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Ingestion and transfer of microplastics in the planktonic food web

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ABSTRACT

Experiments were carried out with different Baltic Sea zooplankton taxa to scan their potential to ingest plastics. Mysid shrimps, copepods, cladocerans, rotifers, polychaete larvae and ciliates were exposed to 10 μm fluorescent polystyrene microspheres. These experiments showed ingestion of microspheres in all taxa studied. The highest percentage of individuals with ingested spheres was found in pelagic polychaete larvae, *Marenzelleria* spp. Experiments with the copepod *Eurytemora affinis* and the mysid shrimp *Neomysis integer* showed egestion of microspheres within 12 h. Food web transfer experiments were done by offering zooplankton labelled with ingested microspheres to mysid shrimps. Microscopy observations of mysid intestine showed the presence of zooplankton prey and microspheres after 3 h incubation. This study shows for the first time the potential of plastic microparticle transfer via planktonic organisms from one trophic level (mesozooplankton) to a higher level (macrozooplankton). The impacts of plastic transfer and possible accumulation in the food web need further investigations.

1. Introduction

Marine debris is a growing global problem posing a threat to a variety of marine organisms through the ingestion of particles and entanglement (Andrady, 2011; Laist, 1987). Plastics are the most common type of marine debris, constituting between 60 and 80% of all marine debris and over 90% of all floating particles (Gordon, 2006). The majority of the studies and reports on marine debris have focused on relatively large debris which is hazardous to marine mammals, birds or fish (Derraik, 2002). Studies carried out during the last decade have, however, pointed out the commonness of plastic microparticles, the so-called microplastics in the marine environment (Magnusson and Noren, 2011; Moore et al., 2001, 2002; Thomson et al., 2004). According to Magnusson and Noren (2011) the concentration of microlitter in the size range of 10–220 μm in the coastal Baltic Sea was up to 4 fibres L^{-1} and 32 other anthropogenic litter particles L^{-1}. The descriptor 10 of the EU Marine Strategy Framework Directive (MSFD, Annex I) emphasizes the importance of decreasing inverse impacts of marine litter – microdebris among them (EC, 2008). If harm is caused to the environment, information on the amount and composition of litter ingested by marine animals is required when assessing the status of European seas (EC, 2010). Microplastics are of concern especially because they can be ingested by a variety of marine organisms, and possibly be also transferred along the food web. The potential toxicity of microplastics is basically due to the additives and monomers they include (Mato et al., 2001; Thompson et al., 2007). Because of their relatively large area to volume ratio microplastics can be effective in absorbing hydrophobic contaminants from the water (Thompson et al., 2007). Ingestion of different type of microplastics can be common, like in the case of the Norway lobster, where 83% of the studied animals were found to include microplastic, constituting mostly of fibres (Murray and Cowie, 2011). Ingested microplastic debris was also identified from 10 species of fish from the English Channel (Lusher et al., 2013). Furthermore, a recent study from the benthic system (Farrel and Nelson, 2013) also gives indications that microplastics that are introduced into the food web by feeding at one trophic level may also transfer to other, higher trophic level organisms.

We focused on the issue of potential threats of microplastics by carrying out simple grazing experiments with fluorescent microspheres and zooplankton. At this point, no attempt to measure ingestion rates was done, just to verify ingestion. In our study we tested experimentally the potential of different Baltic Sea zooplankton organisms to ingest microplastics, to assess the potential of microplastics to enter the planktonic food web. Our hypothesis was that the ingestion of plastic microspheres is a common phenomenon among zooplankton and may assist in the food web transfer of ingested plastics. Our study consists of two parts: 1) direct ingestion experiments with zooplankton, here called as tests, and 2) studies on food web transfer of microplastics, and for both of these we used fluorescent microspheres as surrogates for plastic microlitter. These first ingestion tests were carried out only to confirm which mesozooplankton taxa were the most

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likely to ingest plastic particles, and could thus be used in the following food web transfer experiments. We also studied whether ingested plastic particles would pass through the grazers or instead clog the digestive tract. Although the ingestion of plastic microspheres by zooplankton is a known phenomenon already from the 1980s (Huntley et al., 1983), it has not been tested with all zooplankton taxa, including species that live in both pelagic and benthic habitats. Recently Cole et al. (2013) carried out a study where the ingestion of microspheres by a range of zooplankton from one ecosystem was studied.

