Marine benthic foraminifera and microplastics

Accumulation and effects following short- and long-term exposure

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Exposure of marine benthic foraminifera to microplastics

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Preface

This master's thesis was written as a part of the Marine Biology and Limnology program at the University of Oslo (UiO) during the period from August 2017 to October 2019. The project was performed at the Departments of Biosciences and Geosciences.

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Anna Iudina Grefstad

Abstract

Microplastics contaminate marine environments worldwide, but there is little knowledge of whether and how benthic foraminifera are affected. The aim of this project was to clarify whether microplastics accumulate in and affect benthic foraminifera. Sediment was collected at 163m water depth in the Oslofjord, Norway, on September 2018. Collected sediment was stored in a climate room 7C° at the Department of Biosciences, University of Oslo until further use. Treatments were prepared by adding one of three sized fluorescent polystyrene microspheres (0.5 μm; 1 μm and 6 μm) into containers with 10 mL of gently homogenized sediment. In the control treatment, no microplastic was added to the sediment. Two experiments were performed, exposing benthic foraminifera communities to microplastics for 6 hours and 4 weeks. Following both exposures, rose Bengal-stained foraminifera were identified, counted and the number of specimens with microplastics inside were counted. There was no significant change in community composition after exposure to microplastics (0.5 μm, 1 μm, 6 μm) for six hours or four weeks compared to control. Cluster and multidimensional scaling analyses showed around 85% similarity between samples from the two sampling times. Shannon diversity index of live foraminifera varied from 3.53 to 4.03. In total 17 species ingested microplastic in the six-hour experiment and 21 species ingested microplastic in the four-week experiment. In six-hour and four-week experiments, 8 and 13 species accumulated microplastic in at least three out of five replicates respectively. Most foraminifera did not differentiate between microplastic sizes, but two species differentially accumulated the three sizes of microplastics: *Nonionella turgida* accumulated 6 μm plastic particles more than 1 μm in the six-hour experiment and did not at all accumulate 0.5 μm plastic particles; *Uvigerina peregrine* accumulated 0.5 μm plastic particles more than 1 and 6 μm plastic particles in the four-week experiment. Most of the species accumulated more microplastic after 4 weeks compared to 6 hours. Thirteen foraminifera species accumulated more 0.5 μm microplastic in the four-week experiment than in the six-hour experiment; seven species accumulated more 1 μm microplastic in the four-week experiment than in the six-hour experiment; and ten species accumulated more 6 μm microplastic in the four-week experiment than in the six-hour experiment. Food preferences and test composition of foraminifera affected the accumulation of microplastics, whereas species with high tolerance to organic carbon and or their microhabitat preferences did not appear to influence the

accumulation of microplastics. This study shows that there are differences in the accumulation of microplastics in foraminifera species. Accumulation of microplastics in foraminifera may be an entry of such particles into the marine benthic food webs.

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1. Introduction

1.1. Microplastic in the marine environment

Plastics are synthetic organic compounds that are produced by polymerization. Polymers consist of many repeating units (monomers). Plastics are widely used materials and the production has been increasing since the 1950s (Hammer et al., 2012). About 10% of produced plastic debris enter the ocean from a wide variety of land- and sea-based sources every year (Jambeck et al., 2015). Plastic debris is divided by size into macroplastics (>5mm), microplastics (<5mm) and nanoplastics (< 1000 nm) (Andrady, 2017). There are two sources of microplastics in the sea: direct introduction of manufactured microplastics beads (primary microplastics) and breakdown of macroplastic debris (secondary microplastics) (Andrady, 2011; Browne et al., 2007; Cole et al., 2011; GESAMP, 2015). In the future, the quantity of microplastic in the ocean will increase. Even if the introduction of new plastic debris to the environment would stop, fragmentation of the already present plastic will continue for decades to come (Law & Thompson, 2014; Thompson, 2015).

Plastic as such is biochemically inert and has no direct chemical toxicity. However, it can still have an impact on organisms. The potential harm of microplastics to organisms is related to the ability of a species to ingest and/or interact with it. The negative effect of microplastic on organisms could also conceivably increase with the decreasing particle size (Law & Thompson, 2014; Wright et al., 2013). Small sizes of microplastic make it more available to organisms, increase its levels of reactivity and the ability to interact with biomolecules (Galloway, 2015). Lei et al., (2018) investigated the negative effects of different types and sizes of microplastics on nematode *Caenorhabditis elegans.* They suggest that the toxicity of microplastics is dependent on their size rather than their composition. Of three different sizes of fluorescently labeled polystyrene beads (0.1, 1 and 5 μm), 1-μm particles caused the highest damage to the nematode.

Plastic debris can adsorb contaminants (like persistent organic pollutants), bacteria and/or viruses from the environment and deliver them straight into organisms. Different plastics contain additives such as plasticizers, flame retardants, and antimicrobial agents, which are able to leach from it. These additives are primarily lipophilic, they can penetrate cell membranes, interact biochemically, and cause toxic effects (Andrady, 2011; Hammer et al., 2012). Because of their small size, microplastics are ingested and accumulated by a large variety of organisms (Fig. 1).

Interactions with microplastics have been observed in laboratory studies and there is ample evidence of microplastic ingestion in the natural environment as well (Lusher, 2015; Phuong et al., 2016).

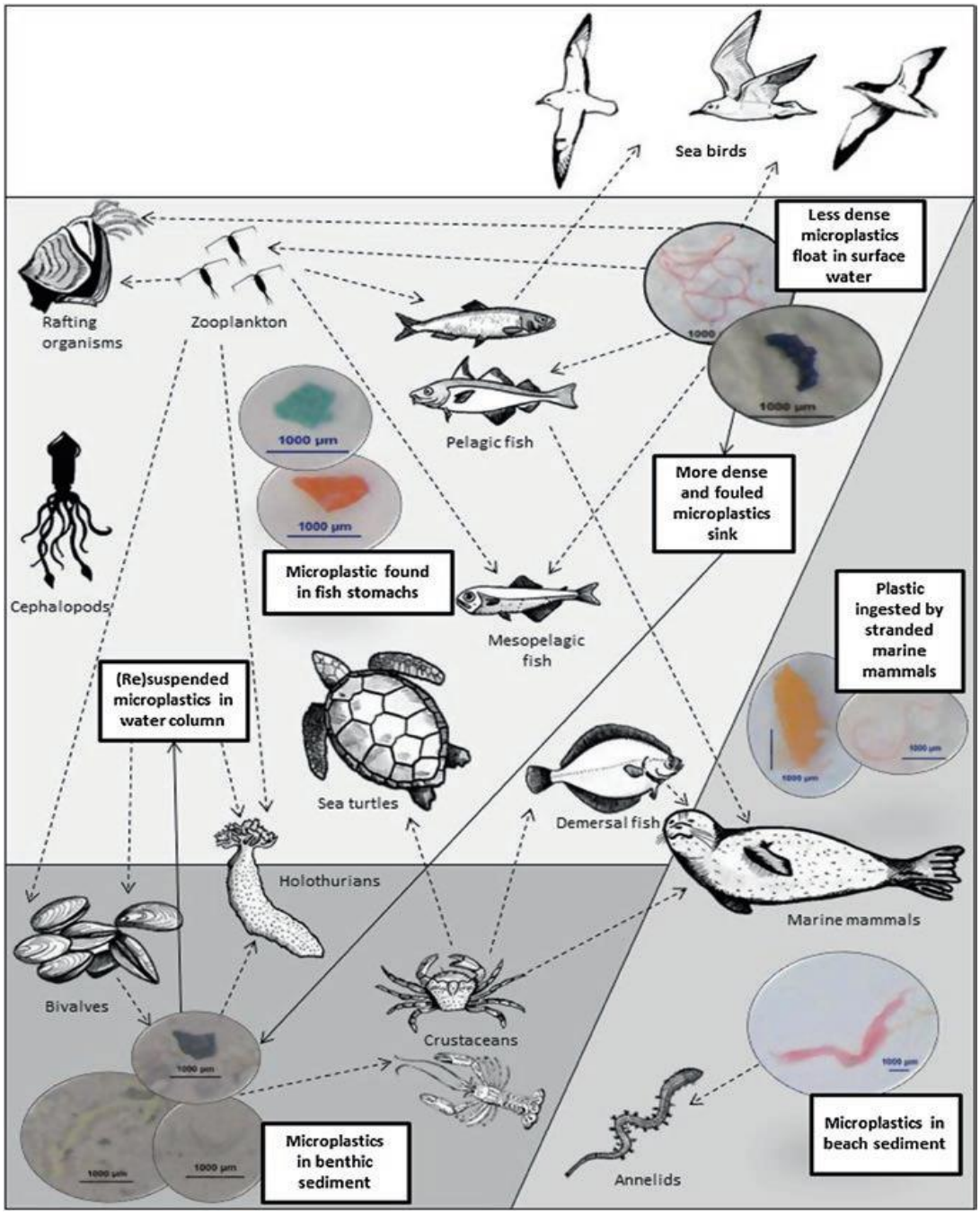

Figure 1. Microplastic interactions in the marine environment including environmental links (solid arrows) and biological links (broken arrows), which highlights potential trophic transfer (Photos of microplastics: A. Lusher) (Lusher, 2015).

Microplastic can be transferred through the food web when predators consume prey contaminated with microplastic. The crab *Carcinus maenas* was fed on mussels *Mytilus edulis* which had been exposed to 0.5-μm polystyrene microspheres. Microplastics were found in the tissues from the stomach, hepatopancreas, ovary, and gills of the crabs. The maximum amount of microplastics were detected after 24 hours, then the number of plastic beads in crab tissues decreased, though some microspheres were still present after 21 days (Farrell & Nelson, 2013). Microplastic was also found inside the gut content of Norway lobster, *Nephrops norvegicus*, collected from Clyde Sea. Later, in a laboratory experiment, *N. norvegicus* was fed fish seeded with strands of polypropylene rope. One hundred percent of the animals had introduced plastics in their stomachs after 24 hours (Murray & Cowie, 2011). The potential of microplastic to be transferred with planktonic organisms from one trophic level (mesozooplankton) to a higher level (macrozooplankton) was shown in an experiment by Setälä et al., (2014). They exposed different zooplankton taxa (copepods, cladocerans, rotifers, polychaete larvae, and ciliates) to 10 μm fluorescent polystyrene microspheres and then offered the zooplankton to mysid shrimps. Already after 3 hours of incubation, zooplankton with microplastics was observed inside the mysid's intestine.

Microplastics are known to enter the very base of the marine plankton food web. Bhattacharya et al., (2010) observed that charged nano-polystyrene beads were absorbed into the cellulose of cell walls for two marine algal species: *Chlorella* sp. and *Scenedesmus* sp. Such absorption inhibited photosynthesis and promoted the production of reactive oxygen species which caused oxidative stress. The ability to ingest microplastic has also been documented for two species of ciliates, *Strombidium sulcatum,* and *Uronema* sp. They ingested plastic microspheres of sizes from 0.5 μm to 1 μm. The rate of uptake of 0.75 μm plastic in *S. sulcatum* was the same as its uptake rate of bacterial cells (Christaki et. al., 1998). A study on zooplankton by Desforges et al. (2015) showed that the calanoid copepod *Neocalanus cristatus* and the euphausiid *Euphausia pasifia* would filter microplastic particles from the water. The rate of ingestion correlated with the concentration and the size of microplastic particles in the environment. Another copepod species, *Calanus helgolandicus,* was also found to ingest microplastic. After 24-hour exposure to polystyrene beads, *C. helgolandicus* started to ingest less food, and a prolonged exposure to microplastic significantly decreased its reproductive output (Cole et al., 2015).

Benthic organisms can encounter and ingest microplastic as well. Thompson et al., (2004) exposed amphipods, barnacles, and lugworms to small quantities of polyvinylchloride (PVC) plastic fragments. All three species ingested microplastic within a few days. Deposit-feeding polychaete worms *Arenicola marina* (lugworm) were maintained in sediments containing microscopic PVC. At a concentration of PVC similar to that found in the natural environment, *A. marina* had significantly depleted energy reserves by up to 50%. The depletion was caused by a combination of reduced feeding activity, longer gut residence times of ingested material and inflammation (Wright et al., 2013a). The suspension-feeding mussel *Mytilus edulis* was fed polystyrene microplastic particles. After ingestion, microplastic accumulated in the gut of the mollusk. After 3 days it was translocated to the hemolymph and persisted there for over 48 days, but no significant biological effect was found (Browne et al., 2008). Deposit- and suspension-feeding holothurians *Holothuria floridana, H. grisea, Cucumaria frondosa* and *Thyonella gemmate* were fed PVC and nylon fragments (0.25 mm -15 mm) in sediment in the laboratory. Microplastics were kept for one week in natural seawater before the experiment. All of the sea cucumbers ingested microplastic at least once during the five feeding trials. Holothurians ingested significantly more plastic fragments than expected (from 2- to 100-fold more). The authors suggested that holothurians were selectively ingesting plastic particles, which may refer to their feeding mode. Plastic ingestion involved both random (the animals had to forage enough to contact particles) and selective (once particles were encountered, they were separated from the sediment) mechanisms (Graham & Thompson, 2009).

Microplastic contaminates marine habitats worldwide, can be encountered by virtually all marine organisms and can be transferred through the food web (Eriksen et al., 2014; Farrell & Nelson, 2013; Lusher, 2015; Murray & Cowie, 2011; Setälä et al., 2014). But physiological and toxicological effects of microplastics need further investigation. It is also required to research how microplastic from benthic sediments affect the infauna. And it is necessary to collect more knowledge about plastic contamination on different marine species (Lusher, 2015).

1.2. Foraminifera

Foraminifera are amoeboid protists. They are abundant and diverse in the oceans, both in planktonic and benthic environments. They play a role of micro-omnivores in the ecosystem, which means that they eat e.g., dissolved organic material, bacteria, detritus, phytoplankton

and/or zooplankton. Foraminifera possess granuloreticulopodia – thin anastomosing pseudopodia with a granular texture. Many foraminifera have a test or a shell that may be organic (not mineralized), agglutinated (constructed of foreign particles cemented together) or composed of calcium carbonate or, in rare cases, silica. A foraminifer's life cycle is characterized by an alternation between sexual and asexual generations. In tropical latitudes, the entire life cycle may take a year, while in temperate and higher latitudes it takes two or more years. Benthic foraminifera occupy a wide range of microhabitats from epibenthic to deep infaunal. Calcareous shells of benthic foraminifera will generally be stored in sediments after their death and will thus form a chronicle of the extant fauna (Armstrong & Brasier, 2005; Sen Gupta & Goldstein, 2006).

Foraminifera are important components of the benthic community food web. They feed at a low trophic level, mainly consuming bacteria and detritus (Gooday et al., 1992; Lipps, 1983). They work as a link between lower and higher trophic levels in the marine benthic food web. Thus foraminifera serve as a food source for both selective and non-selective deposit feeders and specialized predators (Gooday et al., 1992).

The number of toxicological studies with foraminifera as bio-indicators is increasing rapidly. Benthic foraminifera are good subjects for such studies because of their taxonomic diversity, wide distribution, abundance, relatively small size and short reproductive cycles, and at last but not the least, their shells that leave a record of past assemblages, and which often provide morphological or geochemical evidence of previous environmental change (Martinez-Colon et al., 2009; Sen Gupta et al., 2006). Foraminifera are also suitable to be used as bioindicators even under extreme conditions caused by highly variable physicochemical parameters (Martins et al., 2016). In polluted areas, the total abundance of calcareous and agglutinated foraminifera and species diversity can vary as well as abnormalities of tests such as stunted growth, abraded margins and dissolved ornamentations (Nigam et al., 2009). Many studies are available on the effect of different sources of pollution on foraminifera, e.g. sewage outfalls, organic waste, heavy metal pollution, pesticides, oil and agriculture (Alve, 1991b, 1991a; Alve & Olsgard, 1999; Elberling et al., 2003; Nagy & Alve, 1987; Nigam et al., 2009; Schafer et al., 1991). Effects of microplastic on foraminifera are however poorly studied. Recent studies from Japan have shown that agglutinated foraminifera can incorporate microplastic particles inside their test (Tsuchiya & Nomaki, 2019). In another recent laboratory study, benthic foraminifera were fed with polystyrene beads, silicon dioxide, and titanium dioxide particles. In all three experiments,

increased production of neutral lipids and reactive oxygen species was observed, which both are known to be produced by organisms under stress (Bouchet, 2019).

