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Quantifying spatial variation in the uptake of microplastic by mussels using biodeposit traps: A field-based study

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ABSTRACT

Spatial uptake patterns of microplastics (MP) by marine species are largely unexplored under field conditions. A novel "biodeposit trap" that measure uptake and egestion of MP by suspension-feeders through the analysis of their biodeposits, was designed and used to estimate the spatial variation of these processes by mussels in field conditions. Traps containing wild or farmed mussels or control empty shells were deployed at three sites characterised by different MP concentrations and water flow conditions. A different MP dimensional composition was observed between MP pools present in biodeposit and control traps, with the latter shifted towards higher dimensional range (0.05–5 mm). Conversely, mussels accumulated small MP (0.02–0.05 mm) into their biodeposits without any significant difference between wild and farmed specimens. MP uptake rates were on average 4–5 times higher at the site where MP contamination was expected to be highest and where water flow conditions were considered moderate.

1. Introduction

Lack of efficient management and human negligence have led to plastic debris entering all ecosystems on earth and accumulating in the marine environment (Barnes et al., 2009) causing global environmental concern (see Elizalde-Velázquez and Gómez-Oliván, 2021 for an updated review on the risk posed by MP). Microplastics (MP) are small plastic particles from 1 μm to 5 mm with variable shapes (fragments, fibres, films, pellets, foams).

The risks associated with MP contamination can vary in space and/or time not only due to the actual heterogeneous occurrence of MP in the environment, but also with variations in the uptake dynamics of organisms. Although these variable patterns are potentially relevant when assessing the risk of MP to organisms and ecosystems, their impact remains unexplored in real field conditions, particularly at the small spatial scales (meters to hundreds of meters) most relevant for individual organisms (Underwood et al., 2017). This is primarily due to a lack of simple and cheap monitoring methods that allow for the large replication needed to measure MP fluxes and their variability (spatially and temporally) in the field.

Natural organismal uptake of MP is typically assessed by quantifying MP content in the biota soft tissue (e.g. Carbery et al., 2018; O'Connor et al., 2020; Lusher et al., 2017; Rezania et al., 2018), but these classic "snap-shot" approaches do not provide information on the actual exposure over time or the related risks at ecologically relevant conditions. This is because MP in the size range that can be detected in most field studies (>11–20 μm) do not accumulate in the tissues of organisms but are rapidly egested (Piarulli and Airoldi, 2020; Piarulli et al., 2020; Ward et al., 2019), thereby not allowing quantitative variations in MP exposure and uptake to be detected. Alternative dynamic approaches, using radiotracer techniques, have also numerous technical and economic constraints (Lanctôt et al., 2018), which limit their use for extensive and long-term field monitoring programmes. An effective indirect indicator of MP organismal uptake, that can provide an integrated

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measurement over time, is the quantification of synthetic particles in boluses, casts or faeces (Löder and Gerdts, 2015).

In the current study, we developed and tested a novel "biodeposit trap" to estimate the in situ uptake and egestion of MP by mussels and related potential vertical transfer of MP to the sediments. The trap was designed specifically to capture the faeces and pseudofaeces (cumulatively referred to as biodeposit) from sessile benthic suspension-feeding organisms (e.g. mussels or oysters) immediately on production, prior to sinking to the sediments. The method is relatively simple as well as time and cost-effective compared to other available approaches. To validate the system and quantify spatial variations in the uptake and egestion of MP under real field conditions, biodeposit traps containing mussels were deployed in three areas of Ravenna harbour (Italy) differing *a-priori* for potential sources of MP and water flow conditions.

The Mediterranean mussel *Mytilus galloprovincialis,* which is the dominant suspension-feeder naturally occurring on intertidal and subtidal hard substrates in the region (Bacchiocchi and Airoldi, 2003), was used as a model species to test the traps. Particles` uptake by mussels and subsequent biodeposit production as egestion mechanism, is influenced by prevailing environmental conditions including quantity and quality of food available (Mckindsey et al., 2011) and water flow (Cole et al., 1992; Muschenheim, 1987; Newell and Richardson, 2014), and there is generally a positive effect of increasing water velocity on mussel filtration rates.

Recent work has also shown that MP uptake can be influenced by the different ecological traits of species (Piarulli et al., 2020). Since *M. galloprovincialis* is largely farmed in the region using a variety of suspended surfaces (Danovaro et al., 2004; Fabi et al., 2009; Maffei et al., 1996), we also explored if there are any detectable differences in uptake of MP between wild and farmed mussels, where the latter are generally larger and more homogeneous in size than the former.