Fluorescent plastic microspheres have previously been used as surrogates for natural prey to estimate feeding parameters and preferences of nano- and micro-protist and mesozooplankton. Information on the ingestion of plastic spheres exists for unicellular planktonic organisms such as ciliates and flagellates (Borsheim, 1984; Nygaard et al., 1988), copepods (Fernández et al., 2004; Huntley et al., 1983; Paffenhofer and Van Sant, 1985; Wilson, 1973), cladocerans (Bern, 1990; Zánkai, 1994), rotifers (Agasil and Noges, 2005; Ronneberger, 1998) and appendicularians (Fernández et al., 2004) from marine and freshwater environment. A recent study by Cole et al. (2013) confirms these earlier observations and describes the ingestion, egestion and adherence of polystyrene beads with several zooplankton taxa from the Northeast Atlantic.

Similarly, laboratory experiments have shown that nano-sized plastic particles may be ingested by benthic invertebrates, such as lugworms, barnacles, amphipods and mussels (Browne et al., 2008; Thomson et al., 2004). The ingested particles can in theory pass through the gut, or block and accumulate in the digestive tract and mechanically disturb feeding and digestion. Other possible harm connected to the ingestion of microplastics is related to the chemicals that are bound to the particles and may leach to the environment. In the experiments of Browne et al. (2008) microplastics were found translocated from the gut cavity to the circulatory system of blue mussels (*Mytilus edulis*). In such a case where the plastics are retained in an organism also the chemically induced problems are more likely to occur.

2. Material and methods

2.1. Study material

Mesozooplankton was collected in May 2012 and 2013 from Tvärminne Storfjärd, SW coast of Finland (59°50′N, 23°15′E) in the Baltic Sea with vertical hauls (25–0 m) either with a 100 μm plankton net (WP-2 cod end) or a 50 μm net. Net collected plankton material was gently washed into a 20 L plastic bucket and kept in fresh seawater in a temperature controlled room (*in situ* +11 °C) for 24 h to acclimatize in experimental conditions. Macrozooplankton (mysid shrimps) were collected from littoral and pelagial. Three species of mysid shrimps were used in the studies. The littoral species, *Neomysis integer* was collected with a small net attached to a wooden handle from the shore of Tvärminne Zoological Station in May 2013. The net was pulled through *Fucus vesiculosus* along the shore in the littoral zone at approximately 1 m depth to catch the mysids. Pelagic mysid shrimps (*Mysis relicta* and *Mysis mixta*) were collected at night in May 2013 close to the Storfjärd sampling station by towing with a mysid sled with a cod-end on the bottom (ca. 30 m depth) for 5 min (mesh-size 500 μm). The animals were gently washed with water collected from the deep layers to a cool box and kept in a temperature controlled room at *in situ* temperature with aeration in darkness.

2.2. Ingestion of fluorescent microspheres by mesozooplankton and mysid shrimps

We tested the ingestion of microspheres with different mesozooplankton taxa and mysid shrimps (Table 1). Fluorescent 10 μm polystyrene spheres (Polysciences inc.) were used at three different target concentrations (A = 1000, B = 2000 and C = 10 000 particles mL^{-1}). We chose 10 μm microspheres because this size of prey is suitable for several zooplankton taxa from protists to copepods. The zooplankton of the Baltic Sea is generally smaller than in truly marine conditions, and prefers relatively small prey that are in the same size group as flagellates or unicellular phytoplankton cells (Kivi and Setälä, 1995; Setälä et al., 2009). The microsphere concentration was kept relatively low compared to the concentration of natural phytoplankton communities (Kuuppo, 1994; Kononen et al., 2003) in the area to avoid biased results due to too high particle concentrations. The ingestion tests were done either with individually picked animals or with a mixed mesozooplankton community. All three different microsphere concentrations were tested for the copepods *Acartia* spp., *Limnocalanus macrurus* and the mixed plankton community (pilot studies, thus no parallels). Additional tests with the concentration B (2000 microspheres mL^{-1}) were made with the copepod *Eurytemora affinis*, the planktonic larvae of the polychaete *Marenzelleria* spp. and a littoral mysid species, *Neomysis integer*.

