

**SIZE DOES MATTER: EXPOSURE AND EFFECTS OF MICROPLASTICS ON  
THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*).**

A Final Report of the Tibor T. Polgar Fellowship Program

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## ABSTRACT

The global impact plastic pollution has on aquatic ecosystems is rapidly increasing, and there are numerous studies highlighting the negative impacts from microplastic exposure. While the general effects of microplastics are becoming clearer, less is known about the specific impacts of the various polymers that make up plastic. Moreover, many studies show the effects of exposing organisms to microplastics of the same shape and size, which is an inaccurate representation of what organisms are exposed to in the wild. Here, the Eastern Oyster (*Crassostrea virginica*) was exposed to four types of polymers, and its feces, pseudo-feces, and internal tissues were analyzed for microplastics. The results from this study showed plastic particles were present in two main organs - the digestive system, and the gills and mantle. Polystyrene was present in nearly all individuals analyzed, suggesting this type of polymer can increase exposure which may be harmful to filter feeders. Despite the use of their rejection mechanism, oysters did not distinguish polystyrene and polyvinyl chloride from food. Polyethylene was absent in tissues, but was detected in feces, suggesting that *C. virginica* can reject this polymer. Toward the end of the experiment, an accumulation of polyethylene and polyvinyl chloride was documented, suggesting that a longer-term exposure to weathered particles may have a greater impact via biofilm development. Due to their complexity it is necessary for microplastic studies to expose organisms to polymers of various types as well as irregular shapes and sizes. Understanding the potential impacts from diverse polymers is critical for management of waste and can provide important information on which types of plastic may be harmful to organisms inhabiting the environment.

## TABLE OF CONTENTS

Abstract.....	III-2
Table of Contents.....	III-3
List of Figures and Tables.....	III-4
Introduction.....	III-5
Methods.....	III-9
Collection and Maintenance .....	III-9
Experimental Design.....	III-10
Plastic Fragments .....	III-10
Ingestion by <i>Crassostrea virginica</i> .....	III-11
Microplastics in Feces and Pseudo-feces .....	III-11
Dissection and Digestion .....	III-12
Visual Inspection of Microplastics .....	III-12
Reducing Contamination .....	III-13
Statistical Analysis.....	III-13
Results.....	III-13
Discussion.....	III-20
Conclusion .....	III-23
Acknowledgements.....	III-26
References.....	III-27

## LIST OF FIGURES AND TABLES

Figure 1 – The number (Mean + SE) of microplastic fragments found with the digestive system of <i>Crassostrea virginica</i> .....	III-14
Figure 2 – The number (Mean + SE) of microplastic fragments found within the gills and mantle of <i>Crassostrea virginica</i> .....	III-15
Figure 3 – The size range (mm) of microplastic fragments found within <i>Crassostrea virginica</i> tissues .....	III-16
Figure 4 – The number of PVC and PS microplastics found within oysters digestive system.....	III-17
Figure 5 – The number (Mean + SE) of microplastic fragments found within the feces of <i>Crassostrea virginica</i> .....	III-18
Figure 6 – The number (Mean + SE) of microplastic fragments found within the pseudofeces of <i>Crassostrea virginica</i> .....	III-19
Figure 7 – The number of Polyethylene microplastics found within the feces, and pseudofeces of <i>Crassostrea virginica</i> .....	III-19

## INTRODUCTION

Plastic pollution is a major environmental concern for both aquatic and terrestrial ecosystems. Currently, global plastic production is over 300 million tons annually, and more than half of this is manufactured for single use (Lehtiniemi et al. 2018). As a result of mass production, plastics are the largest source of marine debris, and they continue to accumulate in the oceans and potentially threaten organisms at all trophic levels (Lehtiniemi et al. 2018). In general, plastic is a term used to describe synthetic materials that can be easily shaped or molded; however, it is important to recognize that the single-chain organic molecules that make up plastic are complex and vary based on the polymer construction and finished product. The polymers that are used to construct plastic make it virtually impossible to biodegrade on a short time scale, and, as a result, these objects can persist in the environment for many centuries (Green et al. 2018). Many of these synthetic polymers end up in the water as fragments of larger particles, or can arise from cosmetics and synthetic fibers from clothing (Vandermeersch et al. 2015).

Microplastics are defined as small pieces of plastic that range from 0.1  $\mu\text{m}$  - 5mm (Sussarellu et al. 2016; Green et al. 2018) and have been known to have a profound impact on marine biota from all trophic levels (Cauwenberghe et al. 2014). Although microplastics are found in many different forms, they are divided into two categories: primary and secondary. Primary microplastics are manufactured particles added to personal-care products and fillers for industrial applications. Secondary microplastics are fragments of larger plastic pieces physically broken apart through environmental exposure to sunlight and processes in the environment like wind and wave action. Secondary microplastics also include fibers that originate from the degradation of fishing

gear or clothing (e.g., nylon, spandex, etc.) and can enter the environment through wastewater from industries and households.

Once microplastics enter aquatic ecosystems, they can absorb persistent organic pollutants (POPs), such as polychlorinated biphenyls (PBCs) and polycyclic aromatic hydrocarbons (PAH) (Endo et al. 2005), and accumulate in the water column. Moreover, the plastic themselves can release ‘additives,’ which are chemical compounds added during manufacture to improve the performance, functionality, and aging properties of the polymer (Lehtiniemi et al. 2018). Today, some of the most commonly used additives in various plastic materials include plasticizers, flame retardants, and heat stabilizers, which are known to cause endocrine disruption, cancer, and birth defects (Smith and Bertola 2010). Furthermore, because the density of microplastics may vary based on chemical composition of the polymers (Lusher 2015), higher density particles may sink and accumulate in the sediment, whereas low density particles may float at the sea surface (Cauwenberghe et al. 2014). However, it should be noted that weathering processes, as well as turbulence, freshwater input, and mixing may also contribute to the relative distribution of these particles (Lusher 2015).

Today, the presence of microplastics have been reported worldwide and include samples from several marine organisms as well as sediments (Shim and Thompson 2015). Microplastics can be ingested directly by filter feeding and deposit feeding organisms, or indirectly by consumption of prey containing them (Zhang et al. 2019). It is believed that filter feeding species are especially vulnerable to microplastics because of their ability to filter large volumes of water (Sussarellu et al. 2016), thereby increasing their exposure. Several studies have tested the effects of microplastic ingestion in

bivalves (Browne et al. 2008; Von Moos et al. 2012; Cauwenberghe et al. 2014; Sussareullu et al. 2016; Khan and Prezant 2018), as well as the abundance of microplastics within aquatic systems (Eerkes-Medrano et al. 2015; Shim and Thompson 2015). River systems in particular may show specific microplastic characteristics based on the waste sources near the river (Eerkes-Medrano et al. 2015). As a result, estuaries are at a higher risk to microplastics exposure, because of their close proximity to point sources and the small relative size of estuarine systems (Eerkes-Medrano et al. 2015). Aquacultured organisms such as finfish and shellfish are also typically grown in open systems with natural seawater, which makes them extremely vulnerable and more likely to be exposed to any pollutant present in the water column (Cauwenberghe et al. 2014).

Bivalves are suitable model organisms for microplastic studies because they are good indicators of water quality, can be easily sampled, and are highly resistant to stress. In general, suspension feeders process relatively large amounts of water during feeding, which allows them to be exposed to high amounts of harmful materials, ultimately leading to an accumulation of chemical pollutants. Bivalves, like oysters, have a unique mechanism for particle selection in which they sort particles based on their size, shape, palatability or chemical composition (Ward and Shumway 2004; Xu et al. 2017). Typically, particle selection occurs on the gills and labial palps where particles selected are moved from the gills into the bivalve's mouth, where they are eaten, digested and expelled as feces. (Xu et al. 2017). Unwanted particles, which include materials that are too large and dense, small grains of sand, and detritus, are selected by the labial palps and transferred to the mantle cavity as a mucus-bound mass. This mucus-bound material is known as pseduofeces, and although it may resemble actual feces, this unwanted material

is ejected without having passed through the digestive tract (Beninger et al. 1999). Pseudofeces production is an effective mechanism bivalves have that allows the rejection of inorganic particles upon encountering them in their feeding stream; however, an accumulation of microplastics may inhibit their ability to sort and reject unwanted particles (Xu et al. 2017), leading to their accumulation in their tissues (Browne et al. 2008).

Bivalves, such as oysters, can also exhibit immunological problems including neurotoxic and genotoxic physiological responses to microplastic exposure (Avio et al. 2015), which could have a cascading ecological impact to a population. A recent study on microplastics exposure demonstrated strong impacts on the feeding activity, absorption rates, fecundity and offspring growth in oysters (Sussarellu et al. 2016). Another potential impact from the accumulation of microplastics during filter feeding can occur by reduced ciliary movement on the gills, which impacts the oyster's ability to pump water (Xu et al. 2017). Previous studies indicate that there is no accumulation of microplastics in the gut (e.g., Sussarellu et al. 2016); however, in these cases exposure focused on the use of a single type of microbead. The use of one type of polymer is not an accurate representation of what filter feeders are exposed to in nature. More importantly, the shape of a plastic particle can influence the biological effects (Choi et al. 2018) and further studies are necessary to understand the potential impacts from various types, sizes, and shapes of polymers. Overall, the accumulation of plastics may vary and ultimately depend on the chemical characteristics of each polymer. For example, polymers such as polyethylene and polystyrene are believed to absorb a much higher concentration of pollutants than compared to polyvinyl chloride (Rochman et al. 2017). Consequently,



plastic composition may shape exposure levels for pollutants and therefore, understanding the response of filter feeders to different polymers is critical to assess how microplastics may impact species and communities.

In this study, the aim was to improve the understanding of the ingestion of microplastics of varying composition, shapes, and sizes. Experiments were conducted using four common polymers found in the environment including: Polyethylene, Polyvinyl chloride, Polystyrene, and Polypropylene. These polymers are associated with many commonly used plastic products such as bags, bottles, straws, and food containers, as well as many household and automotive products. The objective was to evaluate the ingestion rate of oysters exposed to these four types of polymers, as well as analyze their tissues, pseudofeces, and feces for microplastics.

The null hypotheses tested were 1) all four types of polymers will not be ingested despite differences in chemical composition and 2) polymers will not be present in oyster tissues, feces, or pseudofeces. While it was expected to find microplastic particles present in both pseudofeces and feces, it is predicted that more particles would be found in the feces, which would suggest that oysters are not able to discriminate between plastic and their natural food sources.

## **METHODS**

### Collection and Maintenance

Fifty (50) adult Eastern Oysters (*Crassostrea virginica*) were obtained from Sweet Amalia Oyster Farm in Newfield, New Jersey during June 2018 and transported to the National Oceanic and Atmospheric Administration (NOAA) James J. Howard Marine Sciences Laboratory, where they were placed into two 10 gallon aquarium tanks.

Before initiating experiments, oysters were fed daily for one week with a commercial shellfish diet containing six marine microalgae species – *Isochrysis*, *Pavlova*, *Tetraselmis*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, and *Thalassiosira pseudonana* (Reedmariculture Inc.).

### Experimental Design

After acclimatization, oysters were randomly selected and placed into 2-Liter glass beakers and assigned to one of the following five treatments (N=10/treatment): Control, Polyethylene (PE), Polystyrene (PS), Polyvinyl chloride (PVC), and Polypropylene (PP). To standardize hunger levels, oysters were starved two days prior to the start of feeding experiments. Air circulation and mixing was achieved by attaching glass pipettes to air bubbler tubing. Oysters were fed daily using the commercial shellfish diet either with or without their designated microplastic.

