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Microplastic changes the sinking and resuspension rates of marine mussel biodeposits

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ABSTRACT

Microplastic (MP; < 5 mm) is ubiquitous in marine environments and is likely transported by biotic benthic-pelagic coupling. Mussels are key benthic-pelagic couplers, concentrating particles from the water column into dense and nutrient rich biodeposits. This study examined how MP affects benthic-pelagic coupling processes of mussels exposed to feeding regimes with and without MP by measuring four attributes of biodeposits: 1) morphology, 2) quantity of algal and MP particles, 3) sinking rate, and 4) resuspension velocity. We found interacting effects of particle treatment and biodeposit type on biodeposit morphology. Biodeposits from the algae treatment contained more algal cells on average than biodeposits from the MP treatment. Biodeposits from the MP treatment sank 34–37% slower and resuspended in 7–22% slower shear velocities than biodeposits from the algae treatment. Decreases in sinking and resuspension velocities of biodeposits containing MP may increase dispersal distances, thus decreasing in-bed nutrient input and increasing nutrient subsidies for other communities.

1. Introduction

Plastic is a global anthropogenic pollutant, pervasive across marine systems, and projected to increase in the future (Galloway and Lewis, 2016; Jambeck et al., 2015). It is estimated that only 9% of the plastic produced is recycled (Geyer et al. 2017), and as a result, much of it ends up in waterways via rivers and effluent from coastal populations (Jambeck et al., 2015). Microplastic (MP, 1 μm – 5 mm; Arthur et al., 2009; Hartmann et al., 2019) is a leading source of pollution in marine environments (up to 100,000 particles m^{-3}; Wright et al., 2013) and acts as a sponge and transportation vector for toxics and persistent organic pollutants (Mato et al., 2001; Rios et al., 2007; Engler, 2012; Avio et al., 2015).

Microplastics are ubiquitous in the environment, have been found on surface waters, throughout the water column, and in benthic sediment (Song et al., 2018; Choy et al., 2019). Microplastics are likely transported from surface waters to benthic habitats by biotic and abiotic mechanisms similar to those responsible for plankton transportation and benthic-pelagic coupling. Due to their small size and presence throughout the water column, MP is ingested by numerous animals from multiple functional groups, which can negatively impact physiology (e.g. growth, immune response, and fecundity; Wright et al., 2013; Rist et al., 2016). Ingestion and subsequent digestion and/or excretion can thus affect both the animals and their benthic-pelagic coupling functions.

Mussels are key organisms in benthic-pelagic coupling in both marine and freshwater systems (Graf, 1992; Strayer et al., 1999). As suspension-feeders, mussels are capable of sorting particulate matter based on size, roughness, and chemical composition (Ward and Shumway, 2004; Rosa et al., 2017). As mussels filter and remove particles from the water column, they provide benthic organisms with pelagic resources, such as food and nutrients, that are otherwise unavailable. As an example, mussels concentrate particulate matter into biodeposits that are dense and nutrient rich, thus linking bottom substrate (benthic) to the water column (pelagic; Newell, 2004). However, particles brought into the mussel through the intake siphon are not necessarily ingested—they are size-sorted by the ctenidia (modified gills) and further sorted for preferential ingestion by the labial palps. Particles are either excreted prior to ingestion as pseudofeces or are digested then egested as feces. Both types of mussel biodeposits can concentrate nutrients and particles from the water column that may not otherwise be readily available to benthic organisms (Norkko et al., 2001; Ward et al., 2019).

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Nutrient and particle transport involves more than just water filtration, however. Mussels alter rates of biodeposition and bioresuspension through siphon expulsion (pushing biodeposits away from substrate) and dampening near-bottom hydrodynamics (flow rates decrease within inter-mussel space; Graf and Rosenberg, 1997; Norkko et al., 2001; Carrington et al., 2009). Further, the rate of biodeposition and bioresuspension is also dependent on biodeposit composition and morphology (e.g. Cole et al., 2016). Mussel biodeposits that contain MP, which are typically positively or neutrally buoyant, may sink and resuspend at different rates thus changing the benthic-pelagic coupling functions of mussels (previously documented in zooplankton and larvaceans; Cole et al., 2016, Katija et al., 2017).

Suspension feeding invertebrates ingest a higher quantity of MP compared to other invertebrates (Setälä et al., 2016), and specifically, mussels are known to ingest MP globally with largely unknown long-term consequences (Li et al., 2019). Microplastic concentrations may be influenced by proximity to urban industries and coastlines (Li et al., 2015; Song et al., 2018), which are prominent mussel habitats. As MP become more prevalent in our waters, they may also become more prevalent in mussel diets and biodeposits and thus more readily available to benthic communities that do not usually experience positively buoyant particles like MP (Cole et al., 2016; Katija et al., 2017).

This study focuses on how MP affects aspects of the benthic-pelagic coupling functions of marine mussels, well-known suspension-feeders and foundation species. Specifically, we used feeding trials to quantify how MP affects the morphology and subsequent sinking and resuspension rates of mussel biodeposits. We exposed mussels to feeding regimes with and without MP and measured four attributes of biodeposits (feces and pseudofeces): 1) morphology, 2) quantity of algal cells and MP particles, 3) sinking rate, and 4) resuspension rate. Due to the size and buoyancy of MP in seawater, we hypothesized that mussel biodeposits containing MP 1) sink at a slower rate and 2) resuspend into the water column at a lower water velocity than biodeposits without MP.

2. Methods

2.1. Mussel collection

Pacific blue mussels (*Mytilus trossulus*; 35 ± 2 mm) were collected from Argyle Lagoon (48.519401, −123.013180) on San Juan Island in Washington State, U.S.A. in August 2019. Byssal threads and epibionts were removed upon collection and mussels were acclimatized for 24 h at 11–13 °C in flow-through seawater tables at Friday Harbor Laboratories (FHL), University of Washington. Mussels were starved in 1 μm filtered seawater (FSW) for 24 h prior to experimentation, ensuring that biodeposits released during trials were associated with experimental feeding treatments (Bayne et al., 1979).

