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

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1 Baseline

2 3	Comparative microplastic load in two decapod crustaceans Palinurus
4 5	elephas and Nephrops norvegicus
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42 Abstract

43 44 The present work compares microplastics (MPs) contamination in two charismatic crustaceans: European 45 spiny lobster *Palinurus elephas* and langoustine *Nephrops norvegicus*. Samples (*P. elephas* n=14; *N. norvegicus* n=15) 46 were collected between 76 and 592 m depth, from four sites in west Sardinia, Italy. An extraction protocol 47 was applied on stomachs and intestines, separately, and over 500 particles were further characterized 48 through µFT-IR. We document 100% occurrence in specimens from both species, with P. elephas being 49 significantly more contaminated (9.1 \pm 1.75 vs. 1.66 \pm 0.1 MPs individual⁻¹), ingesting larger microplastics 50 with different polymeric composition. The scavenging-based feeding strategy of both species could explain 51 such exposure to MPs, mostly derived by single-use plastic. The overall results highlight that both species 52 are clearly affected by plastic pollution, being valuable bioindicators and charismatic species that could thus 53 represent excellent flagship species for raising awareness toward the global issue of plastic in the marine 54 environment. 55 56 57

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61 Since 1950s, plastic production generated ca. 5 billion tons of waste, currently dispersed in the 62 environment (Geyer et al., 2017). It has been estimated that 5-8 Mt of plastic move from land to oceans on 63 a vearly basis (Jambeck et al., 2015), with trillions of plastic items currently floating at sea (Eriksen et al., 64 2014). However, the abundance of floating litter on oceans' surface was measured to be lower than what 65 has been forecasted by the most conservative models (Cozar et al., 2014; Eriksen et al., 2014). While recent 66 studies proposed that riverine input might have been overestimated (Weiss et al., 2021), it is widely 67 recognized that the sink of objects represent the major explanation for such discrepancy(Gutow et al., 2018; 68 Ryan, 2015). The existence of an initial floating stage, followed by sink and deposition on the seafloor after 69 a more or less long period of time, renders plastic items capable of reaching also secluded environments 70 such as polar regions and the deep oceans' floor (Bergmann et al., 2017; Jambeck et al., 2015; Peeken et al., 71 2018; Peng et al., 2020). The most emblematic sign of this is a plastic bag documented at ca. 10,900 m depth 72 in the Mariana Trench (Chiba et al., 2018).

73 Processes like biofouling or physical weathering can change specific weight of plastic (Kowalski et 74 al., 2016; Zettler et al., 2013), triggering their sink into the water column. Plastic and microplastics 75 contribute to the so called vertical "pump" and they increase the transfer of organic carbon, organisms and 76 other elements to the ocean depths and, in this respect, ocean floors represent the final sink for plastic 77 particles (Galgani and Loiselle, 2021; Woodall et al., 2014), as demonstrated by the exponential increase in 78 deposition occurred over the last decades (Brandon et al., 2019). Plastic can also slowly degrade through 79 biological, mechanical and physical processes that cause its fragmentation into smaller particles that are 80 called microplastics (MPs), if their dimension is comprised between 1µm and 5mm (Frias and Nash, 2019).

81 Such dimensional range renders these particles particularly suitable for accidental ingestion by 82 marine *biota*, with vagile benthic fauna being particularly exposed compared to other organisms (Bour et 83 al., 2018; Carreras-Colom et al., 2018; Cau et al., 2019a; Murray and Cowie, 2011). The size of MPs particles 84 influences their ingestion and egestion rates and their isolation from digestive tracts of marine organisms, 85 by itself, does not represent a reliable proxy for particle retention (Cau et al., 2020), nor for their 86 accumulation but rather a snapshot of the exposure that organisms experience in the specific environment. 87 Within the European Union, the Marine Strategy Framework Directive evaluates the 88 environmental status of European seas (MSFD; 2008/56/EC) through 11 descriptors, within which marine

