# Analysis of Plastics in Hard Shell Clams (*Mercenaria mercenaria)*

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## Abstract

Plastic is possibly the most harmful debris on our planet. Over time, manufactured pieces of plastic disperse into smaller secondary fragments such as microplastics (MPs) and microfibers (MFs) which are ingested by marine organisms. The objective of this study was to discover how many MPs and MFs are in a *Mercenaria mercenaria*, also known as a hard Shell Clam or a Quahog. Eight clams, four large and four small, were collected from a cove on Snow Island, Harpswell, ME. The four sample sets were filtered on the dock in a filtration system for 0, 24, 36, & 48 hours. A total of 2,129 MPs and 196 MFs were identified among all samples. The small clam in set 3 (Sample 3A) contained the most MFs, and the large clam in set 4 (Sample 4B) contained the highest number of MPs. The smallest clam in the first set that never went through the dock filter had the most microfibers per gram with 6.003 MFs/g and microplastics per gram with 61.281 MPs/g. Therefore, it had the most MP contamination. MP abundance did slightly decrease with increasing time spent in the dock filter as expected. Although, the MP concentrations were not drastically different between the samples. It is important to understand what conditions are and are not likely to affect the organism studied. Hardshell clams are important to understand because as bivalves they act as environmental indicators of pollution through ingestion.

## Introduction

Plastics are distributed widely throughout marine environments. Plastic production has increased exponentially since first produced in the early 1950s, after World War II. When improperly managed, plastic causes drastic environmental damages. Specifically, the plastic pollution enters the ocean through runoff, rivers, wastewater, and direct littering. The fragments are then exposed to ultraviolet rays from the sun and the chemistry of salt water causes slow photodegradation over time. Consequently, the larger macroplastics become many more microplastics (>5 mm) and nanoplastics (>1 μm). Microfibers are also common which are thread particles from synthetic material such as clothing and fishing gear (Luster et al, 2017). There are both primary and secondary microplastics (MPs) and microfibers (MFs). Primary indicates fibers or beads that were created in this small size. Secondary indicates degradation of a larger plastic particle (Rahman, 2019). These plastic particles contaminate our sediment, soil, organisms, and ourselves. When plastic is produced, a range of chemicals are added. Polychlorinated biphenyls (PCBS), polycyclic aromatic hydrocarbons (PAH), and persistent bioaccumulative toxic substances (PBTs) are absorbed by the plastic in the near environment (Lusher et al, 2017).

Additionally, organisms ingest plastics through the food web, specifically trophic transfers. The toxins in these microplastics result in harmful health responses such as heightened immune reactions, decreased growth, and decreased fertility for the organism (Gallo et al, 2018 & Lusher et al, 2017).

Past studies have showcased that marine filter feeders consume microplastics (Cole et al. 2013, Taylor et al. 2016). Bivalves like clams can be an important indicator of pollution and bioavailability (Su et al, 2018). Bioavailability is how successfully a substance is able to be absorbed and used by the organism. Bivalves use bristle structures to strain phytoplankton and zooplankton from the water. Therefore, they cannot tell if a particle is plankton or plastic (Rahman, 2019). It is important to understand how different organisms filter out and/or absorb microplastics to know what a seafood consumer is ingesting. When humans consume bivalves like clams, oysters, scallops, and mussels, they are ingesting the entire organism including the muscle and stomach that has the most MPs in it (Polowski, 2021). Rather when consuming larger fish, the stomach and intestine which have the most MPs in them are not ingested (Barboza et al, 2020).

This study aimed to analyze if wild hard shell clams were able to filter out microplastics and microfibers after being placed in filtered seawater on the dock. In addition, small and large clams were compared to gauge the MP concentrations in this species. It was hypothesized that smaller clams would have higher MP and MF concentrations compared to the large clams. Additionally, it was hypothesized that the longer the clams spent in the dock filter, the lower the MP concentrations would be.

