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The Impact of Microplastics on *Crassostrea virginica* Filtration Efficiency

Jenna Carpenter, Brianna Sierra, and Heather Masonjones[1]
Department of Biology, University of Tampa, Tampa, FL 33606, [1]Faculty Advisor

ABSTRACT

There has been increasing concern among biologists about whether or not microplastics are affecting the health of marine organisms and humans. While some studies have shown microplastic effects on juvenile *Crassostrea virginica (Eastern Oyster)*, there are few studies which focus on how adult *Crassostrea virginica* are affected. This experiment focuses on the ability of adult *Crassostrea virginica* to filter and dispose of different densities of fluorescent polyethylene microbeads obtained from Cospheric Innovations. *Crassostrea virginica*were collected in Tampa Bay and once acclimated, were exposed to treatment concentrations of 0.167 microbeads mL^{-1} and 0.083 microbeads mL^{-1} (500 μm–600 μm) at a density of 1g cm^{-3} over a six hour time interval. An examination of adult oyster clusters exposed to different amounts of microbeads showed the adult oyster's ability to take in microplastics. Microplastics were seen in higher amounts within the 0.167 microbeads mL^{-1} concentration, with a maximum of 52 microbeads observed. Some oysters filtered in microbeads, demonstrating the potential for microplastics to impact oyster health, specifically mass loss. This issue is prevalent in oyster populations and can impact higher trophic level organisms through bioaccumulation. These results demonstrate the issues associated with microplastics and encourage future research to be conducted.

1 INTRODUCTION

Plastics have been increasing in volume within the marine environment, leading to significant issues within the ecosystem. Problems associated with plastics in the marine environment are plastic consumption leading to high rates of mortality among organisms, and plastics that hinder an organism's ability to maneuver and grow specifically among marine mammals (Conkle et al., 2018; Schuyler et al., 2014). Other ways that plastics influence the marine environment are that plastics reduce visibility, concentrate toxic pollutants, and also break down over time to form microplastics (Mato et al., 2001; Paul-Pont et al., 2016).

Microplastics are defined as plastic granules, fibers, beads, etc. that are less than 5 mm in diameter (Barnes et al., 2009). Microplastics enter marine ecosystems through the industrial industry or the breaking down of larger plastics over time (Sussarellu et al., 2016). Plastics are shown to be negatively impacting the environment, as well as the organisms around them. With greater concentrations of microplastics, there comes an issue of organisms possibly ingesting them. Suspension feeders, specifically oysters, are particularly susceptible to filtering in microplastics.

Oysters are important organisms that are present within the marine ecosystem. They act as a nursery for many fish species and invertebrates, providing a place of shelter for smaller and younger organisms from larger predators and are a food source for many. Bivalves (mussels, oysters, clams, scallops) are important with the coastal ecosystem to not only marine life but to humans as well. Oyster reefs that inhabit coastal areas provide a buffer between the shore and rough waves. They also improve water quality through their constant filter feeding by drawing water over their gills, functioning as a natural filter to clean water that is overloaded with nutrients. However oyster reefs are being threatened by a pollution, specifically microplastics.

Microplastics when ingested by oysters have the potential to either cause mechanical or chemical harm to the organism (Bonello et al. 2018). Bivalves typically deal with inorganic or less nutritious matter by means of molding them into a mucous ball and depositing them in the siphon for disposal later on. Little is known about whether or not this process is used for dealing with microplastic consumption in oysters (Waite, 2017; Xu et al., 2017). Oyster reefs are also important habitats and nurseries, as well as a viable food source for larger organisms (Green et al., 2017). The ingestion of oysters by larger organisms can lead to microplastics and nanoplastics being transferred up the food chain (Cole & Galloway, 2015; Green, 2016). Potential impacts for human consumption of oysters that have consumed micro or nanoplastics have not been studied but could develop into a larger issue (Phuong et al., 2018). Microplastics have the ability to draw harmful hydrophobic substances and contaminants to them (Setälä et al., 2016). Examples include concentrating polychlorinated biphenyls (PCBs) as well as already having harmful substances present on the microplastics already such as brominated flame retardants which can be carcinogenic and harmful (Barnes et al., 2009). While the impact of larger plastics has been studied, very little is known about micro and nanoplastic impacts and due to their bioavailability in the marine ecosystem they are becoming a more prevalent issue (Cole et al., 2015).

