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Prevalence and Distribution of Microplastics in Oysters from the Mississippi Sound

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Abstract: Oysters are a foundational part of their ecosystem and research has shown they are negatively impacted by exposure to microplastics (MPs). High MP levels have been documented in waters surrounding oyster reefs, and as filter feeders, oysters can ingest MPs along with their food. Here, we determined MPs (>30 µm) in oysters (*Crassostrea virginica*) from ten sites across the Mississippi Gulf Coast. Further, a subset of these samples was dissected to quantify MPs within specific tissues. Average concentrations ranged from 30.7± 11.5 to 4.7 ± 0.25 putative MPs/g wet weight (ww) of whole tissue, with sites inside bays near population centers displaying higher levels of MPs than those exposed directly to the Gulf. Mantle, gill, and adductor muscle tissues had similar concentrations of putative MPs (15.9 ± 13.4, 11.5 ± 8.6 and 12.8 ± 6.7 MPs/g, respectively), whereas digestive system tissues had lower concentrations (6.8 ± 6.1 MPs/g of tissue). This suggests that most MPs in an oyster likely adhere to external tissues and are not actually ingested. Most of the MPs retained were in the smallest size fraction of 30-90 µm (80%), followed by 125-250 µm (9%), 90-125 µm (8%), and >250 µm (3%). Analysis of samples from Biloxi Bay by µ-FTIR to assess MP composition shows that polyurethane, polyethylene, and polyamide are common, but additional analyses are needed to fully characterize the MP profile across sites. Overall, this work provides much-needed empirical data on the abundances and sizes of MPs in oysters from the Mississippi Sound, as well as the tissues where they reside.

Keywords: *microplastics, oyster condition index, fluorescence microscopy, Gulf of Mexico, Mississippi*

The global nature of microplastics (MPs) as an environmental contaminant has been well documented (Ding et al. 2019; Dodson et al. 2020; Shen et al. 2020; Suaria et al. 2020). The northern Gulf of Mexico is of particular interest for MP contamination, being the outlet of several major rivers including the Mississippi River system. The load of MPs tends to increase as the rivers flow toward the Gulf of Mexico, which acts as a sink for these particles (Scircle et al. 2020a). Thus, it is not surprising that Gulf Coast waters and beaches have high levels of MP contamination compared to many other coastlines worldwide (Wessel et al. 2016; Di Mauro et al. 2017).

While all marine animals are exposed to the MPs in ocean waters, oysters and other filter feeders are particularly vulnerable. This is especially concerning because oysters are foundation species (Ridlon et al. 2021), responsible for the structure and function of oyster reef ecosystems. Oyster reefs serve as nurseries and habitats for other species, act as barriers to protect the shoreline from erosion, and clean the surrounding waters by virtue of the oysters' filter feeding behavior (Beck et al. 2011). Loss of an oyster reef is typically followed by a decrease in biodiversity in the area, causing both environmental and economic damage in areas dependent on commercial fishing, such as the Mississippi Gulf Coast (Beck et al. 2011). Thus, assessing the risk posed to native oyster reefs by MPs is crucial.

Risk assessment is important because some

Research Implications

- Oysters from locations inside bays near population centers had higher average concentrations of MPs.
- Oysters accumulate more MPs on their external tissues than in their digestive system, though the latter gives a snapshot of recently consumed MPs.
- MP concentration in oysters was not correlated with oyster condition index.
- Characterizing the types of MPs present in oysters may provide insight into likely sources of contamination at different sites.
- Lawmakers need to consider federal legislation to address MP pollution in river systems at a national level.

types of MPs can have adverse impacts on oysters. Previous studies have shown that MPs can negatively affect oyster reproduction and energy uptake (Sussarellu et al. 2016; Gardon et al. 2018). More troubling, long term exposure to polystyrene MPs may also result in increased mortality rates (Thomas et al. 2019). Given that previous work has shown high levels of MPs in waters surrounding Mississippi Gulf Coast oyster reefs (Scircle et al. 2020b), this study sought to quantify and characterize the MPs that Mississippi Gulf Coast oysters (*Crassostrea virginica)* ingest and accumulate in their tissues.

While several other studies have assessed MP concentrations in oysters (Li et al. 2018; Keisling et al. 2020; Cho et al. 2021), such studies have been primarily concerned with using oysters for environmental monitoring purposes. As a result, their analyses were focused on whole oysters in order to assess MP levels across a variety of sites. However, such data do not provide information on where those MPs are located inside the oysters, which is crucial to assess potential health risks to both oysters and the humans that eat them. Therefore, another goal of this study was to analyze oyster tissues separately in order to assess whether they contained different concentrations and types of MPs.

Methods

Study Site and Oyster Sampling

Oysters were sampled from ten sites along the Mississippi Gulf Coast (Figure 1), with two of the sites associated with Mississippi Based RESTORE (Resources and Ecosystem Sustainability, Tourist Opportunities, and Revived Economies) Act Center of Excellence (MBRACE) sensor platforms (hereafter called *landers*) and the remaining eight associated with the Mississippi Oyster Gardening Program (MSOGP). GPS coordinates for each site are given in Table 1.

Landers. Oysters were obtained from the Thad Cochran Marine Aquaculture Center in Ocean

Figure 1. Map of oyster collection sites along the Mississippi Gulf Coast. A description of sampling sites is given in Table 1. Sites 1 and 10 correspond to MBRACE landers.

