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Environmentally relevant microplastic exposure affects sediment-dwelling bivalves☆

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ABSTRACT

Most microplastics are expected to sink and end up in marine sediments. However, very little is known concerning their potential impact on sediment-dwelling organisms. We studied the long-term impact of microplastic exposure on two sediment-dwelling bivalve species. *Ennucula tenuis* and *Abra nitida* were exposed to polyethylene microparticles at three concentrations (1; 10 and 25 mg/kg of sediment) for four weeks. Three size classes (4–6; 20–25 and 125–500 μm) were used to study the influence of size on microplastic ecotoxicity. Microplastic exposure did not affect survival, condition index or burrowing behaviour in either bivalve species. However, significant changes in energy reserves were observed. No changes were observed in protein, carbohydrate or lipid contents in *E. tenuis*, with the exception of a decrease in lipid content for one condition. However, total energy decreased in a dose-dependent manner for bivalves exposed to the largest particles. To the contrary, no significant changes in total energy were observed for *A. nitida*, although a significant decrease of protein content was observed for individuals exposed to the largest particles, at all concentrations. Concentration and particle size significantly influenced microplastic impacts on bivalves, the largest particles and higher concentrations leading to more severe effects. Several hypotheses are presented to explain the observed modulation of energy reserves, including the influence of microplastic size and concentration. Our results suggest that long-term exposure to microplastics at environmentally relevant concentrations can impact marine benthic biota.

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1. Introduction

Plastic pollution has been an increasing concern for the past decades due to an exponential increase in the production and release of plastics. Recent estimates indicate that 275 million tons of land-based plastic wastes were generated in 2010, resulting in up to 12.7 million tons entering the oceans (Jambeck et al., 2015). Over 5 trillion plastic items have been estimated to float close to the surface of the oceans (Eriksen et al., 2014) and plastic waste is now found throughout the world's oceans (Van Cauwenberghe et al., 2013). The smallest debris, called microplastics when smaller than 5 mm, are less visible than larger plastics but of large environmental concern. Many studies have shown adverse impacts of microplastics on marine organisms, such as polychaetes (Besseling et al., 2013; Browne et al., 2013; Van Cauwenberghe et al., 2015a; Wright et al., 2013), crustaceans (Lee et al., 2013; Watts et al., 2015; Welden and Cowie, 2016), zooplankton (Cole et al., 2013) and fish larvae (Mazurais et al., 2015). It has also been shown that microplastics have the potential to induce deleterious effects in bivalves, such as altered antioxidant capacity, genotoxicity, neurotoxicity, impacts on the immune system (Avio et al., 2015; Ribeiro et al., 2017) and strong inflammatory responses (von Moos et al., 2012). Impacts at the tissue level were observed as histological changes in the digestive gland of mussels exposed to high density polyethylene (von Moos et al., 2012). Microplastics were also shown to affect reproductive parameters and feeding behaviour in oysters (Sussarellu et al., 2016). Despite their environmental relevance, there appear to be a limited number of studies addressing sediment-dwelling organisms, recently reviewed by Van Cauwenberghe et al. (2015b). Indeed, due to the development of biofilm over microplastic surfaces, most particles will eventually sink and end up in marine sediments (Van Cauwenberghe et al., 2015b, 2013; Woodall et al., 2014). This biofouling phenomenon

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can also increase particle densities to such an extent that even low-density microplastics, such as polyethylene, will eventually be transported to the seafloor (Kowalski et al., 2016) and potentially affect benthic organisms.

It has been shown that marine bivalves can ingest microplastics (Setälä et al., 2016; Van Cauwenberghe et al., 2015a) and adverse impacts on bivalve feeding behaviour (Wegner et al., 2012), reproduction (Sussarellu et al., 2016) and energy budget (Sussarellu et al., 2016; Van Cauwenberghe et al., 2015a) have been reported. Microplastics were also shown to induce inflammatory responses and histological changes in bivalves (von Moos et al., 2012), DNA damage and neurotoxicity (Ribeiro et al., 2017), cytotoxicity (Canesi et al., 2015; Paul-Pont et al., 2016) and altered phagocytic activity (Canesi et al., 2015). *Mytilus edulis* is by far the species most frequently used in microplastic effect studies (Van Cauwenberghe et al., 2015b) and studies involving bivalves with other feeding modes are scarce. However, studies on species presenting different feeding strategies are important as feeding mode influences microplastic ingestion (Setälä et al., 2016) and potentially effects. It has been suggested that deposit-feeders may be particularly vulnerable to plastic debris (Depledge et al., 2013).

In the present study, part of the JPI Oceans' project EPHEMARE, we investigated the effects of long-term exposure to polyethylene (PE) on two widely distributed benthic bivalve species, *Ennucula tenuis* and *Abra nitida*. *E. tenuis* is a sub-surface deposit feeder, feeding in the top 2–3 cm of the sediment (Austen et al., 1998; Widdicombe et al., 2000); *A. nitida* is a surface deposit-feeder, feeding on the sediment surface (Grémare et al., 2004). Due to their feeding mode, these two species will potentially be highly exposed to microplastics present on or close to the sediment surface. We hypothesise that long-term microplastic ingestion may impact bivalve energy reserves and ultimately result in effects at the individual level, such as reduced general condition and modified burrowing behaviour. Despite the growing number of effect studies on microplastics, many knowledge gaps remain. It has been specifically pointed out that despite the importance of particle size, effect studies are too often conducted with one size of microplastics only, at concentrations much higher than those found in the environment (Van Cauwenberghe et al., 2015b). Therefore, to better understand the influence of particle size and concentration, we studied three microplastic size classes, at concentrations including values observed in the environment.

