

## Article

# Microplastics in Atlantic Ribbed Mussels (*Geukensia demissa*) from the Delaware Inland Bays, USA

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**Abstract:** Due to the prevalence of plastic pollution in coastal ecosystems, aquatic organisms are at high risk for accumulating microplastics (MPs). Filter-feeding bivalves, such as mussels and oysters, may be exposed to, and subsequently accumulate, MPs due to the high volume of water they pass through their bodies. This study assessed the levels of MPs within Atlantic ribbed mussels (*Geukensia demissa*), a common filter feeder found along the United States Atlantic Coast, from 12 sites within Rehoboth Bay, Indian River Bay, and Little Assawoman Bay, collectively known as the Delaware Inland Bays. Compositated mussels from each site were digested using potassium hydroxide and filtered. Microplastics were physically identified, sorted based on color, and counted using a digital microscope. Microplastics, almost entirely dominated by synthetic microfibers, were found in all mussels well above laboratory blanks. Across all sites, 40% of microfibers were black, and 27% of fibers were clear. The composite concentrations of MPs ranged from 0.25 to 2.06 particles/g wet tissue, with a mean of  $0.08 \pm 0.06$ . In general, higher concentrations were found in mussels collected at sites that were adjacent to more urbanized land use versus those from rural sites. At two sites, individual mussels, in addition to composites, were analyzed and had MP concentrations ranging from 11 to 69 particles/mussel. This study represents the first evaluation of MPs in this ecologically important coastal species and suggests its viability as a biomonitoring species for microplastic pollution.

**Keywords:** microplastics; bivalves; *Geukensia demissa*; biomonitoring; Atlantic ribbed mussel; microfibers



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## 1. Introduction

### 1.1. Microplastic Pollution

Plastic products are ubiquitous, due in large part to their low cost and high durability. However, because these materials do not readily break down, they persist in the environment long after their intended uses. Microplastics (MPs) are whole or fragmented pieces of plastic that measure less than 5 mm in diameter [1]. Primary MPs originate from pre-production plastic raw material, typically taking the form of microbeads or powders that are utilized to produce plastic products. Secondary MPs, in contrast, are essentially all other microplastics originating from the degradation or fragmentation of larger original plastic pieces.

Microplastics in aquatic environments have been extensively documented worldwide, even in remote regions far from point sources [2,3] For example, it is estimated that MPs comprise 94% of The Great Pacific Garbage Patch, which is 1.6 million square kilometers in size [4]. In addition, a statistical analysis of beach data (average mass per mile of shoreline) from the NOAA Marine Debris Monitoring and Assessment Project

identified several states, including Delaware, as national “hotspots” for marine debris, possibly related to coastal population density, urbanization, and contributions from inland waterways. Approximately 10% of all macro- or microplastic litter enters the oceans [5–8]. While microplastics may enter coastal and marine environments from land, water, and air routes [9], it is estimated that 80% of MP pollution found in the ocean originates from land-based sources, with the mode of transportation oftentimes being river systems [10]. This creates an annual marine capital ecosystem economic burden of USD 3300 to USD 33,000 per ton of plastic [11]. As such, coastal ecosystems are particularly susceptible to MP accumulation. A review article by Li et al. [12] suggested sewage discharge, aquaculture, atmospheric deposition, and surface runoff as some of the main sources of MP pollution in coastal waters.

Due to their prevalence and small size, marine organisms are at risk for ingesting MPs through direct ingestion of these particles, indirect ingestion of contaminated prey items, or by means of respiration [13]. Microplastic accumulation in marine organisms and potential negative effects depend on many factors, such as the size and shape of MPs [14] and target species. Once ingested, MPs may illicit varying effects on aquatic organisms, such as hormonal disruption, neurotoxic effects, impaired feeding ability, and impaired motility, though the mechanisms are poorly understood [15]. Toxicological effects associated with MPs may be due, in part, to the adsorption of heavy metals, hydrophobic organic contaminants, such as polychlorinated biphenyls, pesticides, and polycyclic aromatic hydrocarbons, and pharmaceuticals on their surfaces [16–21].

Microplastic accumulation and its subsequent negative effects can greatly deteriorate the coastal environment and reduce the economic benefits of aquaculture [22]. Characterizing the inventories of these pollutants in aquatic ecosystems is an important step in risk assessment. Although remote sensing technology may be effectively used to track plastic pollution in waters, monitoring MPs provides its own challenges. Optical imagery detects floating macroplastics [23–25]; however, this detection method requires rigorous validation across different geographic regions for reproducibility and replicability. Available European Space Agency’s Copernicus Sentinel-2 satellite data can be used to identify large, floating macroplastics in waterways and might answer questions about sources, trends, and pathways [26]. The high spatial (10 m) and spectral (12 band multispectral) capabilities of Sentinel-2 can be leveraged to distinguish macroplastics from natural floating debris, but monitoring and tracking microplastics remain challenging because of their small size. To assess the presence and concentrations of MPs in aquatic ecosystems, field collection of samples (e.g., soil, water, biota) must be made. In this study, for the first time, MP concentrations within the Atlantic ribbed mussel were evaluated within the Delaware Inland Bays through field collection.

### 1.2. Atlantic Ribbed Mussels

Mussels and other filter-feeding bivalves have been suggested to be effective biomonitoring species for MP accumulation in aquatic ecosystems (e.g., [27–29]). For this study, native Atlantic ribbed mussels (*Geukensia demissa*) were chosen as the target species. They are a stable and ubiquitous species that grow in beds along intertidal marsh habitats along the United States Atlantic Coast. While fully submerged during high tide, they are commonly exposed to the atmosphere during low tide. They have a high tolerance to temperature, moisture, and salinity variation, allowing them to have low mortality rates if faced with environmental stressors [30]. Typically, they can live up to 15 years. By slightly opening their shells, these filter feeders allow water to flow through their gills and either accept or reject particles, such as algae, bacteria, and detritus [31]. Mussels can filter approximately 120 mL of water every minute. Any particles greater than 4 µm can be retained by mussels [32], making them susceptible to exposure and potential accumulation of MPs. The purpose of this study was to assess the presence and extent of MPs within the Atlantic ribbed mussel throughout the Delaware Inland Bays. This

study represents the first published data set on MP concentrations for this species in this ecologically important ecosystem.