2.2. Feeding treatments

Feeding trials followed the methods from our previous clearance rate experiment (Harris and Carrington, 2019). Two feeding treatments were tested, algae and MP + algae. Both particle feeding treatments and all biodeposit experiments were ran simultaneously each day, multiple times. The *algae* treatment used *Dunaliella* spp., grown in culture at FHL, in concentrations ranging 10,000–20,000 cells mL^{-1} between trials (concentration was consistent within trials; algal concentrations in this range were previously shown not to affect CR; Harris and Carrington, 2019). The *microplastic + algae* (or MP) treatment was the same as the algae treatment, but with the addition of fluorescent violet polyethylene spheres 32–38 μm (Item # UVPMS-BV-1.00; Cosphereic; Harris and Carrington, 2019). The spheres were soaked in Tween-20, a surfactant that reduces hydrophobicity and clumping, for 24 h prior to experimentation. Previous experiments confirm this low concentration of Tween-20 does not affect clearance rate of mussels (Harris and Carrington, 2019). Microplastic concentrations ranged from 0 to 675 particles mL^{-1} (MP concentrations in this range were previously shown not to affect CR; Harris and Carrington, 2019). Additional methods and results with polystyrene spheres are presented as supplemental material.

Mussels were placed in treatment containers (3 L; 1 mussel per container with 1 L of aerated FSW) to feed for 1 h. A control container without a mussel accompanied each treatment trial to measure natural particle sinking. Water samples (1.5 mL) were taken from each container at 0, 30, and 60 min to calculate mussel clearance rate. Particle concentrations were quantified with a flow cytometer (Guava C6, EMP Millipore, Hayward, CA) using a RedR vs side scatter plot where the two types of particles fluoresced at different intensities and granularities. Clearance rates were calculated from change in algal concentrations, not MP concentrations, over time. Clearance rate (CR; Lh^{-1}) was calculated with the static system equation, $CR = \frac{Vb}{nt}$, where V is the volume of water (L), b is the slope of the semi-ln plot of algal concentration (particles mL^{-1}) vs. time (h), n is the number of mussels, and t is total clearance time (h; Coughlan, 1969). Natural algae settlement rate, calculated as the CR for the respective control container, was subtracted from initial CR to calculate mussel CR.

2.3. Biodeposit classification and measurements

Biodeposits and associated mussel were collected and transferred to a 200 mL beaker of FSW after experimental feeding treatments where the mussel continued to excrete biodeposits for an additional 24 h. Biodeposits were then selected from each mussel for one of three experimental measurements: particle quantification, sinking rate, or resuspension velocity (0–16 biodeposits per mussel per measurement; experimental measurement sample sizes in Table 1). The quantity of biodeposits collected from each mussel depended on how many were produced. Selected biodeposits were photographed and measured for length and width using ImageJ and volume was calculated (fecal deposit volumes were calculated as cylinders and pseudofecal deposit volumes were calculated as spheres).

All biodeposit classifications were based on morphology (Fig. 1). Feces were classified as having a fixed width, cylindrical shape, and a ribbed line running down the length (due to size and shape of digestive tract). Generally, feces were browner in color than pseudofeces, regardless of particle treatment. Pseudofeces were classified as having an inconsistent shape, often amorphous with particles loosely packed. Generally, pseudofeces were brighter green in color (undigested algae) than feces and had areas of white or clear mucus.

2.4. Particle quantification

Each biodeposit selected for particle quantification was homogenized with a pipette in a 1.5 mL microcentrifuge tube with 0.5 mL FSW. Algal cells (live and whole) and MP particles were counted in each homogenate using a hemocytometer under a compound microscope. There was no MP contamination in biodeposits from algae treatments.

Table 1
Sample sizes of experimental measurements for each combination of particle treatment and biodeposit type. The quantity of mussels exposed to each particle treatment is listed in parentheses and the quantity of each biodeposits measured is listed by type and experiment.

Treatment and experiment	Pseudofeces	Feces
Algae (mussels = 41)		
Quantification	30	53
Sinking rate	32	80
Resuspension	51	80
MP + algae (mussels = 101)		
Quantification	130	168
Sinking rate	125	138
Resuspension	77	238

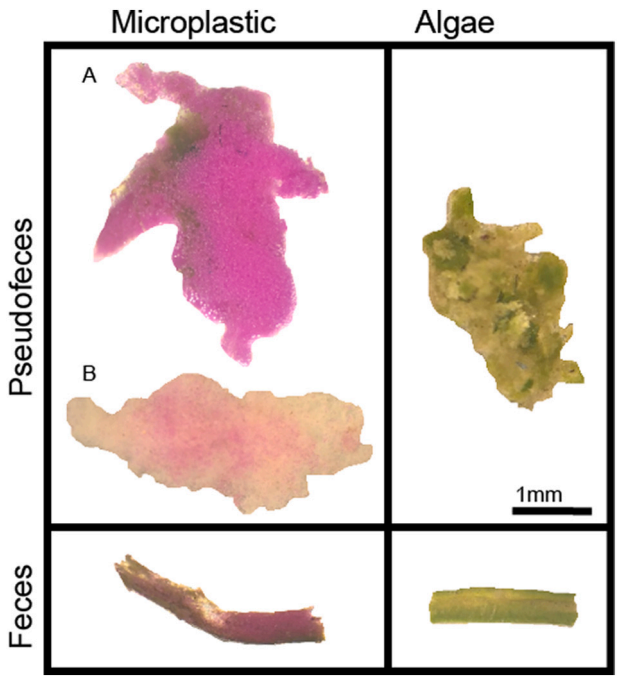

Fig. 1. Examples of biodeposits (pseudofeces and feces) illustrating morphological differences between the microplastic (MP + algae) and algae treatments. Pseudofeces were generally amorphous, containing whole algal cells and MP particles. Pseudofeces with MP were observed with (A) condensed and (B) loose mucus matrices. Feces were generally more compact, with a relatively consistent width (due to gut size).