### Plastic Fragments

Plastic polymers for this experiment were generated and identified using methods from Fries et al. (2013). Plastic fragments made of PP were produced from a blue used children's toy. Fragments were generated by grinding plastic in a commercial mill and storing the material in a glass vial. PE fragments were produced from a yellow mesh produce bag. Fragments were generated by using scissors to cut the mesh in various sizes and storing the material in a glass vial. PS fragments were obtained from a foam packaging material and a scalpel was used to scrape material into various sizes for feeding. PVC fragments were produced from a grey PVC pipe, and similarly to PS, fragments of PVC were obtained by using a scalpel to scrape material.

In order to standardize the amount of plastic introduced into experimental treatments, microplastic polymers were weighed and allocated into 0.0051g samples, labeled, and stored into a separate glass vial. For experimental exposure trials, the daily feeding allotment of commercial shellfish diet was added to each glass vial with their assigned polymer to a total volume of 5ml prior to exposure feeding. Each vial was thoroughly mixed by hand for one minute and then fed to the experimental oysters.

#### Ingestion by *Crassostrea virginica*

For experimental groups, oysters were fed a 5ml mixture of commercial shellfish diet along with their assigned polymer. Similarly, oysters in the control group were fed the same concentration of shellfish diet without any microplastics. Twenty four hours post feeding/exposure, five random oysters (one from each group) was chosen for dissection and analyzed for microplastics. The remaining oysters were left in beakers and fed daily the commercial diet with the assigned microplastic group (5 ml) accordingly for the following ten days. Each subsequent day, an oyster from each group was removed, dissected, and analyzed for the presence of microplastics until all oysters were dissected. There were no water changes. The last group of oysters was sacrificed on day eleven (11).

#### Microplastics in Feces and Pseudofeces

Collection of feces and pseudofeces occurred after feeding during observation hours 1, 8, and 24. In order to avoid mischaracterization during analysis, feces were collected with a dropper only when they were seen actively being released from oysters. No collection occurred when feces were observed at the bottom of the glass container, but release was not seen to minimize any bias associated with mixing of feces and pseudofeces not directly observed. The same method was applied for the collection of

pseudofeces. Identification and recognition of pseudofeces was performed by using a visual confirmation; pseudofeces were released in the form of a mucus ball, whereas feces were loose and had a string-like texture.

### Dissection and Digestion

Before dissection, each oyster was wiped with ethanol using a cotton cloth to clean the external surface of any plastics and then each individual was weighed and measured. Oysters were dissected by tissue types into Gills and Mantle, Labial Palps, Stomach (Digestive system), and Adductor Muscle. Once tissues were dissected out, they were placed into individual glass beakers and wet weight was recorded. Tissue weights were compared among treatments to determine if oyster size or tissue weight differed among treatments potentially biasing the results. Tissue samples were then digested in a 10% Potassium Hydroxide (KOH) solution. To avoid cross contamination among samples, all tools and glassware were rinsed three times between samples and each sample jar was immediately covered with aluminum foil once KOH was added. Finally, each sample jar was incubated for three days at 60 degrees Celsius to allow organic materials to digest. After digestion, solutions were filtered over a 0.45 $\mu$ m glass membrane filter (Whatman) and placed into glass petri dishes to dry for 24 hours.

### Visual Identification of Microplastics

Microplastic particles were visually identified using a compound microscope. Each filter was scanned at 10x magnification and each polymer found was measured along its longest dimension. After measuring each known polymer, a photo was taken to ensure polymers were not counted twice.

### Reducing Contamination

Preventing contamination in microplastic research is a challenge due to the airborne fibers (Cauwenberghe et al. 2014) and possible cross contamination between samples. To prevent contamination in various forms, several strategies were used in this experiment. First, the preparation of microplastics was handled in a separate room using a new 100% cotton lab coat. For processing samples, another new 100% cotton lab coat was worn at all times and before processing, all counters were wiped using deionized water followed by ethanol with a cotton cloth. All equipment used was rinsed three times before use and all sample processing was performed in a closed, isolated, plastic-free room with tacky mats. Procedural blanks were included in every KOH digestion to account for any possible contamination. Blanks were processed in the same manner as oyster tissues.

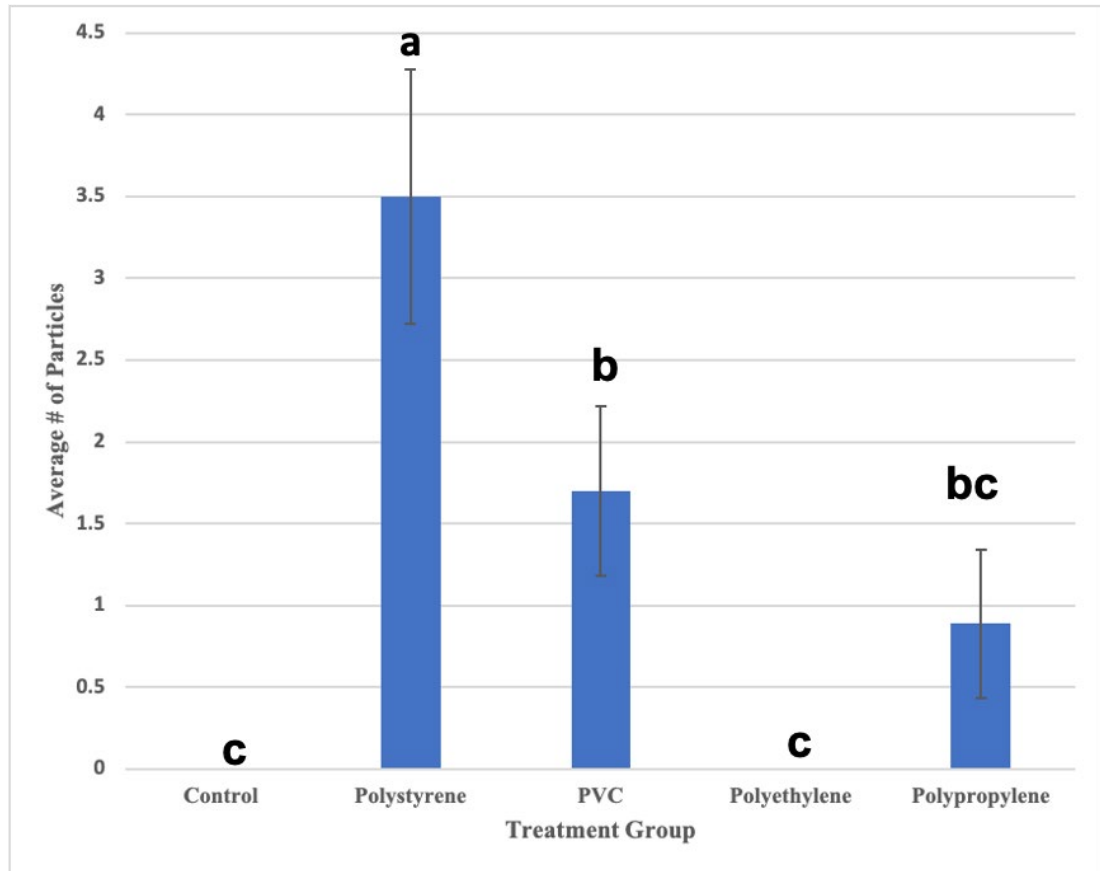
### Statistical analysis:

A one-way ANOVA was performed using the PROC GLM procedure in SAS®, with type of plastic as the independent variable and the number of plastic fragments collected from feces, pseudofeces, and tissue type as the dependent variables. Significance was attributed to comparisons between means with an alpha value set at 0.05 using the LSMEANS Procedure.

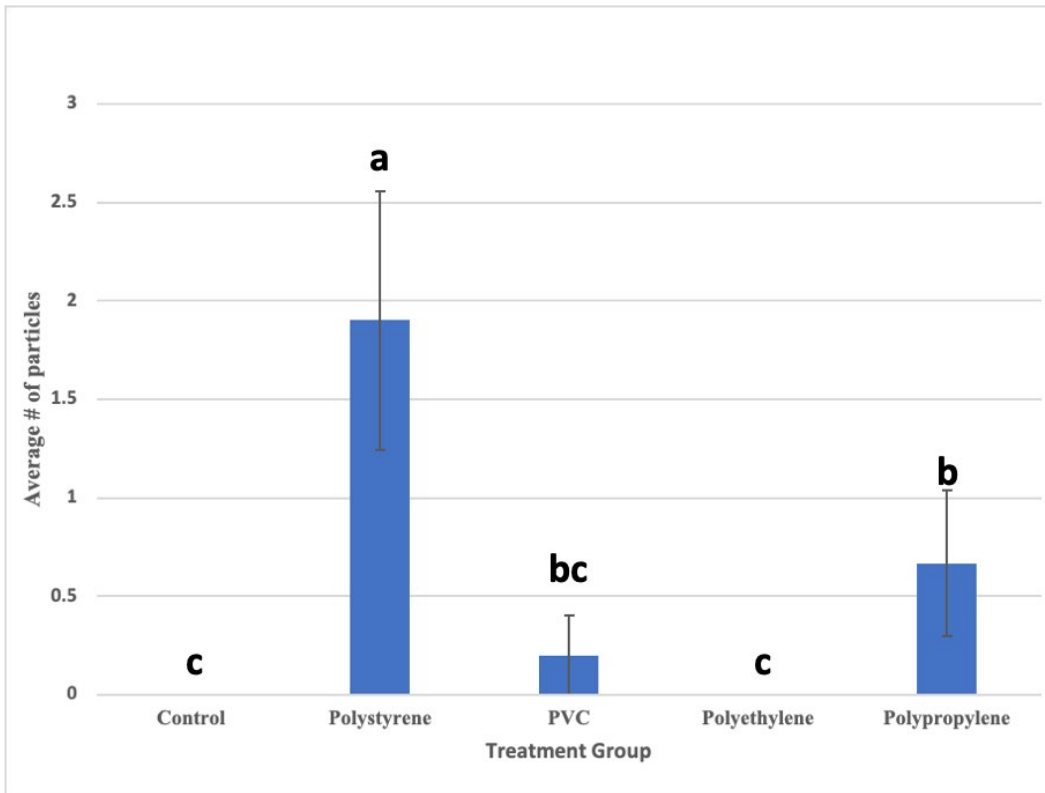
## **RESULTS**

Experimental microplastic fragments were only detected in two of the organs dissected: Digestive System (Figure 1) and the Gills and Mantle (Figure 2) tissues of the oysters, with the digestive system retaining the highest number of microplastics

(Figure 1). It should be noted that organ systems were dissected a whole. As such, it was not possible to differentiate whether plastics in the digestive system were in the lumen or had in fact passed into the digestive tract.

