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Microplastic Fiber Abundance for South Carolina White Shrimp, Litopenaeus setiferus, Across Two Habitats

Adriene Funck
Coastal Carolina University, amfunck@coastal.edu

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By

Adriene Funck

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Louis E. Keiner Director of Honors HTC Honors College	Eric D. Rosch Senior Lecturer Marine Science Department Gupta College of Science

Introduction

Coastal South Carolina is a prime destination for shrimping. The commercial White Shrimp, *Litopenaeus setiferus,* is one of the main species found in South Carolina estuaries and coastline. There are commercial and recreation fishing practices in South Carolina for *L. setiferus,* making it a species of interest for the public. Microplastics are additionally a large interest in the public and scientific community for research and solutions. The threats of plastic pollution are not fully understood in the marine environment, but there has been a boom of research discussing various effects and distributions of microplastics in the ocean recently. Shrimp species have been documented with microplastics in from various regions, such as Malaysia, Ecuador, the Southwest Atlantic, and Indian Ocean from a study using shrimps found in a Singapore market (Curren et al, 2020). In South Carolina, grass shrimp, *Palaemonetes pugio,* was found to contain microplastics, however there are vast differences between *P. pugio* and *L. setiferus* (Gray and Weinstein, 2017). The *L. setiferus* population in South Carolina has yet to be fully analyzed for microplastic contamination, and due to its commercial value, there is a critical need for this research.

White shrimp occupy multiple habitats in South Carolina, including estuarine marsh system and the nearshore coastline. There is evidence of microplastics in the intertidal sediments and sea surface microlayers of two South Carolina estuaries: Winyah Bay and the Charleston Harbor (Gray et al, 2018). Documentation of microplastics in beach sediments from Virginia and North Carolina additionally exist (Dodson et al, 2020). Knowing that microplastics occur in habitats that *L. setiferus* utilize suggests the possibility of shrimp consuming microplastics when foraging, as *L. setiferus* are omnivores and detritivores throughout their life history. It is possible

L. setiferus might consume microplastics from contaminated food items or from the environment directly via repiratory pathways.

The issue of microplastics is additionally important in ecology due to the trophic transfer of microplastics that takes place when predators consume prey that are contaminated with plastics (Ferreira et al, 2019). Microplastics bioaccumulate moving up in trophic levels due to an increased food intake of prey that may have already consumed the microplastics (Ferreira et al, 2019). The feeding preferences of different fish may also play a role in the trophic transfer of microplastics, putting generalist species more at risk if the prey or vegetation they consume is contaminated by microplastics (Peters et al, 2017). Before trophic transfer occurs, microplastics can be toxic to the invertebrates lower on the trophic scale that consume them. Mortality events occurred when *P. pugio* were exposed to microplastics for a short period, and all the deceased individuals were examined and found traces of microplastics to be ingested (Gray and Weinstein, 2017). Another concern of consuming microplastics is the inability to egest the plastics, which has been observed in the lobster species *Nephrops norvegicus* (Murray and Cowie, 2011). Difficulties in excreting microplastics was also observed in the freshwater amphipod *Hyalella azteca*, and there was a decrease in growth due to microplastic concentrations for *H. azteca* as well (Au et al, 2015). These threats posed by microplastics throughout the food web highlight the motivation for studying microplastic abundances at different trophic levels.

The objective of this study was to investigate the abundance of microplastic fibers found in the gastrointestinal tracts of *L. setiferus* in two South Carolina habitats. Two habitats were analyzed to compare how the estuarine and coastal habitats occupied by *L. setiferus* might be affected differently by microplastics. These two sites also represent different food webs that *L. setiferus* occupy and influence. The potential presence of microplastics in *L. setiferus* is important economically as well as for public health and safety from the selling and consumption of contaminated *L. setiferus*.

Methods

Litopenaeus setiferus were collected from two locations in coastal South Carolina. A total of 75 (n=75) shrimp were collected, Garden City beach (n=26) and Oyster Landing (n=49) in the Murrells Inlet Estuary (Fig. 1**)**. Specimens from Garden City were sampled by trawling from a boat, while specimens from Oyster Landing were sampled by seining or with a cast net. Shrimp were brought back to Coastal Carolina University where they were kept frozen until the time of dissection.

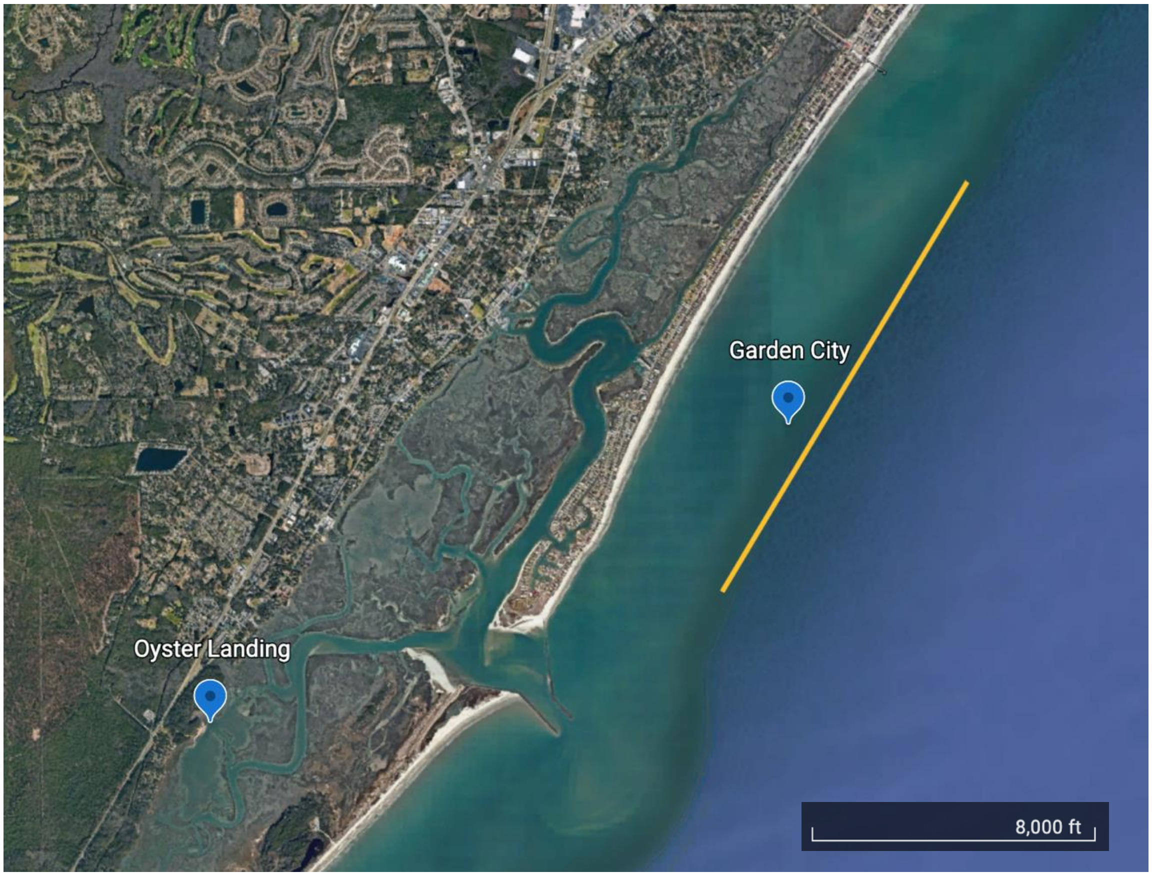


Figure 1. Map of two sampling sites for *Litopenaeus setiferus* collection in South Carolina. The yellow line represents the trawling path.

