Presence of microplastics in benthic and epibenthic organisms: Influence of habitat, feeding mode and trophic level

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Capsule: This field study investigates the influence of three major parameters, habitat, feeding mode and trophic level, on the presence of microplastics in benthic and epibenthic organisms.

Highlights: (between 3 and 5; 85 characters max/bullet point)

- MPs were found in all species sampled
- PE and PP are the polymers most found
- MPs smaller than 200µm account for more than 58% of the total extracted MPs
- MP ingestion is influenced by feeding mode but not habitat or trophic level

Abstract: (300 words max)

Millions of tons of plastic waste have been generated over the past decades due to the exponential production and use of plastics, and the presence of microplastics (MPs) has been reported all over the world's oceans. Field studies have shown that MPs are ingested by various species but important knowledge gaps remain, as most studies focus on pelagic fish species or bivalves used for human consumption. In the present study, we report the presence of MPs in 10 sediment-dwelling and epi-benthic species representative of different habitat, feeding modes and trophic levels. The species analyzed include fish, bivalves, echinoderms, crustaceans and polychaetes. Organisms were sampled in the inner Oslofjord (Oslo, Norway), an area subject to strong anthropogenic pressures. High occurrence of plastic presence was observed, with MPs found in all the sampled species and ingestion frequency reaching up to 65% of positive individuals for some species. In most case, 1 or 2 MPs were found per individual, but some individuals presented up to 7 particles. In total, 8 polymer types were extracted from the organisms, PE and PP being the most found. MP size ranged from 41 µm to fibres as long as 9 mm. Our results indicate that MP ingestion does not seem to be influenced by organisms' habitat or trophic level. However, the presence and polymer type of MPs were significantly influenced by organisms' feeding mode.

Key-words (max 5): Microplastics; field study, ingestion, µFT-IR

1. Introduction

Plastic production has exponentially increased in the past decade (PlasticsEurope, 2017, 2016), resulting in important environmental release. The amount of plastic floating at the surface of the oceans has been estimated to more than five trillions particles, weighing 268 940 tons (Eriksen et al., 2014). Microplastics (MPs), which are commonly referred to as plastic particles below 5 mm in size, originate from two main sources: they can be directly introduced in aquatic environments with microscopic size via runoff or produced by the degradation and breakdown of larger plastic debris already present in the environment (Andrady, 2011). The presence of MPs in marine environments has been reported throughout the world's oceans (Van Cauwenberghe et al., 2013) and in the most remote places (Thompson, 2015), making marine biota very likely to interact with MPs. This has been confirmed by field studies, with MP ingestion being reported for various marine species originating from different locations (Lusher, 2015). Moreover, an increasing number of studies shows that MPs may have harmful effects on organisms, such as physical damages, endocrine disruption, impacts on energy budget, immune system and reproduction, and can act as vectors of pollutants (Bour et al., 2018; Cole et al., 2015; Pittura et al., 2018; Rochman, 2015; Rochman et al., 2014; Sussarellu et al., 2016; Van Cauwenberghe et al., 2015a; von Moos et al., 2012; Wright et al., 2013). It is therefore crucial to have a better knowledge of MP ingestion by organisms in field conditions.

In a recent report focusing on Nordic marine environments, the authors pointed out that the species most studied for the ingestion of MPs in field conditions are fish species and that data on MP ingestion by invertebrates and benthic/epibenthic organisms in Nordic areas is currently scarce (Bråte et al., 2017). A few studies focused on benthic organisms in the North Atlantic: Courtene-Jones et al. (2017) investigated MP ingestion in different benthic invertebrates and observed mostly fiber ingestion. Murray and Cowie (2011) studied contamination in the Norway lobster and found that 83% of the animals contained MPs. Overall, field studies on MP ingestion by benthic or epibenthic organisms are currently very scarce. This substantial lack of studies results in a knowledge gap that needs to be filled, considering that sediments are recognized to be the final sink for most MPs (Van Cauwenberghe et al., 2013, 2015b). Moreover, laboratory experiments have shown that MP can be transferred up to higher trophic levels via the trophic chain (Batel et al., 2016; Mattsson et al., 2015, 2017; Tosetto et al., 2017) and that MP ingestion may depend on organisms feeding mode (Scherer et al., 2017). Therefore, organisms from different trophic levels or feeding guilds might also have different susceptibility to MP ingestion, either direct or via trophic transfer, in the field. However, field studies considering such parameters are rare (Courtene-Jones et al., 2017; Lusher et al., 2013).