As microplastic sinks down to the sea bottom, it will be encountered by foraminifera. Since foraminifera is one of the base components of benthic food webs, it is important to know if all the foraminiferan species would accumulate microplastic and to which extent, and if the size of microplastic matters for its accumulation. In the current study, the accumulation of three differently sized (0.5 μm, 1 μm, 6 μm) polystyrene particles by foraminifera was examined. Two experiments were set up with different exposure times. Short-term exposure of foraminifera community to the microplastics lasted for six hours, and long-term exposure – for four weeks.

1.3. Aims

The overall aim of this project was to clarify whether microplastics accumulate in and affect benthic foraminifera.

The main aim can be subdivided into the following:

- Is there a change in foraminifera community composition after exposure to microplastics (0.5 μm, 1 μm, 6 μm) for six hours and/or four weeks?
- Is there a difference in the accumulation of microplastics (0.5 μm, 1 μm, 6 μm) between foraminifera species?
- Do differently sized microplastics (0.5 μm, 1 μm, 6 μm) accumulate in a similar pattern in different foraminifera?
- Is there a difference in the accumulation of microplastics (0.5 μm, 1 μm, 6 μm) in different foraminifera exposed for six hours and four weeks?
- Does ecologically relevant descriptors of foraminifera (tolerance to organic carbon; microhabitat preferences; food preferences; test composition) explain the accumulation of microplastics?

2. Material and methods

2.1. Sediment sampling

Sediment for the experiments was collected at site IM4X (N 59.645035 E 10.613633, 163m water depth) in September 2018 using the R/V Trygve Braarud (UiO) vessel (Fig. 2). The site used for sampling is located in the outer Oslofjord and was chosen in order to gather a benthic foraminifera community from an unpolluted area. Sediment samples were taken by a Gemini-corer (Fig. 3). In addition to the sediment samples, seawater was collected close to the seafloor at the same site. The upper 2 cm of undisturbed surface sediment were collected from cores and placed in containers. Collected seawater was added to the sediment. The volume of added seawater was approximate twice the volume of the collected sediment. Samples in the containers were stored on ice and transported to the lab. Collected sediments were stored in a climate room 7C° at the Department of Biosciences, University of Oslo. Before starting the experiments, the sediment was transferred to one container and gently homogenized.

Figure 2. Map of the inner Oslofjord (Dolven et al., 2013; Dolven et. al., 2018). IM4X is the site where sediment for the experiments was collected.

Figure 3. Pictures from the sampling campaign; a = R/V Trygve Braarud (UiO); b = Gemini-corer, which was used for sediment core sampling; c = collected sediment core from site IM4X; d = CTD with water sampler.

2.2. Experiment set-up

Two experiments with addition of microplastic particles to the sediments were performed. Both experiments were set up in the same way, but the experimental running time differed. The first experiment lasted for six hours and the second for four weeks. Polystyrene microbeads of three different particle sizes were used in both experiments: Fluoresbrite® YG Microspheres 0.5 µm (2.5% aqueous suspension; 3.64 x 1011 particles/mL; excitation max. = 441 nm; emission max. = 486 nm); Fluoresbrite® Polychromatic Red Microspheres 1.0 µm (2.5% aqueous suspension; 4.55 x 1010 particles/mL; excitation max. = 525 nm; emission max. = 565 nm) and Fluoresbrite® Polychromatic Red Microspheres 6.0 µm (2.5% aqueous suspension; 2.10 x 108 particles/mL; excitation max. = 525 nm; emission max. = 565 nm). In total each experiment contained a set of twenty samples. Five samples were used as a reference without any microplastics added to them. To the remaining fifteen samples microplastics were added. Different sizes of microplastics (0.5 µm, 1 µm, and 6 µm) were added to five replicate samples.

Experimental samples were prepared by transferring 10 mL of gently homogenized sediment into a 40 mL container. To all sediment samples, except the reference samples, one droplet of the microplastic solution was added. After addition of the microplastics, the material in the container was gently mixed to evenly distribute the microplastic particles in the sediment. The final concentration of microplastic in the samples was 1.82*10^9 particles per mL for

Fluoresbrite® YG Microspheres 0.5 µm; 2.275*10^8 particles per mL for Fluoresbrite® Polychromatic Red Microspheres 1.0 µm; and 1.05*10^8 particles per mL for Fluoresbrite® Polychromatic Red Microspheres 6.0 µm. The samples were kept in non-transparent boxes for the experiment periods (six hours and four weeks) to protect them from light and limit algal growth in the chambers (Fig. 4). The boxes were kept in a climate room at a temperature of 7C° throughout the experiments.

For the six-hour experiment, the samples were incubated in five batches. Each batch contained one reference sample and one sample with 0.5 µm, 1 µm and 6 µm microplastic particles. The samples were prepared and added to a batch with twenty minutes intervals. The twenty minutes delay was necessary to keep the incubation time accurate (exactly six hours for every sample), as some time was necessary for processing each sample after the incubation period (washing the sample on a sieve and adding rose Bengal/ethanol mixture (see below)). The first batch was incubated in one day, the next two batches were incubated on the next day and the last two batches were incubated on the third day. The incubation of sample batches in three different days was necessary because the preparation, incubation, and processing of one-two batches of samples took about ten hours. Each batch comprised one chamber for each treatment to avoid batch effects.

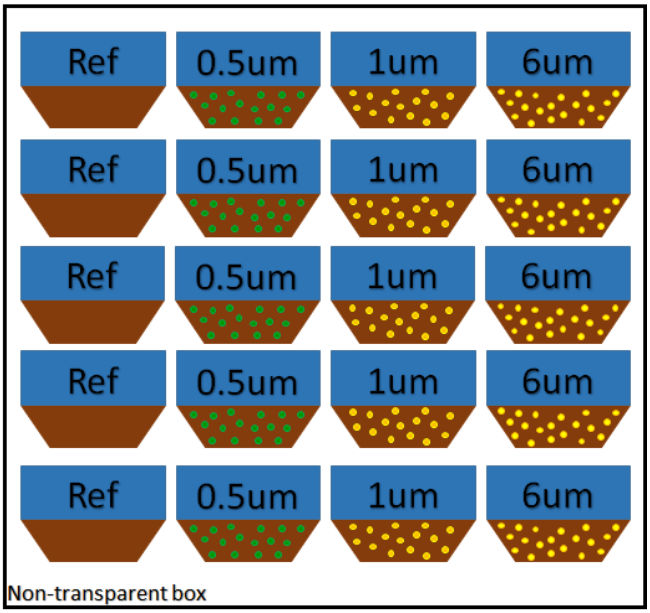

Figure 4. The scheme of the set-up for the six-hour and four-week experiments. “Ref” = reference samples, where no microplastic was added. “0.5um”, “1um”, “6um” = samples to which were added 0.5 µm, 1 µm and 6 µm microplastic particles respectively.

In the four-week experiment, the samples were also prepared in twenty minutes intervals to keep the incubation time accurate (four weeks for every sample). All twenty samples in the four-week experiment were set up in one day.

After the incubation time was over, the samples were gently washed with seawater on three sieves: 500 µm, 250 µm, and 125 µm. Two fractions (250-500 µm and 125-250 µm) were collected and preserved with 70% ethanol and rose Bengal

to stain the cytoplasm (2 g rB/L). The 500 µm sieve was needed to remove debris from the samples. The samples were stored in the rose Bengal/ethanol mixture for two weeks before they were washed again to remove excess stain (Schönfeld et al., 2012).

2.3. Identification of foraminifera and ingested microplastics

The samples were analyzed under a dissecting microscope. Well-stained specimens were considered as living. All living foraminifera from the samples were identified to species level, counted and transferred to slide under a fluorescent microscope "Zeiss Axio Scope.A1" with 10x magnification to check if they contained fluorescent microplastic (Fig.5). Foraminifera with a strong fluorescence signal were considered to be specimens which had ingested microplastics. These specimens were counted and photographed. Under the fluorescent microscope and on the images, 0.5 µm polystyrene particles had green colour, while 1 µm and 6 µm particles had yellow-orange colour.

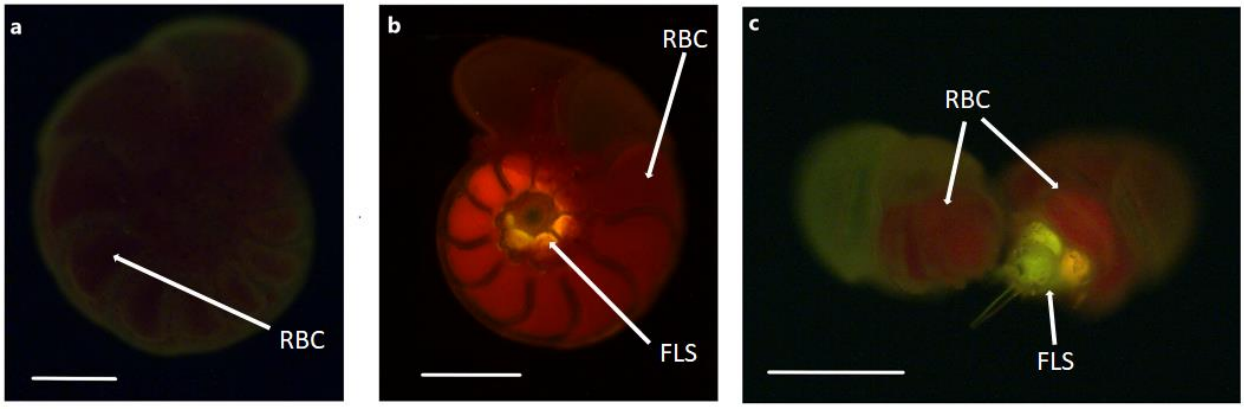

Figure 5. Pictures of foraminifera with and without microplastic inside the cell. FLS = fluorescent signal from the microplastic; RBC = rose Bengal stained cytoplasm. a) *Hyalinea balthica* without microplastic; b) *H. balthica* with microplastic; c) *Bulimina marginata,* on the left - without microplastic and on the right – with microplastic.

2.4. Species grouping

Species were grouped by ecologically relevant descriptors such as tolerance to the organic carbon, microhabitat preferences (vertical distribution in the sediment), feeding strategies and test structure (Table 1). The assignment in the groups has been determined with

the literature and by the advice of experts in foraminifer's ecology. Foraminifera were assigned by the tolerance to the organic carbon to one of five ecogroups according to the marine biotic index AMBI (from the group I to V, where the group I consists of species which are most sensitive to the organic matter enrichment, and group V contains the most opportunistic species) (Alve et al., 2016). By microhabitat preference (vertical distribution in the sediment) foraminifera were divided in epifaunal, shallow infaunal, infaunal and deep infaunal species (Alve & Bernhard, 1995; de Stigter, 1996; Murray, 2003). Feeding strategies of foraminifera are not well studied for many species and the group assignments into phytodetritus feeders and possibly phytodetritus feeders were done for less than a half of all the found species (Gooday, 1988; Gooday & Rathburn, 1999). Based on their test structure foraminifera were grouped into calcareous and agglutinated species (Sen Gupta, 1999).

2.5. Statistical analyses

Cluster and multidimensional scaling (MDS) analyses were performed based on the total abundance of rose Bengal stained foraminifera in each of the samples using Primer-E (Quest Research Limited). Square root transformation was used to minimize the influence of dominating species on the final results. In order to investigate dissimilarities in the data, a correspondence matrix was constructed between all pairs of samples. Based on that resemblance matrix, cluster (S17 Bray Curtis similarity) and MDS analyses were performed. Shannon diversity index (H'(log2)) was also calculated in Primer-E. Kruskal-Wallis tests were used to elucidate the diversity differences between the samples from two experiments and between the four treatments (reference, 0.5 μm, 1 μm, and 6 μm microplastics).

The difference in total abundance of rose Bengal stained foraminifera in samples from four different treatments (reference, 0.5 μm, 1 μm and 6 μm microplastic) and from the six-hour and four-week experiments were tested by Kruskal-Wallis tests using Statistica 12 (StatSoft). In addition, the difference in the number of individuals which ingested microplastic in two experiments was tested by Kruskal-Wallis test. The significance level for all the statistical analyses was $p < 0.05$.

The median numbers of specimens with and without microplastic inside were calculated for the four treatments (reference, 0.5 μm, 1 μm and 6 μm microplastic) based on five replicate samples. Only those of the species which ingested microplastic in three or more replicates gave a

median value higher than "0". Then the ratio of microplastic ingestion (r) was calculated by dividing the number of individuals of one species with ingested microplastic by the total number of all stained individuals of that species found in one sample.

$$r = \frac{N\ forams\ with\ microplastic}{total\ N\ forams\ of\ that\ species}$$

Thus, the ratio of microplastic ingestion shows how many individuals of one species ingested microplastics in relation to the total number of individuals of this species. If the ratio is higher than zero, it means that the species ingested microplastic. The higher the ratio, the more individuals of that species were found with microplastic particles inside.

Ratios, calculated based on the median numbers of individuals, were used for the frequency test. Frequency test was performed in Excel (Microsoft). In addition, microplastic ingestion ratios were compared with the Kruskal-Wallis test using Statistica 12 to clarify whether the ecologically relevant descriptors of foraminifera would describe the ingestion of microplastic (see 2.4 Species grouping).

Ratios of microplastic ingestion were also calculated in every replicate sample for the thirteen foraminifera species. These thirteen species were chosen because they ingested microplastic in at least three out of five replicate samples for any of the three sizes of microplastics. Due to the last, it was considered that these species accumulated microplastic. These ratios were used to create box plots. In addition, Kruskal-Wallis tests were performed based on these ratios using Statistica 12. The Kruskal-Wallis tests were done to elucidate the difference between the ratios of ingesting three sizes of microplastic and the difference in ratios of ingesting each size of microplastic after six hours and four weeks for each of the thirteen species.

Table 1. The grouping of foraminifera species by tolerance to organic carbon (AMBI-index ecogroups), microhabitat preference (vertical distribution in the sediment), feeding strategy and test composition. Epif = epifaunal, Sh inf = shallow infaunal, Inf = infaunal, D inf = deep infaunal, Ph = phytodetritus, P Ph = possibly phytodetritus, aggl = agglutinated, calc = calcareous, n/a = no information (Alve et al., 2016; Alve & Bernhard, 1995; Gooday, 1988; Gooday & Rathburn, 1999; de Stigter, 1996; Murray, 2003; Sen Gupta, 1999).

List of species	AMBI-index ecogroups	microhabitat	feeding strategy	test structure	List of species	AMBI-index ecogroups	microhabitat	feeding strategy	test structure
Adercotryma wrighti	1	Sh inf	Ph	aggl	*Liebusella goesi*	2	Inf	Ph	aggl
Astrononion gallowayi	2	Inf	n/a	calc	*Loxostomum porrectum*	n/a	Inf	n/a	calc
Brizalina skagerrakensis	3	Sh inf	Ph	calc	*Melonis barleeanum*	3	D inf	n/a	calc
Brizalina spathulata	3	Sh inf	Ph	calc	*Nonionella stella*	2	D inf	P Ph	calc
Bulimina marginata	3	Inf	Ph	calc	*Nonionella turgida*	3	Inf	Ph	calc
Cassidulina laevigata	1	Sh inf	P Ph	calc	*Nonionellina labradorica*	n/a	Inf	P Ph	calc
Cibicides lobatulus	1	Epif	n/a	calc	*Pullenia bulloides*	3	Inf	n/a	calc
Cribrostomoides globosum	1	Inf	n/a	aggl	*Quinqueloculina stalkeri*	5	Inf	n/a	calc
Cribrostomoides jeffreysii	n/a	Sh inf	n/a	aggl	*Recurvoides trochamminiformis*	3	Inf	P Ph	aggl
Cribrostomoides nitidum	1	Inf	Ph	aggl	*Reophax bilocularis*	1	Inf	n/a	aggl
Dentalina communis	n/a	Inf	n/a	calc	*Reophax dentaliniformis*	n/a	Sh inf	n/a	aggl
Eggerelloides medius	3	Inf	Ph	aggl	*Reophax fusiformis*	n/a	Inf	n/a	aggl
Eggerelloides scaber	3	Inf	Ph	aggl	*Reophax micaceus*	1	Inf	n/a	aggl
Elphidium excavatum	1	Inf	n/a	calc	*Reophax sp.*	n/a	Inf	n/a	aggl
Epistominella vitrea	2	Sh inf	Ph	calc	*Saccammina sphaerica*	2	Sh inf	n/a	aggl
Glandulina laevigata	n/a	Inf	n/a	calc	*Sigmoilopsis schlumbergeri*	n/a	Inf	n/a	calc
Globobulimina turgida	3	Inf	n/a	calc	*Stainforthia fusiformis*	5	Inf	P Ph	calc
Haplophragmoides bradyi	2	Inf	P Ph	aggl	*Technitella legumen*	n/a	Inf	n/a	aggl
Hyalinea balthica	1	Sh inf	Ph	calc	*Tritaxis conica*	1	Epif	n/a	aggl
Lagena laevis	n/a	Inf	n/a	calc	*Uvigerina peregrina*	3	Inf	Ph	calc
Lagena striata	n/a	Inf	n/a	calc					

3. Results

The calculated diversity indexes (H'(log2)), and counted numbers of rose Bengal stained foraminifera for every sample for the six-hour and four-week experiments are presented in the appendix tables 1-9. The median numbers of specimens with and without microplastic inside, calculated based on five replicates and the ratios of microplastic ingestion calculated based on these medians are presented in the appendix in tables 10 for the six-hour experiment and in table 11 for four-week experiment.