### 1.3. Delaware Inland Bays

The population of the Delaware Inland Bays is constantly growing, attracting more visitors each year to the Bays and nearby beaches. The three bays that make up the Delaware Inland Bays are Rehoboth Bay, Indian River Bay, and Little Assawoman Bay. These three bays have varying land use patterns based on the population size, human activities, and environmental conditions of the area. Rehoboth Bay is a popular recreational area and tourist destination. Both seasonal visitors and permanent residents take advantage of boating and recreational fishing and crabbing during the warmer seasons. Due to its popularity, there is significant coastal development and tourist-related infrastructure that make up the land use in Rehoboth Bay. A relatively large density of residential homes exists along Rehoboth Bay. Because of that, there are many environmental conservation efforts in Rehoboth Bay due to its importance as a habitat for oysters and other estuarine species, including the Atlantic ribbed mussel. Indian River Bay is one of the largest bays in the region, and its land area is similarly used for residential developments and agricultural activities. Little Assawoman Bay is the lesser-developed of the three bays. There are recreational activities that take place, but minimal residential development in the area. There is a greater emphasis on protecting the natural areas and wetlands of this bay from development due to its sensitive ecosystem. The health of the Delaware Inland Bays is essential to the sustainability of their economic contributions and to protect its land use and inhabitants [33]. To assist in that, this study represents the first evaluation of the magnitude and spatial extent of MP pollution in Atlantic ribbed mussels in this important ecosystem.

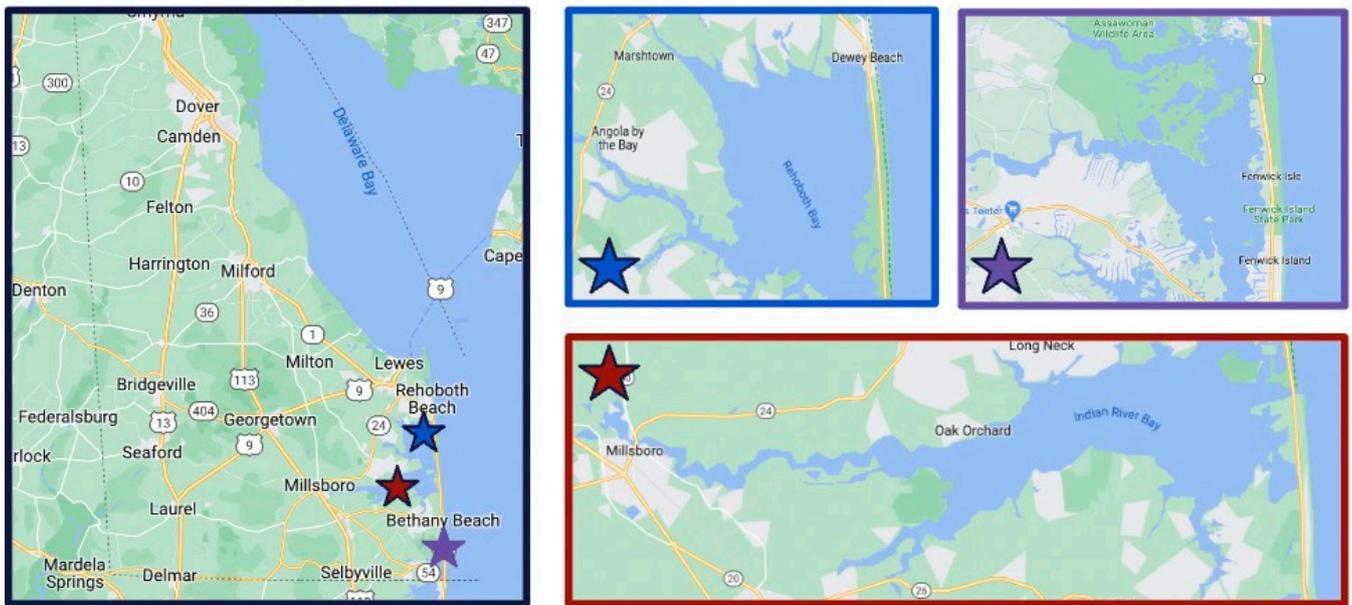
## 2. Methods and Materials

### 2.1. Study Area and Sampling Sites

The Delaware Inland Bays, located in Sussex County in the state of Delaware (USA), comprise approximately 90 Km<sup>2</sup> of three shallow, interconnected coastal bays (Rehoboth, Indian River, and Little Assawoman Bays) separated from the Atlantic Ocean by a narrow barrier island (Figure 1). The waters of Rehoboth Bay and Indian River Bay are tidally connected to the Atlantic Ocean by the Indian River Inlet, while Little Assawoman Bay is connected by the Ocean City Inlet. The depth of the Delaware Inland Bays region is generally less than 2 m, with average depth ranges from 0.9 to 2.4 m. Within all three bays, a semi-diurnal tidal pattern produces an average tidal range of approximately 0.9 m. The land use patterns around the three bays is mostly residential and recreational development. These areas attract both permanent residents and seasonal visitors each year. The land is also used for agricultural-related activities and for commercial and recreational fishing, crabbing, and shellfish harvest. The Delaware Inland Bays support over 35,000 jobs and generate USD 4.5 billion in annual economic activity [33].