89 litter quantification (and microplastics therein) is one of those (Descriptor 10) (Galgani et al., 2013b, 2013a); 90 thus, the necessity and the research for efficient bioindicators is building up constantly across scientific 91 literature (Bonanno and Orlando-Bonaca, 2018; Fossi et al., 2018). This is particularly relevant for the 92 Mediterranean, which is among the most contaminated (or at least most investigated) basins worldwide 93 (Canals et al., 2021). The EU Mission on Restore Our Ocean and Water by 2030 has among its objectives 94 to prevent and eliminate pollution from the Sea, and Mediterranean Basin has been identified for setting a 95 Lighthouse of actions toward plastic pollution. The Mediterranean Sea is estimated to retain 5-10% of the 96 global plastic mass dispersed in oceans (Suaria et al., 2016; Van Sebille et al., 2015), and the resident 97 associated biota showed to diffusely ingest MPs, both in the pelagic and benthic dominium (Cau et al., 2019a). 98 Recent scientific literature emphasized how some decapod crustaceans that show a tight association 99 with the seabed are particularly exposed to MPs: this is the case of Norwegian langoustine Nephrops norvegicus 100 (L. 1758) and European spiny lobster Palinurus elephas (F. 1787). While the former is widely acknowledged 101 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).

105 Crustaceans belonging to the family Palinuridae are among the most highly priced seafood in the 106 world, and their fishery often represent the backbone of export economy in some regions (e.g., Caribbean 107 countries; Higgs et al. 2016). European spiny lobster P. elephas is distributed across the Mediterranean Sea, 108 but also across the eastern part of the Atlantic Ocean, from North Africa to Scotland. Its fishery was first 109 recorded in the 15th century BC, and the popularity of spiny lobsters as gournet food took off in the 19th 110 century and consistently increased till present days, when living specimens of *P. elephas* can be sold at retail 111 prices comprised between 40 and 120€ Kg⁻¹ (Cau et al., 2019b; Groeneveld et al., 2013). With these 112 premises, it is not surprising that European spiny lobster is currently classified as "Vulnerable", by the 113 International Union for Conservation of Nature (IUCN), mostly due to its continuous overfishing (Follesa 114 et al., 2014; Goñi and Latrouite, 2005).

115 *N. norvegicus* (fam. *Nephropidae*) is a benthic decapod inhabiting European temperate and cold waters.
116 Similarly to European spiny lobster, langoustine is a millions of Euros worth fishery resource in Europe,

117 since it is highly appreciated as gourmet seafood either, with a retail price comparable to that of other 118 crustaceans such as lobsters, spiny lobsters or deep sea shrimps (Cau et al., 2019b; Ungfors et al., 2013). 119 Langoustine is a key element in muddy bottoms trophic webs, and it shows a wide bathymetric distribution 120 (up to 800m depth), mostly restricted to deep waters in the Mediterranean area (>200 m depth). The 121 continuous scavenging behaviour on the seabed allows langoustines to interact with other benthic species, 122 but also with sediment-water fluxes and resuspended sediment (Cristo et al., 1998). Because of this, N. 123 norvegicus has been identified as potentially MPs exposed species and documented as reliable bioindicator of 124 MPs contamination of the deep seabed (Carreras-Colom et al., 2022a, 2022b; Cau et al., 2019a; Franceschini 125 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).

133 The present study aims to investigate and compare MPs ingestion in the European spiny lobster P. 134 elephas and the Norwegian lobster Nephrops norvegicus sampled from coastal and deeper habitats from 135 Sardinian waters, in Italy. The analysis of particles in stomachs and intestines was expected to provide 136 additional insights on the role of such benthic crustaceans in modulating the environmental fate and 137 bioavailability of MPs through the ingestion, mechanical fragmentation and egestion process, as recently 138 documented in controlled and wild conditions (Cau et al., 2020; Dawson et al., 2018). These species are 139 commonly and extensively fished for human consumption and, while exhibiting similar feeding strategies 140 in two segregated bathymetric distribution range, were expected to highlight novel insights as to whether 141 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

145 collected from 2 sites from the western coast of Sardinia between 2019 and 2020 (Fig. 1; Table 1), from 146 both artisanal and professional fisheries operating using trammel nets and trawlers, at depths comprised 147 between 76 and 105 m. A total of 14 stomachs and intestines were extracted. Ranges of biometric data (and 148 sex ratio) of analysed specimens were Carapace Length (CL) 25.2 – 41.2 mm for *N. norvegicus* (9 males and 149 6 females), and CL 73 –117.9 mm for *P. elephas* (6 males and 8 females). For *P. elephas* stomach and intestine 150 weight were recorded, separately, and individual weight, which ranged from 350gr to 2.2 Kg.







