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# Subtle ecosystem effects of microplastic exposure in marine mesocosms including fish

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ABSTRACT

For two months, communities in 5.8 $m^3$ outdoor marine mesocosms were exposed to 700 μm sphere-shaped polystyrene (PS) beads in dosages between 0.08 and 80 g/$m^2$. Barnacle (*Semibalanus balanoides*) densities were reduced at dosages of 0.8 g/$m^2$ onwards without following a standard dose response curve. Lugworms and fish (*Solea solea*) ingested PS-beads without accumulating them. Lugworms (*Arenicola marina*) ingested the beads nonselective with the sediment without negative effects. The fish seemed to ingest the plastics only occasionally and at the final sampling day even in the highest dosed mesocosms (>30 beads/$cm^2$) only 20% contained plastic. The condition index of the fish was slightly reduced in mesocosms with dosages of 0.8 g/$m^2$ onwards. No difference in condition was found between fish with and without ingested plastic across mesocosms, illustrating the difficulty to relate plastic ingestion with condition from field data. The fish also ingested mollusks with shells exceeding the size of the PS-beads. Bivalves rejected the PS-beads as pseudofeces, without obvious impact on their condition. Mussel's (*Mytilus edulis*) pseudofeces present an effective matrix to monitor microplastic presence in the water column. Species richness and diversity of the pelagic and benthic community were not affected although, a trend was found that the lower microplastic dosages had a positive effect on the total abundance of benthic invertebrates. In general, the observed effects at even the highest exposure concentrations were that subtle that they will be obscured by natural variation in the field. This underlines the importance of experiments under semi-field conditions for meaningful assessment of the ecological impact of microplastics. This study was performed with the real life, non-toxic, sphere-shaped polystyrene beads as were lost during an actual spill near the Dutch Wadden sea in January 2019. We recommend future mesocosm studies with other types of microplastics, including microfibers, weathered microplastics from sea, and smaller sized particles down to nanoplastics.

## 1. Introduction

Microplastics (<5 mm) are nowadays widely spread in aquatic and terrestrial ecosystems over the globe, which raised the concern that exposure to microplastic poses a potential risk to the health of wildlife, humans, and the environment. Consequently, research on the accumulation and effects of microplastics has boomed during the last decade (Petersen and Hubbart, 2021). Despite this effort, significant uncertainty and discrepancies about microplastics' biological effects still exist. For example, in a systematic literature review, Bucci and co-authors found that the number of laboratory exposure studies with microplastics published in peer-reviewed journals that reported effects (n = 199) is almost equal to the number of studies not showing effects (n = 222) (Bucci et al., 2020). This discrepancy was particularly evident for effects on sub-organism level (macromolecular interactions and cells), on organism level researching the lethal concentration and growth impact, and ecological relevant effects on population size. In addition, most of the effects detected in laboratory studies are caused by very high exposure concentrations that are not capturing levels found in the environment (Bucci et al., 2020; Burns and Boxall, 2018; de Sa et al., 2018). Therefore, additional experimental work considering environmentally realistic exposure scenarios concerning exposure concentrations, types of microplastics and environmental conditions is warranted (Bucci et al., 2020; Burns and Boxall, 2018; de Sa et al., 2018; Green,

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2016; Sendra et al., 2021).

The majority of the laboratory exposure studies with microplastic to date consist of single species experiments with a relatively short exposure time (de Ruijter et al., 2020). With some exceptions, these experiments use virgin microplastics that have not been given time to grow a biofilm, potentially affecting ingestion. During exposure, the test organisms are fed with artificial food (e.g., fish flakes) or cultivated plankton, often offered as a batch. We understand that such studies did not intend to assess natural ingestion rates of microplastics but were focused on the impact of microplastics after ingestion. However as stated by Burns et al. (Burns and Boxall, 2018): 'just because an effect is seen in the laboratory does not mean that the effect will occur in the real environment.' Other authors worry that the lack of clear evidence for ecological effects in nature is due to relatively poor-quality effects studies (de Ruijter et al., 2020). Hence there is an urgent need for more complex chronic exposure studies that better simulate the real-world exposure scenarios in which the test organisms can show their natural behavior and complex community interactions can be integrated. To address this knowledge gap, the current study applied experimental ecosystems (or mesocosms) to test the ecological impact of microplastics under semi-field conditions for the test community while allowing controlled exposure conditions and replication of treatments and controls which are impossible in a real field situation. Since more than two decades freshwater mesocosms that hold a multi-trophic ecosystem are an established tool in the risk assessment of agricultural chemicals, where they are used to bridge the gap between the results obtained in standardized toxicity tests and the more complex field situation (Campbell et al., 1999; De Jong et al., 2008). In parallel, also marine mesocosms have been developed that are nowadays being applied for a wide range of research, considering among other topics: oil spills (Lott et al., 2020; Quade et al., 2022), heavy metals (foekema_et_al_2021; Foekema et al., 2021a; Sharma et al., 2021), climate change (Hall and Lewandowska, 2022; Moreno et al., 2022; Zhou et al., 2022) and nanoparticles (Dauda et al., 2020), or to study the degradation of plastics in the marine environment (Quade et al., 2022). Several papers have also been published on the ecological impact of microplastics in mesocosms, both in freshwater (Michler-Kozma et al., 2022; Redondo--Hasselerharm et al., 2018; Yilcin et al., 2022) and in the marine environment (e.g. (Green, 2016; Green et al., 2016; Setala et al., 2016). In none of these studies fish were included. This is often the case in mesocosm studies as fish's feeding hampers the determination of the impact of the treatment on the invertebrate community (Giddings et al., 2002). To avoid this drawback but still collect data on fish, we added fish in a parallel series of mesocosms that were given the same treatments as the mesocosms we used for determining the impact on the invertebrate community. As far as we are aware this paper describes for the first time, among other topics, the impact of microplastics on fish under semi-field (feeding)conditions.

The goal of the research presented in this paper was to determine how a marine invertebrate community and fish cope under (close to) natural conditions with a dosage series of polystyrene (PS) sphere-shaped beads administered as a single pulse. The outcome was used to assess the potential environmental impact of the 11,250 kg of polystyrene microbeads with a diameter of approximately 700 μm that were lost by the container carrier MSC Zoe during a storm in January 2019. The incident occurred along a transect (53°26.2′N:4°44.6′E to 53°47.2′N:6°28.0′E; www.kustwacht.nl/nl/msczoe) just north of the Dutch part of the Wadden Sea, a nature conservation area that covers the northern coast of the Netherlands until the South Western coasts of Denmark.