2.5. Sinking rate

Sinking experiments were conducted in a 1 L graduated cylinder filled with FSW at 13 °C. Biodeposits were placed a few centimeters below the water surface to avoid complications with surface tension. Biodeposits were initially allowed to sink 10 cm to reduce initial turbulence and to reach terminal velocity, which was measured as the time to sink an additional 10 cm. Approximately ¼ of pseudofeces from the MP + algae treatment floated and were not included in this assay.

Fecal deposit density was calculated using Stokes law, assuming a cylindrical shape: $\rho_c = \frac{w_s \mu \left(\frac{L}{D}\right)^{1.664}}{0.079 g L^2} + \rho$, where w_s is terminal velocity (m s^{-1}; sinking rate), μ is water viscosity (kg m^{-1}), L is length of fecal deposit (m), D is diameter of fecal deposit (m), g is the gravitational constant (m s^{-2}), and ρ is density of water (kg m^{-3}). Pseudofecal deposit density was also calculated using Stokes law, assuming a spherical shape: $\rho_s = \frac{18 w_s \mu}{g D^2} + \rho$, where D is the diameter of pseudofecal deposit (m) (Komar et al., 1981).

Drag was calculated by the equation $F_D = \frac{1}{2} C_D \rho_b A_C {w_s}^2$, where C_D is the drag coefficient (1.15 for a short cylindrical fecal deposit, 0.47 for a spherical pseudofecal deposit; Hoerner, 1958), ρ_b is biodeposit density (calculated above; kg m^{-3}), A_C is biodeposit cross-sectional area (m^2), and w_s is biodeposit terminal velocity (m s^{-1}; sinking rate).

2.6. Resuspension velocity

Mussel biodeposit resuspension velocity was measured in a flume (Rolling Hills Water Tunnel 2436; El Segundo, CA) filled with seawater held at 11–13 °C and flow was manipulated by an external computer. Twenty-four biodeposits were placed 6 cm apart from each other in a 4 × 6 grid pattern at the bottom of the flume working section (40 cm × 40 cm × 2 m, width x height x length). Shear velocity (u_*; cm s^{-1}) was estimated as 10% of free stream velocity (u; cm s^{-1}; Denny 2016). Free stream velocity was ramped up to 3 cm s^{-1} (shear velocity of 0.3 cm s^{-1}) for 10 min and the biodeposits remaining were recorded. This procedure was repeated at progressively higher velocities, up to 64 cm s^{-1} (shear velocity of 6.4 cm s^{-1}) or until all biodeposits left the grid and were resuspended. Some pseudofeces from the MP + algae treatment floated before resuspension trials started and were not included in this assay.

Cumulative probability of resuspension was calculated as a dose-response curve with weighted Weibull I function, $y = c + (d - c) * exp^{-exp * b(\log(x) - \log(e))}$, where y is probability of resuspension, b is steepness of the dose-response curve, c is the lower asymptote, d is the upper asymptote, e is the threshold resuspension (velocity at which 50% of biodeposits resuspended), and x is the shear velocity (Ritz et al., 2015).

2.7. Analysis

All data analyses and graphs were made with computing software R for Mac OS X (version 3.6.2, R Core Team, 2019). Level of significance was set at $\alpha < 0.05$. Homogeneity of variance was confirmed with the Bartlett test and length, width, and volume were natural log transformed for all statistical tests due to the non-normal distribution of the data (Shapiro-Wilk's test). A t-test was used to analyze the difference in clearance rate between particle treatments and linear regression was used to test for an effect of particle concentration on clearance rate.

Biodeposit length, width, volume, biodeposit algal cell concentration, sinking rate, density, drag, and resuspension velocity were analyzed with linear mixed-effects models, where particle treatment (algae and MP + algae) and biodeposit type (feces and pseudofeces) were main effects, their interaction was included, and mussel ID was a random effect. Differences between particle treatment and biodeposit type were evaluated using post-hoc tests (paired contrasts with Bonferroni adjustment). Biodeposit MP particle concentration was evaluated with a linear mixed-effects model with biodeposit type as a fixed effect and mussel ID as a random effect. Weighted Weibull I was used to analyze the dose response curve for shear velocity on the cumulative proportion of resuspended biodeposits. Threshold resuspension (velocity at which 50% of biodeposits resuspended) was calculated for each particle treatment and biodeposit type from the weighted Weibull distributions.

3. Results

Clearance rate did not differ between the two particle treatment groups ($p = 0.4$; t-test) nor was clearance rate dependent on algal or MP concentrations ($p = 0.27$ and $p = 0.28$, respectively; linear regression; data not shown). Average clearance rates for mussels in the algae and MP + algae treatments were 1.6 and 1.4 L h-1, respectively (data not shown).

Biodeposit length was not dependent on particle treatment, biodeposit type, nor the interaction ($p > 0.08$; linear mixed-effects model; Fig. 2a; Table 2). Biodeposit width was dependent on particle treatment ($p = 0.03$) and biodeposit type ($p < 0.001$) and there was no interaction between these effects ($p = 0.74$; linear mixed-effects model; Fig. 2b; Table 2). Specifically, pseudofeces were 59–73% wider than feces and biodeposits from the algae treatment were 7–15% wider than biodeposits from the MP + algae treatment. Biodeposit volume was dependent on the interaction between particle treatment and biodeposit type ($p < 0.001$; linear mixed-effects model; Fig. 2c; Table 2). Pseudofeces were approximately 45% larger than feces, and this difference was amplified in the MP treatment.

