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Project Title:
Microplastic Exposure for Key Ecological species in Coastal South Carolina

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Project Location
ACE Basin/St. Helena Sound along coastal South Carolina

Project Goal:
To improve our understanding of the prevalence and type of microplastics in the stomachs of ecologically significant South Carolina State Wildlife Action Plan Species: Atlantic Menhaden, Mummichog, Striped Mullet, Spot and Southern Flounder in the ACE Basin.

Background

The South Carolina State Wildlife Action Plan (SWAP) denotes the importance of managing essential habitats for marine fishes, suggesting marine fishes would best be protected by managing such habitats instead of focusing on species-specific restoration techniques (SCDNR 2015). One of the most important of these essential fish habitats are estuaries and tidal marshes, which serve as key habitats for all but four marine fish species listed in the SWAP (see Appendix 1-C SCDNR 2015). The value of estuaries and tidal marshes stems from their dynamic nature and unique ecological position of connecting terrestrial, freshwater and ocean ecosystems (Costanza et al. 1997; Abrantes et al. 2015; Auta et al. 2017), which leads to extraordinary levels of local primary and secondary production (Beck et al. 2001; Abrantes et al. 2015; Sheaves et al. 2015).

While a range of anthropogenic activities threaten estuaries and tidal marshes, an emerging threat in these systems is the impact of marine debris, and specifically microplastics (synthetic polymer fragments and fibers < 5 mm in size). Surveys have found the highest regional microplastic (MP) concentrations on the seafloor around estuarine inputs (Galgani et al. 2000; Browne et al. 2010), and around the world relatively high levels of MPs are reported in surface waters of urbanized estuaries in comparisons to other oceanic surface waters (>1 MP L-1; Zhao et al. 2014; McEachern et al. 2019; Leads and Weinstein 2019; Sutton et al. 2019). Increases in urban land use and human populations have been linked to increased MP abundances in freshwater and estuarine systems (Hitchcock and Mitrovic 2019; Yonkos et al. 2014). Stormwater drainage system outfalls, shipping traffic, wastewater and industrial waste discharges, and mismanaged solid waste are all potential sources for macro- and microplastic pollution to nearshore aquatic environments (Yonkos et al. 2014).

Field studies document the presence (absence/detection) and prevalence (counts per individual) for various fish species in coastal waters worldwide (reviewed by Rezania et al. 2018) and in selected major inland river and lake systems (e.g., Great Lakes, McNeish et al. 2018; Pajeu River, Brazil, Silva-Cavalcanti et al. 2017). However, major data gaps exist in many coastal areas of the globe, including coastal U.S. waters off South Carolina (Baechler et al. 2019). Microplastics, because of their small size, may first enter food webs via prey misidentification and accidental consumption, and then through secondary consumption or trophic transfer to multiple trophic levels (Au et al. 2017), from small plankton (Payton et al. 2020) to larger predators like pelagic fishes (Lusher et al. 2013; Peters et al. 2017) and marine mammals (Fossi et al. 2016). Direct ingestion occurs when MP materials, which occupy the same size range as plankton and sediment particles, are mistaken as food or incidentally ingested (Ory et al. 2017); primary biological uptake is therefore dependent on accessibility (size in relation to prey items or mouth opening) and likelihood of encounter (abundance, as determined by sources, hydrodynamics, and polymer buoyancy; and selection or avoidance behavior, as determined by color, shape, or presence of biofilms; Bour et al. 2018). Trophic transfer, via consumption of organisms that have ingested MP themselves, is also possible as demonstrated from quantification of microplastics in field-collected organisms and in controlled feeding studies in artificial food chains.

It is important to understand exposure of estuarine organisms to microplastics to assess potential risk of adverse outcomes to fisheries stocks and even higher order consumers. Interactions with aquatic biota may also have a significant impact on the fate and transport of MPs in the environment (Clark et al. 2016). The consequences of MP contamination for aquatic ecosystems are still unknown (Rochman et al. 2019; Wang et al. 2020), but MP ingestion may result in oxidative stress, cell damage, tissue inflammation, increased gut residence times, and leaching of chemical additives and absorbed contaminants (Vethaak and Leslie 2016; Gray and Weinstein 2017).

To date, efforts to characterize the environmental prevalence of microplastics in South Carolina waterways center around Charleston Harbor. Charleston Harbor represents an estuary highly impacted by urban development, tourism, and commercial port activities with large point sources of MPs to the local watershed being identified as wastewater treatment utilities effluent and local mismanagement of public waste (Gray et al. 2018; Conley et al. 2019). High microplastic concentrations have been reported in the surface water column, ranging between 0.6 and 1 MPs L^{-1} (43 - 104 µm, Payton et al. 2020), and in the sea surface microlayer, between 3 and 36 MPs L^{-1} (63 µm - 5 mm, Gray et al. 2018; Leads and Weinstein 2019). Charleston Harbor's intertidal estuarine habitats also contain elevated microplastic concentrations in sediments, with concentrations observed to range between 3 and 73 particles kg^{-1} that vary both geographically and temporally (Gray et al. 2018; Leads and Weinstein 2019). Yu et al. (2018) sampled MP in beach sands across the Southeastern USA and Caribbean, finding some of the highest concentrations in Charleston Harbor (>300 particles/kg), which was explained in part by the high degree of urban development in the watershed and currents carrying coastal ocean inputs.

Objectives

Build upon our recent work on prevalence of microplastics in finfish of Charleston Harbor by quantifying how MP concentrations vary in the digestive tracts of five SCDNR SWAP species using the ACE Basin. These species include Atlantic Menhaden, Spot, and Striped Mullet, which were also investigated in the Charleston Harbor study, plus two new species in Mummichog and Southern Flounder. Identified objectives include:

1. categorize and quantify microplastics in the digestive tracts of estuarine Atlantic Menhaden, Mummichog, Striped Mullet, Spot and Southern Flounder collected from the ACE Basin,
2. evaluate within year temporal variability of microplastic concentrations within estuaries, and
3. contrast results to a previously completed sister study conducted on a guild of fish species from the highly urbanized Charleston Harbor estuary published by Parker et al. (2020).

As such, the research (i) provides the first quantification of MPs across a number of finfish species, representative of various feeding ecologies and trophic levels, in the ACE Basin; (ii) allows direct comparisons to research conducted in similar systems across the world; and (iii) provide baseline data needed to conduct an ecological risk assessment of potential direct impacts on these species of greatest conservation need and higher-level consumers in the estuarine food web.

Job 1

Categorize and quantify microplastics in the digestive tracts of estuarine Atlantic Menhaden, Mummichog, Striped Mullet, Spot and Southern Flounder collected from the ACE Basin

Methodology

From August 2020 through February 2023, we collected individuals from the targeted five species throughout the ACE Basin. Sample collection leveraged ongoing fishery-independent monitoring programs conducted in the ACE Basin by the SCDNR, namely the SCDNR Inshore Fisheries Trammel Net and Electrofishing surveys. Additional samples were collected via directed field efforts using cast nets, minnow traps, and hook-and-line fishing.

Upon capture, we placed individuals on ice in the field prior to transportation back to the lab where all fish were measured (to the nearest mm total length (TL) and standard length (SL)) and weighed (to the nearest gram). Most fishes ≥100 mm TL were dissected and had their entire digestive tract removed and stored frozen in foil for MP analysis. Fishes <100 mm TL remained whole and were frozen for MP analysis. Digestive tract weights (to the nearest g; n = 353) were obtained where possible. Table 3 provides summary statistics for each of the continuous variables measured (SL, TL, weight, and digestive tract weight) by species and across all species.

We conducted sample processing for microplastic analysis using the best practices for microplastic recovery and reduction of background contamination (Hermsen et al. 2017). Use of plastic equipment was avoided, and all staff wore natural fiber clothing in the lab when working with samples. We followed protocols utilized in Parker et al. (2020). Upon thawing, we rinsed whole fish with filtered deionized (DI) water prior to dissection or digestion to remove any potential external MP contamination. Samples were kept covered with aluminum foil during digestion, drying and storage. Sample processing blanks were run and analyzed alongside biological samples to enable correction of background contamination, if present.

Entire digestive tracts, including internal contents and digestive tissue, were placed inside a beaker with 1 M potassium hydroxide (KOH) solution at approximately three times the volume of the organic biological matter (Foekema et al. 2013; Lusher et al. 2017). Beakers were sealed with aluminum foil and placed on hot plates, set to 80ºC, for a minimum of 24 hours to maintain an internal temperature of 60ºC. Following complete digestion, we sieved the contents through a series of stacked metallic brass sieves (1mm, 500 µm, and 63 µm) to achieve physical isolation. The targeted sizes of MPs for this study ranged from 63 µm to 5 mm. Sieve contents were thoroughly rinsed with filtered DI water (Zhao et al. 2017). Solids left on the 63 µm sieve were carefully backwashed onto a mixed cellulose gridded ester membrane (color: green with black grid, size: 0.45 µm pore, 47 mm diameter) over a glass vacuum filtration funnel to remove liquids (Conley et al. 2019).

To reduce potential contamination, we cleaned laboratory surfaces weekly and wore only 100% cotton clothing and lab coats (Cole et al. 2014). All equipment utilized for processing specimens were thoroughly washed and rinsed with filtered DI water (Hermsen et al. 2017). We ran a sample processing blank (without tissue) with every batch of ten specimen samples (n = 60 total blanks) and examined for MPs. We also collected a lab blank time series (n = 30 total blanks) to evaluate how MP contamination varied with exposure time. Blanks were set out next to the fish dissection table and left for a variable amount of time (five blanks each at 1, 5, 10, 30, and 60 minutes).

We identified microplastics under a stereomicroscope (Nikon SMZ-U, Zoom 1:10) and classified all MPs by color and type as fibers, foams, fragments, or films (Rochman et al. 2019). Microplastics were identified using criteria established for morphology and confirmed via a hot needle test (Lusher et al. 2013, 2017). The established criteria used for MP morphology include homogenously colored, no visible cellular or organic structure, and equal thickness throughout for fibers (Lusher et al. 2017). When manipulated with forceps or metal probe, suspected MPs should not crumble like sediment. Tire wear particles are particles originating from road generated abrasion of vehicle tires (Wagner et al. 2018). Since tire wear particles do not react to a hot needle test, they were classified based on the following criteria: darkly colored (black), elongated or cylindrical in shape, partially or entirely covered with road dust, rough surface texture and rubbery flexibility when manipulated with forceps (Kreider et al. 2010; Leads and Weinstein 2019).

Arithmetic mean MP count values were reported with standard error included (± SE), as well as median and interquartile range (IQR). We assessed differences in the number of MPs among the five species using a Kruskal-Wallis test, as data did not follow a normal distribution. If results of Kruskal-Wallis test suggested significant differences amongst species, we used a Dunn's Multiple Comparison post-hoc test to conduct pairwise comparisons between the species. Results were considered significant when the p-value was < 0.05. Statistical analyses were conducted using R (version 4.3.0). Frequency of occurrence (F) and mean percent (%MN) of different MP types were determined for each species (Graham et al. 2007).

Results

Sample Collection

From August 2020 through February 2023, we collected 611 individual fish throughout the ACE Basin for inclusion in laboratory analyses. These samples represented 104 Atlantic Menhaden, 117 Mummichog, 95 Southern Flounder, 130 Spot, and 165 Striped Mullet (Table 1& Table 2). We collected most samples through routine sampling conducted by the SCDNR Inshore Fisheries Trammel Net (n =167 individuals) and Electrofishing (n = 282 individuals) surveys; we collected the remaining individuals (n = 162) via directed efforts. Fishes were collected from 98 unique sampling stations (Figure 1) divided into six general sampling areas within the ACE Basin, the shoreline between the mouths of the Combahee and Ashepoo Rivers (n = 205), the upper Edisto River (n = 162), the upper Combahee River (n = 133), the lower Edisto River (n = 65), Bennett's Point (n = 26), and Morgan Island (n = 20). Samples were temporally dispersed throughout the year, with 207, 160, 117, and 127 collected during Spring (April-June), Summer (July-September), Fall (October-December) and Winter (January-March) months, respectively (Table 2).

Species Combined

Overall, we found 75% (n = 461) of fishes contained at least one piece of MP in their digestive tract, with an average of 17.5 (± SE of 2.3) MPs fish^{-1} (Table 4). There was evidence of substantial right skew in the data, as the median number of MPs and the interquartile range of MPs fish^{-1} were substantially less and did not include the mean, respectively (Table 4). Similarly, across all species the average number of MPs g^{-1} of fish ($\bar{X} \pm SE$: 0.94 ± 0.14) and average number of MPs g^{-1} of digestive tract ($\bar{X} \pm SE$: 4.2 ± 0.7) relative to their respective medians and interquartile ranges also suggested substantial skew in the data (Table 4). This suggests substantial variability in microplastics burden across individuals, with most individuals containing few microplastics at the time of capture.

Across all five species, four different distinct classes of MPs were identified: foams, fibers, films, and fragments (Figure 2 and Figure 3). Notably, we identified no tire wear particles from the digestive tracts of finfish collected in the ACE Basin. Fibers were present in the highest percentage of stomachs (50%), followed by films (40%), foams (25%) