

**The Impact of Microplastic Ingestion on the Bivalve Filtration Efficiency
of the Hooked Mussel (*Ischadium recurvum*)**

**Matthew Betsill - Computer Science
Juan Gonzalez - Environmental Horticulture & Crop/Soil Sciences
Allison Woods - Computational Modeling and Data Analytics**

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ABSTRACT

Microplastic pollution is an increasing issue as sea animals are observed with pollutants within their bodies and cells. Mussels and other marine bivalves have the capability to filter phytoplanktonic organisms and chemical pollutants, but cannot break down microplastics if ingested. Because bivalves filter pollutants out of the water, many kinds of debris enter their systems. It is hypothesized that microplastics will reduce the efficiency of the *Ischadium recurvum* and its ability to filter toxins that deteriorate water quality. This study will determine the effect of intaking 5 to 50-micrometer diameter plastic on the filtering efficiency of *Ischadium recurvum*. The experiment will prepare two 10-gallon water samples with 34‰ salinity and water turbidity of ~100 NTU from the algae concentration for mussels' environment. Twelve mussels will be collected from the York River to measure the nutrient concentration, dissolved oxygen concentration, water transparency, and chlorophyll concentration to determine the water quality before and after the filtration in both the controlled and polluted environment. A comparison of the two water quality results will determine how microplastics have affected the mussels' filtration. The mussels are expected to completely filter out the contaminants in the control test and experimental trial with microplastic contaminants, albeit at a slower rate. With a bivalve system, mussels can capture particles at a low nutritive value, which will slow down consumption, but leave little filtration difference. The study will provide information for bay restoration projects to utilize different mussels to filter bay water at a higher efficiency.

INTRODUCTION

Since the large scale integration of plastic and other synthetic polymers in the 1950's, global plastic production has increased from 1.5 million tons per year to 250 million tons per year (Claessens 2011). Management of its residual waste from use as single-use packaging as well as higher density, multi-use implementations have accumulated in landfills. Eventually through littering, such plastics accumulated in marine environments. Floating on the surface of the ocean, plastics break down mechanically through exposure to ultraviolet rays and form microplastics (Thompson 2004). Microplastics are less than five millimeters in diameter, making them small enough to infiltrate marine biota such as fish. Fish have been concluded to suffer from intestinal blockage, physical damage, and change in behavior among other implications of microplastic ingestion (Javanovic 2017). These implications do not stop at fish, as any marine animal is subject to microplastic ingestion and inhalation as these microplastic particles can be found in oceans and rivers across the globe. Currently,

microplastics have been documented as having some sort of impact on more than 690 marine species (Gall & Thompson, 2015).

Over the past decades, the oyster population of the Chesapeake Bay has been reduced to roughly 1% of what it formerly was before overfishing. Resultantly, the Hooked Mussel has a key role of cleaning the Chesapeake Bay (Lipicus, 2018). Since the microplastic contents of oceans are ever increasing, the effect of microplastic ingestion on *Ischadium recurvum*'s filtration efficiency can be studied to understand the future of the Chesapeake Bay's water quality.

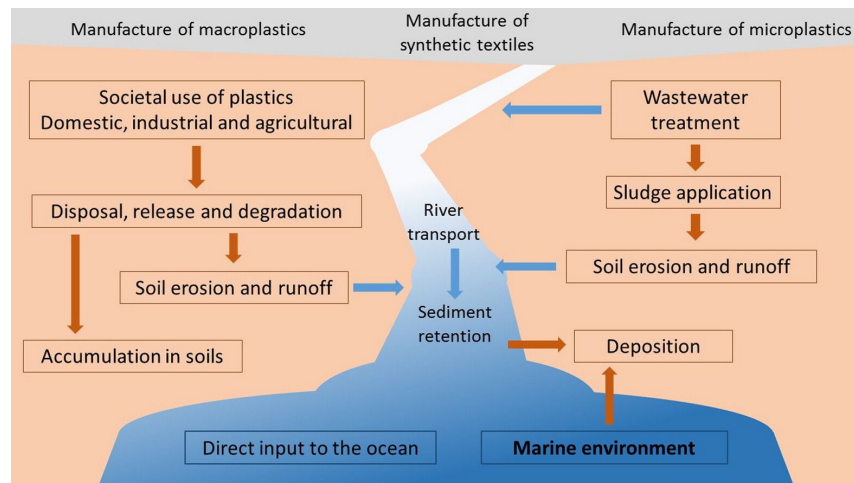


Figure 1: Microplastic Source and Accumulation to Marine Environment (Horton, Walton, Spurgeon, Lahive & Svendsen, 2017)

Figure 1 shows the step by step process that plastics go through in their long lifespan. It is shown that macro-plastics, synthetic textiles, and microplastics themselves are all eventually transported to the marine environment as they have nowhere to be stored so that they aren't able to be washed away and into watersheds.

