Marine microplastic pollution: An interdisciplinary approach to understanding the effects on organisms, ecosystems, and policy

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Abstract

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Microplastics (plastic < 5mm) are ubiquitous in marine environments, from surface waters to benthic sediments. Microplastic in the oceans was first documented in 2004 and our current knowledge of potential biological implications is limited and rapidly growing. Thus far, we know marine organisms are exposed to microplastics in natural settings, ingest microplastics, and experience negative physiological impacts. Many aspects of microplastics such as ingestion fate, extent of trophic transfer, and effect on marine ecosystems remain unknown. Motivated by the need to understand the impact microplastic pollution has on our environment and our lives, I investigated three aspects of the marine microplastic problem: impacts on marine organisms, the ecosystem they support, and the linkages between scientific research and public policy.

In nature, mussels experience a wide range of particle types and concentrations, readily filtering microalgae and abiotic particles other than microplastic. Mussel clearance rate is sensitive to stress, making it a good indicator of stressful conditions and polluted environments. In Chapter 1, I compare mussel (*Mytilus trossulus*) clearance rates when exposed to two different abiotic particles, microplastic and silt, across multiple concentrations. I measure the clearance rates of mussels exposed to increasing concentrations of three particle treatments: Algae, microplastic + algae, and silt + algae. I found that mussel clearance rate was inhibited by high concentrations of microplastics but not silt. In the absence of microplastic, mussel clearance rate was not dependent on the addition of silt, total particle concentration, or algal concentration.

Mussels readily ingest microplastics in natural and laboratory settings, raising concerns about particle fate. Mussels are key benthic-pelagic couplers, concentrating particles from the water column into dense and nutrient rich biodeposits. In Chapter 2, I evaluate how microplastic changes the benthic-pelagic coupling role of marine mussels (*M. trossulus*). I expose mussels to feeding regimes with and without microplastic and measure four attributes of biodeposits: morphology, quantity of algal and microplastic particles, sinking rate, and resuspension velocity. I found biodeposits from the algae treatment contained more algal cells on average than those from the microplastic treatment. Further, biodeposits from the microplastic treatment sank slower and resuspended at slower water velocities than biodeposits from the algae treatment.

To combat plastic pollution, there is sufficient evidence that policies can lead to reduced plastic production and consumption both locally and globally. In Chapter 3, I examine global growth and spread of the marine microplastic field in conjunction with growth and spread of national plastic policies using scientometric and diffusion methods. I conduct systematic literature reviews of marine microplastic papers and national plastic policies through 2019. At a

global level, marine microplastic research and national plastic policies have grown exponentially and remain in the early phases of growth and spatial diffusion. Marine microplastic publication spread at the institution level was best explained by a hybrid of expansion and relocation diffusion, while national plastic policy spread was best explained by expansion diffusion.

Taken together, findings from Chapters 1 and 2 indicate mussels readily filter, ingest, and egest microplastics, demonstrating their ability to transport particles between benthic and pelagic habitats. When exposed to microplastics, decreased clearance rate may result in fewer particles removed from the water column and subsequently available to benthic organisms. Further, decreases in sinking rate and resuspension velocity of biodeposits containing microplastic may increase dispersal distances, thus leading to increased transport of both algal cells and microplastic particles away from mussel beds. While extent of marine microplastic research is not a good indicator of national plastic policies, both the scientific field and national efforts to reduce plastic pollution are spreading globally at exponential rates. Marine microplastic pollution is a local, regional, and global issue that require cross disciplinary attention from researchers and policy makers around the world.

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Dedications

In loving memory of…

My dad, Peter Treistman

His spirit, stories, and photos instilled my love for the ocean (albeit not fishing) and the ever-lasting fashion of bright orange foulies.

My best friend, Alyx Shea

I carry his eccentric spirit, tenacity, and love for weird aesthetics. Thank you for being proud of my science dreams in high school and encouraging pink and purple hair.

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Chapter 1: Impacts of microplastic versus natural abiotic particles on the clearance rate of a marine mussel

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1.1 Abstract

In coastal habitats, mussels are exposed to microplastic (MP; plastic 1µm-5mm) and silt, two abiotic particles that are similarly sized and lack nutrition. The addition of MP or silt may change the functional response of mussels. We measured clearance rate of *Mytilus trossulus* in three particle treatments (algae, MP + algae, and silt + algae) across four concentrations to 1) determine if the effects of MP and silt are similar and 2) disentangle the effects of particle type, particle concentration, and proportion of abiotic particles. Clearance rate decreased by 62% at high MP concentrations (>1,250 particles/ml) but was not affected at equivalent silt concentrations. These findings suggest high MP concentrations inhibit mussel clearance rate, more than expected by changes in particle concentration or the proportion of abiotic particles. As plastic production increases, mussel exposure to MP will increase, potentially reducing energy transfer, benthic-pelagic coupling, and water clarity.

1.2 Introduction

Increased industrialization and urbanization have contributed to increased anthropogenic pollution in coastal habitats, including fertilizers, chemicals, sediment, and microplastics (MP, 1µm-5mm; Arthur 2009; Hartmann et al. 2019). Microplastic is a leading source of pollution, acting as a sponge and a transportation vector for persistent organic pollutants in the ocean (Mato et al. 2001; Rios et al. 2007; Engler 2012; Avio et al. 2015). Organisms from multiple functional groups including suspension-feeders (zooplankton, oysters, mussels), deposit feeders (worms), and free-swimming predators (crabs and fish) ingest MP in laboratory experiments and in natural habitats (e.g. Wright et al. 2013; Frias et al. 2014; Li et al. 2015; Mazurais et al. 2015; Watts et al. 2015; Sussarellu et al. 2016). Of these, suspension-feeding bivalves (mussels and clams) are shown to ingest the highest amount of MP (Setala et al. 2016).

This study focuses on mussels, which are ecosystem engineers and foundation species that feed on microalgae, affect water turbidity, provide habitat heterogeneity, sequester nitrogen, and are vital to the aquaculture industry. Mussel clearance rate (CR) is extremely sensitive to stress (Chandurvelan et al. 2013), making it one of the best biological indicators of stressful conditions and polluted environments (Widdows et al. 1981). Mussels are known to filter and ingest MP in natural habitats (Van Cauwenberghe and Janssen 2014; Li et al. 2016) with unknown long-term outcomes. In short-term laboratory studies (hours to days), however, inert MP elicit negative physiological responses in mussels and other bivalves, including reduced hemocyte production, reduced byssal thread attachment strength, lowered reproductive success, and decreased growth rate of offspring (Browne et al. 2008; Paul-Pont et al. 2015; Rist et al. 2016; Sussarellu et al. 2016; Green et al. 2019). Naturally-occurring and aquaculture-raised

mussels from across the world have been documented to contain MP in tissue, posing potential health problems to ecosystems and humans (e.g. Rochman et al. 2015; Renzi et al. 2018).

In nature, mussels experience a wide range of particle types and concentrations, readily filtering microalgae and abiotic particles other than MP. Often, seston comprises a mix of similarly sized particles including microalgae (< 1-20μm), larger diatoms (2-200μm), and inorganic matter such as silt (2-63μm; Navarro et al. 1996, Ward and Shumway 2004). Smaller MP and silt are similar in many characteristics, including size, and lack of nutritional value. Capturing and processing nutrient poor particles can reduce a mussel's energy budget by increasing feeding costs (sorting abiotic particles) or inducing a false sense of fullness, ultimately leading to less energy allocated to maintenance and growth (Widdows and Johnson 1988; Ward et al. 2019). Silt has been shown to both positively and negatively affect mussel clearance and growth, creating uncertainty in how mussel CR will respond to similar abiotic particles, like MP (e.g. Bayne et al. 1987; Denis 1999; Ward and Shumway 2004*)*.

The effects of abiotic particles are particularly relevant to organisms in coastal habitats where nutrient-poor particles (e.g. silt) are prolific, changing both the total particle concentration as well as seston quality. Typically, the functional response of mussel CR to increasing microalgae concentrations is constant up until a saturation threshold, beyond which CR declines (Figure 1.1; Riisgard et al. 2011). The addition of abiotic particles increases the total particle concentration, but it is unclear if CR is primarily dependent on the aggregate concentration, only the microalgal fraction, or is inhibited by specific types of particles (Figure 1.1). Many MP studies have used extremely high concentrations of MP that often exceed particle saturation and environmental relevance, to test for a threshold effect (e.g. Rist et al. 2016). It is unclear, however, whether the negative physiological responses observed are due to the direct effects of

MP on CR. Negative responses may also be due to the more general effect of increased water turbidity or the proportion of abiotic particles, both of which are known to affect suspension-feeding functional responses (Prins et al. 1991; Riisgard et al. 2011).

Here, we compare algal CR by the mussel, *Mytilus trossulus*, exposed to MP and to silt, a similar abiotic particle, across multiple concentrations. Our research questions were: (1) Do MP and silt particles influence mussel CR similarly? And (2) is mussel CR influenced by the concentration and proportions of microalgae, MP, and silt? We hypothesize (1) mussel CR will be lower in the presence of MP than silt and (2) increasing concentrations and proportions of MP will have a stronger negative effect than increasing concentrations of silt.

1.3 Methods

Mussel collection

Wild Pacific blue mussels, *Mytilus trossulus*, were collected from Argyle Lagoon (48.519401, -123.013180), located on the East side of San Juan Island in Washington State, USA. Individuals with a shell length of 35 ± 2 mm were collected in September - November, 2017 and held in flow through water tables at Friday Harbor Laboratories (FHL), University of Washington. All epibionts and byssal threads were removed prior to experimentation. Mussels were acclimatized at 9-11˚C for a minimum of 48 hours before placement in experimental treatments.

Particle types and concentrations

We measured mussel CR of microalgae (hereafter referred to as algae) as a function of abiotic particle type (MP or silt) and concentration. Note that CR, the volume of water cleared of

particles, has often been used interchangeably with filtration rate (Rosa et al. 2018). All CR trials were conducted in the presence of algae, thus treatments with only algae served as a control for total particle concentration. Each of the three types of particle treatments (algae, MP + algae, and silt + algae) were carried out over a range of particle concentrations. This aimed to control as well as test for the effects of particle concentration, particle type, and proportion of abiotic particles (seston quality; Supplemental Table 1). Total particle concentrations in abiotic treatments were kept within the optimal CR range of 5,000-20,000 particles/ml (Ward et al. 1998; Riisgard et al. 2011). A broader range of algal concentrations (4,000-25,000 particles/ml) was used to control for total particle number. A broader range of abiotic concentrations (0-11,250 particles/ml) was used to examine the effect of the proportion of abiotic particles on CR.

Tween-20, a surfactant, was used to keep MP particles in suspension and was added to all treatments at a concentration of 0.0001%. Preliminary trials confirmed this low concentration of Tween-20 did not affect CR ($p = 0.23$; ANOVA; Supplemental Figure 1; Supplemental Table 2). Preliminary observations of pseudofeces and feces confirmed mussels actively filter, reject, and ingest all particles tested (algae, MP, and silt; Supplemental Figure 2).

The particle treatments were established in 1µm filtered seawater (FSW) as follows (Supplemental Table 1):

Algae: *Dunaliella* spp., grown in culture at FHL, was used due to its size (10-20µm) and chlorophyll fluorescent marker. Mussels were exposed to algal concentrations of 4,000-25,000 cells/ml to test for an effect of particle number on CR independent of abiotic particle type (acted as control). For abiotic particle additions described below, algal concentrations were kept within a constant range (7,000-12,000 cells/ml).

Microplastic + algae: Fluorescent violet polyethylene spheres 32-38µm (Item # UVPMS-BV-1.00 32-38um; Cospheric LLC; Supplemental Figure 3; Mazurais et al. 2015) were soaked in Tween-20 for 12 hours to reduce hydrophobicity before adding to FSW and algae. Microplastic concentrations ranged from 1-2,500 particles/ml, levels that are lower than previously published experiments (e.g. Rist et al. 2016) but do, however, exceed environmental concentrations (Davis III and Murphy 2015; Desforges et al. 2015).

Silt + algae: Silty sediment was collected from Willapa Bay, Washington State from which silt was fractionated to 30-37µm and sterilized in an autoclave. A stock solution of 2.25 x 10^5 particles/ml (counted using a hemocytometer) was diluted to establish concentrations of 1-11,250 particles/ml. Silt concentrations greater than 2,500 particles/ml were used only for analysis of the effect of proportion of abiotic particles in suspension on CR.

Algae and microplastic quantification

Concentrations of algae and MP were quantified with a flow cytometer (Guava C6, EMP Millipore, Hayward, CA), using a RedR vs side scatter plot where the two particle types fluoresced at different intensity levels and granularities (side scatter). Silt did not fluoresce and thus was not counted on the flow cytometer. We categorized MP and silt concentrations into four groups for analyses: Low (1-625), Low-Med (626-1,250), High-Med (1,251-1,875), and High (1,876-2,500 particles/ml).

Measuring Clearance rate, CR

Experimental mussels were starved for 12 hours in 1µm FSW at 9-11˚C. Individual mussels were then placed in 3L plastic containers with 1L FSW and an air stone to circulate and

aerate water. Containers were placed in a 10˚C water bath to maintain constant temperature. Particle treatments were added to each individual container once all mussels were visually identified as open (gaping). A control container with no mussel was used to measure settlement rates of algae and abiotic particles during each set of trials.

Mussels were submerged in treatment containers for one hour. Water samples (1.5ml) were taken every 15 minutes and processed on a flow cytometer to quantify algal concentration over time. Clearance rate, CR, calculations were based solely on the change in algal concentration, not abiotic particles, over time. We used 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 (cells/ml) versus time (hours), n is the number of mussels, and t is total clearance time (hours; Coughlan 1969). Natural settlement rate of algae (control container) was subtracted from initial CR to calculate mussel CR.

Data analysis

All data analyses and graphs were made with computing software R for Mac OS X (version 3.3.3, R Core Team, 2017). Level of significance was set at $\alpha < 0.05$. Trials where CR was negative were not included in statistical analysis (5% of all trials). We confirmed homogeneity of variance with the Bartlett test and square-root transformed CR for all statistical tests due to the non-normal distribution of the data (Shapiro-Wilks test). We randomly chose and ran multiple treatments and concentrations simultaneously each day and pooled data. We used ANCOVA to test for the main and interactive effects of algal cell concentration (covariate) and Tween-20 (fixed effect), on CR. We used a linear regression to test for an effect of algal cell concentrations on CR as well as an effect of abiotic proportion of total particles on CR. We used

a generalized linear model with binomial distribution to test for effects of particle type (MP or silt) and concentration (four levels) on the percentage of mussels feeding. We used two-way ANOVA to test for main and interactive effects of particle type (MP or silt) and concentration (four levels) on CR. We determined significant differences between treatments by post-hoc tests (Tukey's HSD). We used ANCOVA to test for the main and interactive effects of the proportion of abiotic particles suspended (covariate) and particle type (fixed effect) on CR.

1.4 Results

For the algae treatment (cell concentrations ranging 4,000-25,000 cells/ml), mussel CR was highly variable but was not dependent on total particle concentration ($p = 0.08$), the addition of Tween-20 ($p = 0.96$), nor the interaction ($p = 0.73$, ANCOVA; Supplemental Figure 1; Supplemental Table 2). On average, mussel CR was 0.94 ± 0.1L/h ($n = 61$) across all algae + Tween-20 concentrations. Mussels actively filtered (CR > 0.0 L/h) in 95% of trials and the percentage did not depend on particle type or concentration (88-100%; $p > 0.6$, GLM; Figure 1.2).

Mussel CR depended significantly on the interaction between abiotic particle type and concentration ($p = 0.01$, particle type x concentration, two-way ANOVA; Figure 1.3; Table 1.1). Compared to the algae control, high and high-med MP concentrations decreased mussel CR by 62% and 50%, respectively ($p < 0.03$, Tukey's HSD). Low and low-med MP concentrations, however, did not decrease CR ($p > 0.3$, Tukey's HSD). In contrast, mussel CR was unaffected by all silt concentrations tested ($p > 0.2$, Tukey's HSD). Compared to the high silt concentration, high MP concentration decreased mussel CR by 72% ($p = 0.02$, Tukey's HSD). Clearance rate

did not differ between MP and silt treatments for all other concentrations ($p > 0.8$, Tukey's HSD).

There was an interaction between the effects of particle type and the proportion of abiotic particles in suspension on CR ($p = 0.002$, ANCOVA; Table 1.1). Specifically, increasing the proportion of MP particles in suspension significantly decreased CR ($p = 0.0005$, $R^2 = 0.14$, linear regression) while increasing the proportion of silt particles in suspension did not ($p = 0.61$, $R^2 = .01$, linear regression; Figure 1.4; Table 1.1).

1.5 Discussion

Mussel CR was inhibited by high concentrations of MP but not silt, a similarly sized abiotic particle. Only in the high-med and high MP concentrations did mussels slow CR relative to the pure algae treatment (a control for total particle concentration) and only at the high MP concentration did mussel CR slow compared to the equivalent silt concentration. In the absence of MP, mussel CR was not dependent on the addition of silt, total particle concentration, or algal concentration. The proportion of abiotic particles in suspension only affected CR when MP was present.

