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SPECIAL ISSUE-LETTER

Impacts of microplastic vs. natural abiotic particles on the clearance rate of a marine mussel

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Scientific Significance Statement

Microplastic is a rising form of marine pollution and interacts with numerous organisms, such as suspension-feeding mussels. Mussels can filter microalgae, natural sediment like silt, and now, microplastic. Microplastic is known to have some negative effects on mussels at high concentrations but it remains unclear if microplastic inhibits feeding processes. We found evidence that mussel clearance rate (CR) is inhibited under high concentrations of microplastic and not by similar concentrations of other abiotic particle or current levels of microplastics in nature. Decreased mussel CRs could have important consequences at the ecosystem level, such as reducing water clarity and benthic-pelagic coupling.

Abstract

In coastal habitats, mussels are exposed to microplastics (MP; plastic 0.1 μm–5 mm) 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 (CR) 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. CR decreased by 62% at high MP concentrations (> 1250 particles mL^{-1}) but was not affected at equivalent silt concentrations. These findings suggest high MP concentrations inhibit mussel CR, 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.

Increased industrialization and urbanization have contributed to increased anthropogenic pollution in coastal habitats, including fertilizers, chemicals, sediment, and microplastics (MP, 0.1 μm–5 mm; Arthur et al. 2009; Hartmann et al.

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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 (Setälä 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. 2016; 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 (< 1–20 μm), larger diatoms (2–200 μm), and inorganic matter such as silt (2–63 μm; 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 et al. 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 (Fig. 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 (Fig. 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).

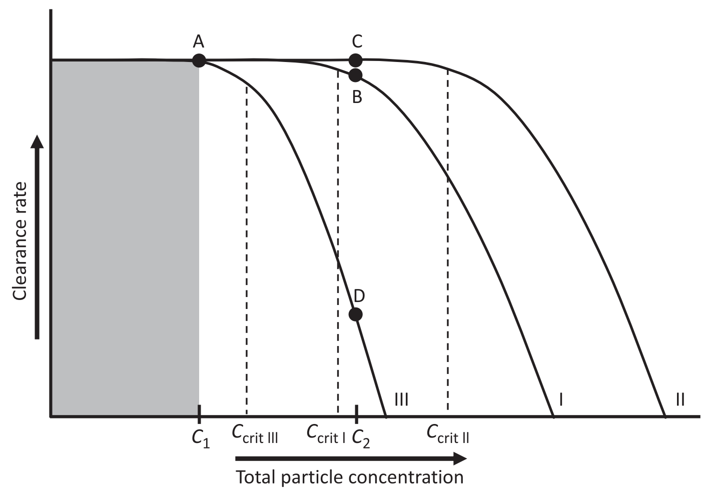

Fig. 1. Conceptual diagram of functional response curves for mussel CR as a function of total particle concentration under different scenarios of mixed particle suspensions. Gray area represents baseline concentrations with only microalgae present. A typical response to increased microalgal concentration 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 CR (A → D).

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.

Methods

Mussel collection

Wild Pacific blue mussels, *M. trossulus*, were collected from Argyle Lagoon (48.519401, −123.013180), located on the East side of San Juan Island in Washington State, U.S.A. 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 h 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; Supporting Information Table S1). Total particle concentrations in abiotic treatments were kept within the optimal CR range of 5000–20,000 particles mL^{-1} (Ward et al. 1998; Riisgard et al. 2011). A broader range of algal concentrations (4000–25,000 particles mL^{-1}) was used to control for total particle number. A broader range of abiotic concentrations (0–11,250 particles mL^{-1}) 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; Supporting Information Fig. S1, Table S2). Preliminary observations of pseudofeces and feces confirmed mussels actively filter, reject, and ingest all particles tested (algae, MP, and silt; Supporting Information Fig. S2).

The particle treatments were established in 1 μm filtered seawater (FSW) as follows (Supporting Information Table S1):

- *Algae*: *Dunaliella* spp., grown in culture at FHL, was used due to its size (10–20 μm) and chlorophyll fluorescent marker. Mussels were exposed to algal concentrations of 4000–25,000 cells mL^{-1} 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 (7000–12,000 cells mL^{-1}).
- *Microplastic + algae*: Fluorescent violet polyethylene spheres 32–38 μm (Item # UVPMS-BV-1.00 32-38um; Cospheric; Supporting Information Fig. S3; Mazurais et al. 2015) were soaked in Tween-20 for 12 h to reduce hydrophobicity before adding to FSW and algae. Microplastic concentrations ranged from 1 to 2500 particles mL^{-1}, levels that are lower than previously published experiments (e.g., Rist et al. 2016) but do, however, exceed environmental concentrations (Davis and Murphy 2015; Desforges et al. 2015).
- *Silt + algae*: Silty sediment was collected from Willapa Bay, Washington State from which silt was fractionated to 30–37 μm and sterilized in an autoclave. A stock solution of 2.25×10^5 particles mL^{-1} (counted using a hemocytometer) was diluted to establish concentrations of 1–11,250 particles mL^{-1}. Silt concentrations > 2500 particles mL^{-1} 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 particles mL^{-1}), low–med (626–1250 particles mL^{-1}), high–med (1251–1875 particles mL^{-1}), and high (1876–2500 particles mL^{-1}).