3.1 Community composition

In the six-hour experiment, in total 39 foraminifera species were identified and in the four-week experiment, 41 species were identified. Species numbers in the samples ranged between 18 and 30. The number of individuals in the samples varied between 231 and 486, where the lowest numbers were observed in the samples from the four-week experiment, and the highest numbers in the six-hour experiment. The total abundance of rose Bengal stained foraminifera in the four-week experiments was significantly lower (Kruskal-Wallis test $H = 21.65$, $p < 0.001$) than in the six-hour experiments (Table 2). But in both experiments, no significant difference of rose Bengal stained foraminifera abundance was observed in samples with different treatments (reference, 0.5 μm, 1 μm and 6 μm microplastic) in six-hour (Kruskal-Wallis test $H = 1.06$, $p = 0.79$) or four-week (Kruskal-Wallis test $H = 2.66$, $p = 0.45$) experiments.

Table 2. Total abundance of rose Bengal stained foraminifera in each sample (indiv./10mL); for each treatment (reference, 0.5 µm, 1 µm and 6 µm microplastic) (indiv./50 mL) and in both experiments (6-hour and 4-week) (indiv./200 mL).

6-hour				4-week			
Ref	0.5 µm	1 µm	6 µm	Ref	0.5 µm	1 µm	6 µm
Total abundance of rose Bengal stained foraminifera in each sample (indiv./10 mL)							
346	354	304	449	357	231	249	302
443	470	381	360	300	286	261	321
404	439	451	351	298	303	340	354
486	429	432	397	384	345	304	273
362	346	423	342	323	284	330	307
Total abundance of rose Bengal stained foraminifera for each treatment (indiv./50 mL)							
2041	2038	1991	1899	1662	1449	1484	1557
Total abundance of rose Bengal stained foraminifera in the whole experiment (indiv./200 mL)							
7969				6152			

The Shannon diversity index (H'(log2)) varied from 3.53 to 4.03. No significant differences were found between the six-hour and four-week experiments (Kruskal-Wallis test: H = 0.28 p = 0.60). Further, no significant differences in diversity were found between treatments in the six-hour (Kruskal-Wallis test H = 2.81, p = 0.42) and four-week (Kruskal-Wallis test H = 4.76, p = 0.19) experiments.

In the cluster analysis, the samples from the six-hour and four-week experiments were similar, separating after the similarity reached around 85% (Fig.6). Multidimensional scaling (MDS) analysis showed the same high similarity between the samples from the six-hour and four-week experiments (Fig.7). Four samples of the six-hour experiment (1-0.5um, 2-1um, 3-6um, and 4-ref) make a cluster and separate from the rest of the samples.

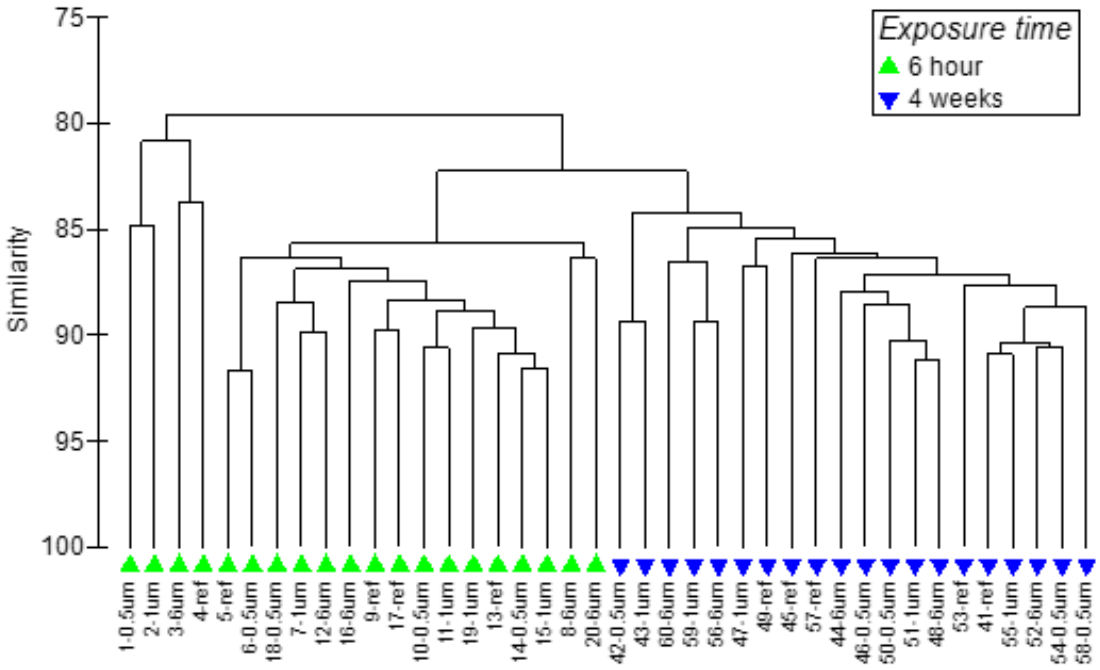

Figure 6. Cluster analyses plot based on rose Bengal stained foraminifera abundance (indiv./10 mL sediment) for all analysed samples. Exposure time to microplastic indicated with colour (green = six-hour exposure; blue = four-week exposure). Transform: square root, resemblance: S17 Bray Curtis similarity.

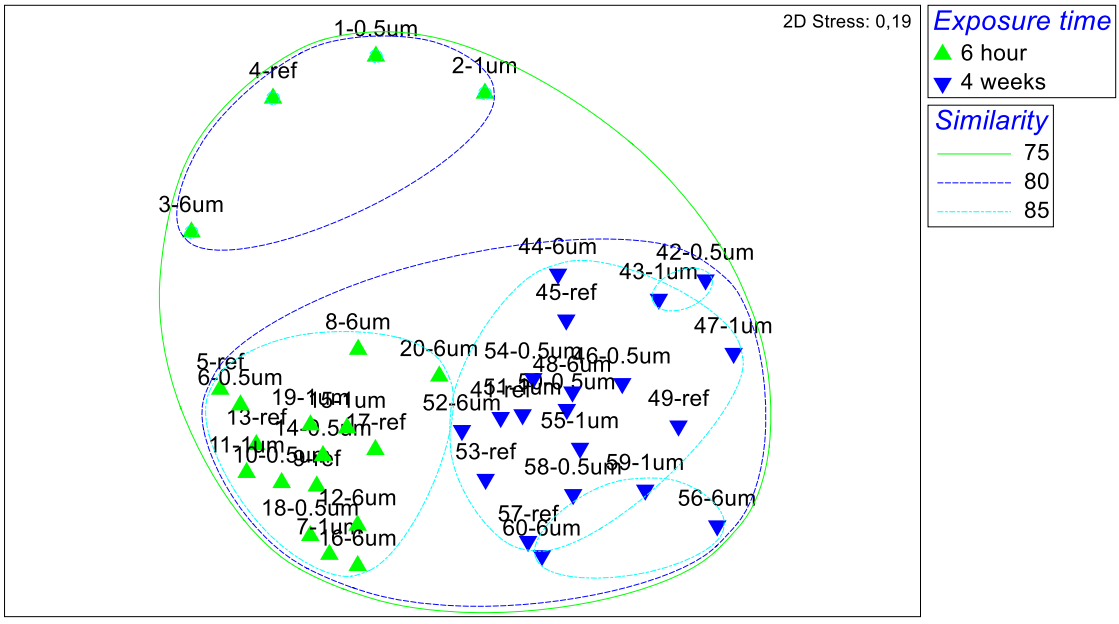

Figure 7. MDS plot based on live foraminifera community composition (indiv./10 mL sediment) for all analysed samples. Exposure time to microplastic indicated with colour (green = six-hour exposure; blue = four-week exposure). Transform: square root, resemblance: S17 Bray Curtis similarity.

3.2. Microplastic accumulation

The total abundance of foraminifera which ingested microplastic is shown in table 3. Almost three times more individuals ingested microplastic in the four-week experiment (1686 individuals) compared to the six-hour experiment (514 individuals). The Kruskal-Wallis test showed that the difference between the number of individuals with microplastic in the six-hour and four-week experiments were significant (Kruskal-Wallis test $H = 21.78$, $p < 0.001$).

Table 3. Total abundance of rose Bengal stained foraminifera which ingested microplastic in each sample (indiv./10 ml); for each treatment (reference, 0.5 µm, 1 µm and 6 µm microplastic) (indiv./50 ml); and in both experiments (6-hour and 4-week) (indiv./200 ml).

6-hour				4-week			
Ref	0.5 µm	1 µm	6 µm	Ref	0.5 µm	1 µm	6 µm
Total abundance of foraminifera which ingested microplastic in each sample (indiv./10 ml)							
0	28	55	72	0	99	104	93
0	35	36	24	0	96	95	103
0	27	19	13	0	124	114	99
0	26	45	28	0	181	78	91
0	22	54	30	0	143	129	137
Total abundance of foraminifera which ingested microplastic for each treatment (indiv./50 ml)							
0	138	209	167	0	643	520	523
Total abundance of foraminifera which ingested microplastic in the whole experiment (indiv./200 ml)							
514				1686			

Seventeen species ingested microplastic in the six-hour experiment and twenty-one species ingested microplastic in the four-week experiment. Individuals of some species had accumulated microplastic (Table 4, group A), whereas for other species only a few individuals ingested microplastic and the majority of individuals of that species had no microplastic inside (Table 4, group B). In the six-hour experiment, 22 species and in the four-week experiment 20 species did not ingest any microplastic at all (Table 4, group C). Figure 8 shows thirteen foraminifera species which accumulated microplastic in at least three out of five replicate samples.

Table 4. All identified foraminifera species in the six-hour and four-week experiments. Group A includes species which accumulated microplastics (individuals of these species ingested microplastic in at least three out of five replicates for any of the three sizes of microplastic). Group B includes the species which ingested microplastic in only one or two replicates. Group C includes species which never ingested any microplastic. The different colours are explained in the discussion (4.2 Microplastic accumulation).

6-hour experiment			4-week experiment		
Group A	Group B	Group C	Group A	Group B	Group C
Brizalina skagerrakensis	*Astrononion gallowayi*	*Adercotryma wrighti*	*Brizalina skagerrakensis*	*Adercotryma wrighti*	*Cibicides lobatulus*
Brizalina spathulata	*Eggerelloides medius*	*Cibicides lobatulus*	*Brizalina spathulata*	*Astrononion gallowayi*	*Cribrostomoides globosum*
Bulimina marginata	*Eggerelloides scaber*	*Cribrostomoides globosum*	*Bulimina marginata*	*Cribrostomoides nitidum*	*Cribrostomoides jeffreysii*
Cassidulina laevigata	*Elphidium excavatum*	*Cribrostomoides jeffreysii*	*Cassidulina laevigata*	*Dentalina communis*	*Epistominella vitrea*
Hyalinea balthica	*Lagena striata*	*Cribrostomoides nitidum*	*Eggerelloides medius*	*Globobulimina turgida*	*Glandulina laevigata*
Nonionella turgida	*Liebusella goesi*	*Epistominella vitrea*	*Eggerelloides scaber*	*Loxostomum porrectum*	*Haplophragmoides bradyi*
Nonionellina labradorica	*Loxostomum porrectum*	*Glandulina laevigata*	*Elphidium excavatum*	*Reophax micaceus*	*Lagena laevis*
Uvigerina peregrina	*Melonis barleeanum*	*Globobulimina turgida*	*Hyalinea balthica*		*Lagena striata*
	Nonionella stella	*Haplophragmoides bradyi*	*Liebusella goesi*		*Pullenia bulloides*
		Lagena laevis	*Melonis barleeanum*		*Quinqueloculina stalkeri*
		Pullenia bulloides	*Nonionella stella*		*Recurvoides trochamminiformis*
		Quinqueloculina stalkeri	*Nonionella turgida*		*Reophax bilocularis*
		Recurvoides trochamminiformis	*Nonionellina labradorica*		*Reophax dentaliniformis*
		Reophax bilocularis	*Uvigerina peregrina*		*Reophax fusiformis*
		Reophax fusiformis			*Reophax sp.*
		Reophax micaceus			*Saccammina sphaerica*
		Reophax sp.			*Sigmoilopsis schlumbergeri*
		Saccammina sphaerica			*Stainforthia fusiformis*
		Sigmoilopsis schlumbergeri			*Technitella legumen*
		Stainforthia fusiformis			*Tritaxis conica*
		Technitella legumen			
		Tritaxis conica			

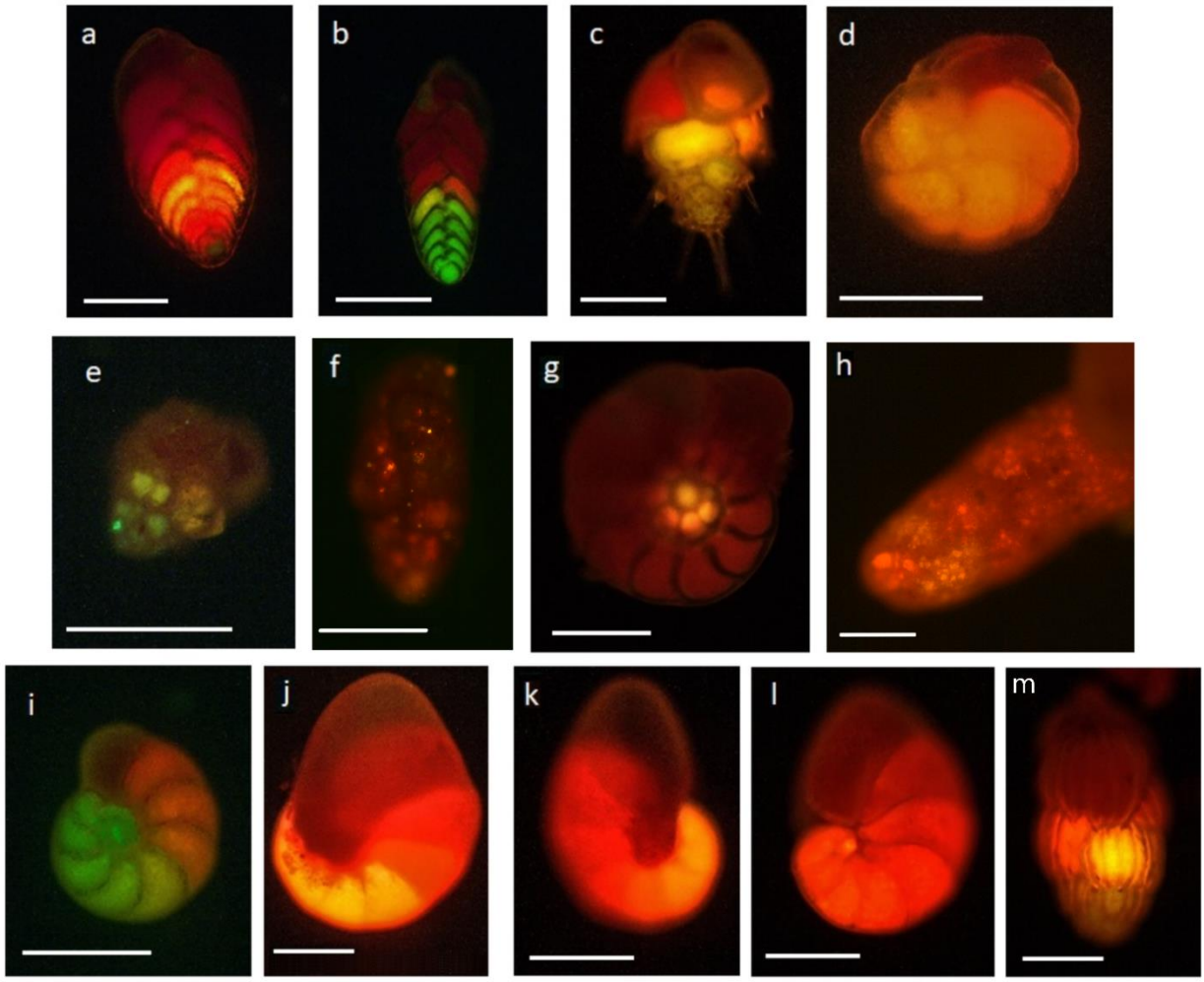

Figure 8. Images of foraminifera which accumulated microplastics; 0.5 µm polystyrene particles coloured green, while 1 µm and 6 µm particles coloured yellow-orange. Magnification = 10x. Scale bar =100 µm; a = *Brizalina skagerrakensis*, 1 µm six-hour experiment; b = *Brizalina spatulata*, 0.5 µm six-hour experiment; c = *Bulimina marginata*, 6 µm six-hour experiment; d = *Cassidulina laevigata*, 6 µm six-hour experiment; e = *Eggerelloides medius*, 0.5 µm four-week experiment; f = *Eggerelloides scaber*, 0.5 µm four-week experiment; g = *Hyalinea balthica*, 1 µm six-hour experiment; h = *Liebusella goesi*, 6 µm four-week experiment i = *Melonis barleeanum*, 0.5 µm four-week experiment; j = *Nonionella stella*, 6 µm four-week experiment; k = *Nonionella turgida*, 6 µm six-hour experiment; l = *Nonionellina labradorica*, 1 µm six-hour experiment; m = *Uvigerina peregrine*, 6 µm six-hour experiment.