Total particle concentrations of algae and silt + algae treatments had no effect on CR (Figure 1.3; Supplemental Figure 1), while the effect of high MP concentrations on mussel CR is most likely inhibitory (e.g., curve III in Figure 1.1). The addition of MP essentially lowers the total particle saturation concentration (C_{crit}) at which mussel CR begins to decrease. This inhibition of CR at high MP concentrations reduces the volume of water mussels clear, which in turn reduces their ability to filter turbid water and energy available from food for processes such as growth, reproduction, and metabolism (Bayne 1976). Microplastics may reduce CR at high

concentrations as a result of unique surface properties that affect the filtration process (Hawkins et al. 1997; Ward and Shumway 2004; Rosa et al. 2017; Ward et al. 2019). Our observations of normal CR in low MP concentrations are consistent with previous reports that mussels readily filter and ingest MP in natural settings (e.g. Li et al. 2015, 2016; Renzi et al. 2018). The ingestion of low concentrations of MP and attached toxics may become more readily bioavailable to benthic communities (through biodeposition) and higher trophic levels (through predation).

In silt treatments, mussel CR did not differ between silt + algae and algae control treatments, across concentrations, or across the proportion of abiotic particles in suspension. While the majority of previous studies indicate physiological responses and growth are high under mixed particle diets, it remains unclear if nutrient-poor particles positively or negatively affect mussel CR (e.g. Bayne et al. 1987, Prins et al. 1991*)*. Further studies using higher concentrations of algae and silt + algae are needed to determine the effect of silt on CR saturation (C_{crit}, curve I versus curve II in Figure 1.1). While mussel CR did not change with increasing silt additions, the added cost of handling nutrient-poor particles could reduce available energy to the mussel. There may be an energetic expense in conditions of low seston quality (high proportion of abiotic particles or low quantity of food available) that reduce CR or increase particle selectivity.

While only the very low-end of the low MP concentration tested in this study may be environmentally relevant, it is important to note that the environmental ranges of MP vary with size. Estimated concentrations of larger MP size classes (~330μm) are low and range 0.26 – 9,200 particles/m^3 in the northeast Pacific Ocean (Davis III and Murphy 2015; Desforges et al. 2015). The concentrations of MP particles in the size range presented here are not known,

however, we can hypothesize that these larger particles break into smaller pieces, therefore smaller particles may be more abundant in the ecosystem. As such, higher MP concentrations may be environmentally relevant and more research is needed in this area.

When considering our findings for the assessment of marine MP pollution on intertidal and benthic organisms, we note that this was not a chronic exposure experiment; mussels were exposed to treatments for only one hour. Future studies could determine if CR responses for each concentration are sustained over time, or if there are chronic exposure effects of the abiotic particles. Examining the long-term effects of MP particles in comparison to other abiotic particles will provide deeper insight into the effects of MP on mussel functional responses and other physiological processes, such as growth or reproduction.

It is likely that increased sediment runoff, water turbulence, and plastic production will lead to increased suspended particulate matter, emphasizing the importance of studying biological implications of biotic and abiotic particles (Gallo et al. 2018). This study suggests that mussel CR is not negatively affected at current MP concentrations. Increased levels of MP, however, may inhibit mussel CR and change the quantity of particles and nutrients that cycle between benthic and pelagic environments. Increased MP may therefore have indirect impacts on the coastal ecosystems that suspension-feeding species support.

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Data Availability Statement

The data that support these findings are available in Dryad at https://doi.org/10.5061/dryad.vn92f3j

1.7 References

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1.8 Tables

Table 1.1 Summary of 2-way ANOVA of the effect of abiotic particle type and concentration on CR and a summary of ANCOVA of the effects of abiotic particle type and proportion of abiotic particles in suspension on CR. Abiotic proportion is the concentration of abiotic particles (microplastic or silt) divided by the total particle concentration. Asterisk (*) indicates statistical significance ($p < 0.05$).

Variable	DF	Sum Sq	Mean Sq	F value	p value
ANOVA					
Abiotic particle type	**1**	**0.44**	**0.44**	**5.17**	**0.03***
Abiotic concentration	3	0.56	0.19	2.21	0.09
Particle type x Concentration	**3**	**0.98**	**0.33**	**3.85**	**0.01***
Residuals	99	8.42	0.09		
ANCOVA					
Abiotic particle type	**1**	**0.61**	**0.61**	**6.64**	**0.01***
Abiotic proportion	1	0.31	0.31	3.35	0.07
Particle type x Proportion	**1**	**0.89**	**0.89**	**9.75**	**0.002***
Residuals	150	13.77	0.09		

1.9 Figures

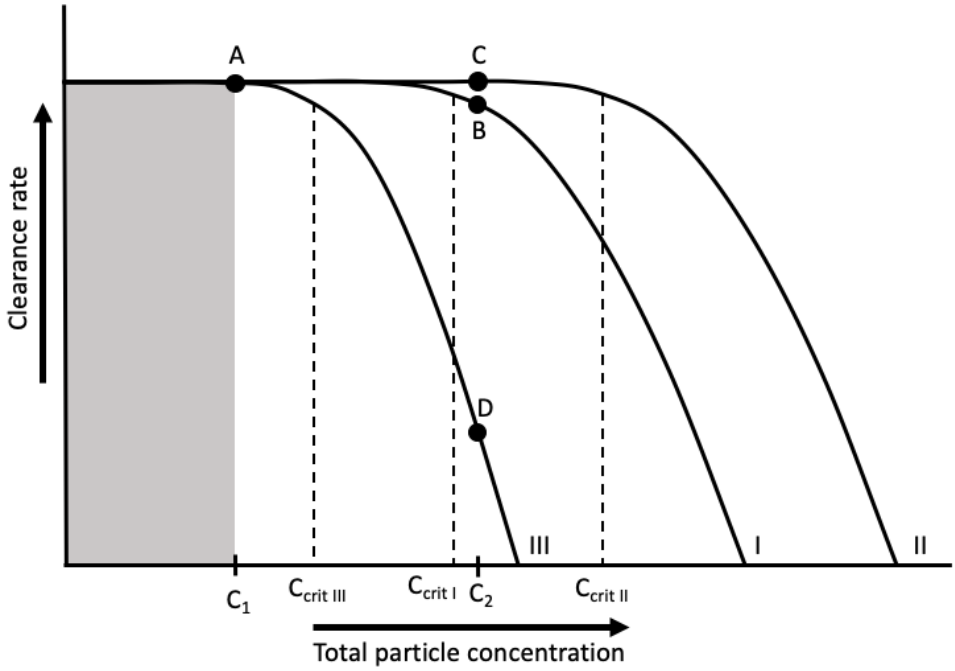


Figure 1.1 Conceptual diagram of functional response curves for mussel CR as a function of total particle concentration under different scenarios of mixed particle suspensions. Grey area represents baseline concentrations with only microalgae present. A typical response to increased microalgal concentration is represented by curve I, where CR is constant at low concentrations, but decreases for concentrations above a critical threshold, $C_{crit\ I}$. If mussel CR is dependent on total particle number, the addition of abiotic particles will follow this response curve (e.g. A → B for an increase from C_1 to C_2). If mussel CR is dependent on only the concentration of microalgae, then the addition of abiotic particles has the effect of shifting the saturation threshold higher ($C_{crit\ II}$). An increase in particle concentration from C_1 to C_2 would not change CR (A → C; curve II). If CR is inhibited by the addition of abiotic particles, then the particle concentration threshold is shifted lower ($C_{crit\ III}$; curve III); Increasing particle concentration from C_1 to C_2 would decrease in CR (A → D).

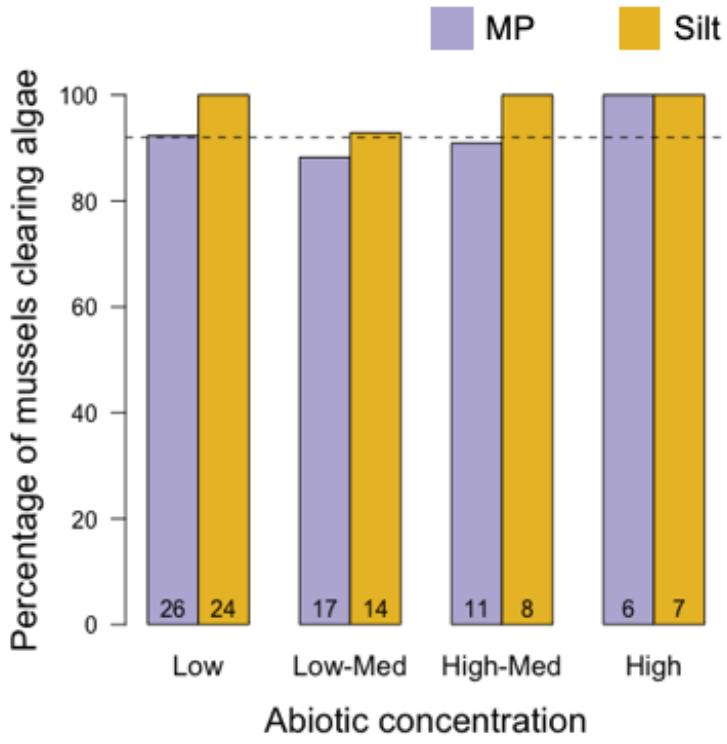


Figure 1.2 Percentage of mussels feeding (CR > 0 L/h) in each abiotic particle treatment as a function of particle concentration. Bars represent absolute percentage of mussels clearing algae across all days. Mussels exposed to control treatment, algae (dashed line), actively cleared algae in 92% of trials. Sample size for each treatment is indicated at the base of each bar. The percentage of mussels suspension-feeding was not affected by particle type or concentration ($p > 0.6$, GLM binomially distributed).

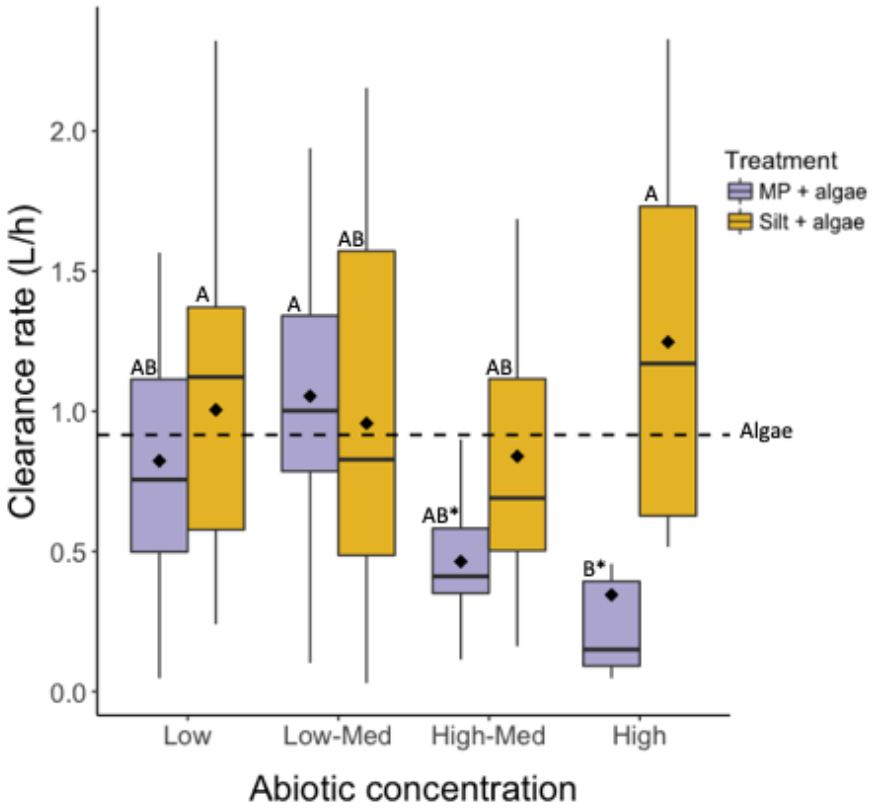


Figure 1.3 Mussel CR as a function of abiotic particle type and concentration. Boxes represent upper and lower quartiles, solid lines within boxes represent median CR, and diamonds represent mean CR. The dashed line represents the mean CR for the algae control treatments across all particle concentrations (0.92 $\pm$ 0.14 L/h). Different letters indicate statistical differences between abiotic treatments within and across particle concentrations. Asterisks (*) indicate a treatment that differed significantly from algae control (dashed line; $p < 0.05$, Tukey's HSD). Sample size ranges 7-26 mussels, see Supplemental Table 1.

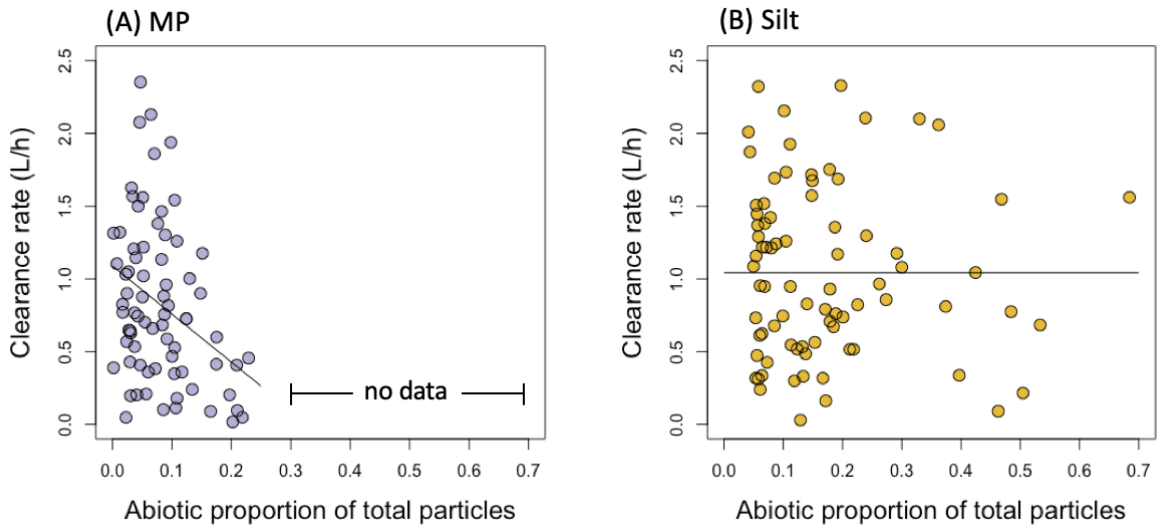


Figure 1.4 Mussel CR as a function of the proportion of abiotic particles in suspension (abiotic particle concentration divided by total particle concentration) for (A) MP and (B) silt. Clearance rate decreased significantly with increasing proportions of MP ($p < 0.001$ $R^2 = 0.14$, linear regression) but not silt ($p = 0.61$, $R^2 = 0.01$). The line for MP (A) is a linear regression and for silt (B) is the average CR across all abiotic proportions (no trend; 1.04 L/h). Clearance rates across increasing abiotic proportions differs between MP and silt ($p = 0.002$, abiotic proportion x particle type, ANCOVA).

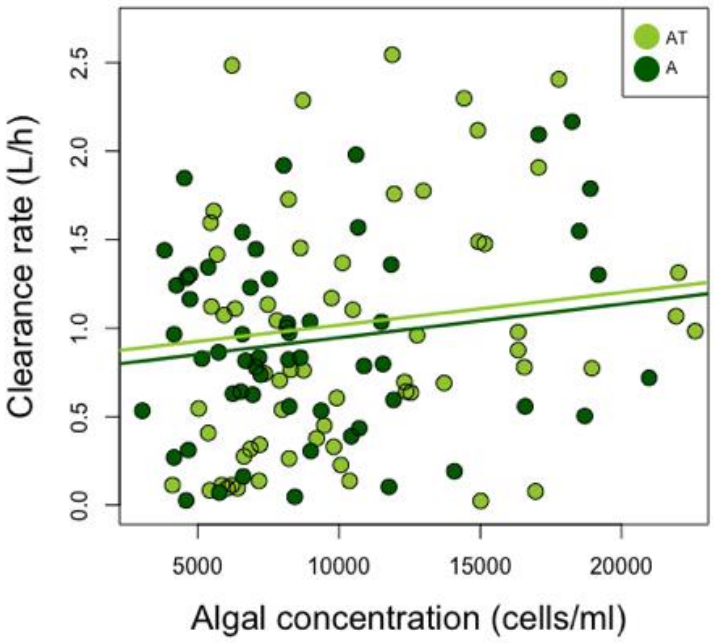
1.10 Supplemental Information

Supplemental Table 1. Matrix of experimental treatments. Different particle treatments and concentrations were processed simultaneously each day and randomized across days. Particle treatment is labeled on the left. Each experimental treatment container was filled with 1L of FSW, 1 mussel, and an experimental treatment. Each experimental treatment was accompanied by a control container with no mussel that accounted for natural particle settlement rate. Algae treatment was tested over a particle range of 4,000-25,000 cells/ml (n = 61). MP + algae treatment consisted of Low (n = 24), Low-med (n = 15), High-med (n = 10), and High (n = 6) concentrations. Silt + algae treatment consisted of Low (n = 24), Low-med (n = 13), High-med (n = 8), and High (n = 7) concentrations. Higher concentrations were tested in the Silt + algae treatment, 2,500-11,250 particles/ml (n = 11), to look at an effect of the proportion of abiotic particles in suspension. Higher concentrations were not tested in the MP + algae treatment due to hydrophobicity of MP particles in high concentrations (would not go into solution).

