1 **Mussels facilitate the sinking of microplastics to bottom sediments and**

2 **their subsequent uptake by detritus-feeders**

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12 **Abstract**

13 Microplastics (MP) are omnipresent contaminants in the oceans, however little is known

14 about the MP transfer between marine compartments and species. Three connected

15 laboratory experiments using the filter-feeding mussel *Mytilus galloprovincialis* and the

16 omnivorous polichaete *Hediste diversicolor* were conducted. As results, mussels

17 significantly removed MP from the water column by incorporating them into biodeposits.

18 This effect was particularly evident for the smallest MP fraction (=41 µm) whose deposition

19 from the water column to the bottom was enhanced by the action of mussels. The

20 incorporation of MP into faecal pellets increased the particles' sinking velocity by about 3-4

21 orders of magnitude. Conversely, the MP presence significantly decreased the

22 depositional velocities of faecal pellets. The MP incorporation into mussels' biodeposits

23 also more than doubled the amount of MP uptaken by *H. diversicolor.* These findings

24 allowed the conclusion that detrital pathways could be a transfer route of MP across

25 marine compartments and food webs, also affecting the distribution of MP in the sediments

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

al., 2020; Piarulli et al., 2019, 2020; Ward et al., 2019). 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

78 conversion of seston into faeces and pseudofaeces supporting a biodeposition-based food

79 web (Zúñiga et al., 2014).

80 We conducted laboratory experiments mimicking as much as possible real environmental

81 condition and using as model organisms the filter-feeding mussel *Mytilus galloprovincialis*

82 and the omnivorous polychaete *Hediste diversicolor*.

83 *M. galloprovincialis* is a dominant component of the marine benthos, endemic to and

84 widely distributed in the Mediterranean Sea (Barsotti and Meluzzi, 1968). It is very

85 common in harbours and open waters, and often a dominant inhabitant on intertidal and

86 shallow subtidal hard substrates, both natural and artificial (Bacchiocchi and Airoldi, 2003).

87 It is also largely cultured for human consumption, using specialized floating rafts, buoys

88 and lines, which serve as recruitment surface for juveniles (Carl et al., 2012). Previous

89 exposure studies on the congeneric blue mussel *M. edulis* showed that nano (1 - 100 nm)

90 or very small (1 - 5 µm) MP can be metabolized, and in some cases also accumulated, at

91 tissue and cell levels (Browne et al., 2008; Von Moos et al., 2012). Conversely, larger MP

92 particles (≥ 6 µm) are often rapidly (ranging from minutes to few hours) excreted via

93 faeces and pseudofaeces (Fernández and Albentosa, 2019; Gonçalves et al., 2019). This

94 fast uptake/egestion of MP can facilitate the transfer of MP to the seafloor and to the

95 associated benthic detritus-feeders.

96 *H. diversicolor* is one of the most common polychaetes in the northern hemisphere

97 sedimentary environments and is often used as indicator species for a variety of

98 contaminants (Giangrande et al., 2005). This species has been described as carnivore

99 and/or scavenger and detritus-feeder (Riisgird, 1989). Its multiple and various feeding

100 strategies make *H. diversicolor* a key player in structuring soft-bottom communities (Rönn

101 et al., 1988), and a potential recipient of MP through various trophic pathways. Studies

102 have shown that mussel biodeposits constitute a high-quality food source for this

103 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$_{SMALL}$ (41 µm) vs MP$_{LARGE}$ (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 *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 *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$_{SMALL}$ and white for MP$_{LARGE}$) and fluorescence (red fluorescence for MP$_{SMALL}$ and blue fluorescence for MP$_{LARGE}$). 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^{-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})

234 compared to PA. The PP fragments were produced by milling PP pellets (Goodfellow

235 Cambridge Ltd. UK) with a pin mill (Alpine C160, Messer group GmbH, DE) into irregular

236 fragments that were subsequently sieved with decreasing mesh size (from 500 to 100).

237 Mussels' FP incorporating different MP types (FP with 1 MP were selected) were obtained

238 by using 3 groups of 10 mussels in 3 separate aquaria. Each group was exposed to

239 contamination from only one type of the 3 tested MP (concentration 0.2 mg L^{-1}), as

240 described in section 2.2. A fourth set of mussels was maintained in filtered seawater in a

241 separate aquarium without MP to produce MP-uncontaminated FP. After 48 h of exposure,

242 FP were removed individually using a Pasteur pipet, and inspected under a

243 stereomicroscope microscope (WILD M8, Leica microsystems) to check for their integrity

244 and for the presence of incorporated MP. MP used in the experiment were recognised due

245 to their distinctive colour and/or fluorescence. Twenty integer FP were selected for each of

246 the 4 treatments (PA_{SMALL}, PA_{LARGE}, PP and no MP). FP were photographed using a

247 mounted camera (Motic BTWB, USA) and images were analysed using imageJ software

248 (Schneider et al., 2012) to determine dimensions and to calculate the equivalent cylindrical

249 volumes and density (Table S2 in Supplementary Information) of each faecal pellet using

250 the Stoke's law modified for use with cylindrical shapes (Komar et al., 1981).

251 The sinking rates of the 4 FP treatments were assessed adapting the established method

252 originally developed by Smayda et al. (1969) to measure the sinking rates of zooplankton

253 FP. The FP were individually transferred with a Pasteur pipet to a 2 L measuring cylinder

254 filled with 20 µm filtered seawater (25 °C, 35 psu). FP were let to sink for the first 10 cm to

255 achieve a constant velocity, and then their descent speed was measured over the

256 remaining 10 cm.

257 Differences in the sinking rates of the 4 groups of FP were tested using one-way ANOVA

258 (n=20 replicated FP per treatment). The data were log (x+1) transformed to meet the

259 criteria for parametric statistics checked with Shapiro and Bartlett's tests (Table S4 in

260   Supplementary Information). Tukey post-hoc test was used for pairwise comparison of the

261   4 groups.

262   The measured sinking rates of the MP incorporated into the FP were further compared

263   with their theoretical sinking rates when free in the water column. Free MP were too small

264   to enable the visual measurement of their sinking rates, therefore their velocity was

265   estimated by using Stoke's law as described by Bach et al. (2012) and Porter et al. (2018),

266   taking into account the physical properties (sizes and densities, Table S1 in

267   Supplementary Information) of the three different MP types (PA_{SMALL}, PA_{LARGE}, and PP) as

268   well as the water temperature and salinity.

269

270   **2.4 Transfer of MP from mussels to polychaetes**

271   To examine whether MP enriched biodeposits produced by mussels can facilitate the

272   uptake of MP by detritivorous polychaetes, a modified version of the previous experimental

273   set-up (described in section 2.2) was used (Figure 2). Eight aquaria containing 5 L of

274   filtered seawater and a 20 mm bottom layer of sand were maintained at 25 °C. Four of the

275   aquaria contained groups of 10 *M. galloprovincialis* and the other four contained groups of

276   10 control empty shells. Based on observations from the previous experiments regarding

277   size selectivity of MP by mussels, MP_{SMALL} fragments (41 µm; conc. 0.2 mg L^{-1}) were

278   chosen as the size class, and prepared in the same manner as described in session 2.2 to

279   establish a biofilm formation prior to use.

280   Following 48 hours of MP exposure, all mussels and control shell groups were removed

281   and replaced by groups of *H. diversicolor* (n=5 per group). After 24 hours, the polychaetes

282   were transferred to aerated 500 ml beakers filled with 0.2 µm pre-filtered artificial seawater

283   for 8 hours to allow the elimination of ingested material, based on the results by Bock and

284   Miller (1999) the depuration time of polichaetes was kept shorter (8 h) than 12 h to avoid

285   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).

312   No MP were found adhering on the shells on alive mussels or control treatments.

313

314   **3.2 Effects of MP incorporation in mussels' faecal pellets**

315   Incorporation in mussels' FP (Figure 4a) accelerated MP sinking rates by over 3 orders of

316   magnitude, compared to the calculated theoretical sinking rates for free MP particles in

317   water, irrespective of MP sizes and/or polymer type (Tables S1 and S2 in Supplementary

318   Information). Averaged (± 1SE) sinking rates of FP contaminated with PA_{SMALL}, PA_{LARGE}

319   and PP were 422 ±61, 393 ±34 and 352 ±35 m day^{-1}, respectively, while theoretical sinking

320   rates of the associated reference free particles were 0.003, 0.03 and 0.03 m day^{-1},

321   respectively.

322   All FP contaminated by MP exhibited significantly (pairwise Tukey's post-hoc test p-values

323   < 0.0001) lower sinking rates compared to uncontaminated FP (Tables S6 and S7 in

324   Supplementary Information). This effect was more pronounced with increasing size and

325   decreasing density of the MP (Figure 4b). However, those differences were quantitatively

326   limited and not statistically significant (Table S7 in Supplementary Information).