The present study aims to provide a better knowledge on MP ingestion by benthic and demersal organisms, by thorough quantification and characterization of ingested MPs. We hypothesized that organism habitat, trophic level and feeding mode could influence MP ingestion by marine organisms. Therefore, we sampled individuals from ten species belonging to different habitats, trophic levels and feeding modes. Sampling was carried out in the inner Oslofjord, an urban area presenting contrasting anthropogenic pressures and uses. After extraction from the

organisms, MP were quantified and characterized for their size, shape, color and composition in order to provide a thorough overview of the characteristics of MPs ingested by marine organisms.

2. Material and Methods

2.1 Sampling

Sampling was carried out in May and September 2017. *Hediste diversicolor* were collected at Jeløya, Norway (59.45N 10.63E), from the shore and close to a small fishing port. Individuals were manually picked up and kept on ice during transport. They were further rinsed with milli-Q water to remove particles attached to the body then individually kept at -80°C. All other species were sampled in the inner Oslofjord, Norway (59.83N 10.50E). Fishes were caught during survey campaigns intended to monitor the fish community in the Oslofjord. Trawling was carried out at 100-110 m depth. Fishes were euthanized and entire digestive tracts were removed from buccal cavity to anus, individually kept on ice during transport then further kept at -80°C. For all other species, sediment was collected with a grab and organisms were manually picked up, kept on ice during transport, further rinsed with milli-Q water and kept at -80°C.

2.2 Sampled species

See Table 1 for species specifications. The polychaete *Hediste diversicolor* is a filter-feeder and deposit-feeder. It is an omnivorous species that scavenges for organic material and detritus on the sediment surface (Moreira et al., 2006) and is one of the most important prey for birds, crustaceans and fish in European estuaries (Rosa et al., 2008). *Trisopterus esmarki* (Norway pout) is present in Norwegian fjords and feeds on crustaceans, amphipods and small fishes. It is a prey for bigger fish species, such as cod and mackerel. Norway Pout is not used in human consumption but is an important target species for fisheries (ICES, 2006). *Enchelyopus cimbrius* (Fourbeard rockling) is found in northern Atlantic Ocean. It forages in the sediment to find and feed on a variety of invertebrates and some fish larvae (Keats and Steele, 1990). *Hippoglossoides platessoides* (long rough dab) feeds on small benthic crustaceans, echinoderms, cnidarians, polychaete, sea urchins and brittle stars. It is a highly commercial species used for human consumption (Johnson, 2005). *Ennucula tenuis* is a protobranch bivalve: it uses its labial palps to select particles while buried in the sediment. It plays an important role in ecosystems due to its bioturbation activity (Austen et al., 1998; Widdicombe et al., 2000). *Ophiura albida* is an omnivorous species of brittle star in the order Ophiurida, present in the top layers of sediments. Rather than hunting, it preys and scavenges on small benthic organisms, rather than hunting. *O. albida* is a prey for number of demersal fishes and is therefore an important link in local food webs (Boos et al., 2010). *Amphiura filiformis* is another species of brittle star living in the sediment. It usually borrows and captures suspended particles by extending its arms at the surface of the sediment (Loo et al., 1996). It is an important part of the diet of many fishes and invertebrates and is an important link between benthic and pelagic environments (Sköld et al., 1994). *Brissopsis lyrifera* (heart urchin) is a non-selective deposit feeder that burrows through the sediment down to a few centimeters and feeds on microorganisms and organic matter adhering to sediment particles (Hollertz, 2002). *Crangon allmani* is a species of shrimp feeding

predominantly on living crustacean and annelids. It is an important item of food for many marketable fish (Allen, 1960). The polychaete *Sabella pavonina* (peacock worm) is present along the coasts of western and northern Europe as well as in the Mediterranean Sea and is found in muddy sediments, sandy or gravelly floors. It filters the water by extending its branchial crown out of its tube (Nicol, 1931).

2.3 MP extraction and characterisation

MP extraction procedure was based on chemical digestion of biological samples followed by density separation and filtration, based on validated protocols previously published (Avio et al., 2015; Dehaut et al., 2016). This procedure has been optimized at UPM (Università Politecnica delle Marche) and presented during a workshop held at UPM in March 2017. The objective was to provide a common procedure to the different laboratories involved in the JPI Oceans' project EPHEMARE, with a purpose of method standardization.

