An Assessment of Microplastic Contamination in Snow Island Oysters (*Crassostrea virginica)*
Oysters as potential bioindicators of the health of Quahog Bay, Harpswell, Maine

Lucinda Medd[a, 1], Katie Lankowicz[b, 2], Isabelle Sée[c, d, 2]

[a] Bates College, Lewiston, Maine, anticipated graduation 2024
[b] Gulf of Maine Research Institute
[c] Quahog Bay Conservancy, Harpswell, Maine
[d] University of Maine, Orono
[1] Author
[2] Project advisor

Submitted in partial fulfillment for Quahog Bay Conservancy environmental conservation internship, summer 2023

Abstract

As filter feeders, oysters are sensitive to water contamination and are at high risk of being impacted by coastal pollution. The state of Maine lacks research on coastal microplastic presence and subsequent effects on ecosystem health. The objective of this study was to survey the microplastic (MP) contamination of Eastern oysters (*Crassostrea virginica*) at Quahog Bay Conservancy's (QBC) *Snow Island* and *Dogs Head* lease sites. We found an average of 0.57 microplastic particles per gram of wet meat in oysters. There was no correlation between MP abundance and meat mass, nor MP abundance and whole oyster mass. Fibers were the dominant identified shape, though fragments and beads were also found. Polyamide, and polyurethane were the most common plastic types identified by LDIR technology from two of our oyster samples. Polyethylene and PVC were also present, but in lower quantities. Looking ahead, these values will serve as baseline data for annual monitoring of oyster health at QBC. Further investigation is needed to determine if there is a correlation between measures of oyster health, like the oyster condition index, and MP presence in *C. virginica*.

Introduction

In an age dominated by the ubiquity of plastic, it is essential that we think about sustainability and the importance of ecosystem monitoring while developing solutions to reduce anthropogenic pollution. The term plastic refers to a sub-category of polymers, while the designation "microplastic" (MP) describes plastic less than 5mm in diameter (GESAMP, 2016). In 2019, 368 million metric tons of plastic were produced globally, and global production outputs are predicted to double by 2040 (Lebreton, 2019). Densely populated coastal areas predominantly serve as the largest contributor of microplastics, producing 80% of plastic in land-based activities. Large-scale plastic production and poor waste management make the ocean the final destination for at least 10% of global plastics (Teng et al., 2021). The degradation time of most microplastics is several hundred years (Chamas et al., 2020). Consequently, the breakdown of plastics over time, underscored by the concurrent production of pre-production plastic pellets, makes MPs the most abundant form of debris in the ocean (GESAMP, 2016). MPs are likely to act as aquatic contaminants, as several heavy metals from the ocean adsorb onto plastic surfaces (Liu et al., 2022). Accidental ingestion of MPs threatens species of all trophic levels; direct ingestion and trophic transfer are the two main pathways for bioaccumulation of microplastics. However, only 23% of countries have carried out research on MPs (Ajith et al., 2020).

As filter feeders, Eastern oysters (*Crassostrea virginic*a) exhibit a sensitivity to water contamination and are at high risk of being impacted by coastal pollution. Oysters are keystone species and create optimal habitat conditions for other organisms (Risgaard, 1988), as well as play an important role in the seafood economy. This keystone species is known to filter both biotic and abiotic particles less than 5 µm in diameter. As sedentary organisms, oysters can be used as a biological indicator species of local water contamination, including MP pollution in marine and estuarine systems (Edge et al., 2014). Because of this, oysters suffer globally from negative effects of anthropogenic activity. A study carried out in-vivo showed that the gills of oysters captured and transported all sizes of microplastics, though they began to expel indigestible matter (like MPs) as pseudofeces within 20 minutes of ingestion (Ward et al., 2019). Therefore, identifying and quantifying MP within an oyster at any given time is a snapshot and possibly fails to give an accurate representation of all MP present in the environment. Although studying microplastics in oysters may not result in an understanding of ecosystem health at large spatial and temporal scales, MPs that are found in oysters do begin to paint a picture of the types of plastics found in a given environment. Moreover, their wide geographical distribution makes them an ideal specimen for global marine MP contamination monitoring.

Currently, the biological effects of MPs on oyster health are understood from laboratory experiments, while little effort has been made to study this effect in natural habitats (Teng, 2021). Natural trophic transfer of MPs increase the risk of poor health of marine organisms and, in turn, for humans (Farrell et al., 2013). Increasing concentrations of MPs are consistent with increasing toxicity to the oyster (Teng et al., 2021). Exposure to microplastics, specifically polyethylene and polyethylene terephthalate, disturbs lipid metabolism, oxidation-reduction processes, and the pentose phosphate pathway in oysters (Teng et al., 2021). At present, oysters at both *Snow Island* and *Dogs Head* lease sites are the first documented cases of *Haplosporidium costale* (SSO) in Maine, suffering high rates of mortality. An investigation of MPs at these sites may provide insight into a correlation between MP presence and overall oyster health and will serve as the first MP assessment in Harpswell, Maine as well as a baseline data set for annual assessment at Snow Island Oyster Company.