327

328   **3.3 Transfer of MP from mussels to polychaetes**

329   After 24 hours, polychaetes exposed to biodeposits in the live mussel treatments ingested

330   significantly more MP (40 %, t-test p-value=0.03, Table S8 in Supplementary Information)

331   than those exposed to free sinking MP in the empty shell controls. On average, the

332   population of polychaetes exposed to the live mussel treatments ingested 57 ±9 MP per

333   unit, while those from the control treatments ingested 24 ±6 MP per unit (Figure 5).

334

335   **4. Discussion**

336   Approximately half of the MP introduced to the marine environment is buoyant, and tends

337   to stay in suspension in the water column (Kooi et al., 2016). This study showed that

13

338 mussels facilitated the deposition of MP (particularly for MP_{SMALL}) from the water column to

339 the bottom by incorporating the MP into biodepostis that resulted in enhanced sinking

340 velocity with respect to free MP. Further, detritus-feeding polychaetes exposed to MP in

341 biodeposits ingested more MP compared to polychaetes exposed to free sinking particles.

342 The mussel-mediated vertical transfer was evident in both the supernatant and at the

343 bottom for MP_{SMALL} (41 µm), which were 15 % more abundant at the bottom of treatments

344 containing live mussels compared to those with control empty shells. This is consistent

345 with the known particle size selection range for mussels, which tends to be more effective

346 for particles smaller than 50 µm (Defossez and Hawkins, 1997; Ward and Shumway,

347 2004). The effect of mussels on the MP vertical transfer was also partially observed for

348 MP_{LARGE} (129 µm), but only at the level of the supernatant, while at the bottom the

349 quantities of MP_{LARGE} were similar between units with alive mussel and controls with empty

350 shells, therefore suggesting that mussels have a sequestrating effect also on MP_{LARGE} but

351 this effect is too small and to be clearly quantified at the bottom. This is ascribable to two

352 main reasons: i) MP_{LARGE} tend to sink fast as free particles due to their large weight ii) the

353 particles' capture efficiency by mussels decreases with increasing particles' size (Ward

354 and Shumway, 2004; Ward et al., 2019).

355 Further, the selection of particles by bivalves, however, does not only depend on the size

356 but also on the shape and chemical composition. Indeed, Ward et al., (2019) showed that

357 fibres larger that 500 µm can also be ingested, therefore, it is likely that mussels could also

358 affect the vertical transfer of MP_{LARGE} of different shapes than those tested in this study.

359 Ultimately, most MP, including low density polymers, are likely to reach the seafloor (Van

360 Cauwenberghe et al., 2015), as demonstrated by the high levels of MP contamination

361 recorded in sediments (Lorenz et al., 2019;Vianello et al., 2013). However, biogenic

362 aggregation in biodeposits by mussels or other bivalves present as stable population in

363 coastal environments or extensively farmed could result in fine-scale heterogeneity of MP

364 distribution in marine sediments, creating concentrated hot-spots of small and highly

365 bioavailable MP. To our knowledge so far this possible biologically-mediated small-scale

366 heterogeneity in MP distribution at the sediment level has never been explored. If proved,

367 it would have important implications both for the ecology of benthic systems and for the

368 management of MP in the sediments.

369 Consistently with the findings by Porter et al. (2018), the measurements of MP sinking

370 rates showed that all the MP incorporated into FP sank 3 - 4 orders of magnitude faster

371 than the same MP as free particles. Although our sinking rates calculations were made in

372 a static experimental system, thereby not fully representing natural conditions, the

373 magnitude of the differences suggests that also under environmental conditions, where

374 oceanographic attenuation (e.g. resuspension) as well as other factors that can alter the

375 MP density (e.g. biofouling) may occur, MP incorporation into FP would decidedly change

376 their fate in the water column.

377 In turn, the incorporation of MP significantly decreased the sinking rates of FP irrespective

378 of polymer type and/or particle size, similarly to what observed by Long et al. (2015) who

379 estimated the effects of MP presence in phytoplankton aggregates. Given the central role

380 that bivalves' faecal matter has in mediating the flux of nutrients and carbon cycling across

381 benthic and pelagic systems (Hewitt et al., 2001; Robert et al., 2013), the observed

382 decelerated sinking rates of FP caused by the presence of MP is of concern. There is a

383 potential for the lowering of nutrient regeneration and availability in the benthic

384 compartment, with unknown consequences at the community and ecosystem levels.

