**Microplastics affect the ecological functioning of an important biogenic habitat**

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**Abstract**

Biological effects of microplastics on the health of bivalves have been demonstrated elsewhere, but ecological impacts on the biodiversity and ecosystem functioning of bivalve-dominated habitats are unknown. Thus, we exposed intact sediment cores containing European flat oysters (*Ostrea edulis*) or blue mussels (*Mytilus edulis*) in seawater to two different densities (2.5 or 25 µg L-1) of biodegradable or conventional microplastics in outdoor mesocosms. We hypothesised that filtration rates of the bivalves, inorganic nitrogen cycling, primary productivity of sediment dwelling microphytobenthos, and the structure of invertebrate benthic assemblages would be influenced by microplastics. After 50 days, filtration by *M. edulis* was significantly less when exposed to 25 µg L-1 of either type of microplastics, but there were no effects on ecosystem functioning or the associated invertebrate assemblages. Contrastingly, filtration by *O. edulis* significantly increased when exposed to 2.5 or 25 µg L-1 of microplastics, and porewater ammonium and biomass of benthic cyanobacteria decreased. Additionally the associated infaunal invertebrate assemblages differed, with significantly less polychaetes and more oligochaetes in treatments exposed to microplastics. These findings highlight the potential of microplastics to impact the functioning and structure of sedimentary habitats and show that such effects may depend on the dominant bivalve present.

**Introduction**

Microplastics contaminate marine habitats across the globe1 and are recognised as a significant environmental challenge requiring urgent management2. It has recently been suggested that they are the most abundant form of solid waste on Earth1 and their abundance is increasing3. Although there is much uncertainty regarding the concentrations of microplastics in the environment, high concentrations in seawater of ~ 3-23 µg L-1 and even up to ~ 4500 µg L-1 have been reported4,5,6 in some heavily contaminated areas. Despite this prevalence, their effects on marine ecosystems are not well understood. Research to date has mostly focused on effects of microplastics on individual species, but effects on assemblages and ecosystem functioning within coastal habitats remain largely unknown7,8.

Previous research has concentrated on organisms that ingest microplastics directly, such as filter-feeders, including marine mussels9,10,11 and oysters12,13. These organisms are typically chosen for exposure experiments due to their great filtration capacity. For example, individual mussels and oysters can filter ~ 0.5-2.514 and ~ 5-2515 L of seawater h-1 respectively. As such, they are very likely to ingest microplastics16, and indeed, specimens from the field have been found to contain microplastics11,17,18. Exposure to relatively high densities of microplastics has been found to alter the respiration rates13, immunology10, reproductive capacity and filtration rates12 of bivalves. Owing to their role as ecosystem engineers, such effects are likely to permeate beyond the individual organism. For example, reefs created by mussels and oysters provide refugia and nursery grounds for other, commercially-important species and can support diverse communities19,20. In addition, filter feeding leads to benthic-pelagic coupling; channelling nutrients from the water column and locally concentrating them via biodeposition (i.e. deposition of faeces and pseudo-faeces). Mussels and oysters can, therefore, enhance the release of limiting inorganic nutrients, such as ammonium, from sediments, fuelling primary productivity of the microphytobenthos (such as diatoms and cyanobacteria) in the sediment, which in turn supports benthic and pelagic food webs21. If microplastics alter the ability of these organisms to filter feed, there may be wider impacts on their associated communities and on the functioning of coastal ecosystems. In addition, biodeposition is a likely mechanism by which suspended microplastics are transported from the pelagic zone onto sediments22. Mussels and oysters may, therefore, locally concentrate microplastics potentially altering biogeochemical processes, the biomass of primary producers and macrofaunal assemblages within the sediment.

In response to concerns of globally increasing plastic pollution, demand for biodegradable plastics has risen, with annual global production predicted to quadruple over the next five years23. It is thought that the replacement of conventional plastics, such as high density polyethylene (HDPE), with biodegradable alternatives, such as polylactic acid (PLA) will reduce the persistence, and therefore the impacts, of plastic pollution24. However, methods developed to assess the rate and extent of biodegradability of plastics in marine environments (e.g. ASTM International D7991-15)25 are still limited in their ability to predict degradation in natural habitats26. The potential for PLA and other bioplastics, or biodegradable plastics to persist as microscopic particles, or to affect assemblages of organisms in the marine environment before they degrade, remains largely unknown. Recently, however, Green, (2016)13 showed that PLA microplastics can lead to alterations in assemblage structure of macrofauna in sandy sediment with oysters.

In this study the effects of microplastics composed of HDPE or PLA, at two densities, on the structure and functioning of bivalve-dominated habitats were assessed using intact sediment cores in outdoor mesocosms, providing controlled, semi-natural conditions. Two experiments were conducted using two common, filter-feeding bivalves; blue mussels (*Mytilus edulis*) and European flat oysters (*Ostrea edulis*). The experiments tested the hypotheses that repeated exposure to biodegradable (PLA) and conventional (HDPE) microplastics in the water column would alter the: (i) filtration rates of the bivalves; (ii) concentration and fluxes of benthic inorganic nitrogen; (iii) biomass of benthic micro-algae; and (iv) diversity and abundance of macrofauna within sedimentary habitats associated with either species of bivalve.

**2. Methodology**

*2.1. Experimental design and set-up*

Two separate mesocosm experiments, one focusing on *M. edulis* and one on *O. edulis* habitats, were set up simultaneously at the outdoor flow-through mesocosm facility at Queen’s University Marine Laboratory, Portaferry, Northern Ireland. Both experiments had the same asymmetric design, with two fixed, orthogonal factors: “Plastic”, with two levels: polylactic acid (PLA) and high density polyethylene (HDPE) and “Dose”, with two levels: 2.5 µg L-1 and 25 µg L-1 seawater. A single treatment, without any added microplastics, was used as a control. To estimate the densities of microplastics in each treatment, water samples were taken from each “Plastic x Dose” treatment on days 1, 26 and 48 and microplastic particles were counted using a haemocytometer (Table S1).For each experiment, all treatments were replicated five times (n = 5, N = 25 per species) for a total of 50 mesocosms. Although the applied doses were relatively high compared with average densities observed and reported in the literature from ~330 µm plankton-net tow samples27, at smaller mesh sizes (50 µm) densities of up to 7800 particles L-1 (equating to ~4500 µg L-1) have been found in heavily contaminated coastal waters6. Furthermore, the densities used in the current study are among the lowest used experimentally to date28 and were chosen to approximately reflect high values currently (2.5 µg L-1) and in the future (25 µg L-1) based on the prediction that global plastic waste input will increase 10-fold by 202527.

The mesocosms were made using clean, opaque 10 L polypropylene buckets (height x diameter = 25 x 25 cm), placed onto large basins (as shown in Green 201613). Each mesocosm had an overflow pipe, allowing drainage directly into the basin. Waste water did not come into contact with other mescosms and each mesocosm was an independent replicate. In order to minimize disturbance to the sediment water interface, mesocosms were equipped with sampling ports, drilled at 0, 1 and 4 cm into the sediment (Figure S1). These ports were plugged until required for nutrient sampling (see section 2.3).

Each mesocosm was filled up to 4 cm depth with an intact core of muddy sediment, collected using a mesocosm with the bottom cut out, from an area (~25 x 25 m) of a nearby shore where *M. edulis* and *O. edulis* were abundant. Sand-filtered seawater, sourced directly from Strangford Lough (54°22’51.1”N; 5°33’04.0”W) was delivered via dedicated, individual hoses to each mesocosm at constant flow rates (~500 mL minute-1), giving an overlying water column of ~8 L and a daily turnover rate of 60 L day-1. The mesocosms were left to acclimatise for 48 h before live *M. edulis* or *O. edulis* were added. *M. edulis* and *O. edulis* were collected from the same shore as the mud and were measured, weighed and allocated randomly to treatments in order to ensure that no biases due to size were introduced into the experiments. The collected *M. edulis* had an initial average (± S.E.M.) wet biomass of 20.1 ± 1.7 g, maximal length of 47.9 ± 0.6 mm, width of 21.5 ± 0.4 mm and height of 23.5 ± 0.3 mm (n = 175). The collected *O. edulis* had an initial average (± S.E.M.) wet biomass of 36.0 ± 5.2 g, maximal length of 63.0 ± 1.6 mm, width of 60.1 ± 1.1 mm and height of 14.9 ± 0.6 mm (n = 50). Dimensions were measured with a calliper. On the 24th of August 2014, seven individuals of *M. edulis* (equivalent to individuals 142.6 m-2) were placed into 25 separate mesocosms and two individuals of *O. edulis* (equivalent to individuals 40.7 m-2) were placed into each of the other 25 mesocosms. These densities were chosen to reflect those high enough to be considered "*M. edulis* dominated" or "*O. edulis* dominated" habitats (i.e. > 30% cover and 5 individuals m-2 for *M. edulis* and *O. edulis* respectively, as defined by OSPAR29). The bivalves were placed on the surface of the sediment to mirror how they occurred locally in the field. There were no significant differences between the biomasses of individuals allocated to the different treatments at the start of the experiment (one-way ANOVA based on averaged dimensions in each mesocosm: *M. edulis*: F4,20 = 0.32, P = 0.861, *O. edulis*: F4,20 = 0.26, P = 0.902).The microplastic particles used in the experiment were of a similar colour (white) and size range, although their volume-weighted mean diameters differed: 65.6 µm (range = 0.6–363 µm) for PLA and 102.6 µm (range = 0.48–316 µm) for HDPE. In order to introduce microplastics into the mesocosms in a realistic manner, a dietary exposure method was used. In brief, microplastics were added to separate cultures (10 L) of the microalgae, *Isochrysis galbana* and left for 3 days with constant aeration. This was long enough for the microplastics to become more neutrally buoyant; i.e. move more freely within the culture containers rather than clinging to the sides or floating on top of the water. Fresh batches of control and microplastic dosed algae cultures were made up weekly. In order to ensure that the concentrations of *I. galbana* did not differ between treatments, algal cells were counted from each culture using a haemocytometer (on days 1, 26 and 48, Table S2). There were no significant differences in the density of *I. galbana* cells between treatments (one-way ANOVA for day 1: F4,20 = 0.21, P = 0.927, day 26: F4,20 = 0.08, P = 0.986 and day 48: F4,20 = 0.28, P = 0.891) and no aggregations of microalgae and microplastics were observed during the experiment. Cultures of *I. galbana* were prepared using seawater (35 psu), which was filtered with 0.45 μm aperture membranes and sterilised with UV light. Every day, each mesocosm received 250 mL of ~2 × 106 cells mL-1 of microalgae containing either 0 (control), 80 or 800 µg L-1 of PLA or HDPE microplastics, equating to final densities in the mesocosms of 2.5 µg L-1 or 25 µg L-1 (i.e. 250 mL diluted by the 8 L mesocosm volume).During feeding the flow of water was stopped for two hours and air bubblers were switched on in order to prevent anoxia and sedimentation of particulates. After this, the water flow in the mesocosms was resumed, replacing each mesocosm with clean seawater. The 2 hour daily exposure was chosen because in aquatic habitats, intermittent (as opposed to constant) exposure of contaminants is more likely to occur and, therefore, may be more environmentally relevant30,31. The experiment ran for 50 days, from the 26th of August until the 14th of October 2014. During this period the mean (± S.E.M) temperature of the water in the mesocosms was 15.4 ± 1.2.

*2.2. Filtration rates of* M. edulis *and* O. edulis

After 50 days, filtration rates were assessed by removing a single, randomly selected individual mussel or oyster from each mesocosm and holding them in separate 500 mL glass beakers with clean seawater each containing 4\*103 cells of *I. galabana* mL-1. Samples of 5 mL were taken after 0, 30, and 60 minutes and suspended algal cells were counted using a coulter counter. Tissue from each replicate was frozen at -20°C and later the dry biomass of each individual was determined by drying at 60°C for 24 h and weighing to the nearest µg to account for body mass. Filtration rates are expressed as the number of cells filtered mg-1 of dry biomass h-1.

