Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: flagship species for deep-sea environments?

Alessandro Cau*[1,2]✉, Carlo Giacomo Avio*[3,4], Claudia Dessì[1], Maria Cristina Follesa[1,2], Davide Moccia[1], Francesco Regoli[2, 3] & Antonio Pusceddu[1,2]

[1]Università degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente, Via Tommaso Fiorelli 1, 09126 Cagliari, Italy

[2] Consorzio Interuniversitario per le Scienze del Mare, CoNISMa, ULR Cagliari, Cagliari, Italy

[3]Università Politecnica delle Marche, Dipartimento di Scienze della Vita e dell'Ambiente, Via Brecce Bianche, 60131, Ancona, Italy

[4] Consorzio Interuniversitario per le Scienze del Mare, CoNISMa, ULR Ancona, Ancona, Italy

*= equally contributing authors.

✉**Corresponding Authors:**

Telephone: +39 349 3862846;

E-mail: alessandrocau@unica.it;

Keywords:

Microplastic, deep-sea biota, feeding strategy, ingestion, bioindicators,

Abstract

Ingestion of microplastics (MPs) has been documented in several marine organisms, but their occurrence in deep-sea species remains almost unknown. In this study, MPs were investigated in two economically and ecologically key crustaceans of the Mediterranean Sea, the Norwegian lobster *Nephrops norvegicus* and the shrimp *Aristeus antennatus*. Both the species were collected from 14 sites around Sardinia Island, at depths comprised between 270 and 660 m. A total of 89 and 63 stomachs were analysed for *N. norvegicus* and *A. antennatus* respectively, and more than 2,000 MPs-like particles were extracted and sorted for identification and characterization by µFT-IR. In *N. norvegicus*, 83% of the specimens contained MPs, with an average abundance of 5.5 ± 0.8 MPs individual^{-1}, while *A. antennatus* showed a lower frequency of ingestion (67%) and a lower mean number of MPs (1.66 ± 0.1 MPs individual^{-1}). Composition and size of particles differed significantly between the two species. The non-selective feeding strategy of *N. norvegicus* could explain the 3 to 5 folds higher numbers of MPs in its stomach, which were mostly composed of films and fragments derived by polyethylene and polypropylene single-use plastic items. Contrarily, most MPs in the stomachs of *A. antennatus* were polyester filaments. The MPs abundance observed in *N. norvegicus* is among the highest detected in Mediterranean species considering both fish and invertebrates species, and provides novel insights on MPs bioavailability in deep-sea habitats. The overall results suggest that both *N. norvegicus* and *A. antennatus*, easily available in common fishery markets, could be valuable bioindicators and flagship species for plastic contamination in the deep-sea.

Capsule:

Two deep sea crustaceans of high economic and ecological relevance showed among the highest MPs ingestion ever reported so far, and are proposed as flagship species for plastic pollution in the deep sea.

1. Introduction

More than 5 trillion plastic items (equivalent to ca. 250,000 tons) are currently estimated to afloat at sea (Eriksen et al., 2014). However, their measured abundance in surface layers seems two order of magnitude lower than that expected from conservative models (Cozar et al., 2014; Eriksen et al., 2014). Besides the potential underestimation due to the methodological aspects related to the use of Manta nets with a mesh size >300 µm, sinking has been suggested to partly explain the discrepancy between the real data and the abundance expected from the models (Cozar et al., 2014; Eriksen et al., 2014). Indeed, a large fraction of plastic particles has been observed to sink to the seafloor (Michels et al., 2018; Woodall et al., 2015).

Processes like biofouling or physical weathering can change the density of plastic (Kowalski et al., 2016; Zettler et al., 2013), and the deepest portion of the oceans may therefore represent the final and largest repository for these particles (Chiba et al., 2018; Woodall et al., 2015). Considering the key role of the sea floor as sink for marine litter (Galgani et al., 2000; Pham et al., 2014; Woodall et al., 2014), research on spatial occurrence of MPs in these environments is of actual importance. The most emblematic sign of this phenomenon is a plastic bag recently documented at ca. 10,900 m depth in the Mariana Trench (Chiba et al., 2018), but several studies have already reported the presence of plastic fragments in marine sediments (Barnes et al., 2009; Van Cauwenberghe et al., 2013; Woodall et al., 2014). However, when considering the deep oceans (>200 m depth), such knowledge is very limited, both in terms of MPs accumulation in sediments (Filgueiras et al., 2019; Van Cauwenberghe et al., 2013; Woodall et al., 2014) and of their ingestion by species living in connection with the sea bottom. In such environments, the persistence of most polymers is enhanced by several environmental peculiarities such as the lack of photo-catalytic degradation, reduced oxygen supply and low temperatures; thus, benthic organisms could be particularly exposed to this threat (Bour et al., 2018; Courtene-Jones et al., 2017; Jamieson et al., 2019; Taylor et al., 2016; Welden et al., 2018). The few available information on MPs in deep sea invertebrates reported the presence of these particles in amphipods, cnidarians, echinoderms and arthropods from depths ranging between 334 m and 10,890 m (Choy et al., 2019; Courtene-Jones et al., 2017; Jamieson et al., 2019; Taylor et al., 2016). The vertical distribution and biological transport of MPs across the epipelagic and mesopelagic zones of the

Monterey Bay demonstrated the highest concentrations of particles between 200 and 600 m, and their subsequent flow to pelagic and benthic food webs (Choy et al., 2019). Similar evidences corroborate that anthropogenic debris is bioavailable even in the deepest parts of the oceans, strengthening the hypothesis that these remote habitats are not excluded from MPs contamination and, apparently, they have never been in the last 40 years (Courtene-Jones et al., 2019).

The Mediterranean Sea represents an excellent area to investigate the fate of MPs in deep-sea environments and their availability to deep-sea biota. Being a semi-enclosed basin with limited outflow of surface waters, densely populated coastlines and intensive sea-based activities, it is estimated to retain between 21% and 54% as number of global MPs particles (3.2 - 28.2*10^{12} particles), equivalent to 5-10% of the global plastic mass (4.8 - 30.3*10^{3} tons) in the oceans (Eriksen et al., 2014; Van Sebille et al., 2015).