2. Material and methods

2.1. Study sites

The study was conducted in July 2017 at three sites in the Ravenna harbour (Fig. 1). This is a semi-enclosed area with moderate water circulation and mixing with the open sea and with varied anthropogenic activities and wastewater sources (Airoldi et al., 2016), which makes it a potential hot-spot for MP contamination. The 3 sites (Fig. S1 in SI) were chosen for their potential differences in point source pollution, as well as differences in water flow conditions which were a priori defined as "high", "moderate" and "low" by considering the known physical and structural characteristics of the anthropogenically-created area (Airoldi et al., 2016). Both point source MP pollution and water flow can affect MP occurrence and filtration rates by mussels. Site A was a docking area in the tourist marina, with moderate water flow and potential inputs of MP from tourist activities. Site B was located nearby an urban wastewater drain at the lower end of the main industrial port channel with high confinement conditions and very low water flow and exchange with open seawaters. Site C was located at the harbour entrance, which had no obvious point sources of MP and was exposed to relatively high hydrodynamics and water exchange with the open sea. Pilot analyses of subsurface seawater from each site (30 L) confirmed the possibility of different levels of MP contamination across 3 sites, with MP concentrations of 0.24, 0.17 and 0.1 MP L^{-1} being determined for sites A, B and C respectively.

2.2. Test organisms

Wild mussels (*M. galloprovincialis*) were collected with a stainless steel scraper from the nearest artificial jetty, seawall or pontoon at each of the three sites. Farmed mussels on the same species were provided by the local aquaculture centre Cooperativa Casa Del Pescatore A.R.L (Cesenatico, Italy). All the experimental preparation (Paragraph S1 in SI) of the mussels was performed directly in the field immediately after collection. A total of 24 groups consisting of 60 specimens of either wild or farmed mussels were created and directly placed suspended in a mesh for 24 h at each site before the start of the experiment according to Van Cauwenberghe et al. (2015) and Ward et al. (2019) to adapt to the exposure conditions. An additional 12 groups of 60 empty double-valve mussel shells chosen of an intermediate size between wild and farmed mussels (4–8 cm, prepared as in Piarulli and Airoldi, 2020) were used as "procedural controls" and followed the exactly the same preparation procedure of the alive mussels. Each group of mussels was subsequently placed into a single biodeposit trap and considered as a single experimental replicate.

2.3. Biodeposit traps

The biodeposit traps (Fig. 1) were inspired by a prototype reported by McKindsey et al. (2009) to quantify the impacts of mussel aquaculture on sedimentary systems, and were designed to collect the

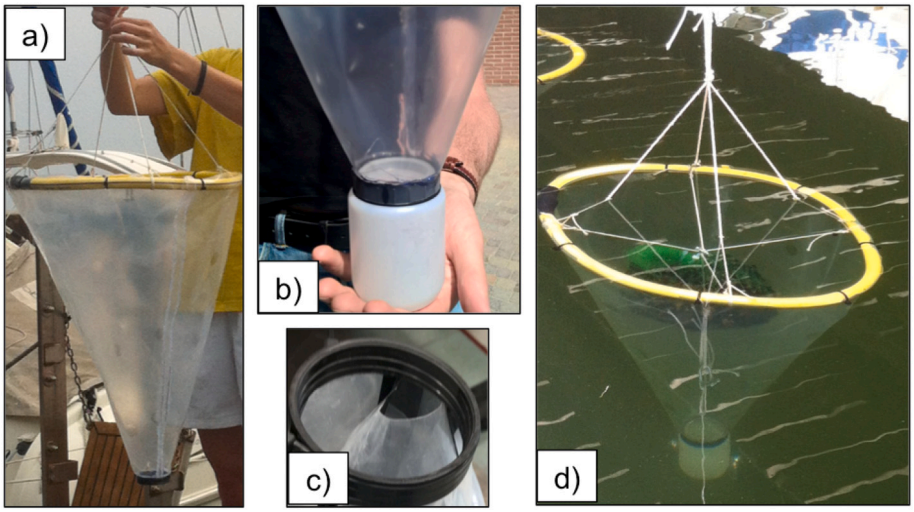

Fig. 1. Photographs showing the different components of a biodeposit trap and how they are deployed. a) Trap before deployment without the jar to collect deposited matter; b) attachment of the jar to the trap; c) narrow end of the trap and internal funnel without the jar; d) field-deployed biodeposit trap with suspended mussels (empty double-valve mussel shells were similarly deployed in control traps).

biodeposit of filter feeders immediately after production, so as to capture any MP eventually ingested. The traps therefore provide an estimate of the uptake of MP in real field conditions and the consequent potential flux to the sediments.

The traps (Fig. 1a) consisted of polyethylene (PE) funnels (70 cm high, 40 cm wide at the upper end and 7 cm to the bottom end) secured with cable ties to rigid polyethylene rings at the upper end, and perforated with three longitudinal series of 4 holes (2.5 cm diameter) allowing the water-flow through the upper part of the funnels where the mussels were located (Fig. 1d). This allowed the mussels to maintain their filtering rates as close as possible to natural conditions. The deposited material (biodeposits in the traps with mussels and detritus in control traps with shells) biodeposits were collected in white 250 mL PE jars screwed into PE rings attached to the bottom (narrower) end of the funnels with professional water-proof glue and cable ties to fill the complete diameter of the jars (Fig. 1b). To directly convey the deposited material into the jars and to avoid accumulation at the jar ledges, internal PE funnels (10 and 3 cm diameter at the upper and bottom ends, respectively) were glued below the last series of holes above the narrow ends of the external funnels and ending directly into the jars (Fig. 1c).