*2.3. Porewater nutrients; ammonium, nitrate and nitrite*

Porewater samples were collected using RhizonTM membranes (Rhizosphere Research Products B.V., The Netherlands) inserted into the sampling ports of the mesocosms. This allowed water to be sampled at the surface (1 cm above the sediment), sediment-water interface (0 cm) and at 1 and 4 cm depths in the sediment. The flow of seawater into mesocosms was stopped and porewater was drawn by attaching a needle to each RhizonTM membrane collecting 10 mL of water directly into sterile vacuum tubes (BD Vacutainer®). Surface water was sampled a second and third time (at 30 minute intervals) to estimate nutrient fluxes. The water samples were stored in the vacuum tubes at 4°C prior to measuring concentrations of ammonium (NH4+), nitrate (NO3-) and nitrite (NO2-) using a Lachat Quick-Chem 8000 flow injection autoanalyser with Lachat methods 31-107-06-1-B (NH4+) and 31-107-04-1-A (NO2- and NO3- nitrate and nitrite). Porewater nutrient concentrations were adjusted for sediment porosity and standardised to dry bulk density. Pools of nutrients were calculated within the depth profile by integrating linear porewater concentration gradients, corrected for porosity, down to 4 cm depth. Concentrations of nitrate and nitrite were too minute (i.e. below the detection limit of ~0.01 mg L-1) to be measured with confidence and were omitted from further analysis.

*2.4. Microalgal biomass on sediment surface*

A benthic fluorometer (BenthoTorch, bbe-Moldaenke GmbH, Schwentinental, Germany32) was used to estimate the biomass of diatoms and cyanobacteria on the sediment surface. Measurements were taken after 48 days, before any disturbance caused by other sampling activties. The BenthoTorch was placed on the surface at three random locations and averaged to serve as a single replicate measurement per mesocosm. Measurements are expressed in µg biomass cm-2. Previous use of the BenthoTorch on similar sediment mesocosms found it to mirror the patterns of chlorophyll-a extraction using solvents33.

*2.5. Infaunal assemblages in the sediment*

Finally, all sediment was removed from each mesocosm and sieved separately through a 500 μm mesh to retain macrofauna, which were placed into containers and topped up with 5% formalin and later enumerated and identified in the laboratory using Hayward and Ryland (1995)34 as a key. Individuals were identified to species level where possible, and the number of taxa (R), the total number of individuals (N) and Shannon-Wiener diversity (H') (with *e* as the base) were calculated as alpha-diversity measurements.

*2.6. Statistical data analyses*

Statistical analysis was done using the R environment (R v3.2.3; R core team 2015). The data were screened for normality (q-q plots, and Shapiro-Wilk tests) and homogeneity of variance (Levene’s test, using the *car* (v2.1-2) package35) to ascertain assumptions for ANOVA. Transformation of some data was necessary to enable them to conform to these assumptions (specific transformations are stated in the results). Data were analysed separately for each of the two experiments (i.e. *M. edulis* and *O. edulis* were not compared in the statistical analyses). Since the design was asymmetrical (i.e. having a single control group for the two factors “Plastic” and “Dose”), the data were analysed by using the mean squares from two independent ANOVAs36 (see Green et al., 201633 for more details on calculations). Briefly, this included partitioning of the variance by calculating: (1) one-way ANOVA with all treatments as separate levels (a=5, n=5, N=25); and (2) a full-factorial two-way ANOVA of “Plastic” by “Dose” without the control (a=2, b=2, n=5, N=20). The residuals of the 1st ANOVA were used to assess differences between the levels within the 2nd ANOVA, allowing the variation associated with controls and that of the other treatments to be distinguished (“C vs. O”), which is contrasted with one degree of freedom36. When a significant effect in the “C vs. O” contrast was found Dunnett’s test was used to contrast the control versus each level of the significant term using the *multcomp* (v1.4-6) package37. Pairwise comparisons for the factors in ANOVA (2) were computed using Tukey HSD tests when the main terms were significant. Statistical significance was assumed at α = 0.05.

Differences in invertebrate assemblage structure among treatments were compared using a two-factor permutational ANOVA based on Bray-Curtis dissimilarities of square root transformed data with 9999 permutations under the reduced model using Type I sum of squares (SS) using PERMANOVA+ add-on (PRIMER-E Ltd. Plymouth, UK). The asymmetrical analyses were achieved by fitting each main effect (“Plastic” and “Dose”) in turn with a Type I (sequential) SS model, then swapping the order of the terms and combining the results of the two analyses38. When a factor was significant, contrasts were used to determine the specific differences. Results of the PERMANOVA were visualised with 2-dimensional ordination using canonical analysis of principal coordinates (CAP)39. Where assemblage structures differed, SIMPER analysis was used to quantify the contribution of different taxa to dissimilarities between treatments.

**3. Results**

*3.1. Effects of microplastics on the filtration rates of bivalves*

Two mussels died during the experiment and were removed. There were no oyster mortalities. When exposed to 25 µg L-1 of PLA or HDPE microplastics, *M. edulis* filtered ~2.4 times less microalgae (*I. galbana*) per hour than when exposed to none of the experimental microplastics (Figure 1a, Table S3, Dunnett's *Control vs 25**µg L-1*: t=2.42, P=0.045). There was no effect of 2.5 µg L-1 of either type of microplastic on the filtration of *M. edulis*. On the contrary, *O. edulis* in the control mesocosms filtered ~7.5 times less microalgae than those in mesocosms with any type or density of microplastic (Figure 1b, Table S3, Dunnett's *Control vs 25 µg L-1*: t=-3.09, P=0.011, *Control vs 2.5 µg L-1*: t=-2.74, P=0.024, *Control vs PLA*: t=-2.51, P=0.038, *Control vs HDPE*: t=-2.74, P=0.024) compared to when not exposed to microplastics.

*3.2. Effects of microplastics on ammonium in sediment porewater*

Concentrations of ammonium increased with depth in the sediment in all mesocosms (Figure 2). Sediment with *M. edulis* had no significantly different ammonium pools and ammonium flux from the surface and was not significantly different between microplastic treatments (Table 1 and S4). Sediment with *O. edulis*, however, contained ~1.8 times more ammonium when no experimental microplastics were present compared with those dosed with either type of microplastic at both densities (Table S4, Dunnett's *Control vs PLA*: t=2.63, P = 0.030; *Control vs HDPE*: t=2.94, P=0.015). In addition, ammonium fluxes from the sediment into the water column were significantly different in mesocosms with *O. edulis* dosed with microplastics than in controls (Tables 1 and S4), however, *post-hoc* tests were unable to determine further significant differences.

*3.3. Effects of microplastics on the microphytobenthos*

The biomass of diatoms was not significantly different between the microplastic treatments for sediments with *M. edulis* or *O. edulis* (Table S4). The biomass of cyanobacteria, however, was significantly less in sediments which contained microplastics with *O. edulis* (Table S4) (but not those with *M. edulis*, Figure 3a), and was ~2 times greater in the controls than in mesocosms dosed with either type or density of microplastics (Figure 3b, Dunnett's *Control vs 25 µg L-1 PLA*: t=4.77, P<0.001; *Control vs 2.5 µg L-1 PLA*: t=3.91, P=0.003; *Control vs 25 µg L-1 HDPE*: t=4.31, P=0.001; *Control vs 2.5 µg L-1HDPE*: t=3.05, P=0.022).

