Clearance rate recovery in the blue mussel (*Mytilus edulis*) after acute exposure to microplastics

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IBIO*4600

February 7th 2020

Introduction

Plastic pollution is one of the major environmental concerns currently threatening every corner of the planet. This is particularly true within marine environments, where UV-B radiation and physical abrasion from wave action cause plastic debris to constantly degrade into smaller fragments, resulting in microplastics (Barnes et al., 2009). Typically characterized by a size smaller than 5 mm (GESAMP, 2015), there are currently over 35,540 tons of microplastics in Earth's oceans (Eriksen et al., 2014). In some intertidal locations, microplastics have been found to make up over 80% of the total plastic debris (Browne et al., 2007). Due to their ubiquitous presence and small dimensions, comparable to those of planktonic organisms, they pose a great threat to a myriad of marine invertebrates that inevitably ingest them through suspension or deposit feeding (Wright et al., 2013; Desforges et al., 2015).

Mussels are benthic organisms of interest due to their broad geographical distribution and accessibility (Bricker et al., 2014). Their extensive filter-feeding activity enables them to filter large quantities of water, thus making them highly susceptible to pollutants in the water column. The uptake of microplastics has been previously studied in blue mussels (*Mytilus edulis*), both in their natural habitat as well as in laboratory settings, with the aim of assessing the effects it can have on their physiology (Browne et al., 2008; von Moos et al., 2012). Due to their irregular shape and tendency to aggregate (Wegner *et al.,* 2012), microplastics are more difficult to digest than food particles. As a result, microplastics ingested by mussels have been shown to accumulate in their digestive systems after three hours of exposure (Browne et al., 2007; von Moos et al., 2012), where they can cause an obstruction of the intestinal tract and further induce adverse effects such as inhibiting gastric enzyme production, reducing feeding stimuli, and increasing the absorption of toxins amongst several other unfavorable impacts (Wright et al., 2013).

However, it remains unclear how microplastics affect their feeding rates, since the several studies that have assessed the problem have obtained highly varied and contradicting results. Furthermore, it is unknown whether complete clearance of the microplastics from their digestive system is possible and how much time it would take for the mussels to recover from the microplastic ingestion. The aim of this study is thus to determine the effect of acute

microplastic exposure on blue mussel (*Mytilus edulis*) clearance rate and to assess how long it will take for the mussels to recover to their original clearance rates.

It is hypothesized that acute exposure to microplastics causes a temporary obstruction of the digestive tract and slows down blue mussel filter-feeding rates until the microplastics are cleared out from their digestive system. If the hypothesis is supported, it is predicted that 1) the clearance rate of algae from the water column (a measure of mussel filter-feeding rate) will temporarily decrease post-acute microplastic exposure, 2) dissected mussels will exhibit the presence and accumulation of microplastics in their digestive tract, and 3) as duration of time following acute microplastic exposure increases, mussel feeding rates should increase until the clearance rate of algae from the water column returns back to original (pre-microplastic exposure) levels.

Methods

Experimental Specimens

Twenty blue mussels (*Mytilus edulis*) were collected from St. Andrews, N.B. and were housed at the Hagen Aqualab at the University of Guelph for the remainder of the experiment. They were on average 28.05 ± 1.02 g. Out of the 20 mussels, 14 were going to be used for the clearance rate experiment, but after being exposed to microplastics one of the mussels was found dead, so data for this mussel was discarded and only the 13 live mussels were considered for data analysis. The remaining six mussels (which were selected at random prior to treatment), were euthanized by freezing a day after microplastic exposure and were dissected for a visual examination of their digestive tracts under a dissecting microscope to confirm the presence of microplastics.

Experimental Set Up

Twenty identical 1.2 L plastic containers were placed in water baths with holes to keep Aqualab seawater cycling through, in order to maintain the temperature constant at 12˚C. Each

individual container was filled with 1 L of seawater and was randomly assigned a blue mussel and an air stone to maintain aeration and to ensure suspension of algae/microplastics. Mussels were left to acclimate in the containers for 24 hours prior to the experiment. Each day of the experiment, the mussels got water changes at 1pm and were then each fed 2 mL of 50% diluted frozen shellfish diet from Reed Mariculture. This amount of food was chosen because it creates a concentration of algae in the water column that is easy to observe under the microscope for particle counts. The feeding period lasted four hours every day until 5pm, when a second water change would take place. This ensured that the mussels followed a strictly controlled feeding schedule. Additionally, the water was tested for ammonia daily to account for this possible confounding variable. Ammonia levels never rose above 1 ppm.