## 2. Materials and methods

### 2.1. General mesocosm set-up

The mesocosms used for this study were set up in identical polyester tanks with a diameter of about 2 m, a depth of about 2 m, and a total volume of 5.9 $m^3$, positioned outdoors at the test facility of Wageningen Marine Research in Den Helder, the Netherlands (52°57′02″N: 4°46′33″E). Each mesocosm was provided with a 15 cm thick layer of fine sea sand (approximately 754 kg or 471 L) and filled to a water depth of approximately 180 cm (approximately 5338 L) with freshly collected Wadden Sea water (52°58′24″N; 04°47′57″E) with a natural plankton community. An air stone was positioned in the center of each mesocosm at 40 cm above the sediment floor to ensure continuous mixing of the water column and facilitate gas exchange. Each mesocosm was inoculated with meiofauna by adding 2 homogenated liter surface silt from a tidal flat in the Wadden Sea (52°56′06″N; 04°59′56″E), that had been sieved through a 5 mm sieve to remove larger organisms. In addition, each mesocosm received 20 cockles (*Cerastoderma edule;* 14.5 ± 1.49 mm, 0.27 ± 0.09 g flesh weight -without shell-), 20 lugworms (*Arenicola marina*; 3.5 ± 0.8 g), 50 periwinkles (*Littorina littorea*; 15.3 ± 0.89 mm, 1.49 ± 0.26 g total weight -with shell-), and 20 mussels (*Mytilus edulis*; 29.8 ± 2.5 mm, 0.73 ± 0.18 g flesh weight). The mussels were packed in an open basket that was suspended at half water depth; all other species were added without enclosures. All organisms mentioned above were collected from tidal flats in the Southwestern part of the Wadden Sea near the area where also the surface silt was collected (52°56′06″N; 04°59′56″E). Based on water currents it could be excluded that this area was affected by the incident with the container carrier. Thirty mesocosms were installed as described above.

During the acclimatization period of five weeks, all 30 mesocosms were connected by tubing, and water was circulated. This acclimatization period is essential to ensure a parallel development of the ecosystems after the mesocosms are disconnected. Duration and conditions of this acclimatization period depend on the mesocosm set-up and season. From years of experience (e.g., Foekema et al., 2015; Foekema et al., 2021a,b; Klok et al., 2014; Wei et al., 2019) we learned that 4–5 weeks is sufficient for these types of mesocosms. At the end of the acclimatization period, the water circulation was stopped, making each mesocosm an independent system and the microplastics were administered as described in detail below (section 2.2). Three weeks later, 15 of the mesocosms received each 20 juvenile soles (*Solea solea,* 14.8 ± 2.2 mm, 0.039 ± 0.019 g) achieved from a commercial hatchery; these mesocosms will now be referred to as 'fish mesocosms'. It was not possible to introduce the fish at an earlier moment in a size/developmental stage that we know is convenient for a mesocosm study (Foekema et al., unpublished data). It was decided to prepare these specific 'fish mesocosms' since the feeding behavior of fish substantially affects the invertebrate community. Therefore, it is recommended not to include free living fish in a mesocosm when the invertebrate community is being studied (Giddings et al., 2002). The 'fish mesocosms' have only been used to investigate the effects on fish. All other data presented in this paper were collected from the mesocosms without fish.

The final sampling of the invertebrate mesocosms took place eight weeks after the administration of the microplastics, the fish mesocosms were terminated the week thereafter. The exposure duration of the fish was thus 6 weeks, while the invertebrate communities in all mesocosms were exposed to the microplastics for 8 weeks. From our experience we know that the variation between replicate mesocosms is relatively low during the first 8 weeks after a sufficient acclimatization period, and then gradually increases with time. Longer test durations therefore reduce the statistical power of the study.

During the final sampling event, sediment core samples and surviving biota were collected and processed as described below. The species selected for this study are representatives of the invertebrates in the Dutch Wadden Sea, and the flatfish that use the area as nursery ground (Reise et al., 2010; Rijnsdorp et al., 1992). They further represent different species traits that might affect their ability to cope with the microplastics. For example, the lugworm is a non-selective sediment feeding worm; mussels and cockles are both suspension feeders, but cockles live in soft sediment while mussels colonize hard substrate that

can be present at other positions in the water column. Sole feeds actively on benthic invertebrates (for other general features of these species see for instance www.marinebio.org).

### 2.2. Microplastics and dosages

The study was performed with industrial polystyrene (PS) sphere-shaped beads with a diameter of 701 ± 112 μm (10%–90% percentile: 539–836 μm) obtained from CARAT GmbH in Germany (product code V2020-0065). Chemical analyses revealed that the beads did not contain flame retardants, plasticizers, or potentially toxic organic chemical additives or contaminants. A description of the analytical procedures and results can be found in Supporting info, Fig. S1. These beads were similar in composition, shape, and appearance to the beads that were lost by the container carrier MSC Zoe in January 2019. This type of PS-beads forms the base material to produce extruded polystyrene foam. Heating the beads to about 100 °C transforms them into a 3–4 mm white ball of polystyrene foam with very high buoyancy. This feature was handy when recovering the beads from sediment samples in this study, as described in the materials and methods section.

The basis for the dosage series was a theoretic scenario that all 11,250 kg of PS-beads that were lost at sea by the container carrier, became distributed homogeneously in 10% of the surface area of the Dutch Wadden Sea. In this situation, 0.08 g, or 326 PS-beads would be present per $m^2$. In the three following treatments, the dosages per mesocosm were each time increased by a factor of 10 resulting in a dosage series of 0; 0.08; 0.8; 8.0, and 80 g PS-beads per $m^2$. Each treatment was triplicated (n = 3). The highest dosage corresponded with more than 300,000 beads per $m^2$. If all PS-beads in this treatment level would either remain suspended in the water column or settle on/in the sediment, the maximum concentrations in these compartments would be 196 beads or 47 mg/L, 1358 beads or 333 mg/kg, respectively (Table 1). These are hypothetical numbers that can only be reached in one compartment when no beads remain in the other compartment.

From their review, Burns & Boxall (2018) conclude that the maximum concentrations of reported microplastics in marine field samples were 17 particles per liter of water (South Korean coast; Song et al., 2015), and 42,560 particles per $m^3$ of sediment (Northern coast of Taiwan; Kunz et al., 2016). The latter would refer to 27 particles per kg for the sediment (specific weight 1.6 kg/L) used in the mesocosms. These maximum field concentrations were thus exceeded in our mesocosm study's second-highest treatment (8 g/$m^2$).

### 2.3. Sampling, sample processing, and data treatment

Every week, temperature, salinity, pH, dissolved oxygen concentration, and turbidity of the water in the mesocosms were determined at half water depth using hand-hold electrodes (Mettler Toledo for pH, Hach-Lange HQ40D for oxygen, Hach-Lange HQ30D for salinity and temperature). Water samples representative for the whole water column were taken employing a water core sampler at four positions (2.5L per position) in the mesocosm for the determination of zooplankton densities (microscope analyses), the chlorophyll-a concentration (BBE Moldaenke algae analyzer), and concentrations of dissolved inorganic nutrients (HACH DR900 colorimeter, with methods 8155-ammonium, 8039-nitrate, and 8048-phosphate).

Every week the baskets holding the mussels were shaken underwater to wash out the accumulated feces and pseudo feces. This material was collected on Day 7 and Day 28 by washing the basket in a bucket. It was then concentrated in a 50 μm-plankton net in a pre-weighted glass beaker and dried for 24h at 60 °C. After dry weight was determined, demineralized water was added, and the beaker was placed in a household microwave oven until the water had boiled for at least 2 min. By then the PS-beads had transformed into white floating beads with a diameter around 2–3 mm, that could easily be collected and counted.

At the termination of the study, the entire mesocosm was sampled. First, the mesocosm water was pumped out over a 1 mm sieve, and all organisms remaining on the sieve were collected. Subsequently, 4 sediment cores (40 mm diameter 15 cm depth) were collected and divided into three depth fractions: 0–1 cm, 1–5 cm, and 5–15 cm. These fractions were pooled per mesocosm and stored refrigerated at approximately 4 °C for determination of the number of PS-beads later. The sediment samples were then transferred to a glass beaker, and demineralized water was added. The beaker was placed in a household microwave oven until the water had boiled for at least 2 min and the PS-beads had transformed into white floating beads with a diameter around 2–3 mm, that could easily be counted. The results were expressed as the number of beads per gram of wet sediment.