*3.4. Effects of microplastics on infaunal assemblages*

There were no significant differences between the structure of infaunal invertebrate assemblages (Figure 4a, Table S5), the diversity indices (Figure 5a, Table S6) nor the abundance of individual taxa (Table S6) in sediments with *M. edulis*. Sediments with *O. edulis*, however, had significantly different assemblage structures in treatments dosed with microplastics, at any density or type of plastic compared to controls (Figure 4b, Table S5) and there were several differences in dominance (Table S7). Although species richness and total abundance did not differ significantly (Table S6), the Shannon-Wiener index (H') was ~2 times greater in controls than in mesocosms dosed with 25 µg L-1 of HDPE microplastics (Figure 5b, Table S6, Dunnett's *Control vs 25 µg L-1 HDPE*: t=0.14, P=0.004). There was a ~3 times greater abundance of *Eteone picta* polychaetes present in sediments not dosed with experimental microplastics than in treatments that received microplastics of either type (Figure 6a, Table S6, Dunnett's *Control vs 25 µg L-1 PLA*: t=3.53, P=0.008; *Control vs 2.5 µg L-1 PLA*: t=3.99, P=0.002; *Control vs 25 µg L-1 HDPE*: t=4.27, P=0.001; *Control vs 2.5 µg L-1HDPE*: t=4.83, P<0.001). On the contrary, sediments in the controls had ~1.9 times fewer *Tubificoides benedii* oligochaetes than those dosed with 25 µg L-1 of either type of microplastic (Figure 6b, Table S6, Dunnett's *Control vs 25 µg L-1*: t=-3.27, P=0.007). There were also ~2.6 times more *Lineus longissimus* nemerteans in sediments when exposed to 25 µg L-1 of PLA than in those exposed to 2.5 µg L-1 of PLA or no microplastics (Figure 6c, Table S6, Tukey's HSD *2.5 µg L-1 PLA vs 25 µg L-1 PLA*: P=0.026, Dunnett's *Control vs 25 µg L-1 PLA*: t=-2.66, P=0.049).

**4. Discussion**

*Mytulis edulis* and *Ostrea edulis* responded differently to contamination with microplastics. The blue mussels filtered fewer algal cells h-1 when exposed to 25 µg L-1 of PLA or HDPE microplastics. This supports findings of Wegner et al. (2012)40 who found decreasing filtration rates with increasing concentrations (constant exposure of 0.1 - 0.3 g) of polystyrene nanoplastics (30 nm), but is in contrast with Browne et al., (2008)9 which found no effect of constant exposure of 0.51 g of 3.0 or 9.6 µm polystyrene microbeads on the filtration rates of *M. edulis* after 48 days. On the contrary, *O. edulis* exposed for 2 hours per day to 2.5 or 25 μg L‑1 of PLA or HDPE microplastics filtered more algae h-1 than when exposed to no microplastics. This is similar to another recent experiment, which found an increase in filtration rates of another species of oyster, *Crassostrea gigas*, in response to constant exposure of 23 μg L‑1 of 6 μm polystyrene microplastics12. From this selection of studies, albeit small, there is a pattern emerging suggesting a trend that mussels filter less and oysters filter more in response to plastic particles. More research is needed, however, to determine whether responses of filtration rates are generally applicable for bivalves in response to microplastics across different environmental contexts and with different polymer types.

Overall, the net filtration rates (corrected for dry weight of animal tissue) were greater for *M. edulis* than for *O. edulis*. Others have also found greater filtration rates (corrected for weight) in mussels than in oysters41,42. This could be because the microalgal concentrations in the filtration measurements were at 4000 cells mL‑1 and *M. edulis* reaches optimum filtration rates at 2000 and 6000 cells43 mL-1, whilst *O. edulis* requires concentrations an order of magnitude greater than this for optimum filtration rates to be reached44. Different bivalves may use different strategies when coping with an increase in particles (which would have occurred with the addition of microplastics). For example, under increased microalgae concentrations, mussels often decrease their filtration rates in order to maintain a constant consumption rate, whilst oysters increase theirs, along with their production of pseudofaeces45.  Due to their importance in benthic-pelagic coupling, such alterations to filtration rates could lead to cascading effects on nutrient cycling and primary productivity in sedimentary habitats. In the current study, this occurred in the oyster-dominated mesocosms.

The pool and flux of ammonium was less in the sediment pore-water with *O. edulis* exposed to microplastics. Although more research is required to ascertain the mechanisms to account for this result, it is possible that microbially-mediated processes which control the production (ammonification) and reduction (nitrification and denitrification) of ammonium were altered by the microplastics. Likely in response to there being less ammonium in the porewater, there was also less biomass of cyanobacteria. In a similar outdoor mesocosm experiment, PLA, HDPE or PVC microplastics (2% of wet sediment weight) directly added to sandy sediment led to reductions in the biomass of benthic diatoms, but not of cyanobacteria33. This difference could be due to the grain size of the sediment. Cyanobacteria are less able to build stable microbial mats on fine (muddy) sediment than they are on coarse (sandy) sediment, whilst diatoms are stable on muddy sediment46. Nano- or micro- plastics have also been found to reduce the productivity of other primary producers. For example, Besseling et al. (2014)47 found that nanoplastics reduced growth of green algae and overall chlorophyll concentrations in laboratory microcosm experiments, and in another study Bhattacharya et al. (2010)48 reported a reduction of photosynthesis by microalgae. Cyanobacteria are key primary producers in sedimentary systems49, vital in food-web dynamics50,51. Together with euglenids and diatoms, they can supply up to 45% of the organic budget of an estuary14 and are important for stabilising sediments52. Decreases in the biomass of primary producers (including cyanobacteria) could, therefore, induce cascading impacts on biodiversity and ecosystem services53.

In the oyster-dominated mesocosms, perhaps in response to the decrease in cyanobacteria, invertebrate assemblage structure was different in all treatments exposed to microplastics compared with controls. Although species richness and the total abundance of infauna were not affected by microplastics, assemblages in *O. edulis* treatments exposed to 25 µg L-1 of HDPE had lower Shannon-Weiner diversity indices, indicating that assemblages were more homogeneous compared with controls. These differences were mostly caused by a greater dominance of oligochaetes, *Tubificoides benedii*, in all treatments with microplastics (which contributed ~30% of the difference between controls and each microplastic treatment, Table S7). Oligochaetes typically respond opportunistically to stressors and have long been considered as indicators of pollution in marine54 and freshwater55 systems. Mesocosms dosed with 25 µg L-1 of PLA or HDPE microplastics also had less *E. picta* (paddle worms). A reduction in the abundance of paddle worms, which are often specialist predators, has been found in response to other stressors, such as nutrient enrichment, in sedimentary systems56. Additionally, the abundance of *Lineus longissimus* (bootlace worms) was greater in treatments with 25 µg L-1 PLA microplastics compared to those with 2.5 µg L-1 or no microplastics. These worms are also a potential indicator species of pollution57. The dominance of opportunistic species, suggests a simplification of the food web in response to high levels of microplastic contamination.

Interestingly, there were no measurable effects of microplastic exposure on infaunal invertebrate assemblages in the sediments with *M. edulis* mussels. This may be due to the different effects of microplastics on filtration activity of the bivalves. For example, the increase in filtration rates of *O. edulis* were likely accompanied by an increase in the production of pseudofaeces. Particles rejected by bivalves as pseudofaeces are embedded in mucous and sink58, possibly increasing the availability of microplastics to the *O. edulis* benthic communities. It is also possible that greater habitat complexity, due to the presence of more shells in the mussel experiment, mitigated any effects of microplastics on ecosystem functioning or on assemblages compared to in the oyster experiment. Habitat structure can influence movement and resource utilisation of organisms and can alter the direct and indirect interactions between species59. In order to fully understand the role of different ecosystem engineers in mediating or exacerbating the effect of microplastics, experiments comparing community level effects with and without ecosystem engineers present are needed. Regardless of the mechanisms, this study shows that microplastics may affect ecosystem functioning and biodiversity but, as has been found for other pollutants60, such effects are context-dependent.

Manipulation of microplastics in field conditions would be difficult and would pollute, therefore, the use of an outdoor mesocosm system, with natural seawater and weather conditions and recruitment of meio- and micro-organisms, provided an ideal compromise between the highly controlled conditions of a laboratory experiment and the realism of a field experiment. The extrapolation of results from any mesocosm experiment should, however, proceed with caution since the assemblages represented in the mesocosms are simplified, compared to the field. For example, pelagic larval recruitment61 and other complex processes involving larger organisms (such as fish), are excluded from the cores62. Regardless, semi-field experiments, such as those using intact cores are a useful technique for evaluating the effects of stressors on infaunal communities63,64,65 and they have been found to produce ecologically relevant data66,67. A similar mesocosm experiment using intact cores and *Ostrea edulis* (in vegetated, sandy rather than muddy sediment) also found alterations to assemblage structure and a reduction in diversity, specifically with less isopods, amphipods and periwinkle snails after 60 days of exposure to 80 µg L-1 of HDPE or PLA microplastics13. Together these two pioneering studies indicate that microplastics could alter benthic assemblages in *O. edulis*-dominated sedimentary habitats if they are repeatedly exposed (even for just 2 hours per day) to concentrations as high as 2.5, 25 or 80 µg L-1. Globally, oyster populations are under threat, and due to overfishing, parasites and disease68, 85% of oyster reefs have been lost world-wide69. Declines in oyster reefs are cause for concern, not only because they provide protein sources and support the fishing industry, but also because of their role in the provision of ecosystem services in the coastal zone70. The current study suggests that microplastics may represent an additional pressure to the organisms living in these already threatened habitats.

*Wider implications and recommendations*

Since microplastics composed of PLA did not rapidly decompose, they caused many of the same impacts as HDPE to *M. edulis* and *O. edulis* and the sediment they inhabit. This, combined with evidence from other studies13,33,71, supports recommendations that the term "biodegradable" should be redefined to ensure that material, such as PLA, that apparently does not rapidly and fully degrade in aquatic habitats does not enter drainage systems as microscale particles such as microbeads72 or enter the environment as larger litter.

Alterations to invertebrate assemblages in oyster-dominated sediment was detected at just 2.5 µg L-1, although this dose is high, it is conservative compared to other recent experimental studies e.g. 1250 - 2500073 μg L-1 and 200 - 480022 μg L-1. It is also much lower than current levels found in some heavily contaminated coasts, for example, ~4500 µg L-1 in Korea6. Current plankton net sampling techniques, however, typically have a lower size limit of ~300 µm, therefore underestimating current densities of microplastics, possibly by between 3 and 6 orders of magnitude when compared to a 10 µm mesh74. Also, given that the cumulative input of plastic waste is expected to increase in the coming decades27 and that the fragmentation of macroplastic litter already present in the environment will continue, concentrations of microplastics are expected to increase75. In the current study, effects on ammonium concentrations and biomass of cyanobacteria occurred at just 2.5 µg L-1. Wider effects of microplastics on nutrient cycling and invertebrate assemblages could, therefore, already be occurring in heavily contaminated oyster-dominated habitats, however more research, including mensurative studies, are needed to ascertain this.

The current study provides ecologically relevant data on the effects of contamination by microplastic of different polymers, focusing on assemblage-level effects and ecosystem functioning. Such data is currently still rare in the literature, but is vital in order to inform policy and prevent damage to ecosystems7. In order to fully assess the ecological impacts of microplastics, however, we also need to test their effects at low concentrations and using realistic mixtures of polymer types (as opposed to one type at a time).

**Figures**

Fig1.TIF

**Figure 1.** Filtration rates of (a) *M. edulis* and (b) *O. edulis* in mesocosms with 2.5 µg L-1 or 25 µg L-1 of PLA or HDPE or with no microplastics (Control) after 50 days. Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data, bars are means (± S.E.M.) with n = 5.

NH4mus+oys.TIF

**Figure 2.** Concentrations ofNH4+ in mesocosms with (a) *M. edulis* or (b) *O. edulis* of surface water (1 cm), the sediment-water interface (0 cm), and 1 & 4 cm into the sediment in mesocosms with 2.5 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend BM.TIF) or 25 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend BH.TIF) of PLA or 2.5 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend HM.TIF) or 25 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend HH.TIF) of HDPE microplastics or no microplastics (Control = C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend C.TIF) after 50 days. Data are means (± S.E.M.) with n = 5.

