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Microplastics in bivalves cultured for human consumption

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ABSTRACT

Microplastics are present throughout the marine environment and ingestion of these plastic particles (<1 mm) has been demonstrated in a laboratory setting for a wide array of marine organisms. Here, we investigate the presence of microplastics in two species of commercially grown bivalves: *Mytilus edulis* and *Crassostrea gigas*. Microplastics were recovered from the soft tissues of both species. At time of human consumption, *M. edulis* contains on average 0.36 ± 0.07 particles g^{-1} (wet weight), while a plastic load of 0.47 ± 0.16 particles g^{-1} ww was detected in *C. gigas*. As a result, the annual dietary exposure for European shellfish consumers can amount to 11,000 microplastics per year. The presence of marine microplastics in seafood could pose a threat to food safety, however, due to the complexity of estimating microplastic toxicity, estimations of the potential risks for human health posed by microplastics in food stuffs is not (yet) possible.

1. Introduction

Plastic debris is ubiquitously present in the world's seas and oceans. Although the consequences of the plastic macrodebris are well known for (vertebrate) wildlife (Derraik, 2002; GEF, 2012), there is increasing evidence that microplastics (plastic particles < 1 mm) also exert an impact on marine biota. Microplastics are the products of the degradation of larger plastic items into smaller fragments (Andrady, 2011; Cole et al., 2011). Additional sources of microplastics include microplastics present in cosmetics (Fendall and Sewell, 2009) and microplastic fibres from synthetic fabrics such as polyester and polyamide (Browne et al., 2011; Dubaish and Liebezeit, 2013). The impact of microplastics on marine organisms will depend on a combination of parameters that determine the position of these particles in the water column. Typically, high-density particles will sink and accumulate in the sediment, while low-density particles float at the sea surface, although biofouling, turbulence and freshwater input may result in vertical mixing (Browne et al., 2007; Kukulka et al., 2012; Morét-Ferguson et al., 2010).

Because of their small dimensions, microplastics become available for ingestion to a wide range of marine organisms. Ingestion has already been demonstrated for organisms at the base of the food chain: a large variety of planktonic organisms, such as copepods, euphausiacea (krill) and larval stages of molluscs, decapods and echinoderms (Cole et al., 2013; Hart, 1991; Lee et al., 2013) will take up microplastics while feeding, as well as other invertebrates, such as polychaetes, bivalves, echinoderms and decapods (Graham and Thompson, 2009; Murray and Cowie, 2011; Thompson et al., 2004). Microplastics can either be ingested directly or indirectly through the consumption of lower trophic level prey (Farrell and Nelson, 2013). This may result in a limited food uptake through the blockage of feeding appendages and the alimentary canal (Cole et al., 2013; Murray and Cowie, 2011). Moreover, ingested microplastics have the potential to be taken up by epithelial cells of the intestinal tract (von Moos et al., 2012) and even translocate through the intestine wall to the circulatory system (Browne et al., 2008) of exposed mussels. Microplastic ingestion does not only cause physical harm but can also act as vectors of additives incorporated during manufacture (e.g. polybrominated diphenyl ethers (PBDE)) and organic pollutants sorbed from the surrounding seawater (e.g. polychlorinated biphenyls (PCBs)) (Teuten et al., 2009) to biota. The ecological significance of this transport was recently questioned by Koelmans et al. (2013). Nevertheless, due to their persistent nature, microplastic abundance in the marine environment will only increase. The increasing scientific evidence that numerous marine (invertebrate) species ingest microplastics is an indication that these microscopic plastic particles are entering the marine food chain. Taking into consideration that the global food supply of seafood, both from capture and aquaculture production, was over 125 × 10^6 tonnes in 2009 (FAO, 2012), consequences for human food safety need to be considered.