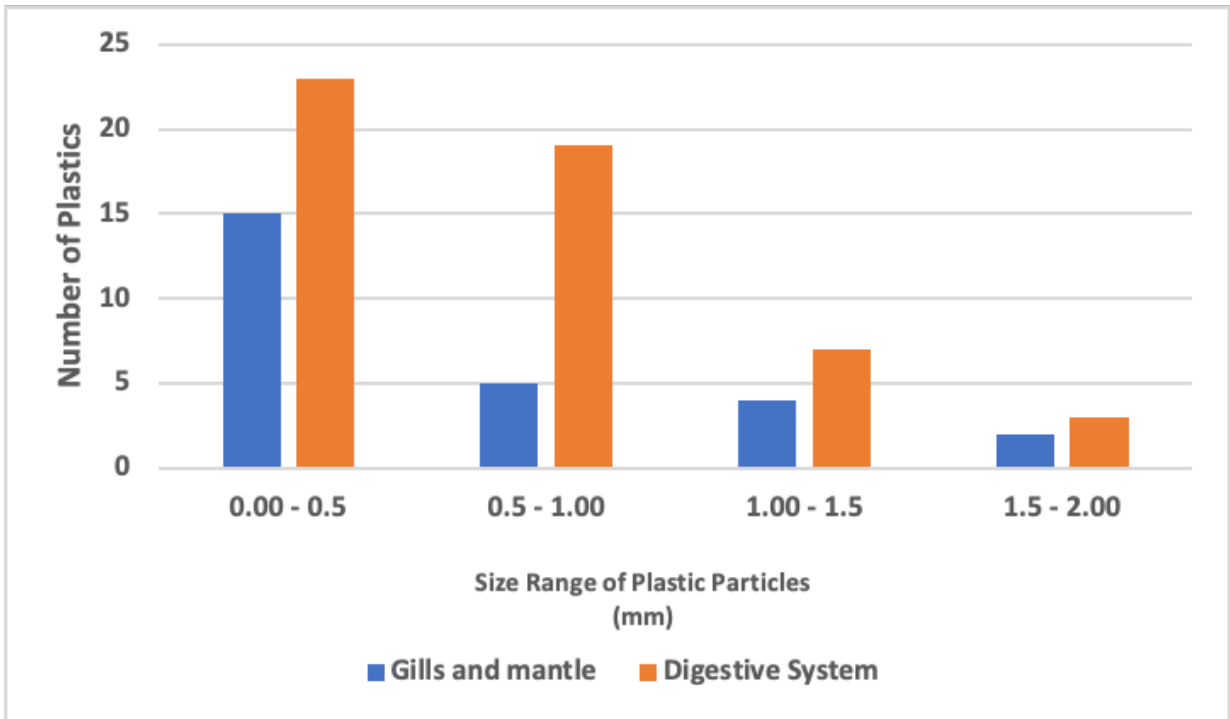


**Figure 1:** The number (Mean + SE) of Microplastic fragments found within the digestive system of *Crassostrea virginica*. Bar graphs with different letters represent statistically significant differences between treatment groups.



**Figure 2:** The number (Mean  $\pm$  SE) of Microplastic fragments found within the Gills and Mantle of *Crassostrea virginica*. Bar graphs with different letters represent statistically significant differences between treatment groups.

No polymer fragments were found within oyster adductor muscles or labial palps for all treatment groups. PS fragments were detected in more than 90 percent of oysters assigned to the PS treatment group, while PE was not found in any of the assigned oyster tissues. Oysters assigned to the PP treatment group ingested the fewest number of total particles (n=14), whereas oysters in the PS treatment group ingested the greatest number of particles (n=47). The size of microplastics documented from oyster tissues ranged from 0.05 to 2mm (Figure 3).

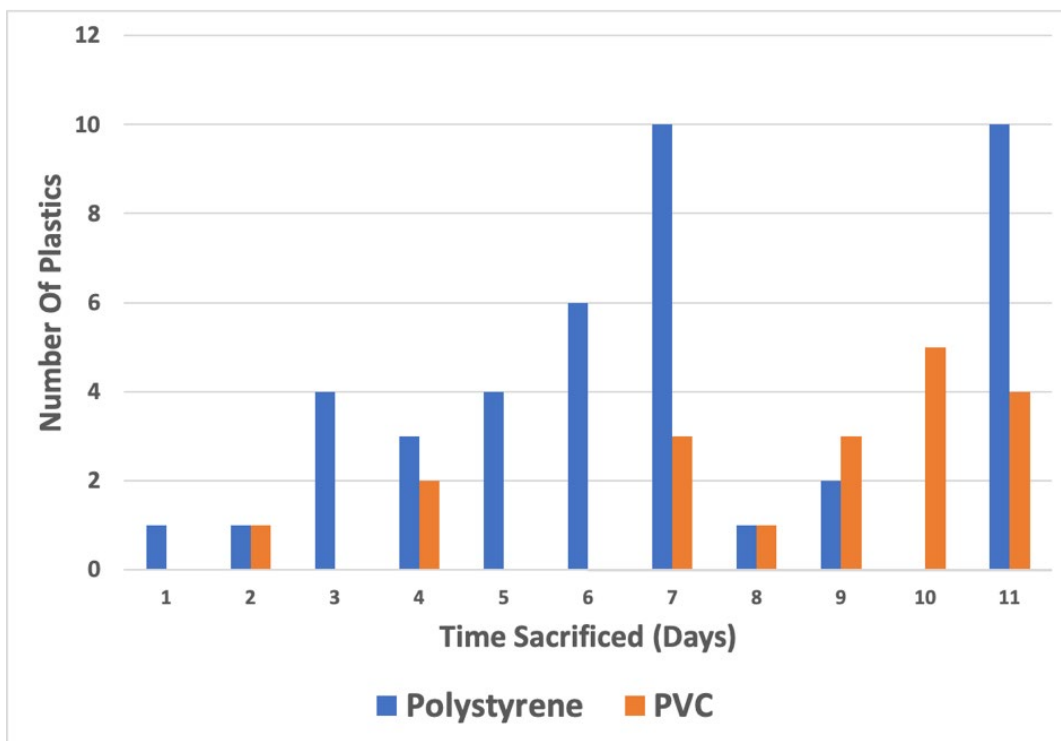


**Figure 3: The size range (mm) of microplastic fragments found within *Crassostrea virginica* tissues.**

An analysis of the relationship between oyster size and individual tissue weights against the number of plastic particles present was conducted. Results showed that no significant differences were present between the oyster size and tissue weights with the number of plastics within oysters' tissues; however, significant differences in the presence of microplastics among oyster treatment groups did occur for the digestive system (Figure 1;  $F_{4,42} = 9.20$ ,  $P < 0.0001$ ) and the gills and mantle tissues (Figure 2;  $F_{4,42} = 5.00$ ,  $P < 0.0022$ ). Oysters fed PS microplastics had significantly more fragments inside the digestive system compared to the other treatment groups (Figure 1). Similarly, there was a significantly greater number of PS fragments in their gills and mantle than any other treatment group (Figure 2). The number of PVC fragments was significantly higher within the digestive system (Figure 1), but was not significantly different from the control group within the gills and mantle (Figure 2). In contrast, PP fragments were significantly higher