Table 1
Percentage of test organisms with ingested microspheres after incubations in different microsphere/prey concentrations. A – C: direct uptake experiments with three microsphere concentrations (A = 1000, B = 2000 and C = 10 000 microspheres mL^{-1}). D = food web transfer experiments with prelabelled copepods and *Marenzelleria* spp. larvae, concentration 100 prey individuals L^{-1}. Incubation times 3 h except for *E. affinis** where the incubation time was 12 h.

Zooplankton taxa	A	B	C	D
Copepoda:				
Acartia spp.	33.3 (n = 27)	28.6 (n = 42)	62.5 (n = 16)	
Eurytemora affinis		47.8 (n = 23)		
*Eurytemora affinis**		67.0 (n = 122)		
Limnocalanus macrurus	28.6 (n = 5)	0 (n = 8)	36.7 (n = 7)	
Cladocera:				
Bosmina coregoni maritima		100 (n = 1)	100 (n = 1)	
Evadne nordmannii		0 (n = 2)		
Polychaeta:				
Marenzelleria spp.		82.4 (n = 17)		
Rotifera:				
Synchaeta spp.		18 (n = 150)		
Mysida:				
Neomysis integer		100 (n = 9)		
Mysis mixta				0 (n = 1)
Mysis relicta				100 (n = 4)
Ciliata:				
Tintinnopsis lobiancoi	55 (n = 20)	85 (n = 20)	85 (n = 20)	

The mesozooplankton experiments were done in 200 mL plastic jars. Units with individually picked mesozooplankton were prepared by picking animals from the net material to the experimental jars with a Pasteur pipette (7–20 ind. jar^{-1}). Units for mixed mesozooplankton were filled with a subsample of the net material and diluted with filtered seawater and transferred into the experimental jars. After the filtering procedures during the preparation of the mixed community, most of the phytoplankton was excluded from the experimental water.

Mysid shrimps were collected with a small sieve from the cooling box and gently washed to the experimental bottles (vol: 1 L, 3 ind. bottle^{-1}) which were then filled with <10 μm filtered seawater. Altogether 5 bottles were prepared, from which individuals from 2 bottles were used for studies on microsphere egestion and 3bottles for stomach content and intestine analyses.

After addition of the test animals, the plastic microspheres were added into the experimental units which were filled completely with filtered seawater to avoid air bubbles, sealed with Parafilm, and placed on a plankton wheel rotating at a speed of 1 rpm at +11 °C in dim light. After 3 h incubation the content of each bottle was carefully poured on a 100 μm sieve, the animals were washed onto petri dishes, where they were counted and evaluated for their condition. The animals prepared for microscopy analyses were fixed with 25% glutaraldehyde. After fixing, they were either picked onto object slides or settled onto Utermöhl settling chambers and investigated with an inverted epifluorescence microscope under blue (FITCH) excitation light (Leica DMIRB, 100 and 200× magnifications). For the detection of microspheres inside the mysid shrimps, the mysids were dissected under a stereomicroscope (Leica Mz 7.5. 6–50× magnification) and their stomach and intestines were removed, stomachs opened and placed onto object slides into a small drop of filtered seawater, covered with a coverslip and examined under an epifluorescence microscope. All copepods, cladocerans and polychaete larvae encountered in the mixed community were examined, but of rotifers that dominated the mixed community, 50 individuals were examined in each microsphere concentration. Since the ingestion of a microspheres was not always possible to identify (especially in the case of copepods), we treated uncertain cases in all experiments as “no ingestion”.

The study of microsphere passage through the copepods (*Eurytemora affinis* adults) was done by incubating the animals as described, and then narcotizing the collected animals with a few drops of carbonated water. After that they were immediately picked onto Utermöhl microscopy chambers to a small drop of particle free seawater with a Pasteur pipette and the number of ingested microspheres was counted under an epifluorescence microscope. After this inspection the animals

were picked again into experimental jars containing particle-free seawater only, and let to recover for another 12 h, after which the microscopy procedure was repeated.

The egestion of ingested microspheres by mysid shrimps (*Neomysis integer*) was studied by sieving individuals from the two experimental bottles, washing them gently with filtered seawater and placing them in Petri dishes with filtered (GF/F) seawater. The mysids were left in the petri dishes in the same incubation area for 12 h. After that they were fixed with 4% formaline and dissected and contents of stomach and intestine studied. The content of the remaining water on the petri dish was also studied under an epifluorescence microscope.

