Mussels facilitate the sinking of microplastics to bottom sediments and their subsequent uptake by detritus-feeders.
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Abstract

Microplastics (MP) are omnipresent contaminants in the oceans, however little is known about the MP transfer between marine compartments and species. Three connected laboratory experiments using the filter-feeding mussel *Mytilus galloprovincialis* and the omnivorous polychaete *Hediste diversicolor* were conducted. As results, mussels significantly removed MP from the water column by incorporating them into biodeposits. This effect was particularly evident for the smallest MP fraction (=41 μm) whose deposition from the water column to the bottom was enhanced by the action of mussels. The incorporation of MP into faecal pellets increased the particles’ sinking velocity by about 3-4 orders of magnitude. Conversely, the MP presence significantly decreased the depositional velocities of faecal pellets. The MP incorporation into mussels' biodeposits also more than doubled the amount of MP uptaken by *H. diversicolor*. These findings allowed the conclusion that detrital pathways could be a transfer route of MP across marine compartments and food webs, also affecting the distribution of MP in the sediments.
**Key words:** Microplastics; Transfer; Vertical flux; Mussels; Biodeposits; Food webs

**Capsule:** Mussels’ biodeposits facilitate MP transfer from the water column to benthic detritus-feeding polychaetes.

1. Introduction

Although no consensus exists on the definition of MP (Frias and Nash 2019), microplastics are commonly identified as plastic particles below 5 mm in size (hereafter MP) (Arthur et al., 2009) including: i) those particles industrially manufactured in very small sizes (Primary MP), and ii) those that originate from the physical, biological or chemical degradation (e.g. ultraviolet radiation, wind or water erosion, microorganisms) of larger pieces of plastic waste released into the environment (Secondary MP) (Carbery et al., 2018). MP are omnipresent and pervasive contaminants, which have been recorded in a variety of aquatic systems (Ivleva et al., 2017). Despite the global interest on this topic there is limited understanding about the distributions of MP in the environment, their sources and sinks, the physical and biological transport mechanisms, and the associated hazards (Katija et al., 2017).

Research has reported the ingestion of MP by aquatic biota across multiple levels of the trophic chain (Ivleva et al., 2017), and its direct trophic transfer from prey to predators (Chagnon et al., 2018; Farrell and Nelson, 2013; Nelms et al., 2018), with also a potential risk of accumulation of associated chemical contaminants across the food web (Carbery et al., 2018; Diepens and Koelmans, 2018; Teuten et al., 2009). The average number of MP found in each organism, however, is usually low (< 1 MP/organism), particularly in invertebrate species (Carbery et al., 2018; Desforges et al., 2015; Devriese et al., 2015; Neves et al., 2015; Piarulli et al., 2020; Van Cauwenberghe and Janssen, 2014). This low MP content could be related to little retention in organisms due to egestion (Le Guen et
Therefore, direct MP trophic transfer via predator-prey interactions could be relatively limited compared to the so far largely underestimated alternative pathway via organic-rich marine detritus accumulated at the seafloor (Clark et al., 2016; Au et al., 2017).

Biogenic aggregates such as marine snow, phytodetritus and faecal matter are ubiquitous and abundant in the marine system (Turner, 2015) and act as a biological pump for the vertical transport of carbon and nutrients from the water surface to the seafloor (Fowler and Knauer, 1986; Turner and Holmes, 2011). The incorporation of MP in biogenic aggregates was first proposed by Moore (2008) and Teuten et al. (2009). More recently Porter et al. (2018) hypothesised that biogenic aggregation could increase the sinking rates of buoyant MP, and enhance their bioavailability to benthic organisms. At the same time, MP could also significantly alter the sinking rates of biogenic aggregates (Cole et al., 2016) affecting the transfer of particulate organic matter (POM) from the water column to the seafloor (Wieczorek et al., 2019). The ecological relevance and generality of these processes, as well as the role played by specific physical-chemical properties of the MP (e.g. size and density) is yet to be empirically explored. Further, most work has focused on planktonic species, while the relevance of biogenic aggregates produced by benthic species has been largely ignored.

