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Assessment of Microplastics in Southeastern Florida Forage Fishes

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HALMOS COLLEGE OF ARTS AND SCIENCES

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By

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Abstract

Microplastics threaten the health of numerous marine organisms at all trophic levels. Currently, the topic is well studied among larger predators such as marine birds, dolphins, pelagic fishes, and even herbivorous organisms such as manatees. However, knowledge of microplastics present in organisms at lower trophic levels is poorly understood. The aim of this study was to assess the presence of microplastics in lower trophic level forage fishes. To gain a clear depiction of microplastics in the forage fishes of South Florida, four locations were sampled. These locations were classified into two categories, urban (Port Everglades and Northern Biscayne Bay) and non-urban (Islamorada and Marathon, in the Florida Keys). Five species were sampled: Striped Mullet, Scaled Sardine, Needlefish, Pinfish, and Irish Mojarra. Every sampled fish except one (n= 248) had microplastics within their systems, with a total of 2,126 pieces found. There was no significant difference in microplastics concentration among forage fish species. However, location had a significant effect on the frequency of microplastics found within the sampled fishes, with Northern Biscayne Bay being greater compared to the other three sample locations. There was no significant difference in microplastic frequency amongst the three feeding habits within the five sampled species. However, as Redfin Needlefish and Pinfish matured, an increase in microplastic frequency was observed. With a frequency of 99.6% of microplastic contamination within the sampled fishes, significant conservation efforts should be warranted.

Keywords: Microplastic, forage fishes, pollution, southeast Florida, marine debris

Introduction

Background

Marine debris threatens the overall health of marine organisms. Anthropogenic litter varies from large sunken ships to small pieces of plastic invisible to the naked eye. The majority of marine debris is in the form of plastics, averaging 8 million metric tons per year (Rodrigues et al., 2018). As plastic begins to degrade, it forms microplastics, or pieces of plastic no larger than 5 mm in diameter (Rodrigues et al., 2018). Microplastics vary immensely based on physico-chemical properties such as size, shape, color, density, and polymer type (Rodrigues et al., 2018) and can be divided into subcategories: granular, fragments, pellets, film, foam, and fibers (Gies et al., 2018; Ngo et al., 2019; Rodrigues et al., 2018). Due to the large variation in types of microplastics, identification of the material/origin can be difficult (Bergmann et al., 2015). In the water, microplastics can float at the surface, stay suspended in the water column, or settle on the seafloor in sand, coral, or vegetated habitats. Once these small particles enter a system, marine life will inevitably encounter them.

The direct and indirect consumption of microplastics in fish threatens an individual's overall fitness (Ferreira et al., 2018). Organisms can directly consume plastic particles, mistaking them for prey. Indirect intake can also occur as a result of plastic being present in the water while a fish is grazing or filter-feeding, and the particles are consumed along with their food (Thompson et al., 2004). The intake of microplastics can cause health issues, including intestinal blockage, alterations to lipid metabolism, and even physical injuries that can result in death (Jovanovic, 2017). Additionally, these plastics can leach toxins known as persistent organic pollutants (POPs) to the surrounding tissues, causing disruptions in the organism's endocrine system (Geyer et al., 2000).

Microplastics are increasingly being recognized as a widespread environmental threat due to their ability to be transferred throughout the food web through prey-predator interactions, ultimately accumulating at higher trophic levels (Garcia et al., 2021; Bergmann et al., 2015). Several studies have assessed the amount of microplastics in large predatory organisms such as seabirds, teleost fishes, and sharks via secondary ingestion (Provecher et al., 2014; Ferreira et al. 2019). Additionally, some herbivorous organisms have been observed to have microplastics present in their systems, including manatees (Bergmann et al., 2015) and sea turtles (Caron et al.,

2018). There is an increased need for research and documentation on the ecological impact of microplastic transferring throughout food webs to better inform resource managers and consumers.

Large carnivorous predators in coastal marine ecosystems such as Common Snook *Centropomus undecimalis* and Constantino Snook *C. mexicanus* are two of many marine organisms previously assessed for microplastic intake. Ferreira et al. (2018) and Ferreira et al. (2019) examined the correlation between microplastic and the maturity of these two snook species in the Gioana Estuary in Brazil. More than half of the collected specimens had ingested microplastics, and as the individual snooks matured, the concentration of microplastic in their intestines increased. The increased concentration of microplastics is thought to reflect the snook's ontogenetic diet change, shifting from invertebrates to larger pelagic fishes. A total of 24 of the 41 undigested fish removed from the snook GI tracts were also contaminated with microplastics, providing evidence that microplastics can transfer between trophic levels in a marine food web.

Historical Basis of the Research Field

In Florida, microplastics are becoming a well-studied topic in large pelagic organisms such as sea turtles, manatees, dolphins, as well as shorebirds (Li et al., 2021; Lusher, 2015). The accumulation of microplastics in predatory organisms is suspected to be due to secondary ingestion via their prey source. However, there is limited information on the presence of microplastics in prey organisms, such as forage fishes, which are a large dietary component for many upper trophic level marine predators. Forage fishes, often known colloquially as "baitfish," are planktivorous or detritivorous pelagic fishes that tend to form schools (Engelhard et al., 2014). Although there is limited published work on microplastics in forage fishes of southeastern Florida, previous research on different taxa shows the presence of microplastics within the same waters (Plee & Pomory, 2020).

Plee and Pomory, (2020) assessed the concentration of microplastics in seagrass beds and surrounding sand flats in the Florida Keys in three species of sea cucumbers. Sea cucumbers are bottom-dwelling invertebrates that are deposit-feeders, and this feeding strategy makes them vulnerable to ingesting microplastics that have settled onto the benthos. All three species assessed contained microplastics, regardless of their surrounding habitat. Further, sea cucumbers

inhabiting seagrass beds had a higher concentration of microplastics than those found in the surrounding sandy areas. Other seagrass-associated organisms such as Irish Mojarra *Diapterus auratus* and Pinfish *Lagodon rhomboides* (Adam, 1976) were both are targeted species in this study. A similar study by Lenz et al. (2016) evaluated the plastic distribution in stomachs of clupeid herrings and Atlantic Cod *Gadus morhua* in the North Sea and Baltic Sea. Herrings feed predominantly on zooplankton and are an important food source for small cod. The authors reported that 23% of their collected herring samples had microplastics present in their stomach contents. Past studies have demonstrated that human population size is directly related to the ratio of microplastics present in coastal waters (Tanaka & Takada, 2016; Kwon et al., 2020). However, how population size influences microplastics in the food web of southeastern Florida is largely unknown and this study intends to evaluate this in several ecologically important forage fishes.

The following study describes and quantifies microplastics found in ecologically important forage fishes of southeastern Florida. Human population size is hypothesized to influence the concentration of microplastics present in the collected specimens. To assess local anthropogenic influences, four locations were sampled: two urban (Port Everglades and Northern Biscayne Bay) and two non-urban (Islamorada and Marathon). Port Everglades and North Biscayne Bay are categorized as urban based on the surrounding human population. According to the U.S. Census Bureau (2021), Broward County (including Port Everglades), has a human population of 1.93 million. Miami-Dade County, the location of Biscayne Bay, has a population of 2.662 million. The non-urban sample locations (Islamorada and Marathon) are both located in Monroe County where the total population is 73,170. Prior work has found that large, urbanized cities are correlated with higher concentration of microplastics in small coastal fishes and their habitats (Tanaka & Takada, 2016; Kwon et al., 2020). Based on this information, it is hypothesized that forage fishes in urban coastal waters will have a higher concentration of microplastics compared to specimens from non-urban locations.

The purpose of this study was to evaluate the prevalence of microplastics within the forage fishes of southeast Florida. While conducting this study, a secondary objective was to identify if a correlation existed between human population density and microplastic concentration. Researching the bioaccumulation of microplastics at lower trophic levels, i.e., prey organisms can give insight into the ability of microplastics to pass through the food web.

Pairing the results of this study to those assessing microplastics in larger predatory organisms of southeast Florida (e.g., Clark et al., *In press*) can help in conservation efforts to preserve southeast Florida fishes.

Methods and Materials:

Research design and analysis:

Five species of forage fishes were collected for microplastic analysis including, Striped Mullet *Mugil cephalus*, Scaled Sardine *Harengula jaguana*, Redfin Needlefish *Strongylura notata*, Pinfish *Lagodon rhomboides*, and Irish Mojarra *Diapterus auratu*s. These species were selected because each has been recorded as prey for piscivorous birds and other marine predators in southeastern Florida (Torres, 2009). Additionally, the selected species are used as bait in both commercial and recreational fisheries, which can later impact human health, through microplastic accumulation in fishes used for food products (Rodriguez-Sierra & Jiménez, 2002; Odum, 1968; Adams, 1976; Arceo-Carranza, 2004; Rossman; 2015).