In this respect, the aim of the present study was to investigate MPs ingestion in the Norwegian lobster (langoustine) *Nephrops norvegicus* (Linnaeus, 1758) and the red shrimp *Aristeus antennatus* (Risso, 1816) sampled from deep-sea sites along the Sardinian coasts. These species, commonly trawled in waters down to 800 m depth, do exhibit different feeding strategies and obtained results were thus expected to provide novel insights on the fate and ingestion of MPs in deep-sea organisms. The tested hypothesis was that geographical and bathymetric parameters (i.e., sites, geographical sub-areas and bathymetric strata), as well as the different feeding strategies of the two species can influence number, shape, size and chemical composition of MPs in their stomachs. The two investigated decapod crustaceans do have relevant ecological roles in the trophic food webs of deep Mediterranean Sea, being also among the most valuable fisheries resource in European Atlantic and Mediterranean waters, with landings worth cumulatively hundreds of millions of Euros (Ungfors et al., 2013). Considering, in addition, their worldwide popularity as seafood, they might be proposed as flagship species, which are defined as “popular, charismatic species that serve as symbol to foster conservation awareness and action” (Bowen-Jones and Entwistle, 2002). In this case, awareness is required in respect to the potential threats of MPs contamination in the deep-sea (Campani et al., 2013; Jacobsen et al., 2010; Lusher et al., 2018).

2. Materials and methods

2.1. Study area and sampling

Samples were collected from 14 sites around the Sardinia island in 2017 (Fig. 1), in the framework of the MEDiterranean International Trawl Survey (MEDITS), at depths comprised between 270 and 660 m. A total of 89 and 63 stomachs were extracted from *Nephrops norvegicus* and *Aristeus antennatus*, respectively (Table 1). Ranges of biometric data (and sex ratio) of analysed specimens were Carapace Length (CL) 25.7-56.4 cm for *N. norvegicus* (54 males and 35 females), and CL 19.4-54 for *A. antennatus* (14 males and 49 females).

2.2. MPs extraction and characterization

Specimens were collected and transported in the laboratory for dissection to avoid the risk of contamination from sampling activities. Each stomach was dissected and stored at -20° until analysis, for which they were dried at 60° C for 24 hours and pottered before extraction. The MPs extraction procedure was based on a density-separation step through a NaCl hypersaline solution (density 1.2 gr cm^{-3}), 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). 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 microscope, photographed and categorized according to shape in: i) fragments; ii) film; iii) pellet/beads; iv) filaments and v) textile fibres. Criteria for shape characterization were the following: fragments included irregular shaped particles, like crystals, powder and flakes, rigid, thick, with sharp crooked edges; pellets were particles with spherical shape, like common resin pellets, spherical microbeads and microspheres; films appeared in irregular shapes, thin and flexible and usually transparent in comparison with fragments; lines or filaments were characterized by regular diameter along the particles and not frayed ends; textile fibres appeared like ribbon, with not regular diameter along the particles and frayed ends.

Once isolated, MPs were measured at their largest cross section under a stereomicroscope. When possible, also the area of particles was estimated, using the ‘length’ and ‘area’ functions of the image analysis software CPCe (Kohler and Gill, 2006).

All extracted particles were characterized using a µFT-IR microscope (Spotlight i200, Perkin Elmer) coupled to a spectrometer (Spectrum Two, 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) .

Before starting the extractions, and between each process step, benches were cleaned with milli-Q water and all the solutions used were pre-filtered through a nitrate acetate membrane with pore size of 0.45µm. Glass and metal materials were used whenever possible and rinsed with prefiltered milli-Q water before 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. Cotton lab coats were used all the time, and special attention was paid to limit the wearing of synthetic clothes. NaCl solution was prepared in distilled water and further filtered on cellulose membranes (0.45µm pore size). Contamination controls were also included (one control for each batch of samples was treated in parallel to samples), consisting of prefiltered hypersaline solution that undertook all the drying, extraction, digestion and sorting steps. Despite such precautions, it was not possible to fully avoid airborne contamination and some textile fibres were found in the control membranes: the µ-FTIR characterization revealed these fibres as non-synthetic and almost constituted of cotton and wool. For this reason, being textile fibres the only category of particles potentially affected by external contamination, they were not included in the presented results.

2.3. Statistical analyses

Differences in MPs content and characteristics between the two species were investigated with uni-variate PERMutational ANalysis Of VAriance (hereafter 'PERMANOVA') (Anderson et al., 2008): sites, geographical sub-regions and bathymetric strata were used, separately, as the unique source of variation in the analysis. In the 3 sites where both the species were collected from the same haul (Table 1, Fig. 1), the potential effect of spatial niche overlap was further tested through uni- and multi-variate PERMANOVA based on Euclidean distance resemblance matrixes.

Statistical analyses revealed that distribution and characteristics of MPs present in *N. norvegicus* and *A. antennatus* stomachs did not vary among geographical sectors or bathymetric strata, nor significant relationships were observed between MPs abundance and specimens' size (data not shown): in this respect, the results for each species will be presented as average values from all the sampling areas.

3. Results

3.1. MPs in Nephrops norvegicus

More than 2,000 particles (textile fibres excluded) were extracted from the 2 species (275 for *A. antennatus* and >1700 for *N. norvegicus*) and sorted for the chemical characterization through µFT-IR. The 83% of all *N. norvegicus* had MPs in their stomachs (i.e., 75 stomachs out of the 89 analysed), and the plastic nature was confirmed for 413 particles retrieved in *N. norvegicus*. The average number of MPs present in the stomachs was 5.5 ± 0.8 MPs individual^{-1} (considering only positive specimens), ranging from 1 to a maximum of 42 (Fig. 2A). Within positive individuals, 52 langoustines (ca. 69%) contained a number of MPs in their stomach comprised between 1 and 5 while 23 individuals (ca. 30%) contained more than 5 MPs. Size of particles ranged between 0.1 and 5 mm (Figure 2B), with 4 particles being >5mm in size and thus classified as meso-plastics (max. 9.5mm in length). The size frequency distribution indicated that particles smaller than 0.5 mm were cumulatively 57% of the total retrieved MPs (Figure 2B), with a second peak of particles between 1 and 2 mm (ca. 14% of the total). The surface was estimated on 358 MPs, mostly films and fragments, which were for ca. 30% below 0.0025 mm^2 and for ca. 85% below 0.2 mm^2 (Fig. 2C); pooling together all the values, MPs within the 75 specimens of *N. norvegicus* had a total surface of 117.3 mm^2.

Plastic films represented 72% of the total MPs abundance, followed by fragments (14%) and filaments (14%, Fig. 3). The μFT-IR analysis revealed the presence of 22 polymers dominated by polyethylene (PE, 60%), followed by polypropylene (PP, 14%), polyamide (PA, 9%) and polyester (PES, 7%; Figure 4). Other polymers (polyurethane PU, polystyrene PS, polyisoprene, silicon, Acrilonitrile-Butadiene-Stirene ABS, and acrilic particles) represented an additional 10% (Figure 4).