Pilot Trials

To ensure that the algae remained mostly suspended in the water column to be easily accessible for the filter-feeding mussels, a pilot trial was conducted prior to the experiment. A visually detectable amount of algae was added to a water-filled container with an air stone. Simultaneously, a second pilot trial was conducted to ensure that the blue mussels would feed in the experimental containers with an air stone present. A single mussel was placed in a separate water-filled container with added food and an air stone. Both containers were left untouched for a 24h period. The next day after a visual inspection, they were both considered successful.

Trials were also run to quantify the amount of algae lost to sedimentation over the 4h feeding period without the presence of mussels, which is needed for the calculation of clearance rate (see formula in next page). The initial concentration of algae in the water column (Cí) was determined by taking a water sample immediately after adding a dose of food to a water-filled container with an air stone, counting the algae particles using a hemocytometer under a microscope, replicating this procedure two more times, and taking the average of the particles counted from the three samples. The final concentration of algae in the water column

(Cf̀) was determined the same way, except samples were collected four hours after the algae was added to the water.

Clearance Rate Trials

The experiment lasted 6 days in total. Water samples were collected twice daily, at 1pm immediately after feeding, and at 5pm once the feeding period was over. On day one, samples were collected for the control (baseline) clearance rates. On day two, mussels were fed as usual but with an added dose of microplastics (concentration of 2.65 mg/L). The microplastic mixture used for this experiment consisted of extra fine non-toxic silver cosmetic glitter (polyethylene terephthalate and polyurethane-33), mixed with the 2 mL of diluted shellfish diet to promote its suspension in the water column. Once again, water samples were collected after the mixture was introduced, as well as 4 hours later. All subsequent feeding periods did not include additional microplastics and remained the same as the control day for feeding, water sample taking, and water changes for days 3-6. On the last day, after the last set of water samples were taken, the remaining 13 mussels were euthanized by freezing, were dissected, and inspected for microplastics in the digestive system.

Each day, the algae particles in the 13 water samples were counted at 1pm (initial concentration of algae, Ci) and at 5pm (final concentration of algae, Cf) using a hemocytometer under a compound microscope. To facilitate this task, photographs for each water sample were taken using an AmScope microscope camera and software. The algae cells were then counted using the photographs. The Ci and Cf values were used to calculate clearance rates (see formula below).

Clearance Rate Calculation

Clearance rate (volume of water filtered in mL per mussel per hour) on each day of the experiment was calculated for each individual mussel using Coughlan's (1969) clearance rate equation, modified to account for algae/microplastic sedimentation rate:

$$CR = \frac{v}{nt}(ln\frac{Ci}{Cf} - ln\frac{Cí}{Cf́})$$

Where: CR = clearance rate (mL/hr), v = volume of container (mL), n = number of mussels, t = feeding time (hours), Ci/Cf = initial/final concentration of algae (cells/mL) with mussels present, and Cí/Cf́ = initial/final concentration of algae (cells/mL) without mussels (to account for the sedimentation rate of particles).

Statistical Analysis

SPSS software was used for statistical analyses. A Shapiro-Wilk test was used to test for normality of the clearance rate data, and a Mauchly's test was used to test for sphericity. Additionally, histograms were used to ensure there were no significant outliers in each day of the experiment. Since both assumptions were met, a parametric repeated measures ANOVA was conducted. This test was chosen because we repeatedly sampled the mussels to determine if there were changes in CR over time after microplastic exposure. Lastly, a post-hoc pairwise comparison was used to determine which days had significantly different clearance rates from each other. α=0.05, such that p-values < 0.05 are considered significant.

Results

Average values of clearance rate ± SED for each day are reported in Table 1.
The repeated measures ANOVA determined that clearance rate was significantly different between days ($F(5, 60) = 4.31$, $p = 0.002$). The post hoc test revealed that the control day (day 1) was only significantly different from treatment day (day 2) ($p=0.017$), and that treatment day was also significantly different from day 4 ($p=0.023$), day 5 ($p=0.005$), and day 6 ($p=0.013$). Day 3, which was the day after the treatment was administered, was only found to be significantly different from day 5 ($p=0.032$).