Algal cell concentration in biodeposits was dependent on the interaction between particle treatment and biodeposit type ($p = 0.04$; linear mixed-effects model; Fig. 3a; Table 3). Biodeposits from the algae particle treatment contained 1.7–1.9 times more algal cells than biodeposits from the MP + algae particle treatment. Microplastic particle concentration in biodeposits was dependent on biodeposit type ($p = 0.001$; linear mixed-effects model; Fig. 3b; Table 3), where pseudofeces

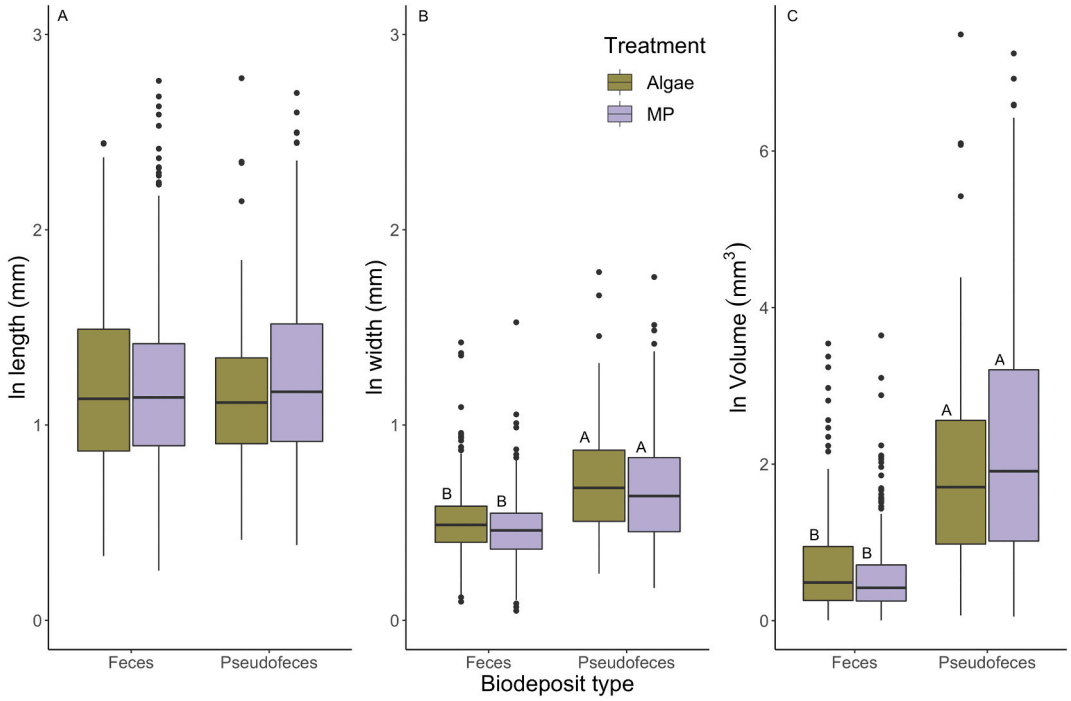

Fig. 2. Morphometric measurements [A) ln length, B) ln width, and C) ln volume] of all biodeposits pooled from the three experiments (quantification, sinking, and resuspension). Green (left) represents the algae treatment and purple (right) represents the MP + algae treatment. Boxes represent upper and lower quartiles and dots represent outliers; solid lines within boxes represent median values. The different letters indicate statistical differences within each morphometric measurement ($p < 0.05$; paired contrasts with Bonferroni adjustment). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary of linear mixed-effects model analyses for biodeposit morphology reported as Type III ANOVA tables. Separate analyses were conducted for biodeposit length, width, and volume. 1202 biodeposits from 129 mussels from all experiments were pooled and were included in these analyses. *P* values estimated through *t*-tests using the Satterthwaite's method. Bold type and asterisk (*) indicates statistical significance ($p < 0.05$).

Dependent variable	Factor	Num DF	Den DF	F value	p value
ln length	Treatment	1	122.57	0.00	0.98
	Biodeposit type	1	1192.88	−0.16	0.69
	Treatment x Type	1	1192.88	3.10	0.08
ln width	**Treatment**	**1**	**109.26**	**4.80**	**0.03***
	Biodeposit type	**1**	**1197.03**	**193.88**	**<0.001***
	Treatment x Type	1	1197.03	0.11	0.74
ln volume	Treatment	1	119.4	0.07	0.79
	Biodeposit type	**1**	**1197.7**	**480.87**	**<0.001***
	Treatment x Type	**1**	**1197.7**	**14.14**	**<0.001***

contained 87% more MP particles than feces.

Biodeposit sinking rate was dependent on particle treatment ($p < 0.001$), and biodeposit type ($p < 0.001$), and there was no interaction between these effects ($p = 0.49$; linear mixed-effects model; Fig. 4a; Table 4). Pseudofeces sank 37–49% slower than feces and biodeposits from the MP + algae treatment sank 34–37% slower than biodeposits from the algae treatment.

Biodeposit density was dependent on biodeposit type ($p < 0.001$) but not on particle treatment ($p = 0.97$), nor the interaction between these effects ($p = 0.60$; linear mixed-effects model; Fig. 4b; Table 4). Feces were 4% more dense than pseudofeces in both particle treatments. Drag was dependent on the interaction of particle treatment and biodeposit type ($p = 0.01$; linear mixed-effects model; Table 4), where pseudofeces from the MP + algae treatment had 2.4–3.7 times more drag than other biodeposits from both treatments.

Biodeposit resuspension velocity was dependent on particle treatment ($p = 0.001$), biodeposit type ($p = 0.01$), and there was no interaction between these effects ($p = 0.1$; linear mixed-effects model; Fig. 5a; Table 4). Pseudofeces resuspended in 4–19% slower shear velocities than feces, and biodeposits from the MP + algae treatment resuspended in 7–22% slower shear velocities than biodeposits from the algae treatment. Resuspension threshold, where 50% of biodeposits resuspended, ranged 0.96–1.03 cm s^{-1} for feces and 0.76–0.95 cm s^{-1} for pseudofeces (MP + algae and algae, respectively; shear velocity; Weibull I distribution; Fig. 5b). For all biodeposits, the cumulative probability of resuspension increased dramatically between 0.5 and 1.5 cm s^{-1} (shear velocity).