Table 1. Oyster sampling sites include those from the Mississippi Oyster Gardening Program (MSOGP) and MBRACE lander sites, with the latter designated by *.

Site	Description	Nearest City	Coordinates
1*	St. Stanislaus High School	Bay St. Louis	30.305025, -89.325315
2	St. Stanislaus High School	Bay St. Louis	30.304968, -89.325304
3	MSOGP site	Bay St. Louis	30.334387, -89.331403
4	MSOGP site	Pass Christian	30.331054, -89.283668
5	Biloxi Maritime Museum	Biloxi	30.392968, -88.857867
6	MSOGP site	Biloxi	30.416986, -88.908997
7	MSOGP site	Ocean Springs	30.418516, -88.836062
8	MSOGP site	Ocean Springs	30.343711, -88.722355
9	MSOGP site	Gautier	30.363593, -88.637757
10*	Grand Bay	Moss Point	30.369654, -88.420534

Springs, MS. Each lander was deployed with about 20-25 oysters on 13 October 2020 along the Mississippi Gulf Coast. Each lander consists of a metal frame resting on top of a rubber tire, which prevents the lander from sinking into the surrounding sediment when deployed (Gledhill et al. 2020). Inside the lander, a milk crate and several trays are used to hold the oysters. Each lander also contains dissolved oxygen, temperature, and conductivity sensors, which continuously monitor environmental conditions. Initially, landers were deployed at ten sites. Unfortunately, the majority of these landers were destroyed in Hurricane Zeta. Only two landers remained following hurricane season, those located at St. Stanislaus High School and in Grand Bay. Thus, we only report lander data from these two sites. Upon collection on 8 December 2020, average oyster wet tissue weights were 21.2 ± 5.4 g at St. Stanislaus and 10.2 ± 1.0 g at Grand Bay.

MSOGP Sites. Additional oysters were sourced from eight MSOGP on 8 December 2020. Briefly, this program helps restore Mississippi's oyster reefs by providing juvenile hatchery-reared oysters for volunteers to raise in cages on private docks until they are old enough to be planted onto oyster reefs. Although they cannot be harvested themselves, the goal is for them to spawn and produce larvae that will re-seed harvestable reefs. Oyster gardening programs also increase public awareness of how oysters improve the water quality and their economic role in Gulf Coast communities ("Mississippi – Oyster Gardening on the Gulf Coast" n.d.). Whereas these oysters were in the Gulf over a longer period (July to December 2020), they tended to be smaller than the lander oysters because they were deployed as oyster spat (newly attached larvae). Upon collection, their average wet tissue weight for each site ranged from 1.93 g to 7.85 g.

Oyster Condition Index Measurements

Condition index (CI) measurements were used to assess the oysters' condition at each site (n = 6-13 per site) following methods from Abbe and Albright (2003). Whole oysters were weighed intact to determine the total wet weight (ww). Each

oyster was then shucked, and the wet tissue was separated, and empty shells were weighed alone. The wet shell cavity volume was then calculated by subtracting the weight of the wet oyster shells from the total wet weight. Following this, the wet oyster tissue was freeze-dried and weighed again to determine the dry tissue weight. The following equation was used to determine the CI for each oyster (Abbe and Albright 2003):

$$CI = \frac{dry\ tissue\ weight}{wet\ shell\ cavity\ volume}$$

Oyster Dissection

In the lab, oysters were assessed based on size to determine which oysters would be dissected and which would be analyzed whole. Due to differences in oyster size between sites, oysters from the two landers and Site 5 were dissected (n = 5 for each site, 15 total), while the smaller oysters from the other sites were analyzed whole (n = 5 for each site; 35 total). Each oyster was shucked with a shucking knife and the mantle pulled back with tweezers to expose the gills (Figure 2). Using tweezers and dissecting scissors, the gills were removed and placed in a labeled 20 mL glass scintillation vial with a foil-lined cap. The mantle was placed in a separate labeled glass vial. A knife was then used to separate the adductor muscle from the shell. Finally, the adductor muscle and heart were separated from the digestive system tissue. The digestive system was placed in one glass vial while the adductor muscle and heart were placed in a separate vial. If the oyster was too small to ensure a clean separation of tissues, it was shucked and placed in a glass vial whole.

Contamination Mitigation Protocols

Sample preparation occurred in a laminar flow hood (AirClean 6000 Workstation) within a HEPA-filtered clean room to reduce the risk of contamination by MPs. Plastic tools were avoided wherever possible in favor of glass and metal tools. All glassware was heat cleaned at 450°C for three hours before use. Additionally, glassware and metal tools were rinsed three times with milliQ water between samples. Reagents were pre-filtered through a 25 µm pore size Monel filter to remove any MPs. Analysts also wore 100% cotton lab coats and nitrile gloves to further reduce contamination risk. Finally, two methodological blanks for each sample run were prepared and used to quantify any contamination that might have occurred despite these precautions. All data reported consist of blank subtracted values.