Finally, all sediment was removed from the mesocosm and washed over a 1 mm sieve, collecting all organisms remaining on the sieve. Fish were immediately transferred to seawater with an overdose of the aquatic anesthetic AQUI-S (active ingredient Isoeugenol), where they were quickly stunned and then died before they were stored at −18 °C. The introduced mussels, cockles, and periwinkles were collected from the sieve and stored at −18 °C directly after sampling. The lugworms were transferred to aquaria with clean seawater without sediment to allow them to empty their intestine overnight. The remaining benthic material on the sieve, consisting of debris and smaller invertebrates, including juveniles of the introduced bivalves and worms, was collected and stored in 4% buffered formaldehyde.

The next day the tissue-dried body wet weight of the individual lugworms was determined, and the worms were stored at −18 °C until further processing. Next, the defecated sediment was collected from the aquaria and examined for PS-beads following the procedure described above for the sediment core samples.

After thawing, the in- and outside of the shell of the mussels, cockles, and periwinkles was rinsed with clean seawater to remove any PS-beads from the outside of the body. Then shell length was measured with a digital caliper, and the whole organism's wet weight was determined. Subsequently, the flesh was removed, and the weight of the empty shell was determined. The difference between whole organism weight and shell weight was used to calculate the flesh weight. Finally, the soft tissue of the bivalves and the worms was placed under a small layer of clean seawater and searched under a stereomicroscope (magnification to 8x) for PS-beads that had entered the intestine or any other tissue.

After being thawed, each fish was tissue dried and weighted, and the total body length was determined on a mm scale. Individual length (L) and weight (W) measurements were used to determine the condition index (K) according to the Fulton formula: $K = 100 \times W/L^b$ (See for details Text S1 in Supporting info). The fish was then placed in a small layer of clean seawater and examined for abnormalities in growth or development, or indications of poor health status under a stereomicroscope. Subsequently, the whole intestine was removed from the body and checked for the presence of PS-beads.

For the characterization of the benthic community first the larger specimens with relative low abundance were collected from the whole

**Table 1**
Dosage of PS-beads added to the mesocosms per $m^2$ and theoretical maximum concentrations in water and sediment when all PS-beads would concentrate solely in that single compartment. Each mesocosm has a 3.14 $m^2$ surface area and contains about 5338 L water and 754 kg (471 L) sediment. The average weight of an individual PS-bead was 0.24 mg. The first column shows the codes used to identify treatments in this paper.

| Code | Dosage | | Water column (max) | | Sediment (max) | |
|---|---|---|---|---|---|---|
| | g/$m^2$ | n/$m^2$ | mg/L | n/L | mg/kg | n/kg |
| 0 g/$m^2$ | 0.0 | 0 | 0 | 0.0 | 0 | 0 |
| 0.1 g/$m^2$ | 0.08 | 326 | 0.05 | 0.2 | 0.3 | 1.4 |
| 0.8 g/$m^2$ | 0.8 | 3259 | 0.47 | 1.9 | 3.3 | 14 |
| 8 g/$m^2$ | 8.0 | 32,590 | 4.7 | 19 | 33 | 136 |
| 80 g/$m^2$ | 80.0 | 325,900 | 47 | 196 | 333 | 1358 |

sample of benthic material. After this representative sub-samples were searched for organisms under a stereomicroscope until sufficient sub-samples were searched to collect more than 200 individual organisms. Collected individuals were identified to species level where possible and counted. Numbers were then corrected for the relative volume of the sub-samples that were investigated and divided by the surface of the mesocosms (3.14 $m^2$) to be expressed as numbers per $m^2$.

### 2.4. Statistical analyses

All statistical analyses were performed with build-in options of the GraphPad PRISM v9.4.0 software package. Each of the three mesocosms per treatment formed an independent replicate. First, it was tested if the data sets conformed to a gaussian distribution using the Shapiro-Wilk test. Time series collected by weekly measurements (e.g., plankton densities) were tested for statistical significance of the treatments by means of a two-way ANOVA. To test for significant treatment effects in data sets that were collected during the final sampling event a one-way ANOVA was applied. When a significant ($p < 0.05$) treatment effect was detected Dunnett's multiple comparisons test was applied as post-test to test the differences between controls (0 g/$m^2$) and individual treatments.

An exception to these general rules was made during the analyses of the benthic community (see section 3.3.5); to investigate the validity of a trend in abundance that was not detected by the one-way ANOVA, the significance of the difference between controls and individual treatments were tested with a two-tailed T-test.

The data sets that distinguished individual fish with and without ingested plastics regardless the mesocosm, did not pass the Shapiro-Wilk normality test. Differences between these groups in body weight, length and condition were therefore tested with the non-parametric two-tailed Mann-Whitney test.

Finally, Pearson r correlation tests and the linear regression option (intercept forced through zero) were applied to test the relations 1) between the number of PS-beads added per mesocosm and the numbers recovered in sediment samples, and 2) between the concentration of PS-beads in the sediment and in the lugworm's feces.

### 2.5. Ethical statement

The use and care of the fish in this experiment complied with Directive 2010/63/EU and was approved by the Dutch "Central Committee Animal experiments".

## 3. Results and discussion

### 3.1. Water characteristics

When the PS-beads were added to the mesocosms (May 13th, 2020), the water temperature was around 13 °C. The maximum of 22 °C was measured 42 days later and the temperature than declined to around 20 °C at the end of the study (July 7th). The average water temperature during the study was 18.6 ± 2.5 °C. The average value for salinity was 28.8 ± 1.2, for pH 8.45 ± 0.16, for dissolved oxygen 98.9 ± 2.3% saturation, and for chlorophyll-a 7.4 ± 4.1 μg/L. These values can be considered normal for the Dutch Wadden Sea in the season the experiment was performed (https://waterinfo.rws.nl). Average concentrations of inorganic nitrogen were 1.09 ± 0.14 mg N/L for all mesocosms during the study. Over 95% of the inorganic nitrogen was present as nitrate. Orthophosphate was detected in average concentrations of 0.12 ± 0.10 mg P/L. None of these values were affected by the treatments.

### 3.2. Exposure conditions

Throughout the study, PS-beads were observed suspended in the water column. With time more of these beads became visually covered with biofilm (Fig. S2 in Supporting info). Concentrations of PS-beads in the water column were not monitored since it would have required large sample volumes to collect reliable data.

The number of PS-beads in the sediment cores at the end of the study indicated that about 85% of the added beads were present on or in the sediment (Correlation Pearson r tests p=<0.0001; Linear regression p=<0.0001, Slope = 0.85 ± 0.02, $R^2$ = 0.9904; Fig. S3 in Supporting info), suggesting that about 15% remained in suspension in the water column. In sediment cores collected from the highest dosed mesocosms 28 ± 2.4 PS-beads per $cm^2$ were present. Due to bioturbation, roughly about 30% of the PS-beads in the sediment ended up deeper than 1 cm and about 10% deeper than 5 cm at the end of the study (Fig. S4 in Supporting info).

### 3.3. Biological and ecological endpoints

#### 3.3.1. Plankton community

Chlorophyll-a concentrations in the water column of the mesocosms ranged between 1.2 and 20.7 μg/L in all mesocosms during the study (Fig. S5 in Supporting info). The fluorescence analyses assigned 60% of the overall chlorophyll concentration to green algae and 35% to diatoms. Differences between treatments and controls were never statistically significant (two-way ANOVA total chlorophyll: F(4, 10) = 2.134; p = 0.1509). The zooplankton community (Fig. S6 in Supporting info) was initially dominated by copepods and their larvae, with numbers steadily increasing during the study to more than 50 individuals per liter in most mesocosms. After day 28, pelagic polychaete larvae emerged, resulting in more than 100 individuals per liter in some mesocosms by the end of the study. For none of these zooplankton groups, nor for total zooplankton abundance, statistically significant differences between treatments were found (two-way ANOVA, Copepods F(4, 10) = 1.480; p = 0.2795; Polychaete larvae F(4, 10) = 0.7901; p = 0.5575; Total abundance F(4, 10) = 0.7043; p = 0.6068).

#### 3.3.2. Barnacles

No adult barnacles were introduced into the mesocosms, but larvae present in the natural seawater settled on the (approximately 10 $m^2$) mesocosm walls, resulting in 10–17 living barnacles (*Semibalanus balanoides*) per $m^2$ in the controls and similar numbers in the 0.08 g/$m^2$ mesocosms (Fig. 1). The effect of treatment was significant (ANOVA F(4, 10) = 5.627; p = 0.0123), and the number of barnacles per $m^2$ was significantly lower in the 0.8 and 80 g/$m^2$ treatments than in the controls (Dunnett's multiple comparisons test DF = 10; p = 0.0075, and p = 0.0200 respectively). The densities in the intermediate treatment, 8 g/$m^2$, did not significantly differ from the controls (Dunnett's multiple

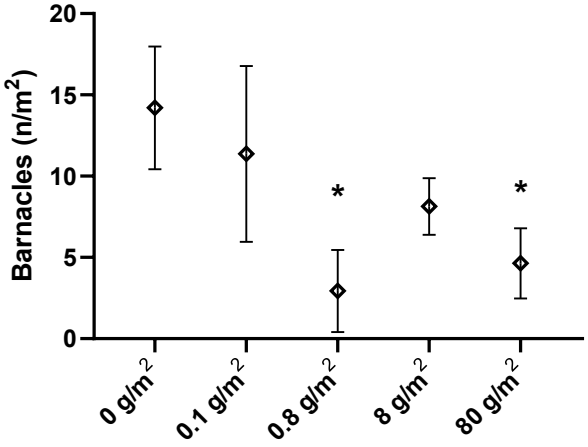


**Fig. 1.** Average density of living barnacles (Semibalanus balanoides) on the mesocosms walls at the end of the study. '*' indicate significant ($p < 0.05$) differences from the controls (0 g/$m^2$).

comparisons test DF = 10; p = 0.1541). Effects of microplastics on barnacles have been reported before, but these were only observed for barnacle larvae exposed to particles with a diameter less than 19 μm (Yu and Chan, 2020b) (Yu and Chan, 2020a), substantially smaller than the 0.7 mm beads used in our study. Other researchers reported a negative impact of (not characterized) polystyrene leachates on settlement of barnacle larvae at an exposure concentration equivalent to 0.004 $m^2$ fresh polystyrene surface per liter of water (Li et al., 2016). In our highest dosed mesocosms, assuming an average surface per PS-bead of 2.02 $mm^2$, the total polyethylene surface was 100 times lower, around 0.0004 $m^2$/L, and 10,000 x lower in the 0.8 g/$m^2$ treatment where the effects on barnacles were first observed. This, in combination with the fact that the impact between the 0.8 and 80 g/$m^2$ treatments seems not dose related, makes it unlikely that in our experiment the barnacle densities were reduced by toxicity of leachates. It remains therefore unclear what caused the reduced barnacle densities.

### 3.3.3. Lugworms

Survival rate (81 ± 11%) and body weight increase (10 ± 7.7%) of the introduced adult lugworms showed no relation with the treatments at the end of the study (ANOVA, Survival F(4, 10) = 1.727; p = 0.2201; Body weight F(4, 10) = 1.102; p = 0.4072; Fig. S7 in Supporting info). In the sediment that the lugworms egested during the night after being sampled, PS-beads were found in treatment-related numbers with a maximum of 35 beads per worm at the highest dosage level (Fig. S8 in Supporting info). When dissecting the lugworms, no remaining beads were found, indicating that the PS-beads did not accumulate internally, which is in line with earlier observations (Besseling et al., 2013). The numbers of PS-beads in the egested sediment were in the same order of magnitude as the numbers in the sediment core samples from the same mesocosms (Linear regression p = 0.0022; slope = 2.1 with 95% CI: 0.9 to 3.3; Fig. 2). This confirms that the lugworm is a non-selective sediment feeder. The two times higher number of beads in the lugworm feces (egested sediment) relative to the ingested (mesocosm) sediment is probably related to the digested organic matter during gut passage, although differences in water content between the sediment samples cannot be excluded.

Since lugworms were the only inhabitants of the mesocosm that could dig more profound than a few centimeters, it is obvious that it was their activity that transferred about 10% of the PS-beads to sediment layers below 5 cm (Fig. S4 in Supporting info). In addition, the lugworms also had a substantial contribution in bringing about 25% of the beads between 1 and 5 cm depth in the sediment, together with other macroinvertebrates, e.g., the cockles. Our data confirm observations by other researchers about the role of lugworms (Gebhardt and Forster, 2018) and other benthic macroinvertebrates (Nakki et al., 2017) in working microplastics to deeper sediment layers.

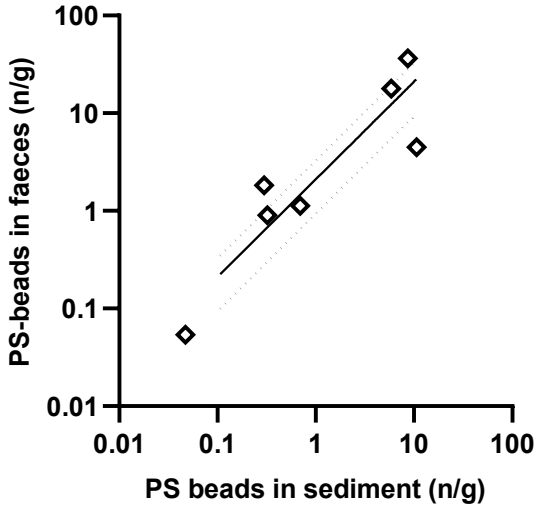


**Fig. 2.** Number (n) of PS-beads in lugworm feces at the end of the study plotted against numbers present in/on the sediment in the same mesocosms; both expressed per gram wet weight. Linear regression with slope = 2.1 with 95% confidence interval 0.9-3.3 (dotted lines), P = 0.0022. Correlation Pearson r test P = 0.0111.

In contrast to other researchers that reported reduced feeding activity (Besseling et al., 2013; Green et al., 2016), and loss of body weight (Besseling et al., 2013) in lugworms due to microplastics, we did not find indications for reduced fitness in our mesocosms. The most likely explanation is that the effects in the other studies were only significant at the highest exposure levels that ranged from 2% (Green et al., 2016) to 7.4% (Besseling et al., 2013), thus at least 50 times higher than our maximum treatment that corresponds to maximum 0.03% (Table 1). Achieving similar concentrations of PS-beads in our mesocosms would have required dosages between 4 and 14.8 kg of PS-beads per $m^2$. It is evident that at such concentrations the sediment structure is affected, and the available food (organic matter) is substantially diluted with plastics. Hence, it is not surprisingly that such a high dosage will affect the benthic community.