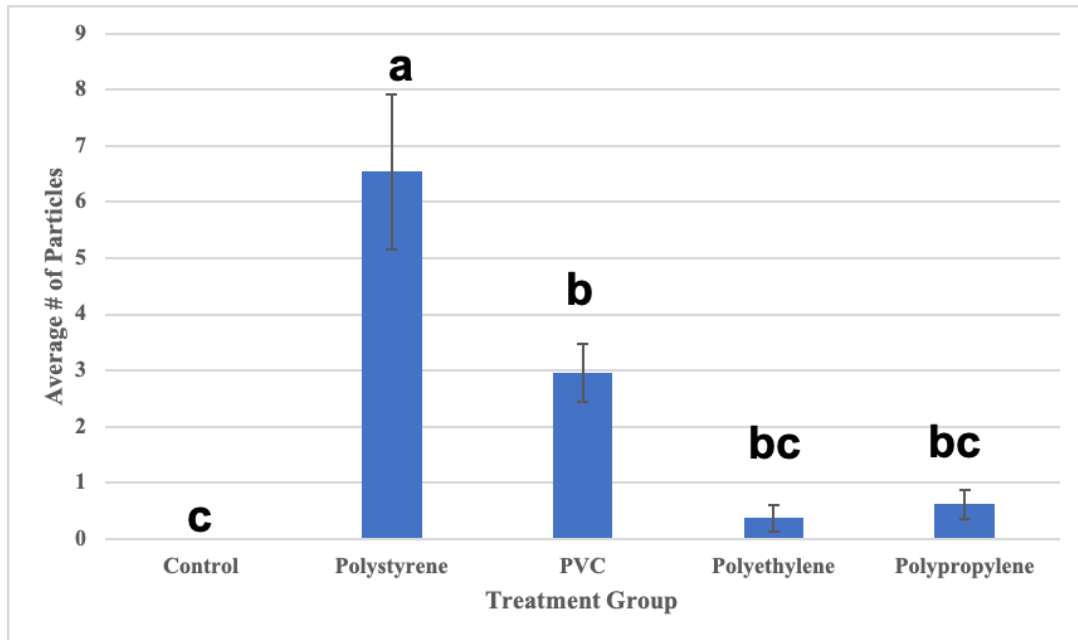
within oyster gills and mantle (Figure 2), but were not significantly different from the control within the digestive system (Figure 1). One interesting change in the consumption and retention of plastic particles, which developed during the experiment, was an increase in the presence of PVC and PS particles within the digestive system as the experiment progressed from Day 1 to Day 11 (Figure 4). This increase over time might reflect ‘aging’ in microplastics where they develop a microbial film once they are introduced to the environment.



**Figure 4: The number of PVC and PS particles found within oysters digestive system during the progression of the experiment.**

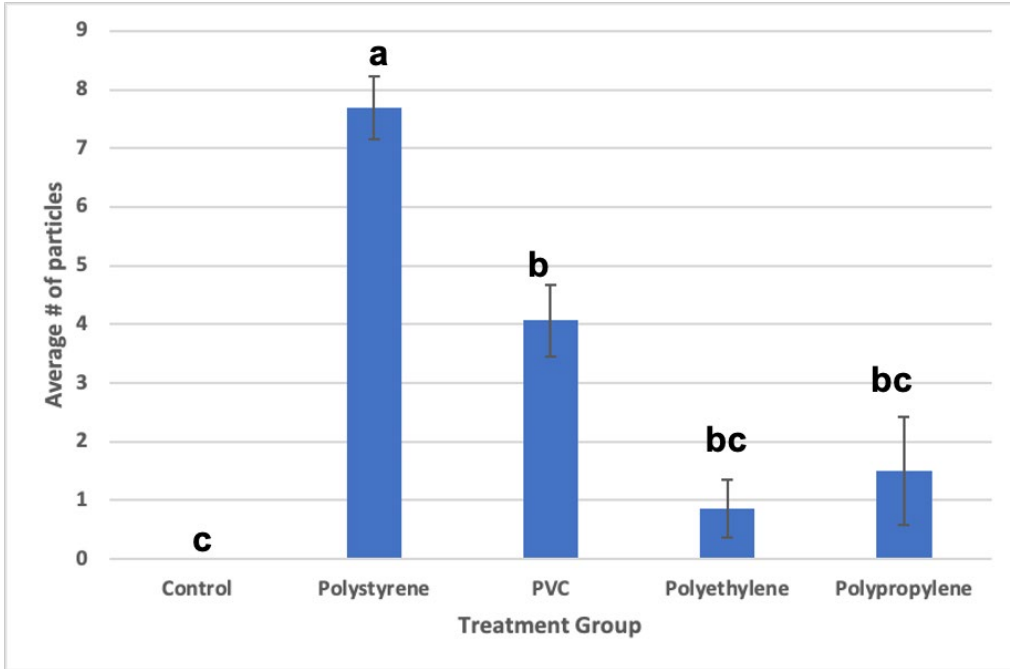
Feces and pseudofeces were collected only during three observation hours 1, 8 and 24. No feces were produced during the 1-hour mark, therefore no collection occurred. By the end of 24 hours, due to decomposition it was impossible to distinguish feces between actual feces or pseudofeces; therefore, these data were removed from the

analysis. However, for the 8-hour observation period, the number of particles in feces varied significantly among oysters assigned to PS, PVC and PE groups (Figure 5;  $F_{4,32} = 12.79$ ,  $P < 0.001$ ).