A total of 260 specimens was the targeted sample size across the five study species. The number of individuals was calculated with the statistical program G*Power (Erdfelder et al., 1996). The expectancy of microplastics being present in this study's samples is high, based on the results of studies such as Wieczorek et al. (2018) and Murray & Cowie (2011). Respectively, these studies found 73% and 62% of their overall specimens contained plastic in their GI tracts. Therefore, the effect size (f^2) was set to 0.6 due to the expectation that more than 50% of the overall sampled individuals would have plastics present in their system. Power was set to 0.8, and the number of predictors was set to "4" to represent the four sample locations. The output sample size was 13 individuals for each species per location. The predicted minimum was 52 individuals for each species of forage fish and 260 total fishes overall.

Data Acquisition:

All four locations (Port Everglades, Biscayne Bay, Islamorada, and Marathon; Fig. 1) were sampled for all five fish species with the use of cast nets, seine nets, pinfish traps, and traditional rod-and-reel gears. Because forage fishes often traveling in schools, it was assumed they have the same food source or were feeding together during the time of capture (Hipfiner et

al., 2018). To avoid potential biases associated with non-random sampling due to forage fish schooling behavior, only three specimens from any individual school were collected and analyzed. At every sample location, five or more schools were sampled per species of fish, ensuring that the sample group was unbiased. With collection methods such as seine fishing, where large areas of water can be sampled at once, each gear deployment was separated by a 50 m distance to minimize the risk of recaptures of previously released fish.

All individuals were immediately euthanized by immersion in MS 222 solution, using guidelines approved and recommended by the American Veterinary Medical Association (AVMA) (Underwood & Anthony, 2020) of 10 g MS 222 per liter of water; specimens were allowed to remain in the solution for ten minutes to ensure humane euthanasia. Individuals were labeled and packaged within Ziploc bags, then kept in long-term frozen storage (-20 °C) until dissection. All collection procedures were approved by the NSU IACUC per protocol 2021.09.DK17.

The weight and fork length of each specimen (mm) was recorded before processing. The gastrointestinal tracts and liver of all the collected samples were removed using a clean scalpel, forceps, and scissors, then weighed. The GI tract and liver were rinsed with DI water, then digested via potassium hydroxide solution (10% KOH) as described by Karami et al. (2016) and Abbasi et al. (2017). After each dissection, the tools used were cleaned with 70% ethanol to ensure no cross contamination occurred (Karbalaei et al., 2020).

All samples were processed at room temperature with 10% KOH, 3:1 body weight volume ratio, in glass mason jars sealed with aluminum foil until all organic matter was dissolved. Then, the remaining liquid was filtered through a 1 μm filter on a Buchner funnel, using a vacuum flask. To assess potential background incidental contamination levels, a blank sample was filtered between each individual fish. Between every fish, the cup of the filter was also rinsed with 70% ethanol to minimize potential environmental contamination (Karbalaei et al., 2020). Filters were then transferred to a petri dish to dry in an AirClean 600 workstation hood. Identification of microplastics was conducted via microscopic visual examination. To further minimize potential environmental microplastic contamination, a hairnet, cotton clothing, and nitrile gloves were worn at all times, and all lab procedures following digestion occurred under the workstation hood.

Once filters were fully dried, a dissecting microscope was used to identify microplastics present, using procedures described by Battaglia et al. (2020). Any particles suspected to be microplastic were digitally photographed (Fig. 2) to document their size, color (light, mid, dark), and type of particle (fiber versus fragment). The lengths of individual microplastics were measured via a 3.5x-180x trinocular stereomicroscope with an LED ring light connected to a 10 MP camera. Using Amscope- AmLite, the longest endpoint to endpoint was measured for each piece. For fibers that appeared curved or twisted, small straight-lined vectors were used to trace the pieces, with the sum of all vectors used for the final length measurement. Any particles that remained unconfirmed pieces of microplastic were inspected via heat testing (Plee & Pomory, 2020). A heated pin was applied to all unconfirmed pieces; if a piece melted and produced a melted plastic aroma, it was considered plastic.

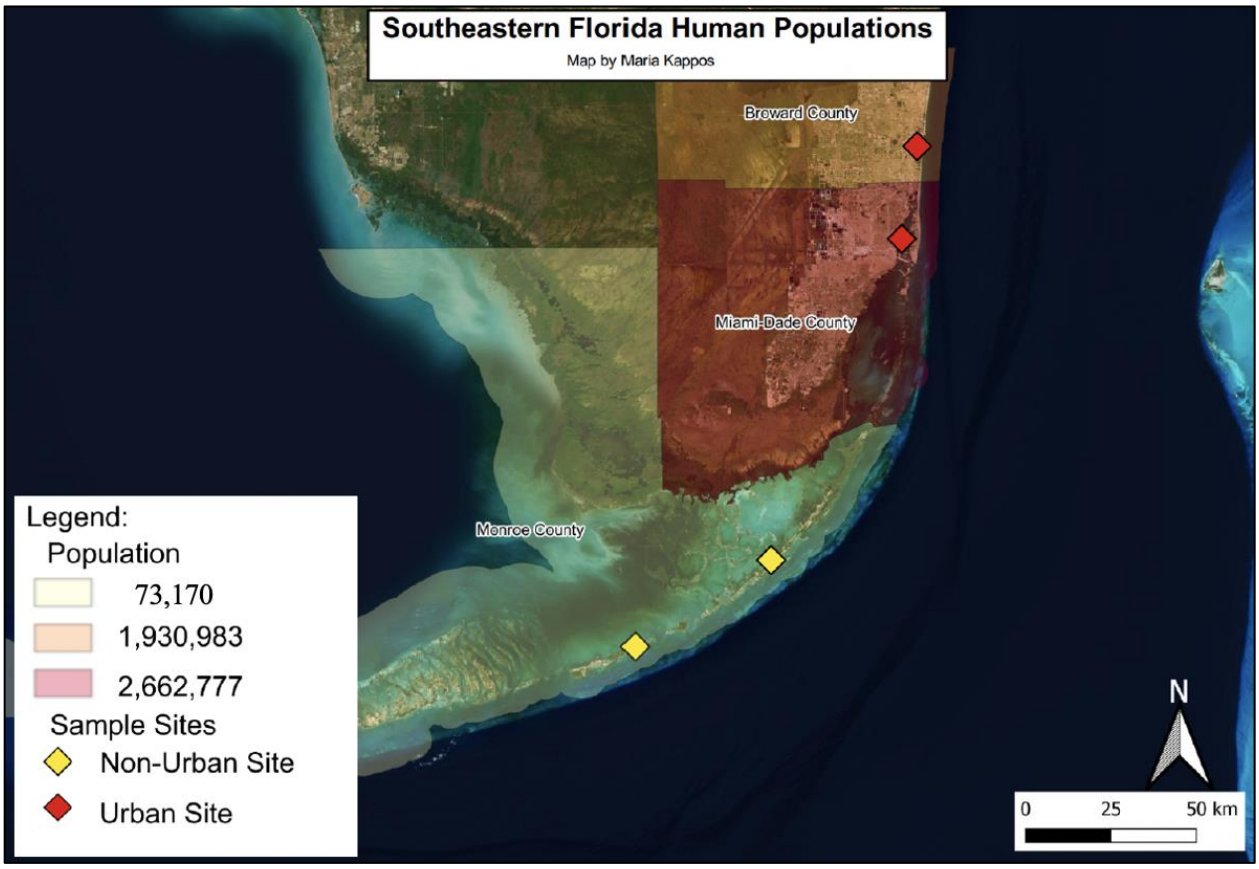

Figure 1. Map of south Florida counties, populations, and sample sites represented.