Aquaculture production of seafood (both finfish and shellfish) is mainly performed in open systems, i.e. in natural seawater. During

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their growth, the cultured organisms are hence exposed to any pollutant present in the seawater, including microplastics. Due to the small sizes of microplastics (i.e. micrometre size range), sampling and extraction from seawater is challenging. As a result, seawater concentrations of microplastics are rather limited in scientific literature, especially when compared to sediment concentrations. Reported seawater concentrations exhibit large spatial variability, ranging from less than one fibre per m^3 (Thompson et al., 2004) to several hundreds of particles and fibres per m^3 (Ng and Obbard, 2006; Van Cauwenberghe et al., 2013). Even though the existing data are too limited to determine a realistic natural concentration of microplastics in seawater, the potential for ingestion by commercially important species, however, remains a cause for concern. Bivalves are of particular interest since their extensive filter-feeding activity exposes them directly to microplastics present in the water column.

In this study, we investigate the presence of microplastics in seafood. To test the hypothesis that cultured bivalves contain microplastics, microplastic load of two widely farmed and commercially important species was determined: the mussel *Mytilus edulis* and the oyster *Crassostrea gigas*, with a global production of 2.1×10^5 tonnes and 6.6×10^5 tonnes in 2010, respectively (FAO, 2012). Any microplastic detected in these cultured animals is a particle that will end up in the human food chain. Therefore, results are discussed in the context of food safety and possible impacts on human health.

2. Materials & methods

Mytilus edulis were acquired directly from a mussel farm in Germany. The organisms were of adult size (5.2 ± 0.4 cm) and were reared for several years in the North Sea. *Crassostrea gigas* were bought in a supermarket and originated from Brittany, France. The oysters were reared in the Atlantic Ocean, and had an average shell length of 9.0 ± 0.5 cm.

Upon arrival at the lab, half of the organisms (*M. edulis*: $N = 36$; *C. gigas*: $N = 10$) were prepared for a three day depuration period, while the other half (*M. edulis*: $N = 36$; *C. gigas*: $N = 11$) was prepared for immediate acid digestion. The organisms assigned to the former treatment were kept in 250 mL glass jars (mussels per three, oysters individually) containing 200 mL filtered artificial seawater (Instant Ocean; 0.8 μm membrane filter, Supor®800, GelmanSciences) for three days to allow them to clear their gut. Prior to use, the glass jars were rinsed three times with filtered deionised water (0.8 μm membrane filter, Supor®800, GelmanSciences). Daily, the water in the test vessels was renewed to ensure that previously egested material, including microplastic particles, would not be ingested again. During this depuration period, starvation and associated retention of particles in the animals' guts, was prevented by daily feeding with the algae *Isochrysis galbana*, which was cultured in clean and sterile conditions.

After three days of depuration for the former organisms, and upon arrival at the lab for the remaining animals, the organisms were removed from their shell and soft tissue wet weight was determined. Subsequently, the soft tissues were destructed as described in Claessens et al. (Claessens et al., 2013). In summary, the animals were left overnight in 69% nitric acid (20 mL for three mussels; 25 mL for one oyster), followed by 2 h of boiling, and dilution (1:10 v/v) with warm (~80 °C) filtered deionised water (0.8 μm membrane filter, Supor®800, GelmanSciences). This solution was subsequently filtered, while still warm, over a 5 μm cellulose nitrate membrane filter (Whatman AE98). After digestion, the filters were dried at 40 °C for 24 h, and analysed for the presence of microplastics using a microscope (Olympus BX41 at magnification 200×). The length and width of the detected particles were determined and, based on the largest dimension (length), every particle was assigned to one of five distinct size classes: 5–10 μm, 11–15 μm, 16–20 μm, 21–25 μm and >25 μm.

Contamination with airborne fibres is a recurring phenomenon in microplastic research (Davison and Asch, 2011; Foekema et al., 2013), and as a result rigorous precautions should be taken while processing samples. In this study, extensive measures were adopted to avoid any contamination while handling and processing samples. A 100% cotton lab coat was worn at all times, all equipment was rinsed three times with filtered deionised water (0.8 μm membrane filter, Supor®800, GelmanSciences) before use and all sample processing was performed in a clean laminar flow cabinet. Additionally, procedural blanks (i.e. samples containing no tissue) were included in every acid destruction performed, to account for any possible contamination.