Fig3.TIF

**Figure 3.** Biomass ofcyanobacteria in mesocosms with (a) *M. edulis* or (b) *O. edulis* and 2.5 µg L-1 or 25 µg L-1 of PLA or HDPE or with no microplastics (Control) after 48 days. Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data and bars are means (± S.E.M.) with n = 5.

Figure4.TIF

**Figure 4.** Canonical analysis of principal coordinates of square root transformed community structure data in mesocosms with (a) *M. edulis* or (b) *O. edilus* and 2.5 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend BM.TIF) or 25 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend BH.TIF)of PLA or 2.5 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend HM.TIF) or 25 µg L-1 (C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend HH.TIF) of HDPE microplastics or with no microplastics (control = C:\Users\Dannielle\Documents\Work\Postdoc_IRC 2013\Portaferry experiments\Mud experiment\Paper files\legend C.TIF) after 50 days, n = 5.

Fig5.TIF

**Figure 5.** Shannon Wiener diversity index inmesocosms with (a) mussels or (b) oysters and 2.5 µg L-1 or 25 µg L-1 of PLA or HDPE or with no microplastics (control) after 50 days Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data, and bars are mean (± S.E.M.) with n = 5.

Fig6.TIF

**Figure 6.** Abundances of (a) *E. picta*, (b) *T. benedii* and (c) *L. longissimus* in oyster treatments with 2.5 µg L-1 or 25 µg L-1 of PLA or HDPE or with no microplastics (control) after 50 days. Data from *M. edulis* mesocosms are not shown. Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data, and bars are mean (± S.E.M.) with n = 5.

**Table 1.** Porewater ammonium pool (µmol dm-3) and flux (µmol h-1) in mesocosm sediment after 50 days with *M. edulis* or *O. edulis* and no microplastics (control), or the two doses of microplastics. Different superscript letters indicate significance between treatments. Data are means (± S.E.M.) with n = 5.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | *M. edulis* |  | *O. edulis* |  |
|  |  | NH4+ pool | NH4+ flux | NH4+ pool | NH4+ flux |
| **Control** | **0 ug L-1** | 436.89 ± 77.63a | 0.14 ± 3.99a | 669.23 ± 80.57a | -57.18 ± 38.61a |
| **PLA** | **2.5 ug L-1** | 293.68 ± 33.29a | -19.68 ± 13.98a | 359.52 ± 108.41b | -19.88 ± 4.52a |
|  | **25 ug L-1** | 493.00 ± 52.43a | -25.58 ± 9.96a | 325.83 ± 54.25b | 2.34 ± 2.54a |
| **HDPE** | **2.5 ug L-1** | 326.25 ± 79.16a | -3.28 ± 8.25a | 322.85 ± 69.08b | -8.76 ± 2.38a |
|  | **25 ug L-1** | 351.19 ± 24.21a | -14.34 ± 6.56a | 450.64 ± 94.07b | -10.42 ± 9.08a |

ASSOCIATED CONTENT

File name “Supporting Material.pdf” containing 10 pages (cover page included), containing 1 figure and 7 tables.

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**Author Contributions**

D.S.G. conceived the idea and designed the experiment. D.S.G. and B.B. carried out the field and laboratory work and analysed the data. All authors contributed to writing the manuscript and all authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interests.

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**References**

1. Eriksen, M.; Lebreton, L. C. M.; Carson, H. S.; Thiel, M.; Moore, C. J.; Borerro, J. C.; Galgani, F.; Ryan, P. G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE* **2014**, *9* (12).

2. UNEP, GRID-Arendal. Marine Litter Vital Graphics. United Nations Environment Programme and GRID-Arendal. Nairobi and Arendal. *www.unep.org* 2016.

3. Law, K. L.; Thompson, R. C. Microplastics in the seas. *Science* **2014**, *345* (6193), 144-145.

4. Reisser, J.; Slat, B.; Noble, K.; du Plessis, K.; Epp, M.; Proietti, M.; de Sonneville, J.; Becker, T.; Pattiaratchi, C. The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. *Biogeosciences* **2015**, *12* (4), 1249-1256.

5. Collignon, A.; Hecq, J. H.; Glagani, F.; Voisin, P.; Collard, F.; Goffart, A. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. *Mar. pollut. bull.* **2012**, *64* (4), 861-864.

6. Kang, J. H.; Kwon, O. Y.; Shim, W. J. Potential Threat of Microplastics to Zooplanktivores in the Surface Waters of the Southern Sea of Korea. *Arch. Environ. Con. Tox.* **2015**, *69* (3), 340-351.

7. Rochman, C. M. Ecologically relevant data are policy-relevant data. *Science* **2016**, *352* (6290), 1172-1172.

8. Browne, M. A.; Underwood, A. J.; Chapman, M. G.; Williams, R.; Thompson, R. C.; van Franeker, J. A. Linking effects of anthropogenic debris to ecological impacts. *P. Roy. Soc. Lond. B Bio.***2015**, *282* (1807).

9. Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol.* **2008**, *42* (13), 5026-5031.

10. von Moos, N.; Burkhardt-Holm, P.; Koehler, A. Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. *Environ. Sci. Technol.* **2012**, *46* (20), 11327-11335.

11. Van Cauwenberghe, L.; Claessens, M.; Vandegehuchte, M. B.; Janssen, C. R. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environ. Pollut.* **2015**, *199*, 10-17.

12. Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M. E. J.; Le Goïc, N.; Quillien, V.; Mingant, C.; Epelboin, Y.; Corporeau, C.; Guyomarch, J.; Robbens, J.; Paul-Pont, I.; Soudant, P.; Huvet, A. Oyster reproduction is affected by exposure to polystyrene microplastics. *P. Natl. Acad. Sci. USA* **2016**, *113* (9), 2430-2435.

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

14. Asmus, H.; Asmus, R. M.; Prins, T. C.; Dankers, N.; Frances, G.; Maass, B.; Reise, K. Benthic-pelagic flux rates on mussel beds - tunnel and tidal flume methodology compared. *Helgolander Meeresun.* **1992**, *46* (3), 341-361.

15. Korringa, P. Recent Advances in Oyster Biology. *Q. Rev. Bio.* **1952**, *27* (3), 266-308.

16. Setala, O.; Norkko, J.; Lehtiniemi, M. Feeding type affects microplastic ingestion in a coastal invertebrate community. *Mar. pollut. bull.* **2016**, *102* (1), 95-101.

17. Li, J.; Yang, D.; Li, L.; Jabeen, K.; Shi, H. Microplastics in commercial bivalves from China. *Environ. Pollut.* **2015,** *207*, 190-195.