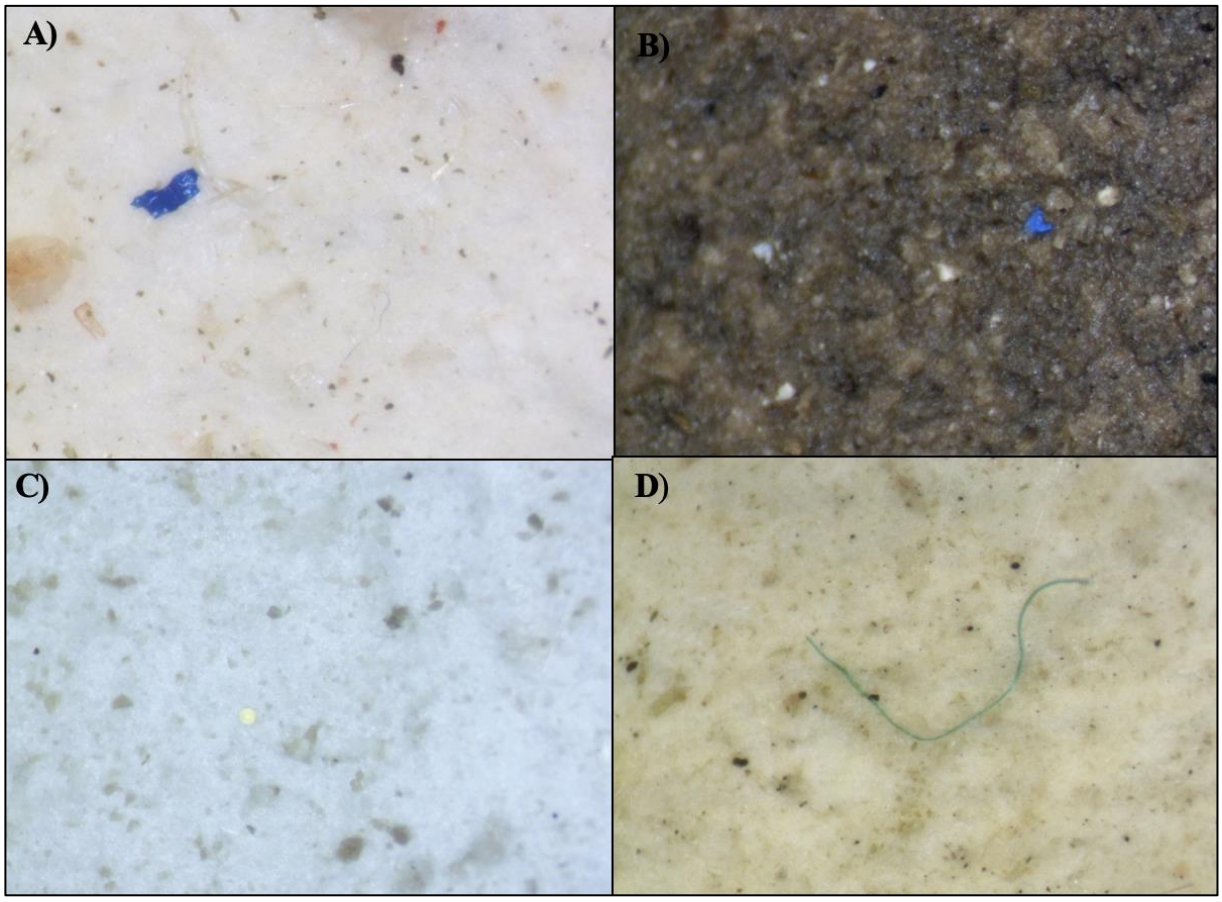

Figure 2. Examples of found microplastics: A) mid colored fragment found in the GI tract of a Scaled Sardine (specimen #196) in Marathon, B) mid-colored fragment found in GI tract of a Stripped Mullet (specimen #211) from Port Everglades, C) light-colored microbead found in GI tract of a Scaled Sardine (specimen #43) from Biscayne Bay, D) mid-colored fiber found in GI tract of a Striped Mullet (specimen #172) from Marathon.

Results:

Microplastics data

A total of 2,126 pieces of microplastics were successfully identified and measured from the GI tracts and livers of 248 fishes. Every specimen except for one was found to have microplastics within their systems (99.6% frequency). The mean number of microplastics present in each fish was 8.57 (± 10.03 pieces). All microplastics found varied in size, shape, and color. The mean length of the microplastics found was 0.93 mm (± 0.98 mm) and the median was 0.56 mm (Fig. 4).

Microbeads, fragments, and fibers were all present in the stomach contents and livers of the forage fishes collected with the most abundant type being fibers, accounting for 1,483 pieces (69.8% frequency) (Fig.11), followed by microbeads accounting for 406 pieces (19.1%), and then fragments with the lowest abundance of 237 pieces (11.1%). The mean sizes of these three different forms of microplastics varied greatly, with the fibers having a mean length of 1.25 mm (± 1.01 mm). Microbeads and fragments were similar in mean length, 0.17 mm (± 0.07 mm) and 0.19 mm (± 0.22 mm), respectively (Fig. 5). 'Light', 'mid', and 'dark' colors were all present in the specimens. The most abundant color was 'light' with 778 pieces (36.6%), followed by 'dark' (35.2%), and then finally 'mid' (28.2%).

Northern Biscayne Bay (BB) was observed to have the highest concentration of microplastics present among the four sample locations with a total of 882 pieces (total fishes sampled, n=65), and a mean of 13.57 pieces per fish (± 15.19 pieces). Fibers accounted for more than half of the total microplastics found in this location (60.2%), followed by microbeads (29.8%), and then fragments (10%). Compared to the other three locations, BB had the largest amount of microbeads, accounting for 120 more beads than the other three locations combined. Islamorada (IM) followed with the second highest amount of microplastics with 500 individual pieces (n=65), and a mean of 7.69 pieces per fish (± 9.11 pieces). Of those 500 pieces, Fibers made up 367 of the total pieces, followed by microbeads with 91 pieces, and lastly, fragments accounted for 42 pieces. Marathon (MA) accounted for 382 pieces of the total microplastics (n=65), and a mean of 5.88 pieces per fish (± 4.38). The majority of the 382 pieces were in the form of fibers (82.5%), with 14.4% fragments, and 3.1% microbeads. Finally, fishes from Port Everglades (PE) had a total of 362 pieces of microplastics (n=54) present within them, for a

mean of 6.83 pieces per fish (±4.85 pieces). Fibers accounted for 74.6%, fragments made up 14.4%, and then microbeads with 11%. All planned sample sizes for each location were achieved except for Pinfish in Port Everglades, which only had one individual captured.

The blanks were excluded from the total number of microplastics to avoid an inaccurate representation of microplastics within the fishes. Of the 248 blank samples, 118 pieces of microplastics were found, averaging 0.48 pieces per sample (±0.72 pieces). Fibers were the most prominent form of microplastics in the blanks by far, accounting for 117 (99.2%) pieces and one fragment (0.8%) (Fig. 11). Not a single microbead was found in any blank.

The GI tracts showed a larger mean of microplastics compared to the mean of microplastics found within the livers. The GI tracts had a total of 1,317 pieces of microplastics (n=248), averaging 5.31 pieces per GI tract (±8.33 pieces). Fibers were the most prevalent with 774 pieces (58.8%), then microbeads accounted for 386 pieces (29.3%), and finally fragments with 156 pieces (11.9%) (Fig. 11). In comparison, the microplastics that were found in the livers totaled 809 pieces (n=248), averaging 3.26 pieces per liver (±3.24 pieces). Again, fibers were the most abundant form of microplastic with 709 (87.6%), followed by fragments totaling 80 pieces (9.9%), then finally microbeads with 20 pieces (2.5%) (Fig. 11).

Macroplastics

Pieces larger than the defined length of a microplastics (<5 mm) were measured but removed for reporting the sampled totals and subsequent analyses. A total of 32 pieces were found that were larger than 5 mm in length. Of the 32, 31 were identified as fibers (96.9%) and one was identified as a fragment (3.1%). Three of the ‘macroplastics’ were found in the blank samples (9.7%), 16 were identified in GI tracts (50%) and 13 were from the liver samples (40.6%). The average length of the ‘macroplastics’ was 6.59 mm (±1.58 mm).

Location vs Total

A one-way ANOVA was utilized to test the effect of sample location on the total microplastics found (2,126 pieces of microplastics found amongst 248 fishes). The data was not normally distributed and did not pass homogeneity assumptions, even after logarithmic and square root transformation, resulting in the use of the non-parametric Kruskal-Wallis test.

There was a significant difference between total microplastics found and location (Kruskal-Wallis chi-squared = 22. 868, p-value = $4.302e^{-05}$, df = 3). The results revealed that North Biscayne Bay was significantly greater than the other three locations (Fig. 7). When the locations are classified into urban and non-urban there are similar results. The 'non-urban' locations (Islamorada and Marathon) are significantly different than the 'urban' locations (Kruskal-Wallis chi-squared = 11.862, df = 1, p-value = 0.0005728).

Species vs Total

A one-way ANOVA was used to test if the different species ingested different amounts of microplastics. As before, the data was not normal and did not pass homogeneity assumptions even after transformation. A non-parametric Kruskal-Wallis test showed that there was no significant difference between species and the total microplastics ingested (chi- square = 1.1553, df = 4, p-value = 0.9034) (Fig. 8). Additionally, a one-way ANOVA was used to evaluate the difference between feeding habits. The five species were split into three feeding habits: pelagic (Redfin Needlefish), filter-feeders/detritivores (Striped Mullet and Scaled Sardine), and benthic invertivores (Irish Mojarra and Pinfish). After running a non-parametric Kruskal-Wallis test, results showed no significant difference in total microplastics within the different feeding habits (chi-squared = 0.60727, df = 2, p-value = 0.7381) (Fig.9).

Bioaccumulation

To understand if microplastics showed patterns of bioaccumulation in this study, a general linear model (GLM) was used to see if individual fish size (e.g., fork length) affected the total number of microplastics found within the fishes. Of the five species sampled, two were found to be significant: Redfin Needlefish (Pr(>|z|)= 0.00428) and Pinfish (Pr(>|z|)= 0.026356).

Species vs Total Microplastics in Liver

A one-way ANOVA was used to test if species influenced the total microplastics found within the liver. The data was not normally distributed and parametric assumptions (e.g., normality and homogeneity of variance) were not met, resulting in the test turning into a GLM. Two of the species showed a significant difference, Stripe Mullet (Pr(>|z|) = 0.000184) and Pinfish (Pr(>|z|)= 0.006891).

