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

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42 **Abstract**

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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  $\mu$ FT-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<sup>-1</sup>), 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.

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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 yearly 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 Loisel, 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) (Galvani 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;  
102 Hara et al., 2020; Joyce et al., 2022a), the latter has only very recently been identified as exposed to MPs  
103 and nanoparticles in the Aegean sea, highlighting the urgent need to provide additional data over a broader  
104 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 *gourmet 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<sup>-1</sup> (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,

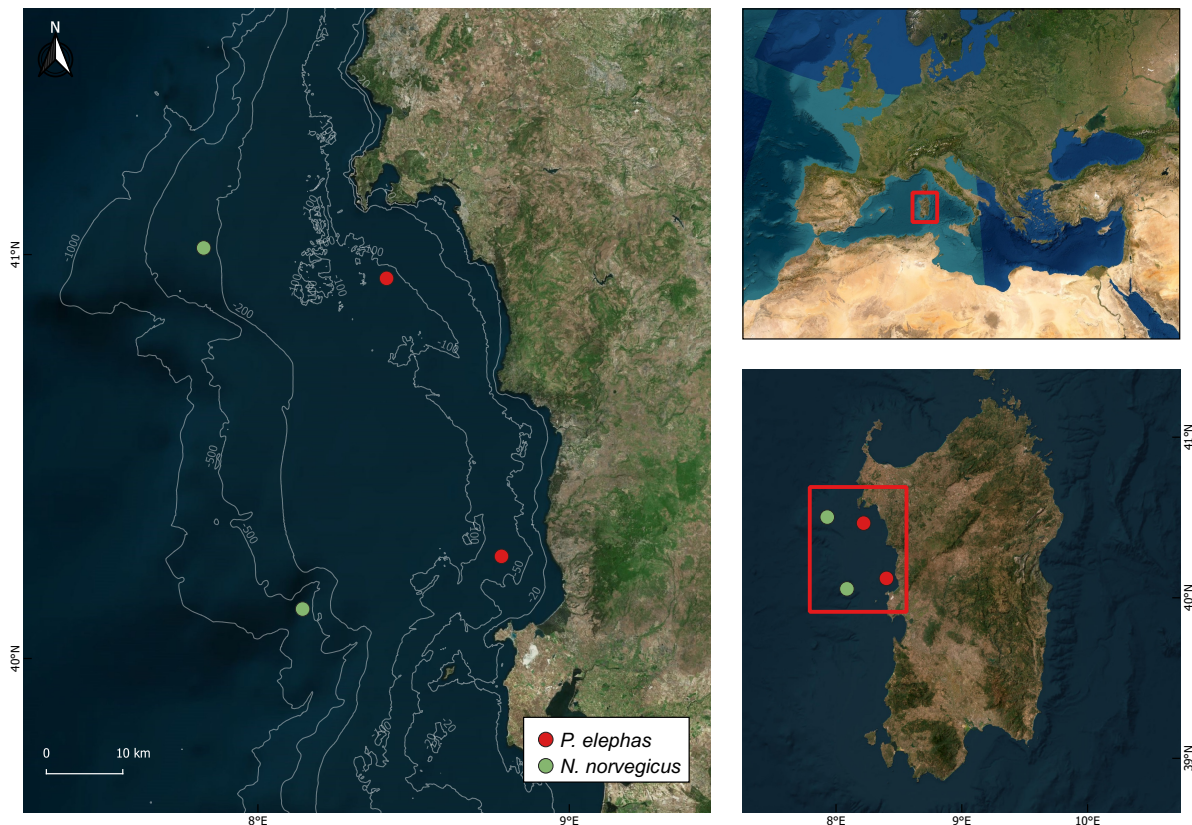
117 since it is highly appreciated as *gourmet* seafood either, with a retail price comparable to that of other  
118 crustaceans such as lobsters, spiny lobsters or deep sea shrimps (Cau et al., 2019b; Ungfors et al., 2013).  
119 Langoustine is a key element in muddy bottoms trophic webs, and it shows 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).

126 Also *P. elephas* is an omnivorous and scavenging species that, contrarily to *N. norvegicus*, dwells in  
127 shallow Mediterranean waters up to 200m depth (Goñi and Latrouite, 2005; Groeneveld et al., 2013). Both  
128 spiny lobster and langoustine are exposed to MPs mostly through their similar trophic habits and, with very  
129 few exceptions (Cau et al., 2020; Joyce et al., 2022b), all available information on particles occurrence in  
130 these species reflects their isolation from stomach contents or through the digestion of the entire digestive  
131 apparatus (Avio et al., 2020; Hara et al., 2020; Joyce et al., 2022a, 2022b; Murray and Cowie, 2011; Welden  
132 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.

142 For *N. norvegicus*, samples were collected from 2 sites around the Sardinia island in 2019 (Fig. 1), in the  
143 framework of the MEDiterranean International Trawl Survey (MEDITS), at depths comprised between  
144 402 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.  
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152  
153 **Figure 1.** Map of the study area where specimens of *N. norvegicus* (green dots) and *P. elephas* (red dots) have been  
154 collected. Bottom-left white bar represents a scale of 10Km.

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156 After collection, specimens were transported in the laboratory using an ice box and placed in cold storage  
157 (-20°) to avoid the risk of contamination from sampling activities. Samples were thawed at room  
158 temperature and each specimen was dissected to remove the stomach and intestine, which were then placed  
159 separately in aluminium foils and stored at -20°, until analysis. Necessary precautions were taken when  
160 handling and processing the samples to prevent aerial and solvent contamination with MPs. Digestion of  
161 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<sup>-3</sup>), 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<sup>-1</sup>. 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

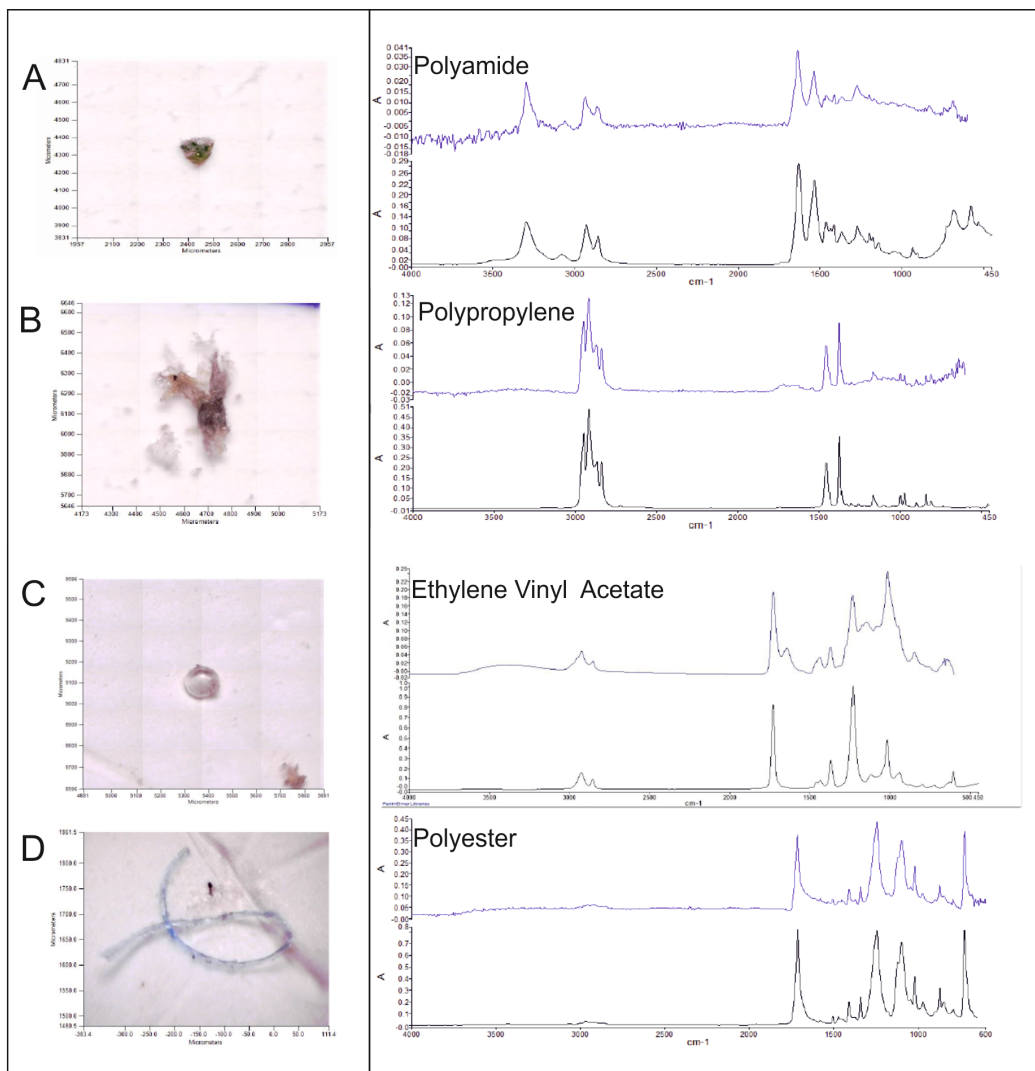
190 whenever possible, rinsed thrice with prefiltered milli-Q water before use and wrapped in aluminium foil  
191 when not in use. After rinsing, all containers were covered with aluminium foils, which were also kept  
192 during digestion, stirring, decantation and filtration steps. After filtration, 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 (0.45 $\mu$ m 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<sup>-1</sup> 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  $\mu$ FT-IR (Fig. 2). After data correction, out



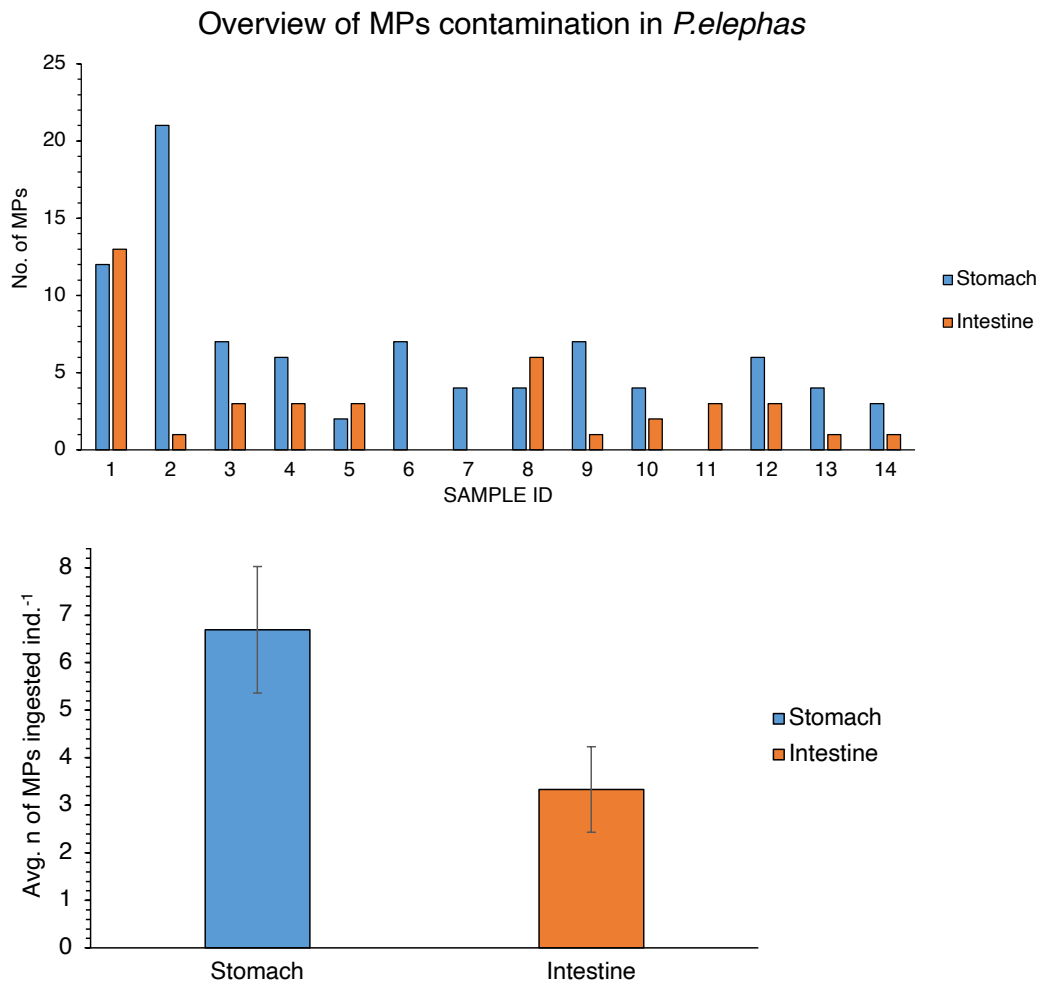
218 of the total number of particles isolated in *P. elephas*, 127 of them were made of plastic, 87 for stomachs  
 219 and 40 for intestines (Fig. 3). All the 14 specimens of *P. elephas* had MPs in their digestive tract (100% of  
 220 occurrence); more in detail, 13 stomachs and 12 intestines, out of 14. The weight of *P. elephas* stomachs  
 221 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  
 222 gr. In both cases, there was no significant correlation between higher weight of stomachs and MPs load.



223

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

227 The average number of MPs was  $9.1 \pm 1.75$  MPs ind.<sup>-1</sup>, 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).



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

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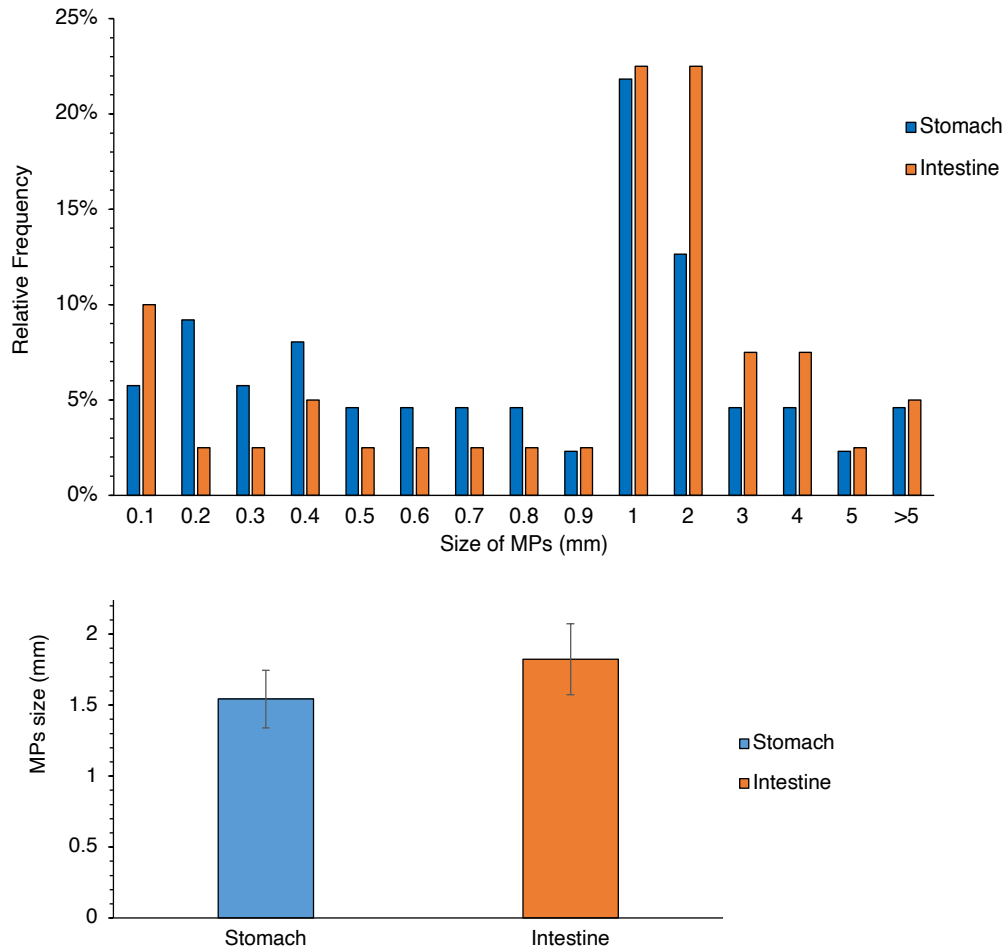
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More in detail, considering only positive individuals, the average number of particles was  $6.7 \pm 1.3$  in stomachs and  $3.4 \pm 0.9$  in intestines (Fig. 3). Among the 14 spiny lobsters, 11 individuals (~79%) showed a cumulative number of MPs 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  $1.63 \pm 0.22$  mm, with those isolated from intestine (avg.  $1.82 \pm 0.22$  mm) being slightly bigger than those found in stomachs (avg.  $1.54 \pm 0.20$  mm; Fig 4). The smallest particles isolated from stomach and intestine were  $41 \mu\text{m}$  and  $64 \mu\text{m}$  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 ( $>5\text{mm}$ ). Out of the total of plastic particles extracted from the 14 individuals, 6 particles were larger than  $5\text{mm}$ , 4 in stomachs and 2 in intestines. Overall, the size-frequency distribution (Fig. 4) showed that approximately

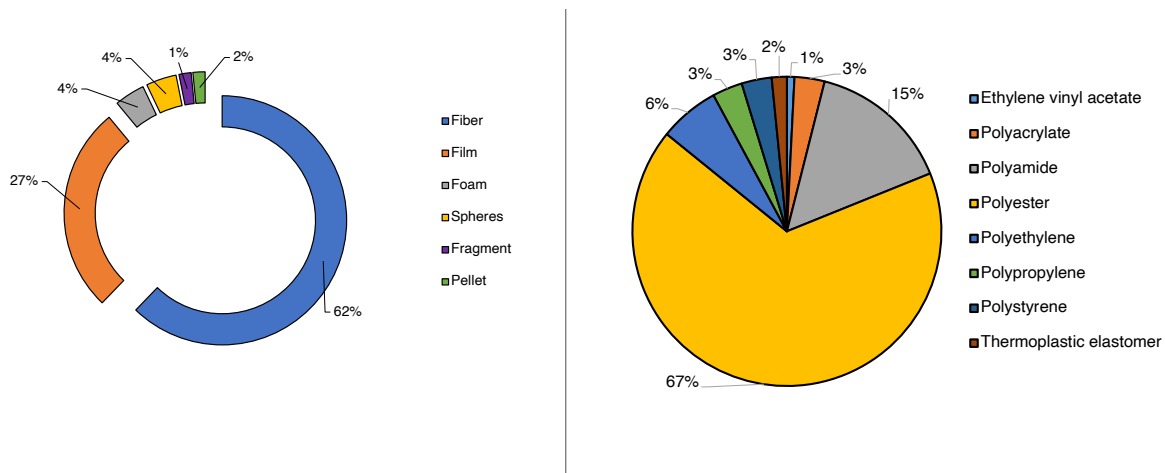
244 50% of the isolated plastic particles were smaller than 1mm, while the most representative size class was in  
 245 the range between 1 and 2 mm, accounting for >20% of the total, in both stomach and intestine (Fig. 4).  
 246



247  
 248 **Figure 4.** Histogram showing the size (mm) frequency distribution of MPs isolated from stomachs and intestines of  
 249 *P. elephas* (upper graph) and histogram showing the average size (mm) of MPs in *P. elephas*.

250  
 251 With respect to the shape, plastic fibers represented the dominant category (62% of the total  
 252 particles), followed by films (27%), foams and spheres (each 4%) and fragments and pellets cumulatively  
 253 accounting for the remaining 3% (Fig. 5). Considering stomach and intestine separately, while the general  
 254 pattern was similar in both compartments, films were more abundant in the stomach, where the relative  
 255 abundance raised to 35%.