Measuring CR

Experimental mussels were starved for 12 h in 1 μm FSW at 9–11°C. Individual mussels were then placed in 3-liter plastic containers with 1-liter 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 1 h. Water samples (1.5 mL) were taken every 15 min and processed on a flow cytometer to quantify algal concentration over time. 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^{-1}) vs. 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-Wilk's test). We randomly chose and ran multiple treatments and concentrations simultaneously each day and pooled data. We used analysis of covariance (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.

Results

For algal treatments (cell concentrations ranging 4000–25,000 cells mL^{-1}), 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; Supporting Information Fig. S1, Table S2). On average, mussel CR was 0.94 ± 0.1 L h^{-1} (n = 61) across all algae + Tween-20 concentrations. Mussels actively filtered (CR > 0.0 L h^{-1}) in 95% of trials and the percentage did not depend on particle type or concentration (88–100%; $p > 0.6$, general linearized model (GLM); Fig. 2).

Mussel CR depended significantly on the interaction between abiotic particle type and concentration (p = 0.01, particle type × concentration, two-way ANOVA; Fig. 3; Table 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). CR 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). Specifically, increasing the proportion of MP particles in suspension significantly

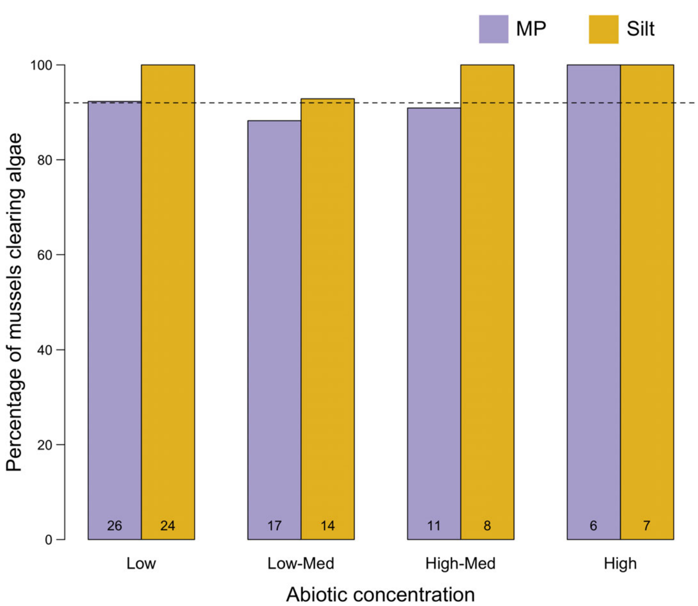

Fig. 2. Percentage of mussels feeding (CR > 0 L h^{-1}) in each abiotic particle treatment as a function of particle concentration. Bars represent absolute percentage of mussels clearing algae across all days. Mussels exposed to control treatment, algae (dashed line), actively cleared algae in 92% of 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).