### 2.2. Sample Collection and Preparation

Atlantic ribbed mussels (*Geukensia demissa*) were collected from the shoreline of 12 sites (4 sites within each bay) within the Delaware Inland Bays on 21 January 2023 (Table 1). Within each bay, sites were selected based on the abundance of intertidal mussel beds and ease of access for sampling. Each site was generally characterized as either rural, intermediate, or urban based on visual evaluation of the adjacent land use (Table 1). A rural designation was given for collection sites that were void of any human influence (e.g., buildings, roads, parking lots, human-made shoreline construction) at a radius of 200 m around the collection site. Intermediate sites had a few, but not dominant, areas of human influence at a perimeter of 100 m. Urban sites had many dominant areas of human influence within a 50 m radius of the collection site.



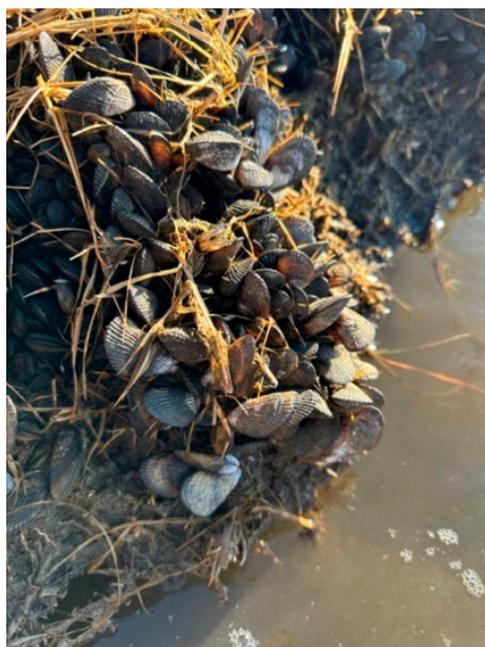
**Figure 1.** Maps showing Rehoboth Bay (blue star), Little Assawoman Bay (purple star), and Indian River Bay (red star), collectively known as the Delaware Inland Bays.

**Table 1.** Collection sites of Atlantic ribbed mussels (*Geukensia demissa*) within the Delaware Inland Bays and adjacent land use based on visual evaluation.

Site Number	Site Location	Coordinates	Adjacent Land Use
1	NW Indian River Bay	38.591868 and 75.215821	Intermediate
2	SW Indian River Bay	38.591367 and 75.126220	Rural
3	NW Assawoman Bay	38.485294 and 75.077185	Rural
4	SW Assawoman Bay	38.483374 and 75.089890	Intermediate
5	SE Assawoman Bay	38.483374 and 75.089890	Urban
6	NE Assawoman Bay	38.497810 and 75.056208	Intermediate
7	SE Indian River Bay	38.588284 and 75.076154	Urban
8	SE Rehoboth Bay	38.628009 and 75.070168	Rural
9	NE Rehoboth Bay	38.669167 and 75.071944	Rural
10	NW Rehoboth Bay	38.690833 and 75.136944	Rural
11	SW Rehoboth Bay	38.635278 and 75.125556	Rural
12	NE Indian River Bay	38.618056 and 75.124444	Intermediate

Sampling occurred over a period of 6 h during an ebbing tidal cycle. Across all sites, the mean water temperature was  $5.7 \pm 0.7$  °C and the mean salinity was  $24 \pm 3$  ppt. At least ten individual specimens of similar visual size were collected from each site (Figure 2). Upon collection, specimens were stored in jars, kept cool, and transferred back to the lab within 2 to 10 h of collection.

Samples were stored at  $-18$  °C until processing. Prior to processing, frozen mussels were thawed for approximately 30 min, after which individual specimens were counted, weighed, and measured. The pre-shucked weight was determined using a standard benchtop scale. Specimen length, from anterior end to posterior end, was measured using a digital caliper. Individual mussels were selected based on similar weight and length and then composited until an approximate weight of 150 g (shell and tissue) was amassed. Whole specimens were then shucked, and tissue was removed from shells using a scalpel, giving an approximate composite tissue mass of 80 g. For each site, composited tissue samples were transferred to a 500 mL beaker for KOH digestion. The choice to use large masses for composited samples was made to ensure MP counts were much higher than laboratory control blanks.



**Figure 2.** A typical bed of Atlantic ribbed mussels (*Geukensia demissa*) exposed to the atmosphere at low tide in the Rehoboth Bay, DE.

For tissue digestion, each composite tissue sample (~80 g) was combined with 100 mL of 10% KOH [34] and heated to 40 °C while stirring for 48–72 h [35]. After digestion, samples were filtered using a vacuum pump filtration system using 47 mm diameter Whatman filter papers (0.45 µm pore size). More viscous samples required multiple filter papers. After filtration of the entire sample, filter papers were washed with deionized water and transferred to a clean, labeled 35 × 10 mm Petri dish, covered, labelled, and stored at room temperature until microscope analysis.

By compositing multiple mussels from each site to create one sample, the ability to discriminate the potential variability in microplastic concentrations between mussels at a given site was lost. To assess this, 5 individual mussels from site 8 and 5 from site 10 were analyzed. Individuals' weights and lengths were measured, and they were subsequently digested and processed in the same process as the composited mussel samples.

### 2.3. Microplastic Identification

A binocular stereo microscope (Leica Zoom 2000, Leica, Deerfield, IL, USA) was used to identify the microplastics present on each piece of filter paper. Surgical tweezers were used to test isolated particles by squeezing them to see if they would break and inspecting for uniformity and opaqueness. If a potential microplastic broke, the particle was not considered an MP, and it was not counted. Using tweezers, individual MPs were placed in a common spot near the edge of the filter paper to allow for easier recognition and analysis. A black Sharpie was used to indicate the spot on the filter paper where the MPs were collated. This was carried out so that when conducting further microscopic analysis it was easier to find where the MPs were collected. Each petri dish was analyzed by two lab members before a final microplastic count was determined. While this may enhance confidence in the enumeration of MPs, very small particles may have been missed, especially if colorless, and particle counts may be slight underestimates of actual numbers. A digital microscope (Keyence VHX-7000, Keyence Corporation of America, Itasca, IL, USA) was used to further confirm the identification of the MPs under enhanced magnification based on recommendations for visual inspection [36]. Additionally, the enhanced magnification also allowed for the identification of MPs that were not previously identified using the stereo microscope. Digital images were collected and stored. The number of MPs, type

(e.g., fiber, fragment, or microbead), and the color of each MP were recorded under the digital microscope.

