Concentration of microplastics in *Geukensia demissa* from Bradley Point, West Haven, CT

Katelyn Zicker, Dr. Gail Hartnett, & Dr. Jean-Paul Simjouw

Department of Biology & Environmental Science, University of New Haven, West Haven, CT

Introduction

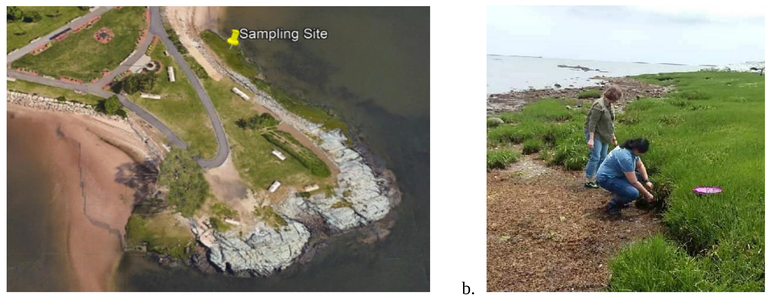
Microplastics (MPs) are pieces of plastic that are between 1 µm and 5 mm in size. There are two types of MPs: primary MPs and secondary MPs. Primary MPs are intentionally manufactured to be that size while secondary MPs are formed from larger pieces of plastic that degrade over time (Talvitie 2017). It is estimated that 808 trillion pieces of primary MPs enter the sewage treatment plants in the United States every day. Even though it is believed that 95% of MPs are not discharged into the bodies of water, that still conservatively leaves 8 trillion pieces of MPs that enter the ecosystem in the final effluent (Rochman 2015). Thus, there is a high bioavailability of primary MPs in the vicinity of effluent pipes. However, due to the size of the plastic, currents can easily disperse them throughout estuaries. Research has shown that organisms ranging from zooplankton to fish to even humans ingest MPs (Cole et al. 2015; Blumenröder et al. 2017). One study calculated that European shellfish consumers could ingest anywhere from 1,800 to 11,000 MPs a year (Cauwenberghe and Janssen 2014).

Experiments have found that MPs are either ingested, or passed over a fish's gills, but it is also possible for MPs to enter organisms through adherence. One study found that 42-59% of the total number of MPs found in bivalves were located in their feet, which are not involved in ingestion or digestion of food. MPs are known to have adverse effects on bivalves such as histological changes, inflammatory responses, reduction in filtering activity, and a change in the cellular oxidative balance (Kolandhasamy et al. 2017). Not only do bivalves in their natural environment contain MPs, but bivalves that are cultured for human consumption have been found to contain them, too (Cauwenberghe and Janssen 2014; Li et al. 2015).

Objectives

The objectives of this study were to determine if *G. demissa* is an indicator species of MPs, to determine if a depuration period does completely remove MPs from mussel tissue, and to refine the methodology of collecting MPs.

Materials and Methods



a. b.

Figure 1 a) Overview of the sampling site at Bradley Point, West Haven, CT. b) Collecting samples in the field.

The mussel were collected from Bradley Point, West Haven, CT on June 7 and July 12, 2018 (Figure 1), and were transported back to the laboratory in seawater from the site. The glassware was cleaned with soap and deionized (DI) water before use to prevent any contamination. The samples were divided into two groups: a depuration group and a non-depuration group. The non-depuration samples were stored in the freezer until analysis and the samples that underwent depuration were placed in individual bowls with artificial seawater for four days. The water was changed daily, and the wastewater was collected and poured into a vacuum filtration system to count the number of MPs on the glass fiber filter (GFF) paper (Figure 2). Samples of the artificial seawater were tested as the blank samples. All samples were analyzed using a similar methodology to that of Li et al. (2015).

The soft tissue of *G. demissa* was removed and each sample was placed in a 1 L glass jar where 250 mL of 30% H_2O_2 was added. The jars were placed in a Thermo Scientific MaxQ 4450 incubating shaker for 24 hours at 65ºC at 80 rpm. Then, the incubating shaker was set for 48 hours at room temperature at 80 rpm; afterwards, 730 mL of filtered 5 M NaCl solution was added to the jars and settled overnight. The MPs were collected from the top layer of solution by using a gravity siphon that lead to a vacuum filter (Figure 3). After the MPs were collected, at least 0.75 L of DI water was filtered through GFF with a pore size of 0.7 µm. The MPs on the filters were stained with a Nile Red (NR) solution of 1 mg of NR/mL of chloroform and counted under a Nikon SMZ 745 dissecting microscope with an Analytik Jena UVP UVGL-58 UV light. Six different types of plastic were cut into MPs and underwent the same methods as the mussels to observe the differences of the materials under UV light (Figure 4).

Materials and Methods

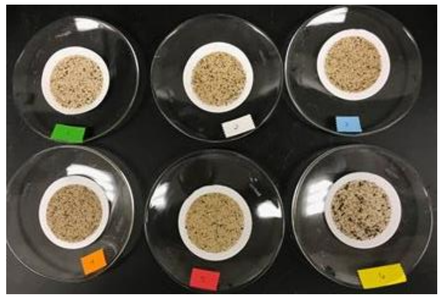


Figure 2 Filter papers after all four days of wastewater passed through the vacuum filtration apparatus.

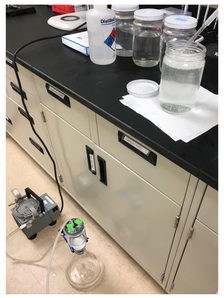


Figure 3 The suctioning apparatus constructed to collect MPs from the samples.

Results

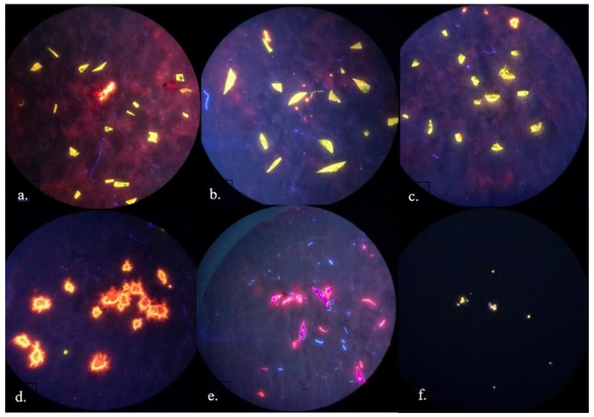


Figure 4 Microscope images of a) low density polyethylene (LDPE) b) high density polyethylene (HDPE) c) polypropylene (PP) d) polystyrene (PS) e) polyethylene terephthalate (PETE) f) copolymer acrylates.

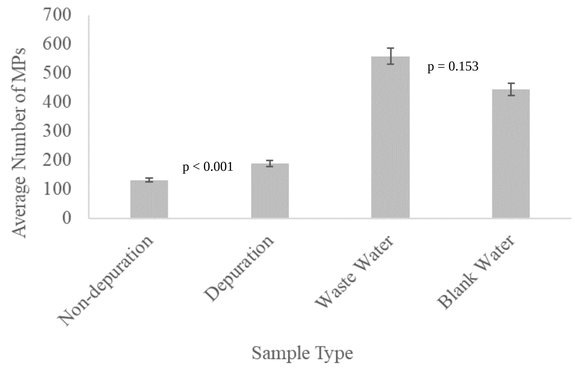


Figure 5 Average number of MPs in each sample type of *G. demissa* with standard deviation bars. An independent samples t-test determined that there was a significant difference between the non-depuration group and depuration group, but not between the waste water and blank water.

Discussion

MPs were present in all samples of *G. demissa* in both the non-depuration and depuration groups. The non-depuration group had an average of 132±32 MPs, and the depuration group had an average of 189±45 MPs. The higher average of MPs in the depuration group can be a result of the artificial seawater containing an average of 444±48 MPs. Cauwenberghe and Janssen (2014) found that the majority of MPs were retained in mussels after a 3 day depuration period, which can be caused by the translocation of MPs into tissues or the circulatory system (Kolandhasamy et al. 2017; Li et al. 2015; Tibbetts 2015). Depending on the species of bivalves and the laboratory conditions the samples are exposed to, the gut retention time is known to range from 1 hour to up to 15 hours (Cauwenberghe and Janssen 2014). Therefore, the high concentration of MPs in the artificial seawater or the retention of MPs could have increased the number of MPs in the depuration group. An independent samples t-test indicated that there was a significant difference between the averages of the two different sample types.

The wastewater collected from the depuration group had an average of 559±115 MPs. One would expect that a high concentration of MPs in the wastewater would correlate to a higher average in the non-depuration group, but this was not the case. This discrepancy could be a result of the artificial seawater containing MPs. Plus, filter papers were not covered to protect against airborne and laboratory contaminants prior to analysis. However, other studies that have included a depuration period in the methodology have not mentioned changing the water throughout the experiment or collecting the wastewater to analyze; thus, there is insufficient data to determine why there was a high concentration of MPs in the wastewater. More research needs to be conducted on mussels exposed to a depuration period. In addition, a methodology to produce artificial seawater without MP contamination needs to be established.

Future research will be conducted to determine the retention time of various concentrations of copolymer acrylates in *G. demissa.*

Conclusions

- The non-depuration group had a lower average of MPs than the depuration group.
- The blank water samples and wastewater samples had high averages of MPs (>400 pieces).
- There was a statistically significant difference between the averages of the depuration and non-depuration groups ($p<0.001$).
- There was not a significant difference between the averages of the wastewater sample and blank water sample ($p=0.153$).
- Future research will be on the retention time of MPs in mussels.

References

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