## Methods

### *Instruments used*

Four 250 mL beakers
Four safety goggles
Latex Gloves
INCU-Shaker Mini
Rocker 300 Vacuum Pump
1 liter vacuum trap
SAS Positive Pressure room (clean room)
Four pyrex glass bowls
Three metal clamps
53 µM Sieve
Three vacuum filter reservoirs
Three vacuum filter funnels
Stainless Steel 3-Station Vacuum Filtration Manifold
Air Science Purair Ductless Fume Hood (negative pressure chemical hood)
Shucking knife
1.0 M sodium hydroxide (NaOH)
Eight Cytiva Whatman GF/F 47 mm filters
Zeiss Stemi 305 Microscope
Eight Corning 20 mm petri dishes
Tin foil
Stainless Steel dissection tray
Ohaus Adventurer Scale

### *Collection of Clam Samples*

The Hard Shell Clams were dug out on a cove on the south side of Snow Island in Harpswell, ME with a fork and put into a clam hod to transport back to the mainland. 8 clams in total were selected: 4 small and 4 large. One set of clams is considered to be 1 small and 1 large clam. Each clam was labeled with its appropriate set number (1-4) and letter: small (A) or large (B). The first set of clams were taken back to the lab where they were shucked and prepared for oxidation. The other three sets were put in a filtration system on the dock at Bethal Point for 24 hrs, 48 hrs, and 72 hrs (Table 1). After each set spent its designated time in the dock filter, they were put in an industrial freezer at 0°F until space opened up to oxidize them in the lab.

| Sample Number | Clam Size | Hrs in Dock Filter | Days in Freezer | Days in NaOH |
|---|---|---|---|---|
| 1A | Small | 0 | 0 | 6 |
| 1B | Large | 0 | 0 | 6 |
| 2A | Small | 24 | 2 | 6 |
| 2B | Large | 24 | 2 | 6 |
| 3A | Small | 48 | 4 | 13 |
| 3B | Large | 48 | 4 | 13 |
| 4A | Small | 72 | 7 | 10 |
| 4B | Large | 72 | 7 | 10 |

*Table 1*: Clam sample data including time in the dock filter, in the freezer, and in sodium hydroxide (NaOH).

***Oxidation of First and Second Set of Clam Samples***

The first set of small and large clams were taken back to the lab immediately after collection to be shucked on a steel dissection tray in the SAS Positive Pressure Room. Gloves were worn for all processes to reduce contamination of MPs, MFs, and protect the analysts. All beakers were labeled with their sample number and cleaned with deionized (DI) water. The empty weight of each beaker was measured prior to sample emplacement. After dissection, each sample was placed into its labeled beakers and weighed to determine the sample wet weight in grams. The following equation was used to calculate the sample weight (g): wet weight - empty beaker weight (Table 2).

The samples were then brought into the Air Science Purair Ductless Fume Hood. 150 mL of 1.0 M sodium hydroxide (NaOH) was poured into each of the 250 mL beakers that the samples were in. Samples were then put into the INCU-Shaker Mini at 60℃ at 130 rpm for 6 - 13 days (Table 1). The second set of clam samples were filtered on the dock in a filtration system for 24 hrs. Once the 24 hrs was over, the two samples were retrieved, wrapped in tinfoil, put in a dated ziploc bag, and placed into the freezer for 48 hours (until space was available in INCU-Shaker). Once retrieved from the freezer, their weights were calculated (Table 2). Then, they were placed into the INCU-Shaker with 150 mL of NaOH. This process was repeated once space opened up in the INCU-Shaker for the third and fourth sample sets (Table 1).

| Sample Number | Clam Size | Sample wet weight (g) | Beaker empty weight (g) | Sample weight (g) |
|---|---|---|---|---|
| 1A | Small | 111.440 | 107.442 | 3.998 |
| 1B | Large | 132.013 | 109.909 | 22.104 |
| 2A | Small | 117.100 | 108.843 | 8.257 |
| 2B | Large | 183.265 | 111.608 | 71.657 |
| 3A | Small | 122.719 | 107.761 | 14.958 |
| 3B | Large | 131.457 | 106.471 | 24.986 |
| 4A | Small | 123.181 | 110.781 | 12.400 |
| 4B | Large | 143.300 | 105.91 | 37.390 |

*Table 2*: Measured wet weight and dry beaker weight of each sample which was used to calculate sample weight (g).