#### 2.4. Quality Control and Quality Assurance

In the field, individuals minimized the presence of synthetic apparel while sampling, and all sampling tools contained no plastic material. Tools were properly cleaned between dissecting mussels from each site. In the laboratory, the presence of synthetic clothing was minimized by using cotton laboratory attire. Glassware was rinsed with distilled water before use. To assess the presence and extent of possible MP contamination of samples during processing, laboratory blanks were conducted. The blanks ( $n = 9$ ) consisted of filter papers processed using the above methodology solely using 1 L of DI water in the absence of mussel tissue. In this study, concentrations are reported as the number of MPs per filter paper.

#### 2.5. Data Analysis

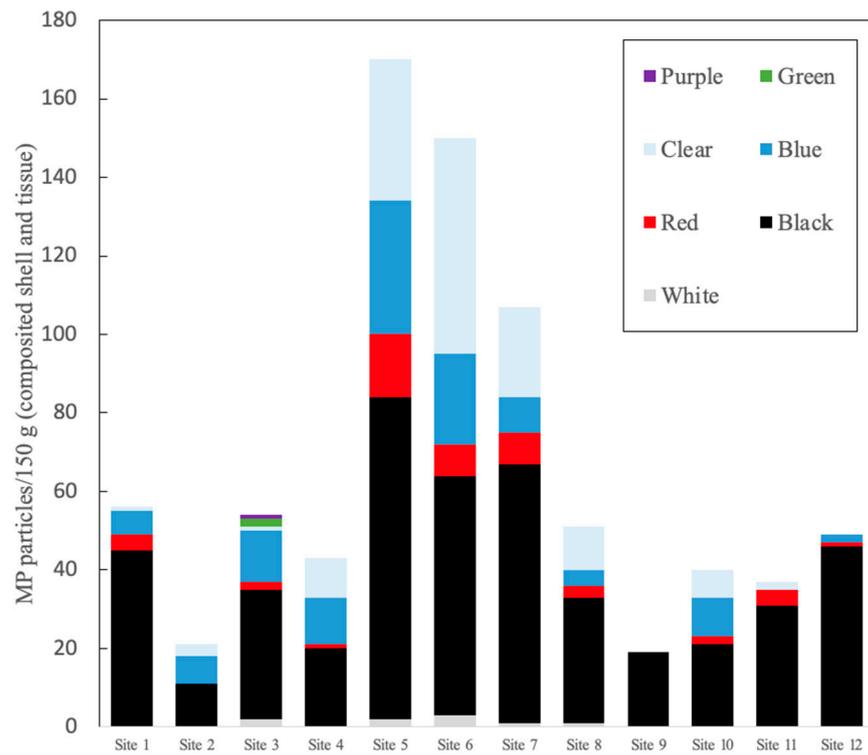
Concentrations of MPs in mussels were calculated and reported in several ways. For composite samples from each site, concentrations were reported as the number of MP particles found for 150 g of each composited sample (shell and tissue). Concentrations were also calculated as MPs per g of tissue (wet). Finally, for sites 8 and 10 where individual mussels, not composites, were analyzed, the concentration was calculated as MP particles per mussel.

A *t*-test was utilized to test if mean mussel concentrations were significantly different from the mean of the blank concentrations. Likewise, at sites 8 and 10, the mean of individual MP concentrations was tested for significance with the mean of the blank concentrations. Finally, a *t*-test was used to assess if the mean concentration from each of the three bays were significantly different from each other. All statistical analyses were performed using the SciPy library package in Python.