Overall, it was found that there was a significant increase in clearance rate four hours after microplastic exposure (from the control clearance rate of 57.6 ± 13.9 mL/hr, to a post-

treatment rate of 135.8 ± 23.1 mL/hr). The average clearance rate therefore increased by about 2.3x after only four hours of exposure. After having peaked post-exposure, the average clearance rate decreased, until it returned back to baseline rate within 52 hours, and stayed constant for the remainder of measurement days (Figure 1). The day following exposure to microplastics, mussels exhibited an "in between" average rate of 109.5 ± 42.6 mL/hr, suggesting that the decrease back to baseline rates was gradual. Clearance rate had already started recovering within 28 hours post exposure. Additionally, the presence of pseudofaeces was detected in each container post-microplastic exposure.

The six mussels that were dissected a day after being exposed to microplastics were all found to contain microplastics in their digestive tracts, though in very small quantities that were nearly negligible. As for the remaining 13 mussels, none of them were found to contain microplastics in the gut at the end of the experiment (four days post treatment), although a few of them had microplastics present in their body cavity.

Table 1. Mean values of clearance rate of blue mussels (*Mytilus edulis*) in mL/mussel/hr with their respective standard error. Day 1 is the control day and day 2 is the microplastic treatment day. Days 3-6 are post treatment. Means do not include data for the 6 mussels that were selected for dissection (n=13).

			95% Confidence Interval	
Day	Mean	Std. Error	Lower Bound	Upper Bound
1	57.687	13.933	27.329	88.044
2	135.818	23.123	85.438	186.198
3	109.526	24.617	55.891	163.162
4	52.180	15.979	17.366	86.995
5	53.374	13.059	24.921	81.826
6	59.245	14.373	27.930	90.560

