.0001$) and NN (GLM; $z = -0.6163$, $p = 0.001$) (Fig. 2).

On the other hand, Catalan samples showed lower ingested nMPs in NN compared to AA (GLM; $z = -0.6539$, $p = 0.025$) but higher loads than in PL (GLM; $z = 0.6787$, $p = 0.021$). Additionally, NN individuals showed a higher prevalence of fibers than PL (GLM; $z = -2.538$, $p = 0.0300$). As observed in the Sardinian samples, the iTL tests in the Catalan samples confirmed the results from the fiber load analysis ($p < 0.0001$), with NN showing lower values than AA (GLM; $z = -7.537$, $p < 0.0001$), but higher than PL (GLM; $z = 7.563$, $p < 0.0001$) (Fig. 2).

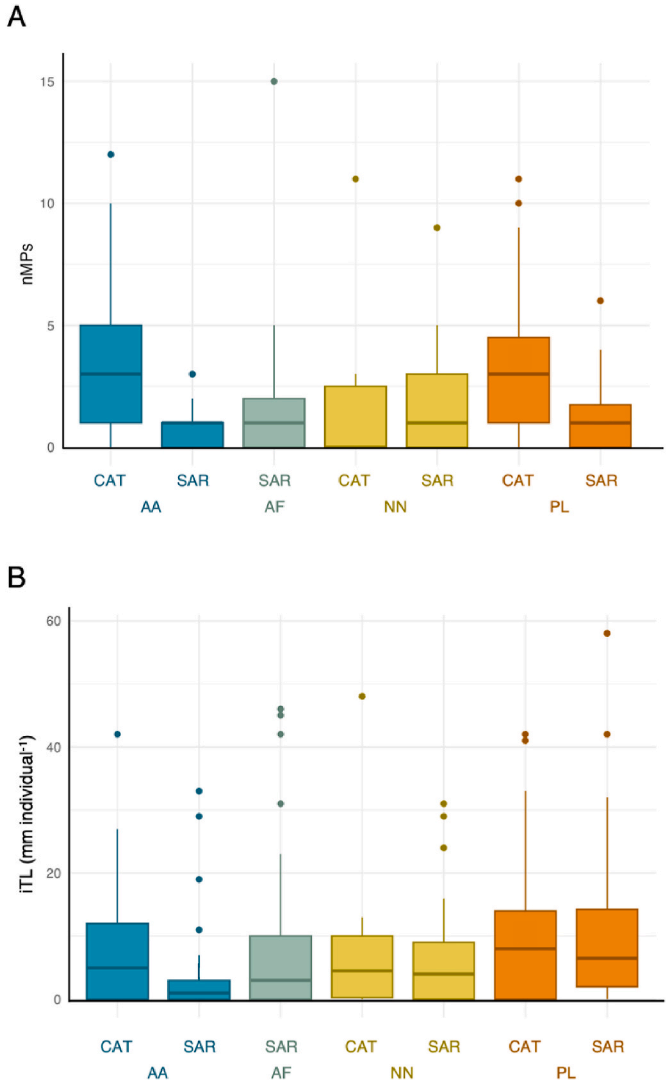

Fig. 2. Interspecific and regional differences, expressed as the number of retrieved plastics (A) and sum of length of the retrieved fibers in each individual (B). Species acronyms: AA (*A. antennatus*), AF (*A. foliacea*), NN (*N. norvegicus*), PL (*P. longirostris*). Location acronyms: CAT (Catalonia), SAR (Sardinia).

3.2. Interregional differences

No significant differences in the prevalence of MPs and tangles between species and locations were observed except for PL, which showed a higher prevalence of tangles in Sardinia (only 3.3% of the individuals) compared to Catalonia (34.4%; $X^2 = 7.67$, $p = 0.006$). Significant variations were observed when accounting for the MPs load through nMPs and iTL. Overall, Catalan individuals showed a two-fold higher fiber load levels (3.69 fibers ind^{-1}) than those from Sardinia (1.62 fibers ind^{-1}). Likewise, the average iTL value was higher in the Catalan group (mean; 12.89 ± 1.48 mm ind^{-1}) than in the Sardinian (5.03 ± 0.76 mm ind^{-1}). Main interregional differences were observed in the number of ingested isolated fibers for each species (AA, PL: GLM; $p < 0.0001$; NN: GLM; $p = 0.0104$). Further analyses reported significant variations in the abundance of tangles for AA (GLM; $p = 0.0050$) and PL (GLM; $p = 0.0058$), with higher values in Catalonia. Significantly higher total lengths were seen in Catalan AA (GLM; $p < 0.0001$) and PL (GLM; $p < 0.0001$) samples while only marginal significance was recorded for NN (GLM; $p = 0.0583$) (Fig. 2).