Methods

Oyster collection and preparation

Five oysters were collected from both *Snow Island* (SI) and *Dogs Head* (DH) lease sites on a weekly basis between June 4 and August 18, 2023. Oysters were collected randomly and labeled *A-E*. All oysters labeled *A* (*B* was used from SI week 2 due to missing data for *A*) were used to survey for microplastic contamination. Whole wet oysters were weighed, then shucked. Oyster shells and meat were separated and weighed, then baked in individual trays at 70ºC for 24 hours (oysters were baked for use in a different project before preparation for MP analysis. Baking was not essential for the digest, but did not affect results).

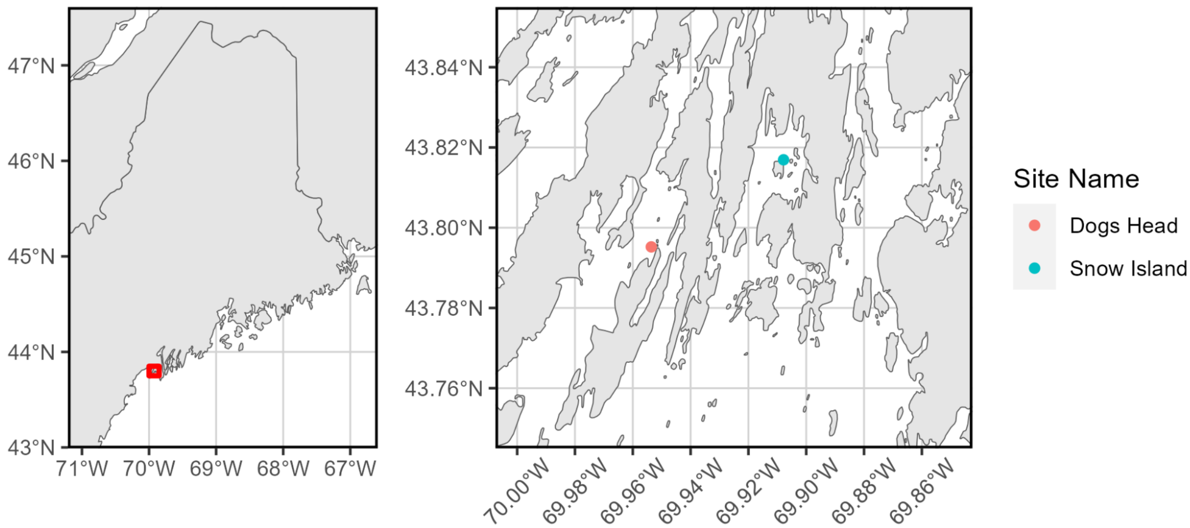


Figure 1. Dogs Head and Snow Island farm sites at Quahog Bay Conservancy in Harpswell, Maine.

Microplastic extraction

KOH digest and first sieve

After baking, dried meat was weighed and placed in a 250mL beaker. To the beaker was added 30mL of 4M KOH. Contents were stirred for one hour at 350 RPM. To the reaction was added 5mL of 30% H_2O_2, stirred for an additional 30 minutes, then left to sit for at least 24 hours, or until thoroughly dissolved. Sample contents were filtered through a 53µm sieve and rinsed with deionized water. The sieve was flipped over and contents were rinsed back into a 250mL beaker with deionized water.

Wet Peroxide Oxidation

In the 250mL beaker with the contents of the sieve, 20mL of 0.05M $FeSO_4$ was added. This solution was formed by adding 7.5g $FeSO_4$ and 3mL H_2SO_4 to 500mL deionized water. To the beaker was added 20mL 30% H_2O_2, allowed to rest for 5 minutes, then stirred for 10 minutes at 120 RPM. Under the fume hood, the beaker was placed on a hot plate at 65ºC until simmer, then cooled. Deionized water was added to the solution to slow the reaction as necessary. The reaction was then stirred at 65ºC for 30 minutes.

Second sieve

The sample was strained through a 53µm sieve and rinsed with deionized water. Using deionized water, the contents of the sieve were rinsed back into the 250mL beaker. Using a Vactrap system, contents were filtered through 1.2 µm-pore filter paper. After vacuum filtration, filter paper was placed into a labeled petri dish with forceps and left to dry under the vacuum hood.