A sub-set of microparticles, selected based on appearance in order to cover the microparticle diversity detected, was analysed using a micro-Raman spectrometer (Bruker Optics 'Senterra' dispersive Raman spectrometer coupled with an Olympus BX51 microscope) to identify plastic type. The Raman spectrometer was operated at a laser wavelength of 785 nm (diode) and high resolution spectra were recorded in three spectral windows, covering 80–2660 cm^{-1}. The microscope has 5×, 20×, and 50× objectives, with spot sizes of approximately 50, 10, and 4 μm, respectively. The instrument is controlled via the OPUS 6.5.6 software.

3. Results

Microplastics were detected in both *Mytilus edulis* and *Crassostrea gigas* (Fig. 1). Due to the rigorous precautions adopted while handling and processing the samples, contamination with (airborne) microplastics was successfully prevented. Indeed, the procedural blanks were completely free of any form of contamination, both fibre- and particle-shaped.

Low numbers of microparticles were recovered from the tissue of both species tested. In *Mytilus edulis* the average microplastic load in the organisms without depuration was 0.36 ± 0.07 particles per gram of soft tissue (wet weight (ww)). After the three day depuration period, only 0.24 ± 0.07 particles g^{-1} ww were recovered (Fig. 2). The same trend was observed in *Crassostrea gigas*: without depuration on average 0.47 ± 0.16 particles g^{-1} ww were found, while microplastic concentrations decreased to an average of 0.35 ± 0.05 particles per gram soft tissue (ww) after depuration (Fig. 2).

Only the particles that had a red or blue colour yielded distinct Raman spectra (Fig. 3). The obvious colouring of these particles is attributed to the presence of pigments, which interfered with the measurements of the plastic type. As a result the spectra obtained were these for the pigments present in the particles, and not those for the plastic type (Fig. 3). Three pigments were measured: haematite (red pigment) and two types of the blue pigment copper phthalocyanine (PB 15:1 and PB 15:3).

The size class frequency distribution of the microplastics detected in the acid digested tissues is presented in Fig. 4. For both species, the three day depuration period resulted in the removal of all (in *M. edulis*) or the majority (in *C. gigas*) of the largest microplastics (i.e. particles >25 μm in length). In *M. edulis* the most abundant microplastics present after gut depuration were the

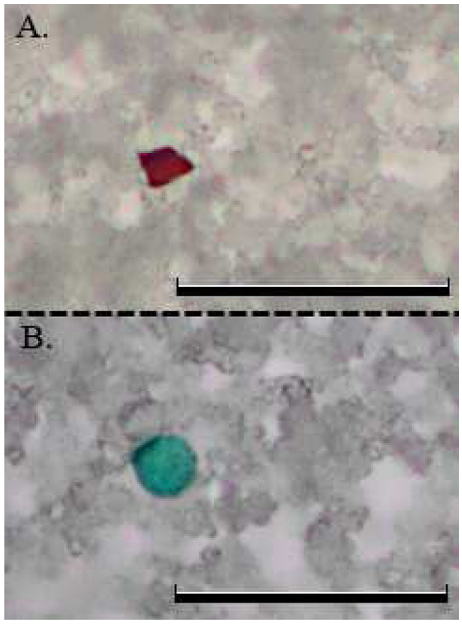

Fig. 1. Microplastics detected in the acid digested *Mytilus edulis* and *Crassostrea gigas*. A. Red particle recovered from *Mytilus edulis*; B. Green sphere detected in the soft tissue of *Crassostrea gigas*. (Scale bar: 50 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

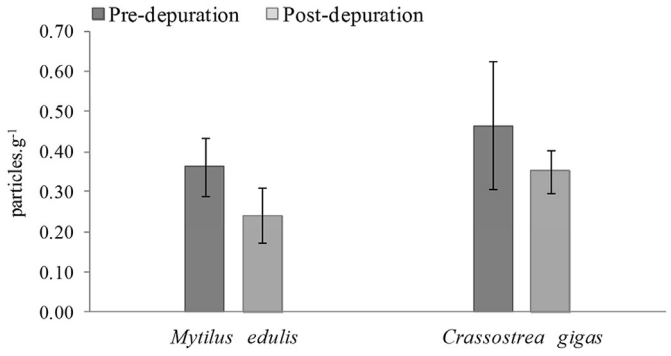

Fig. 2. Average microplastic concentration (particles g^{-1} ww) in the tissues of digested organisms. Before and after a three day depuration period. (Bars represent standard deviation).

particles ranging in size from 5 to 10 μm (50.0%), while in *C. gigas*, the most abundant particles were those in the size ranges 11–15 μm (29.6%) and 16–20 μm (33.3%).