### 3.3.4. Bivalves

The bivalve species deliberately introduced at the start of the study performed well in all mesocosms. At the end of the study, the average survival rate of the introduced cockles per mesocosm was 93 ± 7.0% and not related to treatment (ANOVA, F(4, 10) = 1.879; p = 0.2779; Fig. S9 in Supporting info). Shell length increased from 14.4 mm at the introduction to 22.5 ± 0.5 mm, and flesh weight from 0.27 g to 1.06 ± 0.08 g. Both shell length and flesh weight at the end of the study were not significantly related to the treatments (ANOVA, F(4, 10) = 1.958; p = 0.1772, and F(4, 10) = 0.4919; p = 0.7422 respectively). Survival of the other bivalve, the mussel, was 95 ± 5.2%, and did not relate to treatments (ANOVA, F(4, 10) = 0.4474; p = 0.7723; Fig. S10 in Supporting info). Like the cockles, the mussels showed substantial growth in all mesocosms. Shell length increased from 29.7 mm to 36.56 ± 0.63 mm and showed no relation with treatment (ANOVA F(4, 10) = 0.7497; p = 0.7504; Fig. S10 in Supporting info). Mussel flesh weight increased on average from 0.73 g to 1.67 ± 0.12 g in all mesocosms. Differences between treatments were not significant (ANOVA F(4, 10) = 2.699; p = 0.0925; Fig. S10 in Supporting info).

No PS-beads (or other microplastics) were found inside the bivalve's bodies. This is not remarkable since the size of the PS-beads exceeded about 20 times the maximum size of the seston particles that form the primary food source of these bivalves (Strohmeier et al., 2012Strohmeier et al., 2012). These large particles are thus not ingested but rejected in pseudo feces. Feces and pseudo feces together -from here referred to as (pseudo)feces-accumulated in the baskets that held the mussels. Given the open structure of the baskets it is unlikely that all produced (pseudo)feces was retained, but nonetheless the accumulated amounts and the number of PS-beads therein showed a clear relation with the treatments. The total amount of (pseudo)feces recovered from the mussel baskets was higher on day 7 than on day 28 and was on both days affected by the treatments (ANOVA, F(4, 10) = 5.709; p = 0.0117, and F(4, 10) = 3.662; p = 0.0436 respectively; Fig. S11 in Supporting info). Most material was present in the baskets from the 80 g/$m^2$ mesocosms on both days, and on day 7 the amount of (pseudo)feces in the 80 g/$m^2$ mesocosms was significantly higher than in the controls (Dunnett's multiple comparison test, p = 0.0120). The number of PS-beads that were present in the (pseudo)feces showed a clear relation with the treatment on both days (ANOVA Day 7: F(4, 10) = 50.69; p < 0.0001, and Day 28: F(4, 10) = 23.43; p < 0.0001; Fig. 3). The number of beads per gram (pseudo)feces was similar on day 7 and day 28, with around 1 bead per gram at the lowest tested dosage and over 1000 beads per gram at the highest treatment level. It was not possible in this study to separate feces from pseudofeces, but since the PS-beads were too large to be ingested by the bivalves, feces will not have contributed to the

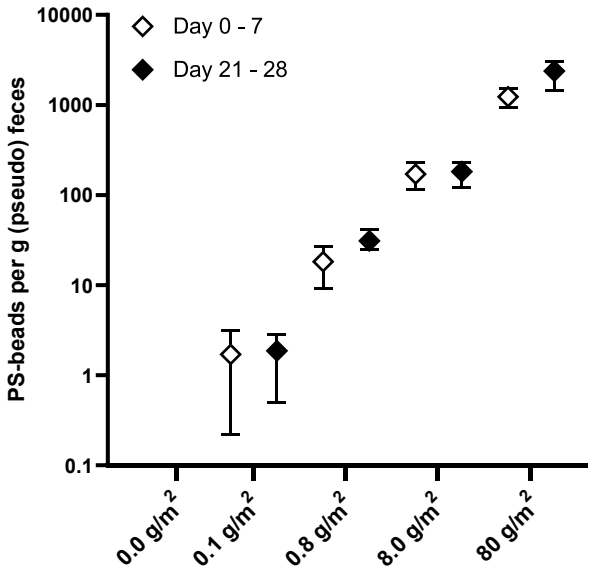


**Fig. 3.** The concentration of PS-beads in mussel's combined feces and pseudo feces for periods Day 0-7 and Day 21-28. Presented on dry weight basis.

number of beads. This result suggests bivalve's pseudofeces as a potential tool to effectively monitor the presence of microplastics in the water column.

### 3.3.5. Benthic community

The invertebrate community present in the sediment at the end of the study consisted of 18–23 species per mesocosm, and this number was not related to treatments (ANOVA, F(4, 10) = 0.5000; p = 0.3416; Fig. S13 supplementary data). The most abundant taxa were *Capitellidae*, *Spionida* (e.g., *Pygospio elegans*), *Corophium volutator*, *Peringia ulvae,* and juveniles of *Ensis* sp. and *Mytilus edulis* (Table S1 in Supporting info). Biodiversity was comparable between mesocosms without an effect of the treatments (ANOVA, Diversity F(4, 10) = 0.2398; P = 0.9094; Evenness F(4, 10) = 0.1398; p = 0.9635; Fig. S13 supplementary data). ANOVA also did not indicate an effect of treatment on total abundance (F(4,10) = 2.149; p = 0.1389), although an upward trend can be seen between the controls and the 0.8 g/m$^2$ treatment (Fig. 4). This trend is driven by the *Annelida,* especially *Captellida* and *Spionida* (Fig. S14 supplementary data), and the mollusks with main contributors *Peringia ulvae* and juvenile cockles (*Cerastoderma edule,* Fig. S15 supplementary data). When tested with the two-tailed T-test, total benthic invertebrate density is significantly (t = 6.193; df = 4; p = 0.0035) higher in the 0.8 g/m$^2$ mesocosms than in the controls, which is also the case for specifically annelids (t = 3.347; df = 4; p = 0.0286), and mollusks (t = 3.016; df = 4; p = 0.0393). Even though ANOVA and related multicomparison post-tests did not indicate impact of treatment on the benthic community, the results of the T-tests suggest that the benthic community experienced some advantages of the treatments up to 0.8 g/m$^2$. Possibly the pelagic larvae of the benthic species experienced less predation from the reduced numbers of barnacles in the 0.8 g/m$^2$ and higher treatments. However, total zooplankton abundance does not indicate reduced predation pressure at treatment levels above 0.8 g/m$^2$ (Fig. S6 supplementary data). Another or additional explanation could be that the presence of suspended PS-beads facilitated biological production in the water column, as biofilm on the beads. After reaching a certain biomass this biofilm may have promoted settlement of the particle, or detached from the bead and sunk to the sediment floor itself. That microplastics have the potential to increase the production or organic matter in the water column and as such can modify the dynamics of organic matter in marine systems has been shown in dedicated experiments (Boldrini et al., 2021; Galgani et al., 2019). In our mesocosms, development and fate of organic matter were not monitored in detail, but the response of the benthic community could be an indication that PS-bead dosages up to 0.8 g/m$^2$ resulted in an increasing flux of organic matter to the sediment floor. At the higher PS-bead concentrations the stimulation of the benthic community seems to disappear which could indicate that nutrient availability restricted the development of enough biomass on all these PS-beads. Dedicated additional research is required to test this hypothesis. Overall, even at the highest treatment level, the PS-beads did not induce adverse negative effects on the benthic community in the mesocosms.