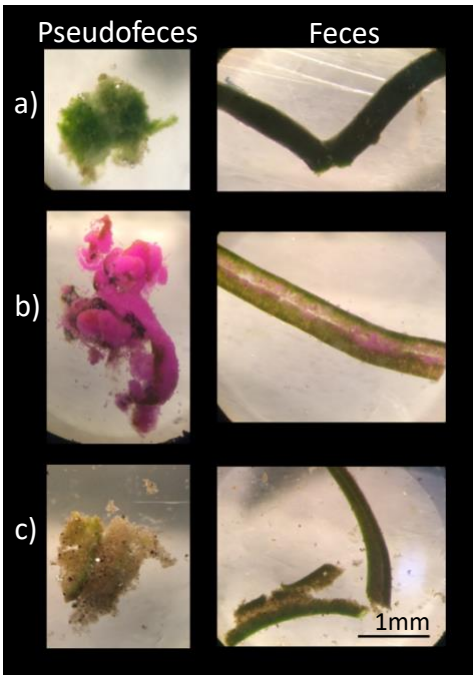
Treatment	Algae (cells/ml)	MP (particles/ml)	Silt (particles/ml)
Algae	4,000 – 25,000 Continuous (n = 61)	0	0
MP +algae	7,000 – 12,000 Continuous	0 – 625 (n = 26)	0
		626 – 1,250 (n = 17)	
		1251 – 1,875 (n = 11)	
		1876 – 2,500 (n = 6)	
Silt + algae	7,000 – 12,000 Continuous	0	0 – 625 (n = 24)
			626 – 1,250 (n = 14)
			1251 – 1,875 (n = 8)
			1876 – 2,500 (n = 7)
			2501 – 11,250 (n =11)

Supplemental Table 2. Summary of ANCOVA of the effect of algal concentrations (4,000-25,000 cells/ml) and Tween-20 on mussel CR.

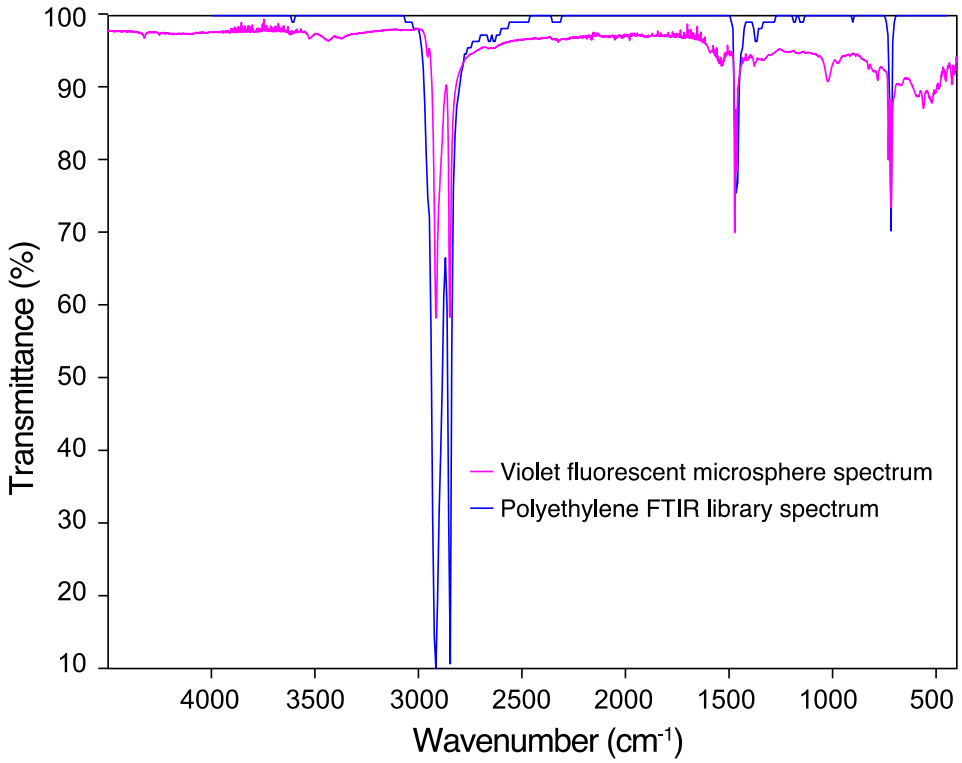
Variable	DF	Sum Sq	Mean Sq	F value	p value
ANCOVA					
Concentration	1	1.43	0.43	3.22	0.08
Tween-20	1	0	$3x10^{-4}$	0.002	0.96
Concentration x Tween-20	1	0.02	0.02	0.12	0.73
Residuals	119	15.73	0.01		



Supplemental Figure 1. Mussel clearance rate as a function of algal concentration, with 0.0001% Tween-20 (AT; light green symbols) and without (A; dark green symbols). Each point represents one individual mussel feeding for one hour. Lines are linear regressions of algae + Tween-20 (AT, $p = 0.19$ and $R^2 = 0.14$, linear regression) and algae (A, $p = 0.23$ and $R^2 = 0.01$, linear regression) treatments. Slopes of CR for algae + Tween-20 and algae treatments did not differ (concentration x presence of Tween-20, $p = 0.73$, ANCOVA).



Supplemental Figure 2. Representative images verifying mussels reject (pseudofeces) and ingest (feces) all experimental particle types. Biodeposits of mussels in a) algae, b) MP + algae, and c) silt + algae treatments. The first column is pseudofeces (filtered and rejected) and the second column is feces (filtered and ingested).



Supplemental Figure 3. Transmittance spectrum of MP beads used in CR experiments, verifying bead composition was polyethylene. Transmittance was measured with a Bruker Vertex 70 Fourier Transform Infrared Spectrometer equipped with an Attenuated Total Reflectance accessory. Violet line represents MP bead and blue line represents known polyethylene transmittance.

Chapter 2: Microplastic changes the sinking and resuspension rates of marine mussel biodeposits

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Keywords: biodeposition, marine, microplastic, foundation species, suspension feeder

2.1 Abstract

Microplastic (MP; < 5mm) 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.

2.2 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; Rist et al. 2016; Wright, Thompson, and Galloway 2013). 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 (Rosa et al. 2017, Ward and Shumway 2004). 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).

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.

2.3 Methods

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 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 hours prior to experimentation, ensuring that biodeposits released during trials were associated with experimental feeding treatments (Bayne et al. 1979).

Feeding treatments

Our 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; concentration previously shown to not affect CR; Harris and Carrington 2019). The ***microplastic + algae*** (or MP) treatment was the same as the algae treatments, 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 – 675 particles mL^{-1} (concentration previously shown to not affect CR; Harris and Carrington 2019). Additional methods and results with polystyrene spheres exposure trials are presented as supplemental material.

Mussels were placed in treatment containers (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 minutes 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 particles, over time.

Clearance rate (CR; L h^{-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.

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 measurements; experimental measurement sample sizes in Table 2.1). 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 (Figure 2.1). 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.

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.

Sinking rate

Sinking experiments were conducted in a 1 L graduated cylinder filled with FSW at 15°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 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: $\rho_c = \frac{w_s \mu (\frac{L}{D})^{1.664}}{0.079 g L^2} + \rho$, 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).

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 x 6 grid pattern at the bottom of the flume working section (40 cm x 40 cm x 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 minutes 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).

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.

Biodeposit length, width, volume, 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 interactions 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.

2.4 Results

Clearance rate did not differ between the two particle treatment groups ($p = 0.4$; t-test); 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; Figure 2.2a; Table 2.2). 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; Figure 2.2b; Table 2.2). 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; Figure 2.2c; Table 2.2). 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; Figure 2.3a; Table 2.3). Biodeposits from the algae treatment contained 1.7-1.9 times more algal cells than biodeposits from the MP + algae treatment. Microplastic particle concentration in biodeposits was dependent on biodeposit type ($p = 0.001$; linear mixed-effects model; Figure 2.3b; Table 2.3), where pseudofeces 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; Figure 2.4a; Table 2.4). 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; Figure 2.4b; Table 2.4). 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 2.4), 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; Figure 2.5a; Table 2.4). 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; Figure 2.5b). For all biodeposits, the cumulative probability of resuspension increased dramatically between 0.5-1.5 cm s^{-1} (shear velocity).

2.5 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 (Figures 2.4 and 2.5). Biodeposit density was dependent on biodeposit type rather than particle treatment, where feces were 4% more dense than pseudofeces for both particle treatments (Figure 2.4b). 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 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 Kasch 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 Figure 2.6. Mussel biodeposits provide important nutrients and particles to other organisms in mussel beds, increasing biodiversity within close vicinity (Norrko 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 (Figure 2.6). 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 $dx = V_x(\frac{dy_1}{V_{y1}} + \frac{dy_2}{V_{y2}})$, 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 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}; Figure 2.4). In this scenario, biodeposits from the MP + algae treatment travelled 34-110% further than biodeposits from the algae treatment (Figure 2.6). 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 (Figure 2.6, 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 suspended in the water column longer at low free stream velocities and may be transported further away from the mussel bed.

Ward and Kacsh (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 digestion 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. We did not measure the rate of biodeposits produced nor the morphology of all biodeposits and suggest future studies to do so. These future measurements may determine how MP affects: 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 (Norrko 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 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 a small, bio-available, and compact package 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, however, 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; Erikson 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 dispersal increases.

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Data Availability Statement

The data that support these findings are available in Dryad at

https://doi.org/10.5061/dryad.nk98sf7s9

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2.8 Tables

Table 2.1 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

Table 2.2 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***

Table 2.3 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***

Table 2.4 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

2.9 Figures

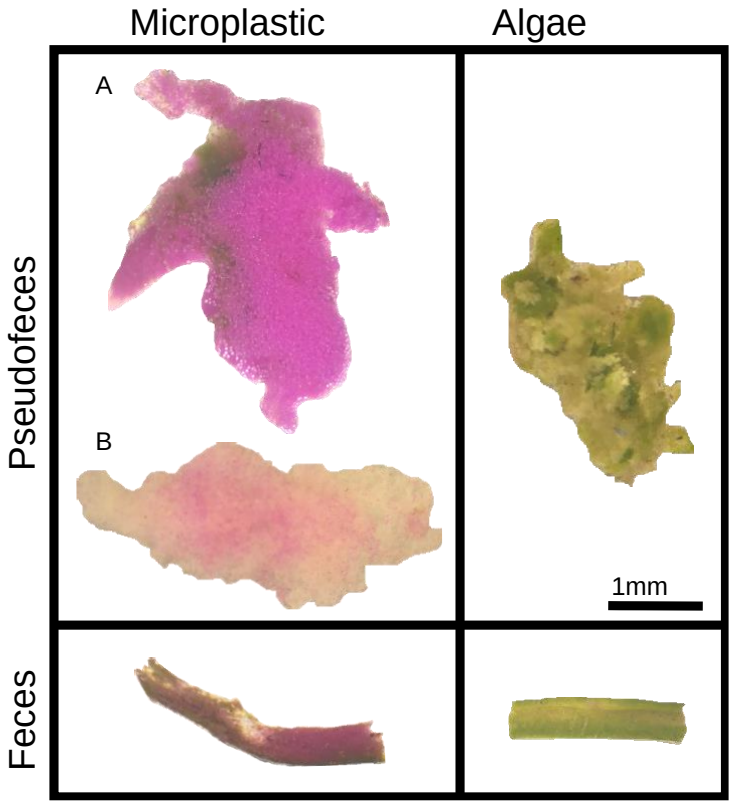


Figure 2.1 Examples of biodeposits (pseudofeces and feces) illustrating morphological differences between the microplastic (MP + algae) and algae treatments. Pseudofeces were generally amorphous, containing whole algal cells and MP particles. Pseudofeces with MP were observed with (A) condensed and (B) loose mucus matrices. Feces were generally more compact, with a relatively consistent width (due to gut size).

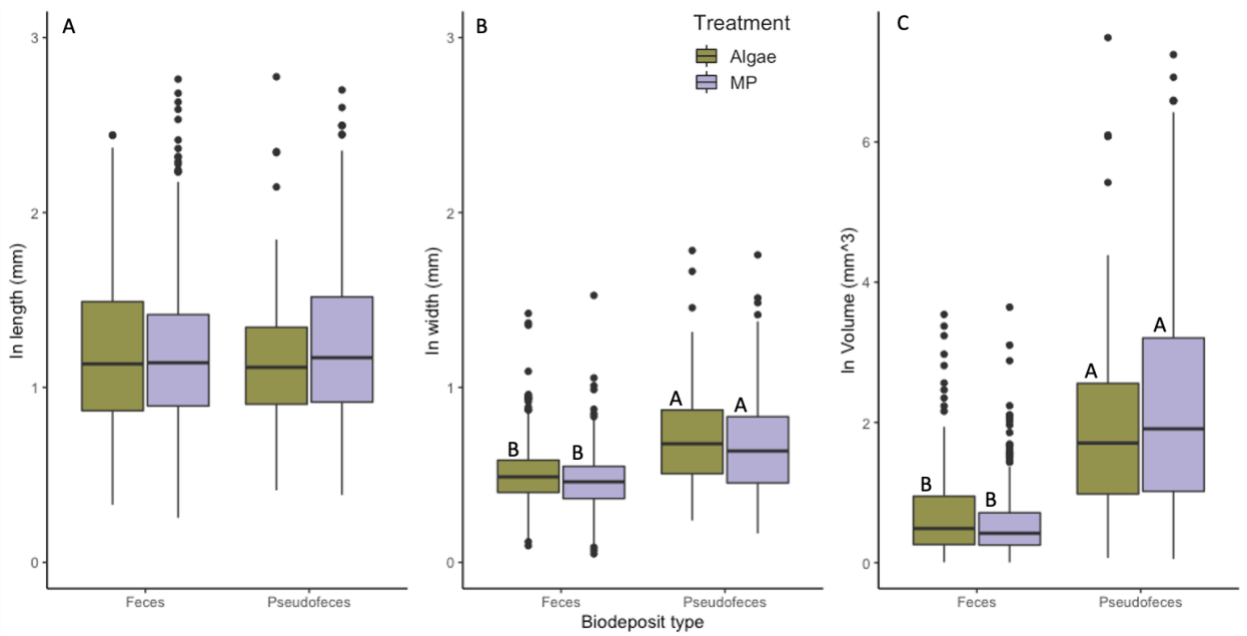


Figure 2.2 Morphometric measurements [A) ln length, B) ln width, and C) ln volume] of all biodeposits pooled from the three experiments (quantification, sinking, and resuspension). Green represents the algae treatment and purple represents the MP + algae treatment. Boxes represent upper and lower quartiles 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).

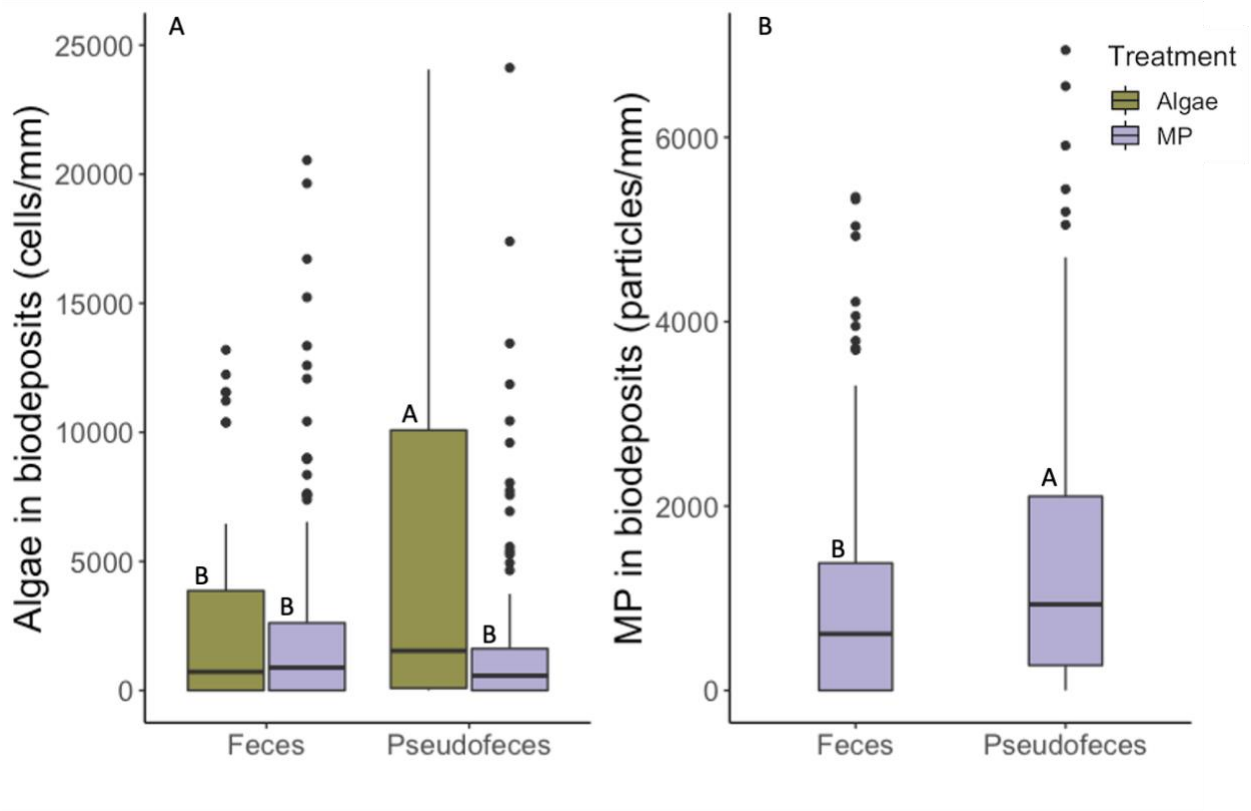


Figure 2.3 Quantitative measurements of A) algal cells and B) MP particles in biodeposits. Green represents the algae treatment and purple represents the MP + algae treatment. Boxes represent upper and lower quartiles and dots represent outliers; solid lines within boxes represent median values. The different letters indicate statistical differences within particle types ($p < 0.05$; paired contrasts with Bonferroni adjustment).

