Comparative microplastic load in two decapod crustaceans Palinurus elephas (Fabricius, 1787) and Nephrops norvegicus (Linnaeus, 1758)

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Comparative microplastic load in two decapod crustaceans *Palinurus elephas* and *Nephrops norvegicus*

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Abstract

The present work compares microplastics (MPs) contamination in two charismatic crustaceans: European spiny lobster *Palinurus elephas* and langoustine *Nephrops norvegicus*. Samples (*P. elephas* n=14; *N. norvegicus* n=15) were collected between 76 and 592 m depth, from four sites in west Sardinia, Italy. An extraction protocol was applied on stomachs and intestines, separately, and over 500 particles were further characterized through μFT-IR. We document 100% occurrence in specimens from both species, with *P. elephas* being significantly more contaminated (9.1 ± 1.75 vs. 1.66 ± 0.1 MPs individual^{-1}), ingesting larger microplastics with different polymeric composition. The scavenging-based feeding strategy of both species could explain such exposure to MPs, mostly derived by single-use plastic. The overall results highlight that both species are clearly affected by plastic pollution, being valuable bioindicators and charismatic species that could thus represent excellent flagship species for raising awareness toward the global issue of plastic in the marine environment.

Since 1950s, plastic production generated ca. 5 billion tons of waste, currently dispersed in the environment (Geyer et al., 2017). It has been estimated that 5-8 Mt of plastic move from land to oceans on a yearly basis (Jambeck et al., 2015), with trillions of plastic items currently floating at sea (Eriksen et al., 2014). However, the abundance of floating litter on oceans' surface was measured to be lower than what has been forecasted by the most conservative models (Cozar et al., 2014; Eriksen et al., 2014). While recent studies proposed that riverine input might have been overestimated (Weiss et al., 2021), it is widely recognized that the sink of objects represent the major explanation for such discrepancy(Gutow et al., 2018; Ryan, 2015). The existence of an initial floating stage, followed by sink and deposition on the seafloor after a more or less long period of time, renders plastic items capable of reaching also secluded environments such as polar regions and the deep oceans' floor (Bergmann et al., 2017; Jambeck et al., 2015; Peeken et al., 2018; Peng et al., 2020). The most emblematic sign of this is a plastic bag documented at ca. 10,900 m depth in the Mariana Trench (Chiba et al., 2018).

Processes like biofouling or physical weathering can change specific weight of plastic (Kowalski et al., 2016; Zettler et al., 2013), triggering their sink into the water column. Plastic and microplastics contribute to the so called vertical "pump" and they increase the transfer of organic carbon, organisms and other elements to the ocean depths and, in this respect, ocean floors represent the final sink for plastic particles (Galgani and Loiselle, 2021; Woodall et al., 2014), as demonstrated by the exponential increase in deposition occurred over the last decades (Brandon et al., 2019). Plastic can also slowly degrade through biological, mechanical and physical processes that cause its fragmentation into smaller particles that are called microplastics (MPs), if their dimension is comprised between 1μm and 5mm (Frias and Nash, 2019).

Such dimensional range renders these particles particularly suitable for accidental ingestion by marine *biota*, with vagile benthic fauna being particularly exposed compared to other organisms (Bour et al., 2018; Carreras-Colom et al., 2018; Cau et al., 2019a; Murray and Cowie, 2011). The size of MPs particles influences their ingestion and egestion rates and their isolation from digestive tracts of marine organisms, by itself, does not represent a reliable proxy for particle retention (Cau et al., 2020), nor for their accumulation but rather a snapshot of the exposure that organisms experience in the specific environment.

Within the European Union, the Marine Strategy Framework Directive evaluates the environmental status of European seas (MSFD; 2008/56/EC) through 11 descriptors, within which marine litter quantification (and microplastics therein) is one of those (Descriptor 10) (Galgani et al., 2013b, 2013a); thus, the necessity and the research for efficient bioindicators is building up constantly across scientific literature (Bonanno and Orlando-Bonaca, 2018; Fossi et al., 2018). This is particularly relevant for the Mediterranean, which is among the most contaminated (or at least most investigated) basins worldwide (Canals et al., 2021). The EU Mission on Restore Our Ocean and Water by 2030 has among its objectives to prevent and eliminate pollution from the Sea, and Mediterranean Basin has been identified for setting a Lighthouse of actions toward plastic pollution. The Mediterranean Sea is estimated to retain 5-10% of the global plastic mass dispersed in oceans (Suaria et al., 2016; Van Sebille et al., 2015), and the resident associated *biota* showed to diffusely ingest MPs, both in the pelagic and benthic *dominium* (Cau et al., 2019a).

Recent scientific literature emphasized how some decapod crustaceans that show a tight association with the seabed are particularly exposed to MPs: this is the case of Norwegian langoustine *Nephrops norvegicus* (L. 1758) and European spiny lobster *Palinurus elephas* (F. 1787). While the former is widely acknowledged as flagship species for MPs contamination across EU waters (Carreras-Colom et al., 2022a; Cau et al., 2019a; Hara et al., 2020; Joyce et al., 2022a), the latter has only very recently been identified as exposed to MPs and nanoparticles in the Aegean sea, highlighting the urgent need to provide additional data over a broader geographical scale (Kampouris et al., 2023).

Crustaceans belonging to the family *Palinuridae* are among the most highly priced seafood in the world, and their fishery often represent the backbone of export economy in some regions (e.g., Caribbean countries; Higgs et al. 2016). European spiny lobster *P. elephas* is distributed across the Mediterranean Sea, but also across the eastern part of the Atlantic Ocean, from North Africa to Scotland. Its fishery was first recorded in the 15th century BC, and the popularity of spiny lobsters as *gourmet food* took off in the 19th century and consistently increased till present days, when living specimens of *P. elephas* can be sold at retail prices comprised between 40 and 120€ Kg^{-1} (Cau et al., 2019b; Groeneveld et al., 2013). With these premises, it is not surprising that European spiny lobster is currently classified as "Vulnerable", by the International Union for Conservation of Nature (IUCN), mostly due to its continuous overfishing (Follesa et al., 2014; Goñi and Latrouite, 2005).