For polychaetes, brittle stars and shrimps, whole organisms were weighted and measured, then processed. For bivalves and heart urchin, organisms were measured, soft tissues and shell/hard body parts were separated and whole soft tissues were weighted and processed. For fish species, entire digestive tracts only (form buccal cavity to anus) were weighted and processed. Samples were digested in 10% KOH heated to 50°C overnight, after which extracts did not contain undigested tissue. Extracts were individually added to 100 ml of NaCl (> 99.5%, NORMAPUR®) filtered hypersaline solution ($d \geq 1.2$ g/cm^3), complemented with NaCl to compensate for sample related dilution, then stirred for 10 minutes, decanted for further 10 minutes and supernatants (approx. 40 ml) were collected. Density separation step was carried out twice and total supernatant filtered under vacuum on a sterile cellulose membrane (8 µm pore size). Membranes were then observed under stereomicroscope and all particles were isolated, photographed, measured at their largest cross section and categorised according to their size, shape and colour. The particles were assigned to three shape categories: fibres, flakes (*i.e.* flat pieces of various shapes) and fragments (*i.e.* every other non-flat particle). Extracted particles were characterized for their polymer composition using a µFT-IR microscope (Spotlight i200, Perkin Elmer) coupled to a spectrometer (Spectrum Two, Perkin Elmer). Following background scans, 16 scans were performed for each particle, with a resolution of 4 cm^{-1}. Spectrum 10 software was used for the output spectra and the polymer identification was performed by comparison with several libraries of standard spectra. Polymers matching with reference spectra for more than 70% were validated, while for polymers with a match comprised 60% and 70% a critical and deepest interpretation of the obtained spectra was performed, according to (Lusher et al., 2013).

2.4 Quality Assurance and Quality Control (QA & QC)

Special attention was paid to limit sample contamination and specific precautions were taken at every step of the process. MP extraction was carried out in a clean room. Work benches were cleaned with milli-Q water before starting the extractions and between each process step. Glass and metal material was used whenever possible and rinsed with 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 milli-Q water. Personal protection equipment was used all the time and special attention was paid to limit the wearing of synthetic clothes. KOH solution was prepared in milli-Q water. NaCl solution was prepared in distilled water further filtered on sterile cellulose membranes (0.45µm pore size). Before digestion, samples were rinsed in milli-Q water and immediately transferred into clean and rinsed glass containers. One contamination control was also added every five samples: it consists of sample-free KOH solution that undertook all the extraction steps in the same conditions as the samples, including overnight heating.

2.5 Statistical analysis

Analysis of variance (One-way ANOVA) was performed to compare the number of MPs per individual based on the species, feeding mode, trophic level and habitat. Level of significance was set at $p < 0.05$; homogeneity of variance was checked with the Cochran test; normal distribution of residues was checked with the Shapiro-Wilk test; mathematical transformation was applied if necessary. When data did not satisfy the assumptions required to perform a parametric ANOVA, Kruscal-Wallis non-parametric test was performed; post-hoc comparison (Dunn's test) was used to discriminate between means of values. Comparison of ingestion frequencies (based on species, feeding mode, trophic level and habitat) was performed using contingency tables and results assessed with the Pearson correlation coefficient. Multivariate statistical analyses (principal component analysis, PCA) were applied to all parameters (number of MP per individual; frequency; polymer composition and shape) in order to discriminate between different species; a threshold factor loading of 0.6 was used as cut-off value.

3. Results

3.1 QA & QC

The analysis of control membranes revealed the presence of blue and red fibres only, perfectly similar in shape. Most of them were small blue fibres (< 1mm; 87.3% of total fibres); other blue fibres were below 3.5 mm in size (4.9% of total fibres) and red fibres were below 1 mm in size (7.8% of total fibres). In total, between 4 and 30 fibres were found per control membrane with an average of 13.5 ±7.3 fibres per membrane. Statistical analysis showed no significant differences between days, indicating consistency in the process. Fibres present on control membranes were isolated and analysed with µFT-IR. Analyses revealed only cotton fibres, suggesting air-born contamination from the lab coat. We conclude that in our conditions, contamination of samples by synthetic particles is negligible, if not absent.