4. Discussion

Our results show that microplastic particles are present in shellfish, more specifically bivalves, cultured for human consumption. *Mytilus edulis* originating from the North Sea contain on average 0.36 ± 0.07 particles g^{-1} tissue at time of consumption (pre-depuration values). When consuming oysters (*Crassostrea gigas*) cultured in the Atlantic Ocean an average of 0.47 ± 0.16

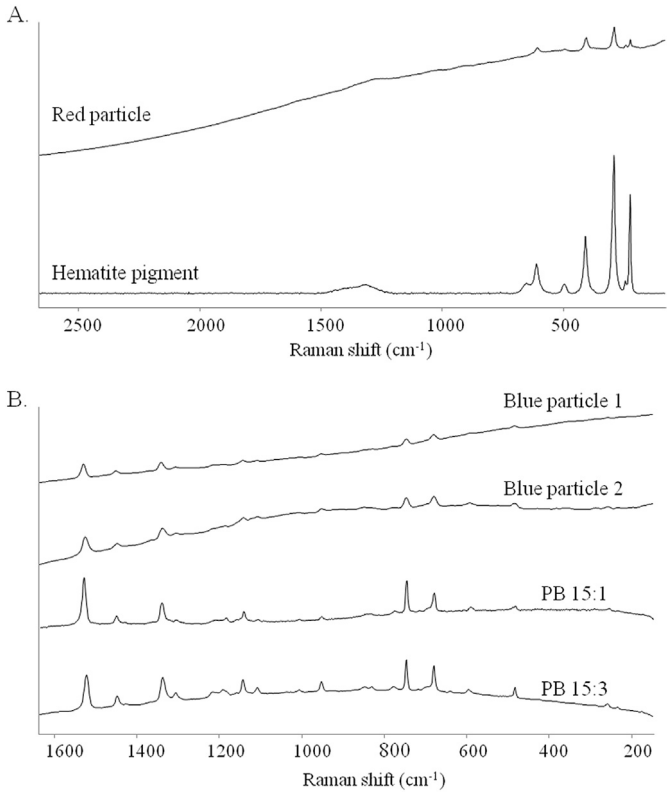

Fig. 3. Identification of microparticles using micro-Raman spectroscopy. A. The Raman spectrum of a red particle extracted from *M. edulis* tissue corresponds to that of the pigment haematite. B. The Raman spectra of two blue particles (particle 1 from *C. gigas* and particle 2 from *M. edulis*) correspond to that of the widely used phthalocyanine dyes PB 15.1 and PB 15:2, respectively.

particles will be ingested per gram of soft tissue. However, due to the use of concentrated HNO_3 during tissue digestion, the microplastic concentrations reported here could be underestimations. Concentrated HNO_3 has a detrimental effect on (nylon) fibres, resulting in the total destruction of this type of microplastic during extraction, resulting in a microfibre extraction efficiency of 0% for this technique (Claessens et al., 2013).

Spectroscopic analysis of a subset of microplastics was performed in an effort to positively identify the detected microparticles as true microplastics. A direct identification (i.e. identification of the plastic type), however, was hindered by the presence of pigments. Processing of the tissue samples in 69% HNO_3 can result in the degradation of the plastic matrix to the extent that the distinct plastic peaks in the spectrum decrease or even disappear (results not shown). This reduced 'plastic signal' is further obscured by the strong signal of the pigments present, hindering the identification of plastic type. Spectroscopic analysis of the blue particles resulted in spectra that correspond with those of phthalocyanine dyes, more specifically copper phthalocyanines. These are synthetic pigments, indicating an anthropogenic origin of these particles. Additionally, these pigments are most commonly used in the plastics industry (Lewis, 2004), which strengthens the assumption that these microparticles are actually microplastics. The second pigment that was positively identified using Raman spectroscopy was haematite, an inorganic red pigment. This mineral iron oxide occurs naturally as a black to grey or brown to dull red mineral (Buxbaum, 1998). The particle that generated the haematite spectrum, however, was bright red indicating this was an anthropogenic particle coloured using haematite as a pigment. The haematite pigment is used in a wide array of applications, including the colouring of plastics. The detection of these pigments in the extracted particles provides with indirect evidence that these particles are of anthropogenic origin, most likely microplastics as these pigments are widely used in plastics. However, as this identification was not successful for all extracted microparticles, only for the blue and red particles, the abundances of microplastic particles reported here may be overestimations as some of the detected microparticles might not be plastic after all.

It is not surprising that seafood contains microplastics: these organisms are cultured in natural conditions. Production of bivalves, such as oysters and mussels, is mainly performed in coastal areas, with the organisms growing on ropes suspended from rafts or on structures built above the seabed. These commercially grown mussels and oysters are not fed by the farmer, they feed on algae naturally present in the seawater. As a result these filter feeders are exposed to any pollutant present in the seawater, including microplastics and other particles, in the same way as their wild counterparts. Ingestion of microplastics of different sizes and shapes by filter feeders has already been demonstrated several times in a laboratory setting (e.g. (Browne et al., 2008; Cole et al., 2013; Thompson et al., 2004; von Moos et al., 2012; Ward and Shumway, 2004)), and has also been detected in wild populations (Van Cauwenberghe et al., submitted). In a recent paper, Mathalon and Hill (2014) detected microfibres in wild and farmed mussels. Farmed mussels had significant higher concentrations of microplastics compared to wild mussels: on average 178 microfibres per farmed mussel compared to an average of 126 microfibres per wild mussel in the most polluted site. These plastic body burdens are 500 times higher than the concentrations in mussels reported in this study. While the use of concentrated HNO_3 in the tissue digestion has detrimental effects on fibres (Claessens et al., 2013), Mathalon and Hill (2014) report a contamination of approximately 100 microfibres per filter.

Part of the mussels and oysters were allowed to clear their gut prior to analysis. In order to achieve gut clearance, the organisms

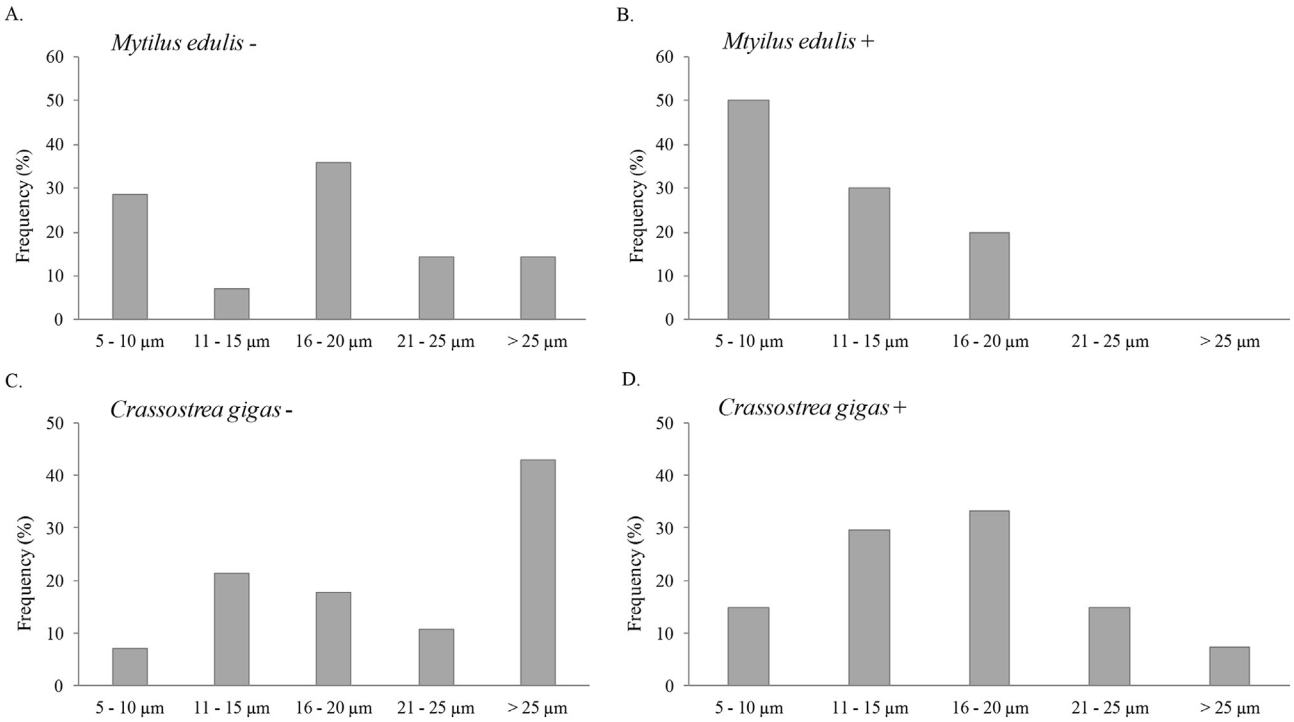

Fig. 4. Size class frequency distribution of microplastics detected in bivalves cultured for human consumption. Assignment to the size classes is based on the largest dimension of the particles. These frequency distributions represent all particles detected per treatment, not per individual. A. *M. edulis* without gut depuration; B. *M. edulis* after a three day gut depuration; C. *C. gigas* without gut depuration; D. *C. gigas* after a three day gut depuration.

were placed in filtered seawater for three consecutive days. Bivalve gut depuration differs greatly, depending on species, temperature and food quantity and quality (Bayne et al., 1987; Hawkins and Bayne, 1984). Typical gut depuration times vary from less than an hour in *Potamocorbula amurensis* (Decho and Luoma, 1991) to up to 15 h in *Mytilus edulis* (Bayne et al., 1987). The three day gut clearance as practiced in this study should hence be sufficient to remove any particles present in the digestive tract. The decreased microplastic body burden observed after three days of gut depuration (Fig. 4) indicates that part of the microplastics detected prior to depuration were present in the digestive tract. The majority of the microplastics, however, appears to be present in the animals on a more permanent basis, since depuration did not result in the removal of these particles. Plastic particles may be retained in the tissues (von Moos et al., 2012) and the circulatory system (Browne et al., 2008), or lodged in the digestive tract (vertebrates e.g. (Denuncio et al., 2011; Lazar and Gračan, 2011; van Franeker et al., 2011); invertebrates (Murray and Cowie, 2011)). The specific removal of larger microplastics as a result of gut depuration might be an indication that the remaining, smaller, particles may have translocated through the gut wall and are subsequently retained in the tissues and circulatory system. Since the largest particles are removed as a result of continued feeding and associated enhanced gut-passage, smaller particles present in the digestive tract should have been egested as well. Especially when considering that in scallop, another filter feeding bivalve, it was demonstrated that larger particles are retained longer compared to smaller particles (Brillant and MacDonald, 2000). As a result, gut retention time is shorter for smaller than for larger particles.

Despite the ever increasing number of scientific reports on the occurrence of microplastics in the marine environment and associated impacts on marine life, this report is the first to report on possible consequences of marine microplastics for humans. The presence of microplastics in seafood is, through entering the human food chain, the first potential direct effect of microplastic pollution on humans. When consuming an average portion of mussels (250 g wet weight) one consumes around 90 particles. An average portion of 6 oysters (100 g ww) contains around 50 particles. Shellfish consumption differs greatly among countries, in Europe for instance mollusc consumption can differ over a factor of 70 between consumers and non-consumers (EFSA, 2011). European top consumers can be found in Belgium (elderly), with a per capita consumption of 72.1 g day^{-1}, while mollusc consumers in France (adolescents) and Ireland (adults) have the lowest per capita consumption: only 11.8 g day^{-1} for both countries (EFSA, 2011). Using the average microplastic concentration detected in this study (i.e. 0.42 particles g^{-1} tissue; average of *M. edulis* and *C. gigas* plastic load without depuration), an annual dietary exposure can be calculated. European top consumers will ingest up to 11,000 microplastics per year, while minor mollusc consumers still have a dietary exposure of 1800 microplastics $year^{-1}$.