N. norvegicus (fam. *Nephropidae*) is a benthic decapod inhabiting European temperate and cold waters. Similarly to European spiny lobster, langoustine is a millions of Euros worth fishery resource in Europe, since it is highly appreciated as *gourmet* seafood either, with a retail price comparable to that of other crustaceans such as lobsters, spiny lobsters or deep sea shrimps (Cau et al., 2019b; Ungfors et al., 2013). Langoustine is a key element in muddy bottoms trophic webs, and it shows a wide bathymetric distribution (up to 800m depth), mostly restricted to deep waters in the Mediterranean area (>200 m depth). The continuous scavenging behaviour on the seabed allows langoustines to interact with other benthic species, but also with sediment-water fluxes and resuspended sediment (Cristo et al., 1998). Because of this, *N. norvegicus* has been identified as potentially MPs exposed species and documented as reliable bioindicator of MPs contamination of the deep seabed (Carreras-Colom et al., 2022a, 2022b; Cau et al., 2019a; Franceschini et al., 2021; Murray and Cowie, 2011).

Also *P. elephas* is an omnivorous and scavenging species that, contrarily to *N. norvegicus*, dwells in shallow Mediterranean waters up to 200m depth (Goñi and Latrouite, 2005; Groeneveld et al., 2013). Both spiny lobster and langoustine are exposed to MPs mostly through their similar trophic habits and, with very few exceptions (Cau et al., 2020; Joyce et al., 2022b), all available information on particles occurrence in these species reflects their isolation from stomach contents or through the digestion of the entire digestive apparatus (Avio et al., 2020; Hara et al., 2020; Joyce et al., 2022a, 2022b; Murray and Cowie, 2011; Welden and Cowie, 2016a).

The present study aims to investigate and compare MPs ingestion in the European spiny lobster *P. elephas* and the Norwegian lobster *Nephrops norvegicus* sampled from coastal and deeper habitats from Sardinian waters, in Italy. The analysis of particles in stomachs and intestines was expected to provide additional insights on the role of such benthic crustaceans in modulating the environmental fate and bioavailability of MPs through the ingestion, mechanical fragmentation and egestion process, as recently documented in controlled and wild conditions (Cau et al., 2020; Dawson et al., 2018). These species are commonly and extensively fished for human consumption and, while exhibiting similar feeding strategies in two segregated bathymetric distribution range, were expected to highlight novel insights as to whether ecologically similar organisms might suffer from different exposure and ingestion of MPs.

For *N. norvegicus*, samples were collected from 2 sites around the Sardinia island in 2019 (Fig. 1), in the framework of the MEDiterranean International Trawl Survey (MEDITS), at depths comprised between 402 and 592 m. Stomachs and intestines were extracted from 15 individuals. For *P. elephas* samples were collected from 2 sites from the western coast of Sardinia between 2019 and 2020 (Fig. 1; Table 1), from both artisanal and professional fisheries operating using trammel nets and trawlers, at depths comprised between 76 and 105 m. A total of 14 stomachs and intestines were extracted. Ranges of biometric data (and sex ratio) of analysed specimens were Carapace Length (CL) 25.2 – 41.2 mm for *N. norvegicus* (9 males and 6 females), and CL 73 –117.9 mm for *P. elephas* (6 males and 8 females). For *P. elephas* stomach and intestine weight were recorded, separately, and individual weight, which ranged from 350gr to 2.2 Kg.

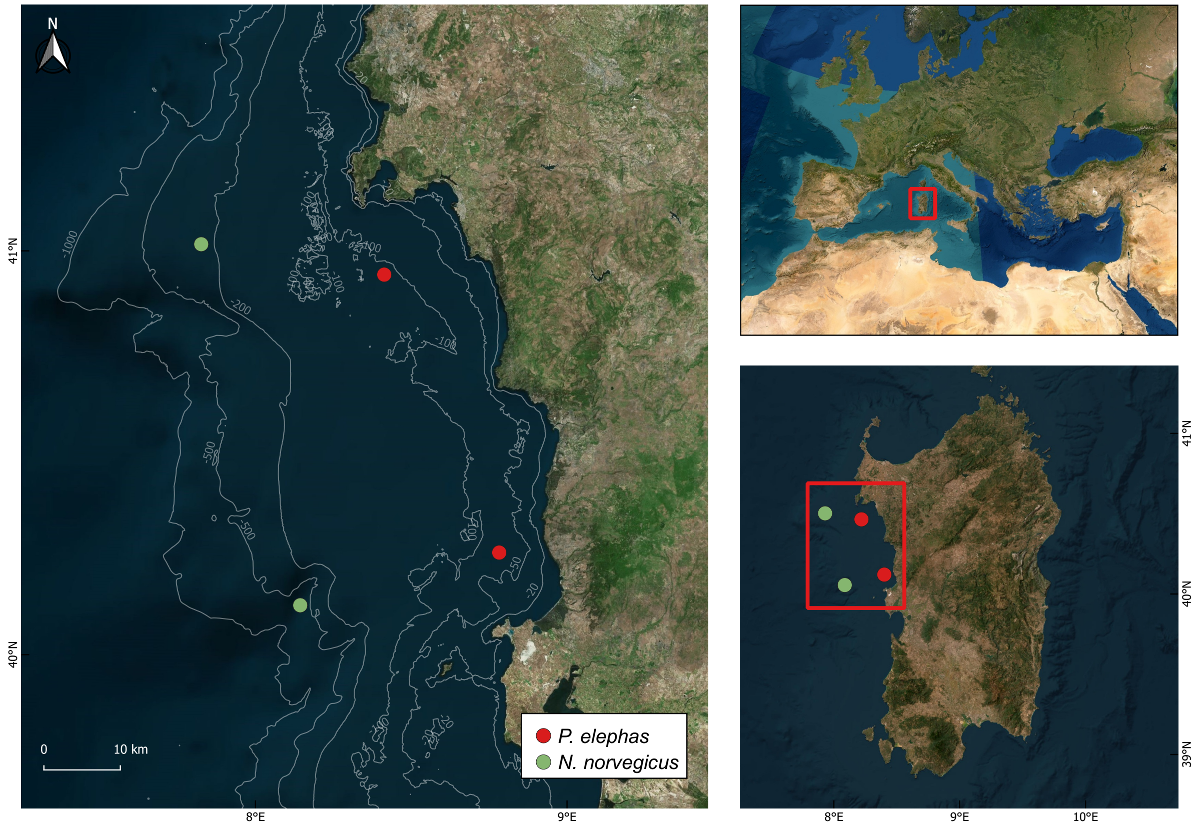

Figure 1. Map of the study area where specimens of *N. norvegicus* (green dots) and *P. elephas* (red dots) have been collected. Bottom-left white bar represents a scale of 10Km.

