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Feeding type affects microplastic ingestion in a coastal invertebrate community

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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

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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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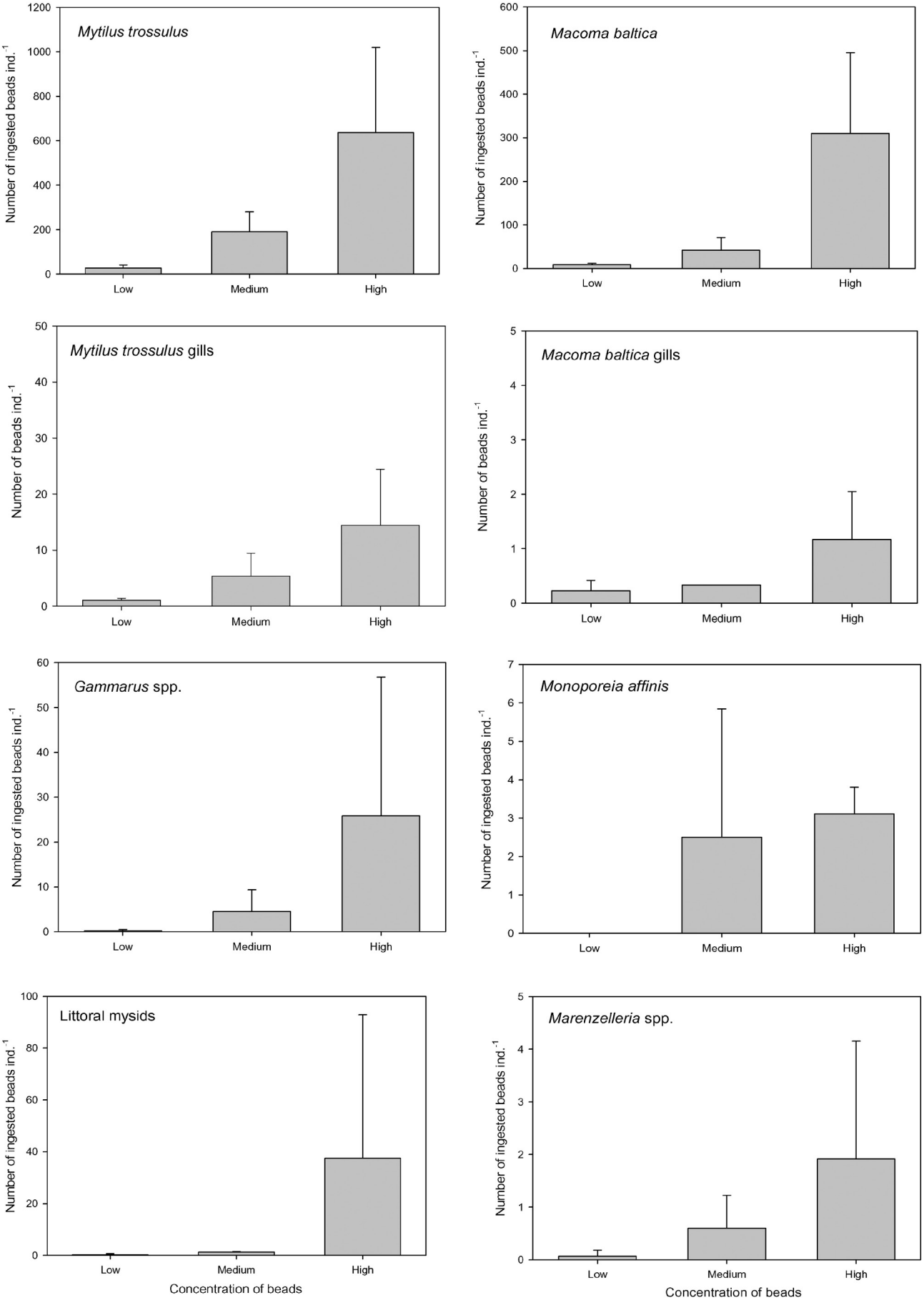