Sample Preparation

Samples were prepared using a modified version of the single pot method previously described (Scircle et al. 2020a). Briefly, each whole oyster

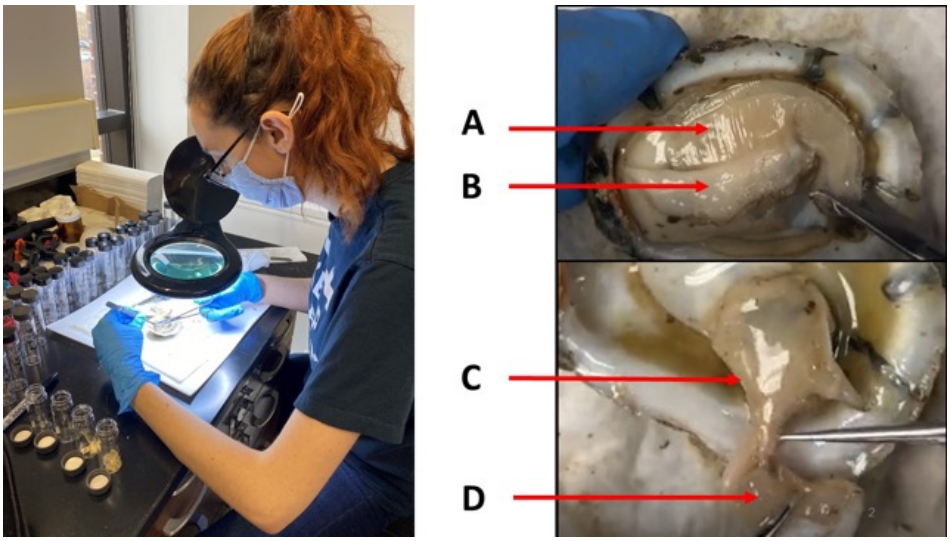

Figure 2. Oyster dissection with mantle (A) peeled back to expose gills (B). Following removal of gills and mantle, the digestive system (C) is separated from the adductor muscle (D) and heart (not visible).

or dissected tissue was weighed and placed in a Mason jar along with 150 mL of 10% w/v KOH solution to digest the biological tissue. A lid was used to cover each jar but not screwed down to allow gases to escape. Samples were then placed in a vacuum oven at 40°C for 24 hours and stirred twice daily. Digestion was performed at 40°C as studies have shown that higher temperatures can cause damage to some polymer types (Thiele et al. 2019). Fully digested samples were removed from the oven while undigested samples were heated for an additional 24 hours. In general, samples with higher masses (>3 g) needed longer digestion times. Once samples had been digested, the solid lids were exchanged for lids with a 57 mm diameter hole. The new lids were placed into the screw band and an 84 mm diameter 30 μm pore Monel filter was placed on top. These were then screwed onto the tops of the jars. Each jar was swirled and turned upside down over a waste bucket and a stream of clean air was applied to the filter to help break the surface tension. After removal of the lids, milliQ water was used to rinse any solids left on the filter back into the Mason jar. A glass vacuum filtration apparatus was used to filter the samples onto 25 mm diameter 30 μm pore Monel filters. During filtration, each jar was rinsed twice with milliQ water to ensure transfer of all MPs. These smaller Monel filters were then rinsed with a 1.63 g/cm^3 density $ZnCl_2$ solution into a 40 mL glass scintillation vial. Each vial was then filled to 30 mL with $ZnCl_2$ solution. The vials were capped and centrifuged at ~1610 G for 12 minutes to separate shell fragments and other inorganic materials. The supernatant was filtered through a 25 mm diameter 10 μm pore polycarbonate filter. The filters were then rinsed with 1 mL of 2% HCl, followed by 5 mL of milliQ water to remove any $ZnCl_2$ precipitate.

Microplastic Analysis by Fluorescence Microscopy

Filters were placed on labeled glass slides and allowed to dry in a laminar flow clean bench. A 10 μg/mL Nile red in methanol solution was used to stain the samples by pipetting 3-4 drops of dye onto each filter. The filters were allowed to dry for ~5 minutes before being covered with a glass cover slip and taped shut. A Nikon Ti2 Eclipse Fluorescence Microscope along with the NIS-Elements application was used to analyze these samples. Filters were imaged in their entirety and the software's object count feature was used to automatically count the number of fluorescing particles above a defined threshold (i = 15000). Each counted object was then manually inspected to ensure that it was a putative MP. Objects with biological features such as striations or intracellular patterning were excluded from the count. Each sample count was then subtracted by the average blank counts of the run to yield the blank-subtracted data.

It is important to note that although fluorescence microscopy is frequently used in MP studies due to its relative low cost and fast analysis times, it does not yield any chemical data about the particles imaged. Although the digestion process, density separation, use of Nile red (a lipophilic dye that preferentially stains plastics), and particle examination (only objects lacking biological features such as cellular structure or striations are counted) minimize false positives, it is still possible to overestimate the number of MPs in a sample. Thus, herein we use the term putative MPs when referring to fluorescence microscopy data.

Determination of MP Compositions by μ-FTIR

Since fluorescence microscopy does not yield information about the chemical identity of the MPs, five samples from two Sites (6 and 7) were prepared for analysis using micro-Fourier transform infrared spectroscopy (μ-FTIR). Polycarbonate filters containing the putative MPs were sonicated for 2 minutes in 30 mL of 50% ethanol. The resulting solution was filtered through a 25 mm aluminum oxide filter (Anodisc). Filters were then dried in a laminar flow clean bench before being analyzed with a Bruker LUMOS II FTIR microscope. Samples were imaged in transmission mode using the FPA detector. A 4-mm square of each filter was analyzed using a resolution of 4 cm^{-1}, 6 scans, and 4 x 4 binning. Data were processed using the OPUS v8.5 and Purency v4.07 software.

Statistics

In order to assess whether statistically significant differences existed between sample sites, one-way ANOVA was utilized. If significant differences were found ($p < 0.05$), post hoc tests

were used to determine which groups gave rise to these differences. Due to having unequal groups in the CI data, Dunn's post hoc test was used for this purpose. Tukey's honestly significant difference (HSD) was used for the MP concentration data as there was an equal number of samples in each group. In order to assess statistical differences between average MP concentration in different types of oyster tissue, a two-way ANOVA analysis followed by Tukey's HSD post hoc test was used. This made it possible to determine differences due to both site and tissue type, as not all dissected oysters came from the same location.

Results and Discussion

Condition Index

The average CI of oysters for sites in this study ranged from 9.3 ± 3.0 to 15.6 ± 2.4, and differed significantly among sites (ANOVA, df = 9, $p < 0.001$), with the lowest values at Site 3 (Bay St. Louis) and the highest at Site 5 (Biloxi) (Figure 3). These values are similar to those of oysters in Alabama and Louisiana Gulf Coast waters (Casas et al. 2017; Leonhardt et al. 2017). We observed no correlation between CI and MP concentration (Pearson's correlation coefficient = -0.13, $p = 0.73$). One limitation to our analysis is that unlike fresh (wet) oyster tissue the freeze-dried oyster tissue used to calculate CI could not be fully digested. As a result, both the CI and MP concentrations could not be determined for the same individual oyster. Instead, we compared the average CI values (n = 6-13) and the average MP concentrations (based on wet weight, n = 5) for each site.

The lack of correlation between CI and MP concentration may be related to the duration of exposure, as MPs have long-term effects. One study found that CI values of oysters continuously exposed to high concentrations of polystyrene MPs increased within the first 10-20 days, but decreased as time went on (Thomas et al. 2019). However, unlike in that study, oysters in this study were not kept in tanks. As such, they were exposed to a variety of environmental conditions, which also may have affected their CI. For example, oysters in areas of low salinity tend to have lower CI values (Leonhardt et al. 2017). This may account for some variability in CI between the sites, as previous work showed much lower salinity levels within Bay St. Louis than at sites on more exposed coastline (Scircle et al. 2020b). This could explain the lower CI values for Sites 3 and 4, which are located inside the bay, compared to Sites 1 and 2 at the bay's entrance. A similar trend, albeit less pronounced, is seen in the Biloxi Bay sites, with

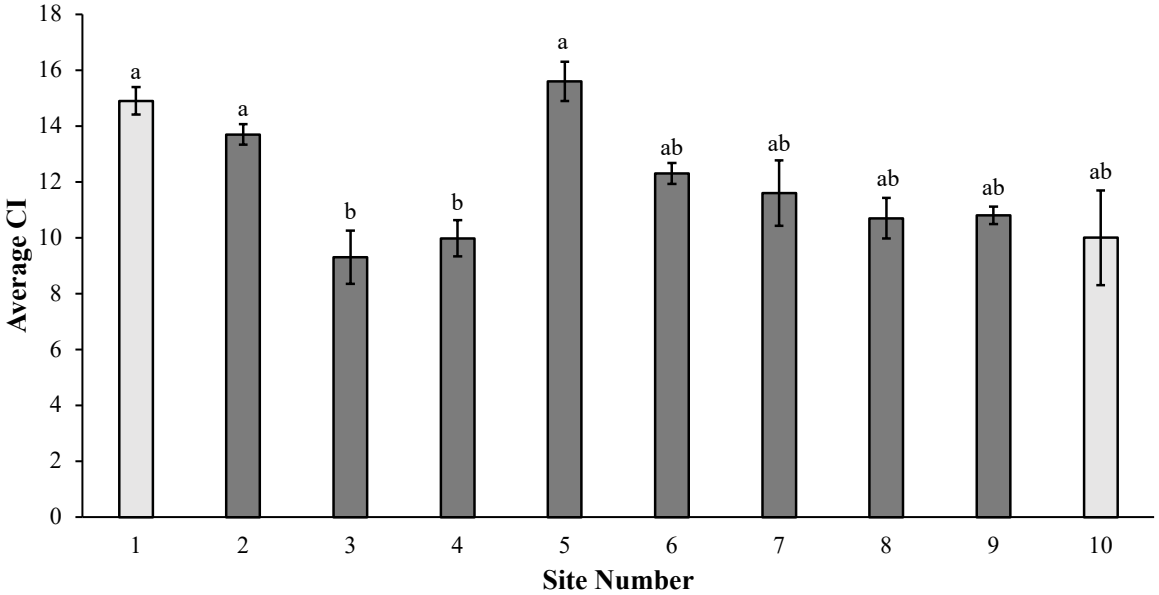

Figure 3. Mean (± SE) condition index of oysters (n = 6-13) from each site (lander sites in light gray and Mississippi Oyster Gardening Program sites in dark gray). Error bars = ± one standard error. Different letters denote means that are significantly different determined via one-way ANOVA followed by Dunn's post hoc test ($p < 0.05$).

Sites 6 and 7 located further in the bay having lower CI values than Site 5.