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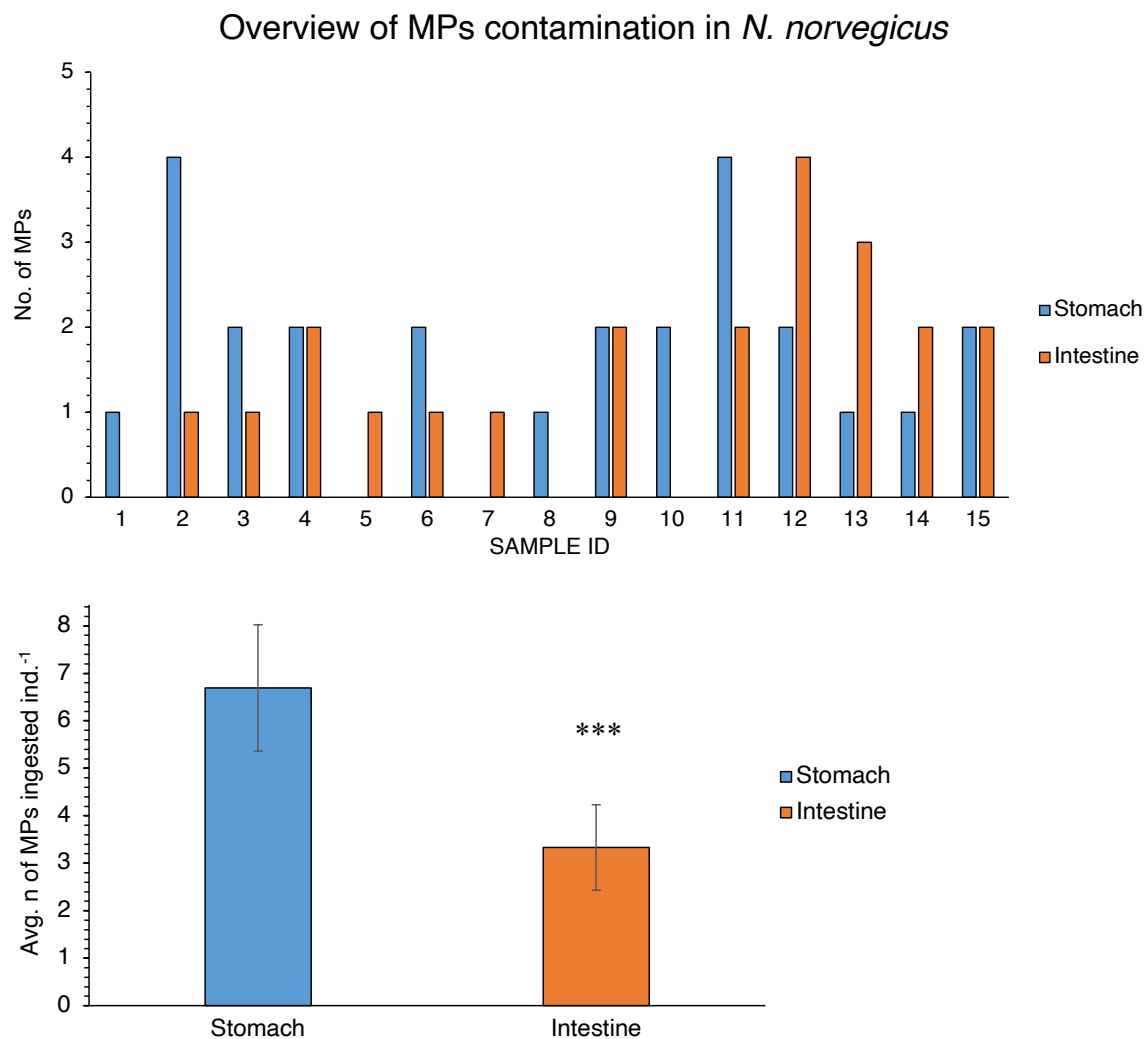
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258 **Figure 5.** Relative abundance (%) in shape and polymeric composition of MPs retrieved in the gastrointestinal tract  
 259 of *P. elephas*.

260

261 The  $\mu$ FT-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).

269 The chemical  $\mu$ FT-IR characterization confirmed as MPs a total of 48 particles isolated  
 270 cumulatively from both stomach and intestine of *N. norvegicus*. Overall, MPs were detected in all individuals  
 271 (100% occurrence; Fig. 6), corresponding to a frequency of ~87% in stomachs and 80% in intestines. The  
 272 average number of particles was on average  $3.2 \pm 0.45$  MPs individual<sup>-1</sup>, ranging from 1 up to a maximum  
 273 of 6 MPs individual<sup>-1</sup> (Fig. 6), without significant differences between stomachs and intestines, showing  $2$   
 274  $\pm 0.26$  and  $1.83 \pm 0.24$  MPs from stomachs and intestines, respectively (Fig. 6).