After collection, specimens were transported in the laboratory using an ice box and placed in cold storage (-20°) to avoid the risk of contamination from sampling activities. Samples were thawed at room temperature and each specimen was dissected to remove the stomach and intestine, which were then placed separately in aluminium foils and stored at -20°, until analysis. Necessary precautions were taken when handling and processing the samples to prevent aerial and solvent contamination with MPs. Digestion of the digestive tract was carried out using a 10% potassium hydroxide (KOH) at 40 °C for 48 h (Hara et al., 2020). The resulting supernatant was filtered using a vacuum pump (VCP130) through 47 mm Sartorius® cellulose nitrate membrane filters (pore size 8μm). The MPs extraction procedure was based on applying the separation procedure on the digestate through a NaCl hypersaline solution (density 1.2 g cm^{-3}), where supernatant solution was collected through glass beaker. For *P. elephas*, since stomachs were full of sand and food material, the obtained solution was again subjected to a second density separation step through a NaCl hypersaline solution, later followed by filtration, partial digestion in diluted hydrogen peroxide (15%), sorting and chemical characterization. The method has been validated and standardized on samples spiked with MPs of different types and sizes (Avio et al., 2015) and used for MPs extraction in the same species targeted by the present study (Avio et al., 2020; Cau et al., 2020, 2019a; Martinelli et al., 2021). When compared with other available methodologies, it showed a recovery yield higher than 90% for particles smaller than 100 μm and 95% for greater ones, with no effects on particle characteristics such as shape or colour. During sorting, all retrieved particles were observed under a stereomicroscope, photographed and categorized according to shape in: i) fragments (small, irregular shaped particles, crystals, rigid, thick); ii) film (irregular shapes, thin and flexible, transparent particles); iii) pellet (cylindrical particles); iv) fiber (elongated, thin, straight particles, frayed ends); v) sphere- like (cubical, sphere); vi) foam (lightweight particles with spongy texture). Once isolated, photographed MPs were measured at their largest cross section under a stereomicroscope using the image analysis CPCe, 'measuring' function (Kohler and Gill, 2006). All extracted particles were characterized using a μFT-IR microscope (Spotlight 200i microscope system coupled with Spectrum Two spectrometer, Perkin Elmer). The measurements were made using the μATR mode. Following back-ground scans, 32 scans were performed for each particle, with a resolution of 4 cm^{-1}. Spectrum 10 software was used for the output spectra and the identification of polymers was performed by comparison with libraries of standard spectra. Polymers matching for more than 70% with the reference spectra were validated, while polymers with a match comprised between 60% and 70% underwent into a more critical interpretation of the spectra (Bour et al., 2018). To reduce background contamination, operators were wearing acid green cotton lab coat to identify possible fibers coming from it and special attention was paid to limit the wearing of synthetic clothes. Before starting the extractions, and between each process step, benches were cleaned with milli-Q water and all solutions used were pre-filtered through a nitrate acetate membrane with pore size of 0.45μm. Glass and metal materials were used whenever possible, rinsed thrice with prefiltered milli-Q water before use and wrapped in aluminium foil when not in use. After rinsing, all containers were covered with aluminium foils, which were also kept during digestion, stirring, decantation and filtration steps. After filtration, membranes were kept in glass petri dishes, previously rinsed with prefiltered milli-Q water. NaCl solution was prepared in distilled water and further filtered (0.45μm pore size). Contamination controls were also included (one control for each batch of samples was treated in parallel to samples), consisting of 10 mL of prefiltered distilled water that undertook all the steps of the protocol. Despite such precautions, it was not possible to fully avoid airborne contamination and some textile fibers were found in the control membranes. We then applied total subtraction of items as correction method, based on a spectral similarity and visual characteristics (Kroon et al., 2018). In brief, fibers were checked with the actual samples and compared, both visually and spectrally. Potential extraneous particles were used to build a visual and spectral contaminant library, against which all sample items were confronted and when particle matched a contaminant or control library item with > 80 % spectral similarity and visual similarity (i.e., same colour, shape, texture), were removed from the dataset. This correction method provides a count of total sample particles minus items confirmed to be contaminant particles.

PERMutational ANalysis Of VAriance ('PERMANOVA'; Anderson et al. 2008) based on Euclidean distance resemblance matrixes (untransformed data) was used to test for significant differences in MPs polymeric composition between the two investigated species. The factor 'Species' (2 levels, fixed) was used as unique source of variation. The n. of particles ind^{-1} of each polymer type was used as response variable. Differences in the number and size of particles between the two species were tested using the Mann-Whitney test. Moreover, within each species, using the same statistical routine, we tested for different contamination descriptors (both in terms of number of particles and size) between stomach and intestine. The PERMANOVA was used to test for differences in the polymeric composition between the two compartments, in this case using the different compartment (i.e., stomach or intestine: 2 levels, fixed) as unique source of variation. Due to the limited number of samples, it was not possible to test for geographical differences within the sub-region object of the study, nor for bathymetric trends.

More than 2,000 particles were extracted from the 2 species (>1,300 for *P. elephas* and >700 for *N. norvegicus*) and sorted for the chemical characterization through μFT-IR (Fig. 2). After data correction, out of the total number of particles isolated in *P. elephas*, 127 of them were made of plastic, 87 for stomachs and 40 for intestines (Fig. 3). All the 14 specimens of *P. elephas* had MPs in their digestive tract (100% of occurrence); more in detail, 13 stomachs and 12 intestines, out of 14. The weight of *P. elephas* stomachs ranged from 4.2 to 24.9 gr while intestines' weight ranged from a minimum of 0.4 gr to a maximum of 10.4 gr. In both cases, there was no significant correlation between higher weight of stomachs and MPs load.