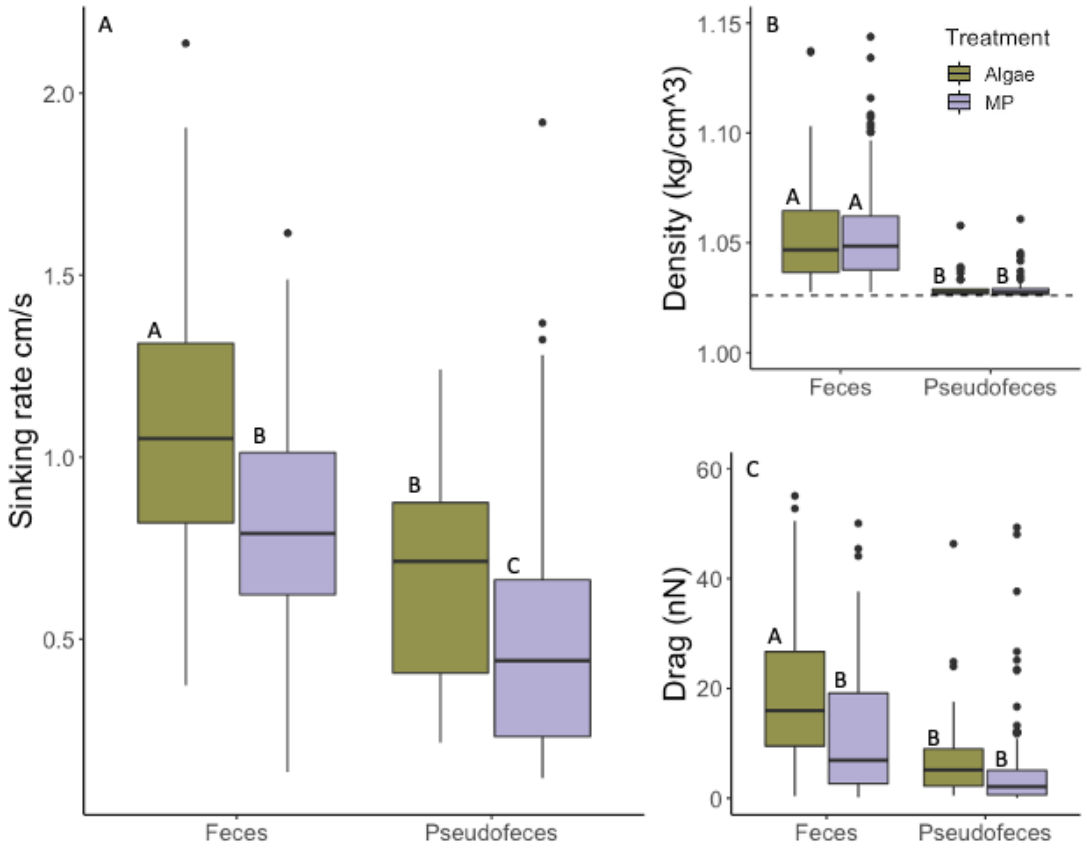


Figure 2.4 The impact of biodeposit type and particle treatment on the A) sinking rate, B) density, and C) drag of mussel biodeposits. Green represents the algae treatment and purple represents the MP + algae treatment. Boxes represent upper and lower quartiles and dots represent outliers; solid lines 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 13ºC for reference.

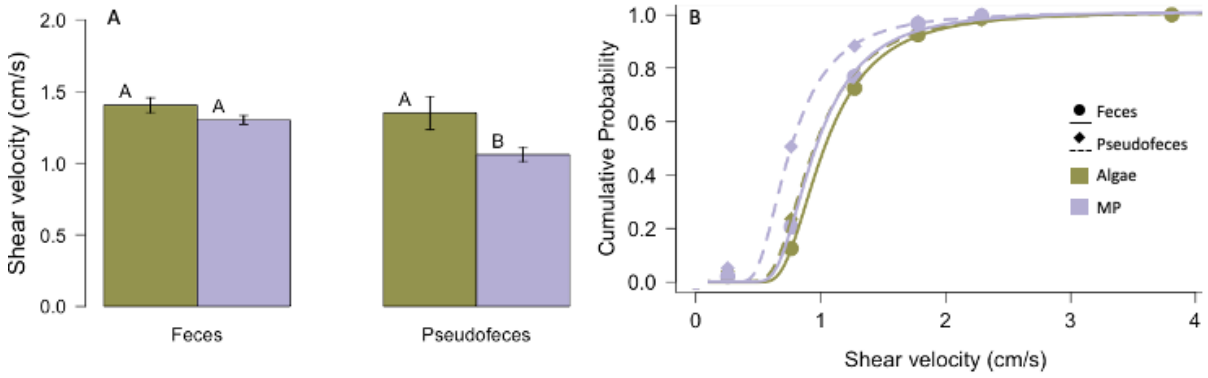


Figure 2.5 The effect of biodeposit type and particle treatment on the shear resuspension velocity of mussel biodeposits. A) Resuspension velocities for each particle treatment and biodeposit type, where bars are mean shear velocity and error bars are standard error. The letters indicate statistical 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.

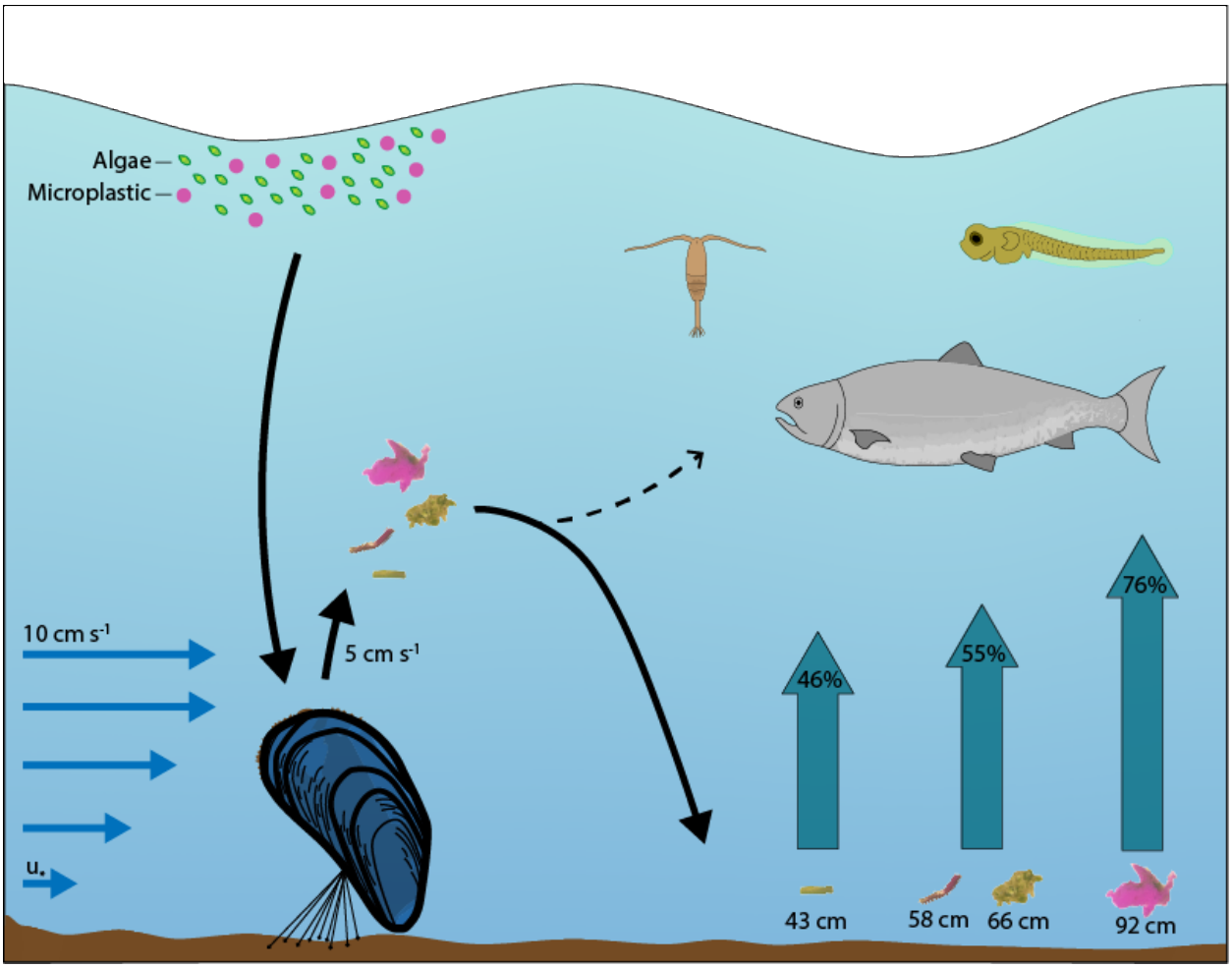


Figure 2.6 Conceptual diagram of biodeposit horizontal displacement under experimental sinking velocities and estimated current and ejection velocities. Solid black lines represent known trajectories of biodeposits, including suspension feeding, ejection, and sinking. Dashed black line represents unknown resuspension before sinking occurs. In 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.

2.10 Supplemental Material

Parallel experiments with polystyrene microplastics (PS), 38-42 μm (Item # PSMS-1.07; Cosphereic), were conducted in January – March 2020. Experimentation and measurements were suspended early due to Covid-19 shutdowns and therefore were not completed nor included with manuscript data. All methods including feeding trials, biodeposit classification and measurements, particle quantification, and sinking rate followed those described in the manuscript for polyethylene microplastics (MP). Clearance rate and resuspension velocity were not measured. Quantity of algae and PS + algae trials and biodeposits measured are reported in Supplemental Table 1. Data analysis and graphs are calculated with algae and PS + algae particle treatments conducted January – March, 2020; manuscript data are not included here.

Results from PS trials

Biodeposit length was not dependent on particle treatment, biodeposit type, nor the interaction ($p > 0.07$; linear mixed-effects model; Supplemental Figure 1a; Supplemental Table 2). Biodeposit width was dependent biodeposit type ($p < 0.001$) and not on particle treatment ($p = 0.05$) nor the interaction between these effects ($p = 0.30$; linear mixed-effects model; Figure 1b; Supplemental Table 2). Specifically, pseudofeces were up to 49% wider than feces. Biodeposit volume was dependent biodeposit type ($p < 0.001$) and not on particle treatment ($p = 0.08$) nor the interaction between these effects ($p = 0.76$; linear mixed-effects model; Figure 1c; Supplemental Table 2). Pseudofeces produced from the PS + algae treatment were up to 7 times larger than feces.

Algal cell concentration in biodeposits was not dependent on particle treatment, biodeposit type, nor the interaction between these effects ($p > 0.05$; linear mixed-effects model; Figure 2a; Supplemental Table 3). Polystyrene particle concentration in biodeposits was dependent on biodeposit type ($p < 0.001$; linear mixed-effects model; Figure 2b; Supplemental Table 3), where pseudofeces contained 4 times more PS particles than feces.

Biodeposit sinking rate was dependent on biodeposit type ($p = 0.004$), and not on particle treatment nor the interaction between these effects ($p > 0.28$; linear mixed-effects model; Figure 3a; Supplemental Table 4). Pseudofeces sank 20-25% slower than feces and biodeposits from the PS + algae treatment sank 10-17% slower than biodeposits from the algae treatment.

Biodeposit density was dependent on biodeposit type ($p < 0.001$) but not on particle treatment nor the interaction between these effects ($p > 0.08$; linear mixed-effects model; Figure 3b; Supplemental Table 4). Drag was dependent on biodeposit type ($p = 0.01$) but not on particle treatment, nor the interaction between these effects ($p > 0.28$; linear mixed-effects model; Figure 3c; Supplemental Table 4).

Supplemental Tables

Supplemental 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 = 28)		
Quantification	10	18
Sinking rate	10	15
PS + algae (mussels = 112)		
Quantification	39	73
Sinking rate	46	58

Supplemental Table 2. Summary of linear mixed-effects model analyses reported as Type III ANOVA tables. Separate analyses were conducted for biodeposit length, width, and volume. 269 biodeposits from 35 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	34.75	3.63	0.07
	Biodeposit type	1	250.35	0.13	0.71
	Treatment x Type	1	250.35	0.38	0.53
ln width	Treatment	1	35.65	4.01	0.05
	Biodeposit type	**1**	**257.22**	**20.11**	**< 0.001***
	Treatment x Type	1	257.22	1.07	0.30
ln volume	Treatment	1	34.91	3.35	0.08
	Biodeposit type	**1**	**251.63**	**127.88**	**< 0.001***
	Treatment x Type	1	251.63	0.09	0.76

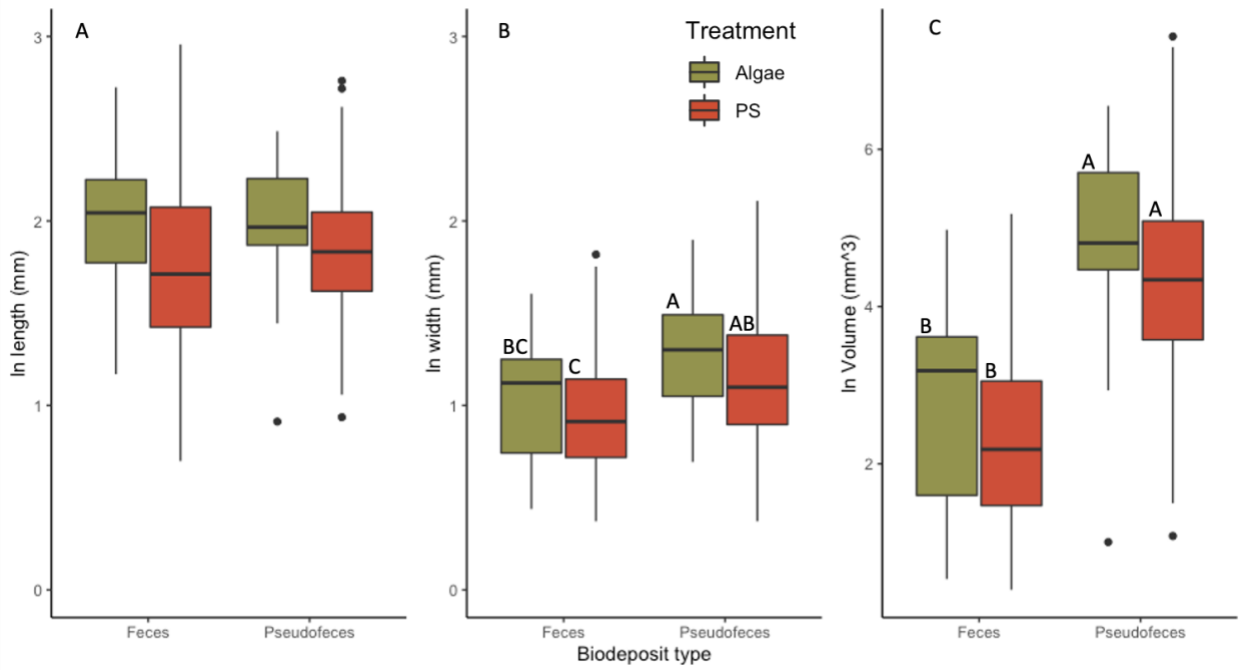
Supplemental Table 3. Summary of linear mixed-effects model analyses reported as Type III ANOVA tables. 140 biodeposits from 35 mussels (from both treatments) were included in the algae cells mm^{-1} analysis and 112 biodeposits from 28 mussels (only from the MP + algae treatment) were included in PS 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	34.85	4.07	0.05
	Biodeposit type	1	127.30	0.44	0.51
	Treatment x Type	1	127.30	0.01	0.91
PS particles mm^{-1}	**Biodeposit type**	**1**	**110**	**31.72**	**<0.001***