Microplastic size

A factorial ANOVA was used to see the relationship between tissue, species, and location on the size of the microplastics found. Due to the data being abnormal and parametric assumptions (e.g., normality and homogeneity of variance) not being met, a GLM was then ran. In regard to the factors individually, only three species were found to significantly influence the size of microplastics. Specifically, Striped Mullet, Pinfish, and Scaled Sardines with Pr(>|z|) values of 5.14e-09, 8.19e-09, and 1.14e-05 respectively. The relationship between tissue and species has an effect on the size of the microplastics. The size of microplastics found in the livers of Striped Mullet, Pinfish, and Scaled Sardines were significantly different than the other two species with Pr(>|z|) values of 0.04084, 0.02972, and 0.02872 respectively. Pinfish showed larger pieces of microplastics than the other sampled species. Whereas, Scaled Sardines and Striped Mullet had smaller pieces (Fig. 12). Lastly, the relationship between species and location was only significant in one pair. The size of the microplastics found within the Pinfish of Islamorada are significantly different (Pr(>|z|) = 0.00189).

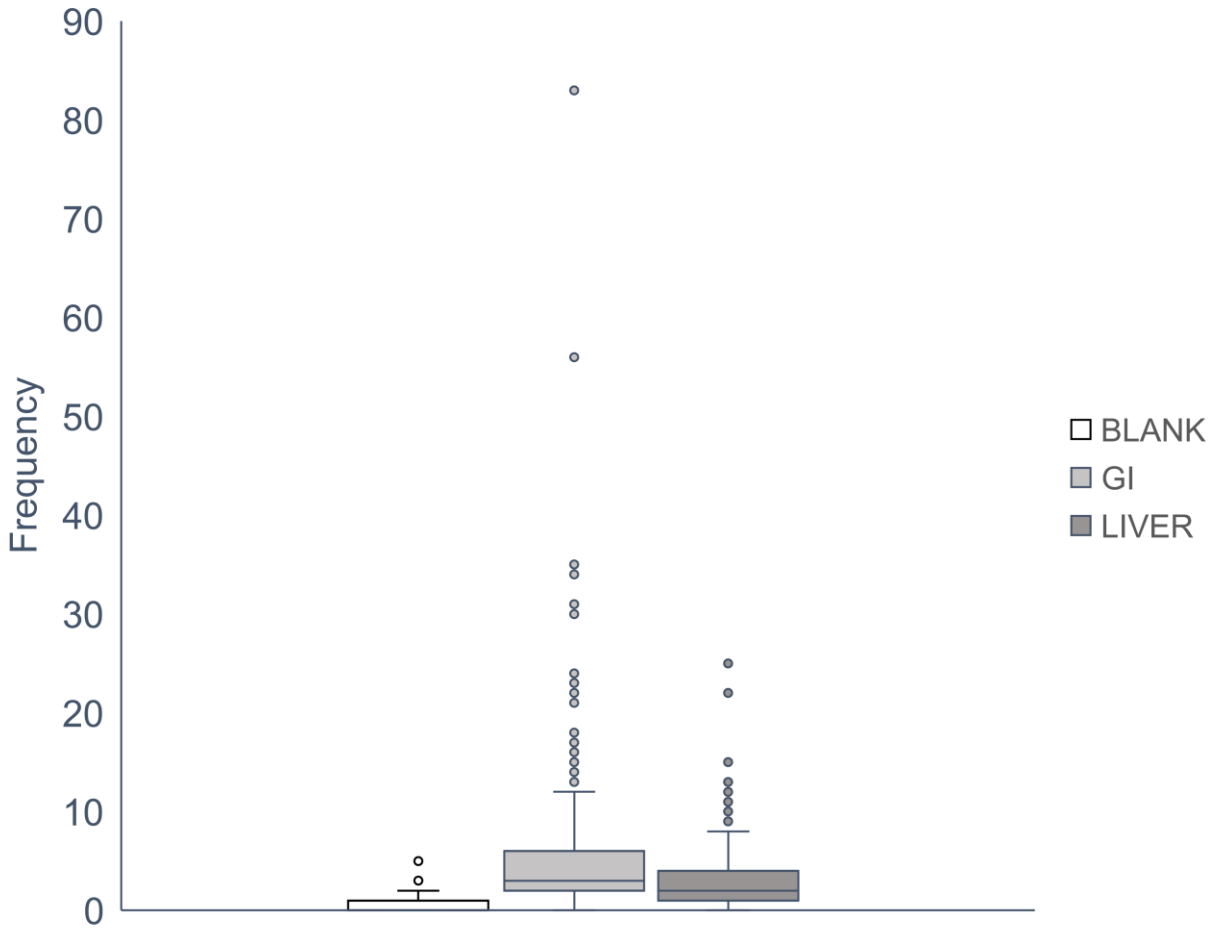

Figure 3. Boxplot of microplastic frequency in Blank, GI tract, and Liver of sampled fishes, all species combined (n=248).

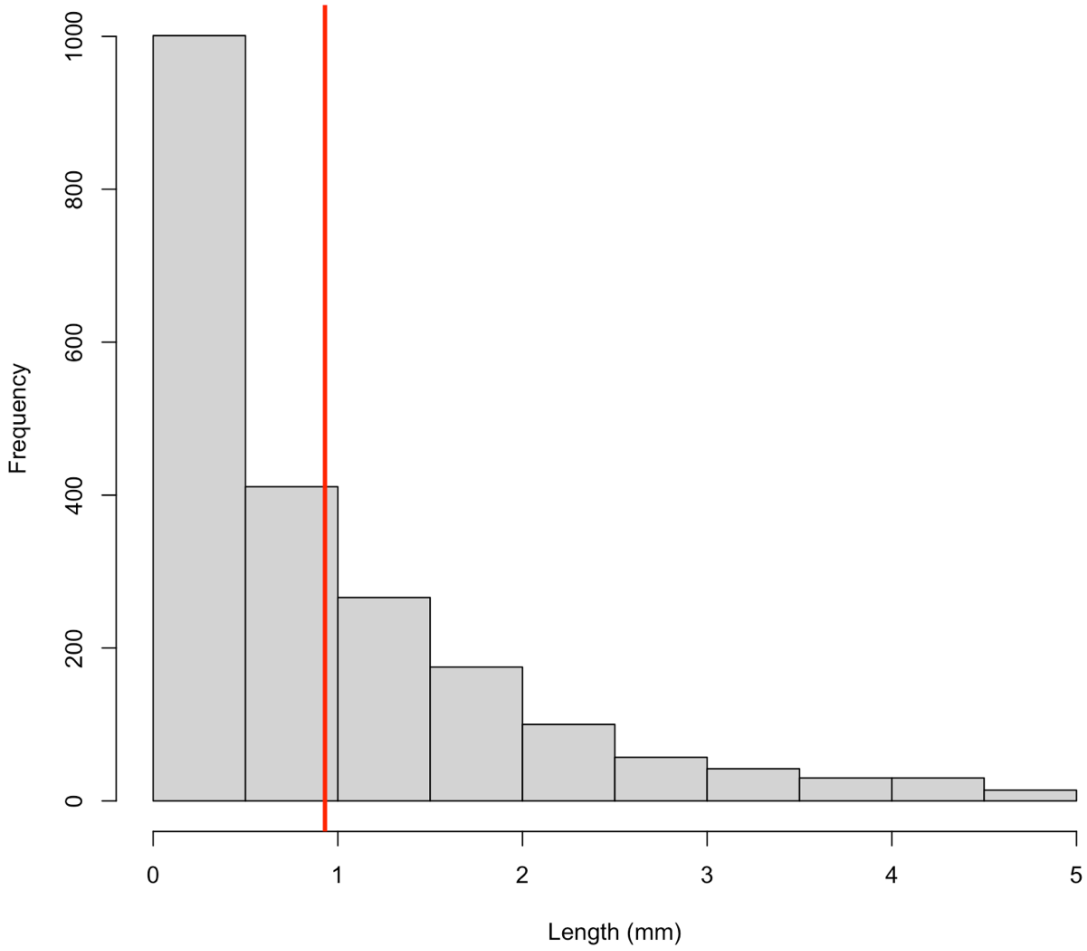

Figure 4. Histogram of size (mm) and frequency of the microplastics, mean line in red. All plastics larger than 5mm in length were excluded, as well as microplastics found in the blank samples.