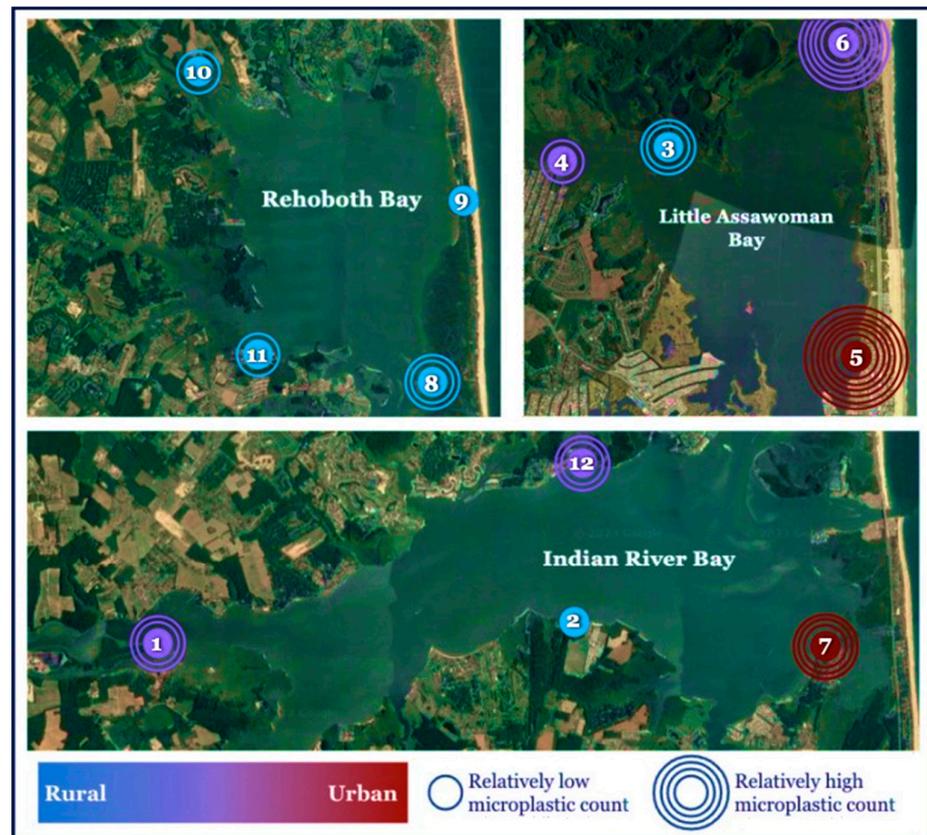
### 3. Results

Microplastics were found in every laboratory blank (ranging from 1 to 11 MPs per filter paper) with a mean and standard deviation of  $4.8 \pm 2.9$  ( $n = 9$ ). All were microfibers, and blue, followed by black, were the most common colors.

Microplastics were found in composited mussel samples from every site above the laboratory blanks. The concentrations ranged from 19 to 173 MPs/150 g (composited shell and tissue), with a mean of  $67 \pm 49$  (Figure 3). All reported MP concentrations were not blank-corrected. To assess statistical significance, the mean of these MP concentrations from the four sites from each of the three bays was calculated. Using Student's *t*-test, the mean of the concentration of MPs in composited samples from each bay was significantly above the mean concentration of the blanks ( $p < 0.1$ ). Three *t*-tests comparing the mean mussel MP concentrations of the three bays (Indian River vs. Little Assawoman, Indian River vs. Rehoboth, Little Assawoman vs. Rehoboth) were performed. Concentrations were significantly different between the Little Assawoman Bay and Rehoboth Bay ( $p = 0.09$ ). The Indian River Bay and Little Assawoman Bay comparison yielded a *p*-value of 0.26, and the Indian River Bay vs. Rehoboth Bay comparison yielded a *p*-value of 0.30, denoting no significant difference in concentration between those bays. Figure 4 identifies land use and MP concentrations at each site, with larger concentric circles representing higher relative abundances of MPs.



**Figure 3.** Stacked bar graph showing the concentration of MPs/150 g (composited shell and tissue) and the abundance of each particle color at each site.



**Figure 4.** Relative MP concentrations (based on 150 g of composited mussel shell and tissue) at each site and adjacent land use classification. Numbers correspond to collection site numbers.

Microfibers were the dominant type of microplastic type observed (96%). Figure 3 presents the distribution of colors of MPs for each site. Of all the MPs identified across all mussel samples, black was the most common color (40%), followed by clear (27%), blue (22%), red (9%), white (1%), and other colors (1%). Digital photos of typical microfibers are presented in Figure 5.



**Figure 5.** Digital microscope images of various microfibers (the dominant MP type) identified in composited mussel samples.

While compositing samples produced MP particle counts in the teens to hundreds, well above laboratory blanks, reporting concentration in terms of MPs/150 g (composited shell and tissue) does not allow for easy comparison to other studies. To facilitate this, concentrations were calculated based on MPs/g wet tissue (Table 2). Concentrations of microplastics ranged from 0.25 to 2.06 MPs/g wet tissue, with a mean and standard deviation of  $0.80 \pm 0.60$  MPs/g wet tissue.

**Table 2.** MP concentrations in Atlantic ribbed mussels at each site in units of MPs/150 g of composited wet tissue and shells and in units of MPs/g wet composited tissue, with corresponding ranges, means, and standard deviation of means.

Site	MPs/150 g Wet Tissue and Shells	MPs/g Wet Tissue
1	56	0.67
2	21	0.25
3	55	0.66

Table 2. Cont.

Site	MPs/150 g Wet Tissue and Shells	MPs/g Wet Tissue
4	43	0.52
5	170	2.06
6	150	1.81
7	107	1.29
8	51	0.62
9	19	0.23
10	40	0.48
11	37	0.44
12	49	0.51
Range	21–170	0.25–2.06
Mean $\pm$ STD	67 $\pm$ 49	0.80 $\pm$ 0.60

To assess variability between individual mussels, at two sites (8 and 10), five mussels were analyzed as individuals. Concentrations ranged from 11 to 69 MPs/individual (shell and wet tissue) at site 8 and 10 to 29 MPs/individual (shell and wet tissue) at site 10 (Table 3). The mean concentrations from each of these two sites, when compared to the mean concentration of the blanks, were significantly different ( $p = 0.003$  for site 8;  $p = 0.0003$  for site 10). Reported concentrations for individual mussels were not blank-corrected.

Table 3. Concentration of MPs/mussel at sites 8 and 10 (both within Rehoboth Bay).

Site	Replicate Number	Mass Whole Mussel (g)	Length of Mussel (cm)	MPs/Individual
8	1	47.45	9.4	23
8	2	44.92	9.2	22
8	3	63.03	10	11
8	4	38.95	8.6	34
8	5	41.86	8.8	69
10	1	31.19	8.1	28
10	2	33.23	7.7	29
10	3	32.03	7.6	13
10	4	32.8	7.8	10
10	5	26.57	7	21
			Mean $\pm$ STD	26 $\pm$ 17

## 4. Discussion

### 4.1. Issues When Identifying and Quantifying MPs

Even with scrupulous efforts to ensure MP-free environments, contamination of samples may occur both in field collection and when processing samples in the laboratory. The use of blank controls is critical in evaluating the extent and magnitude of this contamination. However, a study on 59 MP published articles from 2021 to 2022 found that one in five research studies failed to use blank controls [37]. When blank controls were used and reported, a wide range of methods were used from simply reporting blank levels to use of statistical methods and blank correction of samples based on size, color, and MP type [38]. For example, Noonan et al. [38] reported that almost 60% of studies that used blank controls did not correct sample concentrations. Clearly, the use of applicable blank controls, and the resulting application of them, is still a perplexing and emerging issue amongst researchers characterizing and quantifying MPs in biotic and abiotic samples. As Dawson et al. [39] concluded in their evaluation of the use of blanks in recent MP studies, data analysis within current environmental studies is often nontransparent, particularly the analysis of controls and blanks, suggesting the MP research community establish method harmonization.