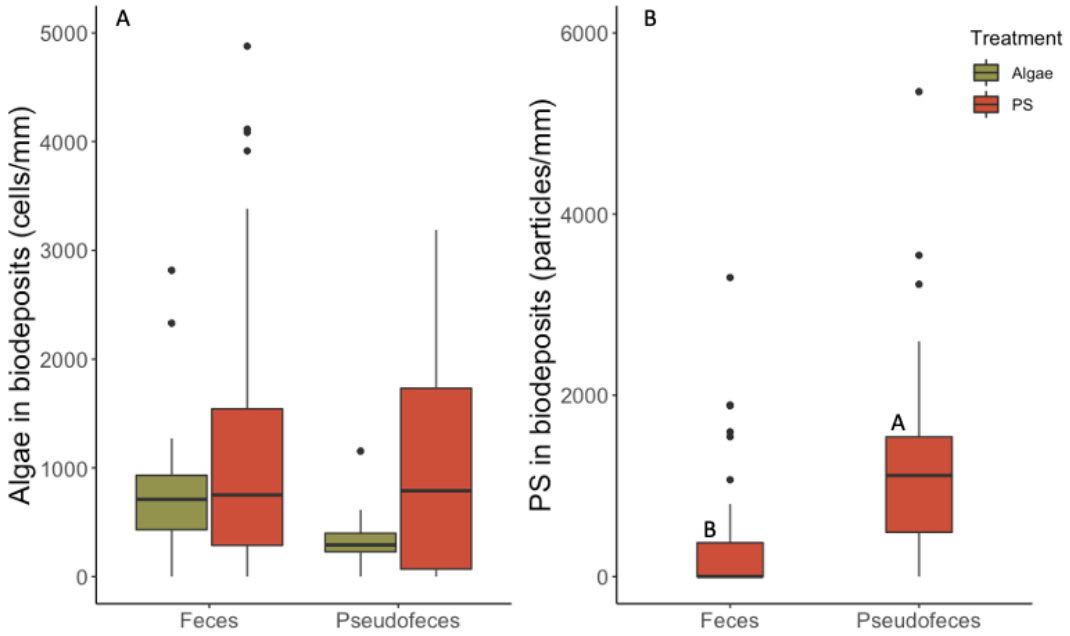
Supplemental Table 4. Summary of linear mixed-effects model analyses reported as Type III ANOVA tables. Separate analyses were conducted for each dependent variable: particle treatment and biodeposit type on biodeposit sinking rate, density, drag, and resuspension. The same group of 129 biodeposits from 35 mussels were used in sinking rate, density, and drag 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**
Sinking rate	Treatment	1	32.63	1.20	0.28
	Biodeposit type	**1**	**117.13**	**8.22**	**0.004***
	Treatment x Type	1	117.13	0.27	0.61
Density	Treatment	1	33.31	0.62	0.44
	Biodeposit type	**1**	**124.65**	**17.85**	**< 0.001***
	Treatment x Type	1	124.65	0.29	0.59
Drag	Treatment	1	33.35	0.00	0.99
	Biodeposit type	**1**	**112.12**	**7.57**	**0.01***
	Treatment x Type	1	112.12	1.14	0.29

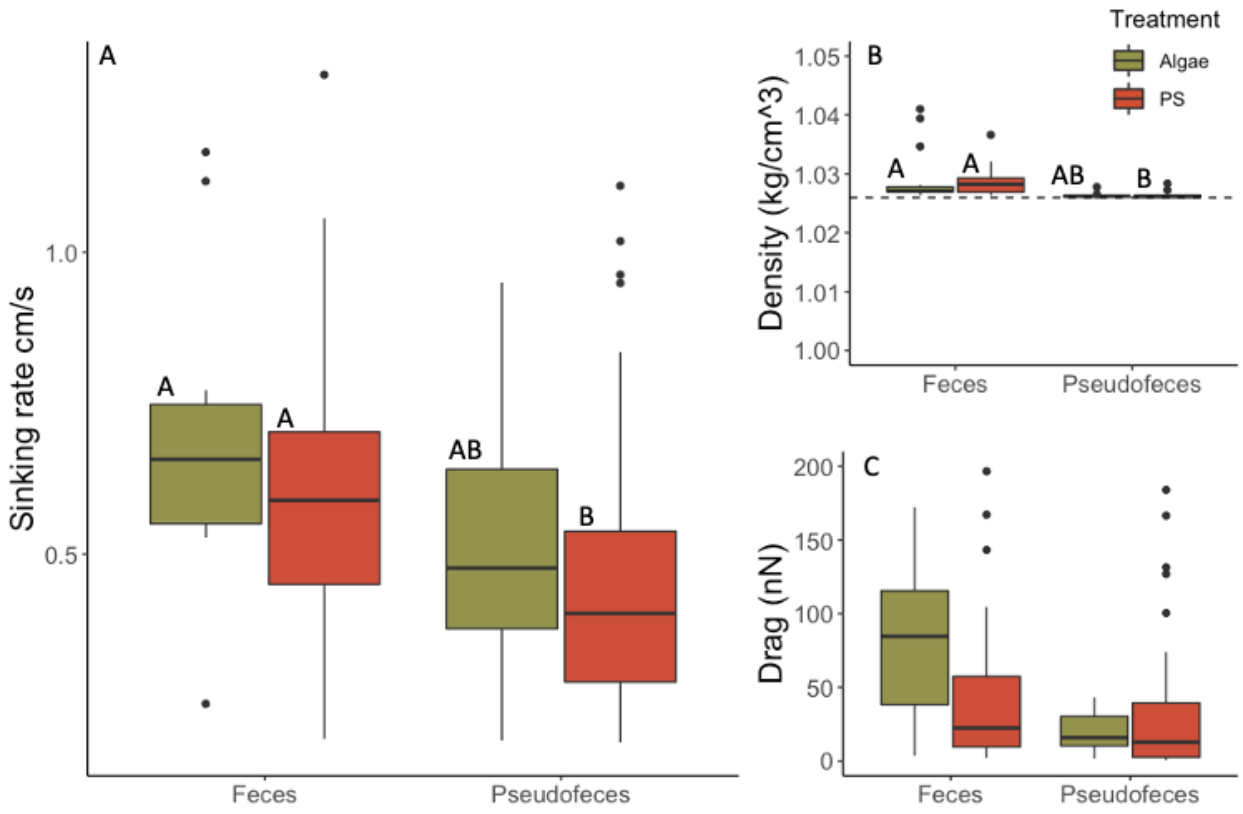
Supplemental Figures



Supplemental Figure 1. Morphometric measurements [A) ln length, B) ln width, and C) ln volume] of all biodeposits pooled from the two experiments (quantification and sinking). Green represents the algae treatment and orange represents the PS + 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).



Supplemental Figure 2. Quantitative measurements of A) algal cells and B) PS particles in biodeposits. Green represents the algae treatment and orange represents the PS + 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).



Supplemental Figure 3. The impact of biodeposit type and treatment on the A) sinking rate, B) density, and C) drag of mussel biodeposits. Green represents the algae treatment and orange represents the PS + 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 13ºC for reference.

Chapter 3: Spatial-temporal growth, distribution, and diffusion of marine microplastic research and national plastic policies

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Keywords: scientometric, scopus, hot spot, pollution, debris

3.1 Abstract

Plastic accounts for 80% of material waste in the ocean. The field of marine microplastic research is relatively new and is growing rapidly, in terms of published papers as well as institutions and countries conducting research. To combat plastic pollution, there is sufficient evidence that policies can lead to reduced plastic production and consumption both locally and globally. We aim to understand how marine plastics research and policies have grown and spread. Specifically, we used scientometric and spatial diffusion methods to best explain how ideas (in this case science and policy) clustered and spread geographically through time. We performed systematic literature searches to determine the spatial and temporal growth of marine microplastic publications and national plastic policies from 1900-2019. We found that more countries adopted national plastic policies than those that have conducted marine plastic research. Doubling times of each temporal growth rate analyzed (research paper, institution, country, and national policy) ranged from 1.1 – 4.05 years. Marine microplastic research has

grown exponentially since 2006, and the topics of inquiry have increased steadily. However, marine microplastic research activity was not a good indicator of a country's resources or motivation toward national plastic policies. Marine microplastic publication spread at the institution level is best explained by a hybrid of expansion and relocation diffusion while national plastic policy spread is best explained by expansion diffusion.

3.2 Introduction

Global plastic production has grown since the late 1940s and today over 300 million tons of plastic is produced globally each year with no slowing in sight (Jambeck et al. 2015; Plastics Europe 2018). As of 2015, over 6,300 megatons of plastic waste was produced and only ~9% was recycled (Geyer et al. 2017). The majority of the plastic is from single use packaging, construction, and textile/apparel (Geyer et al. 2017). Unfortunately, elements that contribute to plastic's popularity, such as flexibility, durability, light weight, and low price, also contribute to the long-lasting environmental impacts. For example, plastic does not readily degrade, but instead breaks into smaller and smaller pieces termed "microplastics" (MP; < 5 mm; Arthur et al. 2009).

In the ocean, material waste in surface, pelagic, benthic, and beach environments is up to 80% plastic (Barnes et al. 2009; Choy et al. 2019). In addition to its overwhelming abundance, plastic acts as a sponge and a transportation vector for persistent organic pollutants (POPs) like DDT and PCB (Rios et al. 2007, Engler 2012; Avio et al. 2015; Jambeck et al. 2015) and pathogens (Bowley et al. 2020). Plastic exposure has been shown to affect marine organisms mechanically due to the physical structure (Browne et al. 2008) and chemically due to the reactivity of attached toxics (Paul-Pont et al. 2016).

The field of marine MP is relatively new, however, and as a result knowledge of potential biological implications is still growing (Barboza and Gimenez 2015). Small plastics were first noted in marine animals in the late 1960s (albatrosses; Kenyon and Kridler 1969) and in marine environments with biological implications in the 1970s (coastal waters; Carpenter and Smith 1972). The term "microscopic plastic," however, was not commonly used until 2004 (Thompson et al.) and was not shortened to "microplastic" until 2006 (Ng and Obbard 2006). Since then,

marine MP research has grown rapidly, in terms of published papers, institutions conducting research, and international collaborations (Barboza and Gimenez 2015). This increase is mainly driven by an increase in laboratory microcosm experiments studying the effects of MP in a controlled environment (Barboza and Gimenez 2015), but there has also been an increase in environmental (sediment and water) and organismal MP contamination studies (wild-caught; Ryan 2015). Thus far, studies found organisms from multiple functional groups, including invertebrates, small vertebrates, large vertebrates, and zooplankton ingest plastic in laboratory experiments and in natural habitats (Thompson et al. 2004, Browne et al. 2008, Wright et al. 2013, Frias et al. 2014, Avio et al. 2015, Cole et al. 2015, Desforges et al. 2015, Li et al. 2015, 2016; Harris and Carrington 2019). Microplastics have the potential to accumulate in the food chain and detrimentally impact marine ecosystems through their physical presence as well as their ability to absorb POPs (Rios et al. 2007; Galloway et al. 2017).

Recently, marine plastic pollution has become a hot topic in the press and among conservation organizations (i.e. the "Plastic or Planet" issue by National Geographic in June 2018). Many of these groups demand a "call to action" for policies to be put into place to reduce both plastic production as well as pollution. Although the field of marine MP is relatively new, there is the overarching conclusion that MPs have negative organismal, social, and economic impacts (Beaumont et al. 2019). To combat these negative effects, there is sufficient evidence that policies can lead to reduced plastic production and consumption both locally and globally (Ryan 2015; Schnurr et al. 2018). Due to the structure of national governance and few international regulating systems, individual countries are left to implement their own versions of plastic policy that are dependent on needs, demands, and trade agreements with neighboring countries.

However, there is international recognition that marine debris is a pervasive pollution issue. Six international marine debris conferences (1984, 1989, 1994, 2000, 2011, 2018; Supplemental Table 1) have occurred and have developed a framework for reducing marine debris in addition to calling upon governments, non-government organizations, industries, and stakeholders to commit to the framework. The framework does not have any legal standing nor does it have incentives (other than environmental preservation) or implementation requirements. The EU is one of the few international organizations to implement regulations with legal standing, as seen with their single-use plastic ban coming into effect in 2021 (European Parliament News, 2019). Broader international regulating organizations (i.e. UN, NATO), however, have made slow (if any) progress implementing a unified approach to marine debris and marine plastic pollution remains a pressing global issue today, in 2020.

As the field of marine MP grows, national and international collaborations between marine MP scientists have increased (Barboza and Gimenez 2015; Pauna et al. 2019) as well as the quantity of organismal, environmental, and review publications (Ryan 2015). Widespread policy is often a result of more scientists conducting studies in more places and reaching out to their local governments (Bromley 2012), emphasizing the importance of scientific research growth to achieve policy spread and growth. Although scientific research and public policy are often funded by different administrative bodies (e.g. agencies and legislature, respectively in the United States), increased collaborations and publications may be a proxy for a country's motivation and resources regarding plastic pollution, resulting in the adoption of national plastic policies.

Given the hot topic of marine plastic and establishment of a marine debris framework, it is unsurprising that national plastic policies are increasing globally (Karasik et al. 2020).

However, the patterns explaining both MP research and national plastic policy growth and geographic diffusion remain unknown. Identifying if and how MP research and national plastic policies experience geographic may establish a link between the two fields. Diffusion patterns arise from expansion or relocation properties that are often a result of external determinants such as motivation, obstacles against innovation, and resources for overcoming the obstacles (Mohr 1969; Berry and Berry 1990). Spatial analyses can therefore be used to determine how ideas (in this case science and policy) spread geographically through time, identify clusters, and lend evidence to distinguishing between diffusion properties.

Diffusive growth typically follows an S-shaped curve, showing the accumulation of adopters over time as entities (e.g. authors, institutions, countries) gain motivation and resources to overcome obstacles, from innovators to majority to laggers (Figure 3.1a; Rogers 1995). In fields that are still growing, like marine MP research or plastic policies, transitions between growth phases may be identified as break points in piece-wise regression analyses (Bornmann and Mutz 2015).

If a diffusion is clustered, spatial analysis can reveal two types of spatial diffusion properties, 1) expansion and 2) relocation (Figure 3.1b-c; Mitchell 2018). Expansion occurs when ideas spread out geographically from one area and when adoption by nearest neighbors is more likely than adoption by non-neighbors. Relocation occurs when there are multiple geographical areas of adoption which can be identified through clustering of dispersed adopters. Hybrid diffusion occurs when both expansion and relocation diffusion are observed at the same time.

Growth and diffusion affect geographical patterns, which can be analyzed for spatial relationships over time with hot spot analyses. Hot spot analyses are common in crime, accident,

and epidemiology research (e.g. Moore and Carpenter 1999; Anderson 2008; Erdogan et al. 2008) and can be useful to visualize event centers over time. If ideas are expanding geographically and institutions or countries are clustered, hot spots will remain in the same locations over time. If ideas are relocating across geographic space, hot spots will shift and jump locations over time. Diffusion is considered hybrid if multiple diffusion properties are evident in visualization. Examining diffusion as a function of time and space can offer insight to how these fields are growing, if and how spread is occurring, and may point towards why they are spreading (Shipan and Volden 2008; Mitchell 2018).

This paper investigates the spatial-temporal growth, distribution, and diffusion of marine MP research and national plastic policies. We use scientometric and spatial diffusion methods to determine how ideas (in this case science and policy) grow spatially and temporally. We propose six independent hypotheses:

H1 *Global marine MP publications are growing exponentially at the paper, institution, and national levels*

H2 *Marine MP papers are increasing in breadth of topics studied*

H3 *Countries implementing national plastic policies are growing exponentially*

H4 *Countries with national plastic policies publish more marine MP papers than countries without national plastic policies*

H5 *Institutional marine MP research growth is in the early majority phase and spread is due to relocation diffusion*

H6 *National plastic policy growth is in the early majority phase and spread is due to expansion diffusion*

3.3 Methods

Marine plastic peer reviewed paper selection

Growth of marine microplastic (MP) publications was compared to other types of plastic research by performing a systematic literature search of peer-reviewed papers from Scopus, Elsevier's abstract and citation database, in April 2020. The search used five sets of keywords: marine AND plastic*, marine AND "plastic bag*", marine AND "single use plastic*," marine AND microbead*, and marine AND microplastic*. The asterisk at the end of a word ensured both the singular and plural forms were considered. Within each of these sets of keywords the "analyze search results" feature was used in Scopus to record the quantity of papers published annually and cumulative number of papers published by country for 1900-2019. We note that many early papers studying mussel feeding physiology used poly-microbeads since the 1980s but were not included in any of the keyword searches (e.g. Ward 1996). Papers were randomly spot-checked to ensure they fit within the keywords, if they did not, they were removed from our selection.

Metadata from marine MP papers were collected from a systematic literature search of peer-reviewed papers from Web of Science in April 2020. The search criteria used were the keywords marine AND microplastic* and all years (1900-2019), the same as the Scopus search. Publishing date, institution of lead author (including latitude and longitude), country of lead author, journal, and title were collected. Papers addressing non-marine MP topics (e.g. table salt or freshwater), highlights, commentary, news features, correspondences, opinion, and review papers were removed.

Each marine MP paper was categorized based on focus topic: chemistry, environment, organism, policy, or review. If a paper studied multiple focus topics, only the predominate one

was recorded. Organism papers were further categorized into functional groups: bacteria, fungus, invertebrate, small vertebrate, large vertebrate, macroalgae, phytoplankton, and zooplankton (includes fish larvae). If a paper studied multiple organisms, all were categorized by functional group and included.

National plastic policy selection

To evaluate plastic policy growth and diffusion, a systematic literature search for national plastic policies implemented through 2019 was conducted. Policy data was collected from Xanthos and Walker (2017), Schnurr et al. (2018), Lam et al. (2018), Plastic Policy Inventory from Duke's Nicholas Institute for Environmental Policy Solutions (2020), and news articles from Wikipedia's "phase-out of lightweight plastic bags" page (April 2020). Country, implementation year, type (plastic bag, microbead, single use plastic; SUP), and level (levy, ban) were recorded. All policies were cross-validated with an internet news search and policies that failed cross-validation were not included. Voluntary national plastic levies and bans were not included. Policies were evaluated at a national level, where countries with multiple levels or types of policies were only counted once in analyses.