4. Discussion

Mussels readily filtered, ingested, and egested algae and microplastic (MP), demonstrating their ability to transport particles between pelagic and benthic habitats. When mussels fed on MP, their biodeposits sank slower and resuspended more readily than biodeposits from the algae only diet. Together, lower sinking and resuspension velocities may result in biodeposits spending more time in the water column, settling further away from mussels, and fewer particles reaching benthic habitats.

Changes in biodeposit morphology due to MP may explain decreases in sinking rate which was dependent on biodeposit type and particle treatment (Figs. 4 and 5). Biodeposit density was dependent on biodeposit type rather than particle treatment, where feces were 4% more dense than pseudofeces for both particle treatments (Fig. 4b). These results may be due to the mucus matrix holding particles together in pseudofeces, occupying volume that is otherwise condensed and digested particles in feces. We observed that mucus matrices appeared more

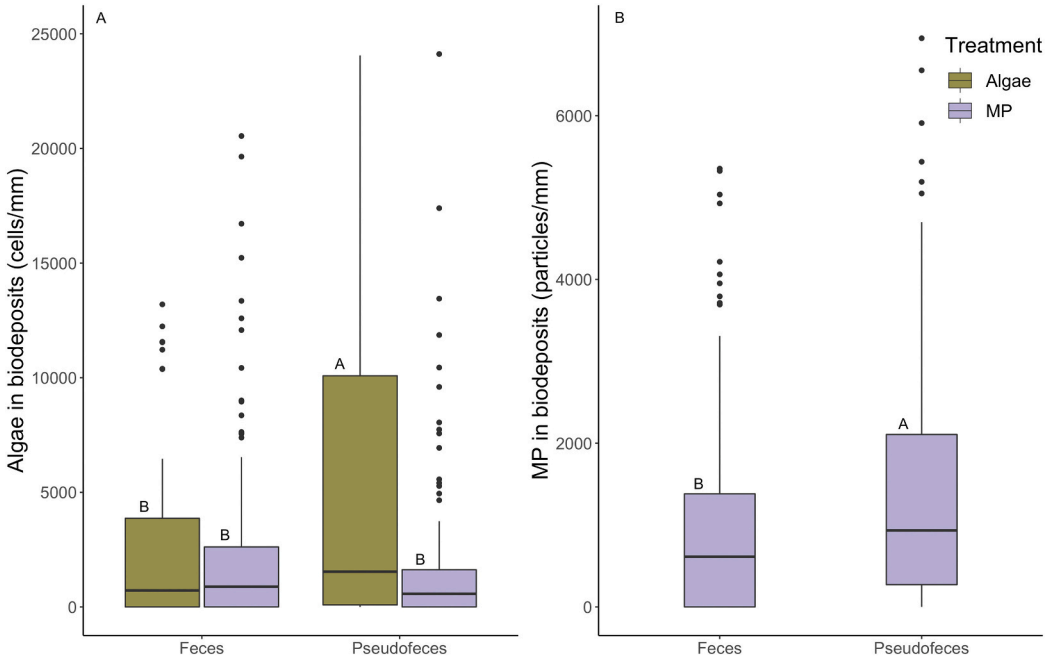

Fig. 3. Quantitative measurements of A) algal cells and B) MP particles in biodeposits. Green (left) represents the algae treatment and purple (right) represents the MP + algae treatment. Boxes represent upper and lower quartiles and dots represent outliers; solid lines within boxes represent median values. The different letters indicate statistical differences within particle types ($p < 0.05$; paired contrasts with Bonferroni adjustment). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Summary of linear mixed-effects model analyses for biodeposit particle concentration reported as Type III ANOVA tables. 381 biodeposits from 102 mussels (from both treatments) were included in the algal cells mm^{-1} analysis and 298 biodeposits from 77 mussels (only from the MP + algae treatment) were included in MP particles mm^{-1} analysis. P values estimated through t-tests using the Satterthwaite's method. Bold type and asterisk (*) indicates statistical significance ($p < 0.05$).

Dependent variable	Factor	Num DF	Den DF	F value	p value
Algal cells mm^{-1}	**Treatment**	**1**	**116.08**	**5.80**	**0.02***
	Biodeposit type	**1**	**366.98**	**8.36**	**0.004***
	Treatment x Type	**1**	**366.98**	**4.50**	**0.04***
MP particles mm^{-1}	**Biodeposit type**	**1**	**293.33**	**11.02**	**0.001***

often in pseudofeces from the MP + algae treatment, which may enhance the buoyant effects of MP. Only mussels that were fed MP produced pseudofeces that floated (observed in both sinking and resuspension experiments), suggesting MP increased buoyancy through either their own buoyancy and/or promoting mucus production. In these cases, MP prohibited pseudofeces from reaching benthic habitats and thus have the potential to negatively affect elements of benthic-pelagic coupling roles of mussels. Microplastics may alter more than just morphology and density of mussel biodeposits in capacities we did not measure, however. Possible explanations for this may be changes in digestion speed, nutrient assimilation, or biodeposit composition (e.g. Prins et al., 1991; Ward and Kach, 2009; Cole et al., 2016; Harris and Carrington, 2019; Ward et al., 2019).

While a complete understanding of the aggregate effects of MP on benthic-pelagic coupling is beyond the scope of this study, we can estimate the combined effects of MP on the processes we did observe using the hypothetical scenario illustrated in Fig. 6. Mussel biodeposits provide important nutrients and particles to other organisms in mussel beds, increasing biodiversity within close vicinity (Norkko et al., 2001). If in-bed biodeposit retention decreases, it could have undesirable impacts on adjacent communities. Conversely, if biodeposit dispersal distance increases, it could become a spatial subsidy for communities further away.