3.2. MPs in Aristeus antennatus

The chemical μFT-IR characterization of extracted particles revealed 70 MPs among those retrieved from *A. antennatus*.

MPs were detected in 42 individuals, corresponding to a frequency of 66.7%; the average number of particles was 1.66 ± 0.11 MPs individual^{-1}, ranging from 1 up to 3 in each stomach (48% and 12 % of positive specimens, respectively; Figure 2A). The size classes of MPs ranged from 0.08 to 5mm, 68% of total particles were <0.5 mm, and 2 particles were meso-plastics, up to 8 mm in length (Figure 2B). The surface of MPs in stomachs of *A. antennatus* was estimated on 68 particles, mostly filaments and fragments; the total surface was 2.7 mm^2, with >98% of MPs falling within the 0.2 mm^2 class (Fig. 2C). Filaments (44%) and fragments (53%) represented the great majority of isolated MPs, and 10 typologies of polymers were identified (Figure 4): PE and PES were the more 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 ca. 9% of total polymers.

The statistical comparison of results obtained in the two species confirmed a significantly higher number of MPs in *N. norvegicus* and a different polymeric composition, either when testing the whole results, or only those obtained from the 3 sites where both the species were simultaneously collected (Table 2).

4. Discussion

While ingestion of MPs in marine organisms and their potential transfer through trophic webs are widely recognized, still limited information is available for the deep-sea fauna (Auta et al., 2017; Avio et al., 2017b; Carbery et al., 2018; Taylor et al., 2016).

This study was focussed on *N. norvegicus* and *A. antennatus*, typical inhabitants of Mediterranean deep-sea environments, which showed very high frequencies of MPs occurrence in the stomachs (>60 and >80%, respectively) as well as high average number of particles (5.5 ± 0.8 and 1.6 ± 0.1 MPs individual^{-1}, respectively). The two species have different feeding strategies and interaction with the seafloor; *N. norvegicus* is a scavenger species with a non-selective feeding behaviour, while *A. antennatus* has a great ability to root in the mud, preying endobenthic and epibenthic small invertebrates, thus showing a narrower spectrum of preys (Carreras-Colom et al., 2018). Moreover, *N. norvegicus* feeds within a small bottom area around its burrows (Sbrana et al., 2019), whereas *A. antennatus* swims in search of food along the continental slope, some tens of meters above the sea bottom. These differences were hypothesized to influence exposure and potential degree of MPs ingestion in the two species, either through direct exposure or via trophic transfer (Desforges et al., 2015). MPs have indeed the same size range of either microplankton and sediment grains, being thus potentially accessible, along with other marine particles, to a variety of organisms characterized by different feeding strategies. In this regard, our results are based on an unprecedented pool of >2,000 extracted particles each characterized by μFTIR, many of which were proved to be of non-polymeric nature (75% for *A. antennatus* and 72% for *N. norvegicus*). Even though future methodological improvements may reduce the number of false positives during the extraction procedure, our results further corroborate the necessity to confirm the polymeric nature of ingested particles when investigating MPs in marine biota (Bessa, 2019; Rivers et al., 2019).

Overall, the chemical analysis of particles in the two crustacean species, highlighted the presence of MPs in 117 individuals, i.e. 70% of the 152 examined, independently on the species and collection area, thus confirming that the deep-sea and its biota are important sink compartments for MPs (Sanchez-Vidal et al., 2018; Woodall et al., 2014). The frequency of MPs in *N. norvegicus* stomachs from the Sardinian sites was very high (86%) and comparable to that observed in the Clyde

Sea, N Atlantic (ca. 85%, (Murray and Cowie, 2011). For *A. antennatus* the percentage of specimens containing MPs was higher in this study than in deep-sea crustaceans from the NW Mediterranean Sea (65% *versus* ca. 40%, (Carreras-Colom et al., 2018), and in deep-sea fish from the Ionian sea (2%) (Anastasopoulou et al., 2013). The possibility that the Tyrrhenian Sea may represent a hot spot of MPs accumulation and bioavailability (Collignon et al., 2014; Fastelli et al., 2016; Suaria et al., 2016), has been documented also in fish from the Giglio Island with an ingestion frequency higher than 40% (Avio et al., 2017a; Collignon et al., 2014; Fastelli et al., 2016; Suaria et al., 2016). At the same time, however, the close proximity of submarine canyons to the investigated areas could represent another explanation for the high MPs availability, since these formations can act both as traps and conduits of marine debris towards the deep-sea (Cau et al., 2018, 2017; Tubau et al., 2015).

The numbers of MPs observed in this study for *N. norvegicus* (5.5 ± 0.8 MPs ind^{-1}) are among the highest documented for sea bottom organisms (Alomar et al., 2017; Alomar and Deudero, 2017; Bour et al., 2018; Welden et al., 2018); interestingly, quite similar levels were only recently measured in the pelagic red crabs and giant larvacean specimens sampled from the deepest portion of the Monterey Bay ecosystem (Choy et al., 2019). Besides the elevated mean abundance of MPs, it is worthy to note that in 70% of the sites at least 1-3 individuals contained more than 10 MPs (with a maximum of 42 MPs ind^{-1}). The abundance of MPs in *N. norvegicus* stomachs are 3-5 folds higher than that observed in *A. antennatus*. Also, frequency, shapes and polymeric composition of MPs differed between the two species. These differences were confirmed when comparisons between species were performed only with specimens collected from the same sites and bathymetric strata, where their food niches overlap. The obtained results allow to corroborate the importance of feeding behaviour in modulating ingestion of MPs, particularly for non-selective scavengers dwelling in deep waters from continental slopes to bathyal plains (Andrades et al., 2019; Courtene-Jones et al., 2017; Jamieson et al., 2019).

The typology of MPs retrieved in *N. norvegicus* from our study area was dominated by films and fragments, a result that may partly depend on the peculiar conformation of the digestive system in these crustaceans. Ingested items and preys are broken up by the gastric mill, a complex of small calcified plates moved against each other for grinding. Triturated particles then pass through a complicated filter apparatus of setae which allow only the fine particles to enter the pyloric stomach and digestive diverticula (Powers, 1973; Yonge, 1924). Since gastric mills of *N. norvegicus* are not designed for cutting flexible and resistant filamentous materials (Welden and Cowie, 2016), our results suggest that, at least some of the ingested MPs may not be eliminated during the digestive process and remain trapped, thus contributing to explain both the elevated number and typology of MPs characterized in this scavenging species.