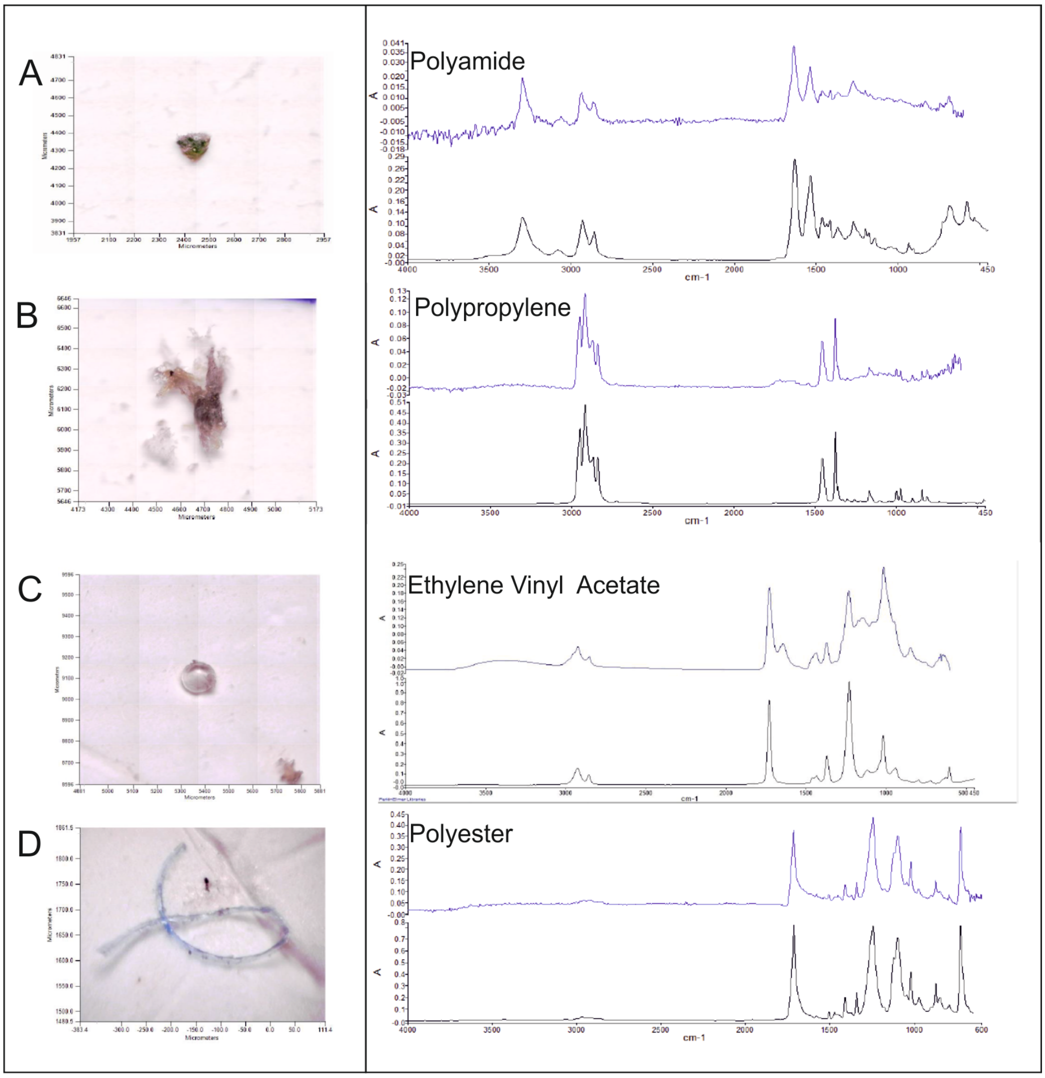

Figure 2. Examples of MPs extracted from *P. elephas* and *N. norvegicus* and corresponding µFT-IR spectra. (A) polyamide fragment; (B) polypropylene particle, (C) ethylene vinyl acetate sphere, (D) polyester fiber. The blue lines represent the characterized particles, while dark lines correspond to the reference spectra.

The average number of MPs was 9.1 ± 1.75 MPs ind. $^{-1}$, ranging from 3 to a maximum of 25 MPs (Fig. 3), with no significant correlation to the weight of stomachs or intestines and with sex or dimensions of organisms (in terms of weight and/or LC).

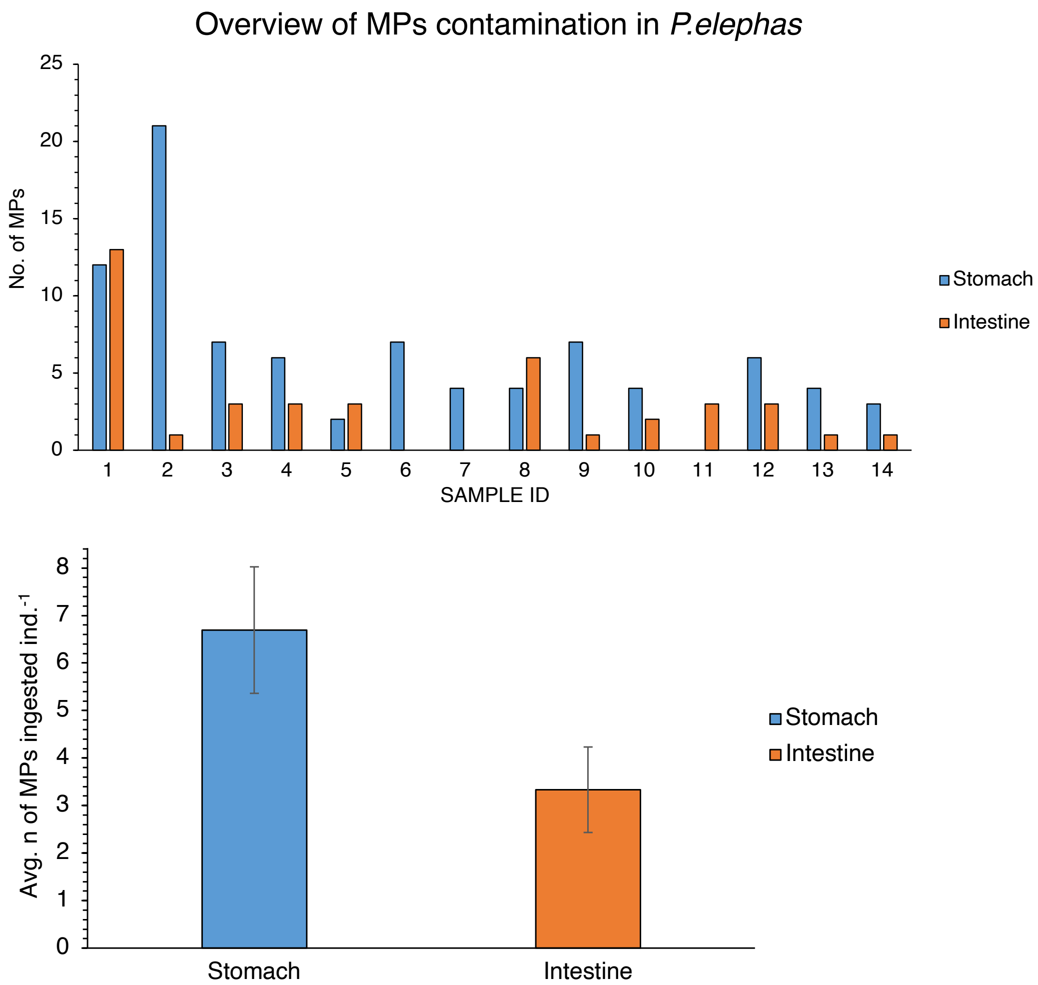

Figure 3. Histogram showing the number of MPs in each sample of *P. elephas* (upper graph) and the average number of particles of MPs (± st. err) isolated from stomachs and intestines.