3.2.1. Frequency test

The frequency test shows that in both experiments the majority of species had ratios equal to 0 (r = 0) (Fig. 9). About 80-85% and 69-76% of the species had r = 0 for three different sizes of microplastic (0.5 µm, 1 µm and 6 µm) in the six-hour and four-week

experiments accordingly. Overall the ratios in the six-hour experiment were significantly lower than in the four-week experiment (Kruskal-Wallis test H = 5.98, p = 0.01).

In the six-hour experiment, about 13% of the species had ratios less than 0.2 ($0 < r \leq 0.2$) for the 0.5 µm microplastic particles and 18% of the species had ratios less than 0.2 ($0 < r \leq 0.2$) for both 1 µm and 6 µm microplastic particles each. About 3% of the species had ratios in diapason from 0.2 to 0.4 for the 0.5 µm and 6 µm microplastics each. And 3% of the species had ratios from in diapason from 0.4 to 0.6 for 1 µm microplastic particles. No species had ratios higher than 0.6.

In the four-week experiment, ratios were higher. There were no species with the ratios less than 0.2 ($0 < r \leq 0.2$). About 7% of the species had ratios in diapason from 0.2 to 0.4 for the 0.5 µm microplastic particles, and 10% of the species had ratios in the same diapason for 1 µm and 6 µm microplastics each. About 14% of the species (0.5 µm microplastics) and about 10% of the species (1 and 6 µm microplastics) had ratios in the diapason from 0.4 to 0.6. In the diapason from 0.6 to 0.8, the ratios had about 7% of the species (0.5 µm microplastics) and about 5% of the species (1 and 6 µm microplastics). Finally, 2% of the species had ratio higher than 0.8 ($0.8 < r \leq 1.0$) for the 0.5 µm and 6 µm microplastics each. Two species, *Elphidium excavatum* and *Liebusella goesi*, had a ratio equal to 1 ($r = 1$) in the four-week experiment.

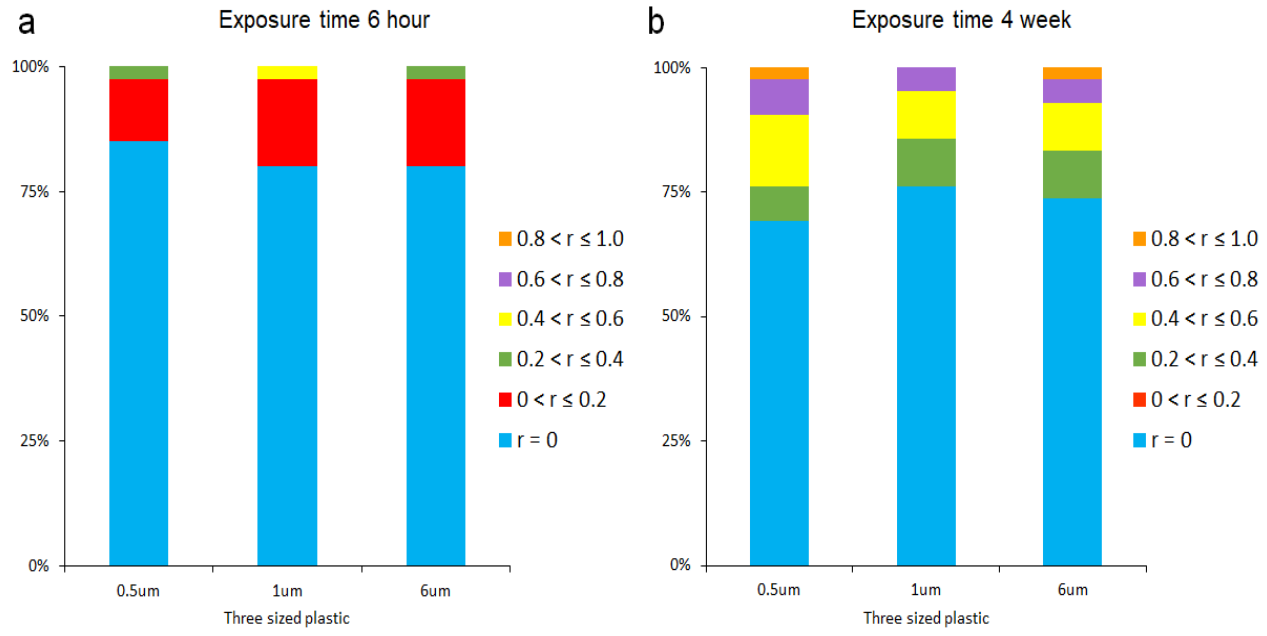

Figure 9. Frequency test of the ratios of microplastic ingestion by foraminifera (r).

3.2.2. Accumulation of three different sizes of microplastic

Figure 10 shows the ratios of microplastic ingestion for the thirteen foraminifera species, which accumulated microplastic in at least three replicates. For most of the species in the six-hour and four-week experiments, there were no significant differences in the accumulation of any of the microplastic sizes (0.5 μm, 1 μm and 6 μm), except for two species. In the six-hour experiment, *Nonionella turgida* only accumulated 1 μm and 6 μm plastic particles and did not accumulate any 0.5 μm microplastic beads (Kruskal-Wallis test $H = 7.62$, $p = 0.02$). In the four-week experiment, *Uvigerina peregrina* accumulated all three sized microplastic particles, but the median of the ratios of microplastic ingestion was highest for the 0.5 μm microplastic particles, lower for 1 μm and the smallest for the 6 μm microplastic (Kruskal-Wallis test $H = 6.70$, $p = 0.035$).

3.2.3. Accumulation of microplastic during six-hour and four-week experiments

The difference in ratios of microplastic ingestion was tested between six-hour and four-week experiments for each microplastic size treatments for the same thirteen foraminifera species. In the four-week experiment, all thirteen species accumulated significantly more of 0.5 μm microplastic particles than in the six-hour experiment. Seven out of thirteen species (*B. spathulata, C. laevigata, E. medius, E. scaber, H. balthica, N. labradorica* and *U. peregrine*) accumulated significantly more of 1 μm microplastic particles. While six species (*B. skagerrakensis, B. marginata, L. goesi, M. barleeanum, N. stella and N. turgida*) accumulated 1 μm microplastic particles in the same way in short- and long-term experiments. Ten out of thirteen species (*B. skagerrakensis, B. spathulata, B. marginata, C. laevigata, E. medius, E. scaber, H. balthica, N. stella, N. labradorica* and *U. peregrine*) accumulated significantly more of 6 μm microplastic particles in the four-week experiment. *L. goesi* also accumulated more microplastic in the longer exposure experiment, even though the difference was not significant, the p-value was on the border of significance ($p = 0.054$). Two species, *M. barleeanum* and *N. turgida,* accumulated 6 μm microplastic particles in the same way in short- and long-term experiments (Table 5).

Table 5. Difference between ratios of microplastic ingestion in six-hour and four-week experiments, Kruskal-Wallis tests. Bold numbers = significantly different.

Species	0.5 um		1 um		6 um	
	H-statistic	*p*-value	H-statistic	*p*-value	H-statistic	*p*-value
Brizalina skagerrakensis	4.81	**0.03**	0.27	0.60	6.82	**0.01**
Brizalina spathulata	5.77	**0.02**	5.77	**0.02**	3.94	**< 0.05**
Bulimina marginata	6.82	**0.01**	2.81	0.09	5.81	**0.02**
Cassidulina laevigata	6.99	**0.01**	6.86	**0.01**	4.84	**0.03**
Eggerelloides medius	7.31	**0.01**	7.76	**0.01**	7.76	**0.01**
Eggerelloides scaber	6.99	**0.01**	7.26	**0.01**	7.76	**0.01**
Hyalinea balthica	6.82	**0.01**	3.94	**0.05**	4.81	**0.03**
Liebusella goesi	5.54	**0.02**	0.02	0.88	3.72	> 0.05
Melonis barleeanum	4.51	**0.03**	0.02	0.88	0.15	0.70
Nonionella stella	6.78	**0.01**	2.01	0.16	5.15	**0.02**
Nonionella turgida	5.54	**0.02**	0.00	1.00	1.89	0.17
Nonionellina labradorica	6.86	**0.01**	5.81	**0.02**	6.86	**0.01**
Uvigerina peregrina	6.82	**0.01**	6.82	**0.01**	6.86	**0.01**

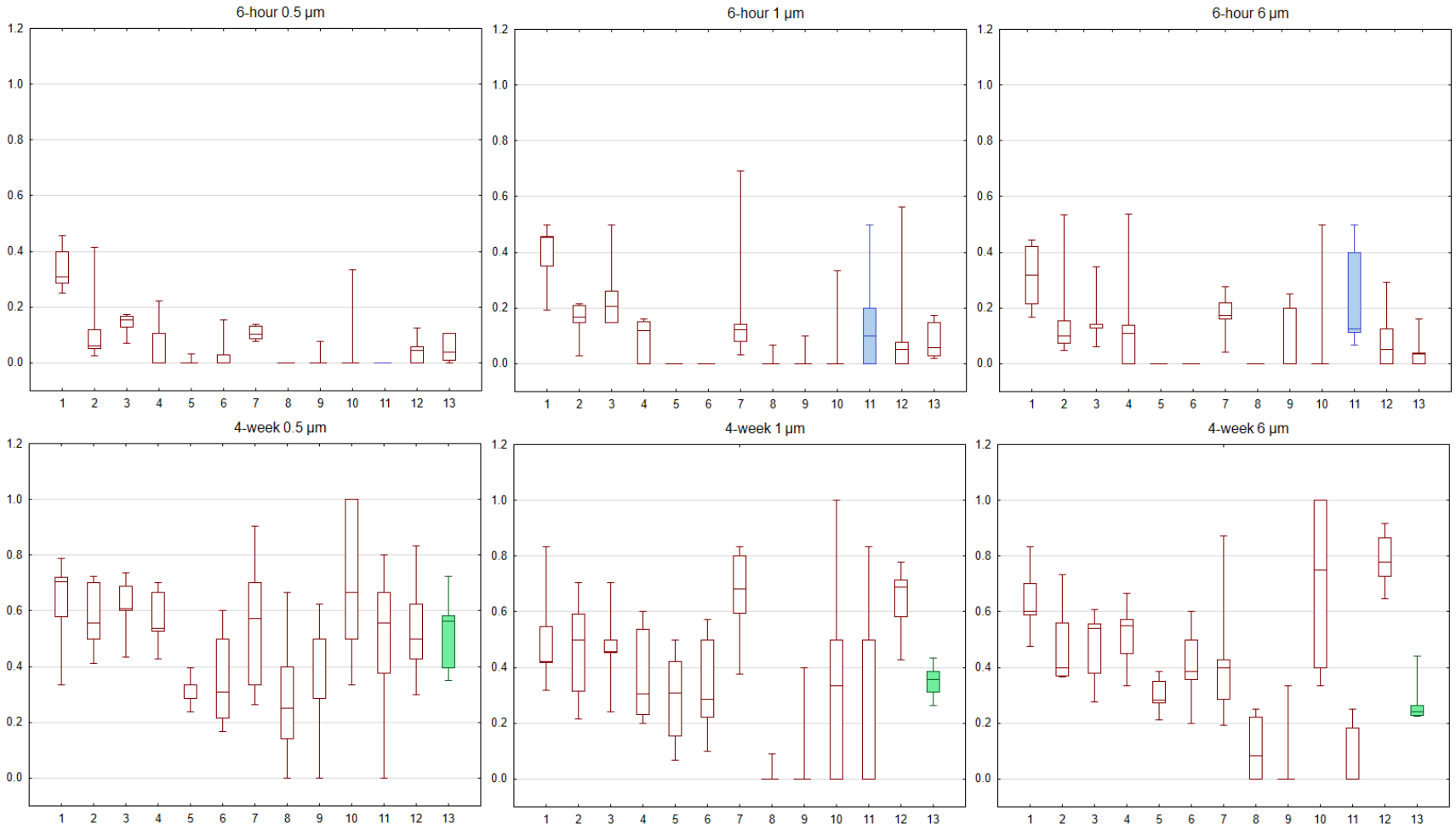

Figure 10. Microplastic accumulation ratios for different species; treatment indicated above graphs. *Nonionella turgida* - blue-coloured and *Uvigerina peregrine* – green-coloured, had a significant difference in the accumulation of three different sizes of microplastic. X scale = foraminifera species; 1 = *Brizalina skagerrakensis*, 2 = *Brizalina spathulata*, 3 = *Bulimina marginata*, 4 = *Cassidulina laevigata*, 5 = *Eggerelloides medius*, 6 = *Eggerelloides scaber*, 7 = *Hyalinea balthica*, 8 = *Liebusella goesi*, 9 = *Melonis barleeanum*, 10 = *Nonionella stella*, 11 = *Nonionella turgida*, 12 = *Nonionellina labradorica*, 13 = *Uvigerina peregrine*; median, 25%-75%, Min-Max.

3.3. Species grouping

No significant differences in the ratios of microplastic ingestion were found in the groups based on AMBI-index in the six-hour experiment. In the four-week experiment a significant difference was found in the ratios of microplastic ingestion based on AMBI-index only for the 0.5 μm microplastic particles (Kruskal-Wallis test H = 11.08, p = 0.03) (Fig. 11 a, b).

Based on the microhabitat preferences (vertical distribution in the sediment), no significant differences were found in the ratios of microplastic ingestion in both six-hour and four-week experiments (Fig. 11 c, d).

In groups based on feeding strategy differences in the ratios of microplastic ingestion were significant in all three treatments (0.5 μm, 1 μm, 6 μm) and in both six-hour and four-week experiments (Fig. 12 a, b):

- six-hour experiment, 0.5 μm – Kruskal-Wallis test H = 9.28, p = 0.01
- six-hour experiment, 1 μm – Kruskal-Wallis test H = 11.27, p = 0.004
- six-hour experiment, 6 μm – Kruskal-Wallis test H = 11.09, p = 0.004
- four-week experiment, 0.5 μm – Kruskal-Wallis test H = 17.39, p = 0.0002
- four-week experiment, 1 μm – Kruskal-Wallis test H = 15.14, p = 0.001
- four-week experiment, 6 μm – Kruskal-Wallis test H = 10.83, p = 0.004

Foraminifera which accumulated microplastic were mainly phytodetritus or possibly phytodetritus feeders.