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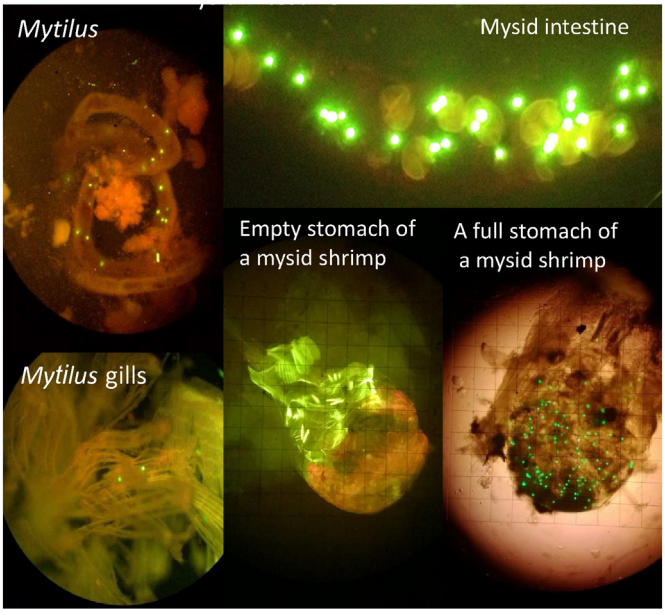

Fig. 2. Microscopy images of ingested microplastic beads.

primarily deposit-feeding animals (*M. affinis* and *Marenzelleria* spp.). The non-parametric analog of Tukey's test was used in post hoc analyses to determine which taxa and groups differed. The non-parametric Related samples Wilcoxon signed rank test was used to compare the number of beads in bivalve digestive tract versus gills.

3. Results

Polystyrene beads were ingested by all species and groups (Figs. 1 and 2). There were significant differences in the amount of ingested beads between the groups (Independent samples Kruskal–Wallis test: $p < 0.0001$). Bivalves (*M. trossulus*, *M. balthica*) contained significantly higher amounts of beads compared with the other groups (Pairwise comparisons: $p = 0.02$ for mysids/*Gammarus*, $p = 0.01$ for *Monoporeia*/*Marenzelleria*) and although *M. trossulus* contained higher amounts of beads than *M. balthica* the difference was not statistically significant. Free-swimming crustaceans (*Gammarus* spp. and littoral mysids) ingested more beads compared with the benthic animals that were feeding only on the sediment surface (*Marenzelleria spp. and M. affinis*, excluding *M. balthica*) (Fig. 1) but the differences were not statistically significant. Beads were found in both the digestive tract and gills of the bivalves (Fig. 2). Significantly higher numbers of beads were however found in the digestive tract (Related samples Wilcoxon signed rank test: $p = 0.008$ for both bivalve species).

The bead concentration affected the number of beads of bivalves (Independent samples Kruskal–Wallis test: $p = 0.027$ for both species) and mysids ($p = 0.026$) (Fig. 1). For all other taxa no significant differences were found. The higher the concentration was in the aquaria the more beads the animals had ingested (Fig. 2). In the lowest concentration the number of ingested beads was very low, close to zero in all species except *M. trossulus* (27.3 ± 14.0 beads ind.$^{-1}$) and *M. balthica* (8.1 ± 3.4 beads ind.$^{-1}$). This was however 2.5–4% of the number of ingested beads in the highest concentration. The intraspecific variation in the number of ingested beads was high especially in the highest concentration, e.g. *M. trossulus* ingested 10–3545 beads ind.$^{-1}$ and *M. balthica* (9–1208 beads ind.$^{-1}$).

In the highest concentration of beads all individuals of both bivalve species, mysids and *Gammarus* spp. contained beads, but only 44% and 33% of *M. affinis* and *Marenzelleria* spp. individuals, respectively (Fig. 3). Bivalves differed clearly from the other groups in the lowest bead concentration. In the lowest concentration 90% of the bivalve individuals contained beads, in other taxa it varied between 0 (*M. affinis*) and 20% of the individuals.