In the lab, each shrimp was thawed under running water and lengths were measured from the anterior rostrum to posterior tail fan in millimeters (mm). Total wet weight was measured using a digital scale in ounces and were later converted into grams (g). The shrimp was then dissected using a sterilized metal scalpel and forceps to remove the gastrointestinal (GI) tract. Each GI tract was placed in a sterilized petri dish and weighed using a digital scale and converted into grams (g). The GI tract was suspended in a solution of deionized water and concentrated bleach to help dissolve organic matter. A blunt probe and fine teasing probe were used to separate the contents of the GI tract to analyze under a dissecting scope following a grid pattern to count the abundance of microplastic fibers. Microplastic fibers were identifiable from other debris in the GI tract due to their coloration and shape. Other forms of microplastic could not be identified without further analysis and a further breakdown of organic material that was not done in this study. All equipment used in this procedure was sterilized between each sample to avoid cross-contamination.

All statistical analyses were conducted using Microsoft Excel. The average length and wet weight and standard deviation for Garden City and Oyster Landing were found, along with a two-sample t-test assuming unequal variance for both length and wet weight at both locations ($p<0.05$). The average abundance and standard deviation of microplastic fibers were found for shrimp collected in Garden City and from Oyster Landing. A two-sample t-test assuming unequal variance was done for the fiber abundance at both locations ($p<0.05$). A linear regression statistical test was done to determine the relationship of *L. setiferus* length and wet weight ($p<0.05$). Linear regressions were also done to determine the relationship of GI tract weight with wet weight and with length ($p<0.05$). The relationship between fiber abundance with length and wet weight were additionally found with linear regression statistical tests ($p<0.05$). For both Garden City and Oyster Landing, the fiber abundance per gram (fiber/g) and fiber abundance per millimeter (fiber/mm) were found, and the average and standard deviation for these values. A two-sample t-test assuming unequal variance was used for the fiber abundance per gram, while a two-sample t-test assuming equal variance was used for the fiber abundance per millimeter ($p<0.05$).

Results

Overall Microplastic Abundance

A total of 110 microplastic fibers were found in the 74 *Litopenaeus setiferus* samples examined. Overall, the average abundance of microplastic fibers was 1.49 ± 1.76 per shrimp. The highest abundance of fibers found was 8 from a shrimp caught at Oyster Landing on April 13th, 2022. There was a total of 29 samples that were not contaminated with microplastic fibers. One specimen was deemed as an extreme outlier with an unlikely result compared to the rest of the data, so this sample was removed from all analyses regarding microplastic abundance. This sample was however included in the analyses regarding body size. The average abundance of fibers for the specimens that were contaminated, without the outlier, was 2.44 ± 1.66 per shrimp. the most common abundance for the shrimp found with microplastics was 1 fiber per individual.

Body Length, Weight, and GI Tract Weight

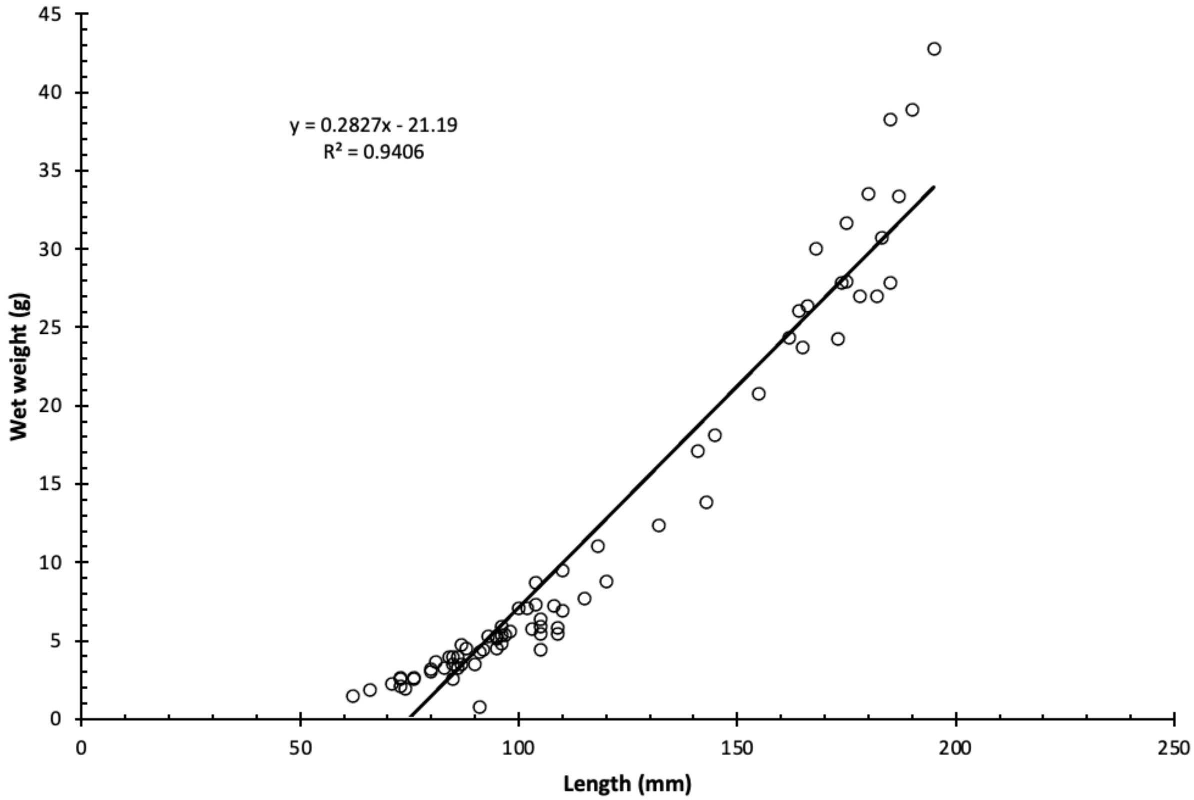
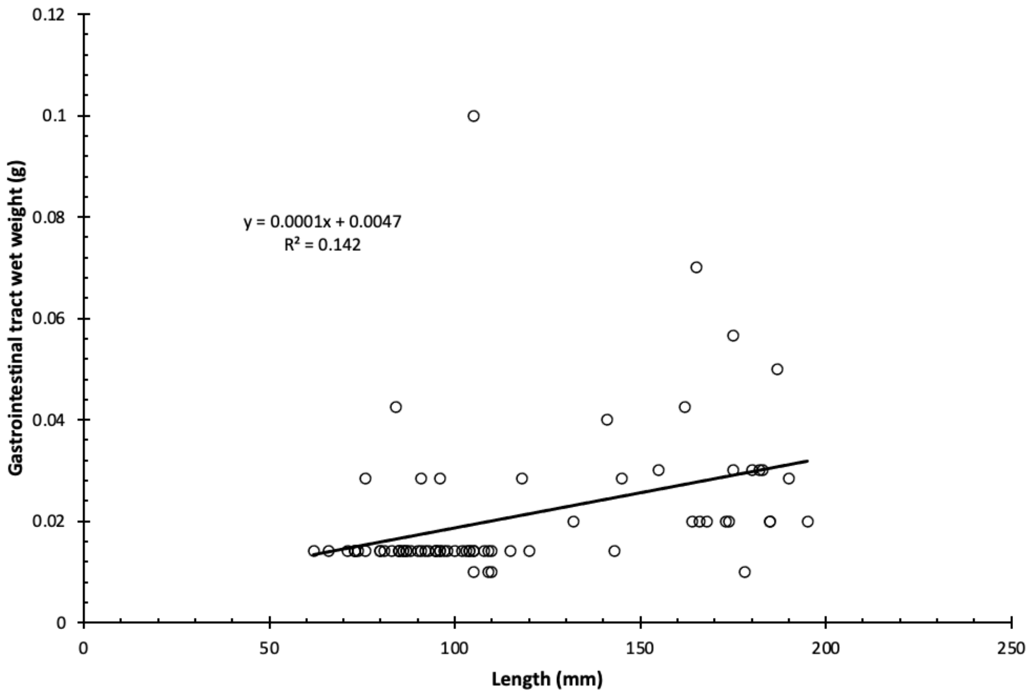