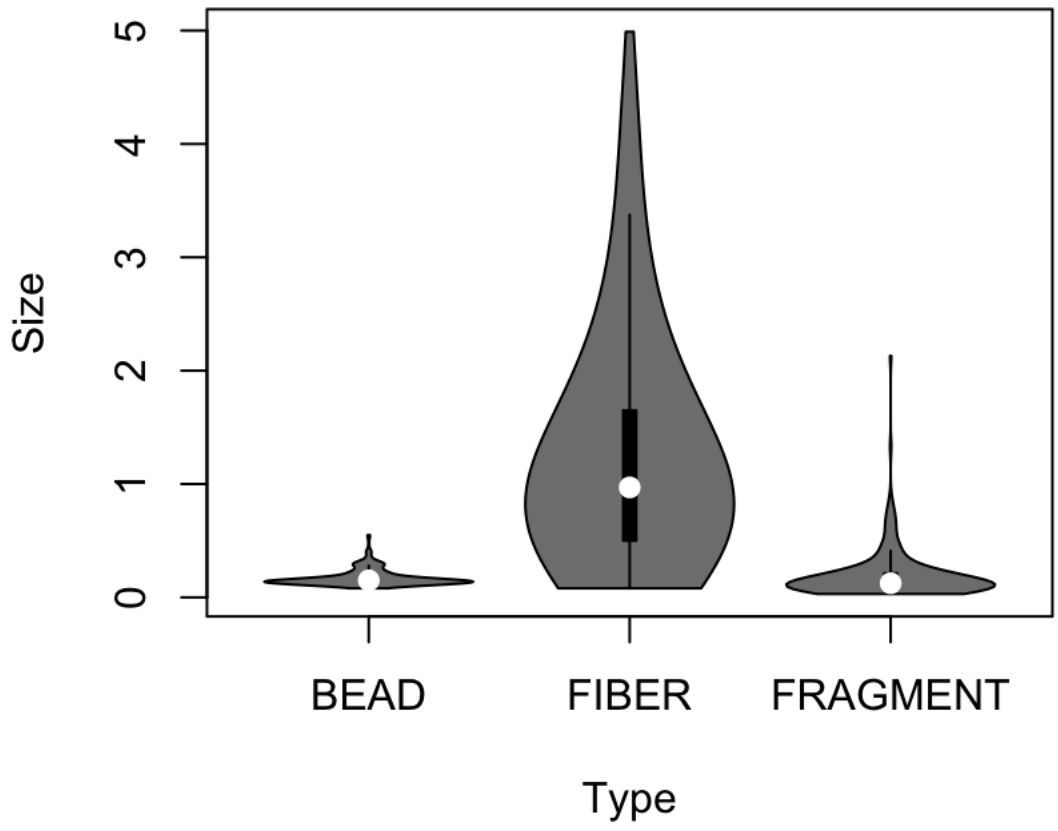

Figure 5. Violin plot of size (mm) distribution amongst microplastic (n=248) type: bead, fiber, fragment. The white circle indicates the median, the bolded rectangle represents the interquartile range, thin vertical line shows the 1.5x interquartile range and the shadowed area shows the probability.

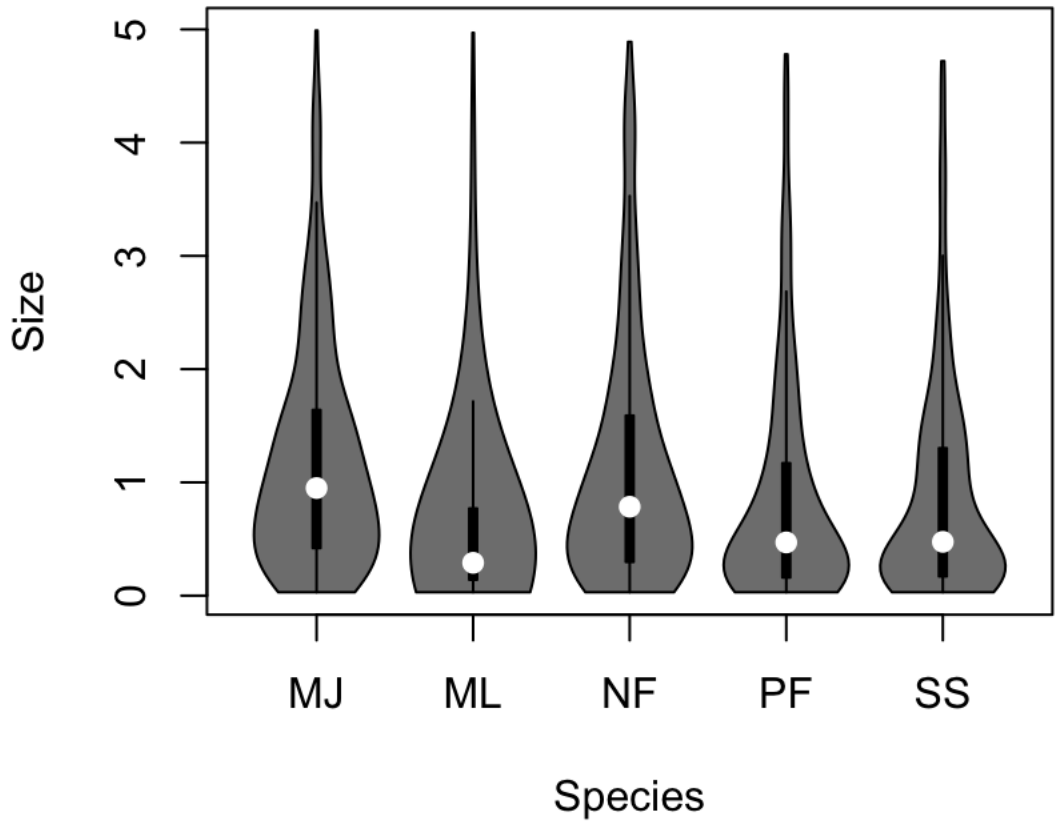

Figure 6. Violin plot of microplastics size (mm) within the five different fish species (n=248). MJ= Irish Mojarra, ML= Striped Mullet, NF= Redfin Needlefish, PF= Pinfish, SS= Scaled Sardine.

Species	FL Range (mm)	FL Mean (mm)	Diet	Source
Redfin Needlefish	119-416	253.21	Silversides, anchovies, crustacians, and insects	Porter & Motta, (2004)
Irish Mojarra	52-256	89.12	Copepods, amphipods, nematodes, shrimp, and ostracods	Kerschner et al., (1985)
Striped Mullet	101-344	232.23	Diatoms, desmids, green algae, crustaceans, nematodes, and invertebrate eggs	Eggold & Motta, (1992)
Scaled Sardine	61-123	83.92	Copepods, pelecypod veligers, and decapod zoea	Modde & Ross, (1983)
Pinfish	75-391	129.63	Algae, seagrass, polychaetes, and crustaceans	Luczkovich & Stellwag, (1993)

Table 1. The fork length (FL) range and mean of all fishes sampled (n=248) with their known diets. The information within this table was used to examine bioaccumulation from prey items to larger fishes within each species.

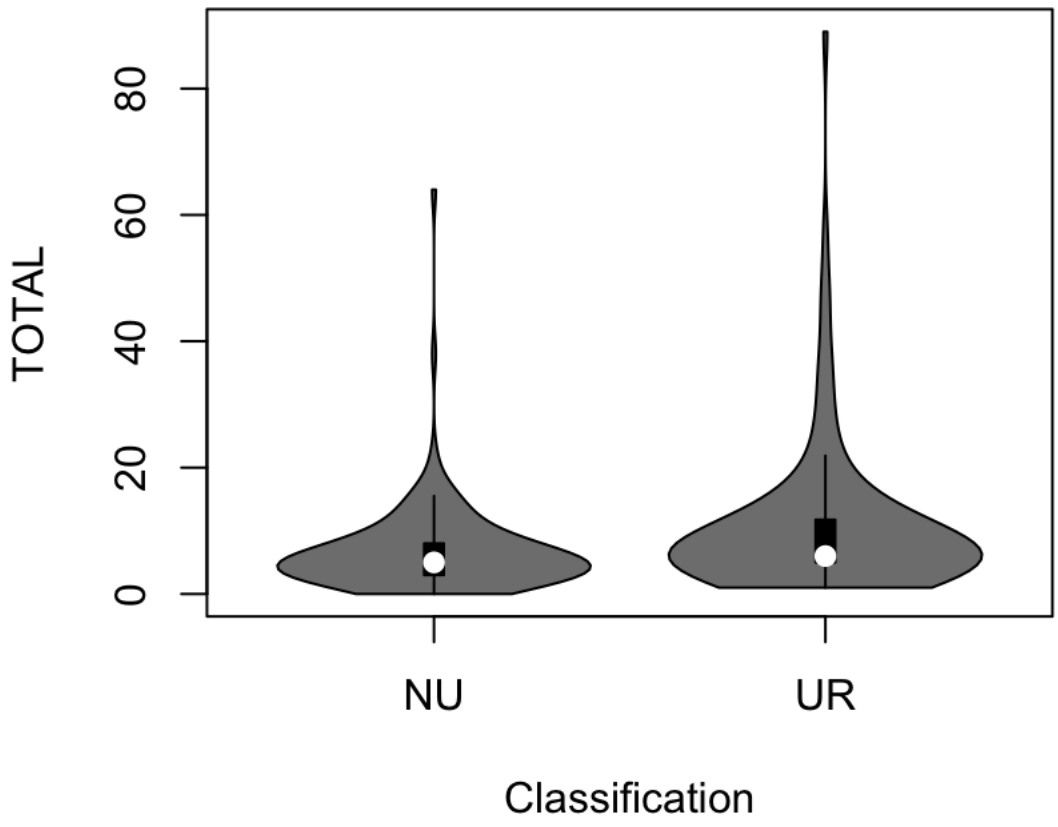

Figure 7. Violin plot of the mean microplastics found within the sampled fishes (n=248). Locations were grouped into classification of non-urban (NU) and urban (UR). The non-urban locations include Islamorada and Marathon, while urban includes Port Everglades and Northern Biscayne Bay. Biscayne Bay had a significantly higher average of microplastics compared to the other three locations, including the other urban location.