3.3. Intraregional differences

Main tests revealed fiber loads, both in terms of nMPs and iTLs, to be strongly influenced by spatial variations at a local scale ($p < 0.0001$). Notably, the samples from Posada showed a significantly lower fiber load than the ones from Carbonara (GLM; $z = -4.074$, $p = 0.0007$) and Teulada (GLM; $z = -3.167$, $p = 0.0192$), considering all species altogether. As for Catalonia, the Tarragona area showed a significantly higher fiber abundance than Vilanova (GLM; $z = 0.5978$, $p = 0.0017$).

Significant differences in iTL were found among sampling locations in both Sardinia and Catalonia ($p < 0.0001$ as output of each pairwise test), except for the Barcelona – Vilanova pairwise comparison (Fig. 3).

4. Discussions

This study provides a novel characterization of MP ingestion in four ecologically and economically significant deep-sea decapod crustaceans, key components of the Mediterranean Sea benthic assemblage. Overall, our results provide further evidence of the high levels of plastic ingestion by decapod crustaceans (Carreras-Colom et al., 2018, 2020, 2022a, 2022b; Cau et al., 2019, 2020), though pointing out variations in the contamination levels among these species. Published literature shows a wide disparity in the abundance and composition of MPs in crustaceans, likely due to the different methodological approaches to isolate and quantify them. For instance, a large number of fragments were reported by Cau et al. (2019, 2020, 2023) in *A. antennatus* and *N. norvegicus* from the Tyrrhenian Sea, while our samples showed a complete absence of such fragments. This discrepancy suggests that various methodologies, such as the digestion of gastrointestinal tract (GIT) contents and density separation protocols (Avio et al., 2015; Karami et al., 2017) might be more effective in identifying the smaller fraction of MPs and fragment-like shapes (Cau et al., 2020; Hara et al., 2020; Joyce et al., 2022). In contrast, visual inspection likely overlooks smaller MPs, focusing instead on larger items like tangled fibers and mesofibers (Carreras-Colom et al., 2022a; Joyce et al., 2023). Additionally, it has been suggested that the presence of small MP particles (<3 mm) may indicate recently consumed items, whereas larger MPs could be retained over longer periods (Joyce et al., 2022). Hence, caution should be exerted when attempting the comparison of studies with different methodological approaches.

In our study, the use of the same method for the detection of the larger MPs fraction has provided a reliable comparative assessment of species-specific ingestion and exposure patterns across different geographical areas. This approach highlights the potential of this

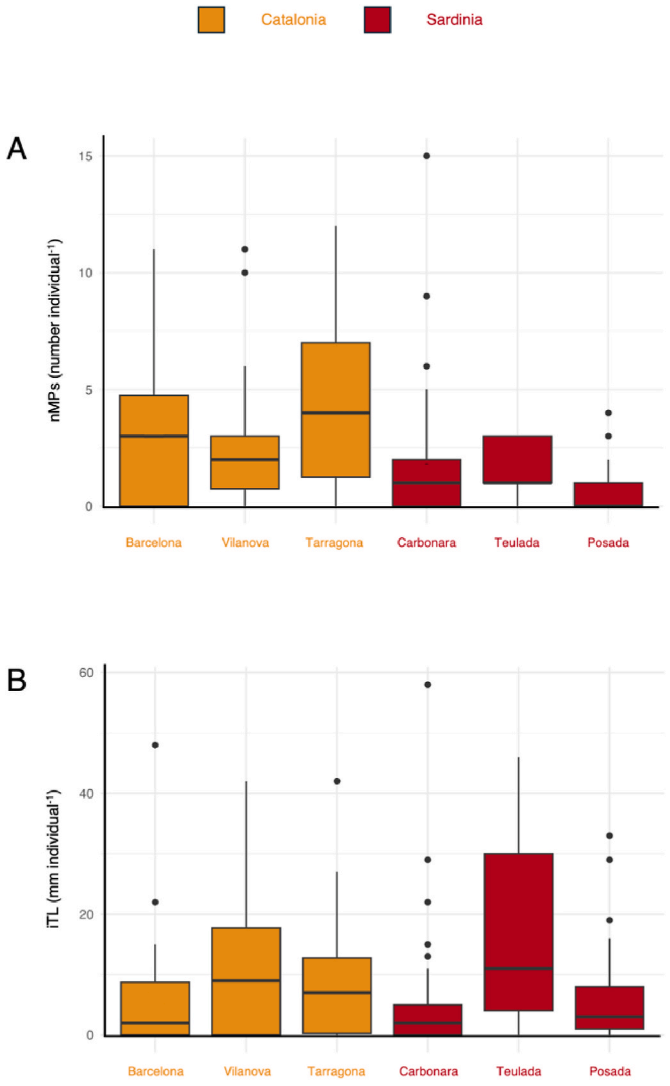

Fig. 3. Interregional differences, expressed as (A) the number of retrieved plastics (nMPs) and (B) sum of length of the retrieved fibers in each individual (iTL). The Catalan sample pool did not include *A. foliacea* because of its extinction within the Balearic basin.

method as a quick, cost-effective, and reliable monitoring tool.

4.1. Interspecific comparison

Regarding the impact on individual species, *N. norvegicus* exhibited similar levels of MPs ingestion across both examined regions (~1.7 MPs per individual). Conversely, *P. longirostris* and *A. antennatus* demonstrated marked regional differences between regions, in both cases reaching average values of 3.4 particles per individual in Catalan waters but notably lower levels in Sardinia (<1 MPs per individual). The MP levels of *A. foliacea* fell within the range observed for *N. norvegicus* in Sardinia, but unfortunately, no Catalan counterpart could be tested. The local extinction of *A. foliacea* in the Balearic Basin during the 1960s, likely driven by environmental changes, has had long-lasting effects on the species' population. This decline is reflected in the extremely low current landings of the species (Cartes et al., 2011; Guijarro et al., 2019; ICATMAR, 2024) which prevented us from performing interregional comparisons. Nevertheless, Sardinian samples suggest that this species could serve as a potential alternative indicator species for monitoring MP pollution on a smaller scale, distributing the sampling stress among different species. Overall, all inspected species proved to be promising indicators, provided that the observed variations are validated with environmental exposure data.

The observed differences in MPs ingestion among the species may result from a combination of factors. It is common to associate MPs ingestion with environmental exposure (Welden et al., 2018; Ohkubo et al., 2020), but other critical factors, particularly in this study were different species were collected in most cases from the same hauls, must be considered, such as the feeding behavior. Although all the inspected species are considered generalist predators, there exist slight differences among them. Most importantly, their diets adapt based on availability and competition, as well as over their lifespan (Cristo and Cartes, 1998; Kapiris, 2012; Fiorentino et al., 2013). For instance, *A. foliacea* presents a preference for pelagic swimmers, a preference partially shared by *P. longirostris* as it alternates a pelagic hunting phase with a digging one (Rainer, 1992; Cartes, 1995; Kapiris, 2004). On the contrary, *A. antennatus* and *N. norvegicus* have a marked rooting behavior and greater interest in buried prey (Cartes and Sarda, 1989; Cartes et al., 1994; Johnson et al., 2013). This allegedly influences the feeding-driven MPs uptake rates, since MPs tend to accumulate in superficial sediment layers (Uddin et al., 2021). The four species share, to some extent, similarities in scavenging activities (Cartes, 1995; Chartosia et al., 2005), suggesting comparable levels of MPs exposure through diets. This is partially supported by our results and is evident in the high levels of plastic fiber ingestion observed throughout the study.