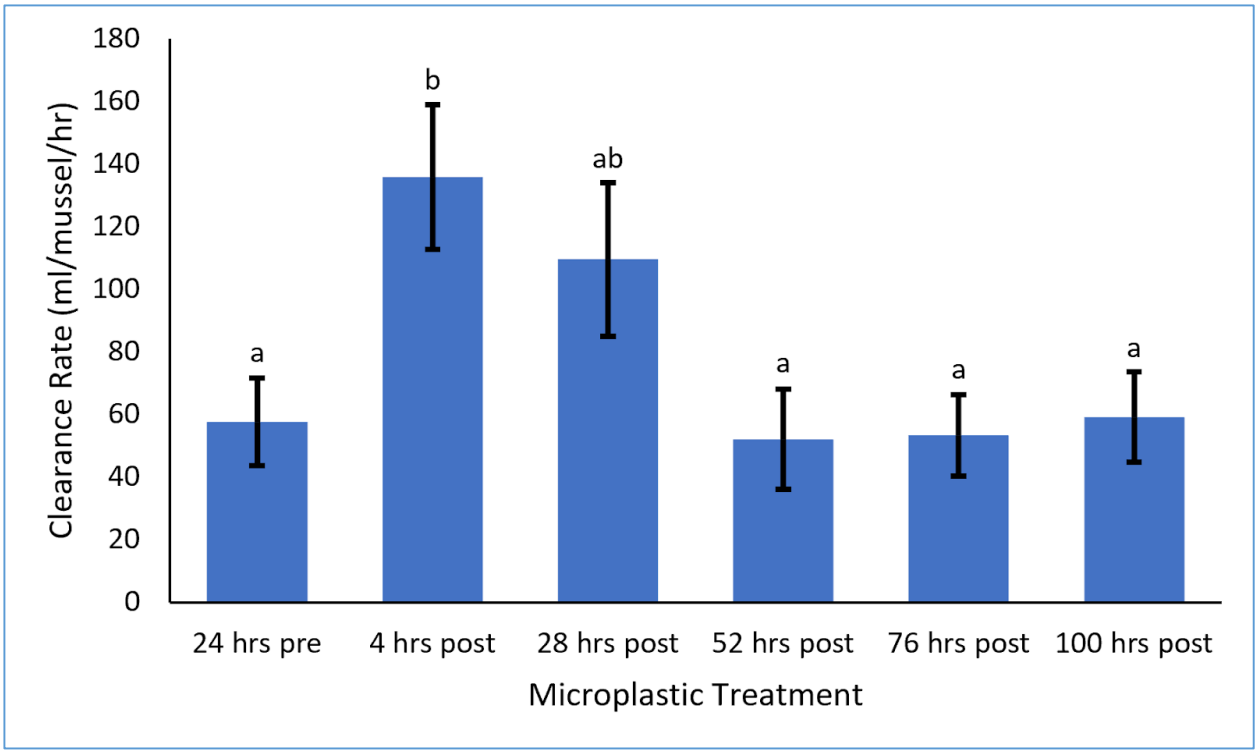

Figure 1. Average clearance rate (mL/mussel/hr) of blue mussels (*Mytilus edulis*) over time after being acutely exposed to microplastics. Significant differences were found between days based on the repeated measures ANOVA, as denoted by different lower-case lettters ($F(5, 60) = 4.31$, $p = 0.002$). Average clearance rate significantly increased (~2.3x) after 4 hours of exposure to microplastics, then decreased gradually until it returned to original clearance rate. Means do not include data for the 6 mussels that were selected for dissection (n=13). Error bars are ± SED.

Discussion

The purpose of the present study was to investigate the effect of a short-term microplastic exposure on the clearance rate of the blue mussel, *Mytilus edulis*, and to determine the duration of time it would take for them to restore their feeding activity back to baseline rates. It was hypothesized that blue mussel filter-feeding rates would be temporarily reduced after an acute exposure to microplastics due to an accumulation of these particles in the digestive tract, and that feeding rates would be restored back to baseline levels once the particles were cleared out of the digestive system. Overall, it was found that the average clearance rate (a measure of mussel filter-feeding rate) increased significantly (~2.3x) after four hours of exposure to microplastics, and then gradually decreased back to baseline levels within

52 hours. Although the clearance rates were re-established over time as predicted, our results contradict the hypothesis that feeding rates would decrease following an acute exposure to microplastics. Additionally, in accordance with our prediction, the six muscles that were randomly selected for dissection immediately post-exposure were found to contain microplastics in their digestive tract. However, the amount was so small that it could not be considered an "accumulation" as it was predicted. Since the hypothesis could not be fully supported by our results, it was rejected.

A possible mechanism that could explain our results, as suggested by Sussarellu et al. (2016), is that mussels increase their feeding rates post-microplastic exposure to overcompensate for the low nutritional value of the microplastics, and enhance mechanical digestion and nutrient absorption efficiency to compensate for the reduced energy intake that results from microplastic interference in the digestive tract. This suggests that mussels are able to differentiate good nutrient sources from bad ones, and are able to modify their feeding behaviour as a result.

Although the presence of microplastics was detected in the digestive tracts of the six mussels that were dissected immediately after microplastic exposure, these were found in very small amounts (in some cases only one particle was detected). This can be attributed to the fact that *Mytilus edulis* has been proven to exhibit particle selection, which occurs at the labial palps (Kiorboe and Mohienberg 1981). Their ability to retain microplastics in their system was also limited by the production of pseudofaeces, a phenomenon that has been observed in previous studies (Wegner et al. 2012; Rist et al. 2016; Xu et al. 2016; Santana et al. 2018). Kiorboe and Mohlenberg (1981) reported that particle selection in filter-feeding bivalves is only possible when pseudofaeces are produced. The presence of pseudofaeces in the current study following the treatment suggest that the mussels used were indeed using a particle-selection strategy to minimize their intake of microplastics. However, the enhanced production of pseudofaeces as a defense mechanism to reject these particles from their bodies requires mucus production, a process that is energetically costly and can thus lead to a depletion of energy reserves (Kiorboe and Mohlenberg 1981). It is currently unknown which properties are responsible for

determining the likelihood of a particle to get trapped in mucus and thus be excreted as pseudofaeces.