In the present study, we explored the role of filter-feeding benthic bivalves in transferring MP from the water column to the sediments and facilitating the subsequent uptake by benthic detritivores. Bivalves have high filtration rates, and capture natural and anthropogenic particles of a wide range of sizes, but have also developed selection mechanisms to eliminate those particles not constituting an energy source (Galimany et al., 2011; Ward and Shumway, 2004). As such they largely contribute to the flux of organic matter from the water surface to the benthic compartment through their continuous...
conversion of seston into faeces and pseudofaeces supporting a biodeposition-based food web (Zúñiga et al., 2014).

We conducted laboratory experiments mimicking as much as possible real environmental condition and using as model organisms the filter-feeding mussel *Mytilus galloprovincialis* and the omnivorous polychaete *Hediste diversicolor*. *M. galloprovincialis* is a dominant component of the marine benthos, endemic to and widely distributed in the Mediterranean Sea (Barsotti and Meluzzi, 1968). It is very common in harbours and open waters, and often a dominant inhabitant on intertidal and shallow subtidal hard substrates, both natural and artificial (Bacchiocchi and Airoldi, 2003). It is also largely cultured for human consumption, using specialized floating rafts, buoys and lines, which serve as recruitment surface for juveniles (Carl et al., 2012). Previous exposure studies on the congeneric blue mussel *M. edulis* showed that nano (1 - 100 nm) or very small (1 - 5 μm) MP can be metabolized, and in some cases also accumulated, at tissue and cell levels (Browne et al., 2008; Von Moos et al., 2012). Conversely, larger MP particles (≥ 6 μm) are often rapidly (ranging from minutes to few hours) excreted via faeces and pseudofaeces (Fernández and Albentosa, 2019; Gonçalves et al., 2019). This fast uptake/egestion of MP can facilitate the transfer of MP to the seafloor and to the associated benthic detritus-feeders.

*H. diversicolor* is one of the most common polychaetes in the northern hemisphere sedimentary environments and is often used as indicator species for a variety of contaminants (Giangrande et al., 2005). This species has been described as carnivore and/or scavenger and detritus-feeder (Riisgird, 1989). Its multiple and various feeding strategies make *H. diversicolor* a key player in structuring soft-bottom communities (Rönn et al., 1988), and a potential recipient of MP through various trophic pathways. Studies have shown that mussel biodeposits constitute a high-quality food source for this polychaetes, with potential application in integrated aquaculture (Jansen et al., 2019) for
mitigation of adverse effects on benthic environments in connection with mussel-farming (Bergström et al., 2019). Furthermore, polychaetes are prey items to a variety of other organisms representing a potential transfer route of MP and associated chemical contaminants to higher trophic levels.

The objectives of this study were: i) to evaluate whether mussels can affect the vertical transport, sinking rate and accumulation of MP onto the seafloor through their incorporation in faeces and pseudofaeces (cumulatively referred in the text as biodeposits); ii) to compare whether the mussel-mediated transfer varies in relation to the size and chemical composition of MP; (iii) to test whether the MP incorporation into biodeposits produced by mussels can increase their subsequent ingestion by *H. diversicolor*, thereby facilitating their transfer across the food web though detrital pathways. We hypothesised that such mechanisms could have a greater magnitude of effect on small sized and/or low density MP compared to large sized and/or high density MP, as reported from planktonic species (Cole et al., 2016; Porter et al., 2018; Wieczorek et al., 2019).