18. Rochman, C. M.; Tahir, A.; Williams, S. L.; Baxa, D. V.; Lam, R.; Miller, J. T.; Teh, F. C.; Werorilangi, S.; Teh, S. J. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci. Rep.* **2015,** *5*, 14340.

19. O'Connor, N. E.; Crowe, T. P. Biodiversity among mussels: separating the influence of sizes of mussels from the ages of patches. *J. Mar. Biol. Assoc. UK* **2007**, *87* (2), 551-557.

20. Grabowski, J. H.; Brumbaugh, R. D.; Conrad, R. F.; Keeler, A. G.; Opaluch, J. J.; Peterson, C. H.; Piehler, M. F.; Powers, S. P.; Smyth, A. R. Economic Valuation of Ecosystem Services Provided by Oyster Reefs. *Bioscience* **2012**, *62* (10), 900-909.

21. Barbier, E. B.; Hacker, S. D.; Kennedy, C.; Koch, E. W.; Stier, A. C.; Silliman, B. R. The value of estuarine and coastal ecosystem services. *Ecol. Monogr.* **2011**, *81* (2), 169-193.

22. Cole, M.; Lindeque, P. K.; Fileman, E.; Clark, J.; Lewis, C.; Halsband, C.; Galloway, T. S. Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets. *Environ. Sci. Technol.* **2016**, *50* (6), 3239-3246.

23. World Economic Forum;The New Plastics Economy - Rethinking the future of plastics. *http://www.ellenmacarthurfoundation.org/publications* 2016.

24. Song, J. H.; Murphy, R. J.; Narayan, R.; Davies, G. B. H. Biodegradable and compostable alternatives to conventional plastics. *P. Roy. Soc. Lond. B Bio.* **2009**, *364* (1526), 2127-2139.

25. ASTM D7991-15, Standard Test Method for Determining Aerobic Biodegradation of Plastics Buried in Sandy Marine Sediment under Controlled Laboratory Conditions, ASTM International, West Conshohocken, PA, **2015**, www.astm.org

26. Nauendorf, A.; Krause, S.; Bigalke, N. K.; Gorb, E. V.; Gorb, S. N.; Haeckel, M.; Wahl, M.; Treude, T. Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. *Mar. pollut. bull.* **2016**, *103* (1-2), 168-78.

27. Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347* (6223), 768-771.

28. Lenz, R.; Enders, K.; Nielsen, T. G. Microplastic exposure studies should be environmentally realistic. *P. Natl. Acad. Sci. USA* **2016,** *113* (29), E4121-E4122.

29. OSPAR Commission website. http://www.ospar.org/work-areas/bdc/species-habitats/mapping-habitats-on-the-ospar-list-of-threatened-or-declining-species-and-habitats.

30. McCahon, C. P.; Pascoe, D. .Episodic pollution: causes, toxicological effects and ecological significance. *Funct. Ecol.* **1990**, *4*,375–383.

31. Handy, R. D. Intermittent exposure to aquatic pollutants: assessment, toxicity

and sublethal responses in fish and invertebrate. *Comp. Biochem. Physiol. C.* **1994**, *107*,171–184.

32. Aberle, N.; Moldaenke, M. B. C.; Wiltshire, K. H. 'Spectral fingerprinting' for specific algal groups on sediments in situ: A new sensor. *Archiv fur Hydrobiologie* **2006**, *167* (1-4), 575-592.

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

34. Hayward, P. J.; Ryland, J. S. *Handbook of the Marine Fauna of North-West Europe*. Oxford University Press: Oxford, U.K., 1995.

35. Fox, J.; Weisberg, S. *An Companion to Applied Regression, Second Edition*. Sage, 2011; http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

36. Underwood, A. *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, U.K., 1997.

37. Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous Inference in General Parametric Models. *Biometrical J.* **2008**, *50* (3), 346-363.

38. Anderson, M. J.; Clarke, R. K.; Permanova+ for Primer: Guide to Software and Statistical Methods PRIMER-E. *Plymouth* 2008.

39. Anderson, M. J.; Willis, T. J., Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* **2003**, *84* (2), 511-525.

40. Wegner, A.; Besseling, E.; Foekema, E. M.; Kamermans, P.; Koelmans, A. A. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). *Environ. Toxicol. Chem.* **2012**, *31* (11), 2490-2497.

41. Pernet, F.; Tremblay, R.; Comeau, L.; Guderley, H. Temperature adaptation in two bivalve species from different thermal habitats: energetics and remodelling of membrane lipids. *J. Exp. Biol.* **2007**, *210* (17), 2999-3014.

42. Mafra Jr, L. L.; Bricelj, V. M.; Fennel, K. Domoic acid uptake and elimination kinetics in oysters and mussels in relation to body size and anatomical distribution of toxin. *Aquat. Toxicol.* **2010**, *100* (1), 17-29.

43. Riisgard, H. U.; Saavedra, I. B.; Feeding Behaviour of the Mussel, *Mytilus edulis*: New Observations, with a Minireview of Current Knowledge. *J.Mar. Biol.* **2011**, *doi:10.1155/2011/312459*.

44. Wilson, J. Retention efficiency and pumping rate of *Ostrea edulis* in suspensions of Isochrysis galbana. *Mar. Ecol. Prog. Ser.* **1983**, *12*, 51-58.

45. Barnes, P. *Shellfish Culture and Particulate Matter Production and Cycling: A Literature Review.* BC Aquaculture Research & Development Committee AE 02.03-02.01, 2006; https://www2.viu.ca/csr/documents/BarnesLiteratureReviewCSR.pdf.

46. Watermann, F.; Hillebrand, H.; Gerdes, G.; Krumbein, W. E.; Sommer, U. Competition between benthic cyanobacteria and diatoms as influenced by different grain sizes and temperatures. *Mar. Ecol. Prog. Ser.* **1999**, *187*, 77-87.

47. Besseling, E.; Wang, B.; Lürling, M.; Koelmans, A. A. Nanoplastic Affects Growth of *S. obliquus* and Reproduction of *D. magna*. *Environ. Sci. Technol.* **2014**, *48* (20), 12336-12343.

48. Bhattacharya, P.; Lin, S.; Turner, J. P.; Ke, P. C., Physical Adsorption of Charged Plastic Nanoparticles Affects Algal Photosynthesis. *J. Phys. Chem. C.* **2010**, *114* (39), 16556-16561.