Analysis

All analyses, maps, and graphs were developed with the computing software R for Mac OS X (version 3.6.1, R Core Team, 2019). The following packages were used: lme4, plyr, ggplot2, rworldmap, maptools, and spatstat. For all tests, level of significance was set at $\alpha < 0.05$.

Semi-ln regression was used to analyze temporal growth rate and doubling time of plastic research papers appearing in Scopus. ANCOVA was used to assess marine MP research from Scopus and Web of Science with source as the main effect and year as the covariate. Chi Squared was used test to analyze the observed distribution of focus categories in marine MP papers as compared to the predicted distributions.

Change in growth rates of marine MP papers, institutions publishing, countries publishing, and countries with national policies were tested for using Davies' test. If a change was indicated, piecewise semi-ln regression analyses were used to estimate the years where the break points occurred. Linear models were used to characterize the temporal growth rate and doubling time of each segment. A t-test was used to compare the quantity of marine MP papers published in countries with and without plastic policies.

Diffusion

Two diffusion properties were compared in both marine MP papers and plastic policies, expansion and relocation. Kernel density estimations for each year (that there were enough papers or policies to do so) were calculated to explore which type of diffusion best explained the spread and clustering of papers and policies over time. Kernel density estimations were plotted each year for latitude and longitude of papers and policies. Papers were evaluated at the institution level and policies at the national level. Hot spot analyses were used to visualize geographic concentrations (Anderson 2009) of institutional marine MP publications over time (spread). Institution coordinates and country centroids coordinates (latitude and longitude) were used for analyses; institution coordinates and country polygons were used for visualizations. All national policies types were assessed together (plastic bags, microbeads, and SUPs).

3.4 Results

Scopus

Marine plastic literature dates back to 1961, when the first study using plastic in marine industries was published (Strickland and Terhune 1961). However, it wasn't for another eight years until the first report of marine plastic pollution was published (Kenyon and Kridler 1969). The field of marine plastic (whether related to industry or pollution) has grown exponentially, doubling on average every 7.7 years, similar to climate change publications and faster than scientific publications as a whole (Table 3.1). Our search of marine AND plastic* literature yielded a total of 7,577 papers; we therefore consider the dataset to serve as a good representation of the broader field (Figure 3.2).

Marine AND "plastic bag*" literature dates back to 1961 where a study used plastic bags as a research tool (Strickland and Terhune 1961). For 30 years, plastic bags were used as pollution confinement measures (oil) or for *in-situ* experiments. The first marine plastic bag record relating to pollution available in Scopus was a study on manatees (Beck and Barros 1991). Marine AND microbead* literature dates to 2004 and the use of fluorescent poly-microbeads in current and water flow studies (Petrisor et al. 2004). It wasn't until 2013 that marine microbeads were recognized as pollution in research (e.g. Fajardo et al. 2013; Eriksen et al. 2013). The field of marine AND "single use plastic*" is the newest, with the first papers published in 2017 (Wagner 2017; Xanthos and Walker 2017). Both of the studies published in 2017 address the efficacy of single use plastic bans rather than any marine aspect. All of the aforementioned fields have grown exponentially with varying doubling times ranging from 1.2 to 8.7 years; the SUPs literature was too recent to include in this doubling rate analysis (Figure 3.2; Table 3.1).

Marine microplastic papers

We used marine MP papers as a manageable case study for investigating spatial-temporal patterns and diffusion properties. Marine microplastic (MP) papers were published more than any other keyword within marine plastic ($p < 0.001$; X^2), exceeding plastic bag, microbead, and SUP publications by eight, 11, and 86 times, respectively (Figure 3.2). There was no significant difference between the quantity of marine MP papers collected from Scopus and Web of Science over time ($p = 0.98$; ANCOVA; Supplemental Figure 1; Supplemental Table 2).

As of the end of 2019, 538 institutions in 64 countries (of 195, not all of which have marine territory) published a total of 1,267 marine MP papers (Table 3.2). Each level of analysis (paper, institution, and country) had a break point where growth rate changed significantly ($p < 0.01$, piecewise semi-ln regression; Figure 3.3, Table 3.1). Marine MP publication rate slowed in 2014 from a doubling time of 1.1 to 1.5 years, institutional publication rate slowed in 2012 from a doubling time of 1.3 to 1.7 years, and country publication rate slowed in 2009 from a doubling time of 1.4 to 3.7 years. We label the institutions and countries from the first publication to the break point as early adopters and from the break point till 2019 as early majority; we acknowledge we cannot separate innovators and early adopters precisely because the field of marine MP research is still growing exponentially (Figure 3.3).

Marine MP papers show increasing trends in quantity both over time and in breadth of topics addressed (Figure 3.4). The largest proportion of papers focused on organisms (34%; Figure 3.4) and within this topic over 32% of papers studied multiple rather than single species. Small vertebrates and invertebrates are the most frequently studied functional groups across all organisms at 50% and 28%, respectively (Table 3.3).

Policies

By the end of 2019, a total of 127 national plastic policies were implemented in 115 countries, placing either a ban and/or levy on plastic bags, microbeads, and/or single use plastics (SUPs; Table 3.4; Supplemental Table 3). Notably, the number of plastic bag policies have tripled globally in the past ten years (2010-2019). Some countries progressed from a levy to a ban of the same type of plastic, some countries have multiple policies regulating different types of plastics, and some have just one type of plastic policy. Here, we examined plastic policies from a national level, where countries with multiple types or levels of policies were only counted once. The growth trajectory of countries adopting national plastic policies exhibited a break point, where adoption rate changed in 2010 from a doubling time of 4.1 to 3.3 years (Piecewise semi-ln regression; Figure 3.3; Table 3.1). As with marine MP papers, adoptions of national policies are still growing exponentially; we labelled countries from the first adopter to the break point as early adopters and from the break point through 2019 as early majority.

Denmark was the first country to adopt a national policy (plastic bag levy) in 1993 and Bangladesh was the first country to successfully adopt a national ban (plastic bags) in 2002. Saint Lucia was the first country to adopt a national single use plastic levy in 2008 and Guyana was the first country to adopt a national SUP ban in 2014. As of 2019, SUP policies only exist in the Caribbean and South America. The United States was the first country to adopt a national plastic microbead ban in 2015. Plastic bag legislation is the most common type of national plastic policy, with only a few countries enforcing microbeads and SUPs policies (Table 3.4; Supplemental Table 3).

More countries adopted national plastic policies than those conducting marine plastic research, and the growth rate differs between the two metrics (115 and 64, respectively; $p < 0.01$;

ANCOVA; Figure 3.3). Further, there is no difference in quantity of marine MP papers published by countries with or without national plastic policies (all types of policies combined; $p = 0.12$; t-test; Supplemental Figure 2).

Diffusion

Institutions publishing marine MP papers have historically been, and continue to be, concentrated in the Northern hemisphere (Figure 3.5). While Europe continues to be a dominate hot spot for MP research, institutions conducting research spread longitudinally over time, flattening the kernel density estimation curve (Figure 3.5). From 2013 to 2019, the quantity of hot spots condenses from four to two, signifying dominant leaders in the marine MP field. These two hot spots are Europe, which continuously maintains high kernel density estimations, and Eastern China, which emerges in 2016 with lower kernel density estimations (Figure 3.5). These estimations and visualizations suggest hybrid diffusion is present, with both expansion as well as relocation diffusion observed.

Conversely, countries with national plastic policies (all plastic policies evaluated together) have historically been, and continue to be, evenly spread across both Northern and Southern hemispheres (Figure 3.6). Kernel density estimates for national plastic policies are highest near zero degrees longitude (Figure 3.6c). Plastic policies were evaluated at the national level and therefore hot spot analyses were not visualized. While more spatially dispersed in 2011, national plastic policies are concentrated across African and European countries as of 2019. When examining kernel density estimations and geographic visualizations, expansive diffusion was predominantly observed.

3.5 Discussion

Marine plastic pollution has been documented in scientific literature since the late 1960s and continues to be a growing research topic and policy concern. We used aspects of scientometric and spatial diffusion research to understand the spatio-temporal growth of marine microplastic (MP) research and national plastic policies. This study shows evidence to support our hypotheses that marine MP publications are increasing exponentially at all levels (*H1*) as well as increasing in breadth of topics studied (*H2*), national plastic policies are increasing exponentially (*H3*), and national plastic policy spread is due to expansion diffusion (*H6*). This study did not find evidence that countries with national plastic policies published more marine MP research (*H4*), nor that marine MP research spread is solely due to relocation diffusion (*H5*). The results point to a heterogenous, hybrid spread of global research activity and policy implementation with different diffusion properties.

Research on marine MPs existed before the keyword was termed, and is encompassed by marine plastics research in general. The term "microplastic" (rather than microscopic plastic or small plastic) is relatively new (2006) and previous research on MPs may have been omitted from our search. We are confident, however, that the collection of marine MP papers we did identify serves as a good case study for growth and diffusion patterns due to its exponential increase and geographic spread. Marine MP research has grown exponentially at the publication, institution, and national levels, doubling every 1.1-1.5 years since 2006, faster than scientific publications as a whole (Table 3.1).

Marine MP research is still in the early phase, including innovators, early adopters, and early majority, as identified by the initial phase of the typical S-curve (Figure 3.1a; Rogers 1995). As of the end of 2019, there appears to be no slowing of publication rate. Perhaps this

trend is in part due to early adopting researchers and institutions (e.g. within United Kingdom and United States) building and expanding individual programs, becoming experts, and forming both domestic as well as international collaborations (Barboza and Gimenez 2015; Pauna et al. 2019), thus promoting the establishment of an early majority. The concept of prolific institutions is supported by kernel density estimates, where Europe starts as a hotspot in 2013, and remains one through 2019 (Figure 3.5). As more papers are published and the late majority starts to adopt, we expect the typical diffusion curve to develop.

Marine MP research exhibited expansive and relocation diffusion. This could be for a multitude of reasons, and we suggest three explanations here. First, scientific research is often collaborative, with the possibility of collaborators at multiple institutions between both neighboring and non-neighboring countries (Barboza and Gimenez 2015; Ryan 2015; Pauna et al. 2019). Second, globalization enables countries to learn about research, policies, and actions of non-neighboring countries, reducing the influential strength of neighbors. Third, research is mainly a product of wealthy countries with prestigious universities, concentrated in select regions. These three ideas, or their combination, could contribute to the hybrid diffusion pattern observed as well as lend insight to diffusion mechanisms at play .

Published marine MP papers focus on a breadth of topics, the most popular being organisms. The most common functional groups studied within organismal papers were invertebrates (e.g. oysters, mussels, crabs) and small vertebrates (i.e. fish), both important in aquaculture and human consumption, and perhaps therefore carry higher economic research incentives. However, there appears to be no relationship between presence or type of national plastic policy and quantity of marine MP papers published by a country. Therefore, we conclude

marine MP research, while potentially having economic incentive, is not a good indication of the country's interest or priority in plastic policies.

At institutional and national levels of marine MP research analyses, the early majority phase is characterized by slower growth than the early adoption phase. Most diffusion literature suggests that later-adopting countries tend to adopt quicker, due to a decrease in perceived risk (i.e. Valente 1995; Albuquerue et al. 2007). We see the opposite trend here, possibly due to a lack of resources to overcome research obstacles. As the field of marine MP has grown, so have the number of species studied, thus reducing novel study organisms that new programs and institutions may research. Further, the advancement in best-practices has called for expensive polymer analysis necessary to validate results for publication (Granek et al. 2020). These aspects of marine MP research present new obstacles to overcome for researchers or institutions trying to get into the field.

The growth of countries researching marine MP is mirrored in the number of countries and researchers taking part in the past six Marine Debris Conferences (Supplemental Table 1) and international groups supporting pollution mitigation (e.g. EU and UN). Currently in the early majority phase, research and policies are being adopted by countries at similar rates (Table 3.1), though this was not always the case. Plastic policies were implemented at the national level since 1993, contributing the high number of countries with policies as opposed to research. It is important to note, however, that there is no limit to the number of papers that can be published while the total number of institutions and countries is relatively fixed. In the coming years we may start to see growth rates of institutions and countries conducting research and adopting policy slow, as they approach saturation, while publication rate remains high.

Countries with marine MP publications and countries with national plastic policies occupy different areas of the world (Figures 3.5 and 3.6). Contrary to marine MP publications, national policies are spread between the Northern and Southern hemispheres, in many developing countries. This trend may be due to difficulty associated with implementing nation-wide policies and the historically ad-hoc, bottom up trend observed with previous plastic bag policy studies (Clapp and Swanston 2009). We also note that while clustering is present, it may have little to do with policy diffusion and more to do with political, economic, and demographic similarities often observed in geographically neighboring countries (Shipan and Volden 2012).

Numerous sources of MPs make it difficult to regulate and mitigate subsequent pollution. At the national level, countries are starting to regulate the most common plastic items. These policies tend to focus on consumer plastic (bags, microbeads, SUPs) rather than production (packaging and industrial material). By directing their attention towards consumer plastics, countries can substantially lower the quantity of low-grade plastic disposal and the subsequent plastic pollution (Schnurr et al. 2018). Clapp and Swanston (2009) explored the emergence of plastic bag legislation in the Southern hemisphere and noted many of these countries established an anti-plastic bag cultural norm. The quick shift in cultural norms and environmentalism was attributed to non-existent or poorly established municipal waste systems often seen in developing countries, so there was no value in recycling plastic bags (Clapp and Swanston 2009).

Populated urban areas place higher demands and burdens on natural resources than rural communities, leading to a greater incentive to manage common-pool resources (e.g. fossil fuels), and issues like pollution (Ehrlich 1968; Stern et al. 1992). Marine plastic pollution affects more than urban areas, however. Microplastics have been found in the Arctic Ocean and Antarctic Peninsula, both of which are places where there are no permanent residents or industries (Cózar

et al. 2017; Lacerda et al. 2019). Unfortunately, rural communities are still inundated by pollution from urban populations. Tiny islands in the Indo-Pacific have more plastic litter on beaches than they have people on the island—all due to ocean currents and global consumerism (National Geographic 2018). While these countries and island nations have passed regulations banning types of plastics, it has not alleviated the issue. Here, we did not examine urban centers, regional plastic policies, or cultural norms, all important aspects when considering how national plastic policies come to fruition. Further, we did not examine diffusion theories (learning, competition, imitation, and coercion) that traditionally explain policy spread.

Marine MPs are a local, regional, and global issue that require cross disciplinary attention from researchers in biological, chemical, environmental, and social sciences. Here, we found that marine MP research has grown since 2006, increasing in topics of research and exponentially in quantity of publications. However, more countries have national plastic policies than published research on marine MPs. We found that aspects of marine MP research explored here were not good indicators of a country's resource or motivation towards national plastic policies and suggest exploring external (motivation, obstacles, and resources for overcoming the obstacles) and internal (e.g. GDP, culture, religion) factors to understand what is driving research and policy diffusion.

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Data Availability Statement

The data that support these findings are available in Dryad at https://doi.org/10.5061/dryad.47d7wm3c2

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3.8 Tables

Table 3.1 Summary of doubling time (years) analyses. A) Other scientific fields, B) Scopus keyword search, C) Marine microplastic papers at the paper, institution, and country level divided into early adopters and early majority, and D) plastic policies at the country level divided into early adopters and early majority. Data on doubling times for other scientific fields were collected from the following sources: Science from Bornmann and Mutz 2015 and climate change from Haunschild et al. 2016.

	Years	Doubling time (years)
A) Field		
Science	1980-2012	24
Climate Change	1980-2014	5-6
B) Scopus keyword search: Marine AND		
plastic*	1961-2019	7.7
"plastic bag*"	1961-2019	8.7
microbead*	2004-2019	3.1
microplastic*	2006-2019	1.2
"single use plastic*"	2017-2019	NA
C) Marine microplastic papers		
Papers	2006-2014	1.1
	2015-2019	1.5
Institutions	2006-2012	1.3
	2013-2019	1.7
Countries	2006-2011	3.4
	2012-2019	3.7
D) Plastic policies		
Countries	1993-2009	4.1
	2010-2019	3.3

Table 3.2 Summary of total institutions and papers published by each country. As of 2019, a total of 64 countries and 538 institutions published a total of 1,267 papers on marine microplastics.