Fig. 2. Linear relationship of *Litopenaeus setiferus* length (mm) and wet weight (g) collected from Garden City and Murrells Inlet, South Carolina from a sample of 75 shrimp. The correlation coefficient is shown. The trendline predicts there is a 0.2827 g increase in wet weight for every 1 mm in length for *L. setiferus*. There is a strong, positive correlation shown for the length and wet weight of *L. setiferus*.

There was a strong, positive correlation for the length and wet weight of *L. setiferus* for all the shrimp studied. The length of the shrimp was estimated to predict 94.05% of its wet weight. The smallest shrimp studied was 62 mm and weighed 1.47 g, which was found at Oyster Landing on April 13th, 2022. The largest shrimp collected from Garden City was on October 18th, 2022, and it measured 195 mm and 42.75 g. There was a statistically significant relationship between the length and wet weight of *L. setiferus (*regression: R^2=0.94, df=74, $p < 0.01$) (Fig. 2).

A.


0.12
0.1
0.08
0.06
0.04
0.02
0
Gastrointestinal tract wet weight (g)
y = 0.0001x + 0.0047
R² = 0.142
0
50
100
150
200
250
Length (mm)

B.

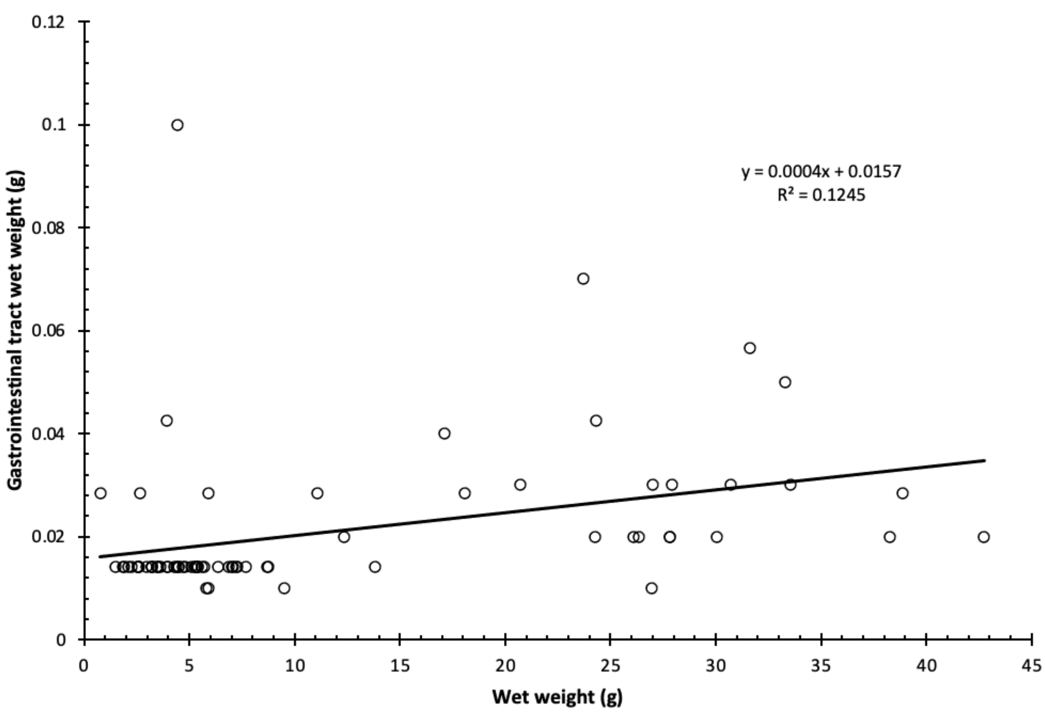
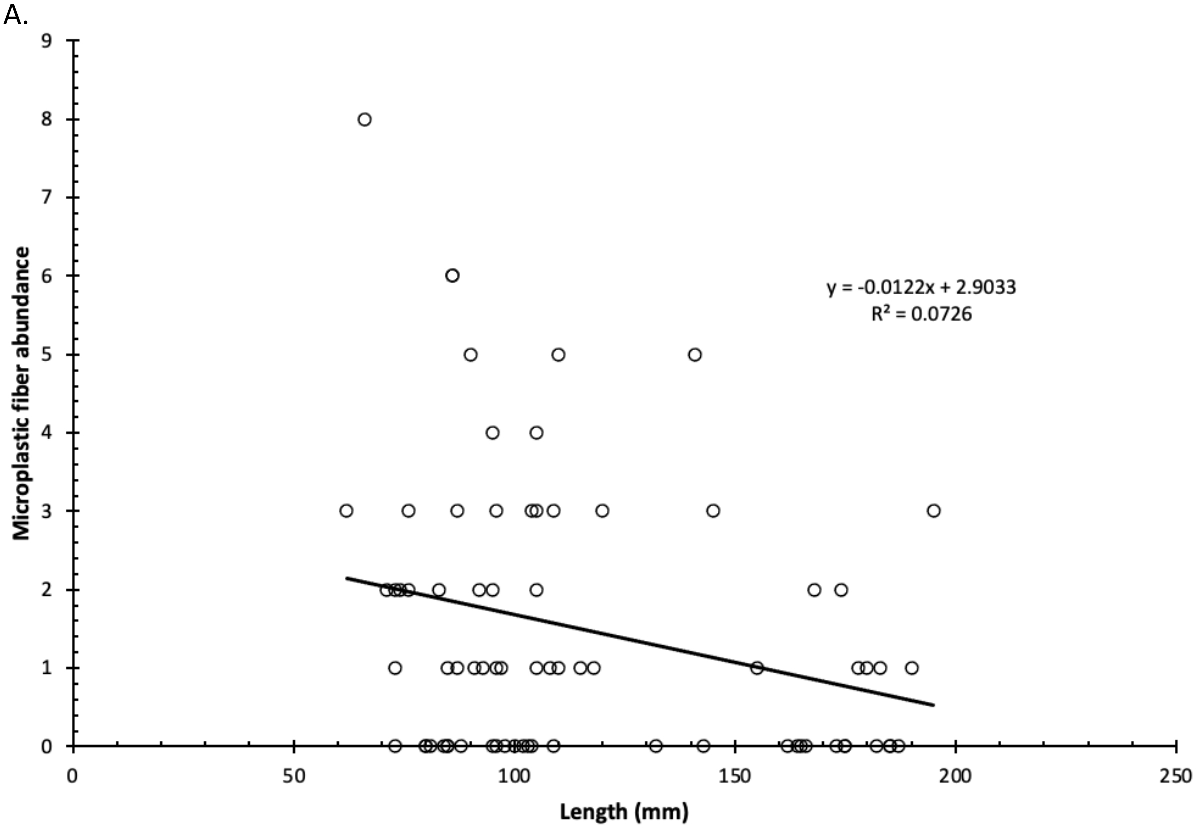