Regarding the polymeric composition, the majority of MPs extracted in *N. norvegicus* were composed by PE and PP, while in *A. antennatus* also PES contributed significantly to the overall polymer composition. These results are in line with data on the global production of plastic (FAO, 2007), suggesting that packaging materials and textile products could be considered as an important source of exposure for deep sea organisms. In addition, our results showed that also non-common polymers were ingested by deep sea crustaceans such as PU, Silicon, acrylic particles and copolymers (e.g. ABS, EVA, PVC, etc) that pooled together constituted approximately 6% of MPs retrieved in *N. norvegicus* and *A. antennatus*. The overall spectrum of polymers obtained in the two species, including both high- and low-density MPs, indicate that specific weight of the polymers is not sufficient to predict their distribution across the water column and availability for the biota. Further, films and filaments have been shown to sink more easily than pellets and fragments (Chubarenko et al., 2016), suggesting that specifically shaped objects, such as disposable plastic bags, could represent a major source of MPs to deep-sea habitats (Chubarenko et al., 2016).

The majority of MPs characterized in this study in the two crustacean species were smaller than 500 µm with an average surface lower than 0.2 mm^2, a result comparable to that obtained in deep-sea fish from the Southern China Sea, which contained transparent, and film- or filament-shaped <0.5 mm particles (Zhu et al., 2019). Despite being limited to the stomach contents, which does not allow to define retention nor accumulation *phenomena* for the two species, our results indicate that both *N. norvegicus* and *A. antennatus* may represent appropriate sentinel species to assess the impact of MPs in deep-sea environments. Considering the high number of retrieved particles, future research effort should be focused also on other tissues that may retain or even accumulate MPs particles.

The use of such charismatic and iconic species as targets of MPs is likely to stimulate actions and proper management policies, providing novel insights for increasing public awareness on plastic pollution in deep sea (Bowen-Jones and Entwistle, 2002; Germanov et al., 2018). The ten criteria indentified for choosing flagship species rely on the common basis of emphasizing local context, cultural significance, traditional knowledge, recognition and ecological role, among the others (Bowen-Jones and Entwistle, 2002). The two crustaceans targeted in the present study belong to the most important stocks in the FAO Major Fishing Areas of European competence, and are also species recognized by local communities, regarded as *gourmet food*, with a considerable market price and human consumption. Based on the overall results of this study, *N. norvegicus* and *A. antennatus* could be adopted as flagship species for MPs pollution in the deep Mediterranean Sea.

Acknowledgements

This study was conducted in the framework of the research project "P.O.R. SARDEGNA F.S.E. 2014-2020 - Asse III "Istruzione e Formazione, Obiettivo Tematico: 10, Obiettivo Specifico: 10.5, Azione dell'accordo di Partenariato:10.5.12". Avviso di chiamata per il finanziamento di Progetti di ricerca – Anno 2017" of the Regione Autonoma di Sardegna.

CGA acknowledges EPHEMARE project (funded by JPI-Oceans) for the financial support of his post-doctoral fellow.

References

Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ. Pollut. 223, 223–229. doi:10.1016/j.envpol.2017.01.015

Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., Deudero, S., 2017. Microplastic ingestion by Mullus surmuletus Linnaeus, 1758 fish and its potential for causing oxidative stress. Environ. Res. 159, 135–142. doi:10.1016/j.envres.2017.07.043

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep. Res. Part I Oceanogr. Res. Pap. 74, 11–13. doi:10.1016/j.dsr.2012.12.008

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: guide to software and statis- tical methods. Plymouth, UK: PRIMER-E Ltd. 214 p.

Andrades, R., Aguiar, R., Silva, A., Teles, D., 2019. Scavenging as a pathway for plastic ingestion by marine animals. Environ. Pollut. 248, 159–165. doi:10.1016/j.envpol.2019.02.010

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environmentA review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176. doi:10.1016/j.envint.2017.02.013

Avio, C.G., Cardelli, L.R., Gorbi, S., Pellegrini, D., Regoli, F., 2017a. Microplastics pollution after the removal of the Costa Concordia wreck: First evidences from a biomonitoring case study. Environ. Pollut. 227, 207–214. doi:10.1016/j.envpol.2017.04.066

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26. doi:10.1016/j.marenvres.2015.06.014

Avio, C.G., Gorbi, S., Regoli, F., 2017b. Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. Mar. Environ. Res. 128, 2–11. doi:10.1016/j.marenvres.2016.05.012

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. 364, 1985–1998. doi:10.1098/rstb.2008.0205

Bergmann, M., Klages, M., 2012. Increase of litter at the Arctic deep-sea observatory HAUSGARTEN. Mar. Pollut. Bull. 64, 2734–2741. doi:10.1016/j.marpolbul.2012.09.018

Bergmann, M., Tekman, M.B., Gutow, L., 2017. Marine litter: Sea change for plastic pollution. Nature 544, 297. doi:10.1038/544297a

Bessa, F. et al., 2019. Harmonized protocol for monitoring microplastics in biota. JPI-Oceans BASEMAN project. doi:10.13140/RG.2.2.28588.72321/1

Bour, A., Avio, C.G., Gorbi, S., Regoli, F., Hylland, K., 2018. Presence of microplastics in benthic and epibenthic organisms: Influence of habitat, feeding mode and trophic level. Environ. Pollut. 243, 1217–1225. doi:10.1016/j.envpol.2018.09.115

Bowen-Jones, E., Entwistle, A., 2002. Identifying appropriate flagship species: the importance of culture and local contexts. Oryx 36, 189–195. doi:10.1017/S0030605302000261

Campani, T., Baini, M., Giannetti, M., Cancelli, F., Mancusi, C., Serena, F., Marsili, L., Casini, S., Fossi, M.C., 2013. Presence of plastic debris in loggerhead turtle stranded along the Tuscany coasts of the Pelagos Sanctuary for Mediterranean Marine Mammals (Italy). Mar. Pollut. Bull. 74, 225–230. doi:10.1016/j.marpolbul.2013.06.053

Carbery, M., Connor, W.O., Thavamani, P., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409. doi:10.1016/j.envint.2018.03.007

Carreras-Colom, E., Constenla, M., Soler-Membrives, A., Cartes, J.E., Baeza, M., Padrós, F., Carrassón, M., 2018. Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp Aristeus antennatus. Mar. Pollut. Bull. 133, 44–52. doi:10.1016/j.marpolbul.2018.05.012

Cau, A., Alvito, A., Moccia, D., Canese, S., Pusceddu, A., Cannas, R., Angiolillo, M., Follesa, M.C., 2017. Submarine canyons along the upper Sardinian slope (Central Western Mediterranean) as repositories for derelict fishing gears. Mar. Pollut. Bull. 123, 357–364. doi:10.1016/j.marpolbul.2017.09.010