The difference in the ratios of microplastic ingestion in the groups based on test structure was significant for all three sizes of microplastic in the six-hour experiment (Fig. 12 c, d). In the four-week experiment appeared more agglutinated foraminifera which ingested microplastic. The difference in the ratios of microplastic ingestion was only significant for 6 μm microplastic particles:

- six-hour experiment, 0.5 μm – Kruskal-Wallis test H = 5.04, p = 0.02
- six-hour experiment, 1 μm – Kruskal-Wallis test H = 7.09, p = 0.01
- six-hour experiment, 6 μm – Kruskal-Wallis test H = 7.09, p = 0.01
- four-week experiment, 0.5 μm – Kruskal-Wallis test H = 3.35, p = 0.07
- four-week experiment, 1 μm – Kruskal-Wallis test H = 3.63, p = 0.06
- four-week experiment, 6 μm – Kruskal-Wallis test H = 4.29, p = 0.04

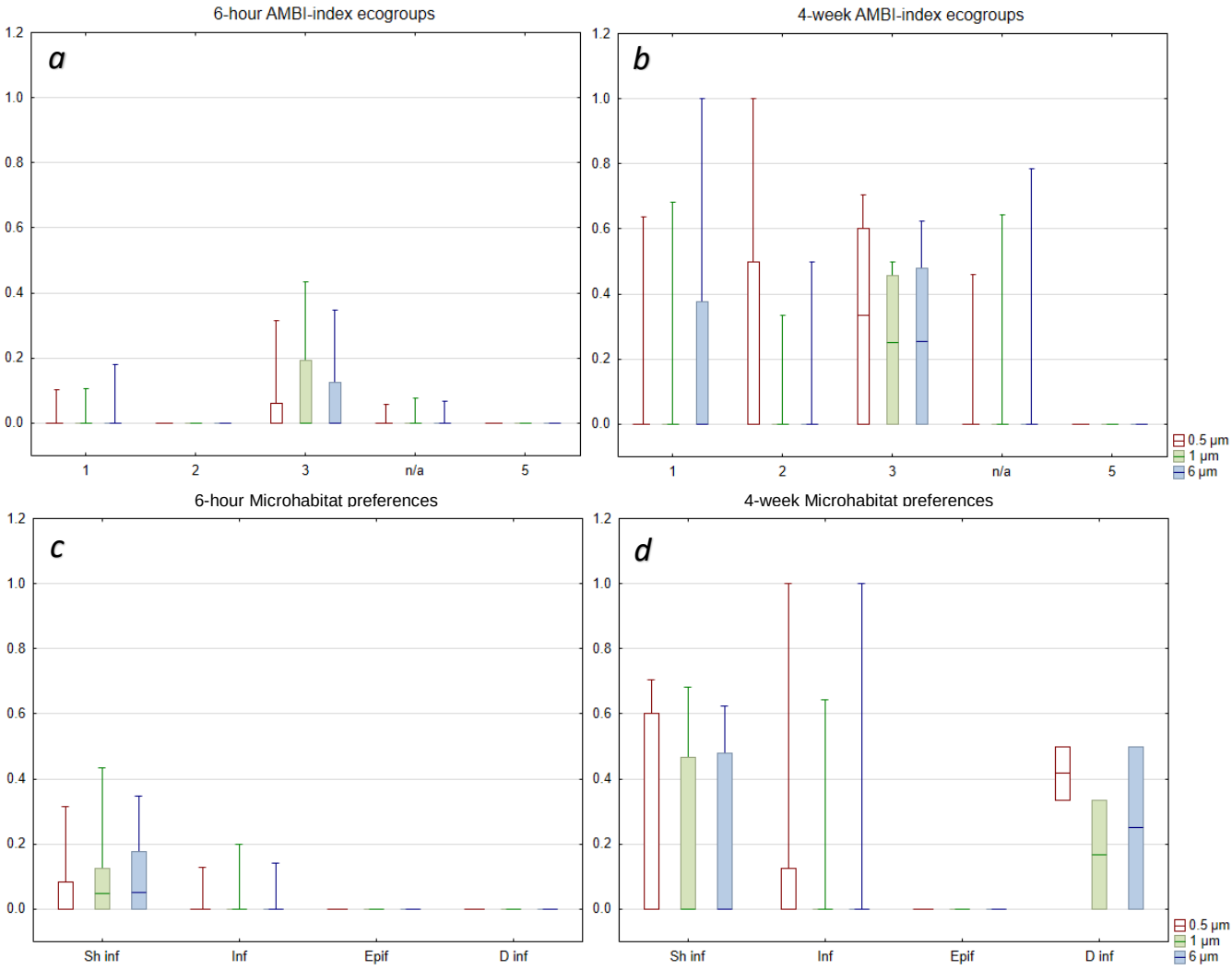

Figure 11. Microplastic accumulation ratios for different ecologically relevant groups; groups indicated above graphs. X scale (a, b) = AMBI-index ecogroups from 1 to 5; 1 = species which are most sensitive to the organic matter enrichment, 5 = the most opportunistic species and n/a = no information. X scale (c, d) = microhabitat preferences (vertical distribution in the sediments); Epif = epifaunal, Sh inf = shallow infaunal, Inf = infaunal, D inf = deep infaunal, median, 25%-75%, Min-Max.

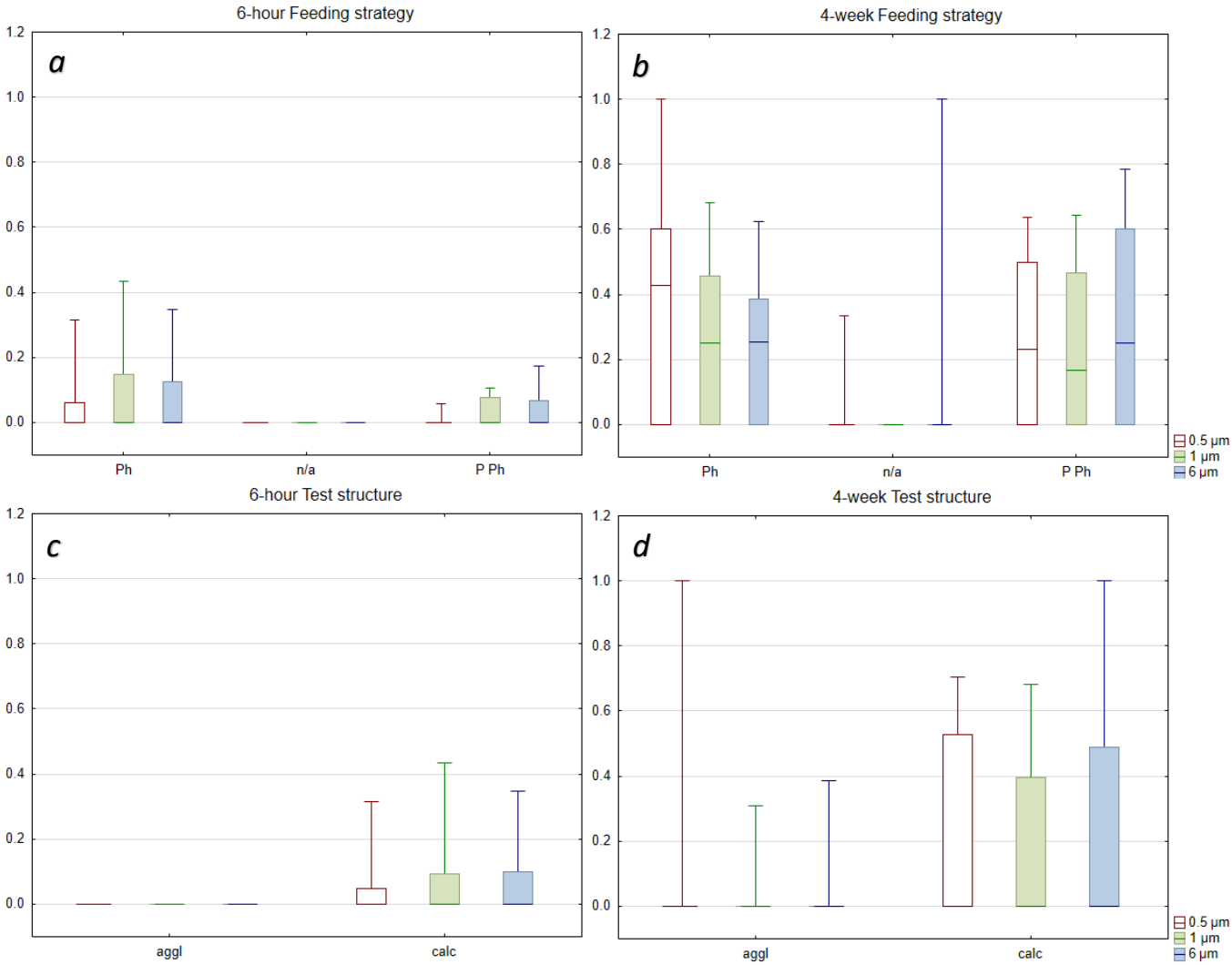

Figure 12. Microplastic accumulation ratios for different ecologically relevant groups; groups indicated above graphs. Y scale = ratio of microplastic ingestion (r). X scale (upper two boxplots) = feeding strategy; Ph = phytodetritus, P Ph = possibly phytodetritus, n/a = no information. X scale (lower two boxplots) = test structure; aggl = agglutinated, calc = calcareous, median, 25%-75%, Min-Max.

4. Discussion

4.1. Community composition

All identified foraminifera are typical for the Oslofjord area (Alve & Nagy, 1990; Murray & Alve, 2016). In the studies of Dolven et al., (2013; 2018), samples were taken from the Oslofjord, including the site IM4X, from which samples were taken for experiments in this study. The Shannon diversity indexes, calculated for the dead foraminifera community at site IM4X in 2009 (H'(log2) = 4.55), and for the live foraminifera community at site IM4X in 2017 (H'(log2) = 4.28) (Dolven et al., 2013; Dolven et al., 2018) were higher than the diversity index calculated in the samples after the experiments (H'(log2) = 3.53 - 4.03). Dead assemblages may have a slightly higher diversity because they represent the average number of species, which lived at the sampled site (Dolven et al., 2018). This can explain the higher diversity index found in 2009. The decrease in foraminifera diversity after the experiments, however, is not likely to be caused by exposure to microplastics since it was observed both in reference samples and in the samples with microplastic addition.

A significant decrease in the total abundance of live foraminifera was observed following a four-week incubation in both reference samples and the samples with microplastic. Perhaps the incubation without extra food addition could affect the foraminifera abundance. However, the abundance of live foraminifera specimens was sufficient and ranged from 231 to 384 in each sample in the four-week experiment. Despite the decrease in the total abundance, the samples from the six-hour and four-week experiments had 85% similarity according to the cluster analysis. In multidimensional scaling (MDS) analysis, four samples of the six-hour experiment (1-0.5um, 2-1um, 3-6um, and 4-ref) made a cluster and separated from the rest of the samples. These were the first four samples prepared in the experiment. It is unclear why these four samples stuck out from the rest of the samples. The number of species and individuals in the samples, as well as the diversity index did not differ from the rest of the samples. However, these samples belonged to four different treatments (reference, 0.5 μm, 1 μm and 6 μm microplastic) and their separation together showed that the design of the experiment was good. It is possible to compare samples from different treatment between each other.

4.2. Microplastic accumulation

The number of foraminifera individuals which ingested microplastic was significantly higher after the four-week experiment than after the six-hour experiment. Nearly all studies on microplastics showed the ingestion of it when microplastics accumulated inside the gut or sometimes inside the tissues of organisms (Lusher, 2015; Wright et al., 2013b). But it did not cross the cell membrane. Foraminifera, however, incorporate microplastic inside their cell (perhaps in a vesicle) or in the test, how it was shown for some agglutinated foraminifera by Tsuchiya & Nomaki, (2019). Microplastics cross the cell membrane and that can be called internalization. Microplastics incorporation was shown for the phytoplankton when the charged nano-polystyrene beads were absorbed into the cellulose of cell walls for algal cells (Bhattacharya et al., 2010).

The number of species which ingested microplastic was higher in the four-week experiment (21 species) than in the six-hour experiment (17 species).

Eight species (*Brizalina skagerrakensis, Brizalina spathulata, Bulimina marginata, Cassidulina laevigata, Hyalinea balthica, N. turgida, Nonionellina labradorica,* and *U. peregrine*) accumulated microplastics in both short- and long-term experiments (Table 4, green-coloured). These species perhaps were not very selective in their food preferences and reacted very fast to the addition of microplastic to the sediments. Already after six hours, they had accumulated microplastic.

Six species (*Eggerelloides medius, Eggerelloides scaber, E. excavatum, L. goesi, Melonis barleeanum,* and *Nonionella stella*) were slow to react to microplastic, and after the six-hour experiment, only a few specimens ingested microplastic (Table 4, red-coloured). But in the four-week experiment, they accumulated microplastic (ingested microplastic in at least three out of five replicates). Microplastics get coated with organic material during a long stay in the seawater (Artham et al., 2009). During the long term experiment, microplastics probably got covered with bacteria or organic material and became more attractive as food for foraminifera. That could be a reason, why these six species ingested microplastic in a higher frequency in the four-week experiment.

Two species (*Astrononion gallowayi* and *Loxostomum porrectum*) ingested microplastic sporadically in both experiments (Table 4, purple coloured).

Four species (A*dercotryma wrighti, Cribrostomoides nitidum, Globobulimina turgida* and *Reophax micaceus*) did not ingest any microplastic in the six-hour experiment and in rare cases were found with microplastic inside of some specimens in the four-week experiment (Table 4, orange-coloured). They needed a longer exposure time to microplastic (more than six hours) to start ingesting it. Maybe with even longer exposure to microplastic these species would also accumulate more microplastic particles. Further research with longer than four-week exposure time to microplastic is needed.

Lagena striata ingested microplastics twice (in one of the replicates for 1 μm and 6 μm microplastics) in the six-hour experiment and did not ingest any microplastic in the four-week experiment. *Dentalina communis* was absent in the six-hour experiment. In the four-week experiment, it ingested once 1 μm microplastic (Table 4, blue-coloured).

Foraminifera species which did not accumulate any microplastic (Table 4, group C, black coloured) probably are more selective with the food they ingest. Perhaps, they never ingested microplastic particles, because microplastics are not attractive for them as a food object

Many species of foraminifera are opportunistic feeders and thus are omnivores, which can consume a wide range of material of appropriate size including bacteria, small algae, plant and fungal fragments, protozoans, crustaceans (Gooday et al., 1992; Lipps, 1983). However, foraminifera have some sort of selectivity in feeding. They prefer to feed on certain bacteria, pennate diatoms, and small chlorophytes. While yeasts, cyanobacteria, dynoflagellates, chrysophytes, and many bacteria are avoided by foraminifera (Gooday et al., 1992).

4.2.1. Frequency test

The frequency test showed that most of the species did not accumulate microplastic in the six-hour (0.5 μm – 85%; 1 μm – 80%; 6 μm – 80%) and four-week (0.5 μm – 69%; 1 μm – 76%; 6 μm – 74%) experiments. For those species which accumulated microplastic, the ratios of ingestion in the six-hour experiments were significantly lower ($0<r<0.6$) than in the four-week experiment ($0.2<r \leq 1$). It means that more specimens in the long-term experiment accumulated microplastic particles.

E. excavatum had a ratio of microplastic ingestion equal to "1" for the 6 μm microplastic particles in the four-week experiment. It was due to a very low abundance of *E. excavatum* specimens found in the samples. No more than 2 individuals of that species were presented in

each of the samples and they always had microplastic particles inside in the four-week experiment. However, because of such a low abundance of that specimens in the samples, *E. excavatum* was not considered as a species which accumulated microplastic (even though it ingested 6 μm microplastic in four replicates in the four-week experiment). *E. excavatum* was excluded from the comparison of accumulation three differently sized microplastics and microplastic accumulation during six-hour and four-week experiments.

L. goesi had a ratio of microplastic ingestion equal to "1" for the 0.5 μm microplastic particles in the four-week experiment. The adult and juvenile individuals were counted separately for this species. The equal to "1" ratio (r=1) was calculated for the adult specimens of *L. goesi*. Juvenile specimens had ratio equal to "0". In further statistical analysis, it was decided to combine juvenile and adult specimen's numbers. The ratios of microplastic ingestion of *L. goesi* varied then from 0.08 to 0.67. These ratios were used in the boxplots, in the comparison of accumulation three differently sized microplastics and in the comparison of microplastic accumulation during six-hour and four-week experiments.

4.2.2. Accumulation of three different sizes of microplastic

No difference in the rates of microplastic ingestion of three sizes of microplastic was found for the most of the thirteen foraminifera species, except for two species (*N. turgida* in the six-hour experiment and *U. peregrine* in the four-week experiment). Overall most of the species, who accumulated microplastic, didn't show any selectivity in sizes of ingested microplastic. Perhaps for foraminifera, which accumulated microplastics, all three sizes used in the experiments were appropriate to ingest. In the electron microscopic research of Heeger (1990), a big variety of particles were found inside the food vacuoles of the foraminifera. Bacteria, silicate structures, pennate diatoms, and unidentified particles in a size range of less than 1 μm up to 25 μm were found inside the food vacuoles. Thus, some foraminifera can ingest particles of the size up to 25 μm. Though it needs further research to clarify whether larger sizes of microplastic (e.g 10-20 μm) could also be ingested by foraminifera.