Many studies have been conducted in order to determine the impact of microplastics on organisms, specifically those that are benthic. These studies have been conducted in the laboratory and under controlled conditions. Evaluating the environmental concentrations of microplastics has been conducted through collection of water samples and the use of plankton tow nets. The environmental concentrations of microplastics within the Old Tampa Bay Area, specifically a site 2.74 km from the *Crassostrea virginica* sampling site was found to contain 1.00 microplastics L^{-1} at a depth of 1 m. A plankton tow net from the same site was found to have 0.0046 microplastics L^{-1} at a depth of 1–2 m and was towed for approximately 3 minutes (McEachern, 2018). No studies have been conducted on microplastic concentrations in oyster reefs, close to the deposition area in the Old Tampa Bay area. Studies of environmental concentrations of microplastics have focused on surface level quantifications of their abundance. Therefore, a larger concentration of microplastics was used within the laboratory setting due to the short time span. In a natural setting, oysters would be exposed to a variety of factors such as sediment deposition, predators, currents, influx of freshwater and marine

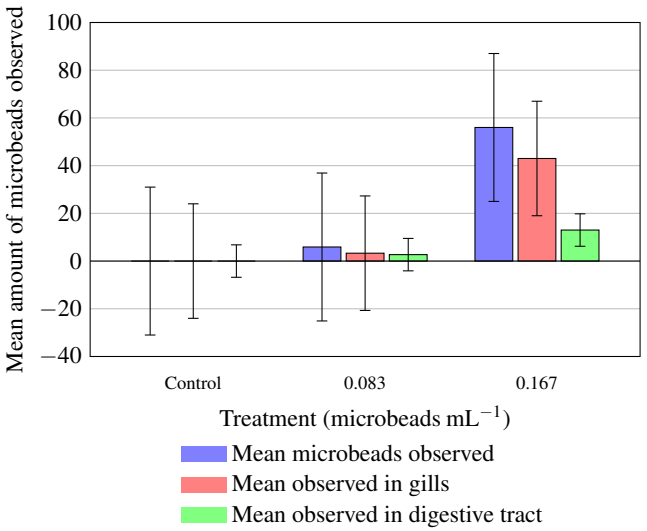

Fig. 1. Mean amount of microbeads observed across three treatment levels of microbeads mL^{-1} in *Crassostrea virginica*. Bars on the graph represents the mean amount of microbeads observed overall, in the gills or in the digestive tract for each treatment . The standard deviation was calculated based on each treatment.

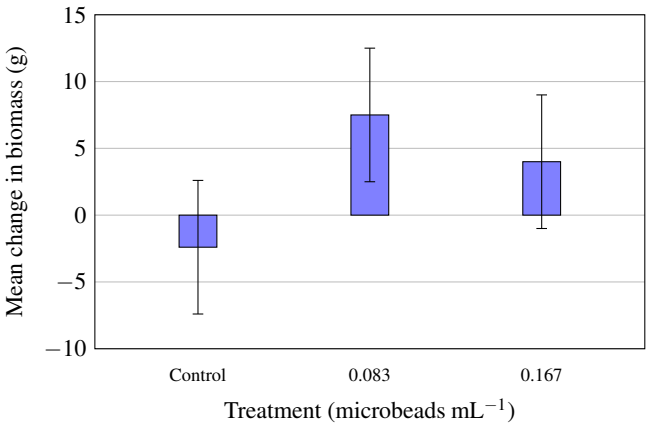

Fig. 2. Mean change in mass observed across three treatment levels of microbeads in *Crassostrea virginica*. Each bar in the graph represents the mean change in mass observed for each treatment.

debris, and competition from other organisms. The accumulation of microplastics within oysters proves to be difficult to observe and in a laboratory setting, many of these environmental parameters would need to be considered if it's to be replicated over a longer time interval.