Cau, A., Bellodi, A., Moccia, D., Mulas, A., Pesci, P., Cannas, R., Pusceddu, A., Follesa, M.C., 2018. Dumping to the abyss: single-use marine litter invading bathyal plains of the Sardinian margin (Tyrrhenian Sea). Mar. Pollut. Bull. 135, 845–851. doi:10.1016/j.marpolbul.2018.08.007

Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., Ogido, M., Fujikura, K., 2018. Human footprint in the abyss: 30 year records of deep-sea plastic debris. Mar. Policy 0–1. doi:10.1016/j.marpol.2018.03.022

Choy, C.A., Robison, B.H., Gagne, T.O., Erwin, B., Firl, E., Halden, R.U., Hamilton, J.A., Katija, K., Lisin, S.E., Rolsky, C., S. Van Houtan, K., 2019. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. Sci. Rep. 9, 7843. doi:10.1038/s41598-019-44117-2

Chubarenko, I., Bagaev, A., Zobkov, M., Esiukova, E., 2016. On some physical and dynamical properties of microplastic particles in marine environment. Mar. Pollut. Bull. 108, 105–112. doi:10.1016/j.marpolbul.2016.04.048

Collignon, A., Hecq, J.H., Galgani, F., Collard, F., Goffart, A., 2014. Annual variation in neustonic micro- and meso-plastic particles and zooplankton in the Bay of Calvi (Mediterranean-Corsica). Mar. Pollut. Bull. 79, 293–298. doi:10.1016/j.marpolbul.2013.11.023

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S.F., Narayanaswamy, B.E., 2019. Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976–2015), a study from the North East Atlantic. Environ. Pollut. 244, 503–512. doi:10.1016/j.envpol.2018.10.090

Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O.M., Narayanaswamy, B.E., 2017. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environ. Pollut. 231, 271–280. doi:10.1016/j.envpol.2017.08.026

Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernandez-Leon, S., Palma, A.T., Navarro, S., Garcia-de-Lomas, J., Ruiz, A., Fernandez-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111, 10239–10244. doi:10.1073/pnas.1314705111

Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. Arch. Environ. Contam. Toxicol. 69, 320–330. doi:10.1007/s00244-015-0172-5

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

FAO, 2007. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fish. Aquac. Tech. Pap. N. 615. Rome, Italy.

Fastelli, P., Blašković, A., Bernardi, G., Romeo, T., Čižmek, H., Andaloro, F., Russo, G.F., Guerranti, C., Renzi, M., 2016. Plastic litter in sediments from a marine area likely to become protected (Aeolian Archipelago's islands, Tyrrhenian sea). Mar. Pollut. Bull. 113, 526–529. doi:10.1016/j.marpolbul.2016.08.054

Filgueiras, A.V., Gago, J., Campillo, J.A., Leon, V.M., 2019. Microplastic distribution in surface sediments along the Spanish Mediterranean continental shelf. Environ. Sci. Pollut. Res.

Galgani, F., Leaute, J.P., Moguedet, P., Souplet, A., Verin, Y., Carpentier, A., Goraguer, H., Latrouite, D., Andral, B., Cadiou, Y., Mahe, J.C., Poulard, J.C., Nerisson, P., 2000. Litter on the sea floor along European coasts. Mar. Pollut. Bull. 40, 516–527. doi:10.1016/S0025-326X(99)00234-9

Germanov, E.S., Marshall, A.D., Bejder, L., Fossi, M.C., Loneragan, N.R., 2018. Microplastics: No Small Problem for Filter-Feeding Megafauna. Trends Ecol. Evol. 33, 227–232. doi:10.1016/j.tree.2018.01.005

Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10, 1–3. doi:10.1038/s41467-019-08316-9

Jacobsen, J.K., Massey, L., Gulland, F., 2010. Fatal ingestion of floating net debris by two sperm whales (Physeter macrocephalus). Mar. Pollut. Bull. 60, 765–767. doi:10.1016/j.marpolbul.2010.03.008

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

Jamieson, A.J., Brooks, L.S.R., Reid, W.D.K., Piertney, S.B., Narayanaswamy, B.E., Linley, T.D., 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. R. Soc. Open Sci. 6, 180667.

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

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

Lacerda, A.L. d. F., Rodrigues, L. dos S., van Sebille, E., Rodrigues, F.L., Ribeiro, L., Secchi, E.R., Kessler, F., Proietti, M.C., 2019. Plastics in sea surface waters around the Antarctic Peninsula. Sci. Rep. 9, 1–12. doi:10.1038/s41598-019-40311-4

Lusher, A.L., Hernandez-Milian, G., Berrow, S., Rogan, E., O'Connor, I., 2018. Incidence of marine debris in cetaceans stranded and bycaught in Ireland: Recent findings and a review of historical knowledge. Environ. Pollut. 232, 467–476. doi:10.1016/j.envpol.2017.09.070

Michels, J., Stippkugel, A., Lenz, M., Wirtz, K., Engel, A., 2018. Rapid aggregation of biofilmcovered microplastics with marine biogenic particles. Proc. R. Soc. B Biol. Sci. 285, 20181203. doi:10.1098/rspb.2018.1203

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

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M., Johns, D.G., 2019. The rise in ocean plastics evidenced from a 60-year time series. Nat. Commun. 10, 1622. doi:10.1038/s41467-019-09506-1

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

Pham, C.K., Ramirez-Llodra, E., Alt, C.H.S., Amaro, T., Bergmann, M., Canals, M., Company, J.B., Davies, J., Duineveld, G., Galgani, F., Howell, K.L., Huvenne, V.A.I., Isidro, E., Jones, D.O.B., Lastras, G., Morato, T., Gomes-Pereira, J.N., Purser, A., Stewart, H., Tojeira, I., Tubau, X., Van Rooij, D., Tyler, P.A., 2014. Marine litter distribution and density in European seas, from the shelves to deep basins. PLoS One 9. doi:10.1371/journal.pone.0095839

Powers, L.W., 1973. Management hurdles for sustainable harvesting of Corallium rubrum. Comp. Biochem. Physiol. 46A, 767–783.