2. Materials and methods

2.1 Specimens collection and preparation

All the experiments were conducted during June and July 2018. Before the start of each experiment, specimens of the mussel *M. galloprovincialis* were collected from the artificial jetties, seawalls or floating pontoons at the marina in Ravenna, Italy (44°29'32.6"N, 12°17'15.2"E, see Airoldi et al. (2016) for a detailed description of the sampling site). The mussels were collected with a stainless steel wall-scraper and transported to the laboratory in a cool container within 30 minutes. Individuals 4-7 cm in size (shell length mean ± SE: 5.6 ± 0.1 cm) were selected to mimic as much as possible natural adult mussels populations that can be find in the field. Each mussel was then, scrubbed and
rinsed to remove any epibiota and possible adhering MP. The mussels were depurated for 24 hours in aerated aquaria filled with artificial seawater without adding any source of food. This was done to allow the mussels to egest any of previously ingested MP and to adapt to experimental conditions (Van Cauwenberghe et al., 2015; Ward et al., 2019).

To isolate the active effects of live mussels on the MP vertical transfer from any potential physical influence of the shells (Kolandhasamy et al., 2018) that may affect the sinking of MP as free particles empty mussel shells were used as controls. These were collected from an intertidal area in close proximity to the mussels’ sampling site. The shells were carefully rinsed with Milli-Q water and scrubbed to remove any external MP, following which valves were partially joined together using professional water-proof glue, creating the physical shape of live mussels.

Unlike mussels, it was difficult to unequivocally identify and collect in the field enough *H. diversicolor* polychaetes of similar size and ontogenetic state. Therefore, we bought commercial specimens of *H. diversicolor* from an aquaculture facility in Venice lagoon, Italy (LESCACHEPESCA SRL, http://www.lescachepesca.eu). The polychaetes were transferred to the laboratory and were kept in depuration for 24 hours (Hentschel, 1998; Van Cauwenberghe et al., 2015) in aerated aquaria with filtered seawater to remove any potential contamination from previously ingested or externally attached MP. The seawater used in all the experiments was collected from the marina in Ravenna and filtered through nylon filters (20 μm mesh, Ø: 47 mm, PLASTOK®) to remove any MP or natural particulate matter > 20 μm.

### 2.2 Effects of mussels on MP vertical transfer

The contribution of mussels to the vertical transfer of MP of different sizes through biodeposition processes was tested by using an experimental set up consisting of 16 rectangular aquaria (5 L vol; 200 x 200 x 400 mm), randomly assigned (n= 4, Figure 1) to
an orthogonal treatment combination of mussels vs. empty shells and exposure to MP\textsubscript{SMALL} (41 μm) vs MP\textsubscript{LARGE} (129 μm). This set-up allowed to compare the vertical fluxes of MP in the presence of live mussels (which could incorporate MP into their biodeposits) to the deposition of sinking free particles in the absence of filtering mussels.

Aquaria were maintained at a constant temperature of 25°C, and portable aerators were used to aerate the water and generate a steady mixing effect. We used separate groups of either 10 individuals of \textit{M. galloprovincialis} or 10 ‘double-valve’ control shells, suspended with a net 15 cm below the water surface in mussels or control aquaria, respectively (Figure 1). This set up mimicked realistic environmental conditions of the Mediterranean sea, where \textit{M. galloprovincialis} growths naturally on a variety of shallow floating substrates or is farmed using floating structures (Zúñiga et al., 2014).