3.2 MPs in field organisms

For this study, we analysed 174 organisms representative of 10 species and of different trophic levels, habitat (*i.e.* benthic, demersal and benthopelagic) and feeding modes (*i.e.* filter-feeders, deposit-feeders and predators). MPs were found in every species, with variable frequency (*i.e.*

percentage of positive individuals; see table 1). The highest ingestion frequency was found in shrimps (*C. allmani*; 65%) and the lowest in Fourbeard Rockling (*E. cimbrius*; 5.9%). One to 7 particles were found per individual, with an average of 1.8 MPs per positive individual (Figure 1). No significant difference was observed between species for the number of MP found per individual. Most MPs found were flakes (45.1%), followed by fragments (23.9%) and fibres (31%) (see Figure 2 for shape repartition depending on the species). The size of extracted MPs ranges between 41 µm and 9 mm (fibre length) and falls into the following categories: below 100 µm (11.8%), 100-200 µm (47%), 300-400 µm (23.5%) and fibres longer than 1 mm (17.6%). Most MPs are blue (36.8%) or transparent (28.3%), but red, green, black, purple and multicolour particles were also found (Figure 3). The vast majority of MPs extracted are polyethylene (PE; 54%) or polypropylene (PP; 16.8%) particles. Polyester, polyamide, polyacrylic, polybutylene terephthalate (PBT), ethylene-vinyl acetate (EVA) and copolymers were also found at lesser proportions (see figure 4 for polymer repartition depending on the species). The PCA carried out on the whole set of parameters produced a two-dimensional pattern explaining 55.7% of total variance (figure 5). Although a quite large percentage remained to be explained, the obtained results indicate a clear separation between species sampled in the fjord (left side) and *H. diversicolor*, sampled on the shore (right side). In addition, fish species (upper side) are quite well separated from other species (lower side). The parameters determining the separation along the PC1 axis were related to some polymer types such as PBT, polyacrylic, copolymers and polyester. On the other side, the average number of MP per individual, the frequency of positive individuals, the occurence of fibers and flakes and polyamide particles determined the separation along the PC2 axis.

Ingestion frequency and the average number of items per positive individual were also analysed depending on organisms feeding mode, habitat and trophic level. Although no statistical differences were found between groups for contamination frequency, some trends can be observed for the feeding-mode: filter-feeders seem less contaminated compared to organisms with different feeding-modes. Moreover, a significantly lower number of items per individual were observed for filter-feeders compared to predators, indicating an influence of the feeding mode on the number of MPs per individual (Figure 6). No significant differences were observed between groups when comparing trophic level or habitat.

We also analysed the influence of feeding mode, trophic level and habitat on the type of MPs present in the organisms. As the majority of MPs extracted are PE or PP particles, MPs were classified in three groups for this analysis: “PE”, “PP” and “other”. Results are presented in Figure 7 and Table 2. They indicate that the number of PE item per individual is influenced by organisms feeding mode, trophic level and habitat: the number of PE items is significantly higher in predators, tertiary consumers and demersal and bentho-pelagic organisms.

4. Discussion

This study presents data on the presence of MPs benthic and epibenthic species, representative of different habitats, feeding modes and trophic levels. With the analysis of 174 individuals

representing 10 species, this is, to our knowledge, the first study including invertebrate and fish species and allowing the analysis of three factors potentially influencing MP ingestion. The first striking result is that MPs were found in every species analysed, providing further evidence for the widespread MP contamination in marine species. Over the 7 invertebrate species analysed, 5 had a ingestion rate equal or above 40%, highlighting the susceptibility of invertebrate species to MP uptake. Although the field of MPs has developed, field studies on the occurrence of MPs in marine invertebrates are still scarce. However, the few studies focusing on invertebrate species show the same tendency: ingestion frequencies were found to be 40% in brittle stars, 73.7% in sea stars, 28.6% in sea snails (Courtene-Jones et al., 2017), 63% in brown shrimps (Devriese et al., 2015), 33.5% in gooseneck barnacle (Goldstein and Goodwin, 2013) and up to 83% in Norway lobsters (Murray and Cowie, 2011). It is interesting to note that the frequency of positive individuals for brown shrimps from the Channel area and the North Sea (Devriese et al., 2015) is very close to our results with the shrimp species, with 63% and 65% of positive individuals, respectively. In the present study, results from fish species vary widely (ingestion frequencies of 5.9%, 25% and 55% for rocklings, Norway pouts and long rough dabs, respectively), reflecting results from the current body of literature. However, the discrepancy in extraction methods used in the different studies does not allow a direct result comparison between studies. On the contrary, in our study results from different species can be directly compared, the same extraction protocol being applied to all the species. Moreover, the use of 10% KOH was found to greatly improve MP recovery while preventing MP impairment (Avio et al., 2015; Dehaut et al., 2016), and the use of 8 µm filters allowed the recovery of the small MP fraction, which was found to be a significant part of the MPs found (58.8% of MPs < 200µm). We can therefore conclude to a good MP recovery in our study.