***Filtration of Clam Samples***

All glassware and tools were rinsed with filtered deionized (DI) water prior to the experiment. Once the samples were oxidized for between 6 - 13 days (Table 1), each sample was taken out of the shaker and put into the clean room to prepare for filtration. Each sample was individually poured through a 53 μm sieve into a bowl below to strain out the NaOH solution. The sieve was then flipped over and backwashed with DI water into a different bowl. After backwashing, each sample in that bowl was carefully poured into a new, clean 250 mL beaker. Samples were then poured into the vacuum filter reservoir, through a 47 mm Cytiva Whatman GF/F filter, and then through a vacuum filter funnel which was all connected by a metal clamp (Figure 1A). The sample proceeded from the funnel into the manifold and then through a plastic tube into a 1- Liter vacuum trap. Three identical filtration stands are connected by a stainless steel stand called a manifold (Figure 1B). The Rocker 300 vacuum pump is connected to a 1-Liter vacuum trap and the manifold by two tubes. Once all samples were filtered, each filter was carefully placed, using tweezers, into its labeled 20 mm sterile petri dish with a cover.

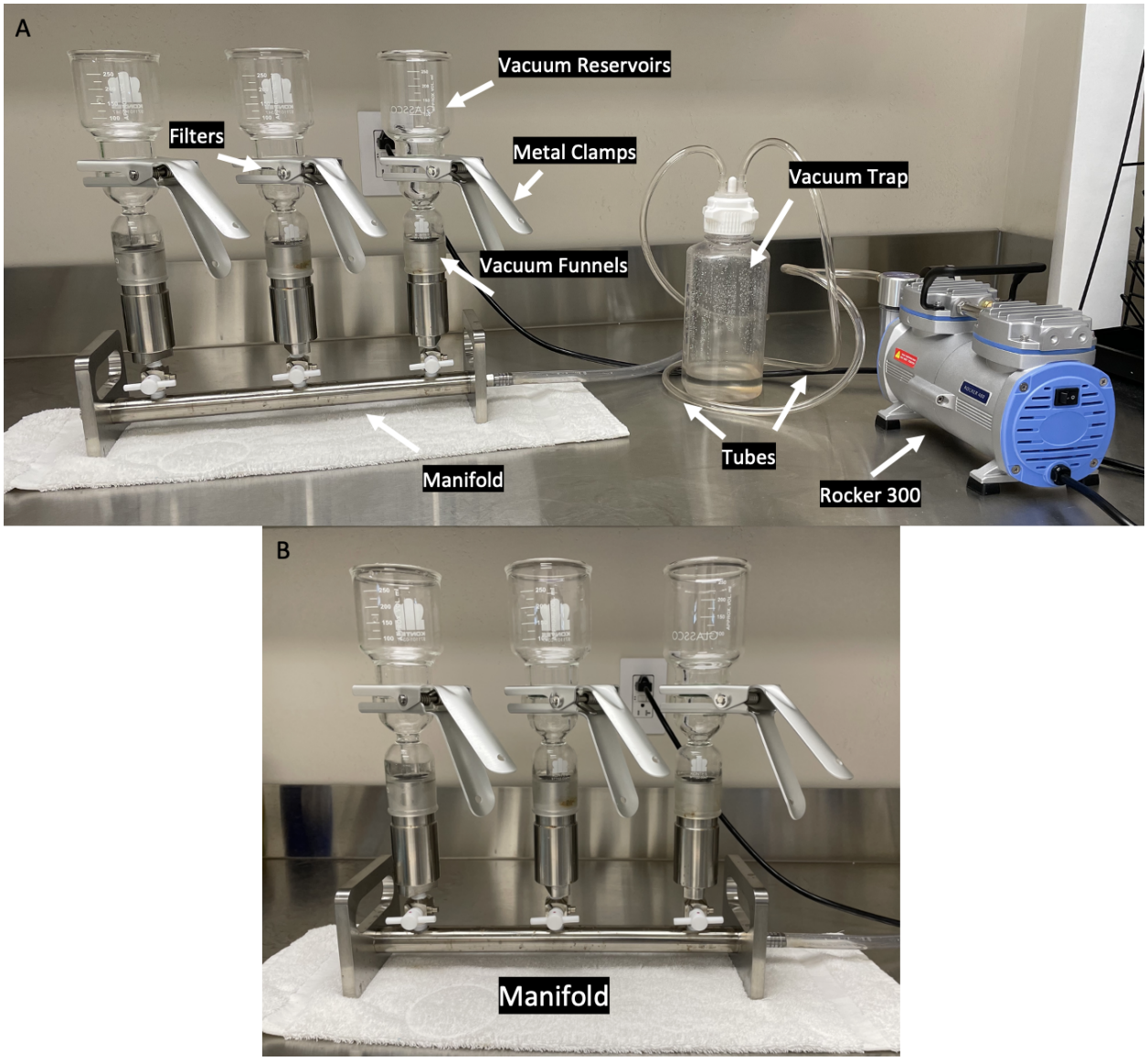


*Figure 1*: Filtration system with the vacuum reservoirs, vacuum funnels, vacuum trap, filters, metal clamps, tubes, and vacuum pump labeled (A). Close up of the manifold from the first image (B).

***Microscopic Counting of Microplastics and Fibers***

To view microplastics and microfibers, each individual sample was placed under the Stemi 305 Microscope in its petri dish without its cover. Between 9 - 12 images were taken of each sample filter to reveal all MPs and MFs. All microscope images were examined on laptops. To identify