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Feeding type affects microplastic ingestion in a coastal invertebrate community

Outi Setälä [a,*], Joanna Norkko [b], Maiju Lehtiniemi [a]

[a] Marine Research Centre, Finnish Environment Institute, P. O. Box 140, FI-00251 Helsinki, Finland
[b] Tvärminne Zoological Station, University of Helsinki, J.A. Palménin tie 260, FI-10900 Hanko, Finland

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ABSTRACT

Marine litter is one of the problems marine ecosystems face at present, coastal habitats and food webs being the most vulnerable as they are closest to the sources of litter. A range of animals (bivalves, free swimming crustaceans and benthic, deposit-feeding animals), of a coastal community of the northern Baltic Sea were exposed to relatively low concentrations of 10 μm microbeads. The experiment was carried out as a small scale mesocosm study to mimic natural habitat. The beads were ingested by all animals in all experimental concentrations (5, 50 and 250 beads mL^{-1}). Bivalves (*Mytilus trossulus*, *Macoma balthica*) contained significantly higher amounts of beads compared with the other groups. Free-swimming crustaceans ingested more beads compared with the benthic animals that were feeding only on the sediment surface. Ingestion of the beads was concluded to be the result of particle concentration, feeding mode and the encounter rate in a patchy environment.

1. Introduction

Litter is one of the most broadly spread environmental hazards in marine environments. Not only does marine litter cause harm to the economy and welfare of people living close to the sea, but it has also negative effects on vulnerable marine ecosystems. Surveys from different marine areas have shown that most of the marine litter consists of different types of plastics (e.g. Kershaw et al., 2011; OSPAR, 2014). This is also the case in the northern Baltic Sea, where results from a beach litter survey showed that on average 56% of all macrolitter items was plastic, and the most common litter type was unidentified plastic fragments, which constituted on average 25.3% of all macrolitter items (Marlin Baltic Marine Litter, 2014).

Microplastics (present categorization <5 mm, Arthur et al., 2009) are either fragmented from larger plastic items (secondary microplastics) or they are already initially and intentionally small (primary microplastics), e.g. abrasive plastic beads found in some personal care products or used in blast-cleaning (Barnes et al., 2009). Microplastics are found worldwide in marine environments where they have been accumulating for several decades (GESAMP, 2012). Microplastics are of concern especially because of their durability and long life-span (very small, not possible to remove from the sea) and their potential to enter marine food webs. Uptake of microplastics can take place via normal ventilation processes (Watts et al., 2014), or they can be directly ingested when mistaken as food (Thompson et al., 2004; Besseling et al., 2013) and can further be transported within different marine food webs (e.g. Eriksson and Burton, 2003; Setälä et al., 2014).

Evidence from the field has revealed ingestion of microplastics by animals occupying different marine habitats, e.g. pelagic and demersal fish (Lusher et al., 2013), bivalves (Mathalon and Hill, 2014), lobsters (Murray and Cowie, 2011), shore crabs (Watts et al., 2014) and lugworms (Van Cauwenberghe et al., 2015). In addition, many marine invertebrates like bivalves, echinoderms, amphipods and zooplankton have ingested plastic microbeads in controlled laboratory incubations (Browne et al., 2008; Graham and Thompson, 2009; Von Moos et al., 2012; Cole et al., 2013; Setälä et al., 2014).

The harm of ingested microplastics may be mechanical (e.g. clogging of the digestive tract, sticking to external surfaces hindering mobility) or chemical. Microplastics may contain harmful additives that have the potential to leach into their environment and cause harm to marine animals (Browne et al., 2013; Nobre et al., 2015). Microplastics can also accumulate harmful hydrophobic substances from the surrounding water (Endo et al., 2005; Rios et al., 2007). The smaller the plastic fragment is, and thus larger its area: volume–ratio, the bigger its adsorption capacity. It has been proposed that these compounds might bioaccumulate in plastic-ingesting organisms, with unknown consequences to the organisms or to the food web (e.g. Teuten et al., 2009; Bowmer and Kershaw, 2010).

Laboratory experiments on microplastic grazing and accumulation in marine organisms have usually been carried out in controlled conditions in small experimental units, where the organisms have been exposed to a known concentration of plastic particles (Browne et al., 2008; Graham and Thompson, 2009; Cole et al., 2013; Setälä et al., 2014). Such studies have given insight into the potential of microplastic

* Corresponding author.
E-mail address: outi.setala@ymparisto.fi (O. Setälä).

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Table 1
Size, number, feeding and habitat type of the animals used in the experiment.

Taxa	Size range (mm)	N of ind. /unit	Feeding type	Habitat
Macoma balthica	15–24	6	Filter-feeder (sediment): phytoplankton, decomposing material	Sediment
Mytilus trossulus	22–28	6	Filter-feeder (water): phytoplankton, decomposing material	Hard surfaces
Gammarus spp.		2	Herbivore: macroalgae, phytoplankton, periphyton	Among vegetation
Mysid shrimps	14–22	4	Omnivore: plankton and sediment surface	Among vegetation
Monoporeia affinis	8–9	6	Deposit feeder/predator: decomposing material, bivalve larvae	Sediment surface
Marenzelleria spp.	15–30	20	Deposit feeder: decomposing material	Sediment

ingestion by various marine organisms, and raised questions regarding the hazards due to microplastic ingestion. How to apply results from laboratory experiments to natural habitats is challenging, because organisms and their habitat interact with each other, as well as different organisms do with each other. One possibility for collecting realistic data is to study the processes in mesocosms. Mesocosm studies aim to mimic natural conditions and they describe especially well predator–prey interactions and driving forces of community dynamics; bottom-up and top-down regulation (e.g. Olsen et al., 2006). Nevertheless, a major challenge in all experimental studies is the concentration of plastic particles that are used as tracers for ingestion. In order to observe effects in short-term experiments (hours to a few days), it may be necessary to use concentrations that exceed natural concentrations of with one order of magnitude or more (Cole et al., 2013; Setälä et al., 2014).

To get a better understanding of the processes that affect microplastic distribution in coastal habitats and the ingestion of microplastics by different organisms, we set up a small-scale mesocosm experiment, where a coastal community consisting of a range of organisms was exposed to different concentrations of microplastics. The study aimed to investigate microplastic ingestion with plastic concentrations closer to natural concentrations than usually tested, and in experimental conditions that mimicked natural environment of a littoral community consisting of dominant invertebrate taxa of the northern Baltic Sea. As we know that the plastic microspheres used in the study would sediment to the bottom, our working hypothesis was that they would be readily available especially for the animals feeding on the sediment surface.

2. Material and methods

2.1. Experimental set up

Experiments were carried out in 20 L aquaria in autumn in a temperature controlled room (11 °C) in darkness, provided with gentle aeration. Sand and mud collected from the vicinity of Tvärminne Zoological Station, situated at the SW coast of Finland, (59° 49′ N, 23° 17′ E) in the northern Baltic Sea, were sieved with 0.5–1 mm sieves, to remove all macrofauna. After that, sand and mud were thoroughly mixed together and 4 L added to each aquarium forming an approx. 10 cm thick layer. The aquaria were filled with 5 L seawater (salinity 5.7, pH 8.4) and two stones and one stem of bladder wrack were added to each aquarium.

The experimental aquaria contained a selection of animals that are common in the coastal zone of the northern Baltic Sea (Table 1) (Bonsdorff, 2006; Lehtiniemi and Nordström, 2008). For the experiments animals were sieved from mud collected with a van Veen grab at 35 m depth (*Marenzelleria* spp. *Monoporeia affinis* and *Macoma balthica*) or collected from the littoral with a hand net (*Gammarus* spp., the mysid shrimps: *Neomysis integer*, *Praunus flexuosus* and *Mytilus trossulus*). The mud-dwelling animals: polychaetes (*Marenzelleria* spp. 20 ind. per aquarium), amphipods (*M. affinis*, 6 ind.) and bivalves (*M. balthica*, 6 ind.) were let to acclimatize to the experimental conditions for 4 weeks, while the other experimental animals were collected one day before the start of the experiment and placed in the aquaria on that same day. For each aquaria 6 mussels (*M. trossulus*), 2 gammarids (*Gammarus* spp.) and 4 individuals of mysid shrimps (mixture of *N. integer*, *P. flexuosus*) were added.

The experiment was started when fluorescent, symmetrically round 10 μm polystyrene beads (Polysciences inc.) were added in three different concentrations (final concentration: 5, 50 and 250 beads mL^{-1}) to the aquaria, with three replicates for each concentration. These beads have proven to be suitable for food web experiments (e.g. Setälä, Cole); they are denser than water (~1.05 g/cm^3, similar to cell densities), are easy to identify from the water and inside animals, and do not form aggregates. Shortly before the start of the experiment a freshly collected mesozooplankton community, collected with 100 μm and 50 μm plankton nets from the pelagial, was added to all aquaria to offer food for the mysid shrimps. The experiment was terminated after 24 h incubation by filtering out the water and picking/sieving the animals.

2.2. Sample processing and microscopy

The ingestion of microbeads was examined from the experimental animals by direct observation with epifluorescence microscopy (Leica DMIRB, and Leitz Diaplan) at 100–200× magnifications. All animals were fixed with 96% ethanol and dissected under a stereomicroscope (Leica Mz 7.5, 6–50× magnification) using different methods. Bivalves: the shell was opened with a sharp knife; tissues were carefully removed and washed by gently shaking them in particle-free water. After that the mantle was peeled off, the gills separated and the rest of the tissue placed in an Utermöhl settling chamber. The separated gills were placed on an object glass and a coverslip positioned on it. *M. affinis* and gammarids were treated in a similar way. The animals were washed by gently shaking them in particle-free water, after which each individual was placed on its side on an object glass and the carapax opened from the back through the whole length of the animal. Once the back was open, the intestine was removed and placed on an object slide for microscopy. Mysids were washed in particle-free water as described, placed on a petri dish, dissected and their intestines and stomachs opened and placed onto object slides into a small drop of filtered seawater and covered with a coverslip. *Marenzelleria* spp. (approx. 1.5–3 cm long) were washed particle free, and each individual was put in a drop of water on an object slide and squeezed firmly with a coverslip. Zooplankton that was added as prey for mysid shrimps was not collected for microscopy.

2.3. Statistical analysis

Due to non-normality of the data set and heterogeneity of variances, the non-parametric Kruskal–Wallis test for independent samples using the statistical program SPSS (Version 22) was first applied in order to investigate if there were differences between the bead ingestion rates among taxa and among offered bead concentrations. For statistical analysis the taxa were further combined to three groups: bivalves, free swimming crustaceans (mysids and *Gammarus* spp.) and benthic,

Fig. 1. Number of ingested beads (aver ± SD) in two bivalve species *Mytilus trossulus* and *Macoma balthica*, littoral mysids, *Gammarus* spp., *Monoporeia affinis* and *Marenzelleria* spp. in three different bead concentrations (Low = 5, medium = 50 and high = 250 beads mL^{-1}). Note the different scales on the y-axes.

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