Changes in sinking rate can be used to calculate how far biodeposits travel in currents before settling onto benthic substrates (Fig. 6). Biodeposit horizontal displacement can be calculated as $dx = V_x * dt$ and vertical displacement can to be calculated as $dy = V_y * dt$, where V_x is the free stream velocity (current; cm s^{-1}), V_y is the vertical velocity (ejection or sinking velocity; cm s^{-1}), and dt is the change in time (s). Combining these two equations and given both initial and temporary upward ejection velocity V_{y1} (upward force is only present while the biodeposit is close to both the mussel's mantle and exhalent siphon), and downward sinking velocity V_{y2}, we can solve for a change in horizontal displacement as $dx = V_x\left(\frac{dy_1}{V_{y1}} + \frac{dy_2}{V_{y2}}\right)$, where y_1 is the upward distance and velocity caused by ejection and y_2 is the downward distance and velocity caused by sinking.

Examining one dispersal distance scenario, we estimated the following parameters: Vy_1 as an ejection velocity of 5.46 cm s^{-1} (for mussels 3.5 ± 0.5 cm; Riisgard et al., 2011), dy_1 as an ejection distance of 1 cm upward (based on Miller et al. 2002), and dy_2 as vertical sinking distance of 4.5 cm (average height of experimental mussels +1 cm). We used a free stream velocity (V_x) of 10 cm s^{-1} and used experimental averages for sinking velocity (V_{y2}; Fig. 4). In this scenario, biodeposits from the MP + algae treatment travelled 34–110% further than biodeposits from the algae treatment (Fig. 6). Pseudofeces contained more MP particles than feces, and are calculated to disperse further away from mussel bed communities. Increased dispersal distance can lead to increased transport of both algal cells as well as MP particles. Communities further away from mussel beds may experience an increase in nutrient subsidies in addition to MP pollution. In wild habitats mussels experience a wide variety of wave action and velocity, varying the net effect of MP on dispersal distance, in-bed nutrients, and benthic-pelagic coupling.

The above scenario is a simplification of the multitude of forces that act upon mussel biodeposits in the wild and does not include resuspension velocity or resuspension threshold. If biodeposits are ejected into free stream velocities that are higher than the velocity needed for resuspension, biodeposits are likely to remain suspended in the water column for an extended period of time (Fig. 6, dashed arrow). Pseudofeces from the MP + algae treatment had the lowest resuspension threshold at a shear velocity of 0.76 cm s^{-1} (free stream velocity of ~7.6 cm s^{-1}) implying that biodeposits from mussels that ingest MP may stay

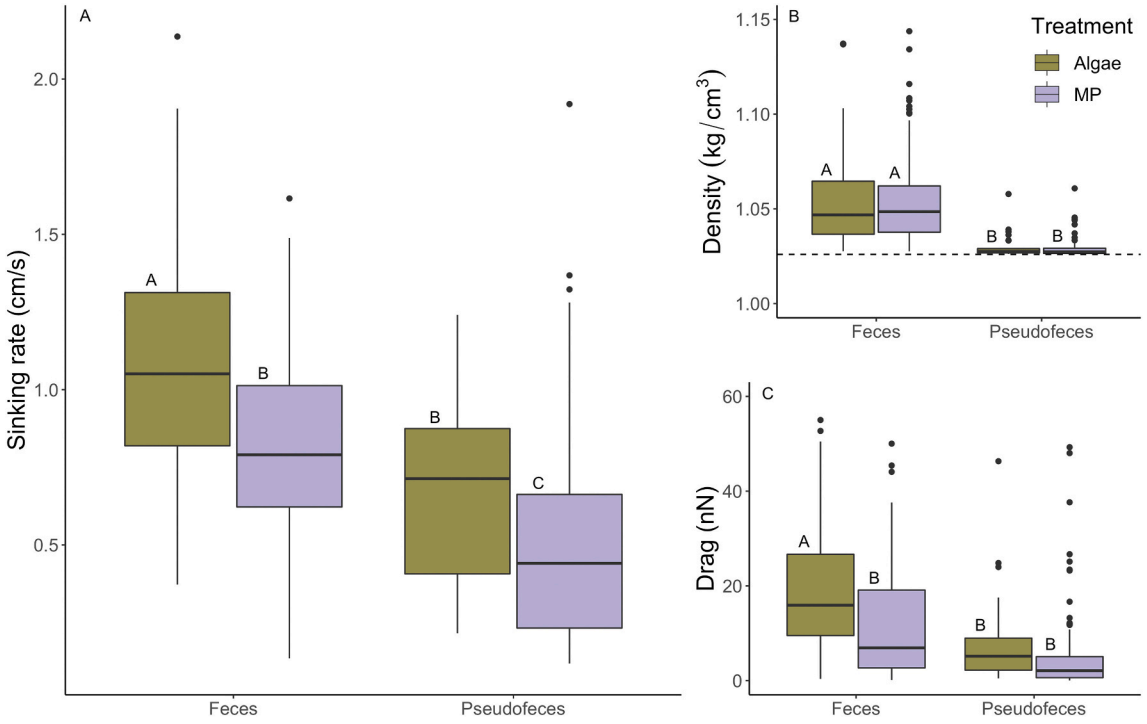

Fig. 4. The impact of biodeposit type and treatment on the A) sinking rate, B) density, and C) drag of mussel biodeposits. Green (left) represents the algae treatment and purple (right) represents the MP + algae treatment. Boxes represent upper and lower quartiles and dots represent outliers; solid lines within boxes represent median values. The different letters indicate statistical differences within dependent measurements ($p < 0.02$; paired contrasts with Bonferroni adjustment). Dashed line in B) represents seawater density at 13 °C for reference. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Summary of linear mixed-effects model analyses for biodeposit sinking, density, drag, and resuspension reported as Type III ANOVA tables. Separate analyses were conducted for each dependent variable: particle treatment and biodeposit type on biodeposit sinking rate, density, drag, and resuspension. The same group of 375 biodeposits from 108 mussels were used in sinking rate, density, and drag analyses. 446 biodeposits from 116 mussels were used in resuspension analysis. P values estimated through t-tests using the Satterthwaite's method. Bold type and asterisk (*) indicates statistical significance ($p < 0.05$).