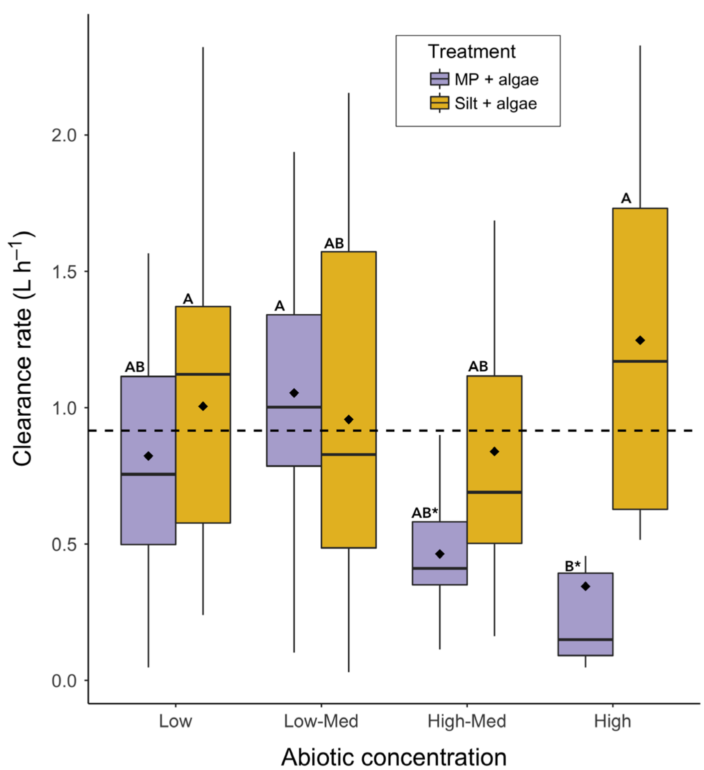

Fig. 3. Mussel CR as a function of abiotic particle type and concentration. Boxes represent upper and lower quartiles, solid lines within boxes represent median CR, and diamonds represent mean CR. The dashed line represents the mean CR for the algae control treatments across all particle concentrations (0.92 ± 0.14 L h^{-1}). 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 Supporting Information Table S1.

Table 1. Top is a summary of two-way ANOVA of the effect of abiotic particle type and concentration on mussel CR. Bottom is 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 (MP 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 × 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 × proportion	1	0.89	0.89	9.75	0.002*
Residuals	150	13.77	0.09	—	—

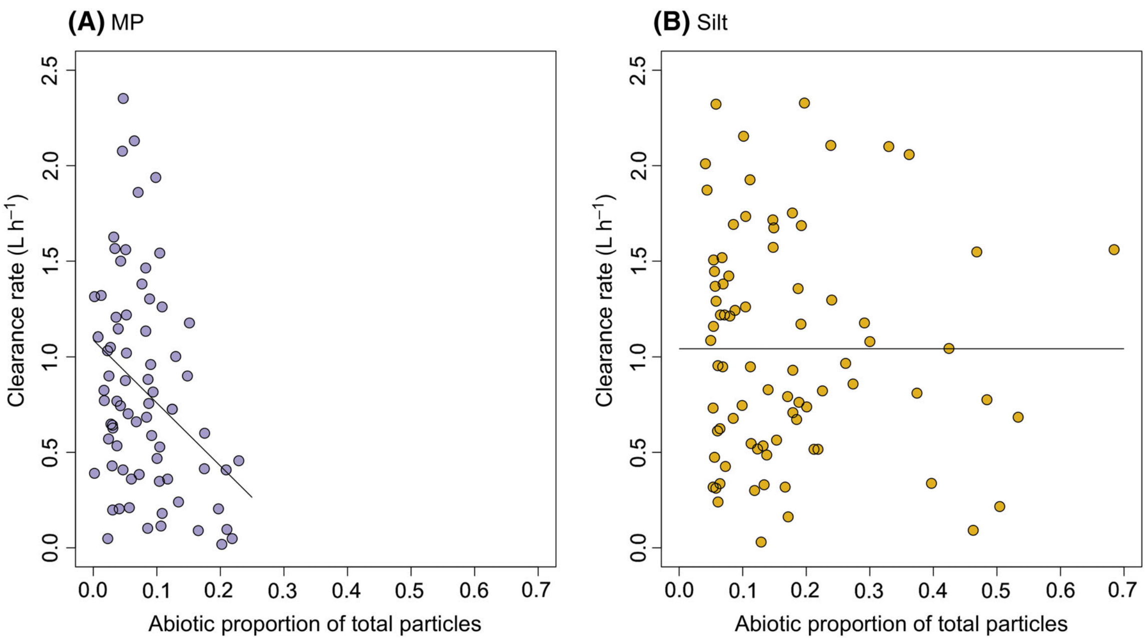

Fig. 4. Mussel CR as a function of the proportion of abiotic particles in suspension (abiotic particle concentration divided by total particle concentration) for (**A**) MP and (**B**) silt. CR decreased significantly with increasing proportions of MP ($p < 0.001$, $R^2 = 0.14$, linear regression) but 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^{-1}). CRs across increasing abiotic proportions differ between MP and silt ($p = 0.002$, abiotic proportion × particle type, ANCOVA).

decreased CR ($p = 0.0005$, $R^2 = 0.14$, linear regression) while increasing the proportion of silt particles in suspension did not ($p = 0.61$, $R^2 = 0.01$, linear regression; Fig. 4; Table 1).

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 (Supporting Information Fig. S1;

Fig. 3), while the effect of high MP concentrations on mussel CR is most likely inhibitory (e.g., curve III in Fig. 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. Microplastics may reduce CR at high concentrations as a result of unique surface properties that affect the filtration process (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 vs. curve II in Fig. 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 (~ 330 μm) are low and range 0.26–9200 particles m^{-3} in the northeast Pacific Ocean (Davis 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; however, 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 1 h. 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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