and count MFs, all threads were considered, especially non-black colored ones. MPs were identified as any dark black fragment. Only the fragments that had dimensions to them were counted, excluding the nanometer-sized dots. Organic matter such as lipids and fatty acids were identified in varying quantities on all of the filters. These were not included in the MP count and were distinguished based on transparency and color. The lipids were slightly transparent and yellowish-green in color. It is inferred that the samples were so dense that they did not have

enough time to oxidize in the NaOH. After counting, close up images were taken on the microscope to better showcase the findings. Additionally, a mm ruler on the microscope ocular was used to measure the length range of MPs and MFs within each sample (Table 3). The number of MPs and MFs per clam set was also calculated by adding the number of each in both of the samples in that set (Table 4). Finally, the number of MFs and MPs per gram in every sample was calculated by dividing the number of MFs or MPs by the sample weight (g) (Table 5).

| Sample Number | Clam Size | Number of Microfibers | Number of Microplastics | Average Microfiber Length (mm) | Average Microplastic Length (mm) |
|---|---|---|---|---|---|
| 1A | Small | 24 | 245 | 3 | 1 |
| 1B | Large | 12 | 347 | 10 | 1 |
| 2A | Small | 21 | 168 | 5 | 0.5 |
| 2B | Large | 23 | 199 | 4 | 0.5 |
| 3A | Small | 48 | 181 | 15 | 1.5 |
| 3B | Large | 19 | 130 | 10 | 1.5 |
| 4A | Small | 39 | 309 | 6 | 0.5 |
| 4B | Large | 10 | 550 | 9 | 1 |

*Table 3*: Total number of counted MPs and MFs, the average measured MP and MF length in each sample.

| Set Number | Total Microfibers | Total Microplastics |
|---|---|---|
| 1 | 36 | 592 |
| 2 | 38 | 367 |
| 3 | 68 | 311 |
| 4 | 65 | 859 |