385 Wieczorek et al. (2019) also observed a longer persistency in the water column of MP-

386 contaminated FP from salps and suggested that MP presence could lower the efficiency of

387 the biologic pump, enhancing water turbidity due to the fragmentation of faecal matter by

388 bacterial activity, with the consequent release of CO_2 directly into the water column. These

389 effects deserve particular attention as bivalve aquaculture is increasing worldwide

390   (Wijsman et al., 2019).

391   The experiment focusing on the subsequent transfer of MP to detritus-feeders, showed

392   that the incorporation into biodeposits significantly enhanced MP uptake by polychaetes,

393   indicating that MP incorporation into biodeposits can be a pathway for the transfer of MP

394   between species with different feeding modes and placed at different trophic levels.

395   *H. diversicolor*, known for its adaptable feeding mode, including detritivory, ingested more

396   than twice of MP when exposed to mussels' biodeposits containing egested MP. This

397   difference does not simply reflect a larger availability of MP in the bottom sediments due to

398   the accelerated sinking but also a qualitative effect. Indeed, the first experiment showed

399   that mussels increased MP abundance by only 15 % compared to controls, while

400   polychaetes ingested 41 % more MP in the presence of mussels. Faeces and

401   pseudofaeces are nutritious (Johannes and Satomi, 1966) and readily consumed by a

402   variety of suspension- and deposit-feeding organisms (Tenore, 1988), thereby aggregation

403   in biodeposits makes MP more bioavailable, facilitating their transfer to consumers. It is

404   likely that ingestion and egestion could also structurally and/or chemically change the MP,

405   for example via fragmentation up to the nanoscale (Dawson et al., 2018) or modification of

406   the biofilm community (Kesy et al., 2016), making the MP more suitable for ingestion.

407   It has been observed that nanoplastics (particularly those between 40 and 50 nm) can

408   irreversibly accumulate in cells and tissues of organisms (Koelmans et al., 2015) with

409   significant potential for direct trophic transfer. Conversely, our experiments with MP ≥ 20

410   μm, which is within the size range detectable in field samples, indicate very fast MP

411   ingestion-egestion rates both by mussels and polychaetes, and very little retention in the

412   soft tissue (< 20% contaminated mussels) consistently with previously reported values by

413   Ward et al. (2019) for *M. edulis,* Piarulli et al., 2020 for various invertebrate species

414   including *M. galloprovincialis* and Mazurais et al. (2015) for fish larvae. This evidence

415   allows the conclusion that direct trophic transfer of MP ≥ 20 μm from contaminated prey to

416 predators could be less relevant than previously hypothesised. It, however, does not

417 exclude the existence of a potential chemical risk of transfer of hazardous substances

418 associated to MP, such as persistent organic pollutants and/or plastic additives (e.g.

419 phthalates). These pollutants can be adsorbed by plastics from the seawater (Rios et al.,

420 2010), and possibly released after uptake by organisms and accumulated in cells and

421 tissue, with a potential for transfer via predator-prey interactions, bioaccumulation at upper

422 trophic levels (Batel et al., 2016) and severe physiological effects (Batel et al., 2018;

423 Pittura et al., 2018). The direct quantification of ingestion-egestion rates of MP and

424 associated contaminants is currently operationally challenging (Lanctôt et al., 2018) and

425 developing cost-effective methods to quantify the real integrated impact over time of MP

426 on organisms at environmental concentrations is a research priority.

427

428 **5. Conclusion**

429 Our 3 connected experiments empirically demonstrated that the incorporation in mussel

430 biodeposits not only accelerates the vertical transport and deposition of MP (particularly

431 those < 50 µm) to the sediments, making MP less available to pelagic and mesopelagic

432 species in natural environments, but also significantly increases MP bioavailability and

433 subsequent uptake by benthic detritivores. Thereby, MP incorporation into faecal matter

434 can constitute a pathway for the transfer of MP across marine compartments and species.

435 Further, the presence of MP decelerates the vertical flux of contaminated faecal pellets

436 and the magnitude of the effect increases with increasing size and decreasing density of

437 the MP. This highlights a potential risk for MP to unbalance the flux of nutrients from the

438 water surface to the seafloor. Further work is urged to characterise the relevant spatial and

439 temporal scales of these processes, and the so far largely unexplored consequences at

440 the ecosystem level.

441

Notes

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