For the MP treatments commercially available polyamide (PA) MP fragments (Environmental Tracing Systems, UK) (Table S1 in Supplementary Information) were used. The MP dimensional range was chosen to favour the egestion of the particles by mussels (Ward et al., 2019) and to use a size detectable range in field samples. The two synthetic particles’ sizes were easily recognisable as they were of different colours (pink for MP\textsubscript{SMALL} and white for MP\textsubscript{LARGE}) and fluorescence (red fluorescence for MP\textsubscript{SMALL} and blue fluorescence for MP\textsubscript{LARGE}). Fragments were used, as opposed to the commonly used spheres, due to the higher prevalence of this MP morphology in the marine environment (Suaria et al., 2016). PA was chosen as polymer because it is slightly denser than the seawater (1.15 g cm\textsuperscript{-3}), thus less susceptible to resuspension after deposition than lower density polymers but not so dense as to immediately fall to the bottom, being therefore available for filtration by mussels. Before use, each MP amount to be added to the aquaria was individually preconditioned into 50 ml falcon tubes containing pre-filtered seawater and incubated for 48 h at room temperature (20°C) with a natural light regime to encourage a natural biofilm formation as described by Wieczorek et al. (2019). The biofilm
formation can affect the MP superficial physical-chemical properties of MP facilitating their ingestion and sink due to enhanced density (Rummel et al., 2017), therefore the biofilm formation was encouraged to mimic as much as possible the environmental MP conditions and dynamics.

The tubes were manually shaken just prior to addition to the respective treatment aquaria to resuspend all MP particles. A mass concentration of 0.2 mg L⁻¹ (corresponding to 1 mg of MP per aquarium) was used for both size classes of MP, corresponding to ~600 MP L⁻¹ for the small class, and ~300 MP L⁻¹ for the large class. These concentrations are in the range of those reported from highly polluted coastal waters (Collignon et al., 2012; Doyle et al., 2011; Goldstein and Goodwin, 2013; Lattin et al., 2004; Moore et al., 2001).

After 48 h of exposure to MP, the aquaria aeration was stopped, the mussels or control empty shells were removed, and suspended and deposited MP were quantified. The aquarium water (supernatant) was slowly pumped out with a portable pump for liquids (D-mail®, IT), filtered (20 μm mesh; Ø: 9 cm; PLASTOK) using vacuum filtration, and the filter used for determining the residual suspended MP fraction. The final 10 mm of water, with any sediment material including biodeposits, was filtered separately to quantify the deposited MP fraction. The removed alive mussels were immediately inspected at the microscope to evaluate the potential adherence of MP on the shells, frozen at -20°C, and a random subsample of 5 individuals from each aquarium (n=8 aquaria with alive mussels, n=40 total number of mussels) was processed to quantify the MP potentially retained in the soft tissue after ingestion. Each mussel was washed with Milli-Q water to remove any external particle, dissected to separate the soft tissue from the shell and weighed (wet weigh) before being digested enzymatically in 100 ml glass beakers, following the protocol described by Piarulli et al. (2019). Briefly, after 24 h incubation in 10 ml of 25 % SDS (SIGMA – ALDRICH®) at 50 °C, 5 mL of lipase and 5 ml of protease/amylase (Biozym F and Biozym SE respectively; Spinnrad®, Bad Segeberg, Germany) were added. All
samples were gently and manually shaken, incubated at room temperature for 96 h, followed by vacuum filtration (20 μm mesh, Ø: 9 cm, PLASTOK®).

The filters of each MP fraction (retained in mussels tissue, suspended in the supernatant, and deposited) were positioned in covered glass petri dishes and dried at room temperature in a glass dryer for 1 week. MP were easily recognised and quantified due to their distinctive colour and fluorescence. MP counts of the dried filters were firstly performed under a Stereomicroscope (WILD M8, Leica microsystems) at 50X magnification, and subsequently validated with also a UV light source (λ 365 nm) (Montserrat et al., 2009) placed to the same microscope in the dark. MP counts were then expressed in distinct units based on the nature of their respective fraction: the ingested as MP ind⁻¹, the suspended as MP L⁻¹ and the deposited as MP cm⁻².

The effect of mussels on the amount of MP in the deposited and suspended fractions was tested separately for the two MP sizes, due to the different initial particle concentrations for the small and large size classes. A Student’s t-test was used after checking for normality and homogeneity with Shapiro–Wilk and Bartlett’s tests respectively and log(x+1) data transformation if required to meet the assumption for parametric statistics (Table S3 in Supplementary Information). Statistical analyses were performed with R studio (v. 0.99.903, R Core Team, 2016), and significance was set at p-value < 0.05. Data were reported as mean ± standard error (SE).