2.3. The food web transfer experiments with zooplankton and mysid shrimps

We tested the food web transfer of microspheres with pre-labelled mesozooplankton (prey) and mysid shrimps (predator). A 50 mL subsample of the collected mesozooplankton net material was incubated with microspheres (conc. 2000 spheres mL^{-1}) in 500 mL glass bottles (sealed, bubble free) attached in a plankton wheel as described earlier. After 12 h the incubation water was gently poured through a 76 μm mesh size net and the animals were washed onto a petri dish with particle-free seawater. Healthy looking copepods and *Marenzelleria* spp. larvae were gently individually picked into 200 mL beakers holding particle free filtered seawater. This procedure was carried out rapidly using very thin glass Pasteur pipettes and picking up only swimming individuals from the surface of the petri dish. At this point no inspection of the presence of the microspheres inside the animals was done to prevent long exposure to handling and egestion. We also wanted to evaluate the share of the copepods/*Marenzelleria* spp. larvae that included fluorescent microspheres from their total abundances. For this purpose a 20 mL subsample of the incubation water was fixed with glutaraldehyde and later examined under an epifluorescence microscope.

The feeding experiments with pelagic mysid shrimps (*Mysis* spp.) on mesozooplankton prey were carried out in glass bottles (Vol: 1 L, 2 ind. mL^{-1}). The concentration of copepods and *Marenzelleria* larvae in each bottle was 100 ind./taxa L^{-1} (Table 1.). The experimental bottles were sealed and attached to the plankton wheel as described earlier. The handling of the pelagic mysids (*Mysis* spp.), as well as the incubation, was done in darkness to prevent eye damage. Bottles were taken from the plankton wheel after 3 h incubation. The incubation was terminated by sieving the mysids out from the experimental bottles and fixing them in 4% buffered formaldehyde solution. After that the mysids were dissected as described above.

3. Results

3.1. Direct ingestion of plastic microspheres by zooplankton

The percentage of individuals that had ingested microspheres varied between different taxa (Table 1. Fig. 1). Many of the *Marenzelleria* spp. larvae had so many plastics inside, that it was not possible to count their exact number (Fig. 2a). The copepod *L. macrurus* did not ingest particles in the concentration B, but then again had ingested particles both in higher and lower concentrations. We also noted that nauplii of *L. macrurus*in the mixed plankton community contained ingested microspheres, but their percentage from all nauplii was not assessed here. Only 2 individuals of *Bosmina coregoni maritima* were among the mixed community, but they both contained several microspheres (Fig. 2b.). *Acartia* spp. copepods ingested microspheres from all concentrations they were exposed to (Table 1). Rotifers (*Synchaeta* spp.) incubated in the lowest concentration did not contain any plastics, while in the higher concentrations B and C ingested sphereswere found. The highest percentage of individuals with ingested plastic microspheres was observed inside of the ciliate *T. lobiancoi* (Fig. 2c) and the littoral mysid *N. integer*(Table 1). All microspheres were found in the mysid intestine, not in their stomachs.

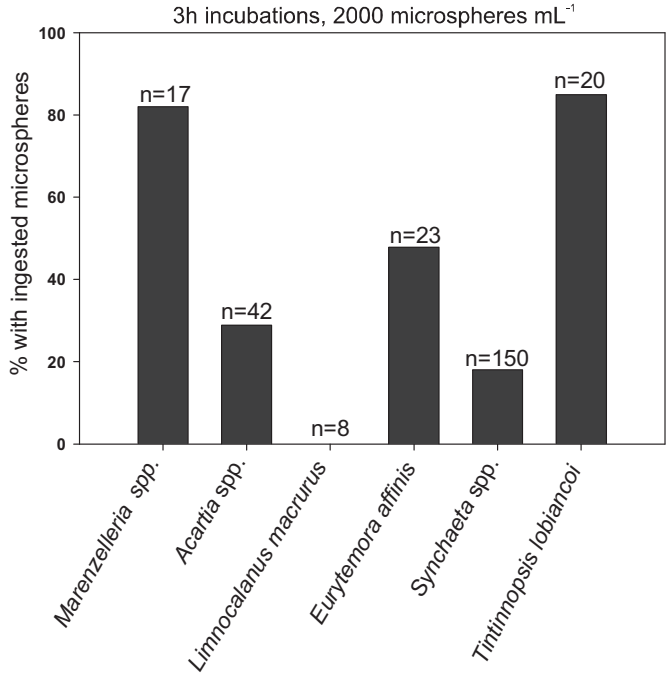

Fig. 1. Proportion (%) of individuals with ingested plastic microspheres.