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., 162 2020). The resulting supernatant was filtered using a vacuum pump (VCP130) through 47 mm Sartorius® 163 cellulose nitrate membrane filters (pore size 8µm). The MPs extraction procedure was based on applying 164 the separation procedure on the digestate through a NaCl hypersaline solution (density 1.2 g cm⁻³), where 165 supernatant solution was collected through glass beaker. For P. elephas, since stomachs were full of sand 166 and food material, the obtained solution was again subjected to a second density separation step through a 167 NaCl hypersaline solution, later followed by filtration, partial digestion in diluted hydrogen peroxide (15%), 168 sorting and chemical characterization. The method has been validated and standardized on samples spiked 169 with MPs of different types and sizes (Avio et al., 2015) and used for MPs extraction in the same species 170 targeted by the present study (Avio et al., 2020; Cau et al., 2020, 2019a; Martinelli et al., 2021). When 171 compared with other available methodologies, it showed a recovery yield higher than 90% for particles 172 smaller than 100 µm and 95% for greater ones, with no effects on particle characteristics such as shape or 173 colour. During sorting, all retrieved particles were observed under a stereomicroscope, photographed and 174 categorized according to shape in: i) fragments (small, irregular shaped particles, crystals, rigid, thick); ii) 175 film (irregular shapes, thin and flexible, transparent particles); iii) pellet (cylindrical particles); iv) fiber 176 (elongated, thin, straight particles, frayed ends); v) sphere- like (cubical, sphere); vi) foam (lightweight 177 particles with spongy texture). Once isolated, photographed MPs were measured at their largest cross 178 section under a stereomicroscope using the image analysis CPCe, 'measuring' function (Kohler and Gill, 179 2006). All extracted particles were characterized using a µFT-IR microscope (Spotlight 200i microscope 180 system coupled with Spectrum Two spectrometer, Perkin Elmer). The measurements were made using the 181 µATR mode. Following back-ground scans, 32 scans were performed for each particle, with a resolution 182 of 4 cm⁻¹. Spectrum 10 software was used for the output spectra and the identification of polymers was 183 performed by comparison with libraries of standard spectra. Polymers matching for more than 70% with 184 the reference spectra were validated, while polymers with a match comprised between 60% and 70% 185 underwent into a more critical interpretation of the spectra (Bour et al., 2018). To reduce background 186 contamination, operators were wearing acid green cotton lab coat to identify possible fibers coming from 187 it and special attention was paid to limit the wearing of synthetic clothes. Before starting the extractions, 188 and between each process step, benches were cleaned with milli-Q water and all solutions used were pre-189 filtered through a nitrate acetate membrane with pore size of 0.45µm. Glass and metal materials were used

190 whenever possible, rinsed thrice with prefiltered milli-Q water before use and wrapped in aluminium foil 191 when not in use. After rinsing, all containers were covered with aluminium foils, which were also kept 192 during digestion, stirring, decantation and filtration steps. After filtration, membranes were kept in glass 193 petri dishes, previously rinsed with prefiltered milli-Q water. NaCl solution was prepared in distilled water 194 and further filtered (0.45µm pore size). Contamination controls were also included (one control for each 195 batch of samples was treated in parallel to samples), consisting of 10 mL of prefiltered distilled water that 196 undertook all the steps of the protocol. Despite such precautions, it was not possible to fully avoid airborne 197 contamination and some textile fibers were found in the control membranes. We then applied total 198 subtraction of items as correction method, based on a spectral similarity and visual characteristics (Kroon 199 et al., 2018). In brief, fibers were checked with the actual samples and compared, both visually and 200 spectrally. Potential extraneous particles were used to build a visual and spectral contaminant library, against 201 which all sample items were confronted and when particle matched a contaminant or control library item 202 with > 80 % spectral similarity and visual similarity (i.e., same colour, shape, texture), were removed from 203 the dataset. This correction method provides a count of total sample particles minus items confirmed to be 204 contaminant particles.

205 PERMutational ANalysis Of VAriance ('PERMANOVA'; Anderson et al. 2008) based on Euclidean 206 distance resemblance matrixes (untransformed data) was used to test for significant differences in MPs 207 polymeric composition between the two investigated species. The factor 'Species' (2 levels, fixed) was used 208 as unique source of variation. The n. of particles ind-1 of each polymer type was used as response variable. 209 Differences in the number and size of particles between the two species were tested using the Mann-210 Whitney test. Moreover, within each species, using the same statistical routine, we tested for different 211 contamination descriptors (both in terms of number of particles and size) between stomach and intestine. 212 The PERMANOVA was used to test for differences in the polymeric composition between the two 213 compartments, in this case using the different compartment (i.e., stomach or intestine: 2 levels, fixed) as 214 unique source of variation. Due to the limited number of samples, it was not possible to test for 215 geographical differences within the sub-region object of the study, nor for bathymetric trends.