*Table 4*: Total number of MPs and MFs per clam set.

| Sample Number | Clam Size | Microfibers per gram | Microplastics per gram |
|---|---|---|---|
| 1A | Small | 6.003 | 61.281 |
| 1B | Large | 0.543 | 15.699 |
| 2A | Small | 2.543 | 20.346 |
| 2B | Large | 0.321 | 2.777 |
| 3A | Small | 3.209 | 12.101 |
| 3B | Large | 0.760 | 5.203 |
| 4A | Small | 3.145 | 24.919 |
| 4B | Large | 0.267 | 14.7098 |

*Table 5*: Calculated microplastics and microfibers per gram in every sample (MP/g, MF/g).

**Results**

In the 8 hardshell clams that were analyzed, a total of 2,129 MPs and 196 MFs were counted. Sample 4B, the large clam in set 4, was observed to have the most MPs at a value of 550 MPs. Sample 3A, the small clam in the third set, had the most MFs at 48 MFs. Out of all the clam sets, set four had the most total MPs: 859 MPs. The set containing the most microfibers was set three with a total of 68 MFs (Table 4).

The clam size and MF abundance seem to be negatively correlated, while clam size and MP abundance are positively correlated. The smaller clams generally have more MFs and the larger clams have more MPs. Throughout all the samples, microfibers ranged from ~3 - 15 mm in length, and microplastics ranged from 0.5 - 1.5 mm in length. Sample 3A, small clam in set three, had the largest average microfiber length at 15 mm. The largest average microplastic length was 1.5 mm in both the large and small clams in set three, Samples 3A & 3B (Table 3).

The microplastics and microfibers per gram within each sample were calculated (Table 5). Sample 1A had the most microfibers with 6.003 MFs/g and microplastics with 61.281 MPs/g. Therefore, sample 1A, the small clam in set 1 that was not in the dock filter, had the most MP contamination. Microplastics abundance per gram decreases from 0 - 24 hours and then slightly increases from 24 - 72 hours in both small and large clams (Figure 2A). The MF concentrations in large clams are relatively consistent. Although, in the small clams there is a significant decrease from 0 - 24 hours and a very slight increase between 24 - 72 hours (Figure 2B).

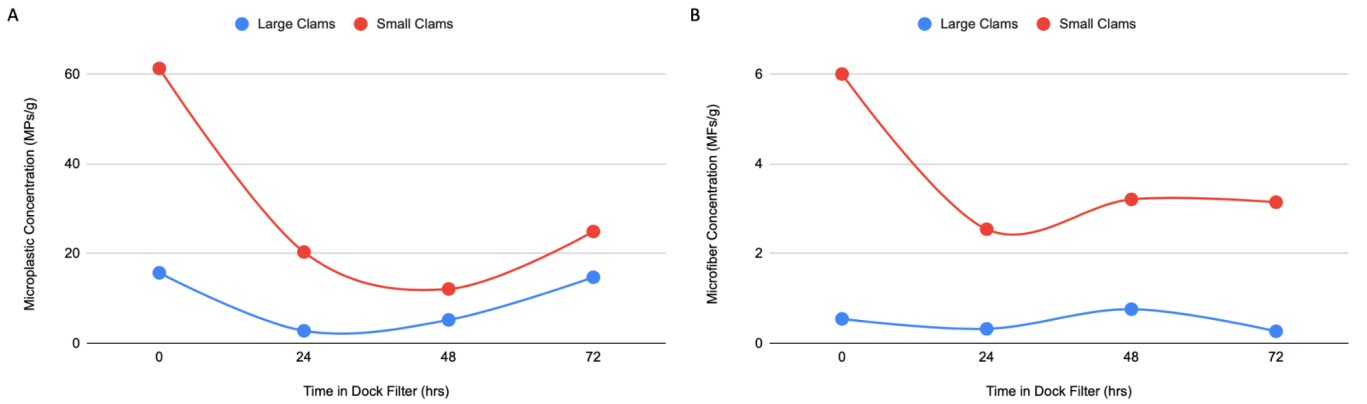


*Figure 2:* Line graph of microplastic concentrations (A) and microfiber concentrations (B) varying over time spent in the dock filter in both large (blue) and small (red) clams.