We also studied the gut passage of ingested microspheres in copepods and mysid shrimps. After 12 h incubation with fluorescent microspheres, 67% of all *E. affinis* individuals had ingested microspheres. After another 12 h incubation, now in particle-free seawater, only 3.7% of all individuals contained microspheres, meaning that the spheres had been egested during the incubation. Egestion of microspheres was also observed when mysid shrimps that had previously ingested microspheres were kept for 12 h in particle free seawater in Petri dishes, after which microspheres appeared in the water.

3.2. Food web transfer of ingested microspheres

A mesozooplankton community was pre-incubated with fluorescent microspheres before offering individually picked *Marenzelleria* spp. and copepods to pelagic mysid shrimps. A subsample from the pre-incubated showed that altogether 43% of the copepods and 86% of the *Marenzelleria* spp. contained ingested microspheres. When the mysid shrimps that had been incubated with the above mentioned prey were more closely examined under the dissecting microscope, we found that there was one *Mysis mixta* and four individuals of *Mysis relicta*. From these all individuals of *M. relicta* had fluorescent microspheres inside their intestines (Fig. 3), but the only *M. mixta* did not.

4. Discussion

The aim of our study was to examine the microplastic transfer in planktonic food webs. Which taxa from the zooplankton community would be the most likely ones to ingest microplastics, and also whether the ingested plastics potentially transfer along the food web? The effective transfer of harmful substances can take place in food webs if the concentrations of pollutants are accumulating at some trophic level. In the case of microplastics, the harm for marine organisms has not been verified in nature. In theory, if plastics are retained inside an organisms, even for a short time, or if additiveds or other harmful substances are leaking from the particles to the organism, accumulation can take place and ingestion of microplastics cause harmful effects.

Our findings from the direct ingestion tests are in agreement with previous zooplankton studies, which have focused on the determination of ingestion rates for different taxa of marine zooplankton. Although feeding experiments have shown that copepods prefer natural prey over polystyrene spheres either by ingesting them at low rates (Huntley et al., 1983) or rejecting them when offered as sole prey (Donaghay and Small, 1979), have plastic microspheres also been successfully used to study in detail the food