2.4. Sampling design and field set up

To estimate MP uptake by mussels and their net contribution to MP deposition, the traps with live mussels producing biodeposits were compared to procedural controls with empty mussel shells. The control samples simulated the physical shape and volume of the live mussels without the active filtration (Piarulli and Airoldi, 2020). Control samples, therefore, included any detritus naturally depositing without the action of mussels. Importantly, they also captured any the MP fraction freely sinking to the bottom in the environment and acted as a way to measure any background contamination for the plastic components of the traps. Both mussels (wild or farmed) and empty shells were positioned into the traps into closed PE nets secured with cable ties at 4 opposite extremes.

The 24 biodeposit traps with either 60 wild or farmed mussels and the 12 control traps containing 60 empty mussel shells were randomly assigned to the 3 study sites, with 4 replicates for each treatment of wild mussels, farmed mussels and control shells. At Sites A and B the traps were placed in a straight line at intervals of approximately 2 m, with each trap suspended by 4 linked ropes departing from the 4 extremes of the trap and tied to a main rope perpendicular to the centre of the larger aperture of the trap and secured to the pier (Fig. 1d). The Traps were suspended at a depth of approximately 40 cm below the water surface (Fig. S1 in SI). At site C it was not possible to deploy the traps from the pier due to uncontrolled accessibility to the public and likelihood of disturbance. Therefore, a system of three horizontal lines suspended with buoys and anchored to the seafloor was used, from which the traps were suspended as at Sites A and B (Fig. S1 in SI). At all sites, the replicate traps for each treatment were deployed in random order. The traps were deployed for 4 h to allow the deposition of either biodeposit or free sinking detritus. Once collected, each jar was immediately transported to the laboratory and frozen at −20 °C before further processing.

2.5. Sample processing and FTIR identification of MP

The content of each jar was vacuum filtered onto pre-weighed 20 μm nylon filters (Ø: 9 cm; PLASTOK, UK). Filters were placed in covered glass petri dishes and dried at room temperature in a glass dryer for 1 week and subsequently weighed to quantify the dry weight of deposited matter in each trap. Very large natural items such as shells or vegetation fragments were manually removed with tweezers during the filtration phase and rinsed with Milli-Q water to allow any MP attached to the items to fall back into the sample.

Inspection of the filters, MP quantification and chemical characterisation was performed by following the same approach and instrumental microscopic and spectroscopical settings described in Piarulli et al. (2020, 2019) and reported in the Supplementary Information. The used methodological approach allowed the detection of MP from 0.02 to 5 mm. Specific QA/QC procedures were applied during all the sample handling and they were applied as described in paragraph S3 of SI. To facilitate subsequent statistical analyses the MP were categorised in 4 size classes (mm): <0.05 (dim1), 0.05–0.5 (dim2), 0.5–1 (dim3), 1–5 (dim4).

2.6. Statistical analyses on MP contents and composition

No MP were found on the air filters or in the procedural blanks, with only natural cotton and cellulose microfibres identified by spectroscopy analyses. Therefore, there was no need to implement any form of blank correction of the actual MP data. Prior to subsequent statistical analyses, the MP data were tested for normality and homogeneity with Shapiro−Wilk and Bartlett's tests respectively. As data were not normally and homogeneously distributed, non-parametric statistical analyses were performed. Differences in the total amount of MP deposited in mussel (wild and farmed) and control traps were tested using univariate permutational analysis of variance (PERMANOVA, Anderson and Walsh, 2013a, b) based on Euclidean distance resemblance matrix, with two orthogonal fixed factors: “treatment” (wild mussels, farmed mussels, and control empty shells) and “site” (Sites A, B and C) and 4 replicates for factor combination. A pairwise post-hoc test was subsequently performed to identify the significantly different levels. The level of significance for the rejection of null-hypothesis was set at P (perm) <0.05, although when PERMANOVA analyses showed marginal significance (P (perm) between 0.05 and 0.07) a post-hoc test was performed anyway as *a-posteriori* permutation-based tests have the statistical power to detect differences between single groups (Hsu, 1996).

Principal component analysis (PCO) based on Bray-Curtis similarities was also performed (Paragraph S4 in SI) to visualise patterns in MP size distribution among traps subjected to different treatments.

The analyses were performed with 9999 permutations using PRIMER 6 & PERMANOVA (Clarke and Gorley, 2015). Data are reported as mean ± SE.