Rivers, M.L., Gwinnett, C., Woodall, L.C., 2019. Quantification is more than counting: Actions required to accurately quantify and report isolated marine microplastics. Mar. Pollut. Bull. 139, 100–104. doi:10.1016/j.marpolbul.2018.12.024

Sanchez-Vidal, A., Thompson, R.C., Canals, M., de Haan, W.P., 2018. The imprint of microplastics from textiles in southern European deep seas. PLoS One 1–13. doi:10.1371/journal.pone.0207033

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

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 37551. doi:10.1038/srep37551

Taylor, M.L., Gwinnett, C., Robinson, L.F., Woodall, L.C., 2016. Plastic microfibre ingestion by deep-sea organisms. Sci. Rep. 6, 33997. doi:10.1038/srep33997

Tubau, X., Canals, M., Lastras, G., Rayo, X., Rivera, J., Amblas, D., 2015. Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: The role of hydrodynamic processes. Prog. Oceanogr. 134, 379–403. doi:10.1016/j.pocean.2015.03.013

Ungfors, A., Bell, E., Johnson, M.L., Cowing, D., Dobson, N.C., Bublitz, R., Sandell, J., 2013. Nephrops Fisheries in European Waters, 1st ed, Advances in Marine Biology. Elsevier Ltd. doi:10.1016/B978-0-12-410466-2.00007-8

Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182, 495–499. doi:10.1016/j.envpol.2013.08.013

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Franeker, J.A. Van, Eriksen, M., Siegel, D., Galgani, F., Law, K.L., 2015. A global inventory of small floating plastic debris. Environ. Res. Lett. 10, 124006. doi:10.1088/1748-9326/10/12/124006

Welden, N.A., Abylkhani, B., Howarth, L.M., 2018. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, Pleuronectes plastessa, and spider crab, Maja squinado. Environ. Pollut. 239, 351–358. doi:10.1016/j.envpol.2018.03.110

Welden, N.A.C., Cowie, P.R., 2016. Environment and gut morphology influence microplastic retention in langoustine, Nephrops norvegicus. Environ. Pollut. 214, 859–865. doi:10.1016/j.envpol.2016.03.067

Woodall, L.C., Robinson, L.F., Rogers, A.D., Narayanaswamy, B.E., Paterson, G.L.J., 2015. Deep-sea litter: a comparison of seamounts, banks and a ridge in the Atlantic and Indian Oceans reveals both environmental and anthropogenic factors impact accumulation and composition. Front. Mar. Sci. 2, 1–10. doi:10.3389/fmars.2015.00003

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317. doi:10.1098/rsos.140317

Worm, B., Lotze, H.K., Jubinville, I., Wilcox, C., Jambeck, J., 2017. Plastic as a Persistent Marine Pollutant. Annu. Rev. Environ. Resour. 42, 1–26.

Yonge, C.M., 1924. Studies on the comparative physiology of digestion II – the mechanism of feeding, digestion and assimilation in Nephrops norvegicus. Br. J. Exp. Biol 41, 343–389.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: Microbial communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146. doi:10.1021/es401288x

Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K., Xia, B., 2019. Microplastic ingestion in deep-sea fish from the South China Sea. Sci. Total Environ. 677, 493–501. doi:10.1016/j.scitotenv.2019.04.380

Table 1. Hauls code, number of samples per haul, geographical coordinates, depth and code of the geographical sub-region where haul was performed (see Figure 1 for legend).

N. norvegicus

ID Hauls	n	Latitude (N)	Longitude (E)	Depth (m)	Area
27MED17	9	40° 59' 75	9° 56' 09	400	2
30MED17	10	41° 09' 29	9° 57' 02	535	2
31MED18	7	41° 11' 98	10° 00' 16	655	2
41MED17	10	41° 09' 39	8° 36' 49	425	3
56MED17	5	40° 10' 68	7° 59' 55	410	5
64MED17	10	40° 01' 34	8° 05' 38	630	5
69MED17	10	39° 51' 93	8° 03' 66	470	5
68MED17	11	39° 30' 95	8° 05' 71	380	6
TEUL_A	8	38° 43’ 30	8° 19’ 39	272	6
TEUL_B	9	38° 31’ 38	8° 31’ 38	604	7

A. antennatus

ID Hauls	n	Latitude (N)	Longitude (E)	Depth (m)	Area
15MED17	9	40° 00' 13	9° 51' 07	625	1
31MED17	8	41° 11' 98	10° 00' 16	655	2
64MED17	10	40° 01' 34	8° 05' 38	630	5
69MED17	10	39° 51' 93	8° 03' 66	470	5
65MED17	6	39° 35' 43	8° 07' 26	550	6
93MED17	10	38° 39' 43	8° 23' 77	580	6
04MED17	10	38° 55' 77	9° 21' 35	590	7

Table 2. Results from the PERMANOVA testing for differences in the number and polymeric composition of ingested MPs between the two species, irrespectively of the sampling site. The same hypothesis was tested also comparing samples from the 3 sites where the two species were collected. Significant p(MC) are reported in bold.

A. antennatus* vs. *N. norvegicus				
Overall MPs ingestion				
Source	**df**	**MS**	**Pseudo-F**	**P(MC)**
Species	1	459.49	16.041	**0.001**
Residual	150	28.65		
Total	151			
Overall MPs composition				
Source	**df**	**MS**	**Pseudo-F**	**P(MC)**
Species	1	235.80	6.118	**0.011**
Residual	113	38.54		
Total	114			
MPs ingestion in same sites				
Source	**df**	**MS**	**Pseudo-F**	**P(MC)**
Species	1	199.43	8.106	**0.005**
Residual	52	24.60		
Total	53			
MPs composition in same sites				
Source	**df**	**MS**	**Pseudo-F**	**P(MC)**
Species	**1**	**83.45**	**2.169**	**0.127**
Residual	**36**	**38.47**		
Total	**37**			