Abundance of MPs by Location on the Mississippi Gulf Coast

Oysters from the ten sites ranged from a high of 30.7 ± 11.5 to a low of 4.7 ± 0.25 putative MPs/g of oyster tissue (Figure 4). Previous studies have shown that the proximity of oyster reefs to urban areas increases the abundance of MPs retained (Li et al. 2018; Cho et al. 2021). Though the most urban sites near Biloxi did have higher MP concentrations, there was only a moderate correlation (Pearson's coefficient = 0.61, p = 0.059) between the number of putative MPs/g of tissue and city population observed in this study. However, there are many factors that influence the circulation inside bays that may also influence MP concentrations and residence time in the water column. Further, both Bay St. Louis (Sites 1-4) and Biloxi Bay (Sites 5-6) had collection sites located inside the bay and at the mouth of the bay, where they would be more exposed to open waters of the Gulf of Mexico. For Bay St. Louis, Sites 1 and 2 at the mouth of the bay did not have statistically significant differences in putative MP concentrations compared to Sites 3 and 4, but they did have lower concentrations (Table 2). Although this seemingly contrasts with previous work, which showed that the waters inside Bay St. Louis had lower MP concentrations than sites located directly on the Gulf, the prior work was conducted during an historic flooding event when freshwater from the Mississippi River was diverted through Lake Pontchartrain into the western Mississippi Sound, including Bay St. Louis (Gledhill et al. 2020; Scircle et al. 2020b).

Our data include an anomalously low concentration at Site 7 located within Biloxi Bay. Site 6, located deep within the bay had the highest MP concentration in this study (30.7 ± 11.5 putative MPs/g of tissue), while Site 5, near open water at the mouth of the bay, had an average putative MP concentration of approximately half that (13.7 ± 1.82 putative MPs/g of tissue). However, Site 7 is also located within the bay but had a significantly (p = 0.007) lower MP concentration (5.8 ± 2.2 putative MPs/g of tissue) compared to Site 6. Unlike Site 5, Site 7 is located within the Old Fort Bayou Coastal Preserve that runs into Biloxi Bay and is likely exposed to lower salinity and less polluted water, probably resulting in this site's low average MP concentration.

While not all of the sites had statistically significant differences in their average MP concentration, a one-way ANOVA analysis (df =

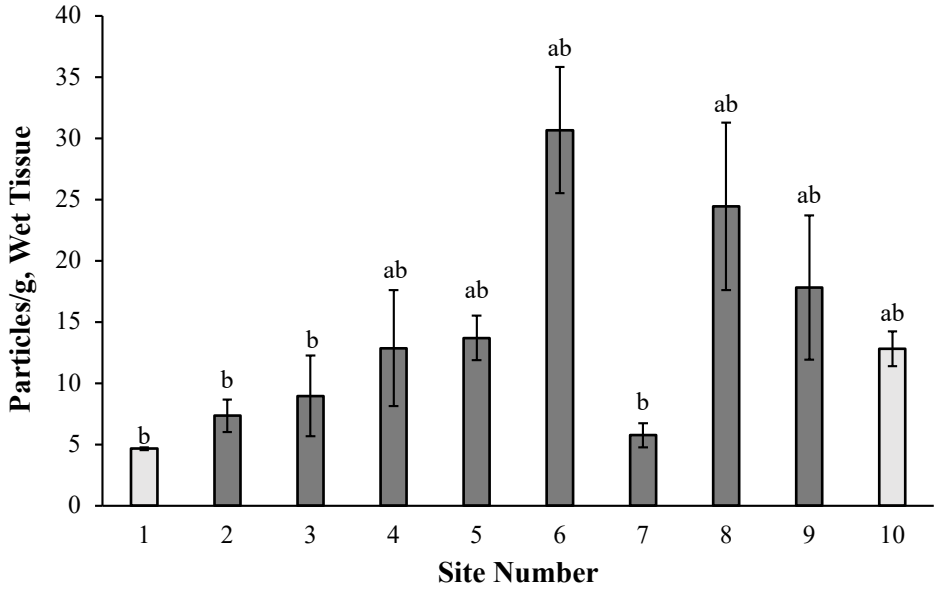

Figure 4. Mean number of putative microplastics per gram of whole oyster tissue by location (n = 5 for each location). Lander sites depicted in light gray, MSOGP in dark gray. Error bars = ± one standard error. Different letters denote means that are significantly different determined via one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

40, p = 0.0113) followed by Tukey's HSD post-hoc tests showed that some did. Specifically, Site 6 was significantly different than Sites 1, 2, 3, and 7 (p = 0.018, 0.005, 0.036, and 0.009, respectively). This suggests that Biloxi Bay and Bay St. Louis do represent distinct environments when it comes to MP concentrations in oysters, potentially because the Bay St. Louis area has a population of roughly one third of the population of Biloxi. Larger populations usually result in a larger amount of plastic waste. When such waste is mismanaged, MPs can find their way into water systems due to stormwater runoff and both household and industrial wastewater. As the Biloxi area has both a larger population and more roadways than the Bay St. Louis area, it is not surprising to see higher MP concentrations in oysters from those sites.

As shown in Figure 5, a two-way ANOVA and Tukey's HSD on the dissected oyster tissue samples showed statistically significant differences when used to assess the effect of both location and tissue type on MP concentration in oysters (tissue type: df = 3, p = 0.0009; site: df = 2, p = 0.005; interaction between tissue type and site: df = 6, p < 0.001). This indicates that there is a large interaction between the tissue in which the MPs localize and the site at which the oyster was located. Post hoc testing showed that Site 2 was significantly different from Sites 5 and 10 (p = 0.0007 and 0.031). However, because Sites 2 and 10 represent MBRACE lander samples and Site 5 is a MSOGP site, these differences could possibly stem from the different durations in the field or oyster age instead of true site differences.