Once inside the human digestive tract, intestinal uptake of the ingested particles may occur. Translocation of various types of microparticulates across the mammalian gut has been demonstrated in multiple studies involving rodents (particle size 0.03–40 µm), rabbits (particle size 0.1–10 µm), dogs (particle size 3–100 µm) and humans (particle size 0.16–150 µm) (Hussain et al., 2001). To date, the M-cells (microfold cells) in the Peyer's patches and other intestinal lymphatic tissue are considered the predominant site of uptake. Using 2 µm latex microspheres in rodents, it was shown that intestinal translocation of microplastics is low (0.04–0.3%) (Carr et al., 2012). However, contrasting reports exist on (i) the upper size limit of particles capable of being translocated and (ii) the magnitude of this type of transport (Hussain et al., 2001). Through the M-cells microplastics can enter the lymphatic system. This transport is governed by particle size: in rats, larger particles (5–10 µm) remained in Peyer's patches, while smaller

particles (<5 μm) were transported systematically into the lymph (Eldridge, 1990).

Unfortunately, in current literature there are no data (neither *in vivo* nor *in vitro*) on the toxicity of (translocated) microplastics in humans. It is, however, likely that these particles can absorb luminal molecules to their surface and carry them into mucosal cells during translocation (Powell et al., 2010). In this way, the ingested microparticles have the potential to enhance gut infectivity or immune-stimulatory properties of the biological agents adsorbed to their surface. Additional toxicity of microplastics potentially arises from the leaching of monomers, additives, and even associated POPs. In literature, several authors reported on concentrations of organic pollutants present in/on marine plastics, mainly resin pellets (Endo et al., 2005; Hirai et al., 2011; Mato et al., 2001; Mizukawa et al., 2013). There is even some evidence of uptake of these adsorbed contaminants into the tissues of birds: both indirect evidence (Ryan et al., 1988; Tanaka et al., 2013) as well as experimental data (Teuten et al., 2009) support plastic-mediated transfer of contaminants to seabirds. These studies, however, focus on larger pieces of plastics (several millimetres in size). Koelmans et al. (Koelmans et al., 2013), however, demonstrated the low significance of this transport from microplastics (400–1300 μm in size) to the invertebrate *Arenicola marina*. Toxicity can also be expected from toxic monomers and additives. Monomers leaching from plastic can cause both acute and chronic effects in humans, such as cancer (e.g. vinyl chloride (Awara et al., 1998)) and neurological effects (e.g. styrene (ATSDR Agency for Toxic Substances and Disease Registry, 2010)). Widely used additives, such as phthalates and bisphenol A (BPA), are know endocrine disruptors and have a toxic impact on both wildlife (Oehlmann et al., 2009) and humans (Hugo et al., 2008), even at low, environmental relevant concentrations. Laboratory studies have shown the transfer of another type of widely used plastic additive: PBDEs or flame retardants. Ingestion of plastics lead to the accumulation of PBDEs in the tissues of lugworms and fish (Browne et al., 2013; Rochman et al., 2013). Furthermore, PBDEs present on ingested plastic, but not in natural prey items, were found in the adipose tissues of oceanic sea birds suggesting the transfer of plastic-derived chemicals to wildlife (Tanaka et al., 2013). As there is a growing body of literature on plastic-associated toxicants and their transfer to exposed wildlife, threats to human health through the consumption of microplastics present in seafood are becoming apparent.

We now established that microplastics are present in mussels and oysters, but likely also other types of seafood may be a source of human microplastic intake. Currently, only a preliminary dietary exposure could be estimated. The hazard posed by microplastics will only become clearer with progresses in effect studies. Due to a lack in dedicated studies, a comprehensive assessment of the hazards associated with microplastics is hindered. As a result, estimations of the potential risks for human health posed by microplastics in food stuffs is not (yet) possible.

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