0.12
0.1
0.08
0.06
0.04
0.02
0
Gastrointestinal tract wet weight (g)
y = 0.0004x + 0.0157
R² = 0.1245
0
5
10
15
20
25
30
35
40
45
Wet weight (g)

Fig. 3. Linear relationship of gastrointestinal tract wet weight of *Litopenaeus setiferus* with (A) length (mm) and (B) wet weight (g) from a sample of 75 shrimp collected from Garden City and Oyster Landing, South Carolina. The correlation coefficient is shown. Trendlines suggest that there is no correlation between the gastrointestinal tract wet weight with length or wet weight for *L. setiferus.*

The most common wet weight measured for the gastrointestinal tracts was 0.014 g overall. The gastrointestinal tract weight did not have a relationship with the length of *L. setiferus* (regression: R^2= 0.14, df=74, p=0.0009) (Fig. 3). The weight of the GI tract additionally did not have a significant relationship with the wet weight of *L. setiferus* (regression: R^2=0.12, df=74, p=0.002) (Fig. 3). This result was likely due to the sensitivity of the digital scale used for the study not having the precision needed to make an accurate conclusion.

Body and GI Tract Size and Microplastic Abundance

A.



B.

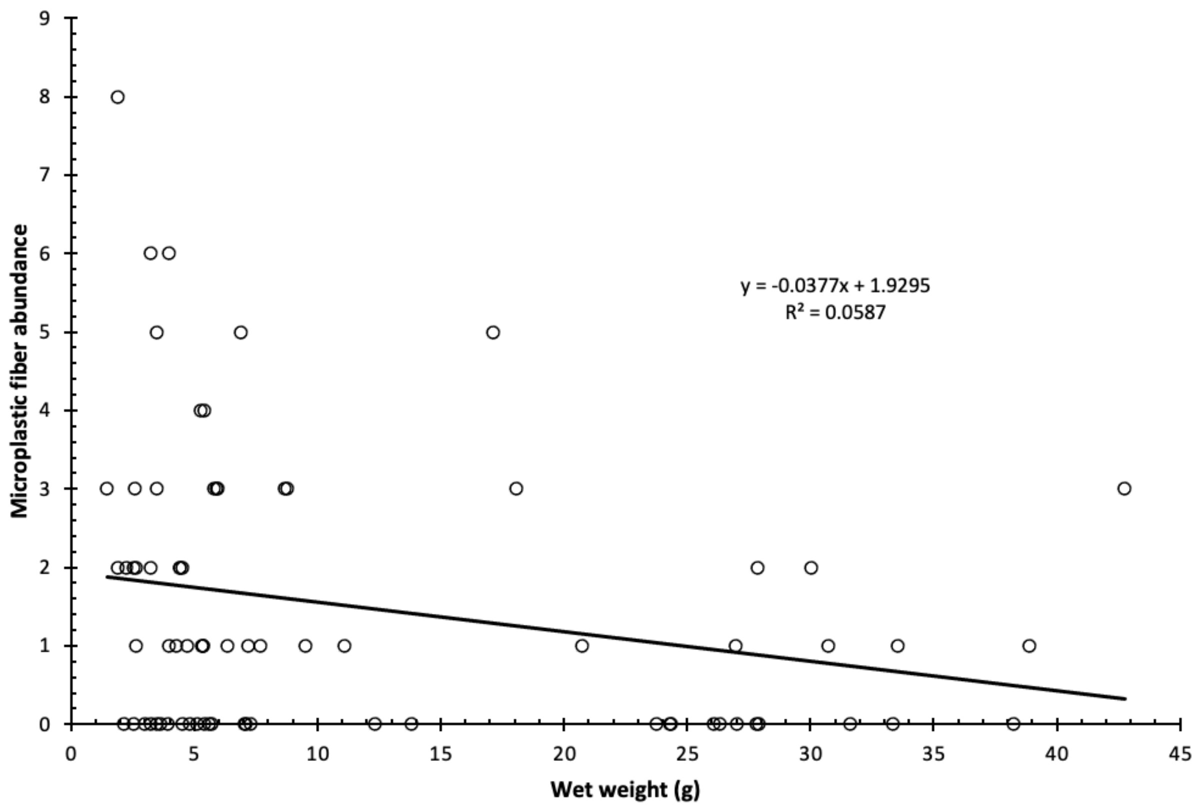


Fig. 4. Linear relationship of microplastic fiber abundance with (A) length (mm) and (B) wet weight (g) for *Litopenaeus setiferus* from Garden City and Oyster Landing, South Carolina (n=74). The correlation coefficient is shown. Trendlines suggest that there is no correlation between abundance of microplastic fibers with length or wet weight of *L. setiferus.*

While there was a relationship for the size of the shrimp, there was not a correlation between size and microplastic fiber abundance. Both the largest and smallest shrimps studied were found with an abundance of 3 microplastic fibers. This means that the length and weight of *L. setiferus* cannot be used to predict the abundance of microplastic fibers in the GI tract. No statistically significant relationship was found between microplastic fiber abundance and wet weight of *L. setiferus* (regression: R^2= 0.05, df=73, p=0.04) (Fig. 4). There was additionally no relationship found between fiber abundance and *L. setiferus* length (regression: R^2= 0.15, df=73, p=0.0008) (Fig. 4). No analysis was done to determine if the weight of the GI tract was correlated to the fiber abundance.

