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

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ABSTRACT

The exponential production and use of plastics has generated increasing environmental release over the past decades, and microplastics (MPs) have been reported across all the oceans. Field studies have documented the occurrence of MPs in several species, but important knowledge gaps still remain. In the present study, we characterized the distribution of MPs in ten sediment-dwelling and epibenthic species representative of different habitat, feeding modes and trophic levels within the inner Oslofjord (Oslo, Norway), an area subjected to moderate anthropogenic pressures. Analysed species included fish, bivalves, echinoderms, crustaceans and polychaetes. MPs were present in all the species with a frequency up to 65% of positive individuals for some species. In most cases, 1 or 2 MPs were found per individual, but some organisms contained up to 7 particles. A total of 8 polymer typologies were identified, with PE and PP being the most common according to our extraction protocol. MP sizes ranged from 41 μm to lines as long as 9 mm. Our results indicate that occurrence of MPs in analysed biota is not influenced by organism habitat or trophic level, while characteristics and typology of polymers might be significantly affected by feeding mode of organisms.

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, weighting 268 940 tons (Eriksen et al., 2014). Microplastics (MPs), commonly referred to as 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 derive by the degradation and breakdown of larger plastic debris (Andrady, 2011). The presence of MPs in marine environments has been reported throughout the world's oceans (Van Cauwenberghe et al., 2013) including the most remote areas (Thompson, 2015), increasing the likelihood of interactions between these particles and marine biota. MP ingestion has been confirmed in a range of field studies and reported for several marine species collected at different locations (see Lusher, 2015, for review).

Experimental studies are also increasingly documenting that MPs may have harmful effects on organisms, including the onset of physical damages, endocrine disruption, impacts on energy budget, immune system and reproduction, (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). Laboratory exposures do not reflect the entire complexity of the environment and experimental doses are often higher than those observed in natural conditions. Nonetheless, the appearance of subtle cellular effects has been recently documented in marine mussels exposed to low doses of MPs (Pittura et al., 2018) supporting the potential risk that these particles, under natural conditions of chronic and multi-stressors exposure, may lead to long-term adverse consequences on organisms' health status.

The ecotoxicological concerns for MPs have also been debated as

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sources of exposure to chemical additives used during manufacturing processes, or adsorbed from the environment (Pittura et al., 2018). It has been demonstrated that MPs can be transferred up to higher trophic levels via prey ingestion (Batel et al., 2016; Mattsson et al., 2017, 2015; Tosetto et al., 2017), and laboratory studies showed that the uptake of these particles is influenced by organisms feeding mode (Scherer et al., 2017). In this respect, species with different ecological characteristics can be expected to have different susceptibility to MP contamination, either through direct exposure or via trophic transfer (Desforges et al., 2015). Although a similar possibility may have great influence on the biological impact of these particles on different species, only a few studies considered the importance of trophic level and feeding mode on MP intake in the field (Courtene-Jones et al., 2017; Lusher et al., 2013). It is therefore crucial to have a better knowledge of the presence and distribution of MPs along marine food webs to enhance our possibility to predict the ecological effects of MPs.

The majority of field data focus on MPs in fish, while more limited are those available for invertebrates, particularly for species not intended for human consumption (Bellas et al., 2016; Desforges et al., 2015; Devriese et al., 2015; Goldstein and Goodwin, 2013; Lusher et al., 2013; Van Cauwenberghe and Janssen, 2014). Also for Nordic marine environments, MP ingestion has been well documented in pelagic fish species, while knowledge is scarce on invertebrates and benthic/epibenthic organisms (Bråte et al., 2017). Among the available studies, benthic invertebrates from the North Atlantic were shown to ingest mostly fibers (Courtene-Jones et al., 2017), 83% of Norway lobster contained MPs (Murray and Cowie, 2011) and these particles appeared generally widespread in invertebrates (Karlsson et al., 2017; Leslie et al., 2017).

Considering that sediments are thought to be the final sink for most MPs (Van Cauwenberghe et al., 2013, 2015b), the present study aims to provide additional insights on MP distribution in benthic and demersal organisms from the inner Oslofjord, an urban area with contrasting anthropogenic pressures and uses (*e.g.* recreational and commercial fishing, sailing, presence of leisure craft, local and international ferries, cruise ships and two sewage water treatment plants). To test the hypothesis that organisms habitat, trophic level and feeding mode could influence the occurrence of MPs in biota, a comprehensive quantification and characterization of extracted particles was compared in terms of size, shape, colour and chemical composition in ten species with different ecological and biological characteristics.

2. Material and methods

2.1. Sampling and species

Sampling was carried out in May and September 2017. Collected species comprise bivalves (*Ennucula tenuis*), brittle stars (*Ophiura albida* and *Amphiura filiformis*), heart urchins (*Brissopsis lyrifera*), polychaetes (*Hediste diversicolor* and *Sabella pavonina*), shrimps (*Crangon allmanni*) and fish (*Hippoglossoides platessoides*, *Enchelyopus cimbrius* and *Trisopterus esmarki*). Species habitat, feeding mode and trophic level are presented in Table 1. *H. diversicolor* individuals were collected at the shoreline close to a small marina at Jeløya, Norway (59.45N 10.63E), manually picked, transferred in glass containers and kept on ice during transport; they were further rinsed with milli-Q water to remove particles attached to the body, then individually stored at −80 °C prior to further treatment. All other species were sampled in the inner Oslofjord, Norway (59.83N 10.50E). Fish were caught trawling at 100–110 m depth during a seasonal survey in the Oslofjord: organisms were euthanized and entire digestive tracts were removed from buccal cavity to anus, individually kept on ice during transport, then further stored at −80 °C. For all other species, sediment was collected with a grab and organisms were manually picked, kept on ice during transport, further rinsed with milli-Q water and stored at −80 °C.

2.2. MP extraction and characterization

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.

MP extraction procedure was based on a slight variation of the protocol proposed by Avio et al. (2015), consisting in a predigestion with KOH (as suggested by Dehaut et al., 2016) before the separation through the NaCl hypersaline solution. The original method was previously validated and standardized on samples spiked with MPs of different types and sizes; in a comparison with other available methodologies, it showed a recovery yield higher than 90% for particles smaller than 100 μm, 95% for greater ones, and no effects on polymer characteristics. Both the variation with KOH and the original method were also cross evaluated within various groups of two JPI Oceans Projects, EPHEMARE and BASEMAN, respectively. The method has already been tested with several types of samples (*i.e.*, turtles, fish, mussels, shrimps, sea urchins), resulting efficient also for brittle stars and heart urchins which were perfectly digested. Therefore, there was no need to test another digestion method and the same protocol was applied for all the species for comparison purpose.

All 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 min, decanted for further 10 min 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 four shape categories: textile fibres (*i.e.* ribbon, not regular along the particles and frayed ends), lines (*i.e.* filament, strands and threads with regular shape and without frayed ends), 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 between 60% and 70%, a more accurate interpretation of the obtained spectra was performed (Lusher et al., 2013).

2.3. Quality assurance and quality control

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 step. Glass and metal material was used whenever possible and rinsed with milli-Q water before use, including during