A two-way ANOVA did show statistically significant differences in the interaction between MP sizes and sampling sites (sites: df = 6, p < 0.001; size range: df = 3, p = < 0.001; interaction between size range and site: df = 18, p < 0.001). Consistent with most MP studies, most of the putative MPs retained were in the smallest size fraction of 30-90 µm (80%) (Figure 6) (Li et al. 2018; Cho et al. 2021; Dehm et al. 2022). The larger size fractions of 90-125 µm, 125-250 µm, and >250 µm, contained 8%, 9%, and 3% of the putative MPs, respectively. While MPs of

Table 2. Oyster condition index and putative microplastic concentrations. * = lander sites.

Site	---------- Condition Index ----------			---- Concentration (MPs/g tissue, ww) ----		
	Average	Standard Error	n	Average	Standard Error	n
1*	14.9	0.49	6	4.66	0.11	5
2	13.7	0.37	11	7.35	1.32	5
3	9.30	0.95	10	8.97	3.30	5
4	9.98	0.65	11	12.9	4.73	5
5	15.6	0.70	12	13.7	1.82	5
6	12.3	0.37	13	30.7	5.14	5
7	11.6	1.17	11	5.76	0.98	5
8	10.7	0.73	11	24.5	6.83	5
9	10.8	0.31	6	17.8	5.90	5
10*	10.0	1.69	7	12.8	1.42	5

all of these sizes are believed to be too large to translocate through tissue, it is worrying that the number of smaller MPs is so much higher than the larger size classes. It is likely that there are even more MP particles in the <30 µm range. While the methodology utilized in this study was not able to measure them, <10 µm MPs are of special concern as they can translocate and may cause damage to oyster tissue (Teng et al. 2021).

Abundances of MPs by Tissue

To assess the risk of MPs to oyster health, it is necessary to determine whether MPs localize in specific tissues, and if so, which ones. To that end, oysters from Sites 2 (St. Stanislaus), 5 (Biloxi Bay), and 10 (Grand Bay) were dissected and their gills, mantles, digestive systems, and adductor muscles/hearts were analyzed separately (Figure 5). The mantle showed the highest average number of MPs (15.9 ± 13.4 putative MPs/g of tissue). The gills and adductor muscle/heart tissues exhibited very similar levels of MPs, with 11.5 ± 8.6 and 12.8 ± 6.7 putative MPs/g of tissue, respectively. The digestive system had much lower levels of MPs, with an average of 6.8 ± 6.1 putative MPs/g of tissue. As these samples had come from multiple

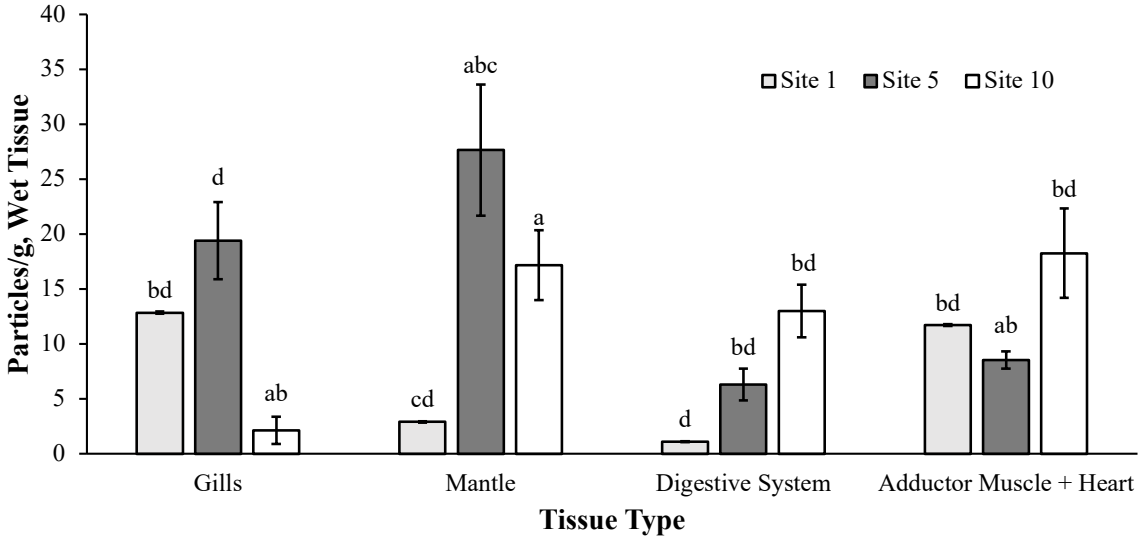

Figure 5. Average number of microplastics per gram of wet tissue by type of tissue (n = 5 oysters from each site, n = 15 for combined data). Each oyster was dissected and analyzed as four separate tissues. Landers data depicted in light gray and white; MSOGP site data in dark gray. Error bars = ± one standard error.