Juvenile oysters are usually chosen for experiments monitoring micro and nanoplastics due to their fast filtration rates (Green et al., 2017). Other organisms, such as mussels and zooplankton have been studied and have shown an impact on the organism's feeding activity, circulatory system and inflammatory responses (Sussarellu et al., 2016). Previous studies have shown the impact on oyster larvae but few studies have investigated adult oysters.

In this study we aim to investigate the effects of microplastics (500 µm–600 µm) on adult *Crassostrea virginica*. The hypothesis tested was that microplastics will not impact the oyster's filtration efficiency and feeding apparatuses. The microbead concentrations used was either 0 microbeads mL^{-1}, 0.167 microbeads mL^{-1}, or 0.083 microbeads mL^{-1} over a six-hour time interval. Each cluster of oysters tested was conducted using 5 adult oysters. The study is important due to the increasing amounts of microplastics in the marine environment and by researching this hypothesis, more knowledge will be gained about filter feeders' ability to deal with exposure to microplastics.

2 MATERIALS AND METHODS

Crassostrea virginica were obtained at the University of Tampa Marine Field Station from sea walls. The sea water from the site was used to hold the oysters in the lab at a salinity of 24 g L^{-1}. The oysters were starved for 48 hours before the start of the experiment. The oysters were moved into separate containers which contained the sea water from the collection site. The mass of the oyster clumps was determined using a Navigator™ scale . Clumps averaging about 5 oysters were obtained from the buckets and placed into three different containers. Each treatment had 10 replicates, totaling 150 oysters across the three treatments. The containers contained 1–3 magnetic stir bars and rested on stirring hot plates which simulated an artificial current. The containers were split based on amounts of microplastic beads, containing concentrations of 0 microbeads mL^{-1}, 0.167 microbeads mL^{-1}, and 0.083 microbeads mL^{-1} each, the individually microplastics being 500 µm–600 µm.

The microbeads were fluorescent polyethylene microspheres obtained from Cospheric Innovations in Microtechnology. The microbeads came with a biocompatible surfactant in order for the microbeads to remain neutrally buoyant in the water column and was prepared using protocol provided by Cospheric. 1 mL of surfactant was used for the 0.083 microbeads mL^{-1} concentration and 2 mL for the 0.167 microbeads mL^{-1} concentration based on the recommended amount of surfactant per gram by Cospheric. Each container contained 6000 mL of salt water and 0.32 mL of phytoplex dilution. The phytoplex was made by Kent Marine and is aquacultured phytoplankton. In the control, just the 0.32 ml of phytoplex dilution was added. For the experimental groups 0.167 microbeads mL^{-1} and 0.083 microbeads mL^{-1} were added along with 0.32 mL of phytoplex dilution to each. The control and experimental groups were then exposed to the conditions for six hours. After the time interval, the oyster clumps were then removed and the mass was determined. Each oyster in the clump was then dissected. The amount of microplastic beads found in the experimental groups were counted after dissection.

Although the microbeads were not observed being filtered by the oysters, dissection of the oysters exposed to 0.167 microbeads mL^{-1} and 0.083 microbeads mL^{-1} found that microbeads were in fact filtered. The data was not found to be evenly distributed due to the values from the all microbead concentrations observed. The variances were not equal due to Levene's test showing $F_{2,27} = 7.7753$, $p = 0.0022$. The mean change in mass between treatments

was found to not be evenly distributed and the variances were not equal ($F_{2,27} = 3.9181$, $p = 0.0321$); therefore nonparametric Wilcoxon/Kruskal Wallis tests were employed for comparisons.

3 RESULTS

Crassostrea virginica were exposed to three treatment levels of microbeads over six-hour time intervals, the treatments specifically being 0 microbeads mL^{-1} as the control, 0.083 microbeads mL^{-1} and 0.167 microbeads mL^{-1}. The null hypothesis was microplastics and nanoplastics will not impact oyster, *Crassostrea virginica*, filtration efficiency and their feeding apparatuses. The null hypothesis was rejected ($x^2 = 23.7929$, $p = 0.0001$, $df = 2$). The control had no microbeads found, while the 0.083 microbeads mL^{-1} and 0.167 microbeads mL^{-1} concentrations found a mean of 5.9 microbeads and 56.4 microbeads respectively.