2. Material and methods

2.1. Materials

Commercially available polyethylene (PE) microparticles (irregular fragments, density 0.96) were provided as dry powder (Micro Powders, Inc.; NY, USA). Three size classes were selected: 4–6 μm; 20–25 μm and 125–500 μm (see Fig. S1 for detailed size distribution). They are hereafter referred to as small, medium and large particles, respectively.

Tris (tris(hydroximetyl)aminomethane, >99.9%), sodium chloride (>99%), phenol (80%), sulfuric acid (95–97%) and tripalmitin (>99%) were purchased from VWR (Oslo, Norway). Triton X-100, TCA (TriChloroAcetic acid, 100%), Bradford reagent, BSA (Bovine Serum Albumin, >98%), chloroform (>99%), dextrose (>99%) and methanol (>99%) were purchased from Sigma-Aldrich (Schnelldorf, Germany).

2.2. Chemical analysis

All microplastics have been analysed for background contamination of three different priority pollutants: benzo [*a*]pyrene (BaP, CAS 50-32-8; Sigma Aldrich; article no. 1002180958; purity ≥96%; Batch: B1760-250 MG; Lot: SLBP 3558V), benzophenone-3 (BP3, CAS 131-57-7; Sigma Aldrich; purity ≥98%; Batch: 59647-50 MG; Lot: BCBL 6989V), and Heptadecafluorooctanesulfonic acid potassium salt (perfluorooctane sulfonate, PFOS, CAS 2785-37-3; Sigma Aldrich; article no. 101637394; purity ≥98%, Batch: 77282-10G, Lot: BCBP6618V). 6 g of PFOS potassium salt contains 5.57 g PFOS isomers. Linear PFOS content is 72%. The selected chemicals represent model compounds for relevant environmental pollutants. They are either used as additives (PFOS) or can be found in plastic because of the production process or adsorption (BaP).

BaP analysis has been conducted according to Larsson et al. (2013). GC vials were filled with 100 ng recovery standard perylene D12 (Chiron) (2 ng/μL in toluene, 50 μL added) and 500 μL of extract transferred from 8 ml vials. In addition, extract vials were rinsed three times with small amount of hexane and added to the sample. The sample volume was reduced to 500 μL using a nitrogen stream. Concentrations of BaP were quantified using a high-resolution GC-MS system (Micromass Autopspec Ultima), separation on a 30 m (0.25 mm i.d., 25 μm film thickness) DB-5MS column (J&W Scientific, Folsom, USA).

The analysis of BP3 has been performed by using an aliquot of the sample (0.3 g) spiked with internal standards (i.e. Benzoic acid-d5, 4-Brombiphenyl etc.) and concentrated with a factor of 10. The sample was extracted with a solvent mixture: ethyl acetate/cyclohexane (1:1). Additionally an external standard calibration, a blank sample and a recovery sample were prepared. The extracts were measured with GC/MS/MS (electron impact ionisation) in MRM mode. Identification and quantification was performed against the retention time and the external calibration using the compound specific selected reaction monitoring (SRM, one quantifier and one qualifier).

Chemical analysis of PFOS has been conducted according to Eriksson et al. (2016). LC vials were prepared with 10 μL of 0.2 ng/μL recovery standard (2 ng of recovery standard was added to empty LC vials). Samples were diluted 10 and 100 times using MeOH (40 v/v %) and ammonium acetate (60 v/v %). Analysis was performed on an Acquity UPLC system coupled to a Xevo TQ-S quadrupole mass spectrometer (Waters Corporation, Milford, USA). A guard column (PFC isolator, Waters Corporation) was inserted between the pump and the injector to prevent contamination from the system. PFOS were separated on 100 mm Acquity BEH C18 column (2.1 mm, 1.7 mm) using MeOH and water as mobile phases, both with 2 mM ammonium acetate.

2.3. Organisms

Abra nitida is a small deposit-feeding bivalve living in the upper 4 cm of muddy sediments. Commonly found in Scandinavian fjords, its distribution ranges from western Finnmark to the Mediterranean Sea and Atlantic coast of Morocco (Wikander, 1980). It is usually an ecologically dominant species when present and plays an important role in the ecosystem functioning due to its feeding mode and bioturbation activity (Wikander, 1980). It also represents an important food item for flatfish (Bellas et al., 2006). *Ennucula tenuis*, formerly named *Nuculoma tenuis*, is a sub-surface deposit feeder largely found in the North Sea and the Bering Sea, where it is widely distributed and common species, and is also found in the Mediterranean Sea ("Marine Species Identification Portal: Nuculoma tenuis,"; Wang et al., 2014). As for *A. nitida*, *E. tenuis* plays an important role in ecosystems due to its bioturbation activity.

A. nitida feeds using its inhalant siphon while buried in the sediment. It extends its siphon at the surface of the sediment and randomly prospects the immediate vicinity of the orifice of the

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