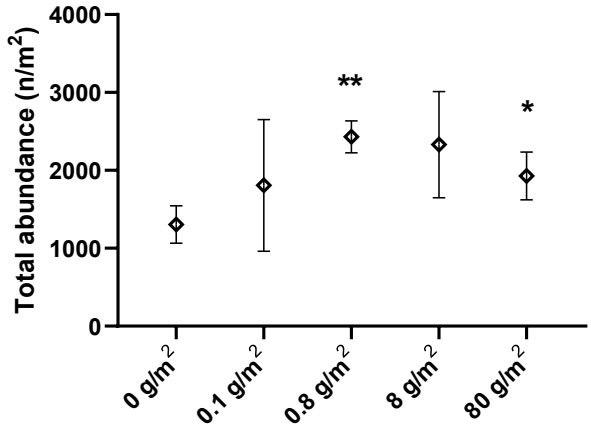


**Fig. 4.** Total abundance of benthic organisms in the mesocosms at the end of the study. Expressed as the average of triplicate mesocosms. With ANOVA no relation with treatment could be detected. '*' ($p < 0.05$) and '**' ($p < 0.01$) indicate significant differences from the controls (0 g/m$^2$) when tested with a two-tailed T-test.

### 3.3.6. Fish

The average survival of the introduced fish (*Solea solea*) per mesocosm was 96 ± 6.7% and not affected by the treatments (ANOVA, F(4, 10) = 0.5156; p = 0.7263; Fig. S12 in Supporting info). In all mesocosms, the fish showed substantial growth resulting in an increased length from 14.8 mm at the introduction, to 39.8 ± 2.1 mm during the final sampling, and a bodyweight increase from 0.04 g to 0.47 ± 0.08 g. Length and bodyweight were not statistically significantly affected by the treatments (ANOVA, Length F(4, 10) = 1.298; p = 0.3348; Body weight F(4, 10) = 1.180; p = 0.3768; Fig. S12 in Supporting info). Although, all fish appeared in good condition during the final sampling, without visual signs of being impacted by the treatment, the calculated condition index (K = 0.15 ± 0.01 for all mesocosms) was treatment-related (ANOVA, F(4, 10) = 3.972; p = 0.0350) with 13–15% lower condition indexes in the treatments 0.8 g/m$^2$ and above, than in the controls. These differences were statistically significant (Dunnett's multiple comparison test with p = 0.0478, 0.0237 and 0.0279 respectively; Fig. 5). As far as we are aware, this is the first time that the impact of microplastics on the condition of fish has been detected under semi-natural realistic exposure conditions.

During the final sampling, fish with ingested PS-beads were present in mesocosms with 0.8, 8.0, and 80 g/m$^2$ (Table S2 supplementary data). Other unnatural materials than the PS-beads were not found in any fish. At the highest exposure concentration, only 20% of the fish contained one or more PS-beads. This indicates a low ingestion frequency as the sediment core samples showed that fish in these mesocosms encountered 28 PS-beads per cm$^2$. Apparently, the sole did not mistake PS-beads for food, but ingestion must have happened by coincidence during foraging.

Visual inspection of the intestines also showed that ingested PS-beads did not accumulate inside the body as they were found in all parts of the digestive tract and were surrounded by natural food items. Among these natural food items were shells of juvenile cockles (*Cerastoderma edule*) or mud snails (*Peringia ulvae*) in sizes that sometimes

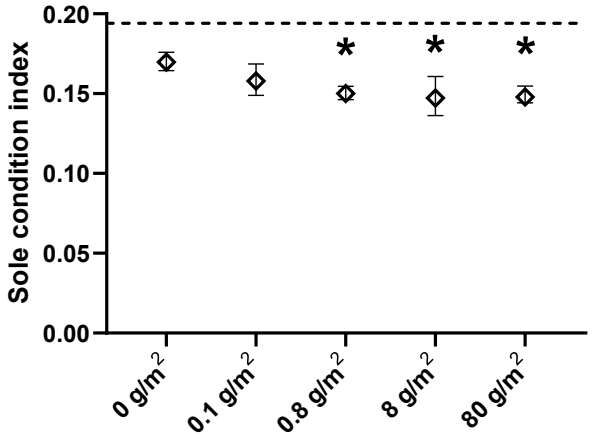


**Fig. 5.** Condition index (K = W*1000/$L^{2.796}$) of Common Sole (*Solea solea*) at the end of the study. Dotted horizontal lines indicate initial values at introduction in the mesocosms. '*' indicate significant ($p < 0.05$) differences from the controls (0 g/$m^2$).

exceeded 4 times the diameter of the PS-beads (Fig. S16 supplementary data), showing that these fish can cope with large non-digestible items. This could make sole, and probably most of the benthic feeding fish less vulnerable to the impact of ingested microplastics than pelagic fish that feed on more easily digestible zooplankton, per the observation that omnivore sea urchins are less sensitive to microplastic ingestion than herbivores (Suckling, 2021).

The presence of relatively large sized non-digestible items like microplastics and shell fragments in the intestine is likely to extend the passage time through the digestive tract and prolong the feeling of satisfaction after a meal, with reduced food intake consequently. This could explain the sole's slightly lower average condition index from the higher treatment levels where accidental ingestion must have happened more regularly. The natural diet of sole reflects prey availability and mainly comprises crustaceans, polychaetes, and mollusks (Molinero and Flos, 1992). Polychaetes are easily digestible, while processing a mollusk with a shell will take more time. Therefore, it seems logical that the impact of an occasionally ingested plastic bead will be more substantial if the diet of the fish consists solely of polychaetes. However, in the field situation, the diet of non-specialized fish will always be diverse. The use of easily digestible food in a laboratory test could however result in an overestimation of the impact of ingested microplastics compared to the field situation. As far as we know negative impact of ingested microplastics in fish has never been established in the field (de Vries et al., 2020; Foekema et al., 2013). Effects have been shown in laboratory tests, where in addition to what has already been said about food quality also questions can be raised about the realism of the exposure conditions. In such tests, the microplastics are often incorporated in food pellets (e.g. (Ahrendt et al., 2020) or fed together with the food (e.g. (Naidoo and Glassom, 2019). Both situations could result in unrealistic ingestion rates either because the food pellets contain much higher plastic concentrations than natural prey, or because microplastics are ingested during the feeding frenzy that occurs when a group of fish is fed once or twice a day, and makes the fish less critical in food selection. In future studies, it is advisable to consider the food choice as a potential effect-modifying factor when assessing the impact of plastic ingestion under artificial (laboratory) conditions. The impact of accidental intake of plastic particles might be less for fish with a diverse diet that naturally already contains more indigestible items.