Microplastic Abundance and Body Size by Location

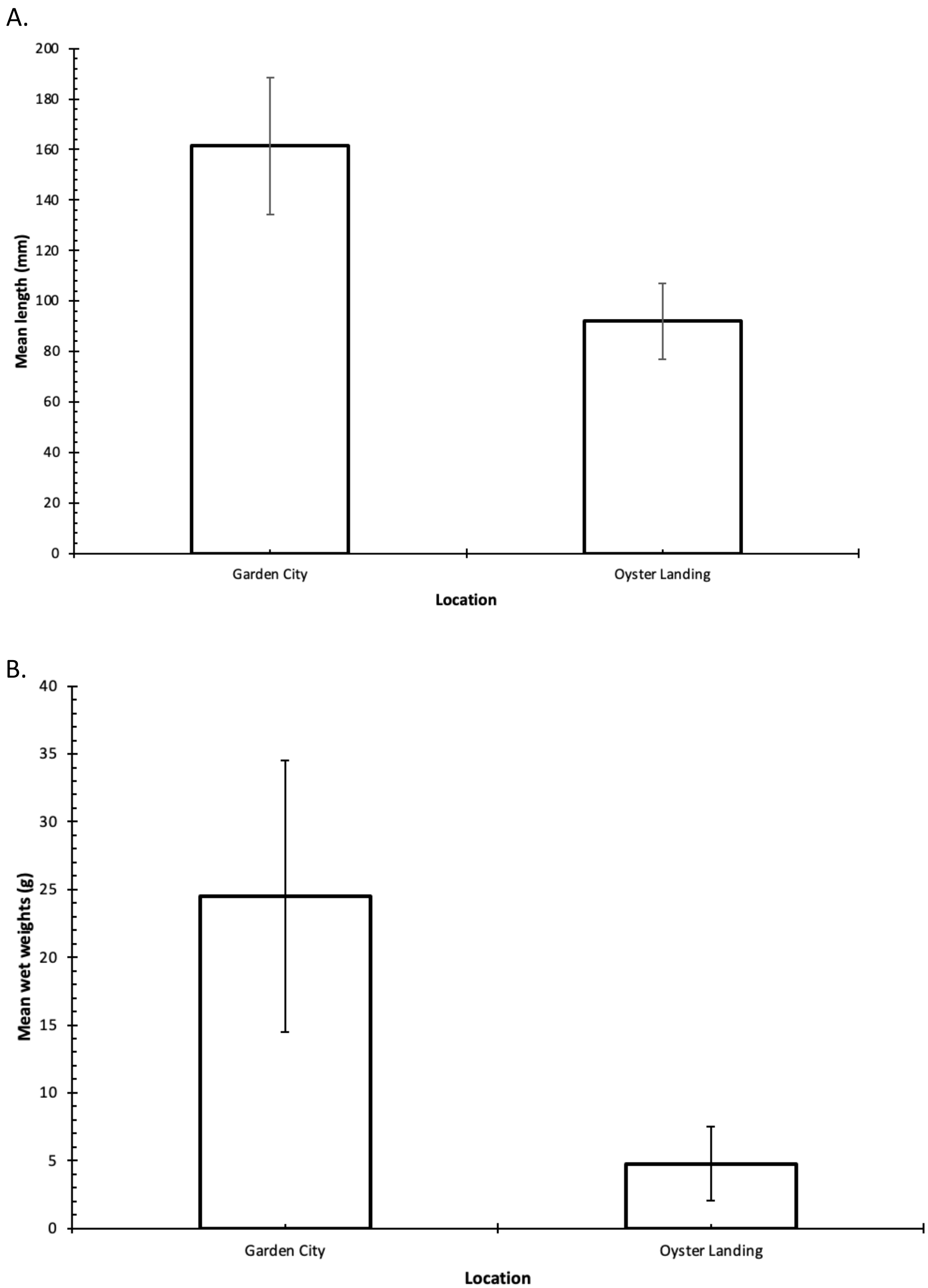


Fig. 5. Mean (A) length (mm) and (B) wet weight (g) of a sample of 75 *Litopenaeus setiferus* from Garden City and Oyster Landing, South Carolina. Error bars show ± 1 standard deviation. Both average length and wet weight for *L. setiferus* were greater at Garden City than at Oyster Landing.

The lengths and wet weights of *L. setiferus* differed between Garden City and Oyster Landing. On average larger body sizes were seen in Garden City than in Oyster Landing. There was a statistically significant difference in lengths of *L. setiferus* at Garden City and Oyster Landing (t-test: df=33, T=12.08, $p < 0.01$) (Fig. 5). Additionally, there was a statistically significant difference in the wet weight of *L. setiferus* between the two sites (t-test: df=27, T=9.83, $p < 0.01$) (Fig. 5).