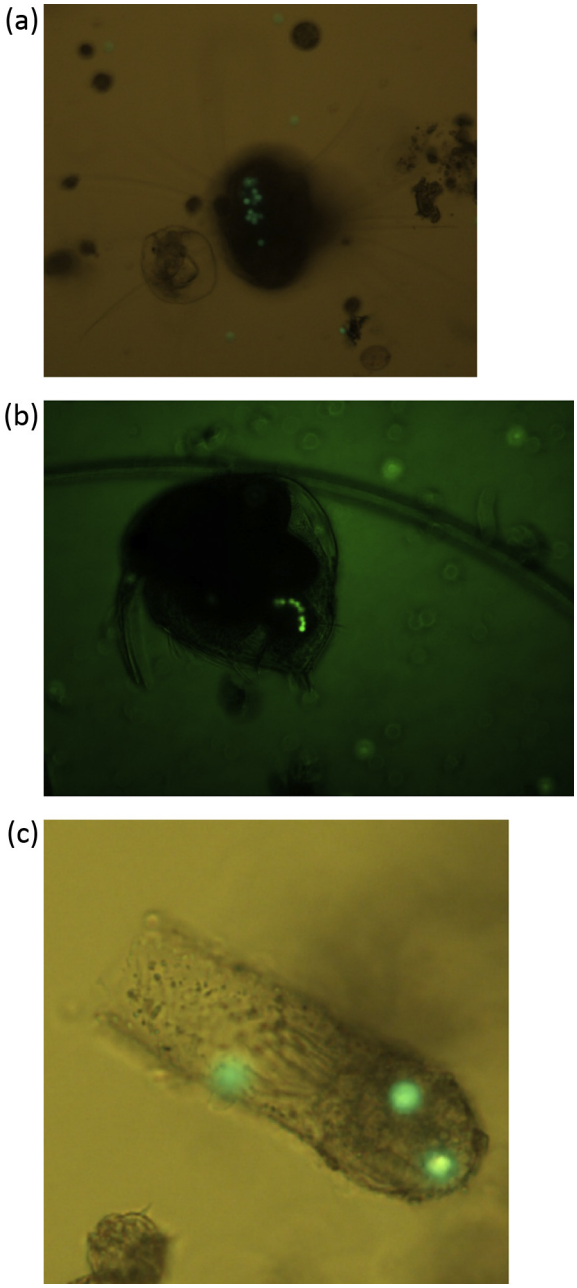

Fig. 2. Zooplankton with ingested fluorescent beads: (a) *Marenzelleria* spp., (b) *Bosmina coregoni maritima*, (c) *Tintinnopsis lobiancoi*.

size selection in copepods (Wilson, 1973) and e.g. cladocerans and rotifers (Agasil and Noges, 2005; Ronneberger, 1998; Zánkai, 1994). In the Baltic Sea studies with different inert particles and planktonic grazers have included ingestion measurements with polystyrene spheres and the pelagic larvae of *Marenzelleria* (Burckhardt et al., 1997), latex spheres and brackish water rotifers (Hlawa and Heerkloss, 1994), starch particles and ciliates and different types of artificial prey (model food particles made of carbohydrates or proteins and polystyrene spheres) and dinoflagellates (Hammer et al., 2001). The most recent study of zooplankton grazing on microspheres is by Cole et al. (2013) from the Northeast Atlantic. As in our study, they observed the ingestion of plastics among different zooplankton taxa and also showed that high concentrations of microspheres (4000–25 000 particles mL^{-1}) have negative effects on their grazing of algae. These findings are important when determining the harm of microplastics to the plankton communities in places with very high plastic contamination.

Our test animals cover all the functional groups in northern Baltic Sea zooplankton: protists (tintinnid), mesozooplankton grazers (herbivorous polychaete larvae, rotifers, cladocerans and copepods) and predatory/omnivorous species (copepods) as well as macroplankton grazers (mysids). We showed that plastic microspheres were widely ingested by various planktonic taxa in the Baltic Sea. From the taxa included in the study the highest percentage of individuals with ingested microspheres was in mysid shrimps, ciliates, and polychaete larvae. Ingestion of plastic in different organisms differs and is dependent on many factors including the size and abundance of particles offered and presence of natural prey. The size of particles that can be captured by a planktonic grazer depends on the feeding mode and specific feeding mechanisms. In general, filter feeding is an adaptation to the exploitation of small particles by a larger organism. The 10 μm microspheres are within the size range of suitable prey estimated by Kivi and Setälä (1995) for *Tintinnopsis lobiancoi* (2.8–23.8 μm) and tintinnid clearance rates in general 1.9–8.4 μl $cell^{-1}h^{-1}$. Although *T. lobiancoi* prefers slightly smaller prey than what was offered for them (estimated average 5.6 μm), they were still able to filter and ingest particles in our study, which are relatively large for an unicellular organism (Fig. 2c). Ciliates in general are important members of the microbial loop that efficiently graze on pico- and nanoplankton and transfer energy and matter within pelagic food webs (Sherr and Sherr, 2002), and have also been found to transfer phycotoxins to zooplankton grazers (Maneiro et al., 2000). In he northern Baltic Sea it has been estimated that planktonic ciliates are able to clear over 100% of the seawater volume during summer periods (Setälä and Kivi, 2003). Based on these results ciliates may turn out to be one very important component in the transfer of microplastics in marine pelagic food webs.

Pelagic larvae of the polychaete *Marenzelleria* spp. prefer planktonic algae and the size of the prey increases with the increasing size of the animals (number of setigers) up to approx

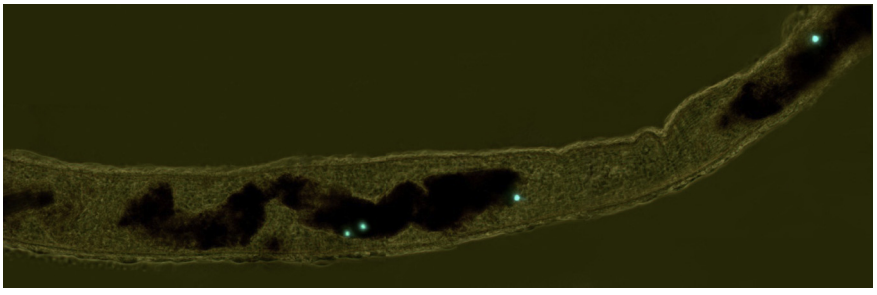

Fig. 3. The contents of a mysid shrimp *Mysis relicta* intestine after 3 h incubation with zooplankton labelled with fluorescent microspheres.