4. Discussion and conclusions

The aim of this study was to investigate the ingestion of plastic microbeads in an experimental set up that would mimic conditions in a coastal habitat. This kind of mesocosm set up gives opportunities to get a better picture of the actual fate of microplastics within a community, instead of experiment carried out with single species.

Our results show clear differences in the microbead ingestion between animal taxa. The behavior and feeding mode of an animal played a major role, largely affecting the number of ingested beads. The lowest number of ingested microbeads was detected in the polychaetes *Marenzelleria* spp. Adult *Marenzelleria* spp. are deposit feeders, feeding on the surface and subsurface layers of the sediment, and living in burrows which, depending on the species, can extend down to 20 cm into the sediment (Zettler et al., 1995, Renz and Foster, 2013). However an earlier study has shown, that *Marenzelleria* spp. transferred small (1 μm) plastic tracer particles only to 1 cm deep into the sediment, while *M. balthica* transported the same particles to 3.5 cm depth (Viitasalo-Frösén et al., 2009). *Marenzelleria* spp. has been found to select smaller particles over the larger ones when particles in the size range of 88–250 μm were offered as food (Bock and Miller, 1999), but we are not aware of any data on the ingestion of particles as small as 10 μm. Since we used small plastic microbeads in our study, we conclude that no selection by *Marenzelleria* spp. was done, and the particles were ingested together with the overall deposited material the worms were feeding on. The number of microbeads found in the guts was thus merely a result of the bead concentration on the sediment surface (encounter rate), bead patchiness and egestion.

M. affinis also ingested a low number of microbeads. It is a small (7–11 mm) amphipod that occupies soft-bottom sediments in the Baltic Sea. It feeds on phytoplankton and other decomposing material, and is also a predator of e.g. bivalve larvae (Ejdung et al., 2000) on the sediment surface (Lopez and Elmgren, 1989). It is a nocturnal swimmer that stays burrowed in the sediment during the day and swims actively in the water column during the night (Lindström and Lindström, 1980). During the experiment *M. affinis* thus had the opportunity to exploit several patches containing microbeads because of this swimming activity. No information of *M. affinis* feeding while swimming in the water column exists, and we assume that it feeds only when it is buried in the sediment. A likely explanation for the low number of ingested

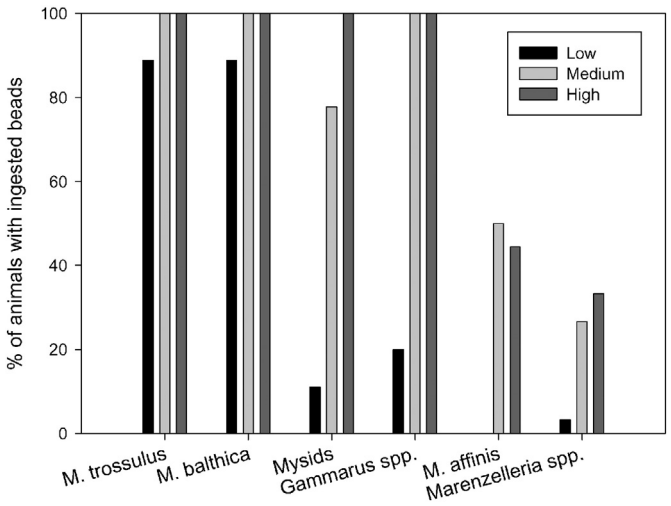

Fig. 3. The proportion (%) of animals with ingested plastic microbeads in the three concentrations (low = 5, medium = 50 and high = 250 beads mL^{-1}).

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microbeads is again the relatively low and patchy concentration of the beads on the sediment surface.