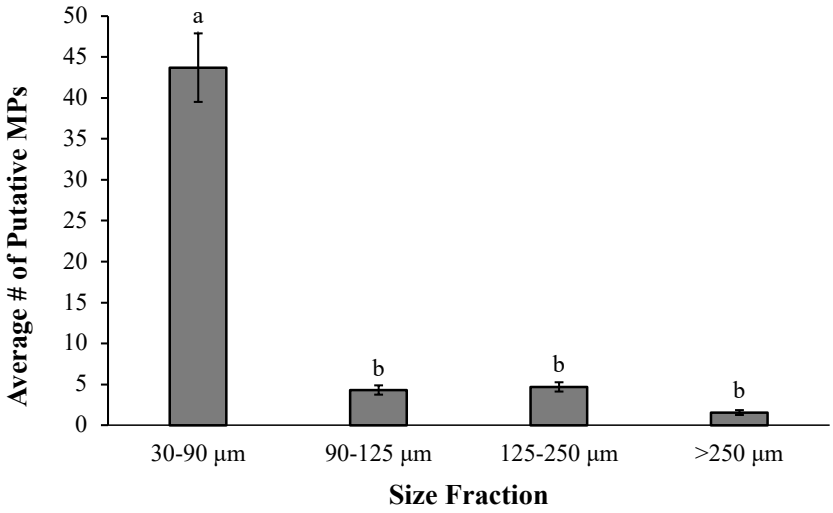

Figure 6. Size distribution of microplastics in oysters (n = 2278 putative MPs). Error bars = ± one standard error.

sites, a two-way ANOVA analysis followed by Tukey's HSD was used to identify the effects of site and tissue type on MP concentrations. Results showed that only the differences between the mantle and digestive system means were statistically significant ($p = 0.0025$). Interestingly, these results also indicated a significant interaction in MP concentrations between the oyster's site of collection and tissue type. At first glance this may seem odd, as we hypothesized that contaminants localize in the same tissue regardless of where an organism is located. However, to understand these results, one must contend with the fundamental nature of MPs as contaminants.

Unlike more traditional contaminants, MPs are not a single element or compound but rather a diverse suite of contaminants. MPs may be made up of many different sizes, shapes, and polymer types, as well as having a variety of chemical additives. Each of these factors could contribute to which tissue the particle ultimately associates with. Moreover, as each site presumably has its own composition of MP particles present in the surrounding waters (Scircle et al. 2020b), it is not surprising that oysters from different locations have putative MPs localizing in different tissues dependent on local MP composition.

As the gills, mantle, and adductor muscle are all exposed to the surrounding water to varying degrees, it is perhaps to be expected that they exhibit higher levels of MPs than the internal digestive system. While it has been shown that smaller (<10 μm) particles can be translocated across tissues in mussels (Browne et al. 2008), this study targeted larger (>30 μm) MPs that are unlikely to translocate. Thus, MPs associated with the gills, mantle, and adductor muscle are likely adhering to the outside of the tissue instead of being embedded within them. Moreover, because the oysters were rinsed with site water in the field, these MPs appear to adhere relatively strongly. Thus, the digestive system tissue represents the best choice for studies targeting MPs consumed by the oysters. Such samples offer a "snapshot" of the particles the oyster had consumed at harvest. Targeting MPs in the digestive system is also important because the MPs enter an environment with substantially different conditions (pH, enzymes, etc.) that may promote desorption and leaching of chemical contaminants from the MPs and that may cause fragmentation, further reducing the size of the MPs. Average MP concentrations in the digestive system were only slightly lower than those reported for whole oysters in China (Li et al. 2018) and were much higher than concentrations reported in oysters and mussels off the coast of Korea (Cho et al. 2021), suggesting that Mississippi oysters have higher overall concentrations than those previously studied.

MP Compositions and Study Limitations

A limitation with observing MPs by fluorescence microscopy is that it does not yield any chemical information that can be used to definitively identify the MP particle. One study comparing results from fluorescence microscopy and μ-FTIR found that fluorescence microscopy overestimates MP abundance by 18-75% (de Guzman et al. 2022). While we sought to address this issue through an automated counting method and a conservative selection approach, it is possible that our counts may still represent overestimates of MP abundances.

Thus, we are currently analyzing the samples used for this work by FTIR microscopy to confirm the particle counts and identify the polymers comprising the MPs. Whereas this will be the subject of a future report, five oysters from two Sites (6 and 7) were analyzed at the time of writing. Our results show that polyurethane, polyethylene, and polyamide are the most common types of MPs in the oysters. Figure 7 depicts a representative sample from this set. A two-way ANOVA did not reveal any statistical differences in polymer types between the two sites (site: $df = 1$, $p = 0.323$; polymer type: $df = 20$, $p = 0.065$; interaction between polymer type and site: $df = 20$, $p = 0.331$). However, as only a portion of each filter was scanned, these results should be considered preliminary.

Because MPs may be unevenly distributed on the filter and because we were unable to scan the entire filter, we cannot yet compare the number of MPs detected by the two methods (fluorescence and μ-FTIR) or make true comparisons between the two sites. Future work will widen the scan area and determine the full MPs profile (abundance, type, size, and shape) across sample sites and for individual tissues.