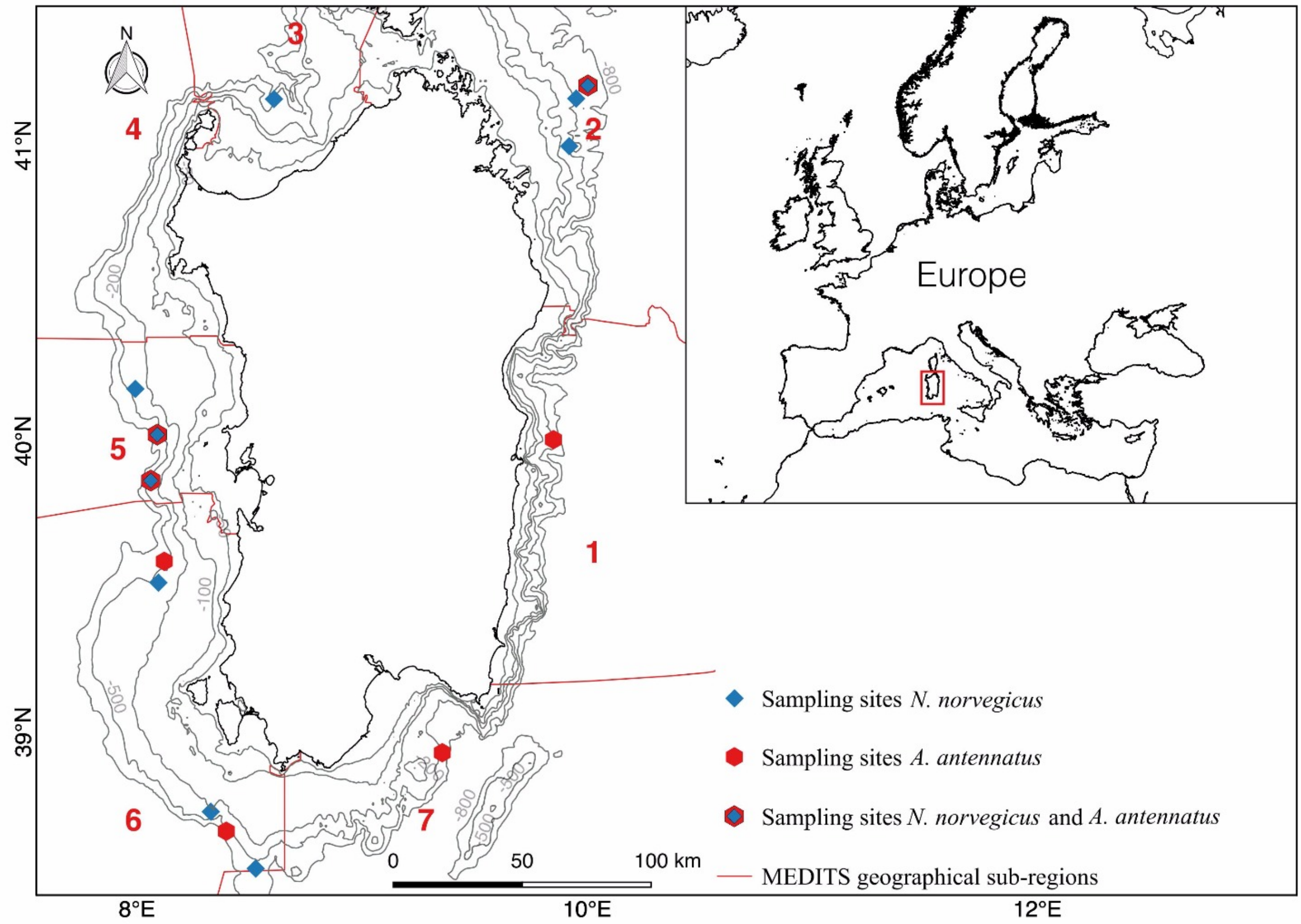

Figure 1. Map of the sampling areas. Red quadrats and blue rhombs refer to sites where samples of *A. antennatus* and *N. norvegicus*, respectively, were collected separately. The blue-contoured red rhombes represent sites where species occurred together.

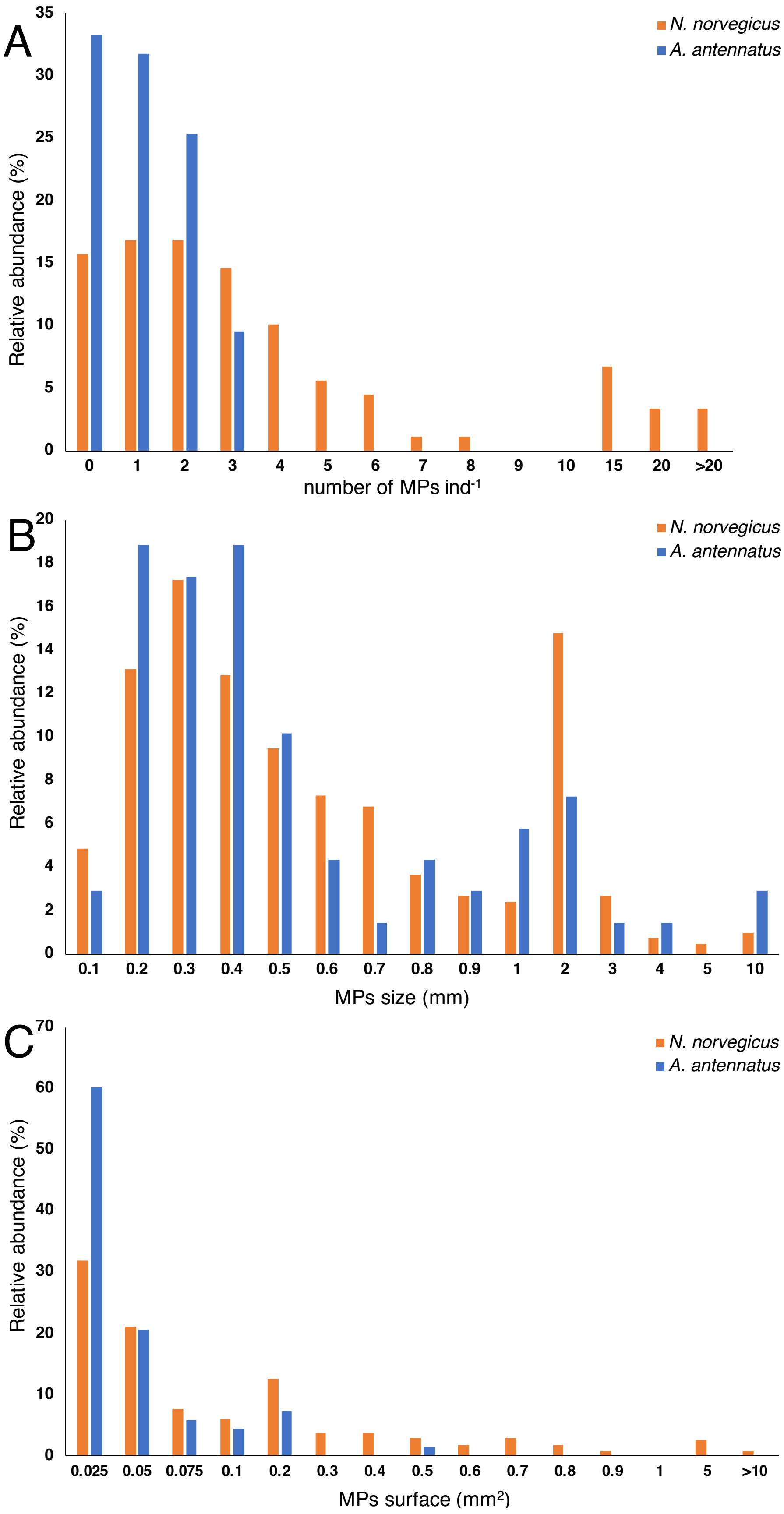

Figure 2. Percentage distribution of: A) number of MPs ingested per individual; B) MPs size (maximum length, in mm); C) area (in mm^2).