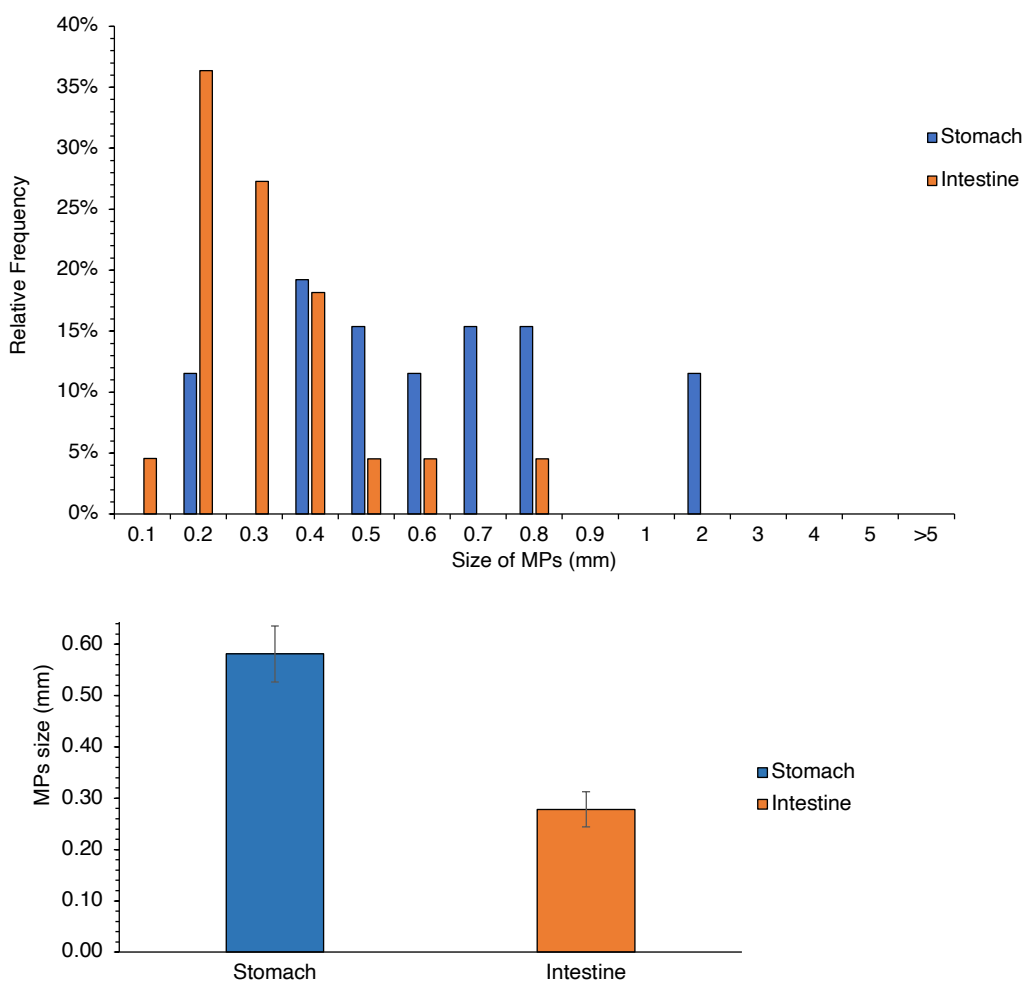


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277 **Figure 6.** Histogram showing the number of MPs isolated from stomach and intestine in each sample of *N.*  
 278 *norvegicus* (upper graph) and histogram showing the average number of MPs ( $\pm$  st. err) isolated from stomachs and  
 279 intestines 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  
 281 average size of  $0.44 \pm 0.3$  mm when considering isolated particles cumulatively. More in detail, 91% of  
 282 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  
 285 significantly smaller ( $0.28 \pm 0.03$  mm) than in the stomach ( $0.58 \pm 0.05$  mm; Fig. 7).

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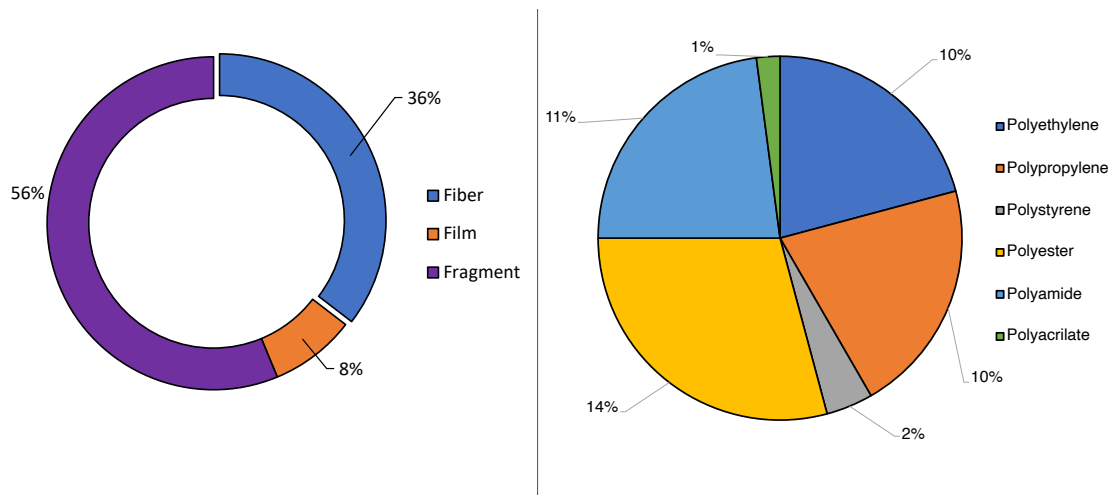
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288 **Figure 7.** Histogram showing the size (mm) frequency distribution of MPs isolated from stomachs and intestines of  
 289 *N. norvegicus* (upper graph). Histogram showing the average size (mm) of MPs particles extracted from stomachs and  
 290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

291

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  
 294 polymers were identified (Fig. 8): PE and PES were the most represented (24% and 39% respectively),  
 295 followed by PP (12%), PS (9%), PA (5%), while PU, acrylic polymers, Ethylene-vinyl acetate (EVA),  
 296 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  
 298 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

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

304

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<sup>-1</sup> 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.

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

342 As previously observed in the Greek study (Kampouris et al., 2023), our results confirmed that the  
343 number of MPs retrieved in *P. elephas* is not influenced by how empty or full are the stomach or intestine,  
344 weight or dimensions of individuals, nor the total weight of the specimen. Interestingly, we also observed  
345 large pieces piece of fishing nets (i.e., up to 6 cm) in the stomach of a specimen collected by means of  
346 trammel nets. That specific individual (sample id=2; Fig. 3) was the one showing the highest n. of particles  
347 ind<sup>-1</sup> (n=25), with red particles of polyamide being dominants (likely fragmented from the ingested net),  
348 supporting the intuition that fishing gears can easily become a source of plastic particles ingestion (Fig. 9).  
349





350  
351 **Figure 9.** Piece of trammel net in the stomach content of *P. elephas*

352  
353 Contrarily to spiny lobster, scientific literature has documented *N. norvegicus* contamination across  
354 different areas and bathymetries, both in the Mediterranean (Avio et al., 2020; Carreras-Colom et al., 2022a;  
355 Cau et al., 2019a; Martinelli et al., 2021) and in the Atlantic (Hara et al., 2020; Joyce et al., 2022a; Murray  
356 and Cowie, 2011). Available literature provided evidence of the wide variety of MPs abundance (n. part ind-  
357 1) in langoustines and results here presented are within the range documented for the Mediterranean, which  
358 is higher than that observed in Atlantic samples.

359 The two crustacean species of this study have similar feeding strategies but different trophic  
360 behaviour since *N. norvegicus* feeds within a small bottom area around its burrows (Sbrana et al., 2019),  
361 whereas *P. elephas* is more mobile and capable of moving for long distance, thus having a larger scale of  
362 representativeness of MPs contamination. With respect to the polymeric composition, the majority of MPs  
363 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 for  
365 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 tract 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).