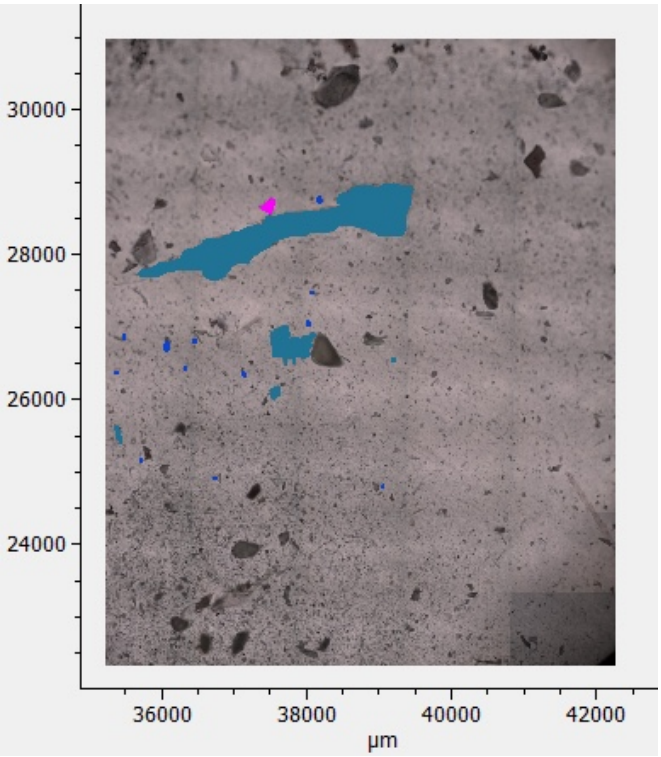

Figure 7. Portion of an Anodisc filter containing microplastics extracted from a Site 6 oyster. Color overlay denotes polymer identity, with light blue signifying polyurethane, dark blue denoting polyamide, and pink representing polyester. Other particles were not identified as plastics. Image created using Purency v4.07.

Conclusions

This study demonstrated that MPs (>30 µm) are retained in relatively high concentrations by Gulf of Mexico oysters along the Mississippi Coast. This is concerning due to the negative impact MPs are known to have on oysters, which are a foundational species for oyster reef ecosystems. Oysters from locations inside bays nearer population centers showed higher average numbers of MPs than those outside of bays, with average concentrations ranging from 30.7 ± 11.5 to 4.7 ± 0.25 putative MPs/g ww of whole tissue. Due to the relatively low concentrations of MPs in the digestive system tissues (6.8 ± 6.1 MPs/g ww of tissue), it appears that most MPs in the oysters are likely adhering to tissues exposed directly to the surrounding water, with lower numbers being ingested. However, given that predators and humans often consume the entire oyster, where MPs are located may not make a difference from a risk standpoint. It remains to be seen if more rigorous washing of oysters can dislodge adhering MPs and decrease MP loads in oysters destined for human consumption. Most of the putative MPs belong to the smallest size fraction studied (30-90 µm). This result is similar to most other MP studies but is still concerning due to potentially higher toxicities of smaller particles. Results from micro-spectroscopy of the extracted MPs indicate that polyester, polyethylene, and polystyrene are the most common types of MPs in the oysters, which is not surprising given their widespread occurrence in the environment. However, more study is needed to fully characterize the MP composition across all sites. Overall, this study demonstrates that MPs are accumulating in the tissues of Gulf Coast oysters, which are consumed by both humans and wildlife.

Recommendations and Policy Implications

While the state of Mississippi does have regulations covering plastic waste disposal in marine waters (Mississippi Code R 2006), this research shows that the current legislation is not sufficient to protect oysters in those waters from MP pollution. One reason for this is that the code only targets plastic disposal from water-going vessels and nearby access areas. Our previous research has shown that the Mississippi River system acts as a funnel for MPs, concentrating and transporting them into nearshore waters of the Gulf of Mexico (Scircle et al. 2020a). As such, current regulations neglect other key sources of MPs and are insufficient to reduce MP pollution in Gulf of Mexico waters.

In order to reduce exposure of Mississippi's oysters to MPs, additional legislation would need to both account for additional sources of MP pollution, such as residential and commercial wastewater and storm-water runoff, as well as be broad enough to encompass all waters flowing into the northern Gulf of Mexico. This brings up two major issues from a legislative perspective. The first is that such legislation may be difficult to enact at a local level. For example, while only covering one potential source of plastic pollution, current Mississippi state law prevents local

municipalities from instituting bans or fees on the use of plastic bags (Mississippi State Legislature 2018). Additionally, Mississippi legislation can only regulate the waters within the state itself. While further legislation may be needed to address MP pollution in Mississippi, such legislation will be ineffective if similar regulations do not govern MP pollution in states upstream of the Mississippi watershed, particularly those on the Mississippi River and its tributaries. While Mississippi may pose an interesting example of this problem due to the impact of MPs on the state's oyster populations, it is far from the only state facing this issue. As such, lawmakers need to consider federal legislation to address both macro- and MP pollution in river systems at a national and global level.

Whereas the problem of MP pollution is ever growing, so too is the awareness of this issue and willingness to address plastic pollution. Recently, Mississippi passed legislation encouraging growth in its recycling sector by recognizing it as a business, not as solid waste disposal (Mississippi State Legislature 2022). While it is far too early to assess what impact this will have on MP concentrations in the Gulf of Mexico, one would hope that an increased focus on recycling could help decrease the number of plastics and ultimately MPs reaching Gulf Coast waters. Further study is needed to evaluate how shifting attitudes and new laws regarding plastic disposal affect MP concentrations in Mississippi oysters.

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Disclaimer

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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