Environmental Pollution 199 (2015) 10–17

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats

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ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form
20 December 2014
Accepted 5 January 2015
Available online

Keywords:
Microplastics
Mytilus edulis
Arenicola marina
Selective uptake
Tissue concentrations
Field conditions

ABSTRACT

We studied the uptake of microplastics under field conditions. At six locations along the French–Belgian–Dutch coastline we collected two species of marine invertebrates representing different feeding strategies: the blue mussel *Mytilus edulis* (filter feeder) and the lugworm *Arenicola marina* (deposit feeder). Additional laboratory experiments were performed to assess possible (adverse) effects of ingestion and translocation of microplastics on the energy metabolism (cellular energy allocation) of these species. Microplastics were present in all organisms collected in the field: on average 0.2 ± 0.3 microplastics g^{-1} (*M. edulis*) and 1.2 ± 2.8 particles g^{-1} (*A. marina*). In a proof of principle laboratory experiment, mussels and lugworms exposed to high concentrations of polystyrene microspheres (110 particles mL^{-1} seawater and 110 particles g^{-1} sediment, respectively) showed no significant adverse effect on the organisms' overall energy budget. The results are discussed in the context of possible risks as a result of the possible transfer of adsorbed contaminants.

1. Introduction

Plastics are present in every aspect of our everyday life. Because of their favourable properties (versatile, lightweight, strong, durable and cheap) they are used in a myriad of applications, ranging from household and personal goods, clothing and packaging to construction materials and transport (Andrady, 2011). This widespread use has driven the annual world production of plastic from 1.7 million tonnes in the 1950s, when mass production of plastics started, to 288 million tonnes in 2012 (PlasticsEurope, 2013). Even though the societal benefits of plastics are undeniable (Andrady and Neal, 2009), there are some serious environmental concerns associated with these materials. While a part of this plastic waste is properly managed (recycled or combusted), Thompson (2006) estimated that 10% of all plastics produced will eventually end up in our oceans and seas.

Once present in the marine environment, plastic marine debris is exposed to degrading forces such as UV-B radiation and physical abrasion by wave action (Andrady, 2011; Barnes et al., 2009). Plastic marine debris items will progressively fragment into smaller and smaller pieces, until they become microplastics. Depending on the author, different definitions for microplastics are used: microplastics have been defined as particles smaller than 5 mm (e.g. Arthur et al., 2009), yet some set the upper size limit at 1 mm (e.g. Browne et al., 2010; Vianello et al., 2013; Dekiff et al., 2014). As we are interested in the ingestion of microplastics by invertebrates, the upper size limit of 1 mm was adopted. The presence of these so-called microplastics has been demonstrated in different marine compartments worldwide such as inter- and subtidal sediments (e.g. Browne et al., 2011; Claessens et al., 2011; Ng and Obbard, 2006; Reddy et al., 2006; Thompson et al., 2004) and in (sub)surface waters (e.g. Collignon et al., 2012; Ng and Obbard, 2006; Thompson et al., 2004). Because of their small dimensions, microplastics have a similar size range as planktonic organisms and other suspended particles, making them available to an array of marine invertebrates (Wright et al., 2013b) commonly not affected by larger marine debris. Many of the latter feed by collecting and sorting particulate matter, applying a feeding strategy that allows

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http://dx.doi.org/10.1016/j.envpol.2015.01.008
0269-7491/

them to trap and ingest anything of appropriate size (Moore, 2008). The uptake of microplastics by these organisms will depend on a combination of parameters (i.e. size, shape and density of the plastic particle) that determine the position of these particles in the water column, and hence the availability to animals. Typically, low-density particles will float in the water column while high-density particles tend to sink and accumulate in the sediment, making them available to filter- or deposit feeders, respectively (Browne et al., 2007). Laboratory experiments have shown that various marine invertebrates (exhibiting different feeding strategies) ingest microplastics: amphipods (detrivores), lugworms (deposit feeders) and barnacles (filter feeders) (Thompson et al., 2004) as well as sea cucumbers (deposit and suspension feeders) (Graham and Thompson, 2009). Experiments focusing on particle selection demonstrated that filter feeding bivalves will ingest polystyrene microparticles (see Ward and Shumway (2004) for more information). Once ingested, microplastics have the potential to translocate from the digestive tract to the circulatory system of the organisms. Browne et al. (2008) showed that in the marine bivalve *Mytilus edulis* ingested polystyrene microspheres (3 and 10 μm) translocated to the circulatory system. Smaller particles seem to undergo translocation more readily than larger ones. von Moos et al. (2012) demonstrated that small plastic particles (>0–80 μm) can accumulate in epithelial cells of the digestive system (more specifically the digestive tubules), where they induce adverse effects, such as a strong inflammatory response, after only 3 h of exposure.