380 In conclusion, we confirm and further extend the awareness of the high exposure of these  
381 crustaceans to MPs, rendering spiny lobsters and langoustines either valuable bioindicators that belong to  
382 the most important stocks in the FAO Major Fishing Areas of European competence, but also species with  
383 socio-cultural relevance within Mediterranean and EU communities. Being regarded as *gourmet food* and  
384 being also amongst the most charismatic, flagship species for citizens, they could trigger and enhance  
385 environmental awareness and consciousness of the vastity of the impact derived from plastic contamination  
386 (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 that  
390 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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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	Latitude (N)	Longitude (E)	Depth (m)
1	<i>P. elephas</i>	9	40° 13' 31"	8° 38' 83"	76
2	<i>P. elephas</i>	5	40° 28' 57"	8° 11' 93"	105
3	<i>N. norvegicus</i>	8	40° 30' 41"	7° 54' 16"	592
4	<i>N. norvegicus</i>	7	40° 16' 08"	7° 49' 58"	402

400

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

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

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### 407 References

- 408 Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark *Galeus melastomus*  
409 *Rafinesque, 1810* in the continental shelf off the western Mediterranean Sea. *Environ. Pollut.*  
410 223, 223–229. doi:10.1016/j.envpol.2017.01.015
- 411 Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: guide to software  
412 and statis- tical methods. Plymouth, UK: PRIMER-E Ltd. 214 p.
- 413 Andrades, R., Aguiar, R., Silva, A., Teles, D., 2019. Scavenging as a pathway for plastic ingestion by  
414 marine animals. *Environ. Pollut.* 248, 159–165. doi:10.1016/j.envpol.2019.02.010
- 415 Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction  
416 and characterization of microplastics in fish tissues: First observations in commercial species  
417 from Adriatic Sea. *Mar. Environ. Res.* 111, 18–26. doi:10.1016/j.marenvres.2015.06.014
- 418 Avio, C.G., Pittura, L., D’Errico, G., Abel, S., Amorello, S., Marino, G., Gorbi, S., Regoli, F., 2020.  
419 Distribution and characterization of microplastic particles and textile microfibers in Adriatic  
420 food webs: General insights for biomonitoring strategies. *Environ. Pollut.* 258, 113766.  
421 doi:10.1016/j.envpol.2019.113766

- 422 Bergmann, M., Wirzberger, V., Krumpfen, T., Lorenz, C., Primpke, S., Tekman, M.B., Gerdts, G.,  
 423 2017. High Quantities of Microplastic in Arctic Deep-Sea Sediments from the HAUSGARTEN  
 424 Observatory. *Environ. Sci. Technol.* 51, 11000–11010. doi:10.1021/acs.est.7b03331
- 425 Bonanno, G., Orlando-Bonaca, M., 2018. Perspectives on using marine species as bioindicators of  
 426 plastic pollution. *Mar. Pollut. Bull.* 137, 209–221. doi:10.1016/j.marpolbul.2018.10.018
- 427 Bour, A., Avio, C.G., Gorbi, S., Regoli, F., Hylland, K., 2018. Presence of microplastics in benthic  
 428 and epibenthic organisms: Influence of habitat, feeding mode and trophic level. *Environ. Pollut.*  
 429 243, 1217–1225. doi:10.1016/j.envpol.2018.09.115
- 430 Brandon, J.A., Jones, W., Ohman, M.D., 2019. Multidecadal increase in plastic particles in coastal  
 431 ocean sediments. *Sci. Adv.* 5, 1–7. doi:10.1126/sciadv.aax0587
- 432 Bray, L., Digka, N., Tsangaris, C., Camedda, A., Gambaiani, D., de Lucia, G.A., Matiddi, M., Miaud,  
 433 C., Palazzo, L., Pérez-del-Olmo, A., Raga, J.A., Silvestri, C., Kaberi, H., 2019. Determining  
 434 suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. *Environ. Pollut.* 247,  
 435 1071–1077. doi:10.1016/j.envpol.2019.01.100
- 436 Canals, M., Pham, C.K., Bergmann, M., Gutow, L., Hanke, G., van Sebille, E., Angiolillo, M., Buhl-  
 437 Mortensen, L., Cau, A., Ioakeimidis, C., Kammann, U., Lundsten, L., Papatheodorou, G.,  
 438 Purser, A., Sanchez-Vidal, A., Schulz, M., Vinci, M., Chiba, S., Galgani, F., Langenkämper, D.,  
 439 Möller, T., Nattkemper, T.W., Ruiz, M., Suikkanen, S., Woodall, L., Fakiris, E., Molina Jack,  
 440 M.E., Giorgetti, A., 2021. The quest for seafloor macrolitter: a critical review of background  
 441 knowledge, current methods and future prospects. *Environ. Res. Lett.* doi:10.1088/1748-  
 442 9326/abc6d4
- 443 Carbery, M., Connor, W.O., Thavamani, P., O’Connor, W., Palanisami, T., 2018. Trophic transfer of  
 444 microplastics and mixed contaminants in the marine food web and implications for human  
 445 health. *Environ. Int.* 115, 400–409. doi:10.1016/j.envint.2018.03.007
- 446 Carreras-Colom, E., Cartes, J.E., Constenla, M., Welden, N.A., Soler-Membrives, A., Carrassón, M.,  
 447 2022a. An affordable method for monitoring plastic fibre ingestion in *Nephrops norvegicus*  
 448 (Linnaeus, 1758) and implementation on wide temporal and geographical scale comparisons.  
 449 *Sci. Total Environ.* 810. doi:10.1016/j.scitotenv.2021.152264
- 450 Carreras-Colom, E., Cartes, J.E., Rodríguez-Romeu, O., Padrós, F., Solé, M., Grelaud, M., Ziveri, P.,  
 451 Palet, C., Soler-Membrives, A., Carrassón, M., 2022b. Anthropogenic pollutants in *Nephrops*  
 452 *norvegicus* (Linnaeus, 1758) from the NW Mediterranean Sea: Uptake assessment and potential  
 453 impact on health. *Environ. Pollut.* 314. doi:10.1016/j.envpol.2022.120230
- 454 Carreras-Colom, E., Constenla, M., Soler-Membrives, A., Cartes, J.E., Baeza, M., Padrós, F.,  
 455 Carrassón, M., 2018. Spatial occurrence and effects of microplastic ingestion on the deep-water  
 456 shrimp *Aristeus antennatus*. *Mar. Pollut. Bull.* 133, 44–52. doi:10.1016/j.marpolbul.2018.05.012
- 457 Cau, A., Avio, C.G., Dessì, C., Follesa, M.C., Moccia, D., Regoli, F., Pusceddu, A., 2019a.  
 458 Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: Flagship species  
 459 for deep-sea environments? *Environ. Pollut.* 255, 113107. doi:10.1016/j.envpol.2019.113107
- 460 Cau, A., Avio, C.G., Dessì, C., Moccia, D., Pusceddu, A., Regoli, F., Cannas, R., Follesa, M.C., 2020.  
 461 Benthic Crustacean Digestion Can Modulate the Environmental Fate of Microplastics in the  
 462 Deep Sea. *Environ. Sci. Technol.* 54, 4886–4892. doi:10.1021/acs.est.9b07705
- 463 Cau, A., Bellodi, A., Cannas, R., Fois, M., Guidetti, P., Moccia, D., Porcu, C., Pusceddu, A., Follesa,  
 464 M.C., 2019b. European spiny lobster recovery from overfishing enhanced through active  
 465 restocking in Fully Protected Areas. *Sci. Rep.* 9, 13025. doi:10.1038/s41598-019-49553-8
- 466 Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., Ogido, M., Fujikura, K., 2018.  
 467 Human footprint in the abyss: 30 year records of deep-sea plastic debris. *Mar. Policy* 0–1.  
 468 doi:10.1016/j.marpol.2018.03.022
- 469 Compa, M., Alomar, C., Wilcox, C., van Sebille, E., Lebreton, L., Hardesty, B.D., Deudero, S., 2019.  
 470 Risk assessment of plastic pollution on marine diversity in the Mediterranean Sea. *Sci. Total*  
 471 *Environ.* 678, 188–196. doi:10.1016/j.scitotenv.2019.04.355
- 472 Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernandez-Leon, S.,  
 473 Palma, A.T., Navarro, S., Garcia-de-Lomas, J., Ruiz, A., Fernandez-de-Puelles, M.L., Duarte,  
 474 C.M., 2014. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci.* 111, 10239–10244.  
 475 doi:10.1073/pnas.1314705111
- 476 Cristo, M., Cartes, J., National, S., 1998. A comparative study of the feeding ecology of *Nephrops*