Microfibers varied in length within all 8 samples. The MFs showcased range from 3 mm - 35 mm (Figure 3). Sample 1A has a dark blue microfiber with a length of 20 mm and a red microfiber with a length of 30 mm (Figure 3A). These were some of the longest and most colorful MFs found in the samples. Red (Figure 3B, C, G), light blue (E), dark blue (A, F, J), purple (I) and black (D & H) MFs were primarily found. One of the smallest MFs seen was in sample 2A (Figure 3D).

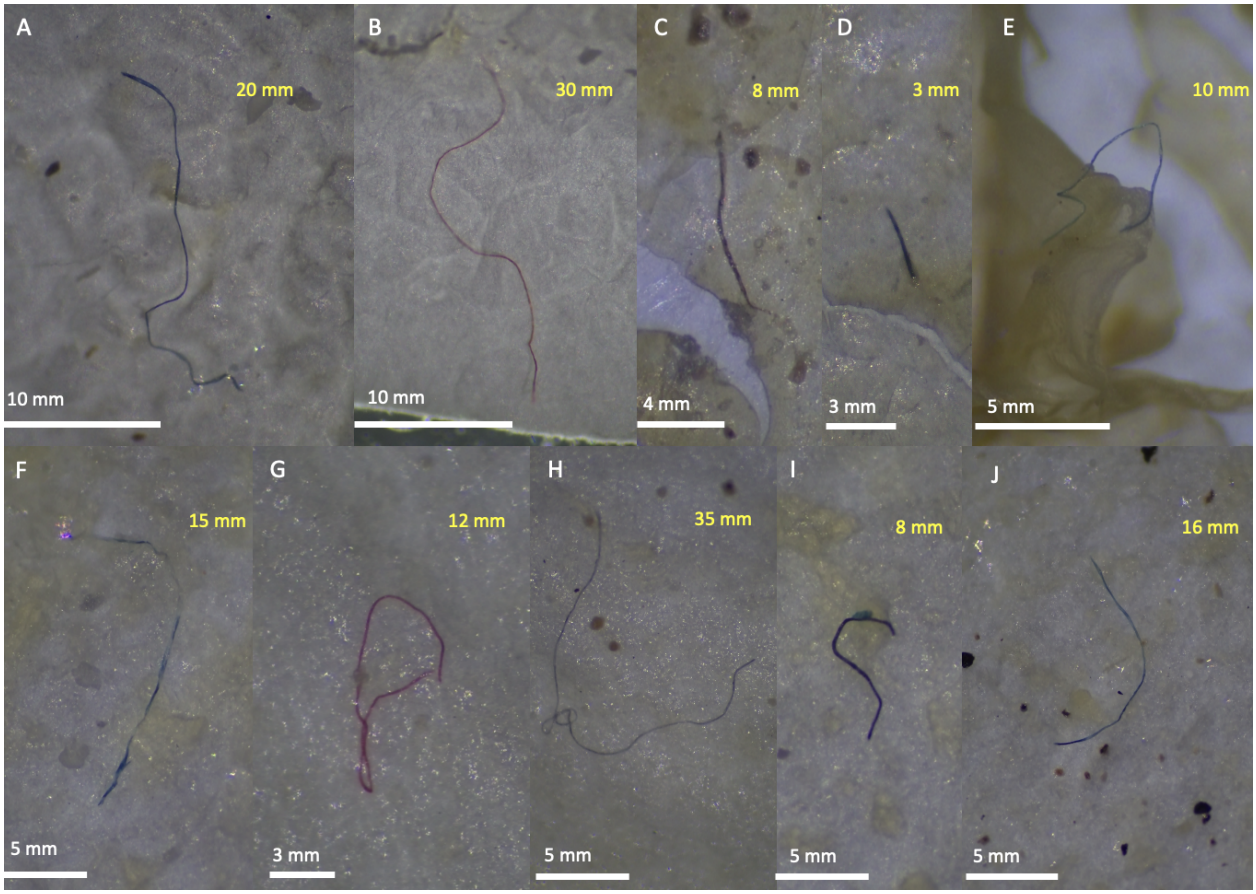


*Figure 3*: Microfibers from all 8 of the samples taken on the Zeiss Stemi 305 Microscope on 4.0 zoom. Sample 1A (A & B), Sample 1B (C), Sample 2A (D), Sample 2B (E), Sample 3A (F), Sample 3 B (G & H), Sample 4A (I), and Sample 4B (J). All sample lengths are shown in yellow and were measured on 1.0 zoom

The MP lengths within all of the samples were all relatively the same size, with some differentiation in color (Figure 4). The MPs identified were primarily black with 1 red and 1 light blue. Sample 3B had the most colorful and intriguing light blue MP measuring 3 mm (Figure 4D). This light blue MP is the largest out of the ones showcased. Sample 2A had the faint red MP with a length of 1 mm (Figure 4C). There were significantly more MPs in these samples than MFs. In sample 4B, 88 MPs were counted in a single image of the top left corner of the filter (Figure 4F).