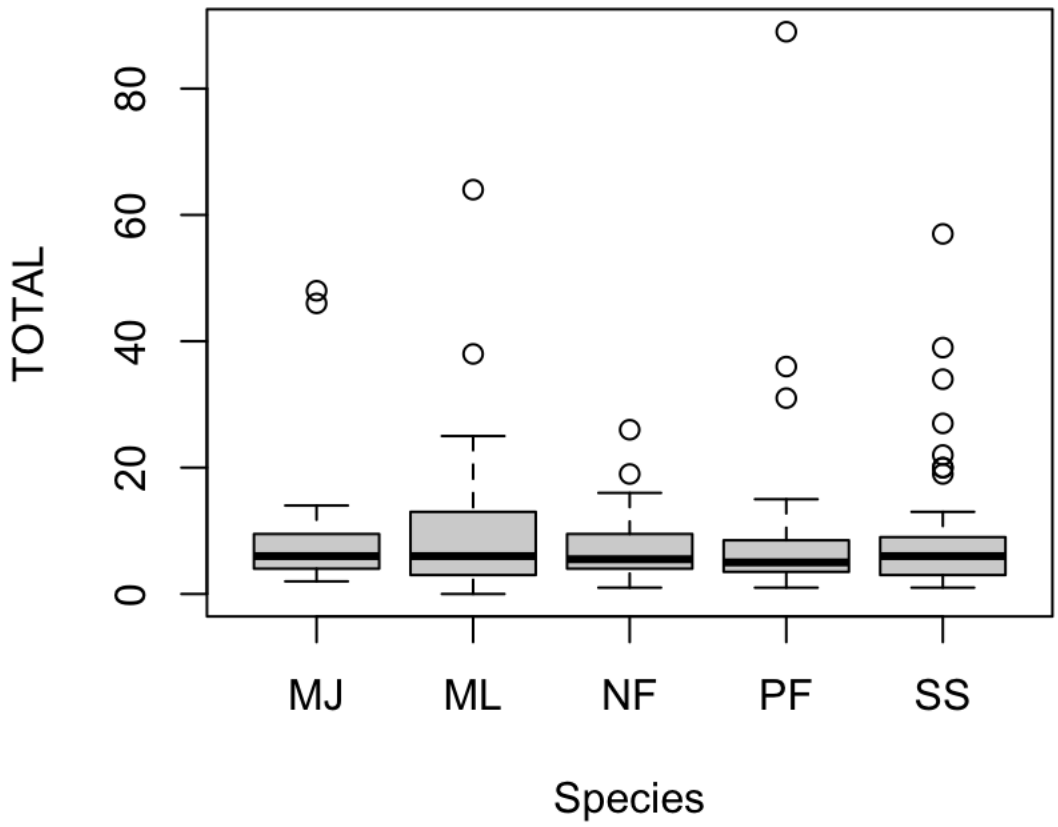

Figure 8. Boxplot of mean microplastics found in all sampled fishes (n=248) organized by species. MJ= Irish Mojarra, ML= Striped Mullet, NF= Redfin Needlefish, PF= Pinfish, SS= Scaled Sardine. None of these fishes were significantly different in mean numbers of microplastics per individual.

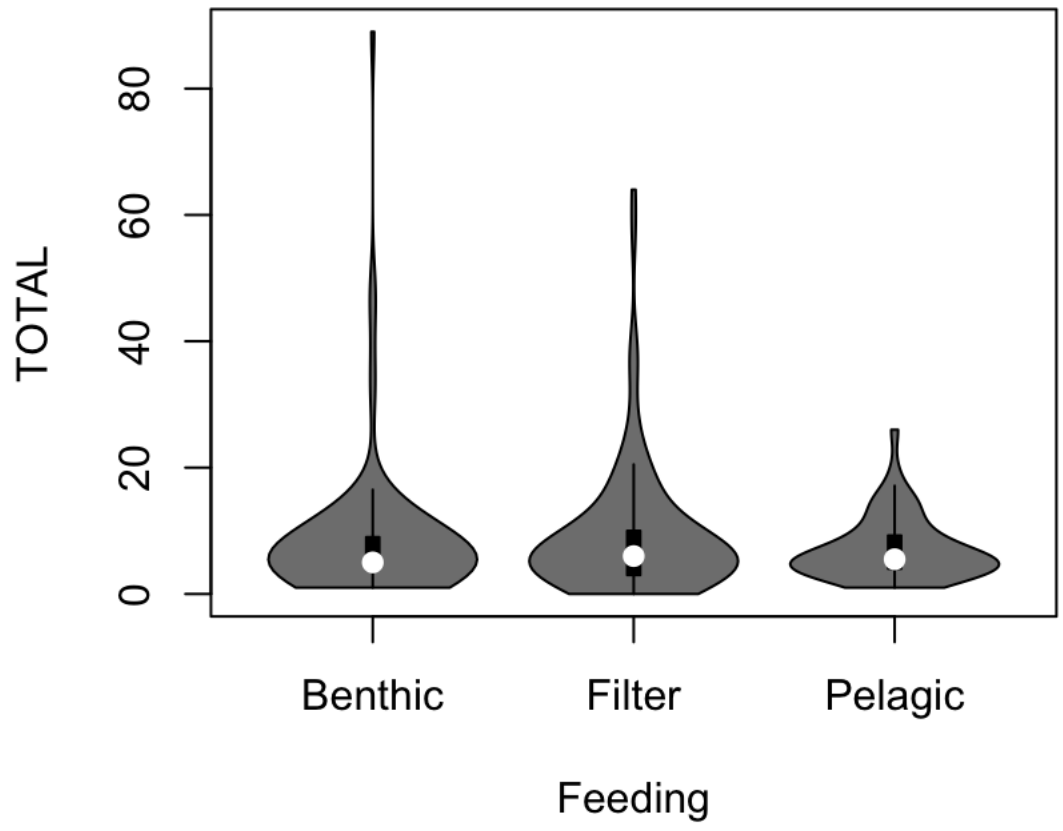

Figure 9. Violin plot of total microplastics within the different feeding habits. Pelagic (Redfin Needlefish), filter-feeders/detritivores (Striped Mullet and Scaled Sardine), and benthic invertivores (Irish Mojarra and Pinfish). Results showed no significant difference between feeding habit in regard to total number of microplastics.

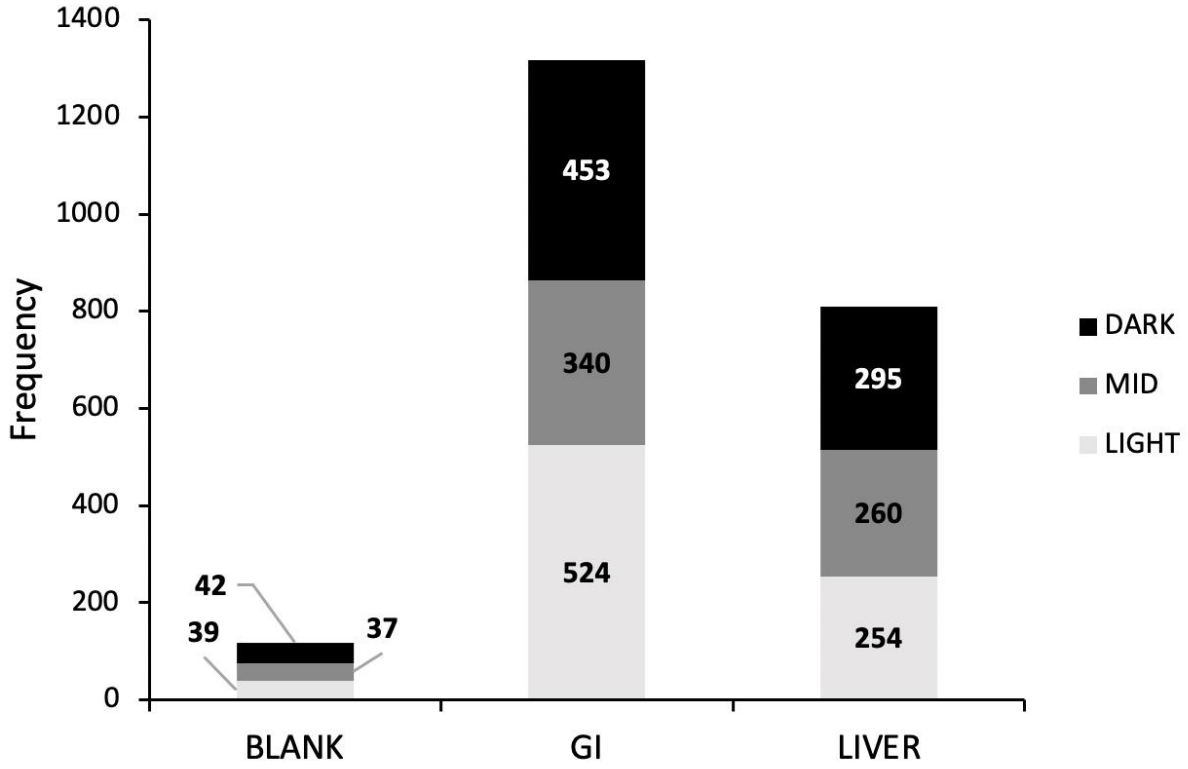

Figure 10. Quantity of colors found within the Blanks, GI tracts, and Livers of sampled fishes (n=248). The numbers in the chart are the n values for that group. Blank: Light (n=39), Mid (n=37), Dark (n=42). GI Tract: Light (n=524), Mid (n=340), Dark (n=453). Liver: Light (n=254), Mid (n=260), Dark (n=295).