2.7. Estimation of MP uptake rates

The biodeposit trap data were converted into an average MP uptake rate per individual mussel ($MP_{\text{mussel}}{}^{-1}\ \text{h}^{-1}$), which was estimated as:

$$MP^{-1}_{\text{mussel}}\ \text{h}^{-1} = \frac{\left[\Sigma_{1-4\ (\text{or}\ 12)}\ \left(MP_{\text{biodeposit}} - MP_{\text{control}}\right)\right]}{N_{\text{i}}} Et^{-1}$$

where $MP_{\text{biodeposit}}$ is the average number of MP in the biodeposit traps with either wild or farmed mussels, MP_{control} is the average number of MP in the control traps, N_{i} is the number of (wild or farmed) mussels per trap (60), and Et the total time of exposure (4 h). Both the uptake rates at each site (averaging the 4 traps per treatment per site) and the average rate for the whole study region (averaging the 12 traps per treatment) were calculated. The uptake rate calculations were made by considering wild and farmed mussels separately.

3. Results

3.1. General overview of MP identified in the traps

None of the polymers used for the experimental set-up or laboratory processing were found in the samples. Therefore, the occurrence of contamination from plastic components in the experimental equipment could be excluded. In the 36 biodeposit or control traps we successfully identified a total of 76 MP. Of these, 62 MP (81.6%) were accumulated in the biodeposit traps, for an average (±1SE) of 2.6 (±0.6) MP trap^{-1}. A

total of 74 MP (97.4%) were identified as polypropylene (PP) fragments, while the remaining 2 MP (2.6%) were polyester (PES) and polyamide (PA) fibres found in site B and C respectively. The size of the MP ranged from 0.02 to 2.1 mm: 25 (32.9%) were < 0.05 mm, 35 (46%) were 0.05–0.5 mm, 11 (14.5%) were 0.5–1 mm and 5 (6.6%) were 1–5 mm.

3.2. Quantitative and qualitative variations in MP

MP in the traps quantitatively differed between sites and between traps with live mussels and controls, but no differences in MP were found between traps with wild and farmed mussels (Table S1 in SI). As shown in Fig. 2a, the average number of MP in the biodeposit traps for both wild and farmed mussels was highest at Site A (6 ± 0.9 and 5.75 ± 1.2 MP trap^{-1} for wild and farmed mussels, respectively). Considerably lower numbers of MP were collected in the biodeposit traps at Site C (0.75 ± 0.7 MP trap^{-1} for wild mussels and 2 ± 1.3 MP trap^{-1} for farmed mussels) and Site B (0.5 ± 0.2 MP trap^{-1} for wild mussels and 0.5 ± 0.5 MP trap^{-1} for the farmed mussels). The amount of MP found in the control traps decreased from Site A (2.5 ± 1 MP trap^{-1}) to site B (1 ± 0.4 MP trap^{-1}) and further to Site C, where no MP accumulated (Fig. 2a). At Site A, both types of mussels deposited significantly more MP compared to controls (Fig. 2a, Table S1 in SI). MP were also found to deposit more in traps with mussels compared to the controls at Site C, but the presence of MP in the biodeposit was numerically too low (<2 particles) to determine if the difference was significant. At Site B, no differences were found in MP quantities between biodeposit traps with mussel and control shells. (Fig. 2a).

The abovementioned patterns were not only quantitative but also qualitative (Fig. 2b and S2 in SI). Although there was relatively high variability among replicate traps of the same treatment, traps containing wild and farmed mussels generally showed an increase in particle number as the particle dimension range decreased. This was evidenced by a dominance of <0.05 mm MP at Site A (3 ± 0.7 and 2.7 ± 1.3 MP trap^{-1} in traps with wild and farmed mussels respectively) and 0.05–0.5 mm at Site C (0.5 ± 0.2 and 2 ± 1 MP trap^{-1} for wild and farmed mussels, respectively). Conversely, MP in control traps exhibited greater dimensional variation with almost no MP < 0.05 mm at Site A (0.25 ± 0.1 MP trap^{-1}) and an higher proportion of particles between 1 and 5 mm, which were almost absent in traps with both wild and farmed mussels (Fig. 2b).

3.3. Estimation of MP uptake rates by mussels

The estimated average MP uptake rates in the harbour of Ravenna for wild and farmed mussels were 0.005 and 0.006 MP h^{-1}, respectively (Fig. 3a). The average uptake rates were very similar between the two types of mussels but varied between sites, with the greatest uptake rates at Site A and no measurable uptake at Site B (Fig. 3b). From a MP dimensional composition perspective, the rate of MP uptake was higher for smaller particles (<0.05 mm and from 0.05 to 0.5 mm), while there was no uptake for particles 1–5 mm (Fig. 3c, d).

4. Discussion

In the current study, we designed a novel field-based trap to collect the MP incorporated into the biodeposits of suspension-feeding mussels immediately after their production. The design of the biodeposit trap is relatively simple and cheap to construct, as well as being easy to deploy in the field. This study has demonstrated that the traps are highly effective in collecting biodeposit samples that were relatively clean from other detritus and therefore simplifying the subsequent sample processing for the isolation and identification of the MP.

Even if in this study biodeposit traps containing mussels were deployed in relatively sheltered estuarine conditions and for relatively

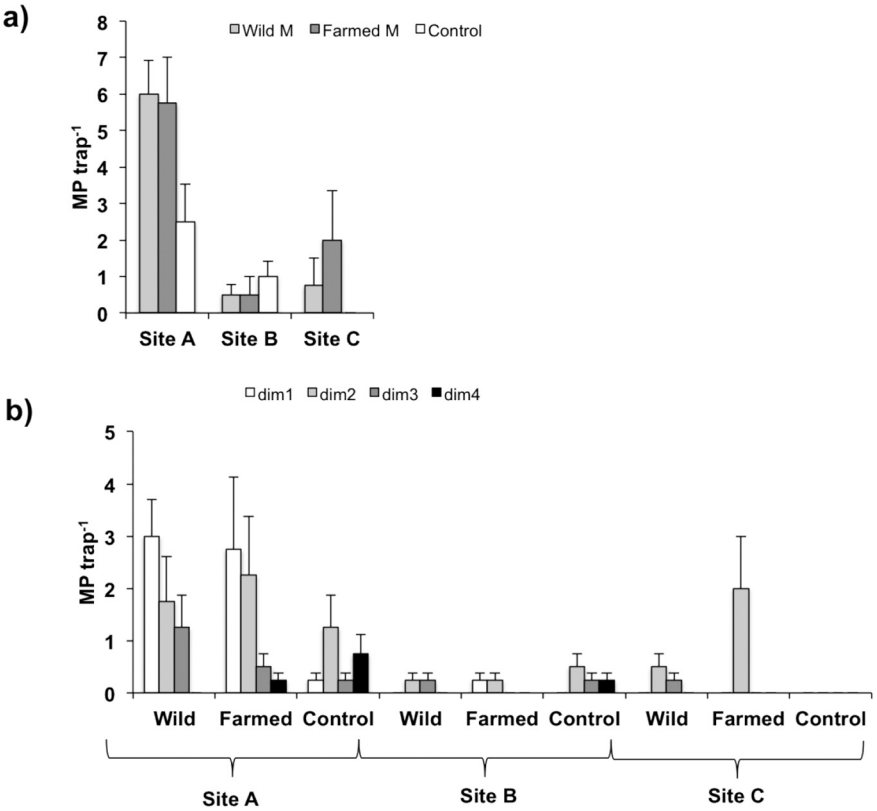

Fig. 2. Average number (±1SE, $n = 4$) of a) total MP in the wild mussel, farmed mussel and control traps at each of three sites (A, B and C), and b) MP in each of the 4 size classes in wild mussel, farmed mussel and control traps at the three sites (A, B and C). Dim1 = MP <0.05 mm; dim2 = MP 0.05–0.5 mm; dim3 = MP 0.5–1 mm; dim4 = MP 1–5 mm.

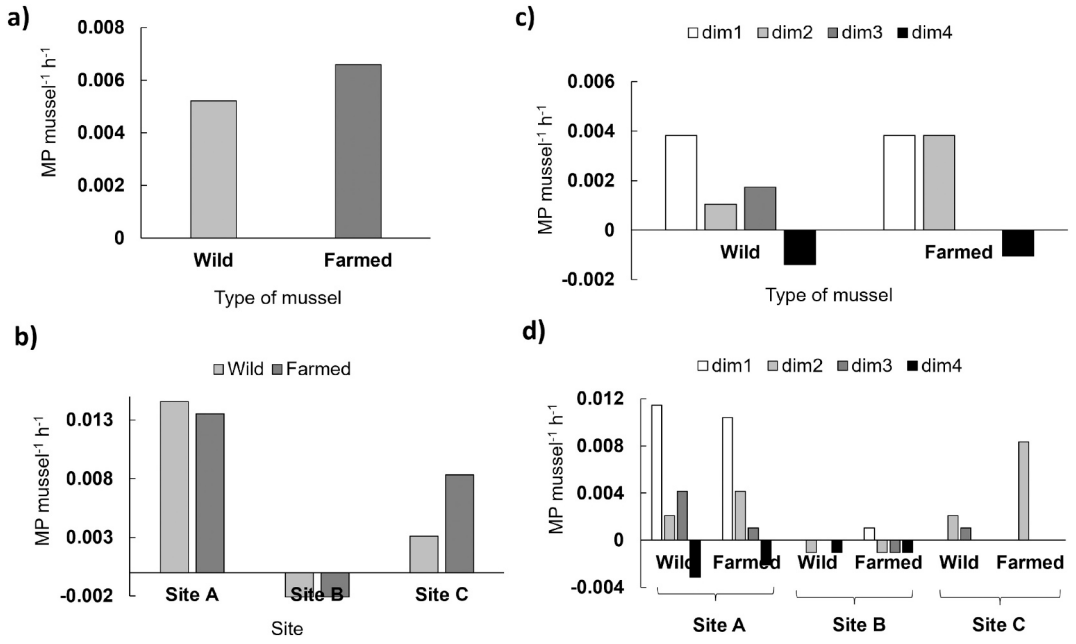

Fig. 3. Uptake rates of MP by individual wild mussels and farmed mussels calculated as the number of MP ingested per hour (MP mussel^{-1} h^{-1}) and plotted as a function of a) cumulative regional MP uptake, b) cumulative site-specific MP uptake, c) regional MP uptake according to the different size classes, d) site-specific MP uptake according to the different size classes.

short time of exposure, the successful outcomes demonstrate that the biodeposit traps could be deployed for longer periods of time and, with appropriate modifications, that the same approach could be applied to other aquatic systems and/or to other relevant benthic filter-feeding organisms (e.g. oysters, ascidians and holothurians). For example, Van Colen et al. (2021) recently presented an application of our biodeposit trap method with the congeneric species *Mytilus edulis* in a more open area along the Belgian coast.

Our results showed there was a significantly higher concentration of the smallest MP (0.02–0.5 mm) in mussel biodeposits traps than in than the control traps. This confirms previous observations from both field and laboratory studies that showed a preferential uptake and further incorporation into biodeposits of MP fragments <100 μm (Piarulli and Airoldi, 2020; Ward et al., 2019; Zhao et al., 2018). This reflects the previously demonstrated tendency of mussels to increase their particle selection (for particles up to ~100 μm) at decreasing particle size range (Møhlenberg and Riisgård, 1978; Tamburri and Zimmer-Faust, 1996; Ward and Kach, 2009; Ward and Shumway, 2004).

The spatial replication of the deployment approach of the biodeposit traps made it possible to estimate how variable the uptake of MP by mussels and the associated vertical transfer from the water column to the sediments via incorporation into biodeposits can be. At Site A, the prevailing water flow conditions were adequate for mussel filtration and the environmental MP concentrations in the water were expected to be high. Using data generated in the study, the transfer of small MP via mussel biodeposits was estimated to be approximately 10 times greater than the transfer rates that would occur via normal sedimentation processes not mediated by the action mussels. This result provides field-based evidence for the mechanism showed by Piarulli and Airoldi (2020) in laboratory-controlled conditions, whereby the incorporation of MP into the biodeposits of mussels enhances the vertical flux of small MP (<50 μm) which would tend to have limited or very slow sinking velocity as "free" particles compared to those of larger dimensions (Piarulli and Airoldi, 2020; Porter et al., 2018). This also confirms the hypothesis that mussels and other suspension-feeding bivalves, when present as stable populations in coastal environments or when extensively farmed, can be responsible for the formation of fine-scale heterogeneity of MP distribution in marine sediments, and formation of accumulation hot-spots of small MP.

Overall, the current study highlighted a large spatial variability in MP uptake by mussels both at small (variability among nearby traps in the order of few meters) and larger (variability among sites in the order of few km) scales according to the study area. The differences in MP quantities between the 3 study sites were significantly larger in the biodeposit traps than in the control traps, which was also reflected by the respective estimated uptake rates. These results confirm our hypothesis that MP uptake and subsequent transfer to sediments reflect not only local MP point sources and availability, but also local water flow conditions.

Marine hydrodynamics influence particle distributions and availability in coastal areas, including MP (Rocha-Santos and Duarte, 2015), as well as controlling the filtration of filter-feeders and their uptake rates of particulate matter (Widdows et al., 2009). The number of MP in mussel biodeposits significantly decreased from the moderately water flow exposed Site A to the very confined Site B with limited water flow. Conversely, Site C, which was the most exposed site, exhibited intermediate MP contents in the collected biodeposit. Higher water flow rates are exert a positive effect on the uptake rates of phytoplankton by mussels due to an increased encounter rate between the mussels and particulate matter (both organic and inorganic particles) (Newell and Richardson, 2014). On the other hand, exposure to very strong water flows can also impair the filtering capacity of mussels (Wildish and Miyares, 1990). Thereby, optimal mussel filtering capacity and increased uptake rates of MP (and particulate matter in general) is most likely to occur in areas characterised by moderate water flows, while reduced uptake occurs in areas where the water flow is either too fast or too slow.

No significant difference was observed in either the amount or properties of MP taken up by wild or farmed mussels at any of the exposure sites. This appears to be in line with the previous findings of De Witte et al. (2014), where MP contamination in wild or farmed *M. edulis* mussels did not reveal any differences in response to similar environmental exposures. Together, these findings indicate that body size may not be a relevant trait affecting filtration rates in mussels, at least within the limited shell size differences in our study (approximately 2 cm; 3–7 and 5–9 cm for wild and farmed mussels, respectively). The biodeposit

trap method seems to be relatively robust to some variability in the size of the used organisms, indicating that the presented approach has good potential for application in more spatially and temporally extensive monitoring campaigns, where the use of a standard organismal size would be advisable but also more difficult to be achieved due to natural variation in the population structure.