49. Currin, C. A.; Levin, L. A.; Talley, T. S.; Michener, R.; Talley, D. The role of cyanobacteria in Southern California salt marsh food webs. *Mar. Ecol.* **2011**, *32* (3), 346-363.

50. MacIntyre, H. L.; Geider, R. J.; Miller, D. C. Microphytobenthos: The ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* **1996**, *19* (2), 186-201.

51. Yamamuro, M. Importance of epiphytic cyanobacteria as food sources for heterotrophs in a tropical seagrass bed. *Coral Reefs* **1999**, *18* (3), 263-271.

52. Stal, L. J. Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. *Ecol. Eng.* **2010**, *36* (2), 236-245.

53. Díez, B.; Ininbergs, K. Ecological importance of cyanobacteria, in Cyanobacteria: An Economic Perspective. In *Cyanobacteria;* Sharma, N. K.; Rai, A. K.; Stal, L. J. Eds.; John Wiley & Sons: Chichester, U.K. 2014.

54. Barnett, B. E. Oligochaetes as indicators of pollution in the Humber estuary, with special reference to *Tubificoides benedeni*. *Environ. Pollut. A.* **1983**, *30* (4), 277-291.

55. Brinkhurst, R. O.; Kennedy, C. R. Studies on the biology of the Tubificidae (annelida, oligochaeta) in a polluted stream. *J. Anim. Ecol.* **1965**, *34* (2), 429-443.

56. O'Gorman, E. J.; Fitch, J. E.; Crowe, T. P. Multiple anthropogenic stressors and the structural properties of food webs. *Ecology* **2012**, *93* (3), 441-448.

57. McEvoy, E. G.; Sundberg, P. Patterns of trace metal accumulation in Swedish marine nemerteans. *Hydrobiologia* **1993**, *266* (1), 273-280.

58. Kiorboe, T.; Mohlenberg, F. Particle selection in suspension-feeding bivalves. *Mar. Ecol. Prog. Ser.* **1981**, *5 (3), 291-296*.

59. Goodsell, P. J.; Connell, S. D. Complexity in the relationship between matrix composition and inter-patch distance in fragmented habitats. *Mar. Biol.* **2008**, *154* (1), 117-125.

60. Mayer-Pinto, M.; Matias, M. G.; Coleman, R. A. The interplay between habitat structure and chemical contaminants on biotic responses of benthic organisms. *PeerJ* **2016**, *4*, e1985.

61. Cowie, R. P.; Widdicombe, S.; Austen, C. M. Effects of physical disturbance on an estuarine intertidal community: field and mesocosm results compared. *Mar. Biol.* **2000**, *136* (3), 485-495.

62. Underwood, A. J., Peterson, C. H. Towards an ecological framework for investigating pollution. *Mar. Ecol. Prog. Ser.* **1988**, *46*, 227-234.

63. Fletcher, R.; Reynoldson, T. B.; Taylor, W. D. The use of benthic mesocosms for the assessment of sediment contamination. *Environ. Pollut.* **2001**, *115* (2), 173-182.

64. Fletcher, R.; Reynoldson, T. B.; Taylor, W. D. Maintenance of intact sediment box cores as laboratory mesocosms. *Environ. Pollut.* **2001**, *115* (2), 183-189.

65. Srivastava, D. S.; Kolasa, J.; Bengtsson, J.; Gonzalez, A.; Lawler, S. P.; Miller, T. E.; Munguia, P.; Romanuk, T.; Schneider, D. C.; Trzcinski, M. K. Are natural microcosms useful model systems for ecology? *Trends Ecol. Evol.* **2004**, *19* (7), 379-384.

66. Bayne, B. L.; Addison, R. F.; Capuzzo, J. M.; Clarke, K. R.; Gray, J. S.; Moore, M. N.; Warwick, R. M. An overview of the GEEP workshop. *Mar. Ecol. Prog. Ser.* **1988**, *46* (1-3), 235-243.

67. Karle, I. M.; Agrenius, S.; Molander, S.; Magnusson, K.; Blanck, H.; Dahl, B.; Hall, P.; Dahllof, I. Verification of a benthic boxcosm system with potential for extrapolating experimental results to the field*. J. Exp. Mar. Biol. Ecol.* **2007**, *353*(2), 265-278.

68. Kamphausen, L.; Jensen, A.; Hawkins, L. Unusually High Proportion of Males in a Collapsing Population of Commercially Fished Oysters (*Ostrea edulis*) in the Solent, United Kingdom. *J. Shellfish Res.* **2011**, *30* (2), 217-222.

69. Beck, M. W.; Brumbaugh, R. D.; Airoldi, L.; Carranza, A.; Coen, L. D.; Crawford, C.; Defeo, O.; Edgar, G. J.; Hancock, B.; Kay, M. C.; Lenihan, H. S.; Luckenbach, M. W.; Toropova, C. L.; Zhang, G. F.; Guo, X. M. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *Bioscience* **2011**, *61* (2), 107-116.

70. Loren, D. C.; Robert, D. B.; David, B.; Ray, G.; Mark, W. L.; Martin, H. P.; Sean, P. P.; Tolley, S. G., Ecosystem services related to oyster restoration. *Mar. Ecol. Prog. Ser.* **2007**, *341*, 303-307.

71. Green, D. S.; Boots, B.; Blockley, D. J.; Rocha, C.; Thompson, R. Impacts of Discarded Plastic Bags on Marine Assemblages and Ecosystem Functioning. *Environ. Sci. Technol.* **2015**, *49* (9), 5380-5389.

72. Rochman, C. M.; Kross, S. M.; Armstrong, J. B.; Bogan, M. T.; Darling, E. S.; Green, S. J.; Smyth, A. R.; Veríssimo, D. Scientific Evidence Supports a Ban on Microbeads. *Environ. Sci. Technol.* **2015**, *49* (18), 10759-10761.

73. Lee, K. W.; Shim, W. J.; Kwon, O. Y.; Kang, J. H. Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod *Tigriopus japonicus*. *Environ. Sci. Technol.* **2013**, *47* (19), 11278-11283.

74. Norén, F.; Norén, K.; Magnusson, K. 2014. Marint mikroplastiskt skräp: undersökning längs svenska västkusten 2013 & 2014. Länsstyrelsen I Västra Götalands län, vattenvårdsenheten.

75. Duis, K.; Coors, A. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Env. Sci. Eur.* **2016**, *28* (1), 1-25.