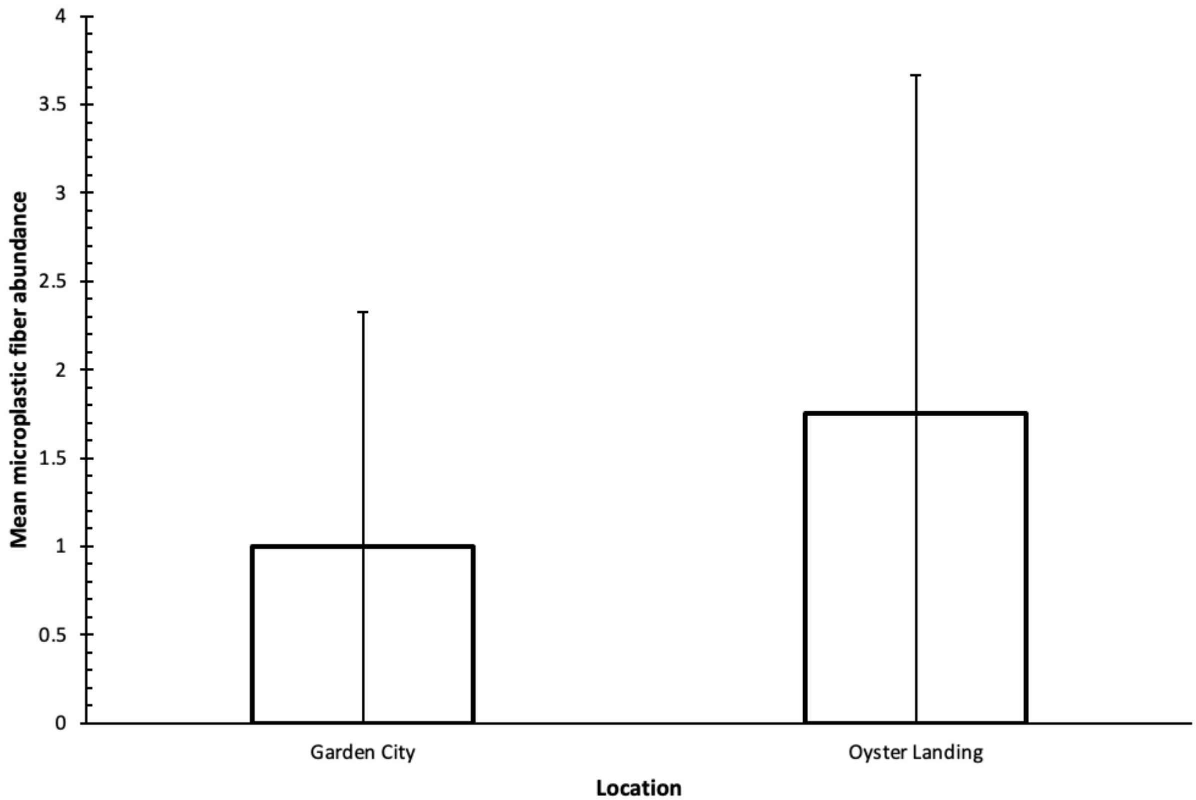


Fig. 6. Mean microplastic fiber abundance found in the gastrointestinal tracts of 74 *Litopenaeus setiferus* from Garden City and Oyster Landing, South Carolina. Error bars represent ± 1 standard deviation. A greater abundance on average of microplastic fibers was found in *L. setiferus* at Oyster Landing than Garden City.

Exactly 50% of the *L. setiferus* from Garden City were found to be clean of microplastics, while only 33.33% were clean from Oyster Landing. A total of 84 microplastic fibers were counted from shrimp collected from Oyster Landing, which is 58 more fibers than what was observed at Garden City. Fibers from Garden City only accounted for 23.64% of the total microplastic fibers observed overall. There was a near statistically significant difference between the average abundance of microplastic fibers found in Garden City compared to Oyster Landing (t-Test: df= 86, T=1.97, p=0.052) (Fig. 6).

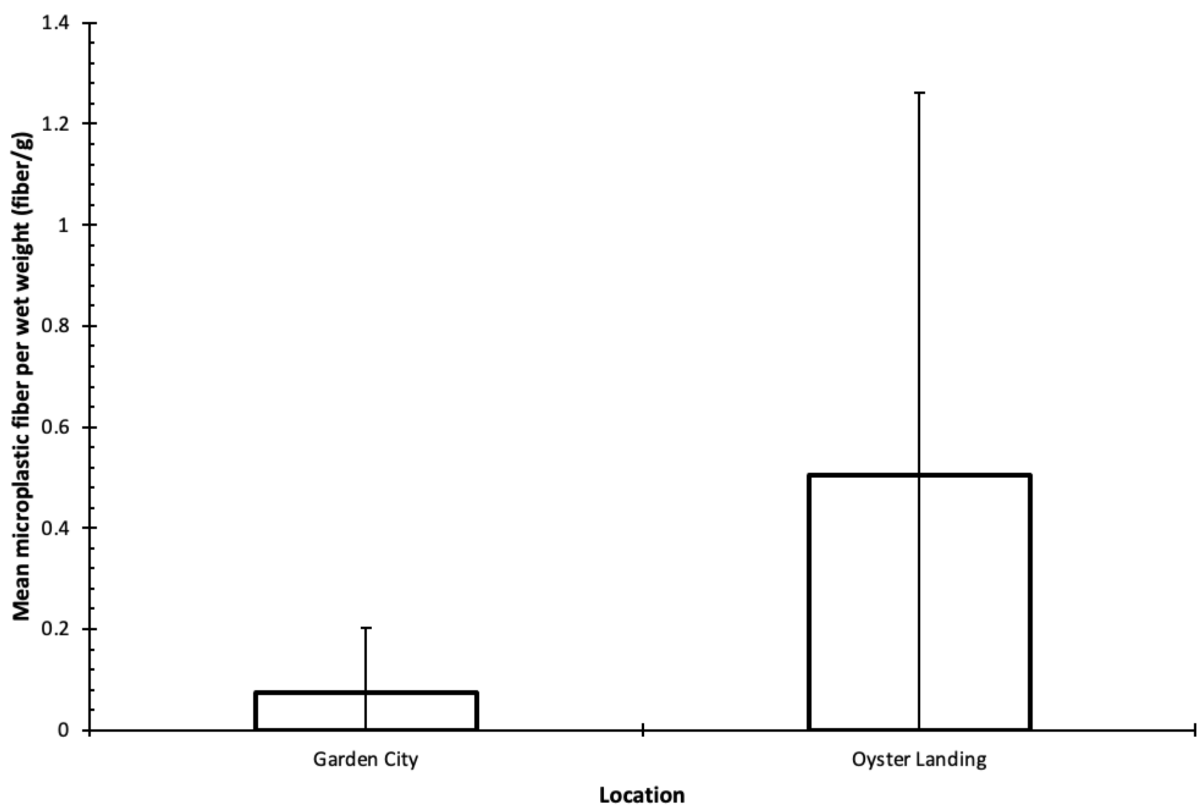


Fig. 7. Mean microplastic fiber abundance per wet weight (fiber/g) of *Litopenaeus setiferus* from Garden City and Oyster Landing, South Carolina. Error bars represent ± 1 standard deviation. Mean fiber abundance per wet weight was greater in *L. setiferus* collected from Oyster Landing than Garden City.

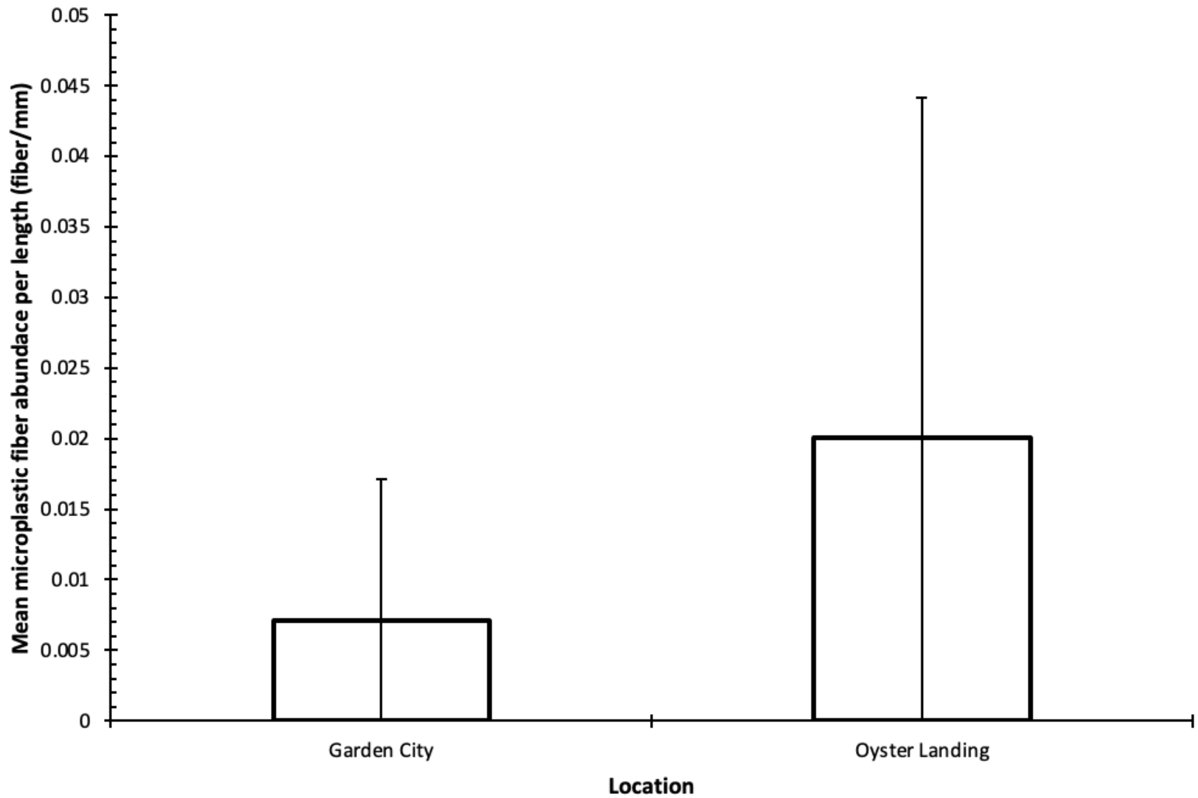


Fig. 8. Mean microplastic fiber abundance per length (fiber/mm) of *Litopenaeus setiferus* from Garden City and Oyster Landing, South Carolina. Error bars represent ± 1 standard deviation. Mean fiber abundance per length was greater in *L. setiferus* collected from Oyster Landing than Garden City.

However, the abundance of microplastic fibers relative to size of *L. setiferus* did differ between Garden City and Oyster Landing. The mean fiber abundance per gram and mean fiber abundance per millimeter was larger for *L. setiferus* collected at Oyster Landing than at Garden

City. There was a statistically significant difference of fiber/g by location (t-test: df=52, T=3.84, p=0.0003) (Fig. 7). There was a statistically significant difference in fiber/mm of *L. setiferus* by location as well (t-test: df= 72, T=2.62, p=0.01) (Fig. 8).

Discussion

Microplastic fibers in shrimp

There is a presence of microplastic fibers in commercial white shrimp *L. setiferus* found in South Carolina. Seventy-four shrimp were examined for microplastics, which resulted in a total of 110 microplastic fibers. Fiber abundance was deemed uncorrelated with the length and wet weight of *L. setiferus*. This is a novel introduction recognizing the presence of microplastics in white shrimp for South Carolina and is likely an underrepresentation of the entirety of microplastic contamination in *L. setiferus*. Only microplastic fibers were counted in this study, but various shrimp species have been documented contaminated with microplastic fibers, spheres, and fragments (Curren et al. 2020). Additional methods would need to be taken to assess the total abundance of all forms of microplastics that exist in the environment and in the gastrointestinal tracts of *L. setiferus*. Also in South Carolina, adult daggerblade grass shrimp, *Palaemonetes pugio*, ingested microplastic fibers, fragments, and spheres under laboratory exposure (Gray and Weinstein, 2017). *P. pugio* were additionally observed with microplastic fibers, fragments, and spheres in their gills (Gray and Weinstein, 2017). Grass shrimp are a smaller species compared to the White shrimp analyzed here, and there is a strong public interest in the microplastic load of white shrimp due to its high commercial value. This is further cause for concern to study the microplastics being consumed by *L. setiferus*. Gray and Weinstein's 2017 study observed an average of 2.3±1.7 particles of microplastics in the guts of *P. pugio* after a three-hour exposure to 93 μm fibers, closely resembling the results of this study for the mean of *L. setiferus* observed with microplastic fibers.

Three shrimp species from Malaysia, Ecuador, the Southwest Atlantic, and the Indian Ocean were observed containing an average of 6 microplastics per individual in the gastrointestinal tracts, with fibers being the most common form (Curren et al. 2020). Their results demonstrate a greater abundance per individual than in this study, again likely due to the greater inclusion of microplastic forms being identified (Curren et al. 2020). They observed differences between species in the amount of microplastics per wet weight (Curren et al. 2020). Abundance per wet weight in this study were compared between locations as only one species of shrimp was examined.

The *L. setiferus* in this study represent estuarine and coastal populations of white shrimp for South Carolina, but there are other invertebrates and fishes of coastal and estuarine areas throughout the world that have been documented ingesting microplastic fibers. In the Clyde Sea, Scotland, lobsters, *Nephrops norvegicus*, were observed with such a magnitude of microplastic fibers that there were formations of tightly packed balls made of fibers in their intestinal tracts (Murray and Cowie, 2011). These abundances and formations were not observed in *L. setiferus*, but it could be of interest for other crustaceans in this region. Fishes ingest microplastics from the prey they consume, demonstrating the trophic transfer of microplastics, and some from the environment itself (Ferreira et al, 2019). Microplastic fibers were the most prevalent form found in snook fishes studied at Brazil, and the abundance of microplastics consumed by snook was found to increase with age as their diet changed to consuming more shrimps and fish (Ferreira et al, 2019). In the Mediterranean Sea, the fish *Boops boops* averaged 3.75 microplastics per fish, and only fibers were observed (Nadal and Deudero, 2016). This is a greater abundance of microplastic fibers than seen in *L. setiferus*, which could be due to the trophic differences of shrimp and *B. boops*. Six other fish species within the Texas Gulf Coast found with microplastics showed fibers to be the most common as well (Peters et al, 2017).

While additional analysis should be more conclusive for the presence of other forms of microplastics, fibers are of particular interest for shrimp, other invertebrates, and fish. One area of concern that could not be analyzed in the present study is the residence time of microplastics once ingested by marine life. The *L. setiferus* samples were removed from their habitat for analysis, so it was not possible to record residence times or egestion. Laboratory studies are able to see these variables after exposing organisms to microplastics. For instance, a freshwater amphipod, *Hyalella azteca*, experienced slower residence times for polypropylene fibers than polyethylene particles (Au et al, 2015). *H. azteca* were able to egest fibers, though at slower rates compared to other plastics particles (Au et al, 2015). The residence time seen in *P. pugio* for microplastic fibers, fragments, and spheres ranged from around one to three days in the gastrointestinal tract and one to two in the gills (Gray and Weinstein, 2017). Death was observed in *P. pugio* exposed to microplastics for acute periods of three hours, and a portion of deaths was attributed to being unable to egest the microplastics from the gut (Gray and Weinstein, 2017). Murray and Cowie, 2011, recorded *N. norvegicus* ingesting microplastics but weren't able to excrete the plastics. This is especially interesting for the present study as it is unknown how long the *L. setiferus* consuming microplastics will take to egest the microplastics, or if death would have soon occurred had they not been collected for examination.

The toxicity of fibers compared to other types is additionally a reason to focus on microplastic fiber contamination. For freshwater amphipod *H. azteca,* polypropylene microplastic fibers were found to be significantly more toxic over polyethylene particles for the residence time differences and observations in decreased growth with an increase of fiber concentration (Au et al, 2015). Fibers were additionally deemed more toxic for *P. pugio* over fragments and spheres, even though fibers were the least abundant in the gut and gills (Gray and Weinstein, 2017). Microplastic fibers greater than 50 μm were the greatest concern for *P. pugio*, and it is unknown how this compares for *L. setiferus* as the lengths of microplastic fibers found could not be measured (Gray and Weinstein, 2017).

Location Differences

Microplastic abundances differed between the two collection sites of this study, with greater abundances observed in specimens collected from the estuarine environment at Oyster Landing than along the coast of Garden City. The difference in fiber abundance per length and wet weight was significant between these sites as well. The two sites represent two different types of environments that commercial shrimp and fish species inhabit. Oyster Landing is a marsh creek system, while Garden City is around 23 feet deep off the coast of the Grand Strand. These differences between locations are likely contributors to the differences in microplastic abundances in *L. setiferus* found at each site. There is more sedimentation and stagnate water in a marsh tidal creek compared to the nearshore ocean, so it is possible that more microplastics will remain at Oyster Landing than in the open ocean at Garden City. Other species have been observed with differing abundances of microplastics based on location. Snooks from the Goiana estuary in Brazil differed in microplastic abundances based on their movement patterns within the estuary at different life stages (Ferreira et al, 2019). They also observed higher concentrations of microplastics associated with areas affected by river runoff and fishing activity, as these are sources for pollutants such as microplastics (Ferreira et al, 2019). Microplastic counts also differed in *Boops boops* depending on location (Nadal and Deudero, 2016). Habitat differences in microplastic ingestion are of concern for commercial species such as *L. setiferus*, because if there are regions with lesser concentrations of microplastics those would be preferred for human consumption of marine species.

The occurrence of microplastics in *L. setiferus* from Oyster Landing and Garden City signifies the presence of microplastics in those environments. The presence of microplastics has been studied in the Winyah Bay and Charleston Harbor estuaries in South Carolina, both south of the Oyster Landing location but similar geologically and oceanographically (Gray et al, 2018). Microplastic fibers were the most abundant form in the Charleston Harbor, and the second most abundant in Winyah Bay (Gray et al, 2018). Greater abundances were observed in intertidal sediments than in the sea surface microlayer overall, which suggests another reason why more microplastic fibers were found from Oyster Landing samples than Garden City (Gray et al, 2018). Differences in watersheds, residence times, flushing rates, and sedimentation rates are all factors contributing to where microplastics can be found in estuarine systems (Gray et al, 2018). Beach sediments from Virginia and North Carolina differed in the distribution of microplastics spatially as well, representing a northern example of the coastal zone of Garden City's beach (Dodson et al, 2020). Microplastic fibers were the most abundant among beach sediments at all the sites examined, highlighting the abundance of fibers that were present in *L. setiferus* from Garden City (Dodson et al, 2020). They also determined that the sediment composition did not affect microplastic distribution (Dodson et al, 2020). This is worthwhile for the Garden City location, though different sediments would be found at Oyster Landing than Garden City, so it is unknown what role the sediment in the marsh plays in microplastic distribution.

A large source for microplastic fibers was found to be from washing clothing (Browne et al, 2011). Testing has found that washing garments can produce over 100 microplastic fibers per liter of effluent, and the proportions of those plastics match with microplastics found in habitats contaminated with microplastics (Browne et al, 2011). The flux of sewage effluent from washing machines can reach multiple habitat types, making it a possible source for the microplastic fibers found in Oyster Landing and Garden City. The degradation of microplastics can also be dependent on the habitat. A study along the Ashley River, South Carolina demonstrated that plastics degrade into microplastics quickly, around 8 weeks, in salt marshes (Weinstein et al, 2016). This suggests that larger debris in salt marshes or estuaries could be releasing microplastics into the environment every tidal cycle (Weinstein et al, 2016).

Implications of the presence of microplastics in shrimp

The presence of microplastics in a commercial species is of importance ecologically not only for the *L. setiferus*, but also other species at higher trophic levels. The trophic transfer of microplastics is a major reason for investigating the abundance of microplastics in smaller species such as *L. setiferus.* Larger predators that consume shrimp will inherently consume any microplastics inside their gastrointestinal tracts, causing the plastics to bioaccumulate in larger species. This is seen in the snook fishes of Brazil as a top predator in their ecosystem (Ferreira et al, 2019). The microplastic abundance in snooks is particularly interesting as their abundances increased as they grew in life stages and diets changed to consuming more Penaeid shrimp (Ferreira et al, 2019). This means that their concentration of microplastics increased with shrimp consumption, and this study demonstrates that there is an abundance of microplastics in one species of Penaeid shrimp. Additional predators along the Texas Gulf Coast that were found with microplastics were associated with the consumption of shrimp as well (Peters et al, 2017). They observed how the foraging preferences of fish influenced the amount of microplastics as well, which is of interest for human consumption of different fish species (Peters et al, 2017).

Knowing the microplastic distribution in fish, or in the prey they consume such as *L. setiferus*, could differentiate which species are cleaner for human consumption.

The potential for the trophic transfer of microplastics can impact humans as well. The marine species that is on the market are likely to contain microplastics as shown by the commercial *L. setiferus*. For shrimp specifically, it is not always the case that the gastrointestinal tract is removed fully for human consumption. Different shrimp species in the Singapore markets were contaminated with microplastics, meaning the shrimps were being sold to the public with microplastics (Curren et al, 2020). The *L. setiferus* here were caught from their habitat so it is not as accessible to the general public for consumption. However, the fishing is a large part of the culture and recreation in South Carolina so there still is risk for human contamination.

The objective of this study was to document the abundance of microplastic fibers in the commercial white shrimp, *L. setiferus*, in South Carolina. These findings act as a background of data for the contamination of *L. setiferus* for microplastics, but further analysis should be done to be inclusive of all types of microplastic. As there is an ever-growing concern for microplastics in the marine environment and marine life, it is important to broaden the research to address what potential solutions could be implemented to prevent further toxicity from microplastics.

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