80 μm, but in a study carried out in the Baltic Sea it was found that most of the ingested prey are however smaller than 20 μm (Burckhardt et al., 1997). The preference of *Marenzelleria* spp. larvae to phytoplankton-sized food was also noticed in our study where they were able to ingest several microspheres (Fig. 2a). No experiments were done to follow the egestion of the particles, but the high feeding activity of these larvae however gives indications that they can be important vectors for harmful effects caused by microplastics.

Planktonic copepods can be vectors for different harmful substances such as phycotoxins (Karjalainen et al., 2005; Lehtiniemi et al., 2002; Setälä et al., 2009; Turner et al., 2000). In our study copepods (*Acartia* spp., *L. macrurus*, *E. affinis*) ingested microplastics, but the percentage of individuals with ingested plastics was lower than in ciliates or polychaete larvae. Moreover, we were not able to see any clear relationship between the microsphere concentration and their ingestion rate in copepods. It is possible that egestion of ingested particles interferes the interpretation of these results, and shorter incubation times would give a better picture of the ingestion activity. Most of the microspheres that were ingested by *E. affinis* were also egested after 12 h incubation in filtered seawater. On the other hand, increased incubation time also increased the percentage of individuals with ingested fluorescent microspheres. This suggests, that despite egestion, continued exposure to a high concentration of microplastics increases their accumulation.

Mysid shrimps are omnivorous feeding on detritus, phytoplankton and zooplankton. They gradually switch diet during growth from a phytoplankton-dominant diet to a zooplankton-dominant one (Kiljunen et al., 2006; Lehtiniemi and Nordström, 2008; Viherluoto et al., 2000) but both of these planktonic components have a role in their diet throughout their life. The littoral mysids used in the microsphere ingestion tests utilise a variety of food items ingesting even small sand particles (Lehtiniemi and Nordström, 2008) thus ingestion of plastic microspheres could happen in nature as well if particles would be available.

All the mysids used in direct feeding experiments ingested plastics. In the study of Bigelow and Lasenby (1991) the ingestion of different sized polystyrene spheres by *Mysis relicta* was also observed, the smallest (0.75 μm) and largest (40–50 μm) being less favoured than intermediate sizes. Direct exposure via feeding on microplastic particles may happen especially with juvenile mysids both in the littoral and pelagic zones. Mysid shrimps from our experiments contained microspheres after being exposed to zooplankton with a history of feeding plastic microspheres. The mysids used in the transfer experiments were adult stages of pelagic mysids, which main diet is formed of zooplankton (Viherluoto et al., 2000). Thus indirect exposure via feeding on zooplankton containing plastics is highly possible.

Trophic transfer of microplastic litter may potentially take place both in benthic and pelagic food webs. A few laboratory studies have so far focused on this issue in benthic systems. Murray and Cowie (2011) carried out a field study where they collected Norway lobsters (*Nephrops norvegicus)* from the Clyde Sea and found plastic fibres in their digestive system. They suggest that these omnivorous feeders can be exposed to plastics via passive ingestion from sediment or via a trophic pathway. To confirm the latter, they carried out laboratory experiments where pieces of fish that were labelled with strands of polypropylene fibres were offered as food for *Nephrops*. In these experiments all the lobsters that had been feeding on the fish offered, also contained strands of polystyrene in their stomachs. In another study (Farrel and Nelson, 2013) the work of Browne et al. (2008) were taken one step further by examining the transfer of plastic microparticles in benthic food webs. Blue mussels (*Mytilus edulis*) were first exposed to a high concentration of 2 μm polystyrene spheres (10^6 spheres mL^{-1}) and then parts of mussel tissue were offered as food for shore crabs. As a result the crabs that had been feeding on mussel tissues contained high numbers of microspheres in their haemolymph and other organs, giving indications that mussels also in natural conditions could be vectors for microplastics in benthic environment.