Dependent variable	Factor	Num DF	Den DF	F value	p value
Sinking rate	**Treatment**	**1**	**119.14**	**24.66**	**<0.001***
	Biodeposit type	**1**	**364.11**	**75.5**	**<0.001***
	Treatment x Type	1	364.11	0.49	0.49
Density	Treatment	1	123.54	0.00	0.97
	Biodeposit type	**1**	**346.7**	**26.91**	**<0.001***
	Treatment x Type	1	346.70	0.27	0.60
Drag	**Treatment**	**1**	**125.48**	**6.03**	**0.02***
	Biodeposit type	**1**	**370.85**	**12.14**	**<0.001***
	Treatment x Type	**1**	**370.85**	**7.61**	**0.01***
Resuspension	**Treatment**	**1**	**69.94**	**11.12**	**0.001***
	Biodeposit type	**1**	**432.93**	**7.03**	**0.01***
	Treatment x Type	1	732.93	2.69	0.10

suspended in the water column longer at low free stream velocities and may be transported further away from the mussel bed.

Ward and Kach (2009) suggest MP cause a false sense of fullness in mussels and remain in the digestive system longer than natural particles, perhaps placing the priority for particle processing and digesting on algal cells. Different types of MP are known to affect the rejection, ingestion, and egestion processes of mussels (Ward et al., 2019). Mussels may experience longer digestion times in the presence of MP, therefore prolonging egestion rates of algal cells and MP particles. Here we suggest mussels, when fed MP, may change their rejection, ingestion, and egestion processes of algal cells as well. This may explain why biodeposits from the MP + algae treatment contained fewer algal cells on average. While this study examined non-aged MP, weathering and biofouling in aquatic environments can alter physical properties of MP (Kowalski et al., 2016) thus changing biodeposit properties in different capacities. We did not measure the rate of biodeposits produced nor the morphology of all biodeposits and suggest future studies to do so with aged and non-aged MP. These future measurements may determine how MP affect: total quantity of biodeposits produced, space limitations in biodeposits (MP particles may displace algal cells), gut retention time, processing time, and processing efficiency.

Mussels are not the only benthic organism to produce nutrient rich biodeposits, however. Sea urchins consume kelp and large detritus, linking pelagic to benthic habitats through messy eating and biodeposits, much like mussels (Dethier et al., 2019). Benthic organisms are generally more efficient at feeding on smaller, finer food particles than larger particles found in the water column (Yorke et al., 2019). Both mussels and sea urchins play a critical role in reducing the size of particles and increasing nutrients in benthic habitats through filter feeding, shredding, and eventual biodeposits (Dethier et al., 2019; Yorke et al., 2019). Mussels, sea urchins, and other organisms with benthic-pelagic coupling functions may be key vectors for MP transport between habitats and functional groups. Here, we demonstrate MP slows mussel biodeposit sinking rates and decreases resuspension velocity, which may lead to a shift in size and quantity of bioavailable benthic food.

Sediment around bivalves has higher concentrations of carbon and nitrogen due to biodeposition, contributing to more diverse macrofaunal communities (Norkko et al., 2001). If biodeposit sinking rates, resuspension velocities, and dispersal distances change due to MP, the concentrations of carbon and nitrogen are likely to decrease in-bed, and

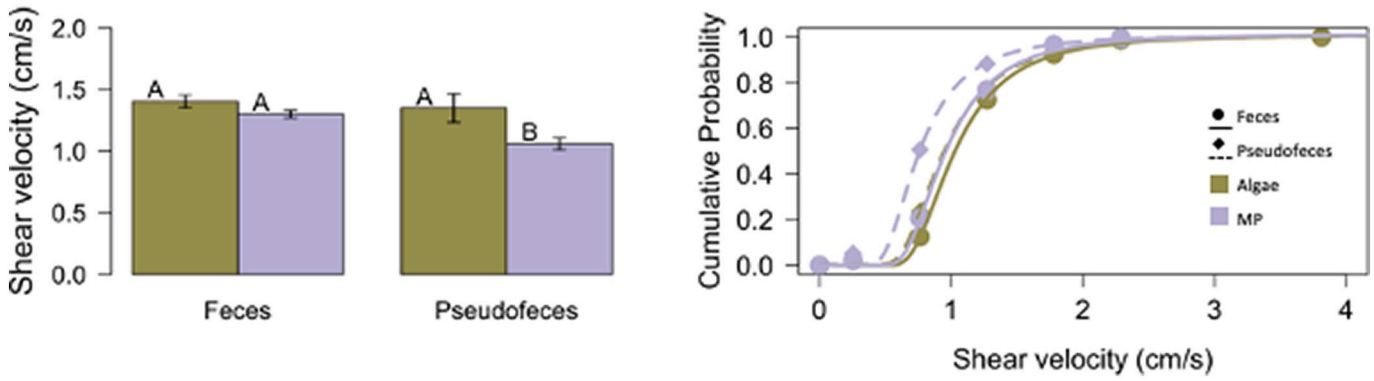

Fig. 5. The effect of biodeposit type and particle treatment on the shear resuspension velocity of mussel biodeposits. A) Resuspension velocities for each particle treatment and biodeposit type, where bars are mean shear velocity and error bars are standard error. The letters indicate statistical difference in shear velocities ($p <$ 001; paired contrasts with Bonferroni adjustment). B) Dose-response curve (weighted Weibull I distribution) where shear velocity is dose and cumulative probability of resuspension is response. Green represents the algae treatment and purple represents the MP + algae treatment, circles and solid lines represent feces and diamonds and dashed lines represent pseudofeces. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