When assessing the ingestion and translocation of microplastics in marine invertebrates, the test organisms are usually exposed to extremely high concentrations of microplastics. For example, in laboratory experiments Thompson et al. (2004) exposed (intertidal) lugworms to 1.5 g of microplastics per litre of sediment, corresponding to 1.17 g microplastics kg^{-1} dry sediment (average sediment density of 1600 kg m^{-3} (Fettweis et al., 2007) and average wet/dry ratio of 1.25). These concentrations seem to be unrealistically high as Claessens et al. (2011), for example, reported an average 0.35 mg microplastics kg^{-1} dry sediment for Belgian intertidal shores (Table 1). In general, experimental microplastic concentrations used in uptake and translocation studies with marine species (Table 2) are much higher (up to 5000 times) than realistic environmental concentrations. While such an approach is often necessary to predict effect concentrations and assess the tested pollutant (especially with regards to emerging pollutants such as microplastics), testing at high, non-natural, concentrations does not provide any information on the current environmental situation, which is equally, if not more, important. Unfortunately, to date, there is only limited evidence (Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014) that organisms in the field take up significant amounts of microplastics and accumulate them.

Here, we examined the presence of microplastics in 'naturally exposed' marine organisms. The blue mussel *M. edulis* and the lugworm *Arenicola marina*, representing different feeding strategies (filter feeder vs. deposit feeder) and different marine compartments (water column vs. sediment), were studied. In addition, to test the hypothesis whether microplastic ingestion adversely affects the energy metabolism, both model species were exposed to high concentrations of microplastics in the laboratory for 14 days after which their energy status was assessed.

Table 1
Average concentrations of microplastics (<1 mm) found in sediments worldwide.

Country	Location	Reported concentration	Normalized concentration[b]	Reference
UK	Beach	Max. 8 items 50 mL^{-1}	Max. 125 items kg^{-1} dry	Browne et al. (2010)
Brazil	Beach	100 items 0.01 m^{-2}[a]	313 items kg^{-1} dry	Costa et al. (2010)
Belgium	Harbour	166.7 items kg^{-1} dry 2.7 mg kg^{-1} dry	166.7 items kg^{-1} dry 2.7 mg kg^{-1} dry	Claessens et al. (2011)
	Continental Shelf	97.2 items kg^{-1} dry 1.1 mg kg^{-1} dry	97.2 items kg^{-1} dry 1.1 mg kg^{-1} dry	
	Beach	92.8 items kg^{-1} dry 0.5 mg kg^{-1} dry	92.8 items kg^{-1} dry 0.5 mg kg^{-1} dry	
Portugal	Beach	Max. 21 items m^{-2} [a]	Max. 1 item kg^{-1} dry	Martins and Sobral (2011)
Italy	Lagoon	1445 item kg^{-1} dry	1445 item kg^{-1} dry	Vianello et al. (2013)
Germany	Beach	Max. 2.3 items kg^{-1} dry	Max. 2.3 items kg^{-1} dry	Dekiff et al. (2014)

[a] Top 2 cm of sand.
[b] Converted using an average sediment density of 1600 kg m^{-3} (Fettweis et al., 2007) and 1.25 as average wet/dry ratio.

Table 2
Microplastic ingestion in laboratory experiments by various marine organisms.

Organism	Microplastic size (μm)	Exposure concentration	Unit	Reference
Echinoderm larvae	10–20	1000–2400	particles mL^{-1}	Hart (1991)
Amphipod	20–2000	1	g indiviual^{-1}	Thompson et al. (2004)
Lugworm	20–2000	1.5	g L^{-1} sediment	
Barnacle	20–2000	1	g L^{-1} seawater	
Mussel	3–9.6	42	particles mL^{-1}	Browne et al. (2008)
Sea cucumber	250–15,000	16.7	g L^{-1} sediment	Graham and Thompson (2009)

2. Materials & methods

2.1. Sampling

Biota, water and sediment were collected at 6 sampling stations along the French, Belgian and Dutch North Sea coast, in late summer of 2011 (Fig. 1). Three of these stations (S3 and S5 in Belgium and S1 in France) are located close to coastal harbours where shipping and industrial activity is high. *M. edulis* (size: 4–4.5 cm) were collected randomly on the local breakwaters. Additionally, two 10 L water samples were taken near the breakwater using a bucket rinsed with filtered deionised water (FDW, 0.8 μm membrane filter, Supor®800, GelmanSciences). *A. marina* (size: 7–11 cm) were collected in the intertidal zone by means of a bait-pump or shovel. The lugworms were rinsed with filtered seawater (FSW, 0.8 μm, Supor®800, GelmanSciences) in order to remove all external sediment, and subsequently transferred per 2 to a jar containing 50 mL FSW. In the area in which the lugworms were sampled, six 0.5 L sediment samples were collected by removing the upper 5 cm with a metal scoop. *A. marina* was not present in all sampling stations: lugworm activity was only visible in S1, S2 and S5. Hence, sediment samples were only collected at these sampling stations.

2.2. Microplastics in environmental samples

The organisms were kept in 250 mL glass jars containing 150 mL FSW (mussels per 3, lugworms per 2) for 24 h after sampling to allow complete gut clearance. During gut clearance, the FSW in which the organisms were kept was changed regularly to prevent re-uptake of egested material. Faeces were collected using a 35 μm sieve. Collected animal faeces were transferred to a 15 mL centrifugation tube and subjected to NaI-extraction (Claessens et al., 2013).

After the 24 h-clearance period, the organisms' soft tissues were