5. Conclusion

This study presented and assessed the effectiveness of a newly developed biodeposit trap, specifically designed for measuring MP uptake and transfer to sediments by mussels in the field. The biodeposit traps were cost-effective and, with minor modifications, could be used across many different aquatic environments and with other suspension-feeding sessile species. The method was applied to dynamically monitor small-scale spatial variations in MP uptake rates for the first time and the results demonstrate that MP uptake and egestion process in mussels can be highly variable within and between nearby sites, potentially reflecting local differences in MP water column concentrations and the prevailing water flow conditions. With many countries currently in the process of developing and implementing MP monitoring programs, their design needs to consider this small-scale heterogeneity in MP distributions to ensure the programs achieve their goals effectively.

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CRediT authorship contribution statement

Stefania Piarulli: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Sara Scapinello:** Methodology, Formal analysis, Investigation. **Giorgia Sciutto:** Investigation, Resources, Methodology, Writing – review & editing. **Silvia Prati:** Writing – review & editing. **Rocco Mazzeo:** Writing – review & editing. **Andy M. Booth:** Writing – review & editing, Funding acquisition, Project administration. **Laura Airoldi:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

Authors declare no competitive financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.113305.

References

Airoldi, L., Ponti, M., Abbiati, M., 2016. Conservation challenges in human dominated seascapes: the harbour and coast of Ravenna. Reg. Stud. Mar. Sci. 8, 308–318. https://doi.org/10.1016/j.rsma.2015.11.003.

Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol. Monogr. 83, 557–574. https://doi.org/10.1890/12-2010.1.

Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol. Monogr. 83, 557–574. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Bacchiocchi, F., Airoldi, L., 2003. Distribution and dynamics of epibiota on hard structures for coastal protection. Estuar. Coast. Shelf Sci. 56, 1157–1166. https://doi.org/10.1016/S0272-7714(02)00322-0.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409. https://doi.org/10.1016/j.envint.2018.03.007.

Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User Manual/. Tutorial. PRIMER-E, Plymouth.

Cole, B.E., Thompson, J.K., Cloern, J.E., 1992. Measurement of filtration rates by infaunal bivalves in a recirculating flume. Mar. Biol. 113, 219–225.

Danovaro, R., Gambi, C., Luna, G.M., Mirto, S., 2004. Sustainable impact of mussel farming in the Adriatic Sea (Mediterranean Sea): evidence from biochemical, microbial and meiofaunal indicators. Mar. Pollut. Bull. 49, 325–333. https://doi.org/10.1016/j.marpolbul.2004.02.038.

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (Mytilus edulis): comparison between commercial and wild types. Mar. Pollut. Bull. 85, 146–155. https://doi.org/10.1016/j.marpolbul.2014.06.006.

Elizalde-Velázquez, G.A., Gómez-Oliván, L.M., 2021. Microplastics in aquatic environments: a review on occurrence, distribution, toxic effects, and implications for human health. Sci. Total Environ. 780, 146551 https://doi.org/10.1016/j.scitotenv.2021.146551.

Fabi, G., Manoukian, S., Spagnolo, A., 2009. Impact of an open-sea suspended mussel culture on macrobenthic community (Western Adriatic Sea). Aquaculture 289, 54–63. https://doi.org/10.1016/j.aquaculture.2008.12.026.

Hsu, J.C., 1996. Multiple Comparisons: Theory and Methods. Chapman & Hall, Ohio, USA (I eds).

Lanctôt, C.M., Oberhänsli, F., Swarzenski, P., Tolosa, I., Metian, M., Al-Sid-Cheikh, M., Catarino, A.I., Cresswell, T., Danis, B., Karapanagioti, H.K., Mincer, T., 2018. Application of nuclear techniques to environmental plastics research. J. Environ. Radioact. 192, 368–375. https://doi.org/10.1016/j.jenvrad.2018.07.019.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G.

Löder, M.G.J., Gerdts, G., 2015. Methodology used for the detection and identification of microplastics-a critical appraisal. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 201–227. https://doi.org/10.1007/978-3-319-16510-3_8.

Maffei, M., Giulini, G., Fabi, G., Fiorentini, L., 1996. Valutazioni comparative su alcuni parametri di qualità in popolazioni di mitili (Mytilus galloprovincialis lamk.) provenienti da differenti condizioni di allevamento e da banco naturale. Biol. Mar. Mediterr. 3, 242–245.

Mckindsey, C.W., Archambault, P., Callier, M.D., Olivier, F., 2011. Influence of suspended and off-bottom mussel culture on the sea bottom and benthic habitats: a review. Can. J. Zool. 89, 622–646. https://doi.org/10.1139/z11-037#.Xr5ze62B1E4.

McKindsey, C.W., Lecuona, M., Huot, M., Weise, A.M., 2009. Biodeposit production and benthic loading by farmed mussels and associated tunicate epifauna in Prince Edward Island. Aquacolture 295, 44–51. https://doi.org/10.1016/j.aquaculture.2009.06.022.

Møhlenberg, F., Riisgård, H.U., 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17, 239–246. https://doi.org/10.1080/00785326.1978.10425487.

Muschenheim, D.K., 1987. The dynamics of near-bed seston flux and suspension-feeding benthos. J. Mar. Res. 45, 473–496.