Country	**Total Papers**	**Total Institutions**
China	178	51
United Kingdom	109	45
Italy	98	33
United States of America	92	60
Germany	85	43
Brazil	57	22
Australia	54	21
France	54	22
Spain	54	23
Portugal	49	9
South Korea	43	13
Netherlands	41	10
Canada	30	15
Belgium	22	6
India	20	15
Ireland	18	8
Denmark	18	4
Japan	17	9
Iran	17	10
Turkey	16	9
Sweden	15	6
South Africa	13	7
Malaysia	13	4
Greece	12	5
Chile	11	3
Russia	11	1
Finland	10	5
Norway	9	5
Mexico	8	7
Indonesia	8	7
Switzerland	7	6
Saudi Arabia	7	2
New Zealand	5	2
Colombia	5	2

Slovenia	5	3
Poland	5	3
Argentina	5	3
Singapore	4	1
Croatia	4	3
Tunisia	4	2
Taiwan	3	3
Austria	3	3
Nigeria	3	3
Qatar	2	1
Israel	2	2
Philippines	2	2
Czech Republic	2	2
Romania	1	1
Uruguay	1	1
Sri Lanka	1	1
Tanzania	1	1
Thailand	1	1
Malta	1	1
Vietnam	1	1
Lithuania	1	1
Bangladesh	1	1
Peru	1	1
Ecuador	1	1
Jamaica	1	1
Egypt	1	1
Algeria	1	1
Trinidad and Tobago	1	1
Bulgaria	1	1
Hungary	1	1

Table 3.3 Frequency of functional groups studied in marine microplastic papers from 2006-2019. Functional groups were determined from paper title, abstract, and results. Papers with multiple functional groups were counted multiple times.

Functional group	Frequency in published papers
Small vertebrate	639
Invertebrate	357
Zooplankton	152
Large vertebrate	59
Bacteria	53
Phytoplankton	16
Fungus	4
Macroalgae	4

Table 3.4 Summary of national plastic policies reported as type as of 2019. A total of 127 national plastic policies were implemented in 115 countries.

Policy Type	Year of first implementation	Quantity of national policies
Plastic Bag	1993	113
Microbead	2015	8
SUP	2008	6

3.9 Figures

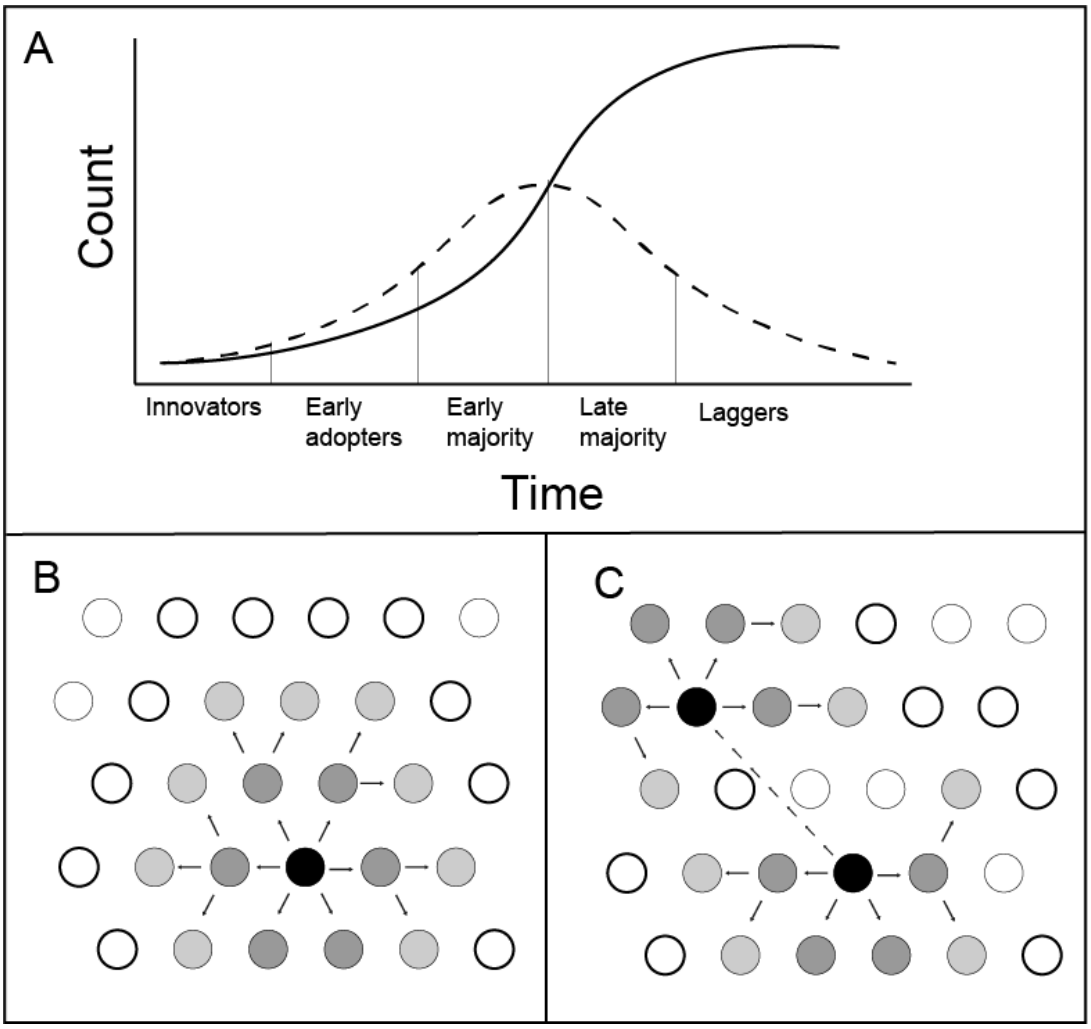


Figure 3.1 Growth and diffusion typically form A) S-shaped curve where the solid line represents quantity growth, the dashed line represents total proportion over time (first derivative), and adoption phases can be assigned. Visualization of spatial diffusion B) expansion and C) relocation. Diffusion can be compared over time and hybrid diffusion can be identified if two types of diffusion occur simultaneously.

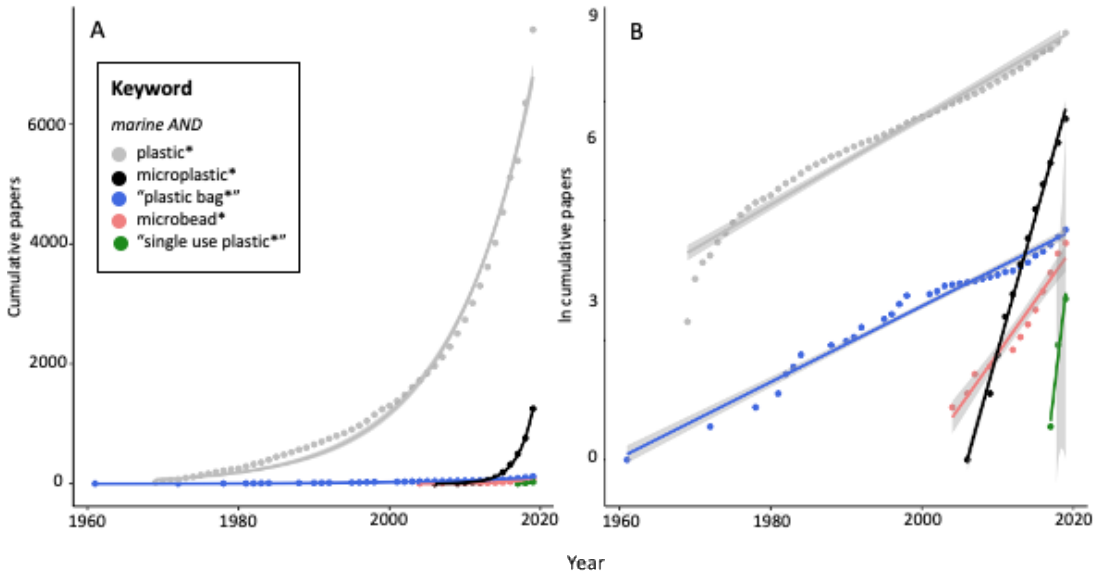


Figure 3.2 Marine plastic papers retrieved from Scopus for various combinations of keywords with publication dates of 1960-2019 as A) cumulative and B) semi-ln cumulative. Slope of lines in B) represent doubling time for each keyword publication rate.

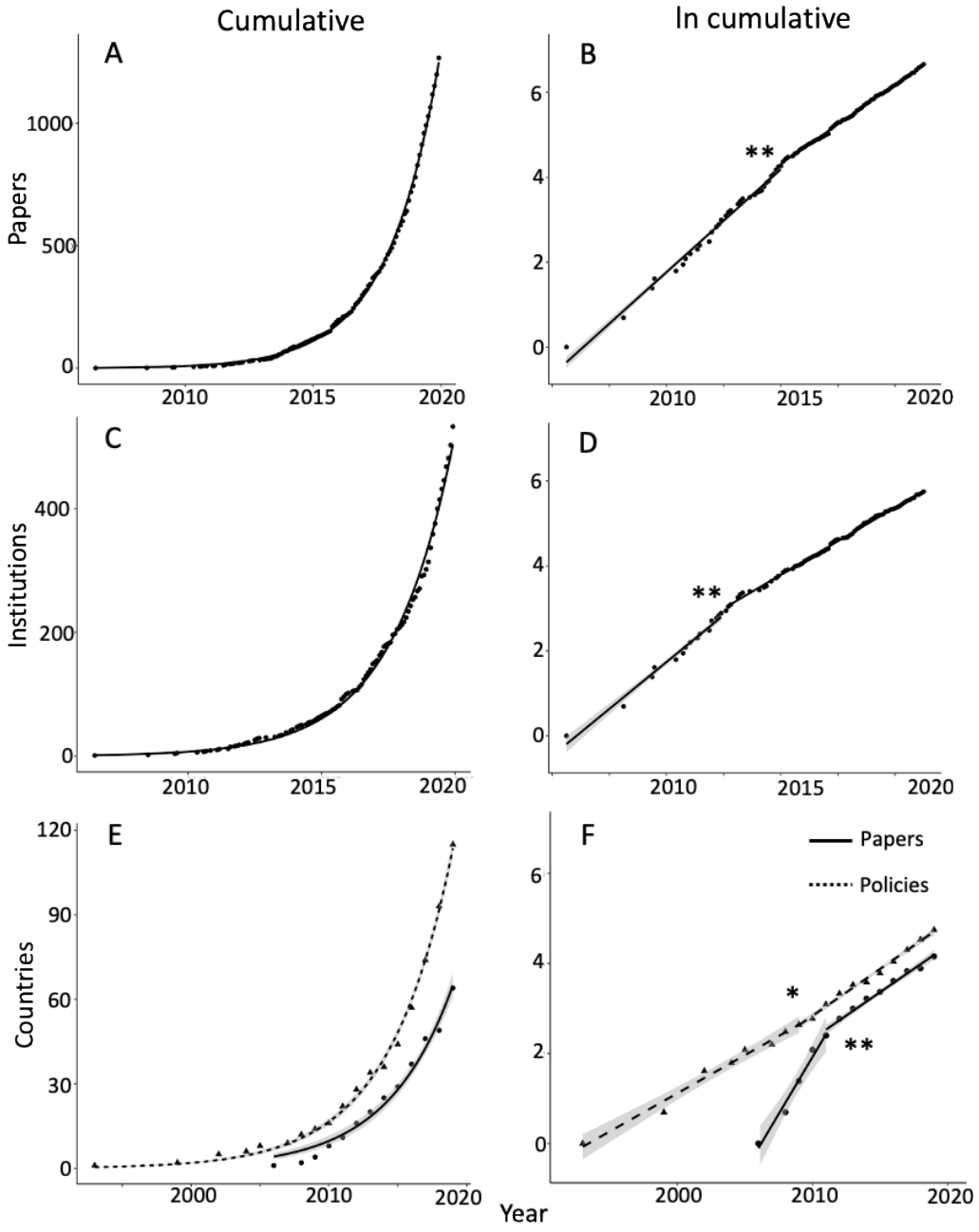


Figure 3.3 Growth of marine microplastic research and plastic policies. A) Cumulative and B) semi-ln cumulative marine microplastic papers; C) cumulative and D) semi-ln cumulative institutions publishing marine microplastic papers; and E) cumulative and F) semi-ln cumulative countries publishing marine microplastic papers and countries with national plastic policies. Papers retrieved from Web of Science for the keywords marine AND microplastic* with

publication dates of 2006-2019. Piecewise regression break points, where slope of line significantly changes, is present in all semi-ln cumulative graphs. One asterisks (*) represents statistical significance of slope change at $p < 0.01$ and two asterisks (**) represents statistical significance of slope change at $p < 0.001$; Davies' Test. Grey shading represents 95% confidence intervals for each line fit.

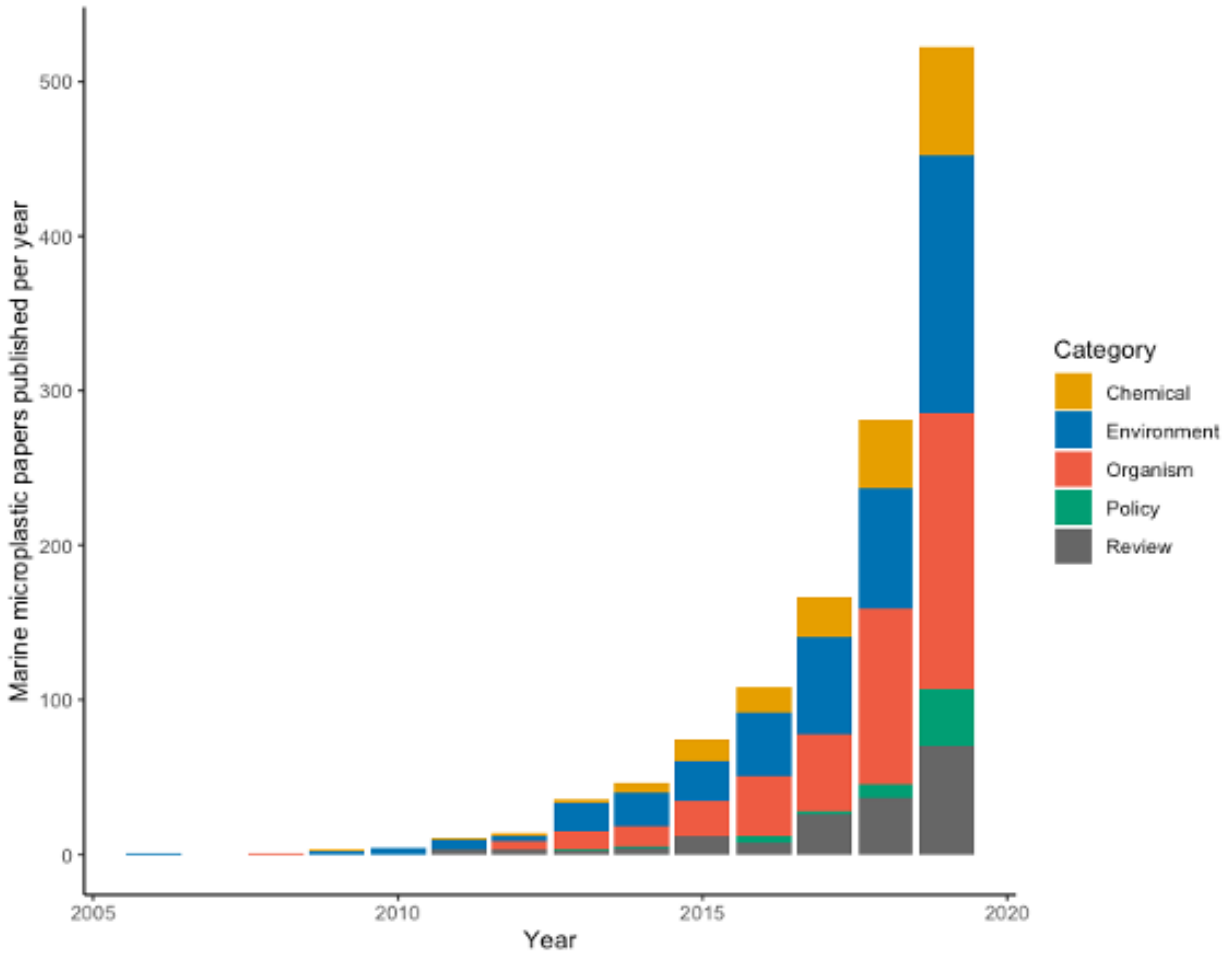


Figure 3.4 Marine microplastic paper publications each year segmented by categories within the field. Papers retrieved from Web of Science for the keywords marine AND microplastic* with publication dates of 2006-2019. Categories were determined based on title and abstract of each paper. Here, each paper was only included once, and the predominate category was assigned.