Except for the two bivalve species, *Gammarus* spp. and mysids contained the highest numbers of ingested microbeads, which indicates feeding activities both on the sediment or *Fucus* surfaces and in the water. During the experiment we observed how their swimming activity gradually mixed the sediment surface and conclude that their activity also contributed to the distribution of the beads in and on the sediment. Mysid shrimps are omnivorous feeding on detritus, phytoplankton and zooplankton (Lehtiniemi and Nordström, 2008), while littoral species of *Gammarus* are mainly herbivorous, grazing on macroalgae, and phytoplankton and periphyton on the surfaces of macroalgae and rocks (Orav-Kotta et al., 2009). Most of the microbeads were sedimenting out from the water column, and were not available for planktonic grazers like cladocerans and copepods thus decreasing the possibility of trophic transfer through zooplankton to mysids. However, mysids feed actively on detritus on the sediment surface, ingesting also sand grains (Lehtiniemi and Nordström, 2008) and could thus easily feed on settled plastic beads as well.

The two bivalves *M. balthica* and *M. trossulus* contained markedly more ingested microbeads than any other animal taxa in our experiment. This is particularly interesting since these species inhabit entirely different habitats/niches in the coastal system, and also use different feeding modes. *M. balthica* is a small (< 30 mm) clam which lives buried in the sediment, where it extends its two long siphons to the sediment surface. *M. balthica* either feeds on organic material which is suspended in the water just above the sediment with its siphon placed at a fixed position, or it can also move its siphon around on the sediment and vacuum deposited particles (Peterson and Skilleter, 1994).

Blue mussel (co-existing species; *M. trossulus* and *Mytilus edulis*) is one of the keystone species in the coastal ecosystems of the Baltic Sea. They form dense communities at salinities above 5 and dominate the shallow hard bottoms (Westerbom et al., 2002). The filtering capacity of blue mussels has been studied experimentally with increasing algal concentrations. For example Clausen and Riisgård (1996) and Riisgård et al. (2011) have demonstrated that *M. edulis* continuously filter the ambient water at a maximum rate when fed an algal concentration between the lower critical level and the upper algal threshold concentration for incipient saturation. Observations on the amount of ingested beads in our study (highest 3545 ind.$^{-1}$, average 635 beads mussel^{-1}) shows that the filtering activity of the blue mussels was not saturated, and also that a part of the microbeads were available suspended in the water due to swimming activities of gammarids and mysids, and aeration of the aquaria.

The intraspecific variation in the number of ingested beads was large as shown by the error bars in the (Fig. 1). This was especially clear in the deposit-feeding animals (*M. affinis*, *Marenzelleria* spp., *Gammarus* spp.) and in mysid shrimps which can switch in feeding between different habitats; sediment surface, the water column. This individual variation was probably partly due to the unequal distribution of the beads on the sediment surface, which had an effect on the feeding success of taxa feeding on the surface. We took great care in building the experimental units as identical as possible, and conclude that the observed differences were mostly created by the activity of the animals themselves. Especially *Monoporeia* and mysids are known to actively stir the topmost 1 cm of the sediment (Viitasalo-Frösén et al., 2009), while *Marenzelleria* spp. can sub duct material several cm down into the sediment (Josefson et al., 2012). If we had used higher concentrations of microbeads, they would have better covered the whole sediment surface, increased the encounter rate and lowered the impact of patchiness, but resulted in unnatural conditions.

Overall, the number of ingested beads increased with the bead concentration. In the highest offered concentration (250 beads mL^{-1}) all individuals of the both bivalve species, mysids and *Gammarus* spp. had beads inside them while less than 50% of the deposit feeding *M. affinis* and *Marenzelleria* spp. had ingested beads. It seems that beads were taken up more effectively by filter-feeding animals or animals utilizing at least partly the water column while feeding. Similar results have already been shown in the previous study from the same area, where the free-swimming planktonic larvae of the Marenzelleria contained high numbers of ingested microbeads (Setälä et al., 2014). In the lowest offered bead concentration the difference between taxa was the highest. In both bivalves still 90% of the individuals had ingested beads while in other taxa the proportion was very low. This clearly shows the efficient filtration capacity of the bivalves compared to crustaceans and polychaetes. Even at low particle abundances the bivalves were exposed to them.