More in detail, considering only positive individuals, the average number of particles was 6.7 ± 1.3 in stomachs and 3.4 ± 0.9 in intestines (Fig. 3). Among the 14 spiny lobsters, 11 individuals (~79%) showed a cumulative number of MPs in their stomach and intestine combined >5. There was no significant difference in the size of particles isolated from stomachs and intestines of *P. elephas*, which had overall an average size of 1.63 ± 0.22 mm, with those isolated from intestine (avg. 1.82 ± 0.22 mm) being slightly bigger than those found in stomachs (avg. 1.54 ± 0.20 mm; Fig 4). The smallest particles isolated from stomach and intestine were 41 μm and 64 μm respectively, while the largest were 9.7 mm and 6.39 mm, both of which outsize the definition of MPs, but rather falling in the class of meso-plastics (>5mm). Out of the total of plastic particles extracted from the 14 individuals, 6 particles were larger than 5mm, 4 in stomachs and 2 in intestines. Overall, the size-frequency distribution (Fig. 4) showed that approximately

50% of the isolated plastic particles were smaller than 1mm, while the most representative size class was in the range between 1 and 2 mm, accounting for >20% of the total, in both stomach and intestine (Fig. 4).

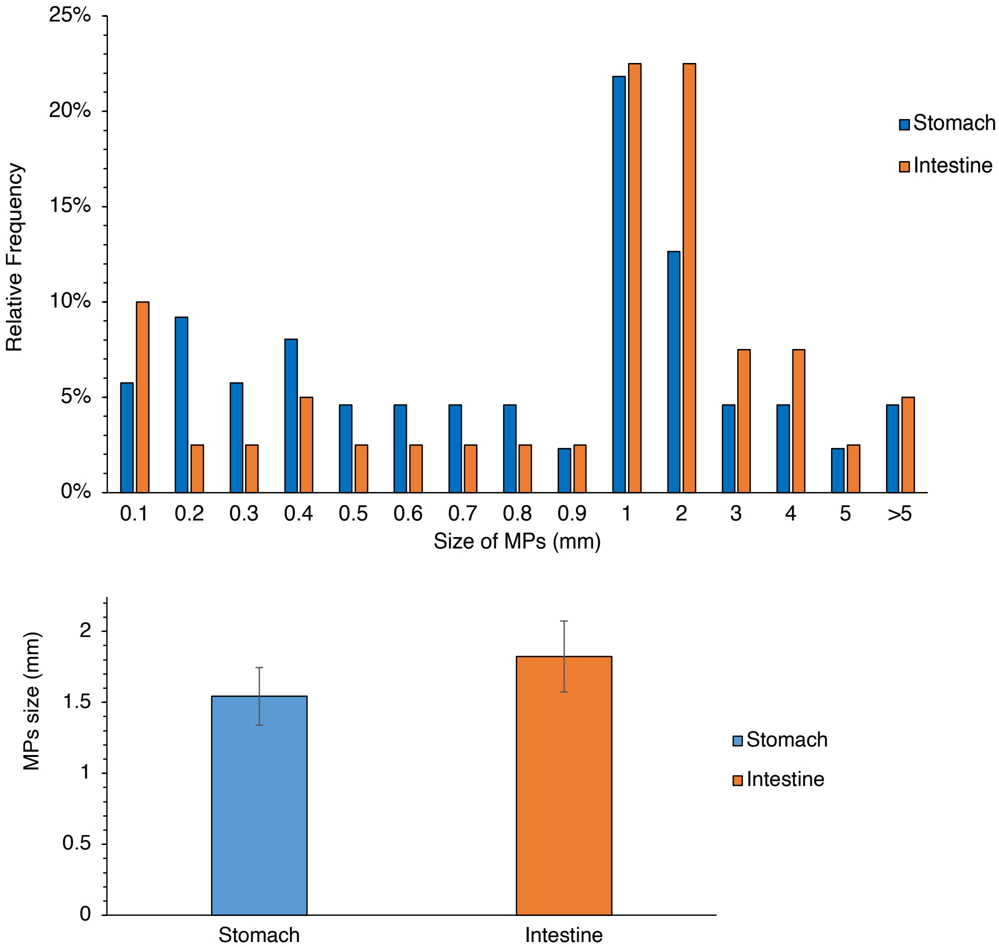

Figure 4. Histogram showing the size (mm) frequency distribution of MPs isolated from stomachs and intestines of *P. elephas* (upper graph) and histogram showing the average size (mm) of MPs in *P. elephas*.

With respect to the shape, plastic fibers represented the dominant category (62% of the total particles), followed by films (27%), foams and spheres (each 4%) and fragments and pellets cumulatively accounting for the remaining 3% (Fig. 5). Considering stomach and intestine separately, while the general pattern was similar in both compartments, films were more abundant in the stomach, where the relative abundance raised to 35%.

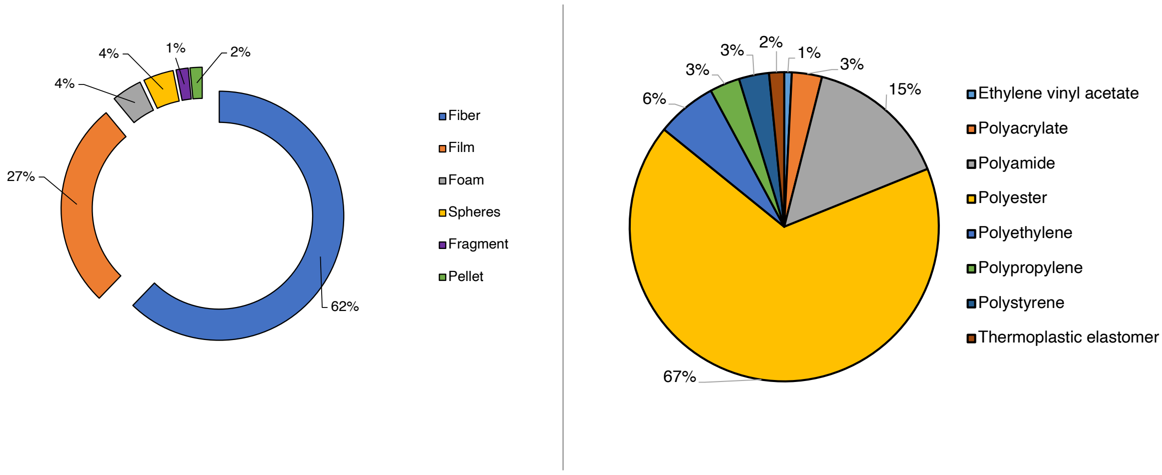

Figure 5. Relative abundance (%) in shape and polymeric composition of MPs retrieved in the gastrointestinal tract of *P. elephas*.