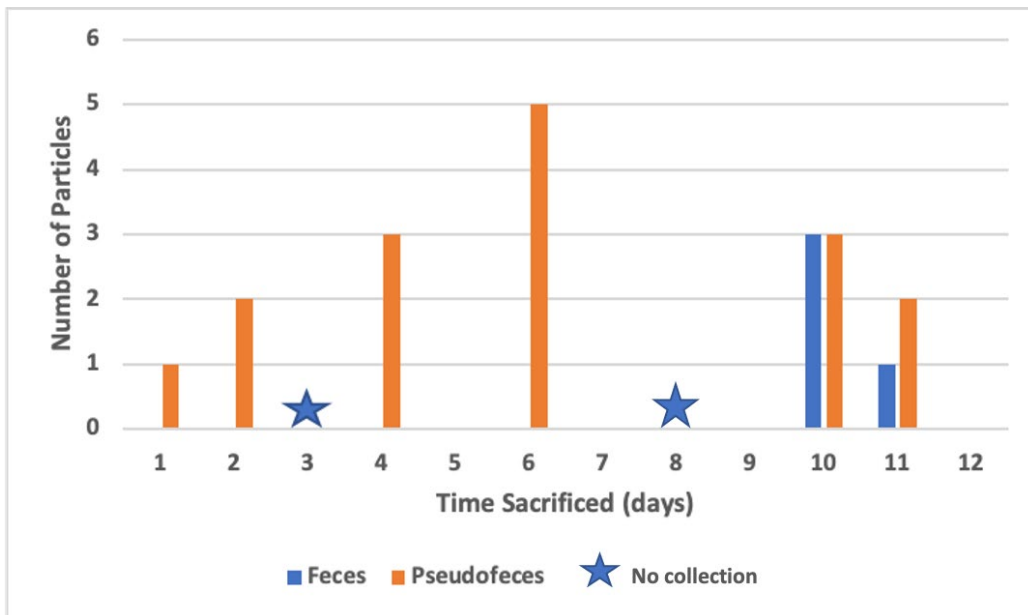


**Figure 5:** The number (Mean ± SE) of microplastics fragments found within the Feces of *Crassostrea virginica*. Bar graphs with different letters represent statistically significant differences between treatment groups.

As in the tissues, the number of PS fragments were significantly higher than any other polymer group. Experimental polymer fragments were found as early as day 1 in pseudofeces. The number of polymer fragments in pseudofeces varied significantly among the treatment groups (Figure 6;  $F_{4,17} = 21.32$ ,  $P < 0.001$ ). Oysters assigned to PS and PVC group had a significantly higher number of particles present than oysters in the PE and PP groups. Initially, PE fragments were only detected in oyster pseudofeces; however, towards the end of the experiment (Day 11) PE fragments were also found in the feces (Figure 7). There were no microplastics found in the feces or pseudofeces of the control group.



**Figure 6:** The number (Mean + SE) of microplastic fragments found within the Pseudofeces of *Crassostrea virginica*. Bar graphs with different letters represent statistically significant differences between treatment groups.



**Figure 7:** The number of Polyethylene microplastics found within the feces, and pseudofeces of *Crassostrea virginica*.

## DISCUSSION

One of the primary goals for this study was to expose oysters to the various types of polymers that are commonly found in the environment. In terms of management, understanding the impacts from various polymers can provide insight on the overall environmental impact a material or process may have. In particular, oysters assigned to the PS group had a significantly higher number of particles than any other treatment group. These results would suggest that PS has the potential to impact oysters and other filter feeding organisms to a greater extent. Moreover, PS was present in feces and pseudofeces, which suggests that oysters were unable to distinguish PS from food and therefore, could not utilize their rejection mechanism effectively. Similarly, an exposure study of polystyrene beads on mussels showed spheres (5  $\mu\text{m}$ ) were found throughout the stomach and intestine of all mussels within the experiment (Khan and Prezant 2018). PS is one of the most commonly used and recycled plastics, with a global production of more than 14 million US tons every year (Chandra et al. 2016). Although it is accepted by some recycling facilities, the shredding processes of recycled polystyrene yields high amounts of secondary microplastics that can ultimately be redistributed into the surrounding environment. Larger pieces of polystyrene can also be easily degraded to smaller pieces due to the combination of many environmental factors such as sun exposure and weathering within the marine environment and thus pose a great impact to the health of the ecosystem.

In contrast, PE was not present in any of the oyster's tissues; however, it was found in both types of feces. It should be noted that PE was only present in feces collected toward the end of the experiment (Figure 7). This could be due to the formation of a biofilm on

accumulated particles in the experimental chambers, which would inhibit the oyster's ability to reject PE. In the environment, once microplastics enter waterways they are quickly conditioned with a layer or film of organic and inorganic substances by adsorption (Rummel et al. 2017). It is through this initial conditioning layer that microorganisms begin to interact with microplastics and ultimately lead to the development of a biofilm (Rummel et al. 2017). Biofilms may contain similar taxa to those which filter feeders may ingest, as well as secrete chemicals that increase the likelihood of the microplastic being mistaken for a food source. A study on copepods showed greater ingestion of aged microplastic beads than pristine microbeads (Vroom et al. 2017), suggesting that organisms are extremely vulnerable to microplastics in the environment due to the aging processes of weathering and biofouling. Moreover, biofilm formation may differ among polymers due to the difference in polymer composition as well as the amount of supplemental chemicals that were added into the polymer during manufacturing (Rummel et al. 2017). For example, Rogers et al. (1994) suggested the higher bacterial count on PE and PVC, as compared to stainless steel, was due to the leaching of additives contributing to biofilm development. These findings concur with the results, which showed that PE was not initially consumed, but as the experiment progressed and a biofilm likely formed on particles, oyster feces showed traces of PE (Figure 7).

Today, much of single use plastic packaging is composed of PE (Plastics Europe 2016) and it is considered the most abundant form of coastal litter; therefore, the greatest source of microplastics in the environment. A major reason PE is favored by manufacturing companies is because it is a "thermoplastic" which means it can be heated

to its melting point, cooled and reheated again without significant damage to the material (GESAMP 2010). However, the same characteristics that make PE and other thermoset materials (PP, PVC, and PS) versatile is what also makes them difficult to dispose of or recycle. Once heated, the chemical composition of the thermoplastic is completely changed and if heated a second time the material will simply burn (GESAMP 2010). Over time, discarded thermoset plastics will end up in landfills or become contributors to marine litter.