Although the condition index of fish from the higher treatments was significantly lower than the controls, no statistically significant differences in weight (non-parametric two-tailed Mann-Whitney test, U = 1478; p = 0.3041), length (U = 1294; p = 0.0949), or condition index (U = 1300; p = 0.1001) were found between all fish without plastics (n = 277) and all fish with plastic ingested (n = 13). This shows that the presence of ingested beads in the fish during the sampling only formed a snapshot, since the beads did not accumulate in individual fish. It explains why it is not possible in field situations to relate plastic ingestion with fish's condition on an individual basis (de Vries et al., 2020; Foekema et al., 2013). Therefore, for a good assessment of the impact of microplastics on fish studies in experimental ecosystems (mesocosms) might be essential.

Our data shows that in a natural situation at least sole is capable to minimize intake of microplastics even at the extremely high exposure levels in our highest treatment. This might be true also for other marine fish species, since the occurrence of ingested microplastic particles in fish is low (Kuhn et al., 2020). Ingested micro-fibers are often reported in high numbers, but unless such studies have been performed with extreme high-quality control these data should be considered with great care (Foekema et al., 2013; Kuhn et al., 2020).

## 4. Conclusions and recommendations

In this mesocosm study, the lowest dosage that induced effects was 0.8 g/$m^2$ (3259 beads per $m^2$) and included statistically significant changes in sole's condition index, reduced barnacle densities and an increased total abundance in the benthic community. All these effects were that subtle that they most likely would be obscured by natural variation in a field situation. The study was performed to assess the impact of an actual PS-beads spill near the Dutch Wadden sea. The 11,250 kg of beads that were lost during that spill could result in a contamination of 0.8 g/$m^2$ (3259 beads per $m^2$) over an area of maximum 15 $km^2$ in absence of water currents. During an extended monitoring program following the spill no such hotspot was discovered, and only less than 10 beads per $m^2$ were detected in Wadden sea sediment. It was therefore concluded that the ecological impact of the spill could not be detectable, which was confirmed by field observations (Foekema et al., 2021a,b).

The results show that except for the barnacles, all organisms were able to cope with even the highest tested concentrations of the 700 μm PS-microbeads (325,900 beads per $m^2$, 196 beads/L) without experiencing substantial negative impact on survival, growth and condition. As indicated in section 2.2, this test concentration was more than 10 times higher than what has been reported as the global maximum values from field monitoring in the marine environment (Burns & Boxall, 2018). Of course the continuous exposure to stably high concentrations of microplastics as occurred in the mesocosms is not likely to occur in the natural marine environment due to continuous water mixing. From that perspective the mesocosms can be regarded a worst-case situation. It can be argued that exposure in the field, albeit to low concentrations, can last longer than 8 weeks. A longer test duration would not necessarily have led to more pronounced effects in our study, since most of the smaller invertebrates in the mesocosm already completed one or more live cycles during the study. For the fish it is likely that the impact of ingested particles becomes less with increasing body size/intestine diameter. Therefore it is not to be expected that a longer exposure would result in more pronounced effects.

Overall, the present research underlines that studies under semi-field conditions with realistic exposure scenarios are essential for meaningful risk assessment of microplastics in the marine environment taking both direct and indirect effects, like affected organic matter fluxes, into account. Of course the outcome cannot simply be translated to the greatly variable other types, compositions, sizes and shapes of microplastics. Therefore, we recommend future mesocosm studies with other types of microplastics, weathered microplastics from sea, and smaller sized particles down to nanoplastics.

## Credit author statement

Edwin M. Foekema: Conceptualization; Funding acquisition;

Investigation; Methodology; Formal analysis, Supervision; Writing - original draft; review & editing.Martijn Keur: Investigation; Writing review & editing, Liesbeth van der Vlies: Investigation; Writing review & editing, Babeth van der Weide: Investigation; Writing review & editing, Oliver Bittner: Investigation; Writing review & editing, AlberTinka J. Murk: Conceptualization; Writing review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wageningen Marine Research reports financial support was provided by Rijkswaterstaat Water Traffic and Living Environment.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120429.

## References

Ahrendt, C., Perez-Venegas, D.J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., Pulgar, J., Galbán-Malagón, C., 2020. Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. Mar. Pollut. Bull. 151.

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). Environ. Sci. Technol. 47, 593–600.

Boldrini, A., Galgani, L., Consumi, M., Loiselle, S.A., 2021. Microplastics contamination versus inorganic particles: effects on the dynamics of marine dissolved organic matter. Environments 8, 14.

Bucci, K., Tulio, M., Rochman, C.M., 2020. What is known and unknown about the effects of plastic pollution: a meta-analysis and systematic review. Ecol. Appl. 30.

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37, 2776–2796.

Campbell, P.J., Arnold, D.J.S., Brock, T.C.M., Grandy, N.J., Heger, W., Heimbach, F., Maund, S.J., Streloke, M. (Eds.), 1999. Guidance Document on Higher-Tier Aquatic Risk Assessment for Pesticides (HARAP). From the SETAC-Europe/OECD/EC Workshop. Lacanau Océan, France, SETAC-Europe, Brussels, Belgium.

Dauda, S., Gabriel, A.M., Idris, O.F., Chia, M.A., 2020. Combined nanoTiO(2)and nitrogen effects on phytoplankton: a mesocosm approach. J. Appl. Phycol. 32, 3123–3132.

De Jong, F.M.W., , B.T.C.M., Foekema, E.M., Leeuwangh, P., 2008. Guidance Document of the Dutch Platform for the Assessment of Higher Tier Studies; Guidance for Summarizing and Evaluating Aquatic Micro- and Mesocosm Studies. RIVM Report 601506009/2008A. RIVM, Bilthoven, Netherlands.

de Ruijter, V.N., Redondo-Hasselerharm, P.E., Gouin, T., Koelmans, A.A., 2020. Quality criteria for microplastic effect studies in the context of risk assessment: a critical review. Environ. Sci. Technol. 54, 11692–11705.

de Sa, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci. Total Environ. 645, 1029–1039.

de Vries, A.N., Govoni, D., Arnason, S.H., Carlsson, P., 2020. Microplastic ingestion by fish: body size, condition factor and gut fullness are not related to the amount of plastics consumed. Mar. Pollut. Bull. 151, 8.

Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in north sea fish. Environ. Sci. Technol. 47, 8818–8824.

Foekema, E.M., Kaag, N., Kramer, K.J.M., Long, K., 2015. Mesocosm validation of the marine No Effect Concentration of dissolved copper derived from a species sensitivity distribution. Sci. Total Environ. 521, 173–182.

Foekema, E.M., Tamis, J.E., Blanco, A., van der Weide, B., Sonneveld, C., Kleissen, F., van den Heuvel-greve, M.J., 2021a. Leaching of metals from steel slag and their ecological effects on a marine ecosystem: validating field data with mesocosm observations. Environ. Toxicol. Chem. 40, 2499–2509.

Foekema, E.M., van der Molen, J., Asjes, A., Bijleveld, A., Brasseur, S.M.J.M., Camphuysen, K., van Franeker, J.A., Holthuijsen, S., Kentie, R., Kühn, S., Leopold, M.F., Kleine Schaars, L., Lok, T., Niemann, H., Schop, J., 2021b. Ecologische effecten van het incident met de MSC Zoe op het Nederlandse Waddengebied, met focus op microplastics. NIOZ Rapport. No. 2021-03.