The 0.083 microbeads mL^{-1} concentration had a mean of 3.3 microbeads observed in the gills and a mean of 2.6 microbeads observed in the digestive tract. The 0.167 microbeads mL^{-1} concentration had a mean of 24.2 microbeads observed in the gills and a mean of 6.88 microbeads observed in the digestive tract (Figure 1).

The change in mass between treatments was significant ($x^2 = 20.1084$, $p = 0.0001$, $df = 2$). The control showed a mean decrease in mass of -2.44 g, and the 0.083 microbeads mL^{-1} treatments showed a mass increase of 7.44 g. The 0.167 microbeads mL^{-1} showed a 4.08 g mean mass increase (Figure 2).

Green coloration was observed in the 0.083 microbeads mL^{-1} and 0.167 microbeads mL^{-1} treatments. Fragmentation of microbeads was seen in the 0.083 microbeads mL^{-1} treatment (Table 1).

Treatment (microbeads mL^{-1})	**Coloration**	**Fragmentation**
0	N	N
0.083	Y	Y
0.167	Y	N

Table 1. Presence of Coloration/ Fragmentation across three treatment levels of microbeads in *Crassostrea virginica*. N indicates no presence, Y indicates presence of coloration/fragmentation of microbeads being observed.

4 DISCUSSION

The null hypothesis was that microplastics will not impact the oyster's filtration efficiency and feeding apparatuses . This study found that adult *Crassostrea virginica* had the ability to take in microplastics which has been seen in other studies (Waite, 2017). Research has found that the intake of microplastics has a negative impact on adult oyster reproduction as well as energy uptake and allocation (Sussarellu et al., 2016). Another study conducted with mussels, utilized microplastics found in human products and found that these microplastics impaired the mussel's digestive glands and gills (Bråte et al., 2018). Other studies incorporating microplastic filtration involved oyster larvae, which produced results that confirmed the intake of microplastics was detrimental to oyster larvae health and growth (Cole & Galloway, 2015). Our study contributes to the overall conclusion that microplastics are able to be taken up by oysters, specifically resulting in a decrease in mass after exposure.

Throughout the experiment, issues encountered were sinking and circulation of microbeads, the overall health of the oysters, and inconsistent clump sizes. The microbeads that came into contact with algae present on the oysters settled on top and did not circulate well even in the presence of bubblers and artificial current. This caused them to not be actively available to the oysters to filter. Originally a test run of each treatment level was run with individual oysters in order to determine if a one-hour time interval versus a twenty-four-hour time interval was enough. One hour was not a sufficient amount of time for the oysters to acclimate in order to start filter feeding, while twenty-four hours resulted in the oysters' overall health deteriorating. A six-hour time interval was decided upon, once the one hour and twenty-four-time interval was deemed insufficient. The oysters were all collected and placed in similar environments, however the buckets of oysters that were used for the control seemed to not be in good health after the trials were conducted due to poor water conditions. When collecting the oysters, many invasive green mussels were found within the clumps but they were removed before the trials were conducted. The clump sizes of the oysters were inconsistent due to the presence of substrate and juvenile oysters. While most of the juvenile oysters were attempted to be removed, it was difficult to separate without impacting the adult oysters. The mass for the control group was found to decrease over the six-hour interval due to the health of the oysters deteriorating. Fragmentation of microbeads was observed in the 0.083 microbeads ml-1 indicating the possible breakdown of microplastics by oysters. The tissues of the oysters were dyed a fluorescent hue of green matching that of the microbeads in the 0.083 microbeads mL^{-1} and 0.167 microbeads mL^{-1} further supporting a possible microbead breakdown.

Future work with microbeads and *Crassostrea virginica* would involve more replicates, investigating fragmentation of microbeads within the digestive tract along with possible chemical/physical factors involved in the breakdown of plastics. The use of an endoscope would provide observational data in terms of observing oysters actively filtering in/ out microbeads. Also investigating irregular shaped microplastics which would more realistically mimic the microplastics found in the natural environment.

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