Microplastics in oceans have tendencies to bond to existing chemical toxins such as lead because of their high surface area to volume ratio. This can create issues when certain contaminated microplastics are ingested by marine biota, on top of the original issue of digesting plastic fragments (Horton, 2017). Due to the small nature of microplastics, most marine life cannot determine their makeup, thus resulting in microplastic being ingested. Since the large, increasing amount of microplastic in the ocean, the debris can be passively ingested because of their ever-present availability in marine waters. Active ingestion also occurs because of the vivid colors often found in plastics indicating a prey item, which a marine animal could inadvertently ingest.

Mussels utilize a bivalve system for water filtration and food ingestion. The Hooked Mussel (*Ischadium recurvum*) found in the Chesapeake Bay is used for water conservation purposes, as mussels are inherently capable of filtering toxins out of water and absorbing them resulting in lesser turbidity and purer water. Mussels do this by utilizing modified gills that are able to absorb dissolved oxygen for cellular respiration and pass food through, ranging from bacteria to plant matter and phytoplankton. These gills are called ctenidia which contain weaves of cilia which can filter out materials in a method not unlike sorting (Bivalve, 2019). Such materials are then digested by the digestive tract which utilize enzymes to break down food particles. Plastics and polymers, however, are unable to be broken down through this process because they are bio inert. They lack the enzymatic pathways necessary to break down the polymers. Because of this, microplastics may be incorporated into the body of bivalves from phagocytosis. In the digestive process, the stomach of the bivalves have sorting areas which determine if a food particle needs to be broken down more in the intestine or if the food particle is ready for the digestive glands based upon its size. If the particle cannot be broken down and offers no nutritional value to the mussel, there will be a loss of energy (Dorit, 1991).

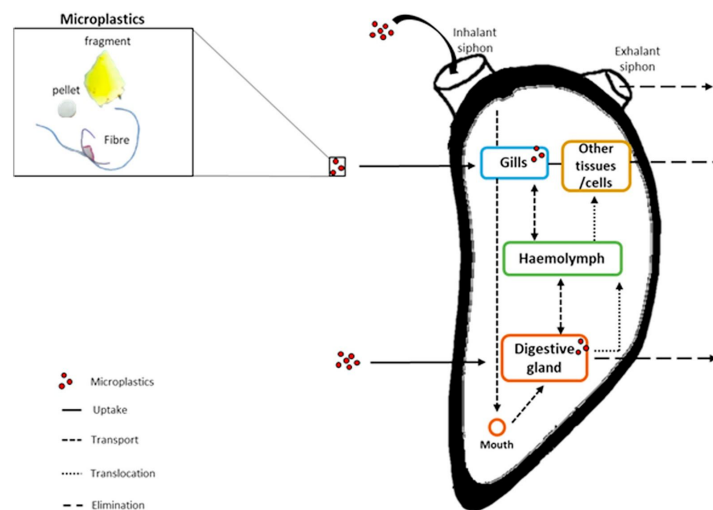


Figure 2: Possible Routes for Uptake, Tissue Distribution, and Elimination of Microplastic in Bivalves (Ribeiro, O'Brien, Galloway, & Thomas, 2019)

Shown in Figure 2, microplastics are ingested through the inhalant siphon and can be found in their gills or their digestive gland because of the varying possible size of the microplastic and how they will interact with the biological systems of the mussel. It is hypothesized that microplastics will reduce the efficiency of the *Ischadium recurvum* and its ability to filter toxins that deteriorate water quality.

METHODS

MUSSEL COLLECTION

A dozen mussels will be collected for each of the two trials. Hooked mussels are typically found in clusters in the Chesapeake Bay at the mouth of the York River at the edge of the water in subtidal kelp beds (Kreeger, pg. 39). According to Kreeger, the best time to collect mussels is in the spring. The first set of mussels will be collected in April and the second set will be collected two weeks later in May. The mussels will be transported to the laboratory in a refrigerated container at four degrees Celsius (Ottaviani). Once in the laboratory, the mussels will sit in clean seawater for three days to allow for gut evacuation (Lobel, 1991).