Galgani, L., Tsapakis, M., Pitta, P., Tsiola, A., Tzempelikou, E., Kalantzi, I., Esposito, C., Loiselle, A., Tsotskou, A., Zivanovic, S., Dafnomili, E., Diliberto, S., Mylona, K., Magiopoulos, I., Zeri, C., Pitta, E., Loiselle, S.A., 2019. Microplastics increase the marine production of particulate forms of organic matter. Environ. Res. Lett. 14, 11.

Gebhardt, C., Forster, S., 2018. Size-selective feeding of Arenicola marina promotes long-term burial of microplastic particles in marine sediments. Environ. Pollut. 242, 1777–1786.

Giddings, J.M., , B.T.C.M., Heger, W., Heimbach, F., Maund, S.J., Norman, S.M., Ratte, H. T., Schafers, C., Streloke, M., 2002. Community-Level Aquatic System Studies - Interpretation Criteria. Societey of Environmental Toxicology and Chemistry (SETAC), Pensacole, FL, USA.

Green, D.S., 2016. Effects of microplastics on European flat oysters, Ostrea edulis and their associated benthic communities. Environ. Pollut. 216, 95–103.

Green, D.S., Boots, B., Sigwart, J., Jiang, S., Rocha, C., 2016. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (Arenicola marina) and sediment nutrient cycling. Environ. Pollut. 208, 426–434.

Hall, C.A.M., Lewandowska, A.M., 2022. Zooplankton dominance shift in response to climate-driven salinity change: a mesocosm study. Front. Mar. Sci. 9.

Klok, C., Wijsman, J.W.M., Kaag, K., Foekema, E., 2014. Effects of CO2 enrichment on cockle shell growth interpreted with a Dynamic Energy Budget model. J. Sea Res. 94, 111–116.

Kuhn, S., van Franeker, J.A., O'Donoghue, A.M., Swiers, A., Starkenburg, M., van Werven, B., Foekema, E., Hermsen, E., Egelkraut-Holtus, M., Lindeboom, H., 2020. Details of plastic ingestion and fibre contamination in North Sea fishes. Environ. Pollut. 257.

Kunz, A., Walther, B.A., Lowemark, L., Lee, Y.C., 2016. Distribution and quantity of microplastic on sandy beaches along the northern coast of Taiwan. Marine Pollution Bulletin 111, 126–135.

Li, H.X., Getzinger, G.J., Ferguson, P.L., Orihuela, B., Zhu, M., Rittschof, D., 2016. Effects of toxic leachate from commercial plastics on larval survival and settlement of the barnacle amphibalanus amphitrite. Environ. Sci. Technol. 50, 924–931.

Lott, C., Eich, A., Unger, B., Makarow, D., Battagliarin, G., Schlegel, K., Lasut, M.T., Weber, M., 2020. Field and mesocosm methods to test biodegradable plastic film under marine conditions. PLoS One 15.

Michler-Kozma, D.N., Kruckenfellner, L., Heitkamp, A., Ebke, K.P., Gabel, F., 2022. Uptake and transfer of polyamide microplastics in a freshwater mesocosm study. Water 14.

Molinero, A., Flos, R., 1992. Influence of season on the feeding-habits of the common sole solea-solea. Mar. Biol. 113, 499–507.

Moreno, H.D., Koring, M., Di Pane, J., Tremblay, N., Wiltshire, K.H., Boersma, M., Meunier, C.L., 2022. An integrated multiple driver mesocosm experiment reveals the effect of global change on planktonic food web structure. Commun. Biol. 5.

Naidoo, T., Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. Mar. Pollut. Bull. 145, 254–259.

Nakki, P., Setala, O., Lehtiniemi, M., 2017. Bioturbation transports secondary microplastics to deeper layers in soft marine sediments of the northern Baltic Sea. Mar. Pollut. Bull. 119, 255–261.

Petersen, F., Hubbart, J.A., 2021. The occurrence and transport of microplastics: the state of the science. Sci. Total Environ. 758, 12.

Quade, J., Lopez-Ibanez, S., Beiras, R., 2022. Mesocosm trials reveal the potential toxic risk of degrading bioplastics to marine life. Mar. Pollut. Bull. 179.

Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E., Koelmans, A.A., 2018. Microplastic effect thresholds for freshwater benthic macroinvertebrates. Environ. Sci. Technol. 52, 2278–2286.

Reise, K., Baptist, M., Burbridge, P., Dankers, N., Fischer, L., Flemming, B., Oost, A.P., Smit, C., 2010. The Wadden sea – a universally outstanding tidal wetland. In: Wadden Sea Ecosystem No. 29.

Rijnsdorp, A.D., Vanbeek, F.A., Flatman, S., Millner, R.M., Riley, J.D., Giret, M., Declerck, R., 1992. Recruitment of sole stocks, *Solea-Solea (L)*, in the northeast atlantic. Neth. J. Sea Res. 29, 173–192.

Sendra, M., Sparaventi, E., Novoa, B., Figueras, A., 2021. An overview of the internalization and effects of microplastics and nanoplastics as pollutants of emerging concern in bivalves. Sci. Total Environ. 753, 15.

Setala, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Mar. Pollut. Bull. 102, 95–101.

Sharma, K.V., Sarvalingam, B.K., Marigoudar, S.R., 2021. A review of mesocosm experiments on heavy metals in marine environment and related issues of emerging concerns. Environ. Sci. Pollut. Control Ser. 28, 1304–1316.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Shim, W.J., 2015. Occurrence and Distribution of Microplastics in the Sea Surface Microlayer in Jinhae Bay, South Korea. Archives of Environmental Contamination and Toxicology 69, 279–287.
Strohmeier, T., Strand, O., Alunno-Bruscia, M., Duinker, A., Cranford, P.J., 2012. Variability in particle retention efficiency by the mussel Mytilus edulis. Journal of Experimental Marine Biology and Ecology 412, 96–102.
Suckling, C.C., 2021. Responses to environmentally relevant microplastics are species-specific with dietary habit as a potential sensitivity indicator. Sci. Total Environ. 751.
Wei, Y.Z., Plath, L., Penning, A., van der Linden, M., Murk, A.J., Foekema, E.M., 2019. The potential impact of underwater exhausted CO2 from innovative ships on invertebrate communities. Int. J. Environ. Res. 13, 669–678.
Yilcin, D., Yalcin, G., Jovanovic, B., Boukal, D.S., Vebrova, L., Riha, D., Stankovic, J., Zdrakovic, D.S., Metin, M., Akyurek, Y.N., Balkanl, D., Filiz, N., Milosevic, D., Feuchtmayr, H., Richardson, J.A., Beklioglu, M., 2022. Effects of a microplastic mixture differ across trophic levels and taxa in a freshwater food web: in situ mesocosm experiment. Sci. Total Environ. 836.
Yu, S.P., Chan, B.K.K., 2020a. Effects of polystyrene microplastics on larval development, settlement, and metamorphosis of the intertidal barnacle amphibalanus amphitrite. Ecotoxicol. Environ. Safety 194.
Yu, S.P., Chan, B.K.K., 2020b. Intergenerational microplastics impact the intertidal barnacle Amphibalanus amphitrite during the planktonic larval and benthic adult stages. Environ. Pollut. 267.
Zhou, Z.Q., Bouma, T.J., Fivash, G.S., Ysebaert, T., van Ijzerloo, L., van Dalen, J., van Dam, B., Walles, B., 2022. Thermal stress affects bioturbators' burrowing behavior: a mesocosm experiment on common cockles (Cerastoderma edule). Sci. Total Environ. 824.