2.3 Effects of MP incorporation on sinking rates of mussels’ faecal pellets

We tested how the incorporation of MP (of different sizes and densities) into faecal pellets (hereafter FP) affects the sinking rates of FP through the water column. Three different polymer types and sizes of MP were used: 41 μm PA fragments (PA_SMALL), 129 μm PA fragments (PA_LARGE), and polypropylene (PP) fragments of a median gran size of 127 μm (Table S1, Supplementary Information), where PP has a lower density (0.92 g cm⁻³)
compared to PA. The PP fragments were produced by milling PP pellets (Goodfellow Cambridge Ltd. UK) with a pin mill (Alpine C160, Messer group GmbH, DE) into irregular fragments that were subsequently sieved with decreasing mesh size (from 500 to 100).

Mussels’ FP incorporating different MP types (FP with 1 MP were selected) were obtained by using 3 groups of 10 mussels in 3 separate aquaria. Each group was exposed to contamination from only one type of the 3 tested MP (concentration 0.2 mg L⁻¹), as described in section 2.2. A fourth set of mussels was maintained in filtered seawater in a separate aquarium without MP to produce MP-uncontaminated FP. After 48 h of exposure, FP were removed individually using a Pasteur pipet, and inspected under a stereomicroscope microscope (WILD M8, Leica microsystems) to check for their integrity and for the presence of incorporated MP. MP used in the experiment were recognised due to their distinctive colour and/or fluorescence. Twenty integer FP were selected for each of the 4 treatments (PA_{SMALL}, PA_{LARGE}, PP and no MP). FP were photographed using a mounted camera (Motic BTWB, USA) and images were analysed using imageJ software (Schneider et al., 2012) to determine dimensions and to calculate the equivalent cylindrical volumes and density (Table S2 in Supplementary Information) of each faecal pellet using the Stoke’s law modified for use with cylindrical shapes (Komar et al., 1981).

The sinking rates of the 4 FP treatments were assessed adapting the established method originally developed by Smayda et al. (1969) to measure the sinking rates of zooplankton FP. The FP were individually transferred with a Pasteur pipet to a 2 L measuring cylinder filled with 20 μm filtered seawater (25 °C, 35 psu). FP were let to sink for the first 10 cm to achieve a constant velocity, and then their descent speed was measured over the remaining 10 cm.

Differences in the sinking rates of the 4 groups of FP were tested using one-way ANOVA (n=20 replicated FP per treatment). The data were log (x+1) transformed to meet the criteria for parametric statistics checked with Shapiro and Bartlett’s tests (Table S4 in
Supplementary Information). Tukey post-hoc test was used for pairwise comparison of the 4 groups.

The measured sinking rates of the MP incorporated into the FP were further compared with their theoretical sinking rates when free in the water column. Free MP were too small to enable the visual measurement of their sinking rates, therefore their velocity was estimated by using Stoke’s law as described by Bach et al. (2012) and Porter et al. (2018), taking into account the physical properties (sizes and densities, Table S1 in Supplementary Information) of the three different MP types (PA\textsubscript{SMALL}, PA\textsubscript{LARGE}, and PP) as well as the water temperature and salinity.

2.4 Transfer of MP from mussels to polychaetes

To examine whether MP enriched biodeposits produced by mussels can facilitate the uptake of MP by detritivorous polychaetes, a modified version of the previous experimental set-up (described in section 2.2) was used (Figure 2). Eight aquaria containing 5 L of filtered seawater and a 20 mm bottom layer of sand were maintained at 25 °C. Four of the aquaria contained groups of 10 \textit{M. galloprovincialis} and the other four contained groups of 10 control empty shells. Based on observations from the previous experiments regarding size selectivity of MP by mussels, MP\textsubscript{SMALL} fragments (41 μm; conc. 0.2 mg L\textsuperscript{-1}) were chosen as the size class, and prepared in the same manner as described in session 2.2 to establish a biofilm formation prior to use.

Following 48 hours of MP exposure, all mussels and control shell groups were removed and replaced by groups of \textit{H. diversicolor} (n=5 per group). After 24 hours, the polychaetes were transferred to aerated 500 ml beakers filled with 0.2 μm pre-filtered artificial seawater for 8 hours to allow the elimination of ingested material, based on the results by Bock and Miller (1999) the depuration time of polychaetes was kept shorter (8 h) than 12 h to avoid the re-ingestion of the previously egested MP. The MP retention in polychaetes soft tissue
has been estimated to be about 0.5 % (Van Cauwenberghe et al., 2015), therefore the MP egested by polychaetes was considered the most representative indirect measure of the MP uptake (Löder and Gerdts, 2015).

Once depurated, polychaetes were removed from the beakers and the depuration water was filtered (mesh size: 20 μm, Ø: 9 cm, PLASTOK®). The filters were dried at room temperature in covered glass petri dishes placed in a glass dryer, and inspected for MP presence under the stereomicroscope. Each group of polychaetes was considered as a single population unit; thereby MP data were expressed as total number of particles per aquarium. Differences in the amount of uptaken (and egested) MP by polychaetes in the presence or absence of mussels and their biodeposits were compared using a t-test (Table S7 in Supplementary Information). Before the analysis, data were checked for normality and homogeneity. No data transformation was needed.

3. Results

3.1 Effects of mussels on MP vertical transfer

After 48 h, the MP_{SMALL} in live mussel treatments showed a significant (t-test p-value=0.006, Table S3 in Supplementary Information) increase (about 15%) in deposited MP compared to control treatments (Figure 3c, Table S4 in Supplementary Information), while no significant (t-test p-value=0.92, Table S3 in Supplementary Information) differences were detected for MP_{LARGE} (Figure 3d Table S4 in Supplementary Information). Suspended MP (Figure 3a and b) was significantly (t-test p-values=0.03 and 0.0004, Table S3 in Supplementary Information) less at the end of the exposure time in live mussel treatments than in controls, for both MP sizes (Table S4 in Supplementary Information).

Occurrence of MP in mussels’ soft tissue was observed only in 15 % (2 individuals with 1 MP and 1 with 3 MP) and 20 % (4 individuals with 1 MP each) of the analysed mussels exposed to MP_{SMALL} and MP_{LARGE}, respectively (Table S4 in Supplementary Information).
No MP were found adhering on the shells on alive mussels or control treatments.

3.2 Effects of MP incorporation in mussels’ faecal pellets

Incorporation in mussels’ FP (Figure 4a) accelerated MP sinking rates by over 3 orders of magnitude, compared to the calculated theoretical sinking rates for free MP particles in water, irrespective of MP sizes and/or polymer type (Tables S1 and S2 in Supplementary Information). Averaged (± 1SE) sinking rates of FP contaminated with PASMALL, PALARGE and PP were 422 ±61, 393 ±34 and 352 ±35 m day⁻¹, respectively, while theoretical sinking rates of the associated reference free particles were 0.003, 0.03 and 0.03 m day⁻¹, respectively.

All FP contaminated by MP exhibited significantly (pairwise Tukey’s post-hoc test p-values < 0.0001) lower sinking rates compared to uncontaminated FP (Tables S6 and S7 in Supplementary Information). This effect was more pronounced with increasing size and decreasing density of the MP (Figure 4b). However, those differences were quantitatively limited and not statistically significant (Table S7 in Supplementary Information).