The amount of microplastic litter in the marine environment has been studied extensively during the past years. The estimates of microplastic abundances vary from low concentrations of 3 particles m^{-3} (Doyle et al., 2011) to very high, hot-spot concentrations of 102 000 particles m^{-3} (Norén and Naustvoll, 2010). The highest reported microplastic concentration is from the Baltic Sea and was measured close to a plastic factory while other results from the Baltic Sea give much lower concentrations, mostly <10 particles m^{-3} (Talvitie et al., 2015, Magnusson and Wahlberg, 2014). We used a series of microbead concentrations in the experiments. The lowest one (5 particles mL^{-1}) is considerably lower than what has been used in previous laboratory studies with microbeads and marine organisms (Graham and Thompson, 2009; Cole et al., 2013; Setälä et al., 2014) and is already closer to natural concentrations although still higher than found in the field. However, there is very little data on the in situ distribution and abundance of microplastic particles of the lower size range (nano-) of the scale that can be ingested by the animals. More information is needed to make reliable comparisons between environmental conditions and experimental studies. Sampling and identifying particles that are less than 30 μm is very demanding, and thus most of the microlitter samples at present are collected with devices like Manta trawl, which collects particles that are one order of magnitude larger (>300 μm). However, pilot samplings indicate that the concentration of the smaller size fractions are higher than the larger ones taken with e.g. Manta trawl.

Very little data on microlitter concentrations from the sediments in the Baltic Sea are available. In a pilot study carried out outside the city of Helsinki (Talvitie et al., 2015) 0–48 fibers and 596–2216 particles sediment kg (ww)$^{-1}$ were found. A thorough study on microplastics methodology and distribution on the German coast of the Baltic Sea (Stolte, 2014) has given estimates of up to 300 fibers and <20 microplastic fragments kg (dw)$^{-1}$. Studies from other seas report microplastic concentrations between 4 particles m^{-2} on deep sea bottoms over 1000 m depth (Van Cauwenberghe et al., 2013) and 621,000 particles kg^{-1} dry sediment on the beaches (Liebezeit and Dubaish, 2012), 166.7 items kg (dw)$^{-1}$ in a harbor (Claessens et al., 2011), showing a great variability both in the amounts of microplastics in sediments and methodologies used. We did not assess the microbead concentration in the sediment of the aquaria, but theoretically, if all added particles would have settled on the sediment surface, the additions would have produced concentrations of 67,667 and 3335 beads m^{-2}.

The results of this study can be further assessed by considering the options for trophic transfer and its potential significance. Both bivalve species of our study, and especially the blue mussels were ingesting high numbers of the beads they were exposed to. The efficiency of bivalves to ingest microlitter has been demonstrated also recently in other marine areas (e.g. Von Moos et al., 2012). From the Baltic Sea it has been assessed that within one year the blue mussel beds filter a water volume equivalent to the whole Sea basin (Kautsky and Kautsky, 2000). Blue mussels thus form important links between pelagic and benthic ecosystems and blue mussel beds are an important food source for vertebrates, like roach (*Rutilus rutilus*) and the common eider (*Somateria mollissima*). Also the Baltic clam *M. balthica* is a prey for benthic fish species such as flounder (Mattila and Bonsdorff, 1998). Thus

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there is no doubt that in case of microplastic exposure, the bivalves will act as an efficient link to higher trophic levels. The invasive spionid polychaetes *Marenzelleria* spp. are presently the most common soft-bottom animals in the Baltic Sea, estimates of its highest abundances exceeding 5000 individuals m^{-2} (Kauppi et al., 2015). Although the number of ingested beads in *Marenzelleria* spp. was markedly lower than in the bivalves they could surely contribute to the trophic transfer of microplastics due to their wide distribution and high abundance also in coastal environments under heavy anthropogenic input as they tolerate eutrophic, oxygen depleted bottoms where microplastic loads are higher compared to open sea ecosystems.

It is thus likely that in hot-spot areas, like urban coastal zones microplastics may become a real and long lasting problem for marine life as they are mistaken as food, are taken up by various common and abundant taxa. Especially filter feeding, benthic animals are at highest risk for being contaminated by microplastics.

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