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Bioturbation transports secondary microplastics to deeper layers in soft marine sediments of the northern Baltic Sea

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ABSTRACT

Microplastics (MPs) are observed to be present on the seafloor ranging from coastal areas to deep seas. Because bioturbation alters the distribution of natural particles on inhabited soft bottoms, a mesocosm experiment with common benthic invertebrates was conducted to study their effect on the distribution of secondary MPs (different-sized pieces of fishing line < 1 mm). During the study period of three weeks, the benthic community increased MP concentration in the depth of 1.7–5.1 cm in the sediment. The experiment revealed a clear vertical gradient in MP distribution with their abundance being highest in the uppermost parts of the sediment and decreasing with depth. The Baltic clam *Macoma balthica* was the only study animal that ingested MPs. This study highlights the need to further examine the vertical distribution of MPs in natural sediments to reliably assess their abundance on the seafloor as well as their potential impacts on benthic communities.

1. Introduction

Extensive production of plastics started in the middle of the 20th century and has been growing ever since (PlasticsEurope, 2013). The wide usage of plastics and poor management practices have resulted in the accumulation of plastic litter in the oceans. Plastics in general are long-lived, and thus persistent in the marine environment (Andrady, 2015). In addition to the concern about macro-sized plastic pollution around the globe, focus has recently shifted towards microplastics (hereafter MPs), which are generally defined as small (< 5 mm) plastic particles that have either been intentionally manufactured to be small (primary MPs) or have fragmented from larger plastic items (secondary MPs) (UNEP, 2016).

MPs have been observed everywhere in the oceans including surface waters (Eriksen et al., 2013; Setälä et al., 2016a), the water column (Kukulka et al., 2012; Reisser et al., 2015), sea ice (Obbard et al., 2014) and the seafloor (Claessens et al., 2011; Van Cauwenberghe et al., 2013). Due to their small size and ubiquitous distribution, MPs may be potentially harmful to marine biota because they can be ingested by a variety of both pelagic and benthic species (Murray and Cowie, 2011; Lusher et al., 2013; Van Cauwenberghe et al., 2015).

The special characteristics of plastic polymers affect their distribution in the sea. Typically plastics less dense than seawater (1.025 g/cm^3), such as common consumer plastics polyethylene and polypropylene, tend to float on the sea surface whereas denser plastic types are suspended in the water column or sink to the seafloor (Andrady, 2011). However, items made of less dense plastic polymers can also eventually sink as a result of biofilm formation (Lobelle and Cunliffe, 2011), after being ingested and subsequently egested in faecal pellets (Cole et al., 2013), or being convoyed with phytoplankton aggregates (Long et al., 2015). Environmental sampling has confirmed that MPs found in or on the seafloor include plastic types that are typically positively buoyant in seawater (Claessens et al., 2011; Vianello et al., 2013). Therefore, the seafloor is proposed to serve as an ultimate sink for marine MPs (Woodall et al., 2014).

Fine-grained soft sediments make up most of the seafloor (Rhoads, 1974), but there is currently little information on the fate of MPs when they reach these habitats. In colonized soft bottoms, animals alter their habitats by influencing the sediment structure in a process called bioturbation (Kristensen et al., 2012). Bioturbation covers all the actions of benthic fauna, such as burrowing, ingestion, defecation and ventilation, that directly or indirectly transport particles or solutes in the sediment matrix (Kristensen et al., 2012). As bioturbation is known to increase the surface area available for particle-exchange between sediment and overlying water (Karlson et al., 2007), we hypothesized that it would also affect the transport of MPs in the sediments. A mesocosm experiment was therefore established in order to investigate how the bioturbation caused by invertebrates in the soft bottom sediments would shape the vertical distribution of secondary MPs on the seafloor.

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2. Material and methods

2.1. Sample collection and experimental set up

The experiment was conducted at Tvärminne Zoological Station (University of Helsinki), southwest Finland, northern Baltic Sea. The sediment and the animals for the experiment were collected close to the station aboard R/V Saduria in February and April 2015 with a van Veen grab at three locations (N59°51′09″ E23°15′25″, depth 7 m; N59°51′16″ E23°15′25″, depth 20 m; N59°51′32″ E23°15′82″, depth 34 m) and with a bottom trawl at one location (N59°51′18″ E23°16′23″, depth 36 m).

The collected sediment was sieved through 1 mm sieve to remove all animals. Sieved sediment from different sampling sites was mixed together to generate a large amount of homogenous sediment, which was then divided into 30 cylinders (height 20 cm, diameter 14 cm) with a movable bottom (Viitasalo-Frösén et al., 2009) and placed in a temperature-controlled room (10 °C). The cylinders were covered with 500 μm steel mesh lids and a hose was placed horizontally above every set of five units. Small holes drilled to the hose allowed a continuous and gentle dropping of ambient seawater (salinity 5–6, temperature 5 °C, oxygen 11.6 mg/L at the start of the acclimatization period) to the units. The sediment in the cylinders was left overnight to settle. The next day 16 individuals of *M. balthica* (mean size 17.3 cm) were added to each of 15 units, and left to acclimatize for 9 weeks. The remaining 15 units served as controls with no animals. During the acclimatization period food was added to all units twice a week (Shellfish diet 1800, Reef Mariculture): feeding was terminated one week prior to the start of the experiment. Polychaete worms (*Marenzelleria* spp.) and amphipods (*Monoporeia affinis*) were collected in April and added to the units containing *M. balthica* one day prior to starting the experiment. The abundances of all the benthic animals used in the experiment were adjusted close to natural densities found in the northern Baltic Sea (Table 1).

Secondary MPs were produced by cutting fishing line (Trilene sensation, Berkley) with a McIlwain™ Tissue Chopper. The diameter of the fishing line was approx. 200 μm and it was cut into three different lengths: 50, 150 and 300 μm. Each size class was cut from a different coloured fishing line, weighted and divided into 30 separate portions using a Mettler Toledo XS205 Dual Range scale. The scale was also used to estimate the concentration of MP additions. Additions to each unit were approximately 490 pieces (50 μm), 880 pieces (150 μm) and 390 pieces (300 μm), which correspond to a concentration of 114,400 pieces/m^2, 880 pieces/L of sediment, 1790 pieces/kg of dry sediment. Relatively high concentration of MPs was used in case there would be problems with their extraction from the sediment. The experiment started when MPs were added to the units. When starting the experiment the water temperature was 6 °C and oxygen content 10.8 mg/L (YSI Environmental ProODO™).

After the experiment had been running for a week, 10 units (5 control units, 5 animal units) were randomly selected and terminated. Sediment from the units was sliced to six layers according to depth (approx. 1.7 cm per slice). The cylinder was lifted on the slicing device (HAPS corer sample ejection aggregate) and a cutting plate was attached on top of the cylinder. When rotating the piston of the device, the sediment in the cylinder was pushed upwards allowing the cutting plate to slice the sediment. The sediment slices were then sealed in ziplock bags and frozen in −20 °C. The rest of the units were terminated and handled in similar manner after two and three weeks.

Table 1
Mean sizes, abundances and densities of individuals added to the animal units. Natural abundances are based on the data of Rousi et al. (2013) taken from the depth of 35 m during years 1993–2007.

	Macoma balthica	*Monoporeia affinis*	*Marenzelleria* spp.
Mean size (mm)	17.3 (SD ± 1.4)	Not measured	20[a]
Individuals per unit	16	3	8
Abundance per unit (m^2)	1038	195	519
Natural abundance (m^2)	200–1100	30–800	8–7000

[a] Estimate, could not been measured due to fragmentation during the preservation.

2.2. Microplastics extraction and sample processing

Frozen sediment slices were thawed at room temperature and animals were handpicked and preserved in 70% ethanol. MPs were extracted from the sediment samples using saturated salt solution (Thompson et al., 2004). The original method was modified by adding solid NaCl to the wet sediment sample according to its volume and maximum solubility (35.7 g NaCl/100 mL sample) to compensate dilution of the solution due to the high water content of the sediment sample. The sample was then mixed and salt allowed to dissolve for 20 min before further processing. This solid NaCl addition raised the density of the salt solution during the first extraction step; however, if solid NaCl is used in processing environmental samples, great care must be taken because the salt can act as an additional source of MP contamination (P.N., personal observation).

Saturated NaCl solution was added until the total volume of the sample was 1 L. The sample was stirred for 1 min and allowed to settle for 8 min. Cleared supernatant was suctioned with a hose through a 100 μm plankton net filter. The small residue above the sediment surface was decanted on a separate 100 μm plankton net filter because it has been observed that most of the MPs are retrieved in the decanting phase (Stolte et al., 2015). These phases were repeated twice without additional solid NaCl to ensure the best possible yield of MPs (Browne et al., 2011; Claessens et al., 2011; Martins and Sobral, 2011). The MPs caught on filters were examined using a stereomicroscope (Leica CLS 150 XE, Schott KL 1500, 0.63–5.0 × magnification). Extraction efficiency calculated from the samples was 49.5% (excluding MPs ingested by study animals). The extraction efficiency was better for bigger particles (300 μm: 83.4%. 150 μm: 43.1%, 50 μm: 34.3%). Most of the extracted MPs (62.2%) were retrieved during the first extraction step; the second extraction step yielded an additional 21.7% of particles and the third 16.1%. Decanting proved to be more efficient compared to suction with a hose in every extraction step; altogether 64% of all the microplastics were recovered when decanting the supernatant residue after suction with a hose.

Grain size analysis was performed separately for all sediment layers of one control unit for background information. Prior to the analysis the salt residue from the density separation was washed away by mixing 1700 mL of pure H_2O with the dried sediment sample and waiting 2 h for the salt residue to dissolve in the water. The supernatant was then removed with a hose and phases repeated once more. Each sample was covered with 6% H_2O_2 for two days and stirred twice a day to digest all organic material in the sediment. Samples were sieved wet through 500, 250 and 63 μm sieves. The material from the sieves was washed into pre-weighted containers and dried at 60 °C to determine the dry weight of each size fraction. Water and the < 63 μm size fraction that passed the smallest sieve was left to settle for two days. The water was then sucked with a hose without disturbing the sediment at the bottom and the sediment was then washed into a pre-weighted container and dried at 60 °C before weighting.

To count the ingested MPs, 75 individual *M. balthica* (5 clams from each unit) and 57 individual *Marenzelleria* spp. (3–5 polychaetes from each unit) were dissected. Because at the end of the experiment *M. affinis* was not retrieved from all the units, 2 individuals from each of 12 units containing them were examined. All the animals were rinsed in a jar containing pure tap water to wash away dirt and MPs that could have been attached on their surfaces. Individual *M. balthica* were measured and rinsed again after being detached from their shells with