3.3 Transfer of MP from mussels to polychaetes

After 24 hours, polychaetes exposed to biodeposits in the live mussel treatments ingested significantly more MP (40 %, t-test p-value=0.03, Table S8 in Supplementary Information) than those exposed to free sinking MP in the empty shell controls. On average, the population of polychaetes exposed to the live mussel treatments ingested 57 ±9 MP per unit, while those from the control treatments ingested 24 ±6 MP per unit (Figure 5).

4. Discussion

Approximately half of the MP introduced to the marine environment is buoyant, and tends to stay in suspension in the water column (Kooi et al., 2016). This study showed that
mussels facilitated the deposition of MP (particularly for MP_{SMALL}) from the water column to the bottom by incorporating the MP into biodeposits that resulted in enhanced sinking velocity with respect to free MP. Further, detritus-feeding polychaetes exposed to MP in biodeposits ingested more MP compared to polychaetes exposed to free sinking particles. The mussel-mediated vertical transfer was evident in both the supernatant and at the bottom for MP_{SMALL} (41 μm), which were 15 % more abundant at the bottom of treatments containing live mussels compared to those with control empty shells. This is consistent with the known particle size selection range for mussels, which tends to be more effective for particles smaller than 50 μm (Defossez and Hawkins, 1997; Ward and Shumway, 2004). The effect of mussels on the MP vertical transfer was also partially observed for MP_{LARGE} (129 μm), but only at the level of the supernatant, while at the bottom the quantities of MP_{LARGE} were similar between units with alive mussel and controls with empty shells, therefore suggesting that mussels have a sequestrating effect also on MP_{LARGE} but this effect is too small and to be clearly quantified at the bottom. This is ascribable to two main reasons: i) MP_{LARGE} tend to sink fast as free particles due to their large weight ii) the particles’ capture efficiency by mussels decreases with increasing particles’ size (Ward and Shumway, 2004; Ward et al., 2019).

Further, the selection of particles by bivalves, however, does not only depend on the size but also on the shape and chemical composition. Indeed, Ward et al., (2019) showed that fibres larger that 500 μm can also be ingested, therefore, it is likely that mussels could also affect the vertical transfer of MP_{LARGE} of different shapes than those tested in this study.

Ultimately, most MP, including low density polymers, are likely to reach the seafloor (Van Cauwenberghe et al., 2015), as demonstrated by the high levels of MP contamination recorded in sediments (Lorenz et al., 2019; Vianello et al., 2013). However, biogenic aggregation in biodeposits by mussels or other bivalves present as stable population in coastal environments or extensively farmed could result in fine-scale heterogeneity of MP
distribution in marine sediments, creating concentrated hot-spots of small and highly bioavailable MP. To our knowledge so far this possible biologically-mediated small-scale heterogeneity in MP distribution at the sediment level has never been explored. If proved, it would have important implications both for the ecology of benthic systems and for the management of MP in the sediments. Consistently with the findings by Porter et al. (2018), the measurements of MP sinking rates showed that all the MP incorporated into FP sank 3 - 4 orders of magnitude faster than the same MP as free particles. Although our sinking rates calculations were made in a static experimental system, thereby not fully representing natural conditions, the magnitude of the differences suggests that also under environmental conditions, where oceanographic attenuation (e.g. resuspension) as well as other factors that can alter the MP density (e.g. biofouling) may occur, MP incorporation into FP would decidedly change their fate in the water column. In turn, the incorporation of MP significantly decreased the sinking rates of FP irrespective of polymer type and/or particle size, similarly to what observed by Long et al. (2015) who estimated the effects of MP presence in phytoplankton aggregates. Given the central role that bivalves’ faecal matter has in mediating the flux of nutrients and carbon cycling across benthic and pelagic systems (Hewitt et al., 2001; Robert et al., 2013), the observed decelerated sinking rates of FP caused by the presence of MP is of concern. There is a potential for the lowering of nutrient regeneration and availability in the benthic compartment, with unknown consequences at the community and ecosystem levels. Wieczorek et al. (2019) also observed a longer persistency in the water column of MP-contaminated FP from salps and suggested that MP presence could lower the efficiency of the biologic pump, enhancing water turbidity due to the fragmentation of faecal matter by bacterial activity, with the consequent release of CO₂ directly into the water column. These effects deserve particular attention as bivalve aquaculture is increasing worldwide
The experiment focusing on the subsequent transfer of MP to detritus-feeders, showed that the incorporation into biodeposits significantly enhanced MP uptake by polychaetes, indicating that MP incorporation into biodeposits can be a pathway for the transfer of MP between species with different feeding modes and placed at different trophic levels. *H. diversicolor*, known for its adaptable feeding mode, including detritivory, ingested more than twice of MP when exposed to mussels' biodeposits containing egested MP. This difference does not simply reflect a larger availability of MP in the bottom sediments due to the accelerated sinking but also a qualitative effect. Indeed, the first experiment showed that mussels increased MP abundance by only 15% compared to controls, while polychaetes ingested 41% more MP in the presence of mussels. Faeces and pseudofaeces are nutritious (Johannes and Satomi, 1966) and readily consumed by a variety of suspension- and deposit-feeding organisms (Tenore, 1988), thereby aggregation in biodeposits makes MP more bioavailable, facilitating their transfer to consumers. It is likely that ingestion and egestion could also structurally and/or chemically change the MP, for example via fragmentation up to the nanoscale (Dawson et al., 2018) or modification of the biofilm community (Kesy et al., 2016), making the MP more suitable for ingestion.