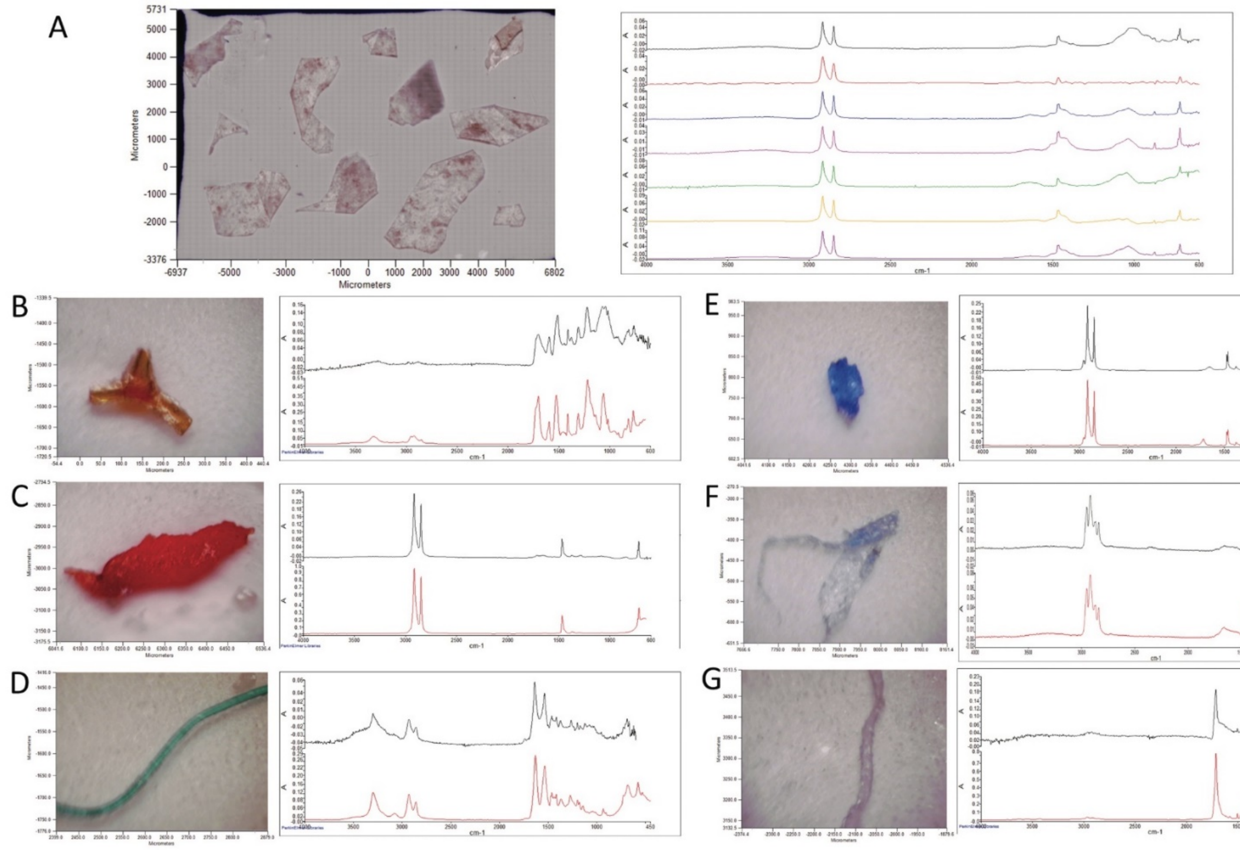

Figure 3. Examples of MPs extracted from *N. norvegicus* and *A. antennatus* and corresponding FT-IR spectra. (A) transparent polyethylene films; (B) polyurethane fragment, (C) red polyethylene film, (D) green polyamide line, (E) polyethylene blue fragments, (F) blue/transparent polypropylene film, (G) transparent polyester line. In picture A each line indicates the spectrum of different particles while from B to G the red lines represent the reference spectra of the polymer, while dark lines correspond to the characterized particles.

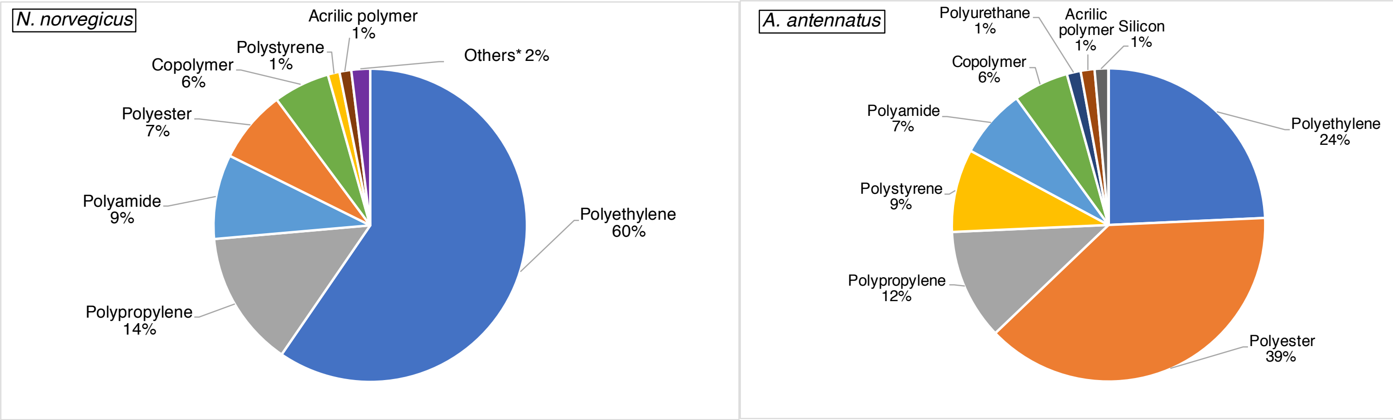

Figure 4. Polymeric composition (%) of MPs retrieved in the stomachs of *N. norvegicus* and *A. antennatus.*

*= Others category for *N. norvegicus* refers to: Polyurethane, Polyisoprene, Polytetrafluoroethylene, Polyvinyl chloride and Silicon pooled together.