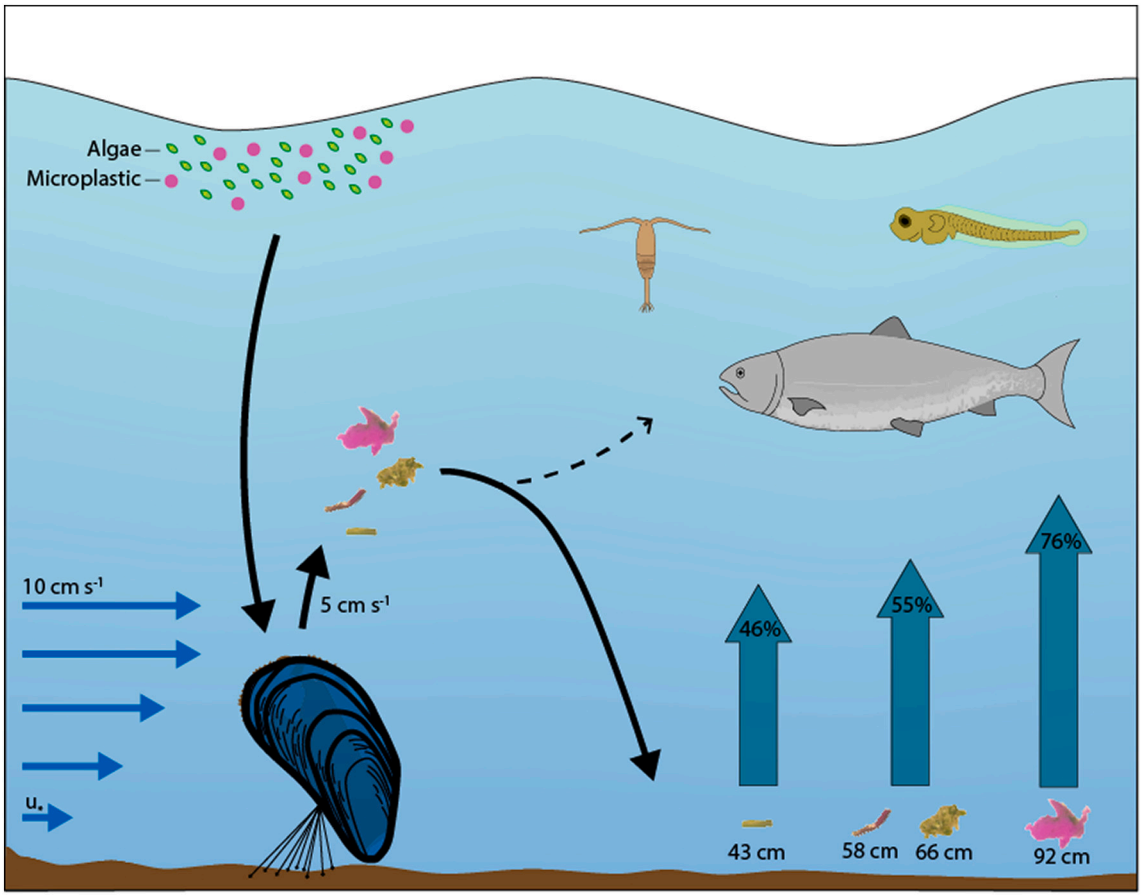

Fig. 6. Conceptual diagram of biodeposit horizontal displacement under experimental sinking velocities and estimated current and ejection velocities. Solid black lines represent known trajectories of biodeposits, including suspension feeding, ejection, and sinking. Dashed black line represents unknown resuspension before sinking occurs. In this scenario with a free stream velocity of 10 cm s^{-1}, mussel biodeposits will travel 43–92 cm away from the mussel. Blue arrows represent 46–76% of biodeposits that will resuspend once settled under this scenario, calculated from weighted Weibull distribution and shear velocity (u_*). Once resuspended into the water column, regardless of mechanism, biodeposits are available for ingestion to pelagic organisms including zooplankton and fish. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

infaunal communities will be affected. Together, sinking and resuspension experiments indicated MP can increase buoyancy, thus creating a mechanism for wide-spread MP dispersal. Other organisms like oysters, barnacles, larvaceans, some fish, and sea urchins contribute to particle and nutrient flux and may also be mechanisms of MP transport to deeper depths. This can give fish, zooplankton, and other pelagic organisms a greater opportunity to ingest small, bio-available, and compact packages of MP. Our findings may help explain how floating or

mid-pelagic MP can be transported across habitats and how the natural biotic pump of microalgal communities, particulate organic matter, and nutrients may be altered by MP. Marine food web analyses may help understand how different organisms contribute to downward particle movement and to what extent nutrient flux may be impacted by MP.

Most quantities of MP tested in this study were higher than environmentally observed concentrations, but they are within the range of expected future concentrations (Jambeck et al., 2015). Previously, studies indicated that the highest concentration of plastics was found in surface waters due to their positive buoyancy (e.g. Cózar et al., 2014; Eriksen et al., 2014). However, recent research indicates high concentrations of MP in the mid-pelagic (i.e. Choy et al., 2019), implying there are likely higher concentrations throughout the water column and available to mussels than previously measured at the surface. Benthic-pelagic coupling organisms may thus play an essential function to MP transport both vertically and laterally through ingestion and egestion mechanisms.

As plastic pollution increases, MP may become more concentrated and bio-available to communities that do not usually experience positively buoyant particles. This study suggests MP changes mussel biodeposit morphology and composition, altering sinking and resuspension rates, and thus changing benthic-pelagic fluxes. Mussels can facilitate trophic transfer of MP through larger and more buoyant biodeposits, which are available for consumption by pelagic organisms. Biodeposits are an important food source for numerous organisms, however, in current and future pollution conditions biodeposits may serve as a vector for MP ingestion to a larger quantity of organisms. Further impacts include MP trophic transfer, bio-magnification and accumulation, and a decrease of infaunal nutrients. Microplastic ingestion is known to cause negative biological consequences and this problem may only get worse as MP dispersal increases.

CRediT authorship contribution statement

Lyda S.T. Harris: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Harsimran Gill:** Investigation, Writing – original draft, Writing – review & editing. **Emily Carrington:** Validation, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2021.112165.

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