The vast majority of extracted polymers were PE (53.9%) and PP (16.8%). They are the most produced polymers and account for more than 49% of the plastic polymer demand in Europe (PlasticsEurope, 2017). They are mostly used in packaging and food packaging, but are also found in fishing gear. The main sources for the other polymers extracted are packaging (EVA and polyester), textile (polyacrylic and polyester), fishing gear (PA) and electronics (PBT). The inner part of the Oslofjord undergoes important anthropogenic pressure due to the proximity with the urban area and to the fishing activity. The polymers extracted from the organisms thus reflect plastic pollution in the fjord and its sources: MPs from consumer items (packaging, textiles) and fishing activity.

Our results show that the presence of MPs was not influenced by organisms' habitat, indicating that although most MPs are expected to sink and end up in sediments (Van Cauwenberghe et al., 2015b; Woodall et al., 2014), this does not necessarily reflect on organismal uptake. Similarly, the absence of significant differences between the different trophic levels indicates that no biomagnification occurs along the trophic chain. Therefore, although MP trophic transfer is possible, high trophic level organisms are not necessarily more exposed than other organisms. The results concerning feeding mode indicate that filter-feeders are less exposed to MPs than deposit-feeders or predators, both in terms of frequency and number of MPs found per organism. A first hypothesis to explain this result is that filter-feeders could be more efficient at

selecting particles they ingest, compared to other organisms. Another explanation would be that less MPs are present in the water column right above the sediment surface, where benthic filter-feeders feed, and rather sink down to the sediment and are therefore available for deposit-feeders rather than filter-feeders. PE particles were significantly more found in predators and tertiary consumers, compared to other groups. This could indicate that this polymer could be more easily transferred between species. It can also be due to the multiple exposure routes to MPs for tertiary consumers and predators (*i.e.* ingestion via preys and via the water/sediment), whereas other species are only exposed via the water or the sediment. It is however important to note that the obtained results only reflect the presence of MPs in organisms at a given time and do not necessarily reflect MP uptake across membranes. Thus, visual inspection of samples from heart urchins and dabs, two species with high levels of MPs, showed important amounts of sediment in the organisms digestive tracts; it is therefore likely that MPs would have been excreted concomitantly with sediment particles. Therefore, even though some species present high levels of MP contamination, results should be interpreted with caution and direct extrapolation to physiological impacts avoided. Moreover, the number of species representing the different habitats, trophic levels and feeding modes is quite limited in the present study. Therefore, although it provides an interesting overview of MP contamination depending on various parameters, studies at larger scales are necessary to confirm the trends observed here.

This field study also provides important input for ecotoxicity studies. First, despite its low density, PE was by far the polymer most found in organisms (53.9% of total MPs). It can be hypothesized that biofouling increased particles density and led them to sink to the sediment (Kowalski et al., 2016). PE is therefore a relevant polymer to study in MP ecotoxicity studies. Secondly, our results tend to show that MP presence in benthic and high trophic level organisms is not higher than in other species. Therefore, although biomagnification has been mentioned as a potential issue with MPs (Au et al., 2017; Law and Thompson, 2014), field data currently do not support this assumption. Finally, MPs extracted from organisms present a wide variety of shape and size, which can highly affect the sorption of organic pollutant (Rochman, 2015). It is therefore important that ecotoxicity studies take more into account this diversity of shape and size when assessing the effects of MPs.

5. Conclusion

MPs were found in every analysed species, highlighting the widespread MP contamination in areas presenting high anthropogenic pressure, such as the inner Oslofjord. Eight polymer types were extracted from the organisms, PE and PP being the most found. The extracted MPs were mostly flakes, followed by fibres and other fragments, and their size ranged from 41 µm to fibres as long as 9 mm, the fraction below 200 µm accounting for more than 58% of the total MPs extracted. This highlights that MP quantification based on water sampling, usually excluding MPs smaller than 333 µm, leads to an important underestimation of the presence of MPs and should be coupled with analyses of biotic samples. Our results indicate that MP ingestion does not seem to be influenced by organisms' habitat or trophic level, while organisms' feeding mode could affect MP uptake. The present study provides important data on MP occurence in benthic

and epibenthic organisms, but larger studies should be conducted to confirm these results and assess potential spatial and temporal variations in MP presence in organisms.

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