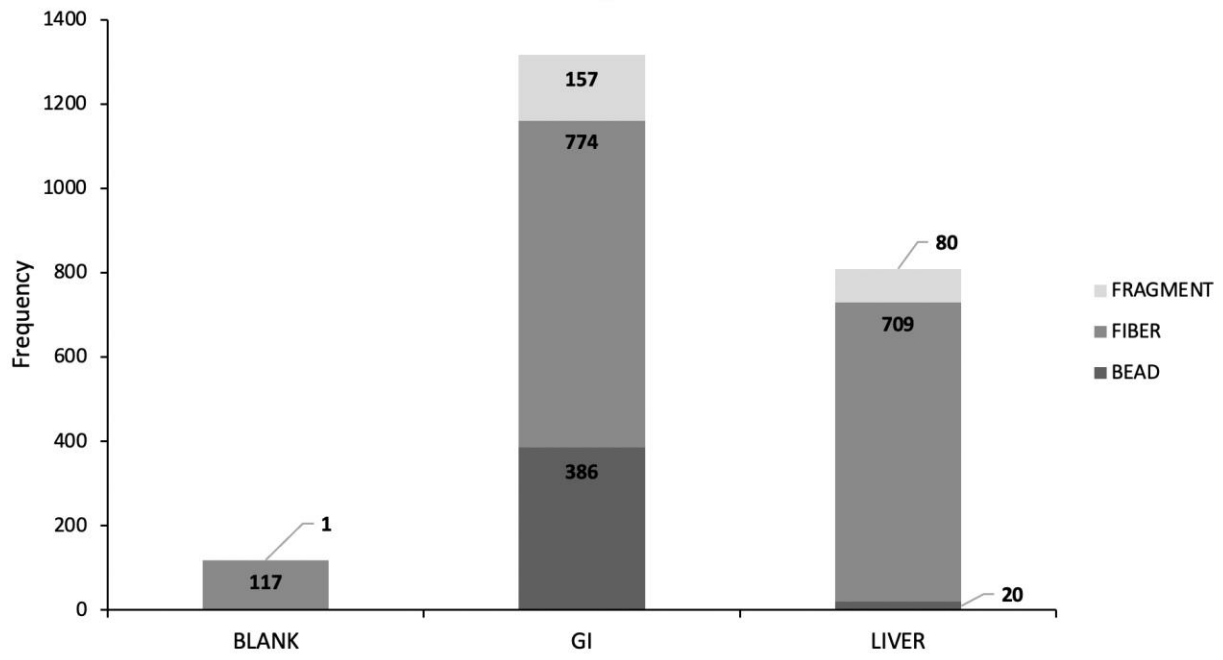

Figure 11. Quantity of type of microplastics found within the Blanks, GI Tracts, and Livers. The numbers in the chart are the n values for that group. Blank: Bead (n=0), Fiber (n=117), Fragment (n=1). GI Tract: Bead (n=386), Fiber (n=774), Fragment (157). Liver: Bead (n=20), Fiber (n=709), Fragment (n=80).

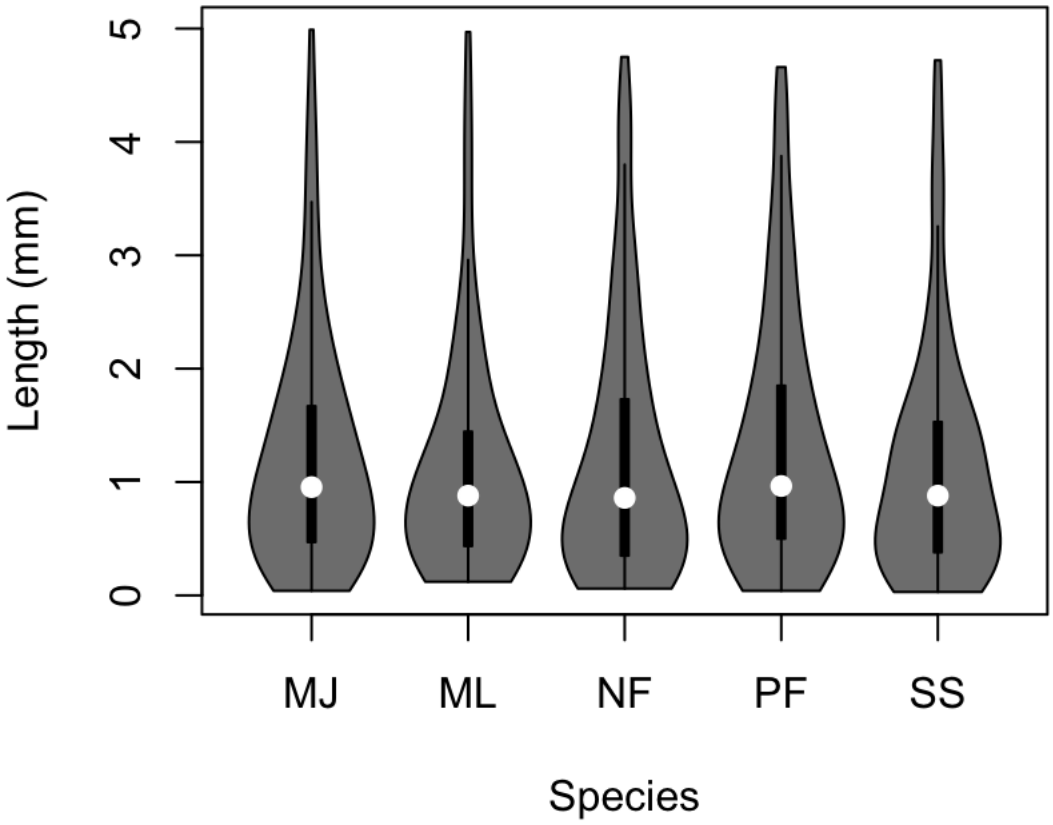

Figure 12. Violin plot of microplastics length within the livers of sampled species. MJ= Irish Mojarra, ML= Striped Mullet, NF= Redfin Needlefish, PF= Pinfish, SS= Scaled Sardine. Pinfish averaging larger pieces of microplastics than the other sampled species. Whereas Scaled Sardines and Striped Mullet averaged smaller pieces.

Discussion

Microplastic Data

A total of 2,126 pieces of microplastics were found in all specimens, with an average of 8.573 pieces per individual (± 10.03 pieces). Only one fish out of 248 samples did not contain microplastics (99.6% found with microplastics). The results are consistent with previous studies. For example, Tanaka & Takada (2016) found a 77% frequency of microplastics within their samples, with an average of 2.3 pieces of microplastics within planktivorous fishes in urban coastal waters of Tokyo Bay. Similarly, Bakir et al. (2020) documented the frequency of microplastics in three species of forage fishes: European anchovy *Engraulis encrasicolus* (57%), Whitehead's round herring *Etrumeus whiteheadi* (72%), and Pacific sardine *Sardinops sagax* (72%). Additionally, in 2015 and 2016, forage fishes of Regina, Saskatchewan, Canada were sampled for microplastics in the GI tracts (Campbell et al., 2017). Their results showed that 73.5% of their total specimens were contaminated with microplastics (Campbell et al., 2017). No previous study has monitored the frequency of microplastics within the forage fishes of southeastern Florida, however, microplastic studies of their predators have recently been published.

Clark et al. (*In press*) assessed microplastics present with seabirds from southeast Florida, reporting a frequency of 97.7% of microplastics within their sampled specimens (n=44) and an average of 14.6 particles per individual seabird. Comparing the finding of Clark et al. (*In press*) and this study supports the hypothesis that microplastics transfer from prey to predator as also seen in other studies (e.g., Ferreira et al., 2018). The accumulation of microplastics among marine organisms can lead to several health risks such as intestinal blockages, alterations to lipids metabolism, and other physical injuries that can result in death (Jovanovic, 2017). The risk of toxicity is another potential impact of microplastics, due to the leaching of POPs (Geyer et al., 2000), which can result in reproductive failure and immune deficiencies (Litz et al., 2007).

Within the blanks, all except one piece, were identified as fibers, showing the ubiquitous nature of microplastics, especially fibers, and their ability to contaminate the air. The single fragment could also have resulted from the wear and tear of the plastic filter cup over time. Additionally, the fact that no microbeads were found in any blank supports the hypothesis of microplastic pollution as a result of sewage drainage into marine environments. Running blanks,

or control samples, is important to ensure the microplastics identified were present in the fish's system and not due to contamination from processing of the samples. The blanks in this study averaged 0.48 pieces per sample (±0.72 pieces), considerably lower than microplastics found in the GI tracts and livers, indicating that the microplastics found within those organs were not a result of outside contamination.

Species vs Total

No significant relationship was found between the fish species and total microplastics found or feeding habits and total microplastics; all five targeted fishes contained similar concentrations of microplastics. In previous studies, Justino et al. (2021) found a difference in microplastics among different feeding habits of their sampled fishes. Detritivores, specifically, were found to have the lowest concentration of microplastics (67% frequency), followed by zoobenthivores with a frequency of 74%, with the highest concentration was found in piscivores (77%). Findings from Justino et al. (2021) findings support the hypothesis that microplastics have the ability to bioaccumulate within larger piscivores towards the top of the food web. However, they assessed the frequency of microplastics between three fishes feed at different trophic levels. In this study, we looked at the microplastic concentration among fishes with different feeding habits but within similar trophic levels (Torres, 2009). The sampling difference may be the reason for conflicting outcomes. For future research, the analysis of microplastics across various trophic levels of southeastern Florida fishes may provide insight on the mechanisms microplastics can be transfer through the trophic levels.

Size of Microbeads

The average size of the microbeads (0.17 mm) is consistent with the average size of microbeads from personal care products (PCPs) (Sun et al., 2020; Guerranti et al., 2019). Microbeads are found in a variety of products such as cleaning products, makeup cosmetics, shower gels, toothpaste, shaving cream, and shampoo (Sun et al., 2020). The pollution of ubiquitous microbead microplastics is typically from primary sources in domestic and industrial locations (Sun et al., 2020). Microbeads have been banned in a number of large, developed nations including Canada, the United States of America, Ireland, the United Kingdom, France, Sweden, the People's Republic of China, South Korea, and New Zealand (Anagnosti et al.,

2020). Unfortunately, other single-use plastics are still widely produced and consumed, adding to an ever-growing amount of marine debris that will degrade down to other forms of microplastics.

Location vs Total

The location had a significant influence on the concentration of microplastics found within the sampled fishes. Northern Biscayne Bay was significantly different than the other three sample locations with 882 total pieces, and a mean of 13.57 pieces per fish. Although Port Everglades was classified as an urban location along with northern Biscayne Bay, the difference in microplastic concentration could be a result of a smaller sample size. However, the large number of microplastics in northern Biscayne Bay still supports the hypothesis that a highly urbanized area would be associated with a higher concentration of microplastics within the sampled fishes.

The north end of Biscayne Bay is highly urbanized and has seen decades of sewage runoff resulting in large amounts of marine debris and chemical pollution (Caccia & Boyer, 2005). Although this is the first study to assess the microplastics within forage fishes of Biscayne Bay, other studies have monitored the concentration of POPs within their predators of Biscayne Bay. For example, Litz et al. (2007) documented POPs in bottlenose dolphins throughout the Bay, finding that males with sighting histories in the northern (urban) portions of the bay had five times higher concentrations of polychlorinated biphenyl (PCB) congeners than the males sighted in the southern (rural) part of the bay. Although this contamination was not linked to microplastic leaching, leaching of POPs from plastic pollution can lead to even higher concentrations within the marine organisms that inhabit the northern part of Biscayne Bay.

Bioaccumulation

Of the five species sampled, two were found to have higher microplastic concentrations as they increase in size: Redfin Needlefish and Pinfish. The increase with size can be associated with the change in diet as the fish matures. As Redfin Needlefish mature, their diet shifts from prey such as insects and crustaceans to larger fishes such as silversides and anchovies (Porter & Motta, 2004). Silversides and anchovies are other forage fishes (Bayfill, 1950; McClatchie et al., 2018) that could be expected to have microplastics within their systems such as the fishes in this study, thus supporting the hypothesis that microplastics have the ability to transfer from prey to

predator. However, Pinfish diets shift differently. As Pinfish grow, their diet shifts from crustaceans to algae and seagrass (Luczkovich & Stellwag, 1993). Seagrass beds are known to have an accumulation of settled microplastics (Plee & Pomory, 2020), this could explain the increase of microplastics in Pinfish as they increase in length.

Statistical Disclaimer

Due to the data being abnormal and not fulfilling parametric assumptions, the results of this study could have a level of error.

Potential Impacted Fisheries

The presence of microplastics in forage fishes will not only impact the overall health of the individual fish but also effect the organisms that prey on them. Specifically in southeastern Florida, these larger predators include the Common Snook, Bonnethead Sharks, Bottlenose Dolphins, White Pelicans, Red Snapper, Vermilion Snapper, Amberjack, and many others (Bethea et al., 2007; Blewett et al., 2006; Chagaris et al., 2015; Whitefield et al., 2012). All the listed predators are large fisheries that the state of Florida heavily rely on (Ault et al., 2005). Trophic transferring can cause a decrease in overall health and fecundity in the predators, resulting in smaller fish stocks. Further research regarding the true implication on overall health and fecundity is necessary for future regulations and conservation efforts to ensure these fisheries are at sustainable levels.

Pinfish in Port Everglades

The original desired sample size for Pinfish was n=13 for Port Everglades. Unfortunately, sampling for Pinfish was difficult, resulting in only one Pinfish captured in this location. To ensure our sample sizes were adequate, we made some adjustments to our G*Power parameters. We expected to find microplastics present in >60% of the stomach content of all specimens based on the findings from similar studies on small fish species based all over the world (Bakir et al., 2020; Campbell et al., 2017; Tanaka & Takada, 2016). The frequency of microplastics present in the sampled fished was 99.6% (n=248). Based on that frequency, the sample size drops to 10 individuals per species per location. Additionally, those sample sizes are based on four predictors (e.g., location) however, we sub-categorized those locations into ‘urban’ and

‘non-urban’ creating two predictors. By doing this sample size drops to 9 fish per species for both ‘urban’ and ‘non-urban’. Bringing the total required sample size to 90, putting this well over with a sample size already at 248. The lack of Pinfish samples within Port Everglades could be a result of seagrass degradation from boating activity. According to Broward County, dredging plans to expand Port Everglades is to occur by 2025 (Port Everglades Department). Plans for future dredging may impact the organisms that rely on seagrass beds for both habitat and food, altering complex ecosystems and food webs (Hallac et al., 2012).

Future Considerations

We encountered several complications during this study, varying from funding restrictions, public fishing access, and time restrictions. Initially, we planned to digest the remaining fish body for microplastics present in separate tissues, including a combination of the skeleton, skeletal muscle, and integument, gills, reproductive system, and GI tract. Unfortunately, the digestion of bone and scales via 10% KOH was very difficult. Previous research suggested that processing the bones on a heated stirrer would help break down the bones (Thiele et al., 2019; Dehaut et al., 2016). The heated stirrer did break down the bone but also created a silt-like mixture which made filtering very time-consuming and required a large number of filters and petri dishes to filter whole samples. We used up to 75 filters and petri dishes for some of the larger Striped Mullet, with some of the smaller fishes using between 10 to 20 filters and periti dishes. Sieving out the bones after tissue appeared to be fully dissolved was the next attempt, however, also required a large amount of time and materials. Although this method did cut down material use the larger fishes still required up to 27 filters for the whole body. We attempted to filter the whole body of 15 fishes. On average, to digest the whole fish, filter, examine filters, and measure any found microplastics required one to two weeks per fish. Thus, we removed the whole fish from this study to save time and resources. Future studies with no time and funding restraints could make this feasible.

Finally, heavily developed locations in southeastern Florida like northern Biscayne Bay have strict trespassing regulations, and we were limited to sampling from bridges, public beaches, and seawalls. Pinfish are a popular baitfish species in the local recreational fisheries and were the most difficult to sample in the Florida Keys, possibly from localize depletion in accessible locations near public fishing access points. For future studies, having access to remote

locations not subjected to localize depletion and public beach access may be the best for sampling locations. In Port Everglades, we were not able to catch our desired sample size of 13 specimens, also likely due to localize depletion and lack of suitable habitat such as seagrass beds within the port.

Conclusion

This was the first study to assess microplastics within the forage fishes of southeastern Florida and was able to conclude that microplastic contamination can be correlated to human population size. Although some plastic bans are in place, the plastics already present within these ecosystems will persist for thousands of years and continue to degrade into smaller pieces increasing the chances of ingestion (Jovanovic, 2016). Significant efforts must be taken to lessen the impact of this pervasive pollutant in order to conserve our southeastern Florida marine organisms.

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