477 norvegicus (L.), (Decapoda:Nephropidae ) in the bathyal Mediterranean and the adjacent  
478 Atlantic 62, 81–90.

479 D'Iglio, C., Di Fresco, D., Spanò, N., Albano, M., Panarello, G., Laface, F., Faggio, C., Capillo, G.,  
480 Savoca, S., 2022. Occurrence of Anthropogenic Debris in Three Commercial Shrimp Species  
481 from South-Western Ionian Sea. *Biology (Basel)*. 11, 1616. doi:10.3390/biology11111616

482 Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M., Bengtson Nash,  
483 S.M., 2018. Turning microplastics into nanoplastics through digestive fragmentation by  
484 Antarctic krill. *Nat. Commun.* 9, 1–8. doi:10.1038/s41467-018-03465-9

485 Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan,  
486 P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic  
487 Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS One* 9, 1–15.  
488 doi:10.1371/journal.pone.0111913

489 Follesa, M.C., Cannas, R., Cau, A., Cuccu, D., Mulas, A., Porcu, C., Saba, S., Cau, A., 2014. Homing  
490 and orientation of *Palinurus elephas* (Fabricius) in three no-take areas of the central-western  
491 Mediterranean: Implications for marine reserve design. *Mar. Freshw. Res.* 66.  
492 doi:10.1071/MF13079

493 Follesa, M.C., Cannas, R., Cau, A., Alessandro, Cuccu, D., Mulas, A., Porcu, C., Saba, S., Cau, Angelo,  
494 2015. Homing and orientation of *Palinurus elephas* (Fabricius) in three no-take areas of the  
495 central-western Mediterranean: implications for marine reserve design. *Mar. Freshw. Res.* 66, 1–  
496 9. doi:10.1071/MF13079

497 Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F.,  
498 Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C.,  
499 Bainsi, M., Cristina, M., Ped, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T.,  
500 Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Bainsi, M.,  
501 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean  
502 biodiversity. *Environ. Pollut.* 237, 1023–1040. doi:10.1016/j.envpol.2017.11.019

503 Franceschini, S., Cau, A., D'Andrea, L., Follesa, M.C., Russo, T., 2021. Eating Near the Dump:  
504 Identification of Nearby Plastic Hotspot as a Proxy for Potential Microplastic Contamination in  
505 the Norwegian Lobster (*Nephrops norvegicus*). *Front. Mar. Sci.* 8, 1–12.  
506 doi:10.3389/fmars.2021.682616

507 Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. *Mar. Pollut.*  
508 *Bull.* 138, 145–147. doi:10.1016/j.marpolbul.2018.11.022

509 Galgani, F., Hanke, G., Werner, S., De Vrees, L., 2013a. Marine litter within the European Marine  
510 Strategy Framework Directive. *ICES J. Mar. Sci.* 70, 1055–1064. doi:10.1093/icesjms/fst122

511 Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kins, S., Liebezeit, G.,  
512 2013b. Guidance on Monitoring of Marine Litter in European Seas. doi:10.2788/99475

513 Galgani, L., Loïselle, S.A., 2021. Plastic pollution impacts on marine carbon biogeochemistry.  
514 *Environ. Pollut.* 268, 115598. doi:10.1016/j.envpol.2020.115598

515 Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. *Sci.*  
516 *Adv.* 3, e1700782. doi:10.1126/sciadv.1700782

517 Goñi, R., Latrouite, D., 2005. Review of the biology, ecology and fisheries of *Palinurus* spp . species  
518 of European waters: *Palinurus elephas* (Fabricius , 1787) and *Palinurus mauritanicus* (Gruvel ,  
519 1911). *Cah. Biol. Mar.* 46, 127–142.

520 Groeneveld, J.C., Goñi, R., Diaz, D., 2013. *Palinurus* species, in: Bruce F. Phillips (Ed.), *Lobsters:  
521 Biology, Management, Aquaculture & Fisheries: Second Edition*. John Wiley & Sons, Ltd, pp.  
522 326–356. doi:10.1002/9781118517444.ch11

523 Gutow, L., Ricker, M., Holstein, J.M., Dannheim, J., Stanev, E. V., Wolff, J.O., 2018. Distribution  
524 and trajectories of floating and benthic marine macrolitter in the south-eastern North Sea. *Mar.*  
525 *Pollut. Bull.* 131, 763–772. doi:10.1016/j.marpolbul.2018.05.003

526 Hara, J., Frias, J., Nash, R., 2020. Quantification of microplastic ingestion by the decapod crustacean  
527 *Nephrops norvegicus* from Irish waters. *Mar. Pollut. Bull.* 152, 110905.  
528 doi:10.1016/j.marpolbul.2020.110905

529 Higgs, N.D., Newton, J., Attrill, M.J., 2016. Caribbean Spiny Lobster Fishery Is Underpinned by  
530 Trophic Subsidies from Chemosynthetic Primary Production. *Curr. Biol.* 26, 3393–3398.  
531 doi:10.1016/j.cub.2016.10.034

532 Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law,  
533 K.L., 2015. Plastic waste inputs from land into the ocean. *Science* (80- ). 347, 768–771.  
534 doi:10.1126/science.1260352

535 Joyce, H., Frias, J., Kavanagh, F., Lynch, R., Pagter, E., White, J., Nash, R., 2022a. Plastics, prawns,  
536 and patterns: Microplastic loadings in *Nephrops norvegicus* and surrounding habitat in the North  
537 East Atlantic. *Sci. Total Environ.* 826, 154036. doi:10.1016/j.scitotenv.2022.154036

538 Joyce, H., Nash, R., Kavanagh, F., Power, T., White, J., Frias, J., 2022b. Size dependent egestion of  
539 polyester fibres in the Dublin Bay Prawn (*Nephrops norvegicus*). *Mar. Pollut. Bull.* 180,  
540 113768. doi:10.1016/j.marpolbul.2022.113768