The existing literature shows that the response of mussel filter-feeding rates to microplastic exposure has been varied. Similarly to the present study, a few researchers have found that mussels exposed to microplastics increased filter-feeding rates compared to mussels that were not exposed to this treatment, although their results were not found to be significant (Jonsson 2016; Santana et al. 2018). In contrast, other studies found a significant reduction in feeding rates after being exposed to both low and high concentrations of microplastics (Wegner et al. 2012; Rist et al. 2016; Xu et al. 2016), with rates dropping as much as 79% in groups that were exposed to severe microplastic pollution levels compared to control groups (Rist et al. 2016). Moreover, several other studies found no significant effect of microplastic exposure on the feeding rates of various bivalves (including mussels), regardless of microplastic concentration (Browne et al. 2008; Green 2016; Redondo-Hasselherharm et al. 2018; Revel et al. 2019). Evidently, these results pertaining to the effect of microplastics on filter-feeding rates are highly varied, with no overall trend. Regardless of the direction of these results, higher concentrations of microplastics in the water column could lead to higher accidental rates of ingestion (despite their particle selection capacity), due to the heteroaggregation of these particles with algae, which makes them more bioavailable to mussels (Wegner et al. 2012). If this is the case, higher algae concentrations may potentially also lead to higher microplastic ingestion (Wegner et al. 2012).

Conclusion

The present study demonstrated that despite the high concentrations of microplastics that were used, the mussels were able to clear the pollutants out of their systems and recover to normal feeding rates within 52 hours, which is indicative of their outstanding ability to filter water. Additionally, this study suggests that blue mussels can recover from an acute exposure to microplastics. However, in the field, mussels are chronically exposed to them, likely for the entire duration of their lives, so it might be of higher relevance to study the long term/chronic

effects of microplastics on their overall biology (e.g. physiology, multi-generational effects), as well as the impacts on food web.

Furthermore, the evidence presented in this study suggests that the presence of microplastics in the water column are eliciting responses in the mussels that are highly energetically costly; increasing feeding rates to compensate for the low nutrient value of microplastics, and enhancing the production of pseudofaeces to reject these foreign particles from their bodies via particle selection. Undoubtedly, these metabolically expensive processes can be detrimental to the organisms in the long term, potentially affecting other aspects of their biology such as growth and reproduction. Due to the highly variable results in the literature, it is imperative to repeat similar experiments under standardized conditions to obtain more reliable and comparable data, and therefore be able to make better predictions of how plastics in the ocean are affecting the life within it.

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Pairwise Comparisons

Measure: CR

(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
1 control	2	-78.131*	28.073	.017	-139.297	-16.966
	3	-51.840	28.849	.098	-114.696	11.016
	4	5.506	19.670	.784	-37.351	48.364
	5	4.313	16.281	.796	-31.161	39.787
	6	-1.559	19.491	.938	-44.026	40.908
2 MP day	1	78.131*	28.073	.017	16.966	139.297
	3	26.292	16.900	.146	-10.530	63.113
	4	83.638*	32.151	.023	13.587	153.689
	5	82.444*	24.302	.005	29.494	135.395
	6	76.573*	26.208	.013	19.469	133.676
3	1	51.840	28.849	.098	-11.016	114.696
	2	-26.292	16.900	.146	-63.113	10.530
	4	57.346	34.177	.119	-17.119	131.812
	5	56.153*	23.121	.032	5.776	106.529
	6	50.281	29.416	.113	-13.810	114.372
4	1	-5.506	19.670	.784	-48.364	37.351
	2	-83.638*	32.151	.023	-153.689	-13.587
	3	-57.346	34.177	.119	-131.812	17.119
	5	-1.193	20.609	.955	-46.096	43.709
	6	-7.065	14.293	.630	-38.206	24.076
5	1	-4.313	16.281	.796	-39.787	31.161
	2	-82.444*	24.302	.005	-135.395	-29.494
	3	-56.153*	23.121	.032	-106.529	-5.776
	4	1.193	20.609	.955	-43.709	46.096
	6	-5.872	20.467	.779	-50.466	38.723
6	1	1.559	19.491	.938	-40.908	44.026
	2	-76.573*	26.208	.013	-133.676	-19.469
	3	-50.281	29.416	.113	-114.372	13.810
	4	7.065	14.293	.630	-24.076	38.206
	5	5.872	20.467	.779	-38.723	50.466

Based on estimated marginal means

*. The mean difference is significant at the .05 level.