Microplastic identification

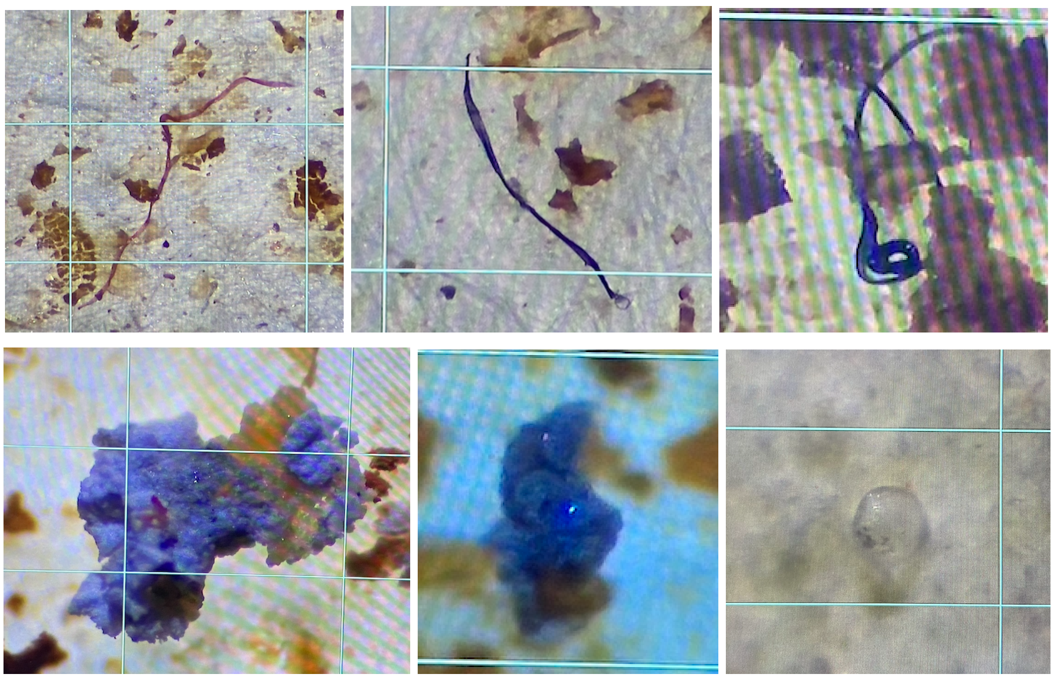
Filters were visualized on 40x using a microscope (Zeiss Stemi 305 CAM Digital Stereo Zoom Microscope) as the primary method to identify isolated microplastic fibers, fragments, and beads. Due to time constraints, only two oysters (SI_2B, DH_7A) were digested and filtered to be analyzed by *Agilent* Laser Direct Infrared (LDIR) technology. *Agilent* microplastic mapping software was used to identify specific types of plastic contaminants within the digested oyster. LDIR gives a confidence value for the identity of the plastic, values >65% were considered viable.

Data analysis

R software was used to develop a map of Quahog Bay, a stacked bar graph and scatter plots.

Results

Visualization under 40X magnification revealed that microplastics were found in all oyster samples. A total of 89 particles were identified as microplastics, ranging in size from 0.5-25 microns. Digital microscopic imaging revealed that particles differed in shape between fibers, fragments, and beads (*Figure 1*). The majority of particles were identified as fibers (72%), followed by fragments (26%) and beads (2%) (*Figure 2*).



(A)

(B)

Figure 1. Optical microscope images (40X magnification) of selected microplastic particles found in oyster samples. Three fibers are shown in row (A), while row (B) shows two fragments and one bead.