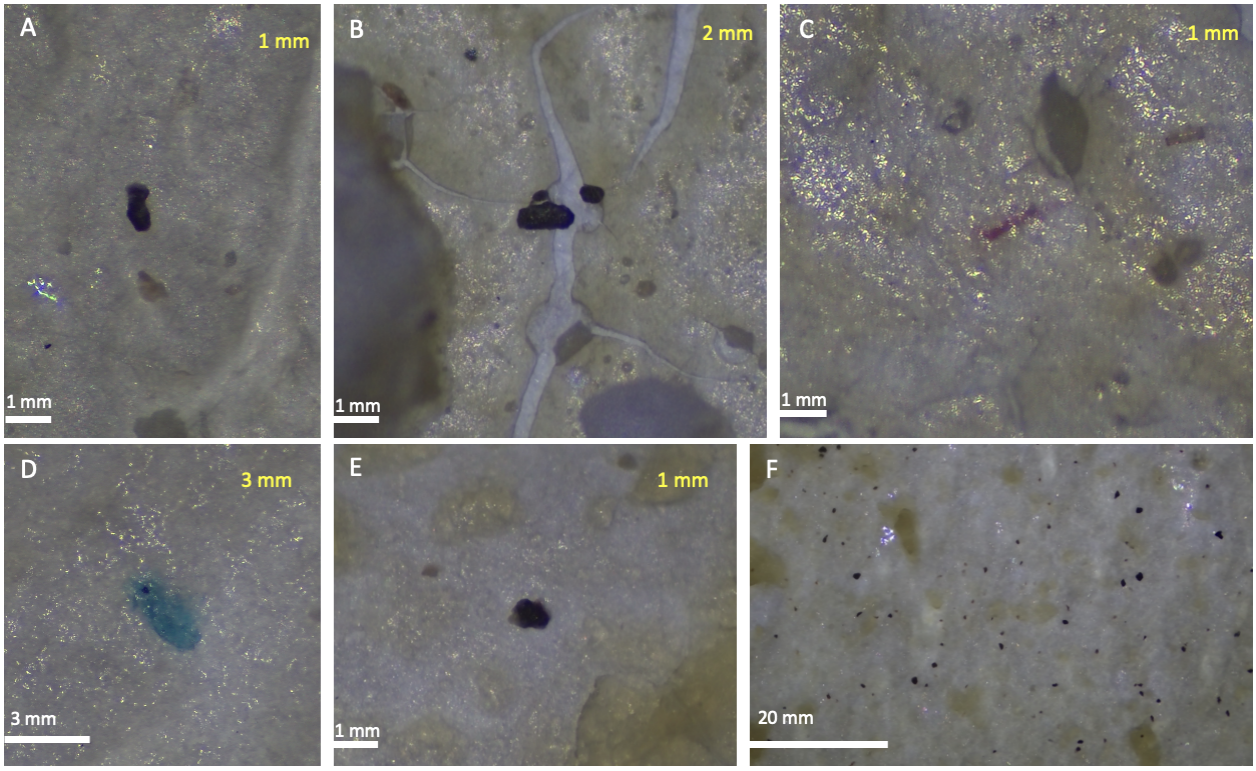


*Figure 4*: Microplastics from samples 1A (A), 1B (B), 2A (C), 3B (D), 4A (E), and 4B (F) taken on the Zeiss Stemi 305 Microscope. Subfigures A - E were taken on 4.0 zoom while E was taken on 1.0 zoom. All sample lengths are displayed in yellow and were measured on 1.0 zoom.

**Discussion**

It was hypothesized that larger quantities of MPs and MFs would be found in the smaller clams, and as the time spent in the dock filter increased, MP/MF concentrations would decrease. The results do not indicate a significant difference between samples to confirm either of these hypotheses. None of the data shows statistical significance. Based on these results, the null hypothesis is accepted. Although, this data indicates that size nor time in a filter are defining factors for measuring MP concentrations. It is significant to understand which parameters do not seem to affect the organism being studied. A larger sample size may have resulted in more significant data and the ability to showcase a trend. The dock filter did not seem to impact the quantity of MPs and MFs measured in the samples. Sample 4B which was a large clam that was in the dock filter for the longest, had the highest MPs. This goes directly against the stated hypothesis. There are many factors other than size that could affect the abundance of MPs in a given sample. A factor to consider is proximity to development or pollution; it could be hypothesized that larger concentrations of MPs may be present in hardshell clams that were adjacent to polluted or urban areas, as opposed to clams that were further removed from development.

It should also be noted that there is some margin of error in our results. Without access to Infrared Spectronomy equipment (LDIR), there is no way of confirming a fragment to be organic or inorganic. In addition, during the oxidation process, the NaOH solution was not able to dissolve the organic matter completely, which resulted in some fatty acids being present on