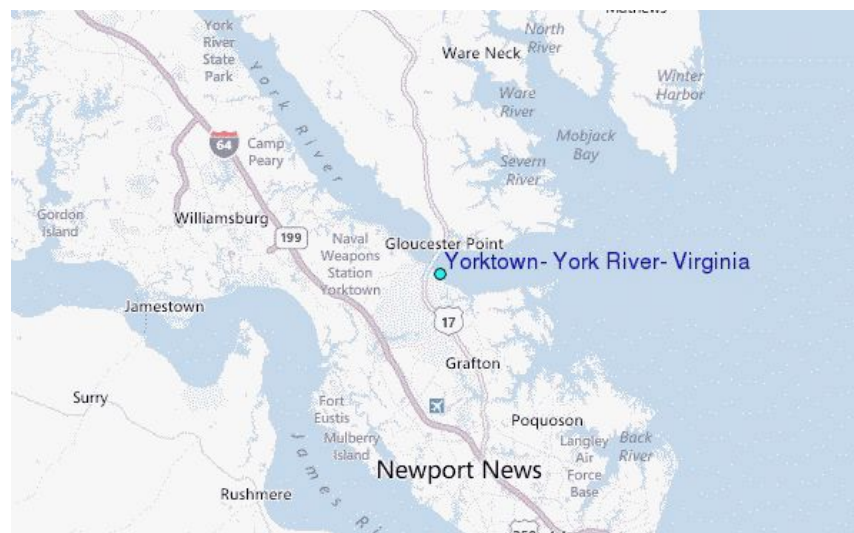


Figure 3: Potential Site for *Ischadium recurvum* Collection (Yorktown)

MUSSEL EXAMINATION AND WATER FILTRATION

Immediately before the acclimatization period, all mussels will be scrubbed to remove epiphytes and other fouling organisms from the valves to ensure a clean slate for each testing. Any externality will contaminate the water source for experimentation and alter the results of algae filtration (Ottaviani et al., 2013). After the mussels were aseptically prepared, the mussels will be distributed into their acclimated tanks to prepare for the control scenario testing.

The experiment will examine the effect of an accumulation of microplastics on the filtration rate of mussels. The filtration rate will be measured by comparing the water quality between the control and microplastic scenario. Beforehand, the water would contain planktonic organisms, which will mimic a lower quality water environment. The water quality measurement will consist of nutrient concentration, dissolved oxygen, water transparency, and chlorophyll-a content. Water with a higher concentration in phosphorus, nitrogen, and nitrate will increase the biomass of algae since these are nutrients essential for plant growth. Dissolved oxygen in water is important for aquatic life. A lower concentration of oxygen in water means algae have consumed the oxygen in the water and other marine organisms cannot live there. A turbidity sensor will be used to indicate the amount of light that penetrates into water. A larger abundance of algae will show lower water transparency. Also, algae use photosynthetic pigments to capture light and oxygen, so a larger chlorophyll concentration will show a larger abundance of algae. A spectrophotometer will be used to compare the standard curve value from the water sample before and after water filtration. Differences in these water quality indicators will determine a change in mussel filtration (Hernández-García et al., 2019).

CONTROL AND MICROPLASTIC EXPERIMENT

The same set of mussels will be used for both the control and test scenario of water filtration. Before the mussels are introduced into the control scenario, a water quality test will be done to have comparative results before the water filtration. Without introducing microplastics, mussels will be placed in the 10-gallon water sample with a set amount of algae concentration and they will be given one hour to filter the water (Figure 4). After the filtration period, another water quality test will be done to have results post-filtration. The differences from the water quality test will be calculated to summarize a final result for the control scenario.



Figure 4: Water Quality Comparison After Mussel Filtration (Ottaviani et al., 2013)

The mussels from the control scenario will be placed into the same conditions, except for the introduction of microplastics in the water. The microplastics will have a 10-micrometer diameter with a spherical shape for rapid ingestion (Martins, Sobral, Costa, & Costa, 2019). The microplastics will also be a tan color to act as an organism to promote ingestion. The water quality measurements will be compared from both experiments to measure how microplastics have affected the mussels' filtration.

POST-PROJECT

After the water analysis, the mussels will be scrubbed clean for a second time and placed in a different tank of clean seawater to allow for gut evacuation to release anything from the experimental procedures. They will then be transported in a refrigerated container back to the site they were extracted from and released into the water. The data should then be analyzed and any conclusions should be drawn.

ANTICIPATED OUTCOMES

After the trials are performed, the results will likely show that the test subject mussels with microplastic contaminants in the tank will not filter water at the same rate as they had when there was no microplastic in the water. The rate of the control versus the filtration rate of the test group with the microplastic in it will likely diverge after a certain amount of time due to the buildup of microplastic in the mussels' digestive tract. Since the Hooked Mussel's respiratory system is largely integrated in its digestive system, with the ctenidia functioning as gills as well as a sorting mechanism for food particles, this buildup will cause *Ischadium recurvum* to undergo more digestive stress (Bivalve, 2019). This digestive stress may translate to a partial blockage of the gills, leading to lesser cellular respiration capabilities. Such a blockage would disrupt many of the systems of the mussel, including the ability to filter water.

The mussels are expected to completely filter out the contaminants in the control test and are also expected to filter contaminants of the experimental trial with microplastic contaminants, albeit at a slower rate as shown in Figure 5. With the microplastic, the Mussels are expected to filter the water at rate of about 75% of the mussels without microplastic, with the rate decreasing near the end due to microplastic accumulation. The mussels are expected to survive with little to no long term effect on their overall welfare.

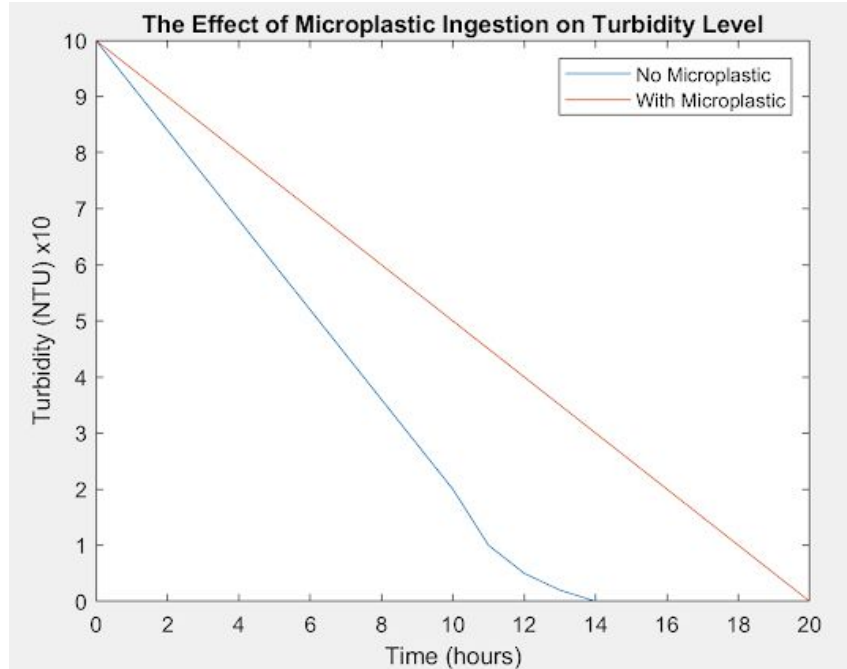


Figure 5: Expected filtration rate change from microplastic ingestion to no microplastic ingestion

LIMITATIONS

Challenges could arise when locating and collecting hooked mussels in the York River region. If mussels cannot be located on the collection day, collectors should try again every three days. If mussels are not located after three tries, the collectors should try the Maryland Chesapeake Bay shoreline and repeat the process. *Ischadium recurvum* can be found in Maryland as well as in the York River (Lobel, 1991). If this fails, search for mussels in the fall season, because the salinity is favorable in the fall for hooked mussels (Lobel, 1991). Hooked mussels can be identified by their one to two inch hook shaped shell (Lobel, 1991). Transportation of mussels to the laboratory could also be an issue, but as long as the mussels are kept in the refrigerated container they should remain healthy for the trip.

ETHICAL CONSIDERATIONS

The National Research Council has specific rules on animal experimentation, but mussels and other bivalves do not require special care based on the Guide for the Care and Use of Laboratory Animals. However, researchers must follow basic rules for experimenting on animals regarding pain inducement. Since the mussels will not experience any pain, this experiment abides by the National Research Council's laws.

The mussels must be transported to and from their natural habitat safely and treated with care while under the supervision of the individual running the experiment.

FUTURE RESEARCH

It would be useful to study larger groups of mussels in more trials of this experiment. It could also be helpful to study the difference in filtration rates between bivalve species and between bivalves living in different ecosystems. For example, mussels living near the National Harbor likely have higher accumulation of microplastics in their digestive systems than mussels that nest off of Yorktown shores because of the amount of microplastics produced in different kinds of cities.

Microplastics are abundant in oceans, bays, rivers, and lakes around the world. While many different kinds of mussels can help remove and break down chemical pollution in their environment, they are not able to break down microplastics. If microplastic accumulation in mussel digestive systems has an effect on their ability to filter other chemicals, the water will become more polluted as time goes on. This would prove that mussels are a temporary fix for existing water pollution and other solutions need to be created. Future research on mussel filtration rates and microplastic concentration within bodies of water, mussels, and other marine organisms would be useful to support the awareness of problems caused by microplastics.

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