Many marine organisms show no specific selection when they are feeding, so they just trap and ingest anything of an appropriate size with which they come in contact with (Moore, 2008; Wright et al., 2013b). Bern (1990), offered polystyrene microplastic beads of four different sizes (2, 6, 11 and 19 μm) to crustacean zooplankton and ^{14}C-labelled alga of equal size.

Bosmina coregoni ingested both 2 μm and 6 μm plastic particles and algal cells non-selectively. But it did not ingest any of 11 μm and 19 μm plastics beads. In other laboratory experiments the larva of a marine polychaete worm, *Galeolaria caespito* were fed microplastic beads. The larvae ingested smaller sized microplastic (3 μm) more than the larger microplastic (10 μm) (Bolton & Havenhand, 1998). However in the study by Christaki et al., (1998), microplastic accumulation in the ciliate *Strombidium sulcatum* suggested a connection between the ingestion of microplastic with the size of microplastic. The clearance rates of plastic microspheres increased linearly as function of prey size.

In the six-hour experiment, *N. turgida* showed the highest ratio of ingestion for the 6 μm plastic particles and did not accumulate any of the 0.5 μm plastic particles. But in a four-week experiment, *N. turgida* showed no significant difference in ratios of ingesting for any of the microplastic particles. Perhaps the selectivity in the accumulation of the microplastic particles only appeared in the short-term experiment and first of all *N. turgida* preferred to capture the largest particles, while during the longer exposure to microplastic, *N. turgida* started to ingest any sized particles. *U. peregrine* showed the opposite pattern. In the six-hour experiment no significant differences in the ratios of microplastic ingestion were observed for all three sizes of microplastic. While in the four-week experiment *U. peregrine* has the highest ratios of ingestion for the smallest 0.5 μm plastic particles and the lowest ratios of ingestion for the biggest 6 μm plastic particles. The reasons for such selectivity are unknown.

4.2.3. Accumulation of microplastic during six-hour and four-week experiments

The smallest microplastic particles (0.5 μm) were accumulated significantly more during the four-week experiment by all the thirteen foraminifera species. The largest microplastic particles (6 μm) were accumulated significantly more during the four-week experiment by most of the species (ten out of thirteen). While half of the species (seven out of thirteen) accumulated significantly more of 1 μm microplastic particles during the four-week experiments than in the six-hour experiment. But the other half of the species (six out of thirteen) accumulated 1 μm microplastic in the same way during both experiments. The reasons why 1 μm microplastics accumulated in a similar way for the six species in the short- and long-term experiments are unknown.

4.2.4. Fluorescent dye

Schür et al., (2019) in their study showed that microplastic beads can leach fluorescence dye. They exposed *Daphnia magna* to the 20 nm and 1000 nm fluorescent polystyrene microplastic beads. After 4 and 24 hours, the fluorescence in the guts and lipid droplets of Daphnids were observed. Nanoplastic particles were visible in the guts of *Daphnia*, but the fluorescence in the lipid droplets was not colocalized with any particles. In addition, the fluorescence in the guts was stable throughout the confocal laser scanning microscopy imaging, while the fluorescence in the lipids quickly faded away. The last is common for fluorescent dyes such as Fluorescein isothiocyanate (FITC). Further, Schür et al., (2019) showed that FITC transfer from the particles to a synthetic matrix.

In our experiments, the microplastic particles were observed inside the foraminifera, even though foraminifera tests scattered much of the fluorescence from the microplastic particles. The fluorescence signal was strong and did not fade away. Based on that, we concluded that fluorescence was connected with the microplastic particles and fluorescence dye did not leach.

4.3. Do some ecologically relevant groups accumulate more microplastics?

Many factors can affect the accumulation of microplastic by organisms like size/type/abundance of microplastic, means of exposure, morphology, physiology, ecology or behavior of organisms and interspecies differences (Phuong et al., 2016; Wright et al., 2013b).

Tolerance to organic carbon probably does not play an important role in the ability of foraminifera to accumulate microplastic. Only in the four-week experiment 0.5 μm microplastic particles were significantly more accumulated by the foraminifera species which belong to the ecogroups 3. To the ecogroups 3 belong species which are moderately tolerant to the organic carbon in the environment.

Microhabitat preferences also seem not to be the defining factor in the ability of foraminifera to accumulate microplastic. However, in the experimental setup, the added microplastic was homogenized in the sediment and, thus, evenly distributed throughout the whole sample. Thereby, microplastic was equally available for all foraminifera, despite their vertical distribution in the sediment.

The feeding strategy of foraminifera, on the other hand, seems to have an important role in their ability to accumulate microplastic. In the six-hour and four-week experiments, all the

species which ingested microplastic were phytodetritus or possibly phytodetritus feeders except for *M. barleeanum* and *E. excavatum,* for whom it is unknown if they can feed on phytodetritus.

The test structure also seems to be an important factor which can affect the accumulation of microplastic by foraminifera. In the six-hour experiment, where the difference in the ratios of microplastic ingestion was significant for all three sizes of microplastic, the majority of species which ingested microplastic had calcareous tests. In the four-week experiment, only 6 μm microplastic particles were accumulated significantly more by calcareous species. However, the p-value for 0.5 and 1 μm microplastic particles were on a border of significance (0.07 and 0.06 accordingly). The majority of the species which accumulated microplastic were still calcareous. Only three agglutinated species *E. medius, E. scaber* and *L. goesi* were regularly found with microplastic inside in the four-week experiment, and rarely – in the six-hour experiment. Another two species *C. nitidum* and *R. micaceus* were found to ingest microplastic once in the four-week experiment.

4.4. Benthic marine food web

There is limited information available on the accumulation of microplastic in marine food webs. Since it is known that organisms at the lower trophic level ingest microplastic, it is likely that microplastics enter the food webs (Wright et al., 2013b). Several works have already shown that microplastics can be transferred from one to other trophic levels (Farrell & Nelson, 2013; Murray & Cowie, 2011; Setälä et al., 2014). Foraminifera occupy the lower trophic level in the benthic marine food web. And it is possible, that they would transfer microplastics to the higher levels. Some organisms, such as e.g. flatworms, polychaetes, mollusks, crustaceans, and fish can ingest foraminifera incidentally during deposit-feeding or grazing. While other organisms such as e.g. nematodes, polychaetes, gastropods, scaphopods, and crustaceans can feed selectively on foraminifera (Gooday et al., 1992; Lipps, 1983). Most evidence of predation on foraminifera are indirect and based on gut-content analyses (Culver & Lipps, 2003). Immunological method indicated that foraminifera were ingested by grenadier fish (*Coryphaenoides armatus*). Eight out of twelve examined fish had antigenic proteins of foraminifera inside of their gut contest, but no foraminifera remains were visually found (Feller et al., 1985). Microplastics have the potential to be transferred from foraminifera to the organisms who prey on them. But further experiments are

needed, to estimate if microplastics actually can be bioaccumulated through the predation on foraminifera.

4.5. What is an environmentally relevant concentration of microplastics?

Experimental exposure to microplastics is not exactly the same as the exposure to microplastics in the environment. The concentration of microplastic particles used in experiments is usually much higher than in the field (Phuong et al., 2016).

The amount of microplastic particles in the Nordic seas were not broadly estimated. Along the Swedish coastline, from the west coast close to the Norwegian border to the southern Bothnian Sea, the number of plastic fragments in the water surface, sampled by pumping water through a 300 μm filter, were estimated around 1 particle per m^3 of water (Strand et al., 2015). Mean microplastic abundance in surface waters of the Swedish west coast collected by manta nets with two different mesh sizes ranged from 150 to 2400 particles per m^3 of water for 80 μm mesh size and 0.01 to 0.14 particles per m^3 of water for 450 μm mesh size (Lusher, 2015). In Skagerrak sea, Sweden, the amount of microplastic, collected by a submersible in situ pump, made up to maximum 102000 particles per m3 of water (Lusher, 2015). In sediments in a transect from the Baltic Sea towards the North Sea were found from 60 to 3600 microplastic particles (from 38 μm to 1 mm) per kg of the sediment (Strand et al., 2015).

The concentration of microplastic particles in Oslofjord waters or sediments is unknown. However, we expect, that the concentration of microplastic in the samples in our experiment, were higher than the concentration of microplastic in the field. Still, in the future, the amount of microplastics in the environment is going to increase (Law & Thompson, 2014; Thompson, 2015). Therefore experiments with exposure to high concentration of microplastic are important.

5. Future studies

Microplastic ingestion by foraminifera is a very poorly studied area. More experiments with different types and sizes of microplastics are needed. It is still unknown to what size limit foraminifera can ingest microplastics. In addition, some foraminifera species accumulate microplastics while others don't. Reasons for such selectivity are not clear. It is also of interest to know where and in what parts of the cell, foraminifera store microplastic. The confocal

microscopy or correlated light and electron microscopy (CTEM) can be used for these purposes. The possibility of microplastic transfer from foraminifera to predators in the food webs is also unknown. More experiments in this field are needed to be performed.

6. Conclusions

Microplastics accumulated inside benthic foraminifera during six-hour and four-week experiments.

No significant change in foraminifera community composition were observed after exposure to microplastics (0.5 μm, 1 μm, 6 μm) for six hours and four weeks. Cluster and multidimensional scaling analyses showed around 85% similarity between samples from the two time points. Shannon diversity index of rose-Bengal stained foraminifera varied from 3.53 to 4.03.

In total 17 species ingested microplastic in the six-hour experiment and 21 species ingested microplastic in the four-week experiment. In the six-hour and four-week experiments, 8 and 13 species accumulated microplastic in at least three out of five replicates, respectively.

Eleven out of thirteen foraminifera species did not differentiate between microplastic sizes, but two species differentially accumulated the three sizes of microplastics: *N. turgida* in the six-hour experiment accumulated 6 μm microplastic particles more than 1 μm and it did not accumulate 0.5 μm microplastic particles. *U. peregrine* in the four-week experiment accumulated 0.5 μm plastic particles more than 1 and 6 μm microplastic particles.

Thirteen foraminifera species accumulated more 0.5 μm microplastic in the four-week experiment than in the six-hour experiment. Seven foraminifera species accumulated more 1 μm microplastic in the four-week experiment than in the six-hour experiment. Ten foraminifera species accumulated more 6 μm microplastic in the four-week experiment than in the six-hour experiment.

Food preferences and test composition of foraminifera species affect the accumulation of microplastic by foraminifera. While tolerance to organic carbon and microhabitat preferences do not seem to influence the accumulation of microplastic.

Microplastics have the potential to enter the marine benthic food webs by being transferred from foraminifera to the organisms who prey on them.

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Appendix

Table 1. The Shannon diversity index, total number of individuals and number of species for each sample in the six-hour and four-week experiments. (H'(log2) = The Shannon diversity index; N = number of individuals; S = number of species.

6-hour					4-week				
Sample №	Treatment	H'(log2)	N	S	Sample №	Treatment	H'(log2)	N	S
1	0.5 um	3.63	354	23	41	ref	3.83	357	25
2	1 um	3.78	304	23	42	0.5 um	3.88	231	26
3	6 um	4.03	449	26	43	1 um	3.79	249	25
4	ref	3.80	346	26	44	6 um	3.85	302	28
5	ref	3.69	443	24	45	ref	3.78	300	25
6	0.5 um	3.77	470	24	46	0.5 um	3.66	286	25
7	1 um	3.70	381	25	47	1 um	3.65	261	24
8	6 um	3.78	360	26	48	6 um	3.72	321	24
9	ref	3.81	404	25	49	ref	3.53	298	18
10	0.5 um	3.61	439	27	50	0.5 um	3.79	303	24
11	1 um	3.75	451	23	51	1 um	3.73	340	23
12	6 um	3.65	351	23	52	6 um	3.83	354	25
13	ref	3.77	486	28	53	ref	3.67	384	24
14	0.5 um	3.82	429	27	54	0.5 um	3.60	345	23
15	1 um	3.87	432	27	55	1 um	3.67	304	24
16	6 um	3.85	397	29	56	6 um	3.96	273	24
17	ref	3.79	362	26	57	ref	3.77	323	27
18	0.5 um	3.79	346	26	58	0.5 um	3.85	284	24
19	1 um	3.84	423	30	59	1 um	3.74	330	23
20	6 um	3.84	342	26	60	6 um	3.80	307	24

Table 2. Six-hour experiment. Counted numbers of rose Bengal stained foraminifera for the reference samples.

	Reference				
Sample number List of species	4 without plastic	5 without plastic	9 without plastic	13 without plastic	17 without plastic
Adercotryma wrighti	22	18	19	15	13
Astrononion gallowayi	4	4	0	3	2
Brizalina skagerrakensis	17	26	27	17	18
Brizalina spathulata	9	28	44	34	29
Bulimina marginata	22	48	32	56	29
Cassidulina laevigata	7	36	23	35	21
Cibicides lobatulus	2	0	1	0	0
Cribrostomoides globosum	0	2	0	3	1
Cribrostomoides jeffreysii	1	0	4	6	3
Cribrostomoides nitidum	2	3	2	2	4
Eggerelloides medius	21	25	30	29	37
Eggerelloides scaber	20	25	33	37	29
Elphidium excavatum	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0
Glandulina laevigata	0	0	0	0	0
Globobulimina turgida	18	8	9	5	4
Haplophragmoides bradyi	4	6	3	3	1
Hyalinea balthica	11	24	21	33	18
Lagena laevis	0	0	0	0	0
Lagena striata	0	0	0	0	0
Leibusella goesi juv.	24	4	10	18	16
Liebusella goesi	4	9	5	6	5
Loxostomum porrectum	2	0	0	1	0
Melonis barleeanum	2	9	11	9	5
Nonionella stella	5	3	5	4	7
Nonionella turgida	12	3	4	5	5
Nonionellina labradorica	24	21	11	14	7
Pullenia bulloides	0	0	0	0	1

Quinqueloculina stalkeri	0	4	0	0	0
Recurvoides trochamminiformis	3	5	4	4	2
Reophax bilocularis	1	0	0	0	0
Reophax fusiformis	2	0	0	1	0
Reophax micaceus	0	0	3	10	4
Reophax sp.	7	5	4	2	5
Saccammina sphaerica	0	0	0	0	0
Sigmoilopsis schlumbergeri	0	0	0	0	0
Stainforthia fusiformis	0	0	1	0	0
Technitella legumen	0	0	0	1	0
Tritaxis conica	0	1	2	2	2
Uvigerina peregrina	100	126	96	131	94

Table 3. Six-hour experiment. Counted numbers of rose Bengal stained foraminifera without and with microplastic for the samples with 0.5 µm microplastic particles added.

	0.5 um plastic									
Sample number	1		6		10		14		18	
List of species	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic
Adercotryma wrighti	21	0	20	0	15	0	16	0	12	0
Astrononion gallowayi	2	0	10	0	1	0	4	0	3	0
Brizalina skagerrakensis	18	6	13	11	12	8	9	4	15	6
Brizalina spathulata	15	2	17	12	36	2	38	1	30	2
Bulimina marginata	39	3	40	8	33	6	34	5	24	5
Cassidulina laevigata	7	2	41	0	20	0	24	0	17	2
Cibicides lobatulus	0	0	0	0	0	0	0	0	0	0
Cribrostomoides globosum	0	0	4	0	1	0	1	0	0	0
Cribrostomoides jeffreysii	1	0	2	0	10	0	8	0	9	0
Cribrostomoides nitidum	3	0	3	0	1	0	5	0	4	0
Eggerelloides medius	4	0	24	0	29	0	30	1	10	0
Eggerelloides scaber	11	2	31	0	35	1	34	0	26	0
Elphidium excavatum	0	0	0	0	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	2	0	0	0
Glandulina laevigata	0	0	0	0	0	0	0	0	0	0
Globobulimina turgida	19	0	12	0	8	0	4	0	8	0
Haplophragmoides bradyi	4	0	1	0	1	0	5	0	1	0
Hyalinea balthica	24	2	31	3	26	4	26	3	25	4
Lagena laevis	0	0	0	0	1	0	0	0	0	0
Lagena striata	0	0	0	0	0	0	0	0	0	0
Leibusella goesi juv.	31	0	14	0	11	0	9	0	4	0
Liebusella goesi	0	0	7	0	7	0	6	0	9	0
Loxostomum porrectum	1	0	0	0	0	0	0	0	0	0
Melonis barleeanum	12	1	6	0	4	0	4	0	4	0
Nonionella stella	0	0	7	0	2	0	5	0	2	1
Nonionella turgida	5	0	2	0	6	0	7	0	3	0
Nonionellina labradorica	18	0	22	1	16	1	14	0	7	1

Pullenia bulloides	1	0	0	0	0	0	0	0	0	0
Quinqueloculina stalkeri	0	0	0	0	1	0	1	0	0	0
Recurvoides trochamminiformis	2	0	6	0	3	0	3	0	4	0
Reophax bilocularis	0	0	0	0	0	0	0	0	0	0
Reophax fusiformis	0	0	0	0	1	0	0	0	1	0
Reophax micaceus	0	0	0	0	1	0	6	0	2	0
Reophax sp.	2	0	3	0	3	0	4	0	8	0
Saccammina sphaerica	0	0	0	0	0	0	0	0	1	0
Sigmoilopsis schlumbergeri	0	0	0	0	0	0	0	0	0	0
Stainforthia fusiformis	0	0	0	0	0	0	0	0	0	0
Technitella legumen	0	0	0	0	0	0	0	0	0	0
Tritaxis conica	2	0	1	0	0	0	2	0	2	0
Uvigerina peregrina	84	10	118	0	128	5	102	12	93	1

Table 4. Six-hour experiment. Counted numbers of rose Bengal stained foraminifera without and with microplastic for the samples with 1 µm microplastic particles added.