Different motilities and extents of feeding areas could play a role as well. Species like *A. antennatus* show higher motility with seasonal and daily migrations along submarine canyons that impact their diet composition (Cartes et al., 1993; Cartes et al., 1994). In contrast, *N. norvegicus* tends to remain close to its burrows, especially during the brooding season (Katoh et al., 2013). Active predatory behaviors and wider movement ranges likely increase the opportunities for plastic ingestion, which could explain the higher values observed in *A. antennatus*, followed closely by *P. longirostris* in the Catalonia region (Cartes, 1994; Kapiris, 2004) and *A. foliacea* in Sardinia (Chartosia et al., 2005).

Differences in the GITs' repletion rates may partially explain the higher MPs loads recorded in *A. antennatus* and *P. longirostris* in Catalonia, as well as *N. norvegicus* in Sardinia, compared to their counterparts, since higher loads of MPs correlate well with higher values of stomach fullness. Nonetheless, seasonal variations could also reflect differences in feeding intensity, driven by resource availability and species-specific reproductive cycles (Nouar et al., 2011; Guijarro et al., 2019). In line with this, it is important to consider resource partitioning amongst co-occurring decapods, a key dynamic in the Mediterranean assemblages (Cartes et al., 2009).Another important factor to consider is community dynamics. To reduce interspecific competition in areas of the continental margin with limited per-capita resources availability, some decapod species tend to partition prey, hunting areas and hunting activity within short (e.g. daily) or long intervals (e.g. throughout their life cycle) (Cartes, 2009; Aguzzi et al., 2009). This strategy may account for intraspecific variations observed between regions. Indeed, species with similar strategies such as *P. longirostris* and *A. antennatus* partition their hunting areas by occupying shallow and deeper areas, respectively. The preference to settle in deeper areas could then enhance the *biota*-MP interactions, as these areas tend to accumulate higher concentrations of MPs (Sanchez-Vidal et al., 2018).

Concerning ecdysis, a potential removal route for ingested MPs in decapods, indices of recent moulting (i.e. soft carapaces) were observed more frequently in Catalonia than in Sardinia. This finding contradicts the higher fiber loads found in the former location. Two possibilities should be considered: (1) ecdysis may be a less effective mechanism of MP removal than previously thought (Welden and Cowie, 2016b) suggesting that the observed loads might be remnants from past retention cycles, or (2) carapace softness may not indicate that molting had just occurred, but rather that it is imminent. In this case, the observed MP loads could result from the accumulation over the entire molting cycle.

Unfortunately, the limited information available on the molting periodicity of these species and the lack of a more consistent and reliable method for its determination prevent further discussion.

Additionally, the characteristics of the gastric mill, particularly the spacing between its components observed in the tested decapods, may influence the retention potential that ultimately determines the observed MP load. According to Patwardhan's (1935a, 1935b) descriptions and Welden and Cowie's (2016b) hypothesis, it is plausible that *N. norvegicus* may exhibit a lower MPs retention than penaeid species (*A. foliacea*, *A. antennatus* and *P. longirostris*). Larger organisms like *N. norvegicus*, which have greater spacing within the gastric mill components, are likely to show reduced retention of MPs when compared to smaller individuals. Beyond the size variation within species, interspecies morphological differences could also be significant. Although our results suggest an overall lower average MPs load in *N. norvegicus*, additional studies covering a broader range of sizes in these species would be necessary to confirm this relationship.

Regardless of its size or morphological variations, the gastric mill is likely to play a key role in the formation of tangles, whose presence has been confirmed in every species and region for the first time. The recorded prevalence (8.64%) of tangles is considered noteworthy. The formation of these tangles is still greatly unknown, it could start from either isolated MPs alone or in conjunction with organic elements (e.g., byssus from Bivalvia and vegetal fibrous remains) and hard fragments (e.g., carapace parts) that were observed to serve as nucleation centers in most tangles. Hence, it is essential to consider that organic materials could eventually serve as influential factors in the health of decapod crustaceans as their presence could play some role not only in the entangling process but in the dimension of the entanglement as well. Concurrently, tangle formation could be a process involving MPs ingestion in multiple species interconnected across one or more levels of the trophic web (Farrell and Nelson, 2013; Crooks et al., 2019), with tangles possibly being transferred from species to species through predation.