The µFT-IR analysis revealed the presence of 8 polymers, within which the dominant was polyester (PES, 67%), polyamide (PA, 15%), polyethylene (PE, 6%), followed by polypropylene (PP, 3%), polystyrene (PS, 3%) and other polymers (ethylene vinyl acetate, polyacrylate, thermoplastic elastomer) cumulatively accounting for the remaining 10% (Fig. 5). Polymeric composition of particles isolated from stomach and intestine did not show any significant difference. The analysis of particles' colours showed a wide heterogeneity, with transparent MPs being the dominant category (29% of the total), followed by blue (23%), red (14%) and black (10%) while remaining colours (green, yellow, purple, brown and others) accounted for the remaining 24% (Supplementary Fig. 1).

The chemical µFT-IR characterization confirmed as MPs a total of 48 particles isolated cumulatively from both stomach and intestine of *N. norvegicus*. Overall, MPs were detected in all individuals (100% occurrence; Fig. 6), corresponding to a frequency of ~87% in stomachs and 80% in intestines. The average number of particles was on average 3.2 ± 0.45 MPs individual^{-1}, ranging from 1 up to a maximum of 6 MPs individual^{-1} (Fig. 6), without significant differences between stomachs and intestines, showing 2 ± 0.26 and 1.83 ± 0.24 MPs from stomachs and intestines, respectively (Fig. 6).

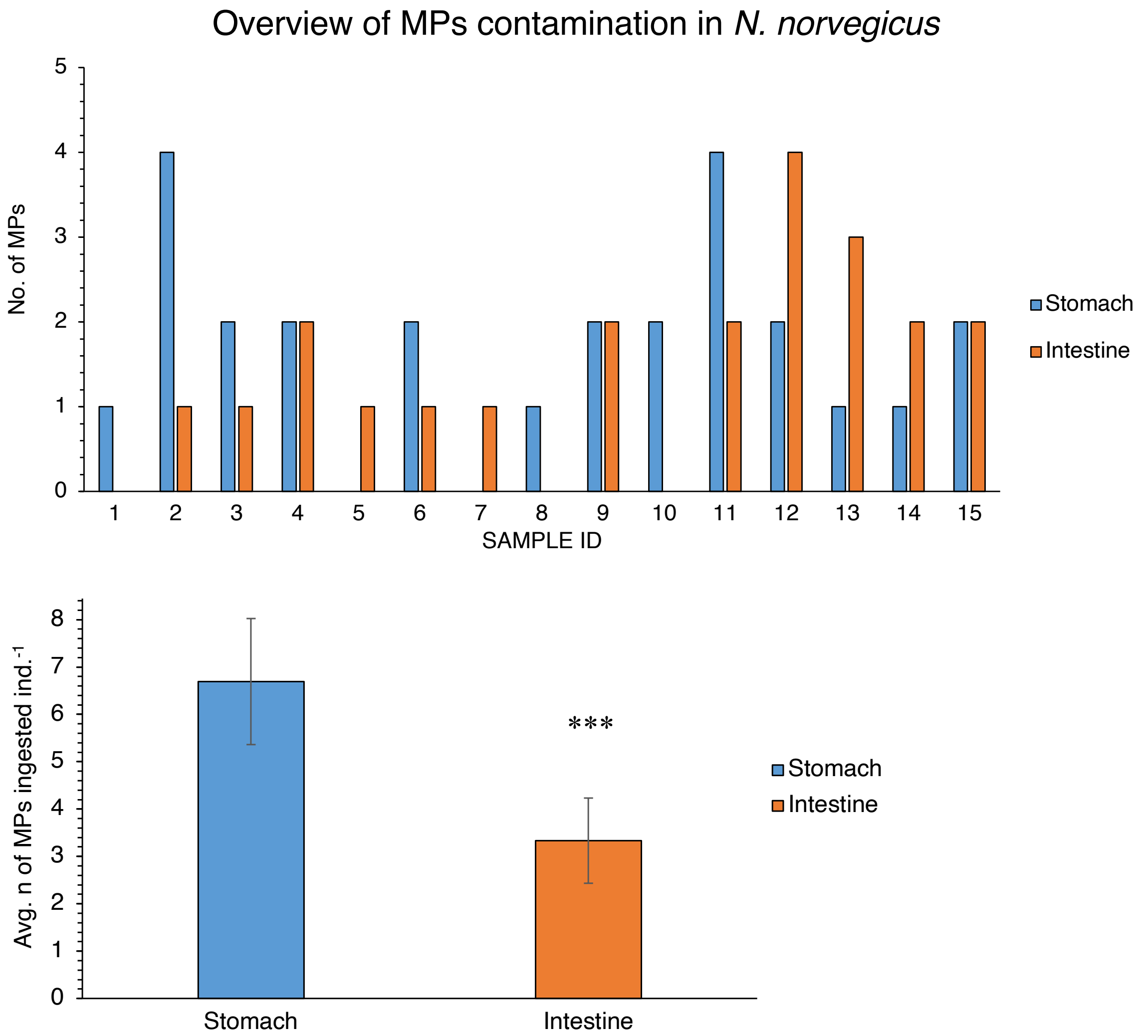

Figure 6. Histogram showing the number of MPs isolated from stomach and intestine in each sample of *N. norvegicus* (upper graph) and histogram showing the average number of MPs (± st. err) isolated from stomachs and intestines of *N. norvegicus* (lower graph).

The size classes of MPs ranged from a minimum of 0.10 to a maximum of 1.20 mm (Fig. 7), with an average size of 0.44 ± 0.3 mm when considering isolated particles cumulatively. More in detail, 91% of the particles isolated from the intestine were smaller than 0.5 mm, while the same range of size comprised only 46% of MPs isolated from the stomach (Fig. 7). Stomach and intestine showed a significant difference in particles size (Mann-Whitney test, $p<0.001$), with those isolated from the intestine being significantly smaller (0.28 ± 0.03 mm) than in the stomach (0.58 ± 0.05 mm; Fig. 7).