Of the 22 composite and individual samples analyzed in this study, 9 procedural blanks were evaluated. Although attempts were made to diminish contamination of samples (e.g., minimizing synthetic fibered clothing, scrupulously rinsing glassware, etc.), all procedural blanks in this study contained MPs. In this study, samples were prepared in a fume hood because of the digestion process and hypothesizing that this would have diminished procedural blank MP levels. However, Noonan et al. [37] recently reported that blank controls from fume-hood-prepared samples were higher compared to those prepared on the laboratory bench or using a laminar flow hood. Munno et al. [38] recommended blank subtracting through a combination of particle characteristics, such as color, morphology, and size fraction, stating that this approach likely results in final MP characteristics that are most representative of the sample. Moreover, although they offer several suggestions, there is still no consensus on the universal method for blank correction. They suggest further work should be conducted to assess other quality assurance and quality control parameters, such as the use of other types of blanks, such as field and matrix blanks, and appropriate methods to determine the limits of detection and quantification.

The current state of knowledge on the most effective methods to reduce contamination and enhance accuracy in MP quantitation is still evolving. Until established protocols are set and promulgated, researchers, including those in this study, continue to define their own guidelines and state caveats. In this study, *t*-tests were used to test for the significance of differences between sample concentrations and those observed in blanks. While all composited and individual samples were significantly above blank concentrations, blank correction, the mathematical process through which MP concentrations found in control blanks are subtracted from the concentrations found in samples, was not performed. Therefore, in this study, reported concentrations are likely overestimated by 3 to 25% of the actual levels based on the concentrations found in laboratory control blanks.

#### 4.2. Dominant MP Types

Microfibers (versus microfragments or microbeads) were the dominant type of MP identified in all mussel samples. Microfibers may be classified as natural (made from plants or collected from animals), semi-synthetic (derived from natural fiber through human-made chemical processes and extruded into fibers), or synthetic (human-made through polymerization of hydrocarbons). Natural polymers, such as cellulose, generally do not withstand KOH digestion as well as synthetic polymers [40], although digestion temperature and other factors may impact this. Chemical confirmation of microfibers through spectroscopic analyses, such as Raman and FTIR [41], were not performed in this study. However, based on the KOH digestion method and the observed thread or filament characteristic surfaces of the MPs under a digital microscope, we are confident that most, if not all, of the isolated MPs were indeed microfibers. However, the reported concentrations of MPs in this study should be considered suspected microplastics, as more definitive characterization using instrumental analyses were not performed.

Synthetic microfibers are globally ubiquitous [42], even in remote areas, such as the deep sea [43] and the Arctic Ocean [44]. Brown et al. [45] quantified polyester, acrylic, polypropylene, polyethylene, and polyamide microfiber contamination on shorelines across the globe. Higher concentrations were observed in more densely populated areas and habitats that received sewage contaminated with microfibers from washing of garments. Through laundering of synthetic textiles, microfibers are formed through the process of shedding [46]. Wastewater treatment plants do not capture these particles, and, subsequently, they are released into the environment through the release of wastewater effluents [47,48] or through land-based applications of wastewater or sludge [49–51]. More recently, atmospheric deposition of microplastics, predominantly microfibers, from urban areas has been shown to be another vector delivering MPs to the environment, though little is known about atmospheric microplastic dispersion and their fate currently [52].

The land usage adjacent to sampled sites within the DE Inland Bays varied from rural to urban, though this system is not located near any heavily urbanized or industrialized

sites. While historically, wastewater treatment discharges may have impacted this system, currently only a very small percentage of discharge waters are released to the waters of the DE Inland Bays. Most of the wastewater is land applied to areas not adjacent to these three bays. Land-based application of wastewaters and atmospheric deposition may represent vectors for the delivery of microfibers to this system, though quantifying the significance of their contribution is daunting if not impossible. Other industrial dischargers, such as food production farms, remain very low and are likely not significant factors in releasing microfibers. Evaluating MPs in salt marsh sediments, Lloret et al. [53] found that microfragments had a local origin, whereas microfibers were likely transported from large-scale areas. The study area is largely void of sources that would deliver microfragments and may explain this high abundance of microfibers. However, for this very limited study, it remains impossible to identify sources of these microfibers.

Across all samples in this study, both composite and individuals, microbeads were extremely rare. Microbeads, being primary MPs, are spherical, small plastic beads most often used in consumer products as a form of grit [54]. Their presence in aquatic ecosystems has elicited concern among the scientific community [55]. The United States legislature banned the use of microbeads in rinse-off personal care products through the Microbead-Free Waters Act of 2015 [56]. This may explain, in part, the relative paucity of these types of MPs in mussels in this study, despite the ability of bivalves to accumulate microbeads, which induce deleterious effects [57,58].

Additionally, very few MPs were characterized as microfragments. Microfragments, secondary MPs, are made of small pieces of weathered or fragmented hard plastics from a variety of sources. In recent bioaccumulation studies, they were found less than microfibers. For example, microfibers were the dominant type of MP in both the water and bivalves analyzed (97% and 93%) [59]. However, within the bivalves, microfragments made up a larger proportion of observed microplastics (7% present in bivalves vs. 3% in water) [59]. This may be due to their tendency to settle at a higher rate within water than microfibers [60]. Their relative scarcity in this study likely suggests minimal local sources of microfragments in the Delaware Inland Bay ecosystem, though adjacent water and sediment MP concentrations would be needed to confirm this [53].

#### 4.3. Comparison to Other Data Sets

Li et al. [27] provided an extensive literature review of field and laboratory studies published from 2014 to 2018 documenting MP concentrations in mussels. Most of these field studies documented MPs in *Mytilus edulis* and *Mytilus galloprovincialis*. While the authors proposed the use of mussels as target species to monitor MP pollution, it was noted that the lack of a standardized approach, as well as temporal and spatial variability, make these studies incomparable. More recently, Bom and Sa [61] provided a systematic review of 93 published articles reporting MP concentrations in 70 species of bivalves worldwide, with mussels (*Mytilus* spp.) and oysters (*Crassostrea* spp.) being the predominant genus studied. Similar to the review by Li et al. [29], the authors noted that due to varying methodologies used in the digestion of tissues from organisms and the range of procedures used to identify the MPs, comparisons between the results of different studies were difficult. In addition, they noted that many studies, including this current research, did not report MP concentrations in the adjacent environment (water column and/or sediment) and were unable to correlate that with accumulated MP concentrations within organisms.

To augment these prior extensive literature reviews and summaries, select studies published from 2021 to the present evaluating MP concentration in various species of estuarine or marine mussels are summarized and compared to this study (Table 4). For example, Sparks et al. [62] evaluated MPs in mussels at three sites in Cape Town Harbour and the Two Oceans Aquarium in Cape Town, South Africa, finding an average concentration of MPs in mussels predominantly composed of black and grey filaments, as in this current study, of  $6.27 \pm 0.59$  MPs/individual ( $3.05 \pm 1.09$  MPs/g soft tissue wet weight). Evaluating MPs in commercial mussels (*Mytilus galloprovincialis*) from the Apulia

Region in Italy, Dambrosia et al. [63] found an average value of  $1.59 \pm 0.95$  MPs/g and  $6.51 \pm 4.32$  MPs/individual where blue polyamide fragments, sized 10–500  $\mu\text{m}$ , were the most prevalently. Marques et al. [64] observed mussel (*Mytilus* spp.) MP concentrations ranging from 0.54 to 3.0 MPs/g, with a significant percentage (50%) being microfibers, though not as high as this current study (97%). Mercogliano et al. [65] analyzed farmed mussels from the Tyrrhenian Sea and the Adriatic Sea, observing an average abundance of 3.8 items/individual and 0.5 MPs/g of tissue, with black the most represented color, as observed in this current study. In two species of commercially important mussels with different ecological traits (*Amarilladesma mactroides* and *Brachidontes rodriguezii*) collected from Argentinian beaches, black and blue microfibers were found to be the most abundant type of MPs, with concentrations ranging from 0.15 to 0.5 MPs/g wet weight [66]. Finally, Cho et al. [28] used filter-feeding bivalves from Korean coastal environments as bioindicators to identify the national contamination level and characteristics of microplastics. They found mean concentrations of  $0.33 \pm 0.23$  MPs/g ( $1.21 \pm 0.68$  MPs/individual) in oysters/mussels. The levels of MPs in bivalves were relatively high in urbanized areas with a wide diversity of polymer types compared with those in non-urbanized areas, suggesting that bivalves reflect the MP characteristics of the surrounding waters in which they live.

**Table 4.** Comparison of MP concentrations and types identified in various mussel species from recent studies (2021–2023) to this study.

Organism/Species	Location	Reported Concentrations	Types of MPs Identified	Reference
Mussels	Cape Town Harbour and Two Oceans Aquarium, South Africa	$6.27 \pm 0.59$ MPs/individual $3.05 \pm 1.09$ MPs/g soft tissue wet weight	Black and grey filaments most prevalent	Sparks et al. [62]
Commercial Mussels ( <i>Mytilus galloprovincialis</i> )	Apulia Region, Italy	$6.51 \pm 4.32$ MPs/individual $1.59 \pm 0.95$ MPs/g	Blue polyamide fragments most prevalent	Dambrosia et al. [63]
Mussels ( <i>Mytilus</i> spp)	Portuguese coast	0.54 to 3.0 MPs/g	Microfibers the most abundant shape (50%)	Marques et al. [64]
Farmed Mussels	Tyrrhenian Sea and Adriatic Sea	3.8 MPs/individual 0.5 MPs/g of tissue	Black MPs most prevalent	Mercogliano et al. [65]
fCommercial Mussels ( <i>Amarilladesma mactroides</i> and <i>Brachidontes rodriguezii</i> )	Argentina	0.15 to 0.5 MPs/g wet weight	Black and blue fibers of <0.5 and 0.5–1 mm the most abundant	Truchet et al. [66]
Oysters, Mussels	South Korean Coastline	Oysters/Mussels $1.21 \pm 0.68$ MPs/individual $0.33 \pm 0.23$ MPs/g	Colorless fragments smaller than 300 $\mu\text{m}$ most prevalent	Cho et al. [28]
Mussels ( <i>Geukensia demissa</i> )	Delaware Inland Bays, United States	$0.80 \pm 0.60$ MPs/g wet weight $26 \pm 16$ MPs/individual	96% microfibers; black MPs most prevalent	This Study

Although many factors may influence MP concentrations, such as location, species, extraction methodology, and methods used to characterize particles, the mean MP concentrations ( $0.80 \pm 0.60$  MPs/g wet weight) found in this study were similar to those of recent studies summarized in Table 4. However, this study found a mean MP concentration in individual mussels of  $26 \pm 16$  MPs/individual, which was higher than those reported in other recent studies. Doucet et al. [67] studied microfibers within freshwater mussels from rural tributaries of the St. John River, Canada. Concentrations were higher in smaller mussels compared to larger mussels of the same species, likely attributed to larger quantities of water being filtered on a weight-normalized basis. The relatively small Atlantic ribbed

mussel, compared to larger species that dominate the literature, may explain the higher individual MP concentrations obtained in this study. In addition to this, as mentioned in previous comparisons of MPs in mussels [27,61], due to variations in certain factors, such as species, location sampled, and laboratory methods used to isolate and characterize MPs, it is not unusual to observe these variances in concentrations when comparing to previous studies. To date, only two publications have included MPs in the Atlantic ribbed mussels, both of which did not quantify concentrations in this species but rather focused on MP concentrations in biodeposits or adjacent mussel bed sediment [68,69]. While this study represents the first published concentrations of MP in this mussel species (*Geukensia demissa*) and these data will be helpful for future studies characterizing MP levels in particular species, locations of sample collection will likely impede direct comparisons to future data sets. Despite this, quantifying MP concentrations in this species can be used to monitor the magnitude of MP contamination and infer spatial differences in coastal systems like the DE Inland Bays.