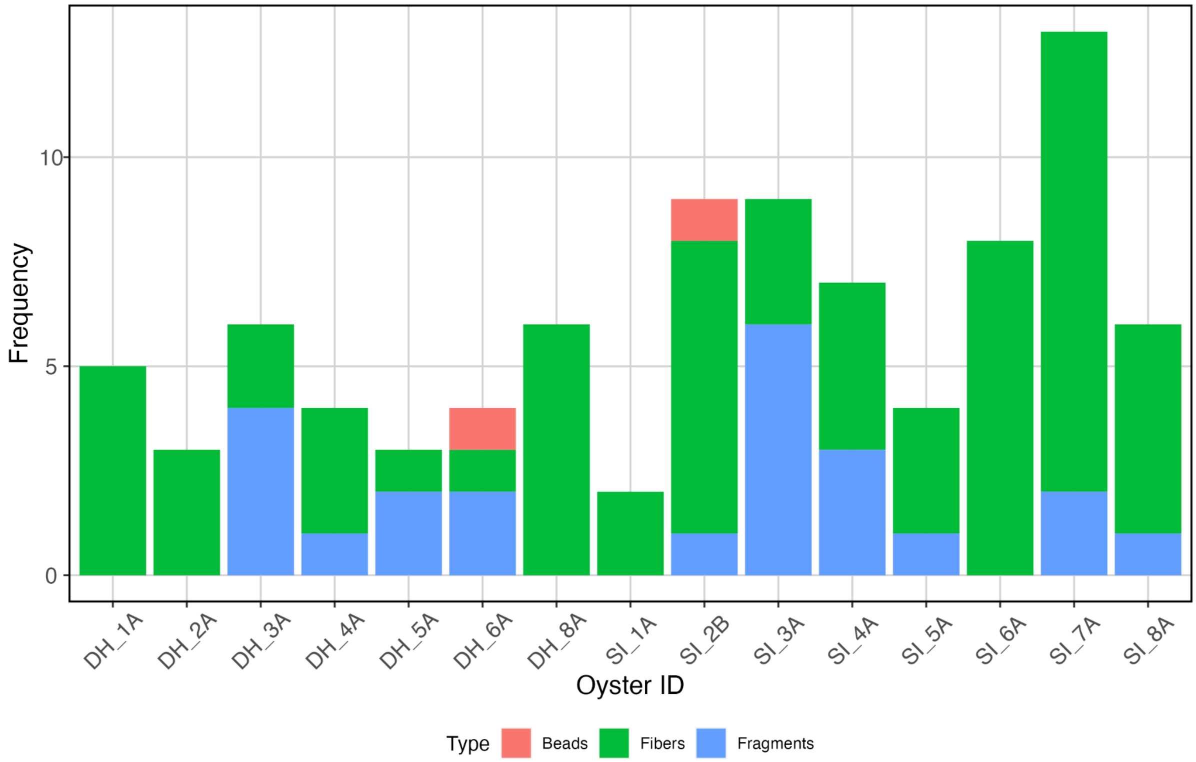


Figure 2. Frequency of microplastic particles found in oyster samples. Beads, fibers, and fragments are shown in pink, green, and blue respectively.

Across both lease sites, the average abundance of microplastics was 0.57 particles/gram meat and 0.13 particles/gram whole oyster. No significant correlation was found between particle abundance and oyster meat mass (*Figure 3*), nor between particle abundance and whole oyster mass (*Figure 4*).

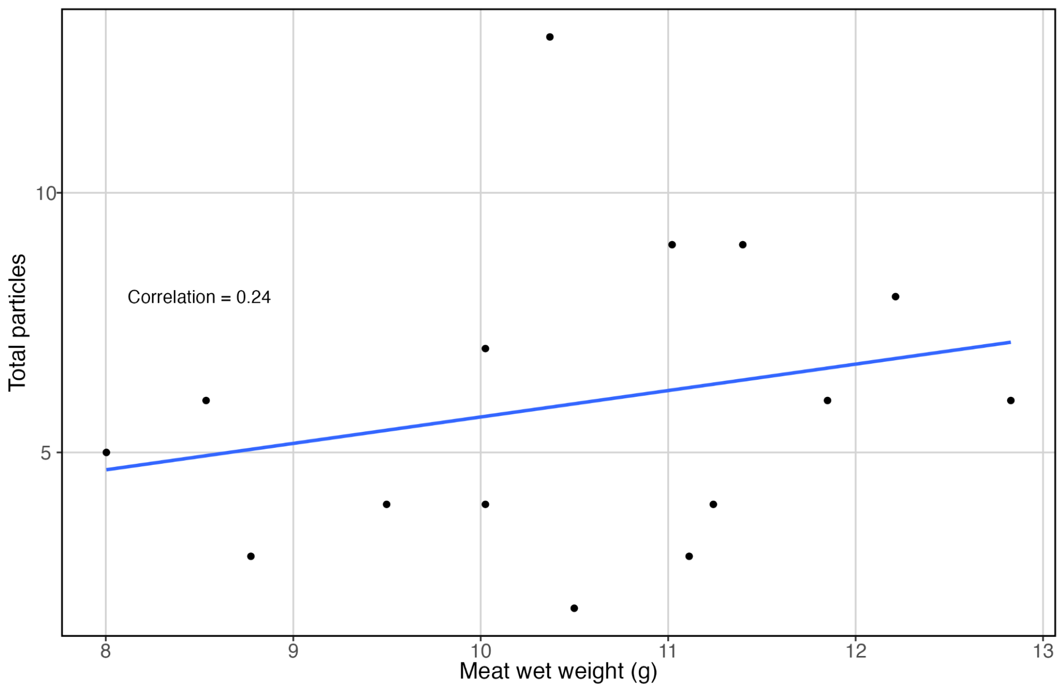


Figure 3. Scatter plot shows MP presence per gram of wet oyster meat. There is a correlation of 0.24, which is not a significant value.