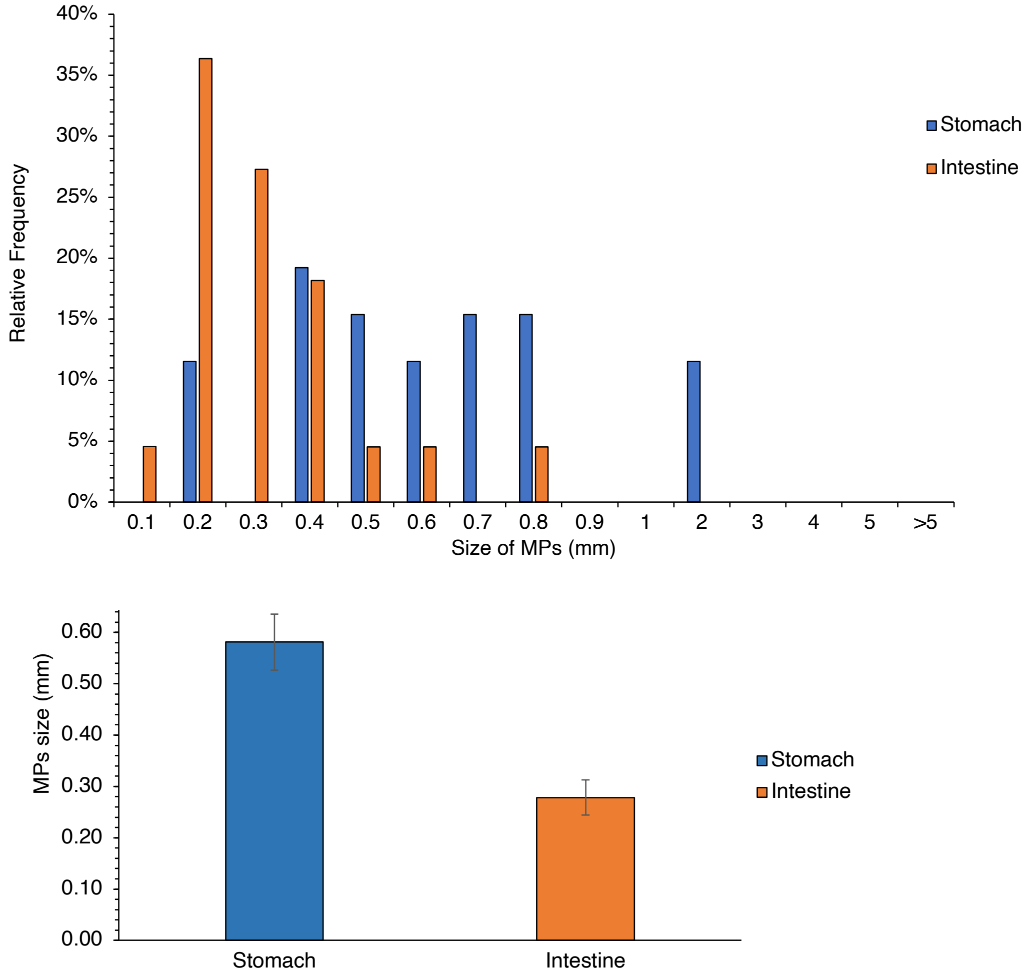

Figure 7. Histogram showing the size (mm) frequency distribution of MPs isolated from stomachs and intestines of *N. norvegicus* (upper graph). Histogram showing the average size (mm) of MPs particles extracted from stomachs and intestines of *N. norvegicus* (lower graph).

The pattern of shapes showed the dominance of fragments (56%), followed by fibers (36%) and films (8%) (Fig. 8), with no significant difference among stomachs and intestines. Overall, 6 typologies of polymers were identified (Fig. 8): PE and PES were the most represented (24% and 39% respectively), followed by PP (12%), PS (9%), PA (5%), while PU, acrylic polymers, Ethylene-vinyl acetate (EVA), silicon and copolymers cumulatively accounted for approximately 9% of total polymers. There was no significant difference in polymeric composition between stomach and intestine. The most dominant color was transparent (59% of total particles), followed by black (23%) and white (6%); green, yellow, and red particles cumulatively accounted for the remaining 6% (Supplementary Fig. 2).

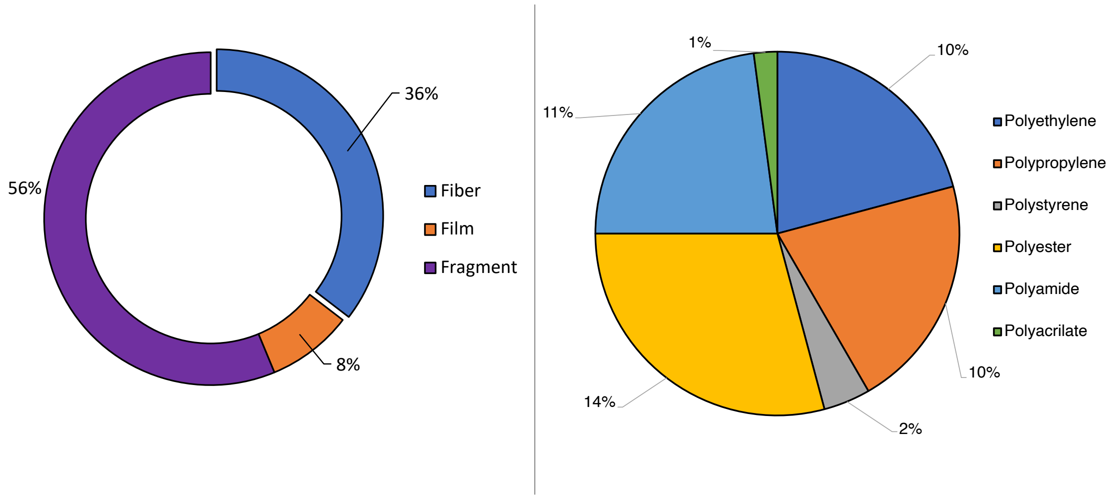

Figure 8. Relative abundance (%) in shape and polymeric composition of MPs retrieved in the gastrointestinal tract of *N. norvegicus*.

The comparison of the two species confirmed a significantly higher number of MPs in *P. elephas*, compared to *N. norvegicus* (M-W, $p<0.001$), and different polymeric composition (PERMANOVA, $p<0.001$, Table 2).