All things considered, no evidence of obstruction was observed, even in cases involving the retrieval of large ball tangles (category 4), the presence of both stomach and intestinal contents suggested effective food transit or no appetite loss. This observation is aligned with recent findings that report no significant adverse effects of MPs ingestion on wild marine crustacean decapods at histological nor biochemical levels (Carreras-Colom et al., 2020, 2022b), despite the large number of ingested MPs.

4.2. Geographical comparison

Obtained results are in accordance with previous spatial trends reported for *A. antennatus* in the same area (Carreras-Colom et al., 2020), which identified the Barcelona metropolitan area as a significant hotspot for MPs. Additionally, the Catalan sites are located within low-energy and microtidal continental shelves, with low resuspension rates that possibly ease the transport towards the benthic layer and, consequently, their ingestion by benthic organisms (Palanques et al., 2002). On the other hand, in Sardinia, the lower abundance of MPs in Carbonara, despite being under the influence of the most densely populated area of Sardinia might be attributed to the comparatively lower population density to Barcelona, in addition to the action of the hydrodynamic currents in the area that may prevent MP sedimentation (Cau et al., 2024).

With specific focus on the two regions taken individually, two different patterns of MPs distribution were identified. Sardinian samples showed higher abundance in areas bordering more densely populated zones like Carbonara Valley, while the Posada site had lower contamination, possibly due to its farthest position from relevant macrolitter hotspots (Franceschini et al., 2021).

On the contrary, Catalan specimens revealed the highest fiber load along the southern sampling site, Tarragona, which is the furthest from Barcelona city, the most anthropized area. Therefore, the area between the city of Barcelona and Tarragona might act as a transition zone for MPs with the main current of the area (the Northern Current) conveying them southwards (Font et al., 1988). These patterns put a light on the complexity of MPs dynamics, as high nMPs concentration did not necessarily translate to elevated iTL values.

Indeed, nMPs and iTL did not show a high correlation, especially in Sardinia ($R^2 = 0.59$). A stronger correlation was observed in Catalonia ($R^2 = 0.72$), although cases of inverse relationship between iTL and nMPs still occurred in some sampling areas. Clear examples include Teulada, which exhibited the highest iTL values despite the relatively lower nMPs, and Barcelona, where high nMPs we observed alongside low iTL. These observations highlight how using the abundance of items could represent an oversimplistic measurement of the impact if not properly coupled to the characterization of the items size.

5. Conclusions

This study presents, for the first time, a comprehensive evaluation of microplastic ingestion levels in four ecologically and commercially significant crustaceans, spanning two distinct regions in the Mediterranean Sea. By addressing this critical knowledge gap, a consistent methodology was used, enhancing data comparability and providing novel insights into regional and species-specific patterns of microplastic exposure. Biological and ecological features are discussed as potential drivers of enhanced or reduced MP ingestion rates. Indeed, variations were related to species-specific differences in the environmental exposure, feeding habits and intensities and morphological characteristics of the species, together providing a more detailed understanding of MP ingestion patterns across species and locations. Our findings underscore once again that decapods may be among the most affected organisms by MP ingestion, as evidenced by the average of 2.4 MPs ingested by each individual and the prevalence of tangled fiber tangles (about one individual out of five had a tangle in the GIT). Anyway, no evidence of gut obstruction was detected. As a future perspective, it is advisable to acknowledge that these species face multiple threats beyond microplastic ingestion, including the broader impacts of global change and that it is crucial to understand their coping strategies. Also, continued monitoring is essential to fully understand the cumulative impacts of these stressors, ensuring effective protection and management for these ecologically and economically significant crustaceans.

CRediT authorship contribution statement

L. Chiacchio: Writing – original draft, Methodology, Investigation, Formal analysis. **A. Cau:** Writing – review & editing, Validation, Conceptualization. **A. Soler-Membrives:** Writing – review & editing, Validation, Conceptualization. **M.C. Follesa:** Writing – review & editing, Resources. **A. Bellodi:** Writing – review & editing, Resources. **E. Carreras-Colom:** Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ester Carreras-Colom reports financial support was provided by European Union - NextGenerationEU. If there are other authors, they 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.envres.2025.120962.

Data availability

Data will be made available on request.

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