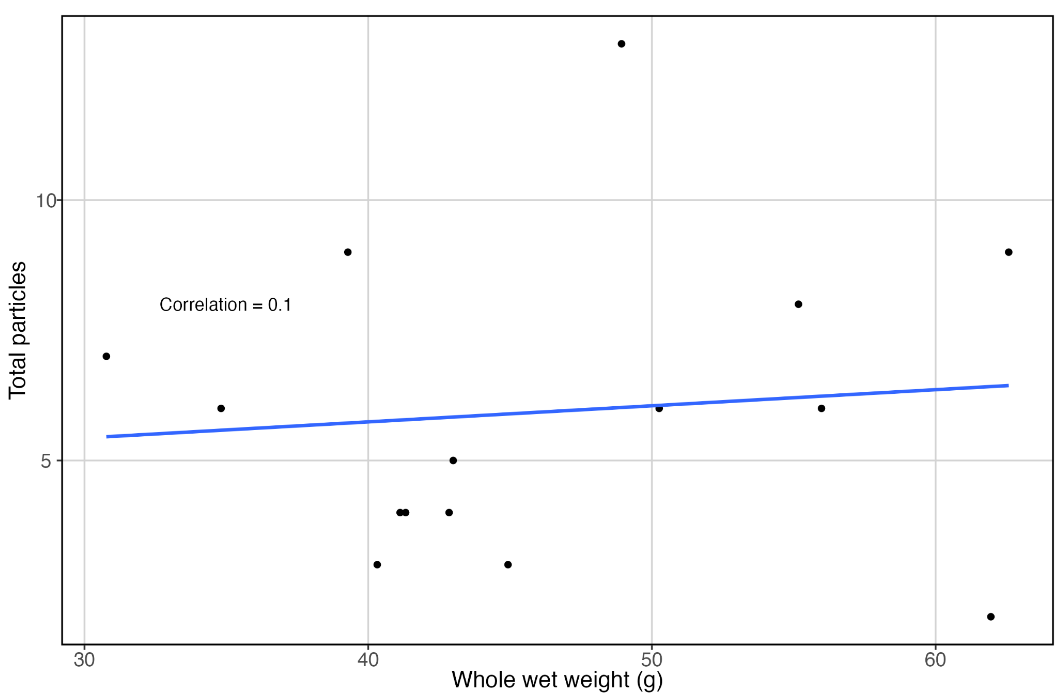


Figure 4. Scatter plot shows MP presence per gram of whole oyster. There is a correlation of 0.1, which is not a significant value.

Analysis by laser direct infrared spectroscopy (LDIR) showed the presence of 20 polymeric types of plastic. Of the 89 identified particles, only those with a confidence value >65% were considered.

Among the most common plastics present were rubber, polyamide, polyurethane, and acrylonitrile. Polyethylene, alkyd varnish, and polyvinyl chloride (PVC) were also found in high abundance.

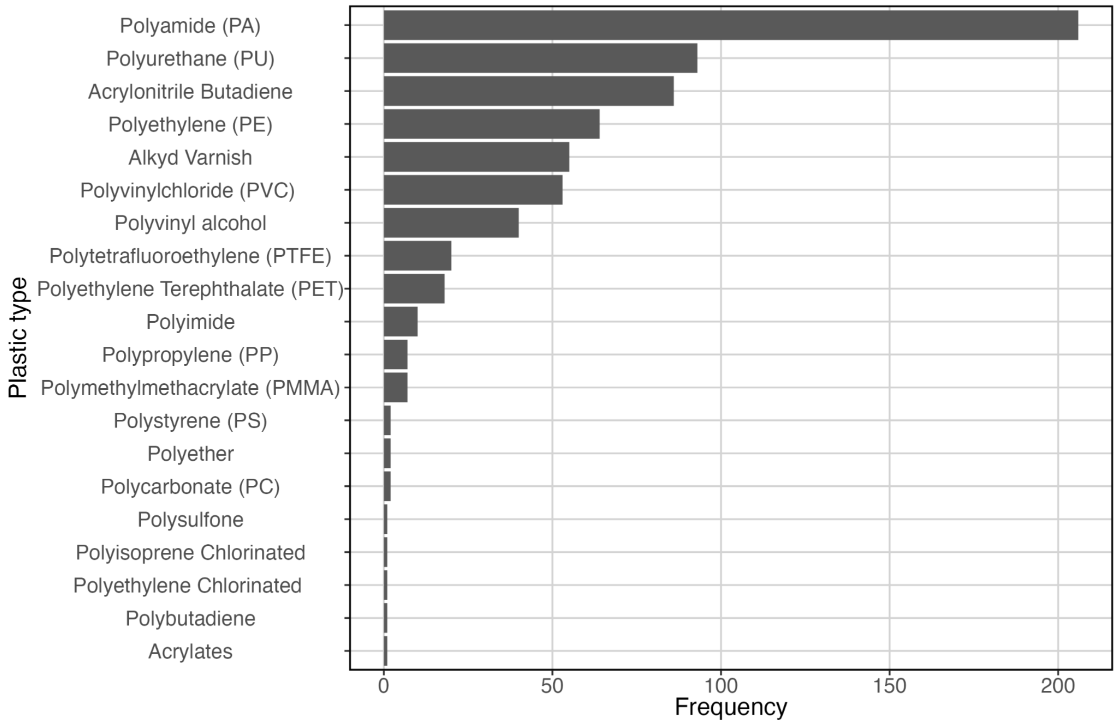


Figure 5. Frequency of plastic types (LDIR accuracy >65%) found in a Snow Island lease oyster.

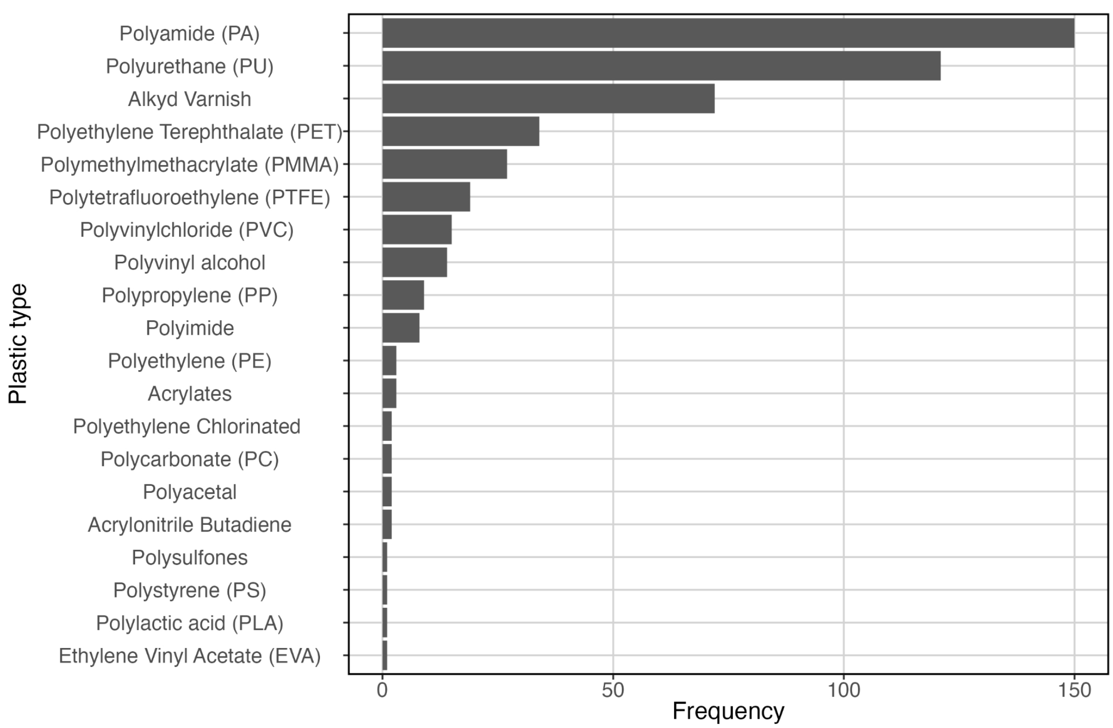


Figure 6. Frequency of plastic types (LDIR accuracy >65%) found in a Dog's Head lease oyster.

Discussion

Consideration of MP presence in Snow Island and Dog's Head oysters can be used to generate an understanding of possible pollutant sources in Quahog Bay. Historically, oysters have been used as bioindicators of water quality and habitat health. Despite their ability to reject indigestible material, we found MP contamination in 100% of sampled oysters. Although contamination quantity and type are subjective to the time of sampling, our results give a snapshot in space and time. A MP presence of 100% indicates that there is a constant flow of MPs in the water column and/or that some plastics are not expellable.

A larger sample size would paint a more accurate picture of MP pollution composition, but our findings suggest that Quahog Bay is contaminated with hazardous MPs, which may have subsequent effects on aquatic life within the bay. Three different shapes of MP were identified (*Figure 1*), suggesting the presence of multiple different pollution sources. The most common shape was fiber, which aligns with multiple other findings (Baroja, 2021; Covernton, 2019).

The prominence of black and red fiber contamination (*Figure 2*) may originate from oyster bags, which are red and black fibrous plastic bags in which the oysters are transported and sold. Plastic products are widely used for aquaculture activities, making the industry a MP contributor; plastic from Snow Island Oyster cages, boat, gloves, etc. are all reasonably possible sources of MP contamination. That said, some aquaculture farms are beginning to transition from plastic equipment to cedar grow boxes, an option worth investigating.