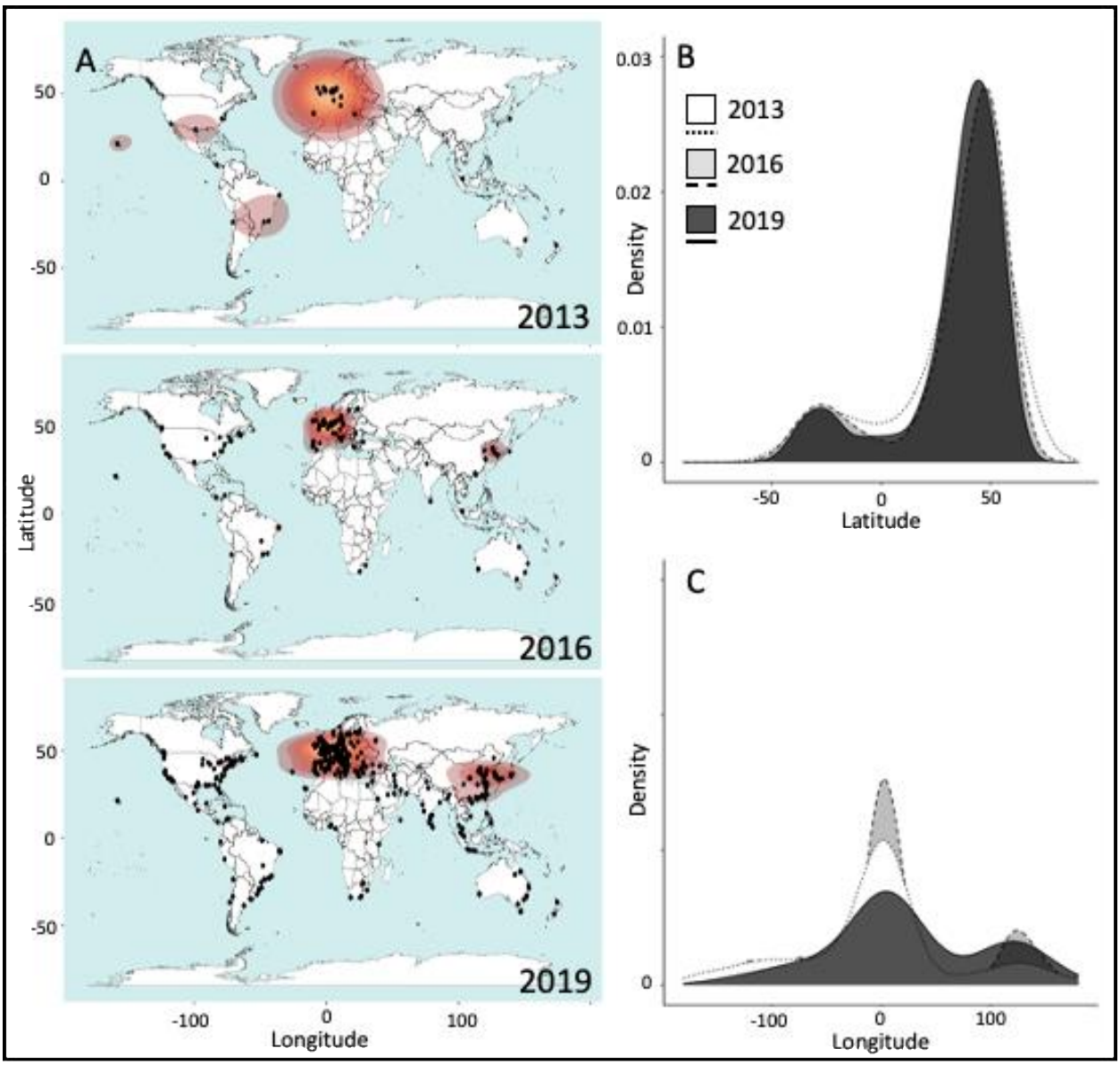


Figure 3.5 Geographic visualization of institutions publishing marine MP papers through A) hot spot analyses where red symbolizes hot spots of institutions that published papers in 2013, 2016, and 2019, B) kernel density estimations of published MP papers across latitudes and years, and C) kernel density estimations of published MP papers across longitudes and years. Colors in A) are a visualization of hot spots associated with kernel density estimation in B-C.

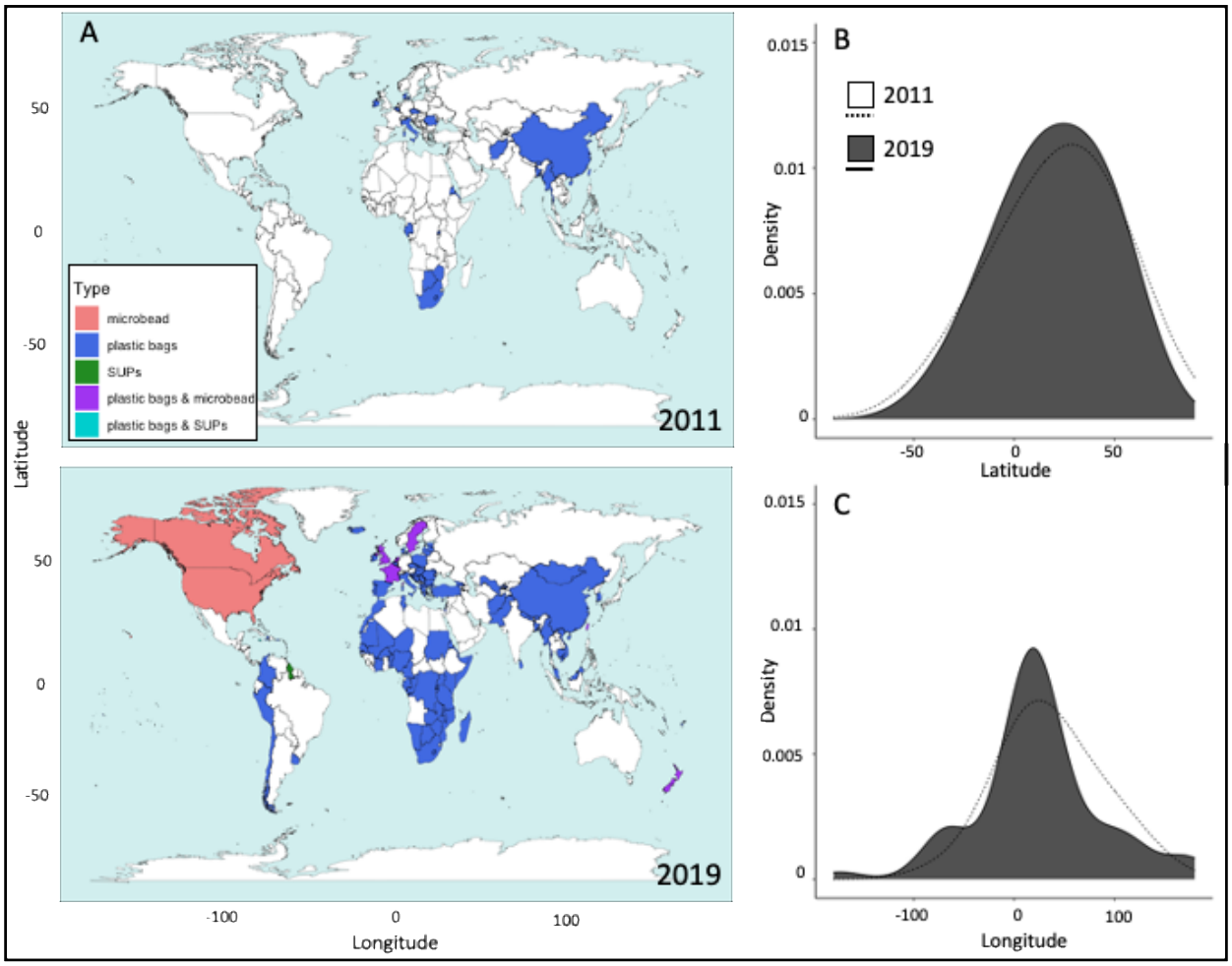


Figure 3.6 Geographic visualization of national plastic policy types in A) 2011 and in 2019. Both bans and levies are included and not differentiated in this geographic visualization. Color corresponds to the type of national plastic policy. Country centroids were used to analyze kernel density estimations of national plastic policies across B) latitudes and C) longitudes in 2011 and 2019.

3.10 Supplemental Information

Supplemental Table 1. Summary of the six International Marine Debris Conferences.

Year	Conference Location	Attending countries
1984	Honolulu, HI, USA	8
1989	Honolulu, HI, USA	10
1994	Miami, FL, USA	26
2000	Honolulu, HI, USA	20
2011	Honolulu, HI, USA	38
2018	San Diego, CA, USA	54

Supplemental Table 2. Summary of ANCOVA of the effects of year and systematic literature source on quantity of retrieved marine microplastic publications. The two sources were Scopus and Web of Science for the years 2006-2019. Asterisk (*) indicates statistical significance.

	DF	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Year	**1**	**116.80**	**116.80**	**3213.951**	**<0.001***
Source	1	0	0	0.001	0.981
Year : Source	1	0	0	0.53	0.821
Residuals	21	0.76	0.04		

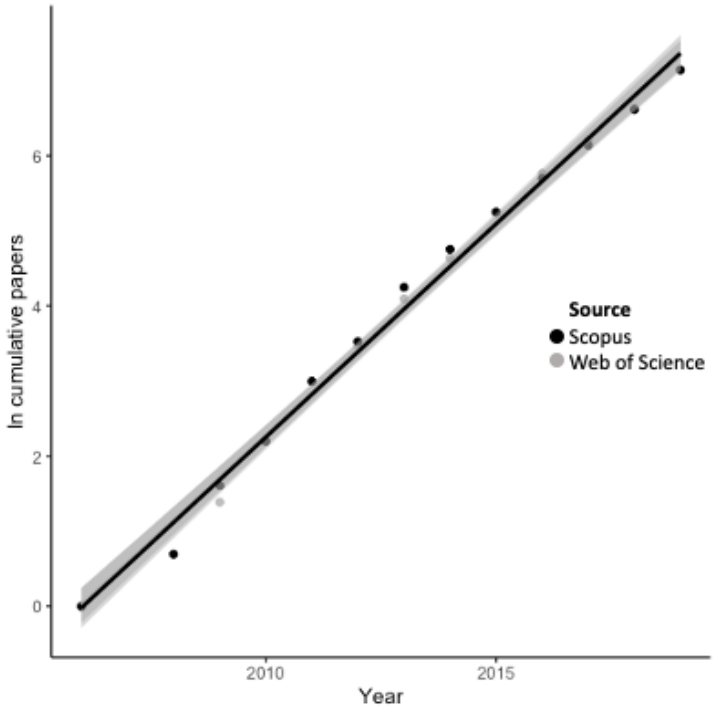
Supplemental Table 3. Summary table of national plastic policies implemented by each country. As of 2019, a total of 115 countries implemented one or more type of plastic policy. Asterisks (*) indicates the policy was ineffective and later revoked. Note: Not all listed are recognized by the United Nations. Those not recognized were not included in our analyses.

Country	**Year**	**Level**	**Type**
Denmark	1993	levy	plastic bags
Bhutan	1999	ban	plastic bags*
Bangladesh	2002	ban	plastic bags
Ireland	2002	levy	plastic bags
Taiwan	2002, 2018	levy, ban	plastic bags, SUPs
South Africa	2004	levy	plastic bags
Botswana	2005, 2018	levy, ban	plastic bags
Eritrea	2005	ban	plastic bags
Belgium	2007	levy	plastic bags
China	2008	levy	plastic bags
Rwanda	2008	ban	plastic bags
Saint Lucia	2008	levy	SUPs
Malta	2009	levy	plastic bags
Romania	2009, 2018	levy, ban	plastic bags
Gabon	2010	ban	plastic bags
Zimbabwe	2010	ban	plastic bags
Afghanistan	2011	ban	plastic bags
Czech Republic	2011	levy	plastic bags
Italy	2011	ban	plastic bags
Myanmar	2011	ban	plastic bags
Republic of the Congo	2011	ban	plastic bags
Bulgaria	2012	levy	plastic bags
Haiti	2012	ban	plastic bags
Hungary	2012	levy	plastic bags
Mali	2012	ban	plastic bags
Serbia	2012	levy	plastic bags
Vietnam	2012	levy	plastic bags
Ivory Coast	2013	ban	plastic bags*
Mauritania	2013	ban	plastic bags
Niger	2013	ban	plastic bags
Macedonia	2013	levy	plastic bags*

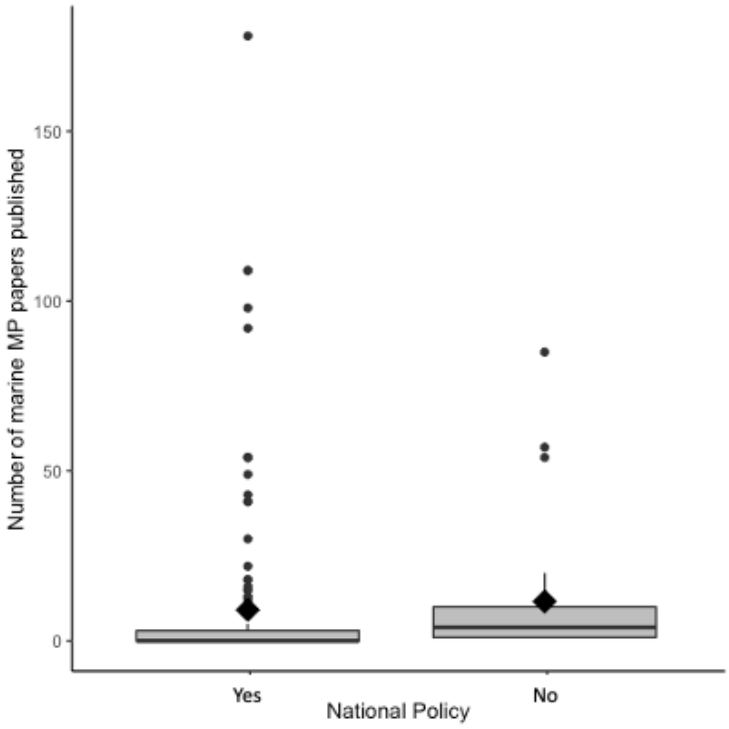
Pakistan	2013	ban	plastic bags
Tonga	2013	levy	plastic bags
Cameroon	2014	ban	plastic bags
Guyana	2014	ban	SUPs
Burkina Faso	2015	ban	plastic bags
Gambia	2015	ban	plastic bags
Hong Kong S.A.R.	2015	levy	plastic bags
Madagascar	2015	ban	plastic bags
Malawi	2015	ban	plastic bags
Portugal	2015	levy	plastic bags
United Kingdom	2015, 2018	levy, ban	plastic bags, microbead
United States of America	2015	ban	microbead
Djibouti	2016	ban	plastic bags
Estonia	2016, 2017	levy, ban	plastic bags
France	2016, 2018	ban	plastic bags, microbead
Guinea Bissau	2016	ban	plastic bags
Maldives	2016	ban	plastic bags
Mauritius	2016	ban	plastic bags
Monaco	2016	ban	plastic bags
Morocco	2016	ban	plastic bags
Mozambique	2016	levy	plastic bags
Nepal	2016	ban	plastic bags
Netherlands	2016	levy, ban	plastic bags, microbead
Puerto Rico	2016	ban	plastic bags
Senegal	2016	ban	plastic bags*
Andorra	2017	ban	plastic bags
Antigua and Barbuda	2017	ban	plastic bags
Benin	2017	ban	plastic bags
Cambodia	2017	levy	plastic bags
Cape Verde	2017	ban	plastic bags
Colombia	2017	ban	plastic bags
Fiji	2017	levy	plastic bags
Georgia	2017	ban	plastic bags
Israel	2017	levy	plastic bags
Kenya	2017	ban	plastic bags
Malaysia	2017	ban	plastic bags
Marshall Islands	2017	ban	plastic bags
Palau	2017	ban	plastic bags
Seychelles	2017	ban	plastic bags, SUPs

Slovakia	2017	levy	plastic bags
Sri Lanka	2017	ban	plastic bags
Tunisia	2017	ban	plastic bags
Albania	2018	ban	plastic bags
Bosnia and Herzegovina	2018	levy	plastic bags
Canada	2018	ban	microbead
Cyprus	2018	levy	plastic bags
Democratic Republic of the Congo	2018	ban	plastic bags
Greece	2018	levy	plastic bags
Lithuania	2018	levy	plastic bags
Luxembourg	2018	levy	plastic bags
New Zealand	2018, 2019	ban	plastic bag, microbead
Poland	2018	levy	plastic bags
South Korea	2018	ban	plastic bags
Moldova	2018	ban	plastic bags
Somalia	2018	ban	plastic bags
Spain	2018	levy	plastic bags
Sudan	2018	ban	plastic bags
Sweden	2018	levy, ban	plastic bags, microbead
Togo	2018	ban	plastic bags
Uganda	2018	ban	plastic bags
Vanuatu	2018	ban	plastic bags
Zambia	2018	ban	plastic bags
Bahrain	2019	ban	plastic bags
Barbados	2019	ban	SUPs
Burundi	2019	ban	plastic bags
Chile	2019	ban	plastic bags
Croatia	2019	levy	plastic bags
Dominica	2019	ban	plastic bags
Grenada	2019	ban	SUPs
Iceland	2019	levy	plastic bags
Jamaica	2019	ban	plastic bags, SUPs
Latvia	2019	levy	plastic bags
Mongolia	2019	ban	plastic bags
Namibia	2019	levy	plastic bags
Nigeria	2019	ban	plastic bags
Peru	2019	levy	plastic bags
Samoa	2019	ban	plastic bags
Slovenia	2019	levy	plastic bags

Tanzania	2019	ban	plastic bags
Turkey	2019	levy	plastic bags
Tuvalu	2019	ban	plastic bags
Uruguay	2019	ban	plastic bags
Uzbekistan	2019	levy	plastic bags
Vatican City	2019	ban	plastic bags



Supplemental Figure 1. Semi-ln cumulative number of marine plastic papers retrieved from Scopus (grey) and Web of Science (black) from 2006-2019. There is no statistical difference between quantity of papers retrieved from the two databases ($p = 0.98$; ANCOVA; Supplemental Table 2).



Supplemental Figure 2. Number of marine microplastic papers published by countries with (yes) and without (no) national plastic policies. Papers were collected from Web of Science with the keywords marine AND microplastic* from 2006-2019. There was no statistical difference in publication quantities between countries with and without national plastic policies ($p = 0.62$, t-test).