216 More than 2,000 particles were extracted from the 2 species (>1,300 for *P. elephas* and >700 for *N.* 217 *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.

227 The average number of MPs was 9.1 ± 1.75 MPs ind. ⁻¹, ranging from 3 to a maximum of 25 MPs

228 (Fig. 3), with no significant correlation to the weight of stomachs or intestines and with sex or dimensions

229 of organisms (in terms of weight and/or LC).



Overview of MPs contamination in P.elephas



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.

234 More in detail, considering only positive individuals, the average number of particles was 6.7 ± 1.3 235 in stomachs and 3.4 ± 0.9 in intestines (Fig. 3). Among the 14 spiny lobsters, 11 individuals (~79%) showed 236 a cumulative number of MPs in their stomach and intestine combined >5. There was no significant 237 difference in the size of particles isolated from stomachs and intestines of *P. elephas*, which had overall an 238 average size of 1.63 \pm 0.22 mm, with those isolated from intestine (avg. 1.82 \pm 0.22 mm) being slightly 239 bigger than those found in stomachs (avg. 1.54 ± 0.20 mm; Fig 4). The smallest particles isolated from 240 stomach and intestine were 41 µm and 64 µm respectively, while the largest were 9.7 mm and 6.39 mm, 241 both of which outsize the definition of MPs, but rather falling in the class of meso-plastics (>5mm). Out 242 of the total of plastic particles extracted from the 14 individuals, 6 particles were larger than 5mm, 4 in 243 stomachs and 2 in intestines. Overall, the size-frequency distribution (Fig. 4) showed that approximately 244 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).

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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*.

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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*.

261 The µFT-IR analysis revealed the presence of 8 polymers, within which the dominant was polyester 262 (PES, 67%), polyamide (PA, 15%), polyethylene (PE, 6%), followed by polypropylene (PP, 3%), 263 polystyrene (PS, 3%) and other polymers (ethylene vinyl acetate, polyacrylate, thermoplastic elastomer) 264 cumulatively accounting for the remaining 10% (Fig. 5). Polymeric composition of particles isolated from 265 stomach and intestine did not show any significant difference. The analysis of particles' colours showed a 266 wide heterogeneity, with transparent MPs being the dominant category (29% of the total), followed by blue 267 (23%), red (14%) and black (10%) while remaining colours (green, yellow, purple, brown and others) 268 accounted for the remaining 24% (Supplementary Fig. 1).

The chemical μ FT-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).



Overview of MPs contamination in N. norvegicus

- 281 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
- 283 only 46% of MPs isolated from the stomach (Fig. 7). Stomach and intestine showed a significant
- 284 difference in particles size (Mann-Whitney test, p<0.001), with those isolated from the intestine being
- significantly smaller (0.28 \pm 0.03 mm) than in the stomach (0.58 \pm 0.05 mm; Fig. 7).
- 286

²⁷⁶

²⁷⁷Figure 6. Histogram showing the number of MPs isolated from stomach and intestine in each sample of N.278norvegicus (upper graph) and histogram showing the average number of MPs (± st. err) isolated from stomachs and279intestines of N. norvegicus (lower graph).

²⁸⁰ The size classes of MPs ranged from a minimum of 0.10 to a maximum of 1.20 mm (Fig. 7), with an



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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).

292 The pattern of shapes showed the dominance of fragments (56%), followed by fibers (36%) and films

293 (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
- 297 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
- 299 particles cumulatively accounted for the remaining 6% (Supplementary Fig. 2).
- 300



301

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

305 The comparison of the two species confirmed a significantly higher number of MPs in *P. elephas*, 306 compared to *N. norvegicus* (M-W, p<0.001), and different polymeric composition (PERMANOVA, 307 p<0.001, Table 2).