It has been observed that nanoplastics (particularly those between 40 and 50 nm) can irreversibly accumulate in cells and tissues of organisms (Koelmans et al., 2015) with significant potential for direct trophic transfer. Conversely, our experiments with MP ≥ 20 μm, which is within the size range detectable in field samples, indicate very fast MP ingestion-egestion rates both by mussels and polychaetes, and very little retention in the soft tissue (< 20% contaminated mussels) consistently with previously reported values by Ward et al. (2019) for *M. edulis*, Piarulli et al., 2020 for various invertebrate species including *M. galloprovincialis* and Mazurais et al. (2015) for fish larvae. This evidence allows the conclusion that direct trophic transfer of MP ≥ 20 μm from contaminated prey to
predators could be less relevant than previously hypothesised. It, however, does not exclude the existence of a potential chemical risk of transfer of hazardous substances associated to MP, such as persistent organic pollutants and/or plastic additives (e.g. phthalates). These pollutants can be adsorbed by plastics from the seawater (Rios et al., 2010), and possibly released after uptake by organisms and accumulated in cells and tissue, with a potential for transfer via predator-prey interactions, bioaccumulation at upper trophic levels (Batel et al., 2016) and severe physiological effects (Batel et al., 2018; Pittura et al., 2018). The direct quantification of ingestion-egestion rates of MP and associated contaminants is currently operationally challenging (Lanctôt et al., 2018) and developing cost-effective methods to quantify the real integrated impact over time of MP on organisms at environmental concentrations is a research priority.

5. Conclusion

Our 3 connected experiments empirically demonstrated that the incorporation in mussel biodeposits not only accelerates the vertical transport and deposition of MP (particularly those < 50 μm) to the sediments, making MP less available to pelagic and mesopelagic species in natural environments, but also significantly increases MP bioavailability and subsequent uptake by benthic detritivores. Thereby, MP incorporation into faecal matter can constitute a pathway for the transfer of MP across marine compartments and species. Further, the presence of MP decelerates the vertical flux of contaminated faecal pellets and the magnitude of the effect increases with increasing size and decreasing density of the MP. This highlights a potential risk for MP to unbalance the flux of nutrients from the water surface to the seafloor. Further work is urged to characterise the relevant spatial and temporal scales of these processes, and the so far largely unexplored consequences at the ecosystem level.
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Notes
The authors declare no competing financial interest.

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References
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