Newell, C.R., Richardson, J., 2014. The effects of ambient and aquaculture structure hydrodynamics on the food supply and demand of mussel rafts. J. Shellfish Res. 33, 257–272. https://doi.org/10.2983/035.033.0125.

O'Connor, J.D., Mahon, A.M., Ramsperger, A.F.R.M., Trotter, B., Redondo-Hasselerharm, P.E., Koelmans, A.A., Lally, H.T., Murphy, S., 2020. Microplastics in freshwater biota: a critical review of isolation, characterization, and assessment methods. Glob. Chall. 4 https://doi.org/10.1002/gch2.201800118.

Piarulli, S., Airoldi, L., 2020. Mussels facilitate the sinking of microplastics to bottom sediments and their subsequent uptake by detritus-feeders. Environ. Pollut. 266 https://doi.org/10.1016/j.envpol.2020.115151.

Piarulli, S., Scapinello, S., Comandini, P., Magnusson, K., Granberg, M., Wong, J.X.W., Sciutto, G., Prati, S., Mazzeo, R., Booth, A.M., Airoldi, L., 2019. Microplastic in wild populations of the omnivorous crab *Carcinus aestuarii*: a review and a regional-scale test of extraction methods, including microfibres. Environ. Pollut. 251, 117–127. https://doi.org/10.1016/j.envpol.2019.04.092.

Piarulli, S., Vanhove, B., Comandini, P., Scapinello, S., Moens, T., Vrielinck, H., Sciutto, G., Prati, S., Mazzeo, R., Booth, A.M., Van Colen, C., Airoldi, L., 2020. Do

different habits affect microplastics contents in organisms? A trait-based analysis on salt marsh species. Mar. Pollut. Bull. 153 https://doi.org/10.1016/j.marpolbul.2020.110983.

Porter, A., Lyons, B.P., Galloway, T.S., Lewis, C., 2018. Role of marine snows in microplastic fate and bioavailability. Environ. Sci. Technol. 52, 7111–7119. https://doi.org/10.1021/acs.est.8b01000.

Rezania, S., Park, J., Md Din, M.F., Mat Taib, S., Talaiekhozani, A., Kumar Yadav, K., Kamyab, H., 2018. Microplastics pollution in different aquatic environments and biota: a review of recent studies. Mar. Pollut. Bull. 133, 191–208. https://doi.org/10.1016/j.marpolbul.2018.05.022.

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends Anal. Chem. 65, 47–53. https://doi.org/10.1016/j.trac.2014.10.011.

Tamburri, M.N., Zimmer-Faust, R.K., 1996. Suspension feeding: basic mechanisms controlling recognition and ingestion of larvae. Limnol. Oceanogr. 41, 1188–1197. https://doi.org/10.4319/lo.1996.41.6.1188.

Underwood, A.J., Chapman, M.G., Browne, M.A., 2017. Some problems and practicalities in design and interpretation of samples of microplastic waste. Anal. Methods 9, 1332–1345. https://doi.org/10.1039/c6ay02641a.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ. Pollut. 199, 10–17. https://doi.org/10.1016/j.envpol.2015.01.008.

Van Colen, C., Moereels, L., Vanhove, B., Vrielinck, H., Moens, T., 2021. The biological plastic pump: evidence from a local case study using blue mussel and infaunal benthic communities. Environ. Pollut. 274 https://doi.org/10.1016/j.envpol.2020.115825.

Ward, J.E., Kach, D.J., 2009. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. Mar. Environ. Res. 68, 137–142. https://doi.org/10.1016/j.marenvres.2009.05.002.

Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Bio. Ecol. 300, 83–130. https://doi.org/10.1016/j.jembe.2004.03.002.

Ward, J.E., Zhao, S., Holohan, B.A., Mladinich, K.M., Griffin, T.W., Wozniak, J., Shumway, S.E., 2019. Selective ingestion and egestion of plastic particles by the blue mussel (Mytilus edulis) and eastern oyster (Crassostrea virginica): implications for using bivalves as bioindicators of microplastic pollution. Environ. Sci. Technol. 53, 8776–8784. https://doi.org/10.1021/acs.est.9b02073.

Widdows, J., Pope, N.D., Brinsley, M.D., Gascoigne, J., Kaiser, M.J., 2009. Influence of self-organised structures on near-bed hydrodynamics and sediment dynamics within a mussel (Mytilus edulis) bed in the Menai Strait. J. Exp. Mar. Bio. Ecol. 379, 92–100. https://doi.org/10.1016/j.jembe.2009.08.017.

Wildish, D.J., Miyares, M.P., 1990. Filtration rate of blue mussels as a function of flow velocity: preliminary experiments. J. Exp. Mar. Bio. Ecol. 142, 213–219. https://doi.org/10.1016/0022-0981(90)90092-Q.

Zhao, S., Ward, J.E., Danley, M., Mincer, T.J., 2018. Field-based evidence for microplastic in marine aggregates and mussels: implications for trophic transfer. Environ. Sci. Technol. 52, 11038–11048. https://doi.org/10.1021/acs.est.8b03467.