Globally, MPs have been identified in 94% of sampled oysters, with an average of 1.41 ± 0.33 per gram of soft tissue wet weight (Wooton et al., 2022). Although our results yielded lower than global average abundance (0.57 particles/g oyster tissue), low abundance is not the same as low risk for oyster health. In a risk assessment, researchers in South China determined that microplastic pollution assessments should not solely consider abundance, but also toxicity to humans and oysters (Li et al., 2021). Future studies in Quahog Bay could assess the human hazard levels of identified microplastics using the Polymer Hazard Index (Yuan et al., 2022). Plastics found in oysters at both lease sites include PE, PA, and PET (*Figures 5, 6*), which all have hazard ratings of two (out of five), moderate toxicity (Yuan et al., 2022). PA is commonly known as nylon and likely to originate from fishing line and other net-like equipment. Also found within the oysters were low-density polymers such as PP and PE which tend to float on the water surface and are particularly harmful to oyster health (Teng, et al., 2021).

One study considered long-term, low-dose exposure of MP to Pacific oysters and found a significant correlation to oyster health index (Thomas, 2019). As MP doses increased in concentration, oyster health index values dropped. A higher concentration of microbead presence was shown to increase mortality amongst juvenile oysters (Thomas, 2019). Knowing this, it would be valuable to continue both the Quahog Bay Conservancy oyster condition index in addition to this microplastic survey over the next decade to assess the health of the bay and the concentration of pollution.

This investigation occurred between June and August, 2023, the busiest time on the water in Maine. As a seasonal study, our results make for a narrow scope of understanding of MP presence in the bay. Oyster samples were placed under the flow hood and covered with glass or tin foil to avoid cross-contamination. During transport to the microscope however, samples were uncovered and exposed to the environment. Cotton clothing was worn while working with the samples. In future MP experiments, bright colored clothing (eg. pink or lime green) could be worn for easy identification of cross-contamination. Identification of MP was done under a digital microscope (4x), and a MP particle was only recorded if the team member was confident it was a plastic. If a particle could be broken with tweezers, it was not considered plastic. For future intern studies, it would be more accurate to use FTIR technology to identify specific MPs after the initial LDIR mapping scan, and avoid the digital microscope altogether. The Shaw Institute in Blue Hill, Maine is the only institute in Maine with a focused microplastic research program, and would be a good partner in a collaborative effort to develop reliable identification methods and help understand MP pollution in Quahog Bay as well as the effects on cultured oysters.

Conclusion

Oysters are potential bioindicators of the microplastic contamination in Quahog Bay, Harpswell, Maine. The average MP abundance per gram of wet meat in oysters *C. virginica* was 0.57 pieces. Fibers were the dominant shape, though fragments and beads were also found. Polyamide and polyurethane were the most common plastic types identified by LDIR technology. Polyethylene, PVC, and PET were also present, but in lower quantities.

Due to the hazardous nature of many synthetic polymers, we recommend a continual evaluation of the MPs in Quahog Bay. Additionally, Snow Island Oysters could consider transitioning to cedar grow boxes instead of plastic bags. The Harpswell Recycling and Transfer station offers free compost and recycling, and it would be beneficial to implement a sort system at Quahog Bay Conservancy to engage with the effort to reduce microplastic presence in the bay.

Acknowledgements

This study was made possible by the Quahog Bay Conservancy (QBC) internship program and its founder, Pat Scanlan. For his help with sample preparation and LDIR analysis, I thank Nash Holley (Bates College, 2024). For her help with statistical analysis and for her continual support throughout the summer, I thank Katie Lankowicz, PhD (Gulf of Maine Research Institute). For their support, I thank Isabelle Sée (QBC), Tait Nygaard (QBC), Dora Chaison-Lapine (Warren Wilson College, 2024), Sasha Milsky (Union College, 2022), and Hannah Orton (Bates College, 2024).

References

Ajith, Nithin et al. “Global Distribution of Microplastics and Its Impact on Marine Environment—a Review.” Environmental science and pollution research international 27.21 (2020): 25970–25986. Web.

Baroja, E., Christoforou, E., Lindström, J., & Spatharis, S. (2021). Effects of microplastics on bivalves: Are experimental settings reflecting conditions in the field? *Marine Pollution Bulletin, 171*, 112696. doi:https://doi.org/10.1016/j.marpolbul.2021.112696

Covernton, G. A., Collicutt, B., Gurney-Smith, H. J., Pearce, C. M., Dower, J. F., Ross, P. S., & Dudas, S. E. (2019). Microplastics in bivalves and their habitat in relation to shellfish aquaculture proximity in coastal British Columbia, Canada. *Aquaculture Environment Interactions, 11*, 357-374. Retrieved from https://www.int-res.com/abstracts/aei/v11/p357-374/

Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H., . . . Suh, S. (2020). Degradation Rates of Plastics in the Environment. *ACS Sustainable Chemistry & Engineering, 8*(9), 3494-3511. doi:10.1021/acssuschemeng.9b06635

Do, V. M., Dang, T. T., Le, X. T. T., Nguyen, D. T., Phung, T. V., Vu, D. N., & Pham, H. V. (2022). Abundance of microplastics in cultured oysters (Crassostrea gigas) from Danang Bay of Vietnam. *Marine pollution bulletin, 180*, 113800. doi:https://doi.org/10.1016/j.marpolbul.2022.113800

Edge, K. J., Dafforn, K. A., Simpson, S. L., Roach, A. C., & Johnston, E. L. (2014). A biomarker of contaminant exposure is effective in large scale assessment of ten estuaries. *Chemosphere, 100*, 16-26. doi:https://doi.org/10.1016/j.chemosphere.2014.01.001

Everaert, G., Van Cauwenberghe, L., De Rijcke, M., A. Koelmans, A., Mees, J., Vandegehuchte, M., Colin R. Janssen. (2018). Risk assessment of microplastics in the ocean: Modelling approach and first conclusions. *Environmental Pollution*, Volume 242, Part B, pg 1930-1938, https://doi.org/10.1016/j.envpol.2018.07.069.

Farrell, & Nelson, K. (2013). Trophic level transfer of microplastic: Mytilus edulis. *Environmental Pollution (1987)*, *177*, 1–. https://doi.org/10.1016/j.envpol.2013.01.046

GESAMP (2016) Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. In: Kershaw PJ, Rochmann CM (Eds.), Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Rep Stud GESAMP No. 93: 220

Lebreton, L., & Andrady, A. (2019). Future scenarios of global plastic waste generation and disposal. *Palgrave Communications*, *5*(1), 1-11.

Li, Y., Chen, G., Xu, K., Huang, K., & Wang, J. (2021). Microplastics Environmental Effect and Risk Assessment on the Aquaculture Systems from South China. *International Journal of Environmental Research and Public Health*, *18*(4), 1869. MDPI AG. Retrieved from http://dx.doi.org/10.3390/ijerph18041869

Liu, Q., Wu, H., Chen, J., Guo, B., Zhao, X., Lin, H., Li, W., Zhao, X., Lv, S., & Huang, C. (2022). Adsorption mechanism of trace heavy metals on microplastics and simulating their effect on microalgae in river. *Environmental research*, *214*(Pt 1), 113777. https://doi.org/10.1016/j.envres.2022.113777

Riisgaard, H.U. 1988. Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Marine Ecology. Progress Series 45: 217–223.

Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., . . . Zhao, J. (2019). Microplastic in cultured oysters from different coastal areas of China. *The Science of the total environment, 653*, 1282-1292. doi:10.1016/j.scitotenv.2018.11.057

Teng, J., Zhao, J., Zhu, X., Shan, E., & Wang, Q. (2021). Oxidative stress biomarkers, physiological responses and proteomic profiling in oyster (Crassostrea gigas) exposed to microplastics with irregular-shaped PE and PET microplastic. *The Science of the total environment, 786*, 147425. doi:10.1016/j.scitotenv.2021.147425

Walker, T. R., & Fequet, L. (2023). Current trends of unsustainable plastic production and micro(nano)plastic pollution. *TrAC Trends in Analytical Chemistry, 160*, 116984. doi:https://doi.org/10.1016/j.trac.2023.116984

Ward, J. E., Zhao, S., Holohan, B. A., Mladinich, K. M., Griffin, T. W., Wozniak, J., & Shumway, S. E. (2019). Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (Mytilus edulis) and Eastern Oyster (Crassostrea virginica): Implications for Using Bivalves as Bioindicators of Microplastic Pollution. *Environmental Science & Technology, 53*(15), 8776-8784. doi:10.1021/acs.est.9b02073

Wootton, N., Sarakinis, K., Varea, R., Reis-Santos, P., & Gillanders, B. M. (2022). Microplastic in oysters: A review of global trends and comparison to southern Australia. *Chemosphere, 307*, 136065. doi:https://doi.org/10.1016/j.chemosphere.2022.136065

Wu, F., Wang, Y., Leung, J. Y. S., Huang, W., Zeng, J., Tang, Y., Chen, J., Shi, A., Yu, X., Xu, X., Zhang, H., & Cao, L. (2020). Accumulation of microplastics in typical commercial aquatic species: A case study at a productive aquaculture site in China. *The Science of the total environment*, *708*, 135432. https://doi.org/10.1016/j.scitotenv.2019.135432

Yuan, Z., Nag, R., & Cummins, E. (2022). Ranking of potential hazards from microplastics polymers in the marine environment. *Journal of Hazardous Materials, 429*, 128399. doi:https://doi.org/10.1016/j.jhazmat.2022.128399

Thomas, M., Jon, B., Craig, S., Edward, R., Ruth, H., John, B., Dick, V. A., Heather, L. A., & Matthew, S. (2019). The world is your oyster: low-dose, long-term microplastic exposure of juvenile oysters. *Heliyon*, *6*(1), e03103. https://doi.org/10.1016/j.heliyon.2019.e03103