The idea behind these experiments is simply that if an organism ingests microlitter particles, it can also act as a vector for litter transfer to higher trophic level organisms. The importance of this link depends on the organism itself as well litter abundance and type: do the particles pass the first-step organisms' digestive system, as seems to be the case of *E. affinis* in our study, or are the particles accumulating within the organism.

It is likely that microplastic transfer in the marine food webs has similar linkages as the transfer of many other harmful substaces like hazardous chemicals or phycotoxins that are produced by harmful algae (HABs). The toxic transfer of phycotoxins has been under an extensive research, because it has effects on human health and economy. Phycotoxins are mostly carried within the toxic alga, and transferred to the first step consumers in plankton or benthos, like bivalves or crustaceans, by feeding. Several studies have emphasized the role of benthic organisms in this process. For example diarrethic (DSP) and paralytic (PSP) shellfish poisonings are caused by algal toxins that efficiently accumulate in shellfish and can cause hazardous symptoms in human consumers (Campbell et al., 2005; Mons et al., 1998; Teegarden and Cembella, 1996). Besides benthic systems, algal toxins can also be transferred along the trophic food web via the so-called non-traditional vectors (Deeds et al., 2008), which include planktonic crustaceans as well as some fish species (Teegarden and Cembella, 1996; Turner et al., 2000; White, 1981). In the studies of White (1980, 1981) herbivorous zooplankton was found to be a vector for algal toxins to fish, causing fish kills. Fish themselves may also act as vectors for waterborne toxins, as was found by Geraci et al. (1989) who studied the death of 14 humpack whales (*Megaptera novaeangliae*), and concluded that the cause of death was PSP via its main prey, mackerel (*Scomber scombrus*).

Toxins produced by HABs pose a serious problem to marine life globally, while the hazardous effects of microplastics in marine food webs are still unknown and thus speculative. It is possible that microplastics, or the toxins that are related to microplastics can accumulate in marine food webs, but the magnitude or actual impact has to be assessed. In the Baltic Sea harmful microalgae can in bloom conditions reach concentrations from 10^3 to 10^6 cells L^{-1}, or higher (Klöpper et al., 2003; Hällfors et al. 2011; Witek, 2004; Hakanen et al., 2012), and can potentially be used as food for many filter feeding organisms. Compared to those concentrations the estimates on all micro-sized litter in the plankton of coastal Baltic Sea are relatively low, in the range of approximately 10–100 particles L^{-1} (Magnusson and Noren, 2011). The harmful effects of microlitter in plankton may thus also be a magnitude or two smaller, at least if short lived pelagic zooplankton is the main vector. Benthic organisms may, however be more efficient in harvesting and also accumulating microlitter. For example the blue mussels, which have been estimated to have the capacity to filter the whole water volume of the Baltic Sea within a year (Kautsky, 1981), are abundant and also have a life span of several years. As shown by laboratory studies, they may also accumulate foreign particles in their tissues (Browne et al., 2008; Farrel and Nelson, 2013). Likewise also other benthic organisms with a longer life span are likely to be exposed to that part of microlitter which is sedimenting out from the pelagic system and accumulating on and in the sediment with organic material. These could for example be bivalves that feed on the sediment surface with their siphon, or sediment burrowing polychaetes such as *Marenzelleria* spp. or other worms.

5. Conclusions

This study shows the potential of different zooplankton taxa to ingest foreign particles, like plastic microspheres. Although the particles were egested in mysid shrimps and copepods, we cannot exclude the possibility for accumulation of harmful substances from the ingested particles during teheir passage in the gut. Experiments with mesozooplankton and mysid shrimps also showed that particles may transfer within the food web. Mysid shrimps were exposed to the microspheres not only directly, but also indirectly, which implies that there are several alternate routes for microplastic transfer in the pelagic food webs. Both mysids and polychaete larvae live partially in the pelagial and partly in benthic realm (mysids are nektobenthic animals, polychaete larvae settle to the bottom and live in the sediments as adults) having potential to transfer microplastics between the food webs of these environments. Based on our studies we conclude that high concentrations of microplastic litter has the potential to enter marine food webs. Therefore we suggest that their abundance and distribution should be monitored in order to implement e.g. the MSFD and their sources and management options studied to achieve good environmental status in marine ecosystems.

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