	1 um plastic									
Sample number	2		7		11		15		19	
List of species	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic
Adercotryma wrighti	16	0	8	0	32	0	21	0	17	0
Astrononion gallowayi	4	1	4	0	0	0	1	0	2	0
Brizalina skagerrakensis	13	11	13	7	21	5	12	10	11	11
Brizalina spathulata	15	3	42	11	33	1	29	5	29	8
Bulimina marginata	17	6	35	9	40	7	18	18	29	5
Cassidulina laevigata	7	0	23	0	15	2	17	3	21	4
Cibicides lobatulus	0	0	1	0	0	0	0	0	0	0
Cribrostomoides globosum	0	0	0	0	0	0	1	0	2	0
Cribrostomoides jeffreysii	0	0	5	0	10	0	5	0	6	0
Cribrostomoides nitidum	8	0	1	0	1	0	2	0	1	0
Eggerelloides medius	8	0	17	0	27	0	43	0	36	0
Eggerelloides scaber	12	0	21	0	35	0	23	0	22	1
Elphidium excavatum	0	0	0	0	0	0	0	0	1	0
Epistominella vitrea	0	0	0	0	0	0	0	0	1	0
Glandulina laevigata	0	0	0	0	0	0	0	0	0	0
Globobulimina turgida	17	0	9	0	9	0	5	0	5	0
Haplophragmoides bradyi	2	0	1	0	3	0	5	0	2	0
Hyalinea balthica	4	9	18	3	30	1	34	3	29	4
Lagena laevis	0	0	0	0	0	0	0	0	0	0
Lagena striata	0	0	0	0	0	0	0	0	1	0
Leibusella goesi juv.	14	0	8	0	19	0	12	0	15	0
Liebusella goesi	6	0	7	1	4	0	7	0	3	0
Loxostomum porrectum	0	0	0	0	0	0	0	1	0	0
Melonis barleeanum	9	1	5	0	5	0	9	0	7	0
Nonionella stella	0	0	2	0	4	0	4	0	2	1
Nonionella turgida	2	2	1	0	5	0	4	1	9	1
Nonionellina labradorica	7	9	29	0	18	1	12	1	9	0

Pullenia bulloides	0	0	0	0	0	0	0	0	0	0
Quinqueloculina stalkeri	0	0	0	0	0	0	1	0	1	0
Recurvoides trochamminiformis	8	0	2	0	4	0	10	0	5	0
Reophax bilocularis	0	0	0	0	1	0	0	0	0	0
Reophax fusiformis	1	0	0	0	0	0	0	0	2	0
Reophax micaceus	1	0	1	0	3	0	7	0	1	0
Reophax sp.	0	0	6	0	5	0	3	0	4	0
Saccammina sphaerica	0	0	0	0	0	0	0	0	0	0
Sigmoilopsis schlumbergeri	1	0	0	0	0	0	0	0	0	0
Stainforthia fusiformis	0	0	0	0	0	0	0	0	0	0
Technitella legumen	0	0	0	0	0	0	0	0	0	0
Tritaxis conica	3	0	2	0	0	0	2	0	5	0
Uvigerina peregrina	74	13	84	5	108	2	100	3	91	19

Table 5. Six-hour experiment. Counted numbers of rose Bengal stained foraminifera without and with microplastic for the samples with 6 μm microplastic particles added.

	6 um plastic									
Sample number	3		8		12		16		20	
List of species	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic
Adercotryma wrighti	33	0	6	0	10	0	8	0	7	0
Astrononion gallowayi	6	0	2	0	0	0	4	0	3	0
Brizalina skagerrakensis	20	16	17	8	15	3	11	3	11	8
Brizalina spathulata	7	8	39	2	25	2	38	7	27	3
Bulimina marginata	15	8	30	5	45	3	34	5	24	4
Cassidulina laevigata	6	7	19	0	13	0	25	4	33	4
Cibicides lobatulus	0	0	0	0	0	0	0	0	0	0
Cribrostomoides globosum	1	0	0	0	1	0	1	0	2	0
Cribrostomoides jeffreysii	0	0	8	0	4	0	8	0	6	0
Cribrostomoides nitidum	5	0	4	0	0	0	2	0	1	0
Eggerelloides medius	36	0	28	0	19	0	31	0	25	0
Eggerelloides scaber	22	0	15	0	28	0	26	0	10	0
Elphidium excavatum	0	0	0	0	0	0	1	1	0	0
Epistominella vitrea	0	0	0	0	0	0	0	0	1	0
Glandulina laevigata	0	0	0	0	0	0	0	0	0	0
Globobulimina turgida	15	0	4	0	6	0	6	0	8	0
Haplophragmoides bradyi	7	0	3	0	1	0	3	0	3	0
Hyalinea balthica	13	5	24	5	23	1	26	5	18	5
Lagena laevis	0	0	0	0	0	0	1	0	0	0
Lagena striata	0	0	0	0	0	0	0	1	0	0
Leibusella goesi juv.	16	0	11	0	6	0	8	0	13	0
Liebusella goesi	19	0	4	0	8	0	4	0	5	0
Loxostomum porrectum	1	0	1	0	0	0	1	0	0	0
Melonis barleeanum	8	0	6	0	4	1	3	1	2	0
Nonionella stella	3	3	2	0	4	0	6	0	3	0
Nonionella turgida	14	1	7	1	3	2	1	1	8	1
Nonionellina labradorica	17	7	8	0	19	1	11	0	14	2

Pullenia bulloides	0	0	2	0	0	0	0	0	0	0
Quinqueloculina stalkeri	5	0	1	0	0	0	0	0	0	0
Recurvoides trochamminiformis	6	0	2	0	2	0	3	0	7	0
Reophax bilocularis	0	0	0	0	0	0	0	0	0	0
Reophax fusiformis	0	0	1	0	0	0	0	0	1	0
Reophax micaceus	8	0	0	0	3	0	2	0	2	0
Reophax sp.	5	0	7	0	3	0	13	0	1	0
Saccammina sphaerica	0	0	0	0	0	0	0	0	0	0
Sigmoilopsis schlumbergeri	0	0	0	0	0	0	0	0	0	0
Stainforthia fusiformis	0	0	0	0	0	0	0	0	0	0
Technitella legumen	0	0	0	0	0	0	0	0	0	0
Tritaxis conica	1	0	0	0	2	0	1	0	0	0
Uvigerina peregrina	88	17	85	3	94	0	91	0	77	3

Table 6. Four-week experiment. Counted numbers of rose Bengal stained foraminifera for the reference samples.

	Reference				
Sample number	41	45	49	53	57
List of species	without plastic	without plastic	without plastic	without plastic	without plastic
Adercotryma wrighti	13	10	9	16	12
Astrononion gallowayi	12	6	5	2	5
Brizalina skagerrakensis	19	12	17	13	21
Brizalina spathulata	28	23	29	26	22
Bulimina marginata	28	41	24	36	34
Cassidulina laevigata	14	16	21	18	13
Cibicides lobatulus	0	0	0	0	0
Cribrostomoides globosum	2	1	0	0	0
Cribrostomoides jeffreysii	5	2	1	4	2
Cribrostomoides nitidum	2	3	0	2	3
Dentalina communis	0	0	0	0	0
Eggerelloides medius	43	32	39	45	28
Eggerelloides scaber	8	4	9	17	22
Elphidium excavatum	0	0	0	0	1
Epistominella vitrea	0	0	0	0	2
Glandulina laevigata	0	0	0	0	0
Globobulimina turgida	11	3	5	9	1
Haplophragmoides bradyi	4	3	2	8	2
Hyalinea balthica	31	23	28	33	29
Lagena laevis	0	0	0	0	0
Lagena striata	0	0	0	0	1
Leibusella goesi juv.	12	10	7	16	8
Liebusella goesi	1	1	0	2	1
Loxostomum porrectum	0	0	0	1	0
Melonis barleeanum	8	6	0	1	5
Nonionella stella	5	0	3	3	5
Nonionella turgida	4	7	6	4	3
Nonionellina labradorica	14	20	15	13	8

Pullenia bulloides	0	0	0	0	0
Quinqueloculina stalkeri	0	0	0	0	0
Recurvoides trochamminiformis	4	2	5	3	7
Reophax bilocularis	0	0	0	0	0
Reophax dentaliniformis	0	0	0	0	0
Reophax fusiformis	0	0	0	0	0
Reophax micaceus	1	1	0	5	1
Reophax sp.	0	2	0	0	0
Saccammina sphaerica	0	0	0	0	0
Sigmoilopsis schlumbergeri	1	3	0	3	2
Stainforthia fusiformis	0	0	0	0	0
Technitella legumen	0	0	0	0	0
Tritaxis conica	2	3	0	0	3
Uvigerina peregrina	85	66	73	104	82

Table 7. Four-week experiment. Counted numbers of rose Bengal stained foraminifera without and with microplastic for the samples with 0.5 µm microplastic particles added.

	0.5 um plastic									
Sample number	42		46		50		54		58	
List of species	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic
Adercotryma wrighti	4	0	11	0	13	0	14	0	13	0
Astrononion gallowayi	2	0	4	0	2	0	8	0	4	1
Brizalina skagerrakensis	5	12	10	5	8	11	5	13	4	15
Brizalina spathulata	6	14	15	15	10	7	8	21	12	15
Bulimina marginata	8	12	13	10	11	17	5	14	9	20
Cassidulina laevigata	3	7	4	3	9	10	4	8	6	7
Cibicides lobatulus	1	0	0	0	0	0	0	0	0	0
Cribrostomoides globosum	1	0	0	0	0	0	0	0	1	0
Cribrostomoides jeffreysii	1	0	6	0	1	0	4	0	4	0
Cribrostomoides nitidum	2	0	2	0	1	0	1	0	0	0
Dentalina communis	0	0	0	0	0	0	0	0	0	0
Eggerelloides medius	18	9	22	11	29	19	32	10	20	8
Eggerelloides scaber	4	6	3	3	9	4	11	3	10	2
Elphidium excavatum	0	0	0	2	0	0	0	0	0	0
Epistominella vitrea	0	0	0	0	0	0	0	0	0	0
Glandulina laevigata	0	0	0	0	0	0	0	0	0	0
Globobulimina turgida	2	2	3	0	3	0	9	2	10	0
Haplophragmoides bradyi	3	0	5	0	7	0	6	0	1	0
Hyalinea balthica	14	5	14	7	9	12	6	14	2	19
Lagena laevis	0	0	0	0	0	0	0	0	0	0
Lagena striata	0	0	0	0	0	0	0	0	0	0
Leibusella goesi juv.	6	2	4	0	10	0	6	0	5	1
Liebusella goesi	0	2	0	1	2	0	1	1	0	1
Loxostomum porrectum	0	0	2	0	3	0	0	0	0	0
Melonis barleeanum	1	1	2	0	5	2	1	1	3	5
Nonionella stella	1	2	1	1	2	1	0	2	0	1
Nonionella turgida	6	0	5	3	1	2	1	4	4	5

Nonionellina labradorica	8	6	7	7	7	3	9	15	1	5
Pullenia bulloides	2	0	0	0	0	0	0	0	0	0
Quinqueloculina stalkeri	0	0	0	0	0	0	0	0	0	0
Recurvoides trochamminiformis	2	0	2	0	5	0	3	0	4	0
Reophax bilocularis	0	0	0	0	0	0	0	0	0	0
Reophax dentaliniformis	1	0	0	0	0	0	0	0	0	0
Reophax fusiformis	0	0	0	0	0	0	0	0	0	0
Reophax micaceus	0	0	2	0	2	0	0	0	0	3
Reophax sp.	0	0	0	0	0	0	0	0	0	0
Saccammina sphaerica	0	0	0	0	0	0	0	0	0	0
Sigmoilopsis schlumbergeri	0	0	0	0	0	0	1	0	2	0
Stainforthia fusiformis	0	0	0	0	0	0	0	0	0	0
Technitella legumen	0	0	0	0	0	0	0	0	0	0
Tritaxis conica	2	0	1	0	2	0	1	0	1	0
Uvigerina peregrina	29	19	52	28	28	36	28	73	25	35

Table 8. Four-week experiment. Counted numbers of rose Bengal stained foraminifera without and with microplastic for the samples with 1 µm microplastic particles added.

	1 um plastic									
Sample number	43		47		51		55		59	
List of species	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic
Adercotryma wrighti	2	0	7	0	20	0	8	0	8	0
Astrononion gallowayi	4	0	8	0	8	0	7	2	1	1
Brizalina skagerrakensis	7	5	1	5	15	7	11	8	5	6
Brizalina spathulata	11	3	9	13	11	11	13	6	8	19
Bulimina marginata	8	19	13	11	18	15	22	7	11	11
Cassidulina laevigata	8	2	6	9	16	7	10	3	6	7
Cibicides lobatulus	0	0	0	0	0	0	0	0	0	0
Cribrostomoides globosum	4	0	1	0	0	0	0	0	0	0
Cribrostomoides jeffreysii	2	0	3	0	2	0	5	0	0	0
Cribrostomoides nitidum	2	0	1	0	1	0	1	0	1	0
Dentalina communis	0	0	1	1	0	0	0	0	0	0
Eggerelloides medius	22	16	33	6	20	20	42	3	27	12
Eggerelloides scaber	3	4	5	2	6	6	7	2	18	2
Elphidium excavatum	0	0	0	0	0	1	0	0	0	2
Epistominella vitrea	0	0	0	0	0	0	0	0	0	0
Glandulina laevigata	0	0	0	0	0	0	0	0	0	0
Globobulimina turgida	4	0	2	0	3	0	4	0	10	0
Haplophragmoides bradyi	3	0	3	0	3	0	2	0	4	0
Hyalinea balthica	7	15	3	15	15	9	13	19	7	28
Lagena laevis	0	0	0	0	0	0	0	0	0	0
Lagena striata	0	0	0	0	0	0	0	0	0	0
Leibusella goesi juv.	10	0	12	0	8	0	6	0	10	0
Liebusella goesi	3	0	1	0	2	0	2	0	1	1
Loxostomum porrectum	1	0	0	0	0	0	0	0	1	0
Melonis barleeanum	2	0	2	0	4	0	3	2	4	0
Nonionella stella	1	1	0	2	2	0	4	0	2	1
Nonionella turgida	1	5	3	0	3	3	3	0	5	0

Nonionellina labradorica	5	11	2	7	4	10	5	7	12	9
Pullenia bulloides	0	0	0	0	0	0	0	0	0	0
Quinqueloculina stalkeri	0	0	0	0	0	0	0	0	0	0
Recurvoides trochamminiformis	3	0	6	0	7	0	1	0	6	0
Reophax bilocularis	0	0	0	0	0	0	0	0	0	0
Reophax dentaliniformis	0	0	0	0	0	0	0	0	0	0
Reophax fusiformis	0	0	1	0	0	0	0	0	0	0
Reophax micaceus	0	0	0	0	3	0	0	0	0	0
Reophax sp.	1	0	0	0	0	0	1	0	0	0
Saccammina sphaerica	0	0	0	0	0	0	0	0	0	0
Sigmoilopsis schlumbergeri	0	0	0	0	0	0	2	0	6	0
Stainforthia fusiformis	0	0	0	0	0	0	0	0	0	0
Technitella legumen	0	0	0	0	0	0	0	0	0	0
Tritaxis conica	1	0	0	0	0	0	1	0	0	0
Uvigerina peregrina	30	23	43	24	55	25	53	19	48	30

Table 9. Four-week experiment. Counted numbers of rose Bengal stained foraminifera without and with microplastic for the samples with 6 µm microplastic particles added.

	6 um plastic									
Sample number	44		48		52		56		60	
List of species	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic	without plastic	with plastic
Adercotryma wrighti	13	0	9	0	14	0	5	2	9	0
Astrononion gallowayi	4	0	6	0	6	0	3	2	2	0
Brizalina skagerrakensis	7	10	6	9	11	10	3	7	2	10
Brizalina spathulata	12	7	15	10	19	11	4	11	11	14
Bulimina marginata	13	8	12	15	26	10	11	13	11	17
Cassidulina laevigata	5	10	11	9	9	11	6	3	6	8
Cibicides lobatulus	0	0	0	0	0	0	0	0	1	0
Cribrostomoides globosum	0	0	0	0	0	0	0	0	0	0
Cribrostomoides jeffreysii	1	0	6	0	4	0	1	0	0	0
Cribrostomoides nitidum	2	0	1	0	4	0	0	0	1	1
Dentalina communis	0	0	0	0	0	0	0	0	0	0
Eggerelloides medius	32	12	38	15	26	14	26	7	16	10
Eggerelloides scaber	2	3	8	5	12	3	8	8	9	5
Elphidium excavatum	0	1	0	1	0	0	0	1	0	0
Epistominella vitrea	1	0	0	0	0	0	0	0	0	0
Glandulina laevigata	0	0	0	0	0	0	0	0	0	0
Globobulimina turgida	7	0	4	0	7	0	4	0	10	0
Haplophragmoides bradyi	3	0	3	0	7	0	6	0	1	0
Hyalinea balthica	10	4	8	6	15	10	25	6	4	27
Lagena laevis	0	0	0	0	0	0	0	0	0	0
Lagena striata	0	0	0	0	0	0	0	0	0	0
Leibusella goesi juv.	6	2	13	0	5	0	8	1	7	0
Liebusella goesi	3	0	2	0	6	0	8	3	5	1
Loxostomum porrectum	1	0	1	0	0	0	0	1	0	0
Melonis barleeanum	6	0	2	1	7	0	3	0	7	0
Nonionella stella	0	1	2	1	0	1	1	3	3	2
Nonionella turgida	9	2	6	0	4	0	3	1	6	0

Nonionellina labradorica	4	14	2	13	1	11	6	11	3	8
Pullenia bulloides	0	0	0	0	0	0	0	0	0	0
Quinqueloculina stalkeri	0	0	0	0	0	0	0	0	0	0
Recurvoides trochamminiformis	10	0	5	0	5	0	5	0	1	0
Reophax bilocularis	0	0	0	0	0	0	0	0	0	0
Reophax dentaliniformis	0	0	0	0	0	0	0	0	0	0
Reophax fusiformis	1	0	0	0	0	0	0	0	0	0
Reophax micaceus	1	0	0	0	1	0	2	0	5	0
Reophax sp.	1	0	0	0	1	0	0	0	0	0
Saccammina sphaerica	0	0	0	0	0	0	0	0	0	0
Sigmoilopsis schlumbergeri	0	0	0	0	2	0	7	0	5	0
Stainforthia fusiformis	0	0	0	0	0	0	0	0	0	0
Technitella legumen	0	0	0	0	0	0	0	0	0	0
Tritaxis conica	2	0	1	0	1	0	0	0	2	0
Uvigerina peregrina	53	19	57	18	62	18	37	11	43	34

Table 10. Six-hour experiment. Calculated median values, standard error and ratios of microplastic ingestion for a rose Bengal stained foraminifera from four different treatments – reference, 0.5 μm, 1 μm and 6 μm microplastic.

	Reference		0.5 um plastic					1 um plastic					6 um plastic				
	median		median		median			median		median			median		median		
List of species	without plastic	standart error	without plastic	standart error	with plastic	standart error	ratio	without plastic	standart error	with plastic	standart error	ratio	without plastic	standart error	with plastic	standart error	ratio
Adercotryma wrighti	18	1.57	16	1.66	0	0.00	0.00	17	3.92	0	0.00	0.00	8	5.69	0	0.00	0.00
Astrononion gallowayi	3	0.75	3	1.58	0	0.00	0.00	2	0.80	0	0.20	0.00	3	1.12	0	0.00	0.00
Brizalina skagerrakensis	18	2.26	13	1.50	6	1.18	0.32	13	1.79	10	1.20	0.43	15	1.95	8	2.38	0.35
Brizalina spathulata	29	5.70	30	4.77	2	2.06	0.06	29	4.35	5	1.78	0.15	27	6.47	3	1.29	0.10
Bulimina marginata	32	6.31	34	2.85	5	0.81	0.13	29	4.55	7	2.35	0.19	30	5.60	5	0.84	0.14
Cassidulina laevigata	23	5.31	20	5.56	0	0.49	0.00	17	2.79	2	0.80	0.11	19	5.22	4	1.34	0.17
Cibicides lobatulus	0	0.40	0	0.00	0	0.00	0.00	0	0.20	0	0.00	0.00	0	0.00	0	0.00	0.00
Cribrostomoides globosum	1	0.58	1	0.73	0	0.00	0.00	0	0.40	0	0.00	0.00	1	0.35	0	0.00	0.00
Cribrostomoides jeffreysii	3	1.07	8	1.87	0	0.00	0.00	5	1.59	0	0.00	0.00	6	1.67	0	0.00	0.00
Cribrostomoides nitidum	2	0.40	3	0.66	0	0.00	0.00	1	1.36	0	0.00	0.00	2	1.04	0	0.00	0.00
Eggerelloides medius	29	2.68	24	5.25	0	0.20	0.00	27	6.30	0	0.00	0.00	28	3.19	0	0.00	0.00
Eggerelloides scaber	29	2.97	31	4.39	0	0.40	0.00	22	3.67	0	0.20	0.00	22	3.78	0	0.00	0.00
Elphidium excavatum	0	0.00	0	0.00	0	0.00	0.00	0	0.20	0	0.00	0.00	0	0.22	0	0.20	0.00
Epistominella vitrea	0	0.00	0	0.40	0	0.00	0.00	0	0.20	0	0.00	0.00	0	0.22	0	0.00	0.00
Glandulina laevigata	0	0.00	0	0.00	0	0.00	0.00	0	0.00	0	0.00	0.00	0	0.00	0	0.00	0.00
Globobulimina turgida	8	2.48	8	2.54	0	0.00	0.00	9	2.19	0	0.00	0.00	6	2.13	0	0.00	0.00
Haplophragmoides bradyi	3	0.81	1	0.87	0	0.00	0.00	2	0.68	0	0.00	0.00	3	1.10	0	0.00	0.00
Hyalinea balthica	21	3.61	26	1.21	3	0.37	0.10	29	5.44	3	1.34	0.09	23	2.63	5	0.80	0.18
Lagena laevis	0	0.00	0	0.20	0	0.00	0.00	0	0.00	0	0.00	0.00	0	0.22	0	0.00	0.00
Lagena striata	0	0.00	0	0.00	0	0.00	0.00	0	0.20	0	0.00	0.00	0	0.00	0	0.20	0.00
Leibusella goesi juv.	5	0.86	7	1.53	0	0.00	0.00	6	0.81	0	0.20	0.00	5	3.18	0	0.00	0.00
Liebusella goesi	16	3.43	11	4.60	0	0.00	0.00	14	1.81	0	0.00	0.00	11	1.98	0	0.00	0.00
Loxostomum porrectum	0	0.40	0	0.20	0	0.00	0.00	0	0.00	0	0.20	0.00	1	0.27	0	0.00	0.00
Melonis barleeanum	9	1.62	4	1.55	0	0.20	0.00	7	0.89	0	0.25	0.00	4	1.20	0	0.24	0.00

Nonionella stella	5	3.14	2	2.48	0	0.24	0.00	2	3.96	0	1.71	0.00	3	2.22	0	1.30	0.00
Nonionella turgida	5	0.66	5	1.24	0	0.20	0.00	4	0.75	1	0.20	0.20	7	0.76	1	0.60	0.13
Nonionellina labradorica	14	1.59	16	0.93	1	0.00	0.06	12	1.39	1	0.37	0.08	14	2.51	1	0.20	0.07
Pullenia bulloides	0	0.20	0	0.20	0	0.00	0.00	0	0.00	0	0.00	0.00	0	0.45	0	0.00	0.00
Quinqueloculina stalkeri	0	0.80	0	0.24	0	0.00	0.00	0	0.24	0	0.00	0.00	0	1.08	0	0.00	0.00
Recurvoides trochamminiformis	4	0.51	3	0.68	0	0.00	0.00	5	1.43	0	0.00	0.00	3	1.17	0	0.00	0.00
Reophax bilocularis	0	0.20	0	0.00	0	0.00	0.00	0	0.20	0	0.00	0.00	0	0.00	0	0.00	0.00
Reophax fusiformis	0	0.40	0	0.24	0	0.00	0.00	0	0.40	0	0.00	0.00	0	0.27	0	0.00	0.00
Reophax micaceus	3	1.83	1	1.11	0	0.00	0.00	1	1.17	0	0.00	0.00	2	1.50	0	0.00	0.00
Reophax sp.	5	0.81	3	1.05	0	0.00	0.00	4	1.03	0	0.00	0.00	5	2.30	0	0.00	0.00
Saccammina sphaerica	0	0.00	0	0.20	0	0.00	0.00	0	0.00	0	0.00	0.00	0	0.00	0	0.00	0.00
Sigmoilopsis schlumbergeri	0	0.00	0	0.00	0	0.00	0.00	0	0.20	0	0.00	0.00	0	0.00	0	0.00	0.00
Stainforthia fusiformis	0	0.20	0	0.00	0	0.00	0.00	0	0.00	0	0.00	0.00	0	0.00	0	0.00	0.00
Technitella legumen	0	0.20	0	0.00	0	0.00	0.00	0	0.00	0	0.00	0.00	0	0.00	0	0.00	0.00
Tritaxis conica	2	0.40	2	0.40	0	0.00	0.00	2	0.81	0	0.00	0.00	1	0.42	0	0.00	0.00
Uvigerina peregrina	100	7.90	102	8.04	5	2.38	0.05	91	5.95	5	3.28	0.05	88	3.26	3	3.17	0.03

Table 11. Four-week experiment. Calculated median values, standard error and ratios of microplastic ingestion for a rose Bengal stained foraminifera from four different treatments – reference, 0.5 μm, 1 μm and 6 μm microplastic.

	Reference		0.5 um plastic					1 um plastic					6 um plastic				
	median		median		median			median		median			median		median		
List of species	without plastic	stand art error	without plastic	stand art error	with plastic	stand art error	ratio	without plastic	stand art error	with plastic	stand art error	ratio	without plastic	stand art error	with plastic	stand art error	ratio
Adercotryma wrighti	12	1.22	13	1.82	0	0.00	0.000	8	2.97	0	0.00	0.000	9	1.61	0	0.40	0.000
Astrononion gallowayi	5	1.64	4	1.10	0	0.20	0.000	7	1.36	0	0.40	0.000	4	0.80	0	0.40	0.000
Brizalina skagerrakensis	17	1.72	5	1.12	12	1.69	0.706	7	2.42	6	0.58	0.462	6	1.59	10	0.58	0.625
Brizalina spathulata	26	1.36	10	1.56	15	2.23	0.600	11	0.87	11	2.79	0.500	12	2.48	11	1.12	0.478
Bulimina marginata	34	2.99	9	1.36	14	1.78	0.609	13	2.50	11	2.04	0.458	12	2.87	13	1.63	0.520
Cassidulina laevigata	16	1.44	4	1.07	7	1.14	0.636	8	1.85	7	1.33	0.467	6	1.12	9	1.39	0.600
Cibicides lobatulus	0	0.00	0	0.20	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.20	0	0.00	0.000
Cribrostomoides globosum	0	0.40	0	0.24	0	0.00	0.000	0	0.77	0	0.00	0.000	0	0.00	0	0.00	0.000
Cribrostomoides jeffreysii	2	0.73	4	0.97	0	0.00	0.000	2	0.81	0	0.00	0.000	1	1.12	0	0.00	0.000
Cribrostomoides nitidum	2	0.55	1	0.37	0	0.00	0.000	1	0.20	0	0.00	0.000	1	0.68	0	0.20	0.000
Dentalina communis	0	0.00	0	0.00	0	0.00	0.000	0	0.20	0	0.20	0.000	0	0.00	0	0.00	0.000
Eggerelloides medius	39	3.23	22	2.69	10	1.96	0.313	27	3.99	12	3.12	0.308	26	3.66	12	1.44	0.316
Eggerelloides scaber	9	3.27	9	1.63	3	0.68	0.250	6	2.63	2	0.80	0.250	8	1.62	5	0.92	0.385
Elphidium excavatum	0	0.20	0	0.00	0	0.40	0.000	0	0.00	0	0.40	0.000	0	0.00	1	0.24	1.000
Epistominella vitrea	0	0.40	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.20	0	0.00	0.000
Glandulina laevigata	0	0.00	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Globobulimina turgida	5	1.85	3	1.69	0	0.49	0.000	4	1.40	0	0.00	0.000	7	1.12	0	0.00	0.000
Haplophragmoides bradyi	3	1.11	5	1.08	0	0.00	0.000	3	0.32	0	0.00	0.000	3	1.10	0	0.00	0.000
Hyalinea balthica	29	1.69	9	2.32	12	2.50	0.571	7	2.19	15	3.14	0.682	10	3.61	6	4.21	0.375
Lagena laevis	0	0.00	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Lagena striata	0	0.20	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Leibusella goesi juv.	10	1.60	6	1.02	0	0.40	0.000	10	1.02	0	0.00	0.000	7	1.39	0	0.40	0.000
Liebusella goesi	1	0.32	0	0.40	1	0.32	1.000	2	0.37	0	0.20	0.000	5	1.07	0	0.58	0.000
Loxostomum porrectum	0	0.20	0	0.63	0	0.00	0.000	0	0.24	0	0.00	0.000	0	0.24	0	0.20	0.000

Melonis barleeanum	5	1.52	2	0.75	1	0.86	0.333	3	0.45	0	0.40	0.000	6	1.05	0	0.20	0.000
Nonionella stella	3	0.92	1	0.37	1	0.24	0.500	2	0.66	1	0.37	0.333	1	0.58	1	0.40	0.500
Nonionella turgida	4	0.73	4	1.03	3	0.86	0.429	3	0.63	0	1.03	0.000	6	1.03	0	0.40	0.000
Nonionellina labradorica	14	1.92	7	1.40	6	2.06	0.462	5	1.69	9	0.80	0.643	3	0.86	11	1.03	0.786
Pullenia bulloides	0	0.00	0	0.40	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Quinqueloculina stalkeri	0	0.00	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Recurvoides trochamminiformis	4	0.86	3	0.58	0	0.00	0.000	6	1.12	0	0.00	0.000	5	1.43	0	0.00	0.000
Reophax bilocularis	0	0.00	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Reophax dentaliniformis	0	0.00	0	0.20	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Reophax fusiformis	0	0.00	0	0.00	0	0.00	0.000	0	0.20	0	0.00	0.000	0	0.20	0	0.00	0.000
Reophax micaceus	1	0.87	0	0.49	0	0.60	0.000	0	0.60	0	0.00	0.000	1	0.86	0	0.00	0.000
Reophax sp.	0	0.40	0	0.00	0	0.00	0.000	0	0.24	0	0.00	0.000	0	0.24	0	0.00	0.000
Saccammina sphaerica	0	0.00	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Sigmoilopsis schlumbergeri	2	0.58	0	0.40	0	0.00	0.000	0	1.17	0	0.00	0.000	2	1.39	0	0.00	0.000
Stainforthia fusiformis	0	0.00	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Technitella legumen	0	0.00	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000	0	0.00	0	0.00	0.000
Tritaxis conica	2	0.68	1	0.24	0	0.00	0.000	0	0.24	0	0.00	0.000	1	0.37	0	0.00	0.000
Uvigerina peregrina	82	6.44	28	4.95	35	9.22	0.556	48	4.47	24	1.77	0.333	53	4.58	18	3.78	0.254