308 The ingestion of MPs by marine organisms and patterns of potential transfer through 309 trophic webs are increasingly being documented (Carbery et al., 2018). Due to MPs ubiquity, from sea 310 surface to the bottom, a consistent increase of scientific literature is highlighting contaminated organisms 311 that could be potentially adopted as surrogate descriptors of MPs contamination: sharks, jellyfish, 312 crustaceans, mammals and fishes (Alomar and Deudero, 2017; Bray et al., 2019; Carreras-Colom et al., 313 2022a; Compa et al., 2019; Fossi et al., 2018; Macali et al., 2018; Sbrana et al., 2022). Similarly, to fishermen 314 using suitable gear to target various species according to their peculiar features, different bioindicators are 315 representative of specific compartments of the marine environment, according to their biology and ecology. 316 In our case, we focused the attention on *P. elephas* and *N. norvegicus*, typical inhabitants of Mediterranean 317 benthic environments across a very wide bathymetric range, from a few meters to ca. 200 m depth in case 318 of P. elephas and from ca. 200 m up to 800 m depth for N. norvegicus. While showing different movement 319 patterns, with langoustines being more static compared to spiny lobsters (Follesa et al., 2015; Mulas et al., 320 2022; Sbrana et al., 2019), the two species share the same scavenging behaviour, which has been highlighted 321 as the trophic strategy that most likely expose benthic organisms to the accidental ingestion of MPs 322 (Andrades et al., 2019). Our results confirmed these species as highly exposed to MPs ingestion, with an

323 occurrence of particles in 100% of analysed specimens. Nonetheless, the number of MPs observed in P. 324 elephas was much lower, up to one order of magnitude, compared to those reported in the only available 325 study that documented ca. 250 MPs ind-1 in samples of this charismatic species from NW Aegean sea 326 (Kampouris et al., 2023). The two studies showed similar polymeric composition, with different abundance 327 of PA and PVC as principal difference, that could be likely representative different level of contamination 328 of investigated areas and sites. Scavenging crustaceans are known for being representative of local 329 contamination and the different polymeric composition of isolated particles compared to Aegean samples, 330 might suggest that different quantities and qualities of polymers characterize Sardinian benthic habitats. 331 With respect to the extraction protocol, both the present study and Kampouris et al. (2023) used a pre-332 digestion and density separation based approach, which has been used on several organisms, including 333 decapod crustaceans (Avio et al., 2020; Cau et al., 2019a): the slight adaptations to the peculiar necessities 334 of MPs extraction in *P. elephas* (e.g., a further density separation step for full stomachs with lot of detritus), 335 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).



- 351 Figure 9. Piece of trammel net in the stomach content of *P.elephas*
- 352

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 364 observations that highlighted packaging materials and textile products as the major source of exposure for365 benthic organisms.

366 The peculiar gastrointestinal tract of N. norvegicus can act as a bottleneck for ingested MPs (Welden 367 and Cowie, 2016b), with larger ones being retained and accumulated in the stomachs that are not designed 368 for cutting flexible and resistant filamentous materials such as fibers (Carreras-Colom et al. 2022a): on the 369 contrary, smaller particles can be easily egested. Recent evidence also documented that the action of the 370 gastric mill of langoustine can be responsible for the fragmentation and re-distribution of smaller 371 'secondary' MPs in the environment, thus modulating and extending their environmental path (Cau et al., 372 2020). Since the gastric mill is a common feature of these species, we tested if also P. elephas could eventually 373 modulate the environmental fate of MPs in benthic environments. Our results do not support this 374 hypothesis for spiny lobster since particles were significantly larger than those found in N. norvegicus but did 375 not show any significant difference among stomach and intestine. Results here presented are the first 376 available on the extraction of MPs from the two parts of the digestive trait of P. elephas and, despite being 377 based over a limited number of samples, suggest that biologically mediated fragmentation of MPs particles 378 might not occur in *P. elephas*. On the contrary, the significant differences in particles size between stomach 379 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).

387

388 Declaration of competing interest

389 The authors declare that they have no known competing financial interests or personal relationships that390 could have appeared to influence the work reported in this paper.

391 Data availability

392 Data will be made available on request.

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- 396
- 397
- 398 Table 1. Number of individuals, geographical coordinates and average depth of trawls conducted in the 3 sampling
 399 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

401 Table 2. Output of the PERMANOVA routine, testing for differences in the polymeric composition of the402 particles retrieved from the specimens of *N. norregicus* and *P. elephas*.

POI YMERIC COMPOSITION			
P. elephas vs. N. norvegicus			
Source df MS Pseudo-F P(MC			
Species 1 79.78 8.82 0.00			
Residual 50 9.042			
Total 51			
eferences			

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