The ingestion of MPs by marine organisms and patterns of potential transfer through trophic webs are increasingly being documented (Carbery et al., 2018). Due to MPs ubiquity, from sea surface to the bottom, a consistent increase of scientific literature is highlighting contaminated organisms that could be potentially adopted as surrogate descriptors of MPs contamination: sharks, jellyfish, crustaceans, mammals and fishes (Alomar and Deudero, 2017; Bray et al., 2019; Carreras-Colom et al., 2022a; Compa et al., 2019; Fossi et al., 2018; Macali et al., 2018; Sbrana et al., 2022). Similarly, to fishermen using suitable gear to target various species according to their peculiar features, different bioindicators are representative of specific compartments of the marine environment, according to their biology and ecology. In our case, we focused the attention on *P. elephas* and *N. norvegicus*, typical inhabitants of Mediterranean benthic environments across a very wide bathymetric range, from a few meters to ca. 200 m depth in case of *P. elephas* and from ca. 200 m up to 800 m depth for *N. norvegicus*. While showing different movement patterns, with langoustines being more static compared to spiny lobsters (Follesa et al., 2015; Mulas et al., 2022; Sbrana et al., 2019), the two species share the same scavenging behaviour, which has been highlighted as the trophic strategy that most likely expose benthic organisms to the accidental ingestion of MPs (Andrades et al., 2019). Our results confirmed these species as highly exposed to MPs ingestion, with an occurrence of particles in 100% of analysed specimens. Nonetheless, the number of MPs observed in *P. elephas* was much lower, up to one order of magnitude, compared to those reported in the only available study that documented ca. 250 MPs ind^{-1} in samples of this charismatic species from NW Aegean sea (Kampouris et al., 2023). The two studies showed similar polymeric composition, with different abundance of PA and PVC as principal difference, that could be likely representative different level of contamination of investigated areas and sites. Scavenging crustaceans are known for being representative of local contamination and the different polymeric composition of isolated particles compared to Aegean samples, might suggest that different quantities and qualities of polymers characterize Sardinian benthic habitats. With respect to the extraction protocol, both the present study and Kampouris et al. (2023) used a pre-digestion and density separation based approach, which has been used on several organisms, including decapod crustaceans (Avio et al., 2020; Cau et al., 2019a): the slight adaptations to the peculiar necessities of MPs extraction in *P. elephas* (e.g, a further density separation step for full stomachs with lot of detritus), would hardly justify such discrepancies.

The spiny lobster *P. elephas* laks a significant body of literature on MPs contamination, since less than 100 specimens have been processed so far in the whole Mediterranean, making difficult to establish if MPs contamination of this species in the Mediterranean area can be as heterogeneous as per other crustaceans such as *A. antennatus*, *A. foliacea* or *N. norvegicus*, with very different levels of MPs ingestion according to the geographic areas and sites (Carreras-Colom et al., 2022a, 2018; D'Iglio et al., 2022; Hara et al., 2020; Joyce et al., 2022a).

As previously observed in the Greek study (Kampouris et al., 2023), our results confirmed that the number of MPs retrieved in *P. elephas* is not influenced by how empty or full are the stomach or intestine, weight or dimensions of individuals, nor the total weight of the specimen. Interestingly, we also observed large pieces piece of fishing nets (i.e., up to 6 cm) in the stomach of a specimen collected by means of trammel nets. That specific individual (sample id=2; Fig. 3) was the one showing the highest n. of particles ind^{-1} (n=25), with red particles of polyamide being dominants (likely fragmented from the ingested net), supporting the intuition that fishing gears can easily become a source of plastic particles ingestion (Fig. 9).

Figure 9. Piece of trammel net in the stomach content of *P.elephas*

Contrarily to spiny lobster, scientific literature has documented *N. norvegicus* contamination across different areas and bathymetries, both in the Mediterranean (Avio et al., 2020; Carreras-Colom et al., 2022a; Cau et al., 2019a; Martinelli et al., 2021) and in the Atlantic (Hara et al., 2020; Joyce et al., 2022a; Murray and Cowie, 2011). Available literature provided evidence of the wide variety of MPs abundance (n. part ind^{-1}) in langoustines and results here presented are within the range documented for the Mediterranean, which is higher than that observed in Atlantic samples.

The two crustacean species of this study have similar feeding strategies but different trophic behaviour since *N. norvegicus* feeds within a small bottom area around its burrows (Sbrana et al., 2019), whereas *P. elephas* is more mobile and capable of moving for long distance, thus having a larger scale of representativeness of MPs contamination. With respect to the polymeric composition, the majority of MPs extracted from both *N. norvegicus* and *P. elephas* were composed by PE, PES and PP confirming previous observations that highlighted packaging materials and textile products as the major source of exposure for benthic organisms.

The peculiar gastrointestinal tract of *N. norvegicus* can act as a bottleneck for ingested MPs (Welden and Cowie, 2016b), with larger ones being retained and accumulated in the stomachs that are not designed for cutting flexible and resistant filamentous materials such as fibers (Carreras-Colom et al. 2022a): on the contrary, smaller particles can be easily egested. Recent evidence also documented that the action of the gastric mill of langoustine can be responsible for the fragmentation and re-distribution of smaller 'secondary' MPs in the environment, thus modulating and extending their environmental path (Cau et al., 2020). Since the gastric mill is a common feature of these species, we tested if also *P. elephas* could eventually modulate the environmental fate of MPs in benthic environments. Our results do not support this hypothesis for spiny lobster since particles were significantly larger than those found in *N. norvegicus* but did not show any significant difference among stomach and intestine. Results here presented are the first available on the extraction of MPs from the two parts of the digestive trait of *P. elephas* and, despite being based over a limited number of samples, suggest that biologically mediated fragmentation of MPs particles might not occur in *P. elephas*. On the contrary, the significant differences in particles size between stomach and intestine of *Nephrops norvegicus* corroborated the hypothesis described in Cau et al. (2020).

In conclusion, we confirm and further extend the awareness of the high exposure of these crustaceans to MPs, rendering spiny lobsters and langoustines either valuable bioindicators that belong to the most important stocks in the FAO Major Fishing Areas of European competence, but also species with socio-cultural relevance within Mediterranean and EU communities. Being regarded as *gourmet food* and being also amongst the most charismatic, flagship species for citizens, they could trigger and enhance environmental awareness and consciousness of the vastity of the impact derived from plastic contamination (Cau et al., 2019a; Kampouris et al., 2023).

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.

Data availability

Data will be made available on request.

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Table 1. Number of individuals, geographical coordinates and average depth of trawls conducted in the 3 sampling sites.

Site	Species	n. of individuals	Latitudine (N)	Longitude (E)	Depth (m)
1	*P. elephas*	9	40° 13’ 31”	8° 38’ 83”	76
2	*P. elephas*	5	40° 28’ 57”	8° 11’ 93”	105
3	*N. norvegicus*	8	40° 30' 41"	7° 54' 16"	592
4	*N. norvegicus*	7	40° 16' 08"	7° 49' 58"	402

Table 2. Output of the PERMANOVA routine, testing for differences in the polymeric composition of the particles retrieved from the specimens of *N. norvegicus* and *P. elephas*.

POLYMERIC COMPOSITION				
P. elephas vs. N. norvegicus				
Source	**df**	**MS**	**Pseudo-F**	**P(MC)**
Species	1	79.78	8.82	**0.001**
Residual	50	9.042		
Total	51			

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