#### 4.4. Regional Differences in MP Concentrations

Between all twelve sites, wet-weight MP concentrations did not vary considerably. In this study, only Little Assawoman Bay had significantly higher concentrations than Rehoboth Bay. In general, collection sites adjacent to more urbanized land use (sites 5, 6, and 7) had higher concentrations, particularly in Little Assawoman Bay, compared to sites adjacent to more rural land use. While proximity to direct sources of MPs, such as runoff from non-permeable surfaces, may deliver more MPs to the water body, other factors, such as wind direction, tidal influences, and currents, may be important. Prevailing easterly winds may build up more MPs along the eastern portions of the DE Inland Bays, possibly explaining some of the higher concentrations at these sites. Further studies are needed to understand the complexities of water flow in possibly determining spatial differences in MP accumulation in sessile organisms like bivalves.

#### 4.5. Potential for Trophic Transfer of MPs

Within aquatic ecosystems along the United States Atlantic Coast, the primary predator of ribbed mussels is the crab. Under laboratory feeding studies, Seed [70,71] documented blue crab (*Callinectes sapidus*) and mud crab (*Panopeus herbstii*) predation on Atlantic ribbed mussels. Similarly, Lin [72] used laboratory studies to evaluate blue crab predation and found mussels with stronger attachment strength or those buried deeper in the sediment had lower mortality. Using both laboratory and field experiments, Lin [73] reported mud crab (*Panopeus herbstii*) predation on Atlantic ribbed mussels (*Geukensia demissa*) at two sites in coastal North Carolina. In field studies, Lin [74] evaluated the survivorship of ribbed mussels due to predation primarily from blue crabs, finding predation by terrestrial predators (e.g., wading birds, raccoons) had little, if any, impact.

Though not evaluated in this study, predation may represent a vector for trophic transfer of MPs from ribbed mussels to crabs. Using an extensive literature search, Guillory and Elliot [75] identified ninety-three species predators of blue crab zoea, megalopae, and juvenile/adults, including invertebrates, fish, reptiles, birds, and mammals. Though smaller blue crabs are subject to higher predation rates than larger blue crabs, further trophic transfer of MPs may occur. Additionally, bivalves may eliminate MPs within feces and pseudofeces [76,77], providing an additional vector for trophic transfer to benthic organisms. Studying a bed of Atlantic ribbed mussels in NJ, Khan and Prezant [68] found ingestion of MPs and subsequent rejection in feces and pseudofeces decreases the buoyancy of plastics, suggesting biodeposits are a source of MPs for benthic deposit-feeding organisms. Jenkins [69] found that the presence of Atlantic ribbed mussel in Jamaica Bay marshes enhanced MP content in marsh sediments, suggesting that salt marshes with significant mussel populations may act as a sink for MPs within urban estuaries. Future research characterizing both abiotic (e.g., water, sediment) and biotic (e.g., multiple species from varying trophic levels) sources should be performed. Lastly, unlike other species of mussels,

human ingestion of Atlantic ribbed mussels as a food item is very rare. Unlike documented human health concerns for MP exposure through ingestion of seafood (e.g., [78,79]), human exposure to MPs via ingestion of this species may be ignored.

#### 4.6. Future Research

The findings from this study allow for multiple paths for further investigation. In particular, future studies should include MP characterization and quantification of both water column and sedimentary phases to augment data from mussels and verify their use as a biomonitoring species. This also will allow for a more comprehensive evaluation of the mechanisms of MP accumulation and removal within this mussel species. Future studies may also evaluate the link between MP exposure and the growth/reproductive health of the Atlantic ribbed mussel, as there has been some evidence to indicate a link between microplastic exposure and a decrease in offspring performance in oysters [80] and juvenile growth in mussels [81]. Finally, future studies should revisit the Delaware Inland Bays ecosystem, as improved methodology, particularly regarding blank controls and their use to establish more accurate data sets, could establish further data on the extent and magnitude of microplastic pollution in this area with greater resolution to begin to identify the factors contributing to it.

### 5. Conclusions

As the population of the Delaware Inland Bays region continues to grow, development of its natural lands adjacent to its aquatic resources continues to increase. Having value in both its commercial (e.g., aquaculture, recreational) and natural resources, there is a growing need to understand the myriad of stressors impacting this aquatic ecosystem, including levels of MPs. This study, though limited in its scope, represents the first published data set for MP concentrations in a filter-feeding aquatic species within the DE Inland Bays. While the Atlantic ribbed mussel is largely ignored as a food item for humans, shellfish aquaculture centering on other species, such as oysters, is an important re-emerging industry in the Delaware Inland Bays. Accumulation of plastics in oysters may pose a risk to both oyster and human health. In oysters, exposure to MPs during the growing process can reduce the reproductive success of organisms [80], cause growth delays in early larval development [82], and delay larval settlement [83]. Microplastic exposure and its subsequent negative effects may greatly reduce the economic benefits of aquaculture [22]. This study confirmed MP concentrations in Atlantic ribbed mussels; however, it is likely that oysters accumulate MPs similarly. Thus, data from this study and others to follow will play a critical role in biomonitoring studies to assist environmental risk management of the natural and commercially important waters of the Delaware Inland Bays.

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