Longer-term and short-term exposures of specific polymers may also result in adverse biological impacts. A study on the polychaete worm, *Arenicola marina*, showed a significant reduction in its feeding activity and the gut passage time of sediments due to the chronic exposure of sediments containing PVC (Wright et al. 2013). Similarly, a 52-day exposure to PE showed a significant reduction in the attachment strength and production of byssal threads in the blue mussel, *Mytilus edulis* (Green et al. 2018). In the current study, the number of PVC particles began to increase toward the end of the experiment (Figure 7), suggesting a longer-term exposure may have a larger impact on oysters.

Exposing organisms to various size particles may help us understand the different pathways microplastics may undergo within an organism. Smaller size particles can translocate within an organism by passing through the cell membranes and once taken up, they can be retained for long periods of time (Browne et al. 2007). In this study, polymers from each treatment group with the exception of PE were confined to the stomach, gills and mantle. Although biological impacts from microplastic ingestion were not investigated in the present study, a study on *Crassostrea gigas* showed significantly

higher energy usage due to digestive interference from polystyrene microplastics in the gut (Sussarellu et al. 2016). While the present study was unable to discriminate plastics in the different segments of the oysters' digestive tract to confirm ingestion, Khan and Prezant (2018) found that the mussel species *Geukensia demissa* did ingest similar sized and shaped microplastics, and they were present in digestive tubules, suggesting active retention of PS. Previous studies discovered that oysters can ingest particles between 5 to 30 $\mu$ m (Baldwin and Newell 1995); however the results from laboratory studies like this one, as well as field experiments (Cauwenberghe et al. 2014), clearly show they can ingest larger particles. The results of the current study are consistent with those of previous studies (Browne et al. 2007; Koehler et al. 2008; Xu et al. 2017), which suggest that smaller particles are more likely to be ingested by filter feeders (Figure 3). These results also demonstrate a clear difference in the inability of oysters to discriminate PS from natural food resources, and that aging of microplastics reduces their ability to reject plastic particles. Consequently, aged microplastic presence in the environment could have substantial impacts on oyster survival and growth.

## CONCLUSION

Oysters are ecosystem engineers and provide a wide range of ecosystem services such as water filtration, food, and habitat for many organisms (Beck et al. 2011). Oyster reefs also provide shoreline stabilization and coastal protection from natural disasters, and yet despite their importance, oyster habitats are continuously declining due to human-induced threats (Beck et al. 2011). Given that plastic waste is expected to continue to increase (Andrady 2011) and the amount of macroplastic fragmentation is

already happening, concentrations of microplastics will continue to heavily pollute the environment. Although oysters were observed rejecting and ejecting polymers like PE, expelled waste and pseudofeces will ultimately draw down microplastics from the water column, introducing them to the sediments. Subsequently, this exposes benthic epifauna and infauna to these microplastics and the contaminants that they may carry (Galloway et al. 2017). Microplastic bioavailability to marine animals is clear and, since they are so ubiquitous and present throughout the environment, it would be wise to include them in monitoring and models to predicate how their transport and accumulation may change over time.

The results from this study clearly indicate that filter feeders such as oysters are extremely vulnerable to ingestion of microplastics. Although oysters are more likely to ingest smaller particles, this study shows that they are capable of ingesting larger particles as well, which could result in digestive blockages within the oyster. Larger pieces may also inhibit their ability to further reject polymers and as a result, microplastics may have cascading impacts to individuals and the population. It is clear that microplastics are a continuing and accelerating threat to the environment, but still so much is yet to be understood.

Although there are many studies surrounding microplastics and the environmental impact they pose, there is still so much unknown and room for more studies with improved methodologies. There is a need for research to standardize methods using similar terminology to reduce confusion and to aid comparison among studies. Preventing and reducing contamination is also a major challenge due to the presence of fibers in the atmosphere. More importantly, laboratory studies need to represent environmental



conditions by using similar concentrations and irregular shapes and sizes of particles to accurately understand the biological effects of microplastics. Studies using chemical analyses such as Raman spectroscopy are also needed to accurately identify fibers as plastic or nonsynthetic materials (Remy et al. 2015).

There is a need for exposure studies to evaluate differences between longer-term exposure and short-term exposure on feeding and health of organisms. In this study, PVC began to accumulate toward the end of the experiment, and PE, which was not consumed initially, was observed in both feces and pseudofeces toward the end of the experiment indicating aged plastics may harbor biofilms which make plastic particles indistinguishable from food items. This suggests that longer-term exposure to PVC and PE would have a greater impact on bivalves than ‘fresh’ particles. Moreover, there is little information on the fate of microplastics and whether particles are deposited in deep-sea sediments (Choy et al. 2019) or limited to the shelf and coastline regions. Vertical movement of various types and sizes of microplastics is also unknown, yet an important research topic since microplastics with biofilms may sink, but once the biofilm is removed through processes like digestion, the particles may become buoyant again (GESAMP 2010). Overall, there is a need for further studies to evaluate the absorption and desorption rates between pollutants and microplastics, and whether this process is reversible (GESAMP 2010). For example, in regions where persistent, bioaccumulative and toxic substances concentrations are high, microplastics can readily become vectors and transport these toxic substances into cleaner remote regions. Notably, the majority of microplastic studies frequently concentrate on particles of the same size, because there is a significant amount of time, effort, and expense needed to process and analyze samples

(Lehtiniemi et al. 2018), but this does not mirror conditions in the real world. This study demonstrates several key findings and highlights the importance of investigating various polymers, including polymers with irregular shapes and sizes to ensure results are as accurate and unbiased as possible and to mimic real world conditions. Smaller particles have the potential to impact different organisms including individuals at lower trophic levels, whereas larger particles can cause blockages and inhibit biological processes like swimming and feeding behaviors (Choi et al. 2018). Also, this study showed how oysters preferentially selected against some type of plastics, but as plastics age, they lose the ability to distinguish them as non-food resources. This means that research into certain polymers, like PE and PVC, require longer-term exposures and biofilm development to fully understand the potential impacts these substances have on organisms and the environment.

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