541 Kampouris, T.E., Syranidou, E., Seridou, P., Gagoulis, K., Batjakas, I.E., Kalogerakis, N., 2023. MPs  
542 and NPs intake and heavy metals accumulation in tissues of *Palinurus elephas* (J.C. Fabricius,  
543 1787), from NW Aegean sea, Greece. *Environ. Pollut.* 316, 120725.  
544 doi:10.1016/j.envpol.2022.120725

545 Kohler, K.E., Gill, S.M., 2006. Coral Point Count with Excel extensions (CPCe): A Visual Basic  
546 program for the determination of coral and substrate coverage using random point count  
547 methodology. *Comput. Geosci.* 32, 1259–1269. doi:10.1016/j.cageo.2005.11.009

548 Kowalski, N., Reichardt, A.M., Waniek, J.J., 2016. Sinking rates of microplastics and potential  
549 implications of their alteration by physical , biological , and chemical factors. *Mar. Pollut. Bull.*  
550 109, 310–319. doi:10.1016/j.marpolbul.2016.05.064

551 Kroon, F., Motti, C., Talbot, S., Sobral, P., Puotinen, M., 2018. A workflow for improving estimates  
552 of microplastic contamination in marine waters: A case study from North-Western Australia.  
553 *Environ. Pollut.* 238, 26–38. doi:10.1016/j.envpol.2018.03.010

554 Macali, A., Semenov, A., Venuti, V., Crupi, V., D’Amico, F., Rossi, B., Corsi, I., Bergami, E., 2018.  
555 Episodic records of jellyfish ingestion of plastic items reveal a novel pathway for trophic  
556 transference of marine litter. *Sci. Rep.* 8, 6105. doi:10.1038/s41598-018-24427-7

557 Martinelli, M., Gomiero, A., Guicciardi, S., Emanuela, F., Straffella, P., Angelini, S., Domenichetti, F.,  
558 Belardinelli, A., Colella, S., 2021. Preliminary results on the occurrence and anatomical  
559 distribution in wild populations of *Nephrops norvegicus* from the Adriatic Sea. *Environ. Pollut.*  
560 278, 334509. doi:10.1016/j.envpol.2021.116872

561 Mulas, A., Sbaraglia, S., Bellodi, A., Bitetto, I., Carbonara, P., Carugati, L., Cau, A., Marongiu, M.F.,  
562 Pascale, N., Porcu, C., Zupa, W., Follesa, M.C., 2022. Movement patterns analysis as a tool in  
563 Fully Protected Areas design: Influence of relocations on travelled distances of *Palinurus*  
564 *elephas* (Fabr. 1787) in Sardinian FPAs (central-western Mediterranean). *Mar. Environ. Res.*  
565 182, 105766. doi:10.1016/j.marenvres.2022.105766

566 Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus*  
567 (Linnaeus, 1758). *Mar. Pollut. Bull.* 62, 1207–1217. doi:10.1016/j.marpolbul.2011.03.032

568 Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpfen, T., Bergmann, M.,  
569 Hehemann, L., Gerds, G., 2018. Arctic sea ice is an important temporal sink and means of  
570 transport for microplastic. *Nat. Commun.* 9, 1505. doi:10.1038/s41467-018-03825-5

571 Peng, G., Bellerby, R., Zhang, F., Sun, X., Li, D., 2020. The ocean’s ultimate trashcan: Hadal  
572 trenches as major depositories for plastic pollution. *Water Res.* 168, 115121.  
573 doi:10.1016/j.watres.2019.115121

574 Ryan, P.G., 2015. Does size and buoyancy affect the long-distance transport of floating debris?  
575 *Environ. Res. Lett.* 10. doi:10.1088/1748-9326/10/8/084019

576 Sbrana, A., Cau, A., Cicala, D., Franceschini, S., Giarrizzo, T., Gravina, M.F., Ligas, A., Maiello, G.,  
577 Matiddi, M., Parisi, A., Sartor, P., Sbrana, M., Scacco, U., Valente, T., Viva, C., Russo, T.,  
578 2022. Ask the shark: blackmouth catshark (*Galeus melastomus*) as a sentinel of plastic waste on  
579 the seabed. *Mar. Biol.* 169, 1–17. doi:10.1007/s00227-022-04084-1

580 Sbrana, M., Zupa, W., Ligas, A., Capezzuto, F., Archonita, C., Follesa, M.C., Gancitano, V., Guijarro,  
581 B., Isajlovic, I., Jadaud, A., Markovic, O., Micallef, R., Peristeraki, P., Piccinetti, C., Thasitis, I.,  
582 Carbonara, P., 2019. Spatio temporal abundance pattern of deep-water rose shrimp, *Parapenaeus*  
583 *longirostris*, and Norway lobster, *Nephrops norvegicus*, in European Mediterranean waters. *Sci.*  
584 *Mar.* 83S1, 1–10.

585 Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F.,  
586 Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface

587 waters. *Sci. Rep.* 6, 37551. doi:10.1038/srep37551  
588 Ungfors, A., Bell, E., Johnson, M.L., Cowing, D., Dobson, N.C., Bublitz, R., Sandell, J., 2013.  
589 *Nephrops Fisheries in European Waters*, 1st ed, *Advances in Marine Biology*. Elsevier Ltd.  
590 doi:10.1016/B978-0-12-410466-2.00007-8  
591 Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Franeker, J.A. Van,  
592 Eriksen, M., Siegel, D., Galgani, F., Law, K.L., 2015. A global inventory of small floating  
593 plastic debris. *Environ. Res. Lett.* 10, 124006. doi:10.1088/1748-9326/10/12/124006  
594 Weiss, L., Ludwig, W., Heussner, S., Canals, M., Ghiglione, J.-F., Estournel, C., Constant, M.,  
595 Kerhervé, P., 2021. The missing ocean plastic sink: Gone with the rivers. *Science* (80-. ). 373,  
596 107–111. doi:10.1126/science.abe0290  
597 Welden, N.A.C., Cowie, P.R., 2016a. Environment and gut morphology influence microplastic  
598 retention in langoustine, *Nephrops norvegicus*. *Environ. Pollut.* 214, 859–865.  
599 doi:10.1016/j.envpol.2016.03.067  
600 Welden, N.A.C., Cowie, P.R., 2016b. Long-term microplastic retention causes reduced body  
601 condition in the langoustine, *Nephrops norvegicus*. *Environ. Pollut.* 218, 895–900.  
602 doi:10.1016/j.envpol.2016.08.020  
603 Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A.,  
604 Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for  
605 microplastic debris. *R. Soc. Open Sci.* 1, 140317. doi:10.1098/rsos.140317  
606 Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: Microbial  
607 communities on plastic marine debris. *Environ. Sci. Technol.* 47, 7137–7146.  
608 doi:10.1021/es401288x  
609