the filters, especially in the larger clams. The plastic fragments were identified and counted by observing the shape and makeup of a given fragment, and making an educated guess on whether it was organic or inorganic. It is possible that in the process of identifying particles on the filters of the larger clams, some undissolved lipids that resembled MPs were counted towards the total MPs present in the sample. With more advanced equipment, a more exact number can be determined, and a larger sample size could produce different trends.

**Conclusion**

Microplastics have thus far been found in the majority of marine macro-organisms. As filter feeders, hardshell clams are especially susceptible to external pollution through ingestion. This also means that hardshell clams are exceptional environmental indicators. Quahog Bay contains a healthy hardshell clam population that is frequently harvested and eaten by its residents. The intention of this study was to observe the presence of microplastics in hardshell clams, while also detecting any trends present in the microplastic concentrations of each sample. The results of the study conveyed valuable information on the abundance of microplastics in hardshell clams, despite being out of line with the initial hypotheses of the study. Knowing what conditions these clams are able to withstand and how they are affected is an important factor that can be applied in future research. More investigation is needed to fully understand how microplastics are ingested by hardshell clams, and how hardshell clams are affected by these plastics. If correlation can be found between hardshell clam health and microplastic abundance, the stress that plastic ingestion causes to clams could be quantified. In future studies, larger sample sizes are recommended. In order to understand how microplastics are affecting humans, it is vital to understand their impact on the organisms that humans consume.

**Credit Authorship Contribution Statement**

*Teagan Cunningham*: Methodology, Investigation, Writing - original draft, review, and editing.
*Nash Holley*: Methodology, Investigation, Writing - original draft, review and editing
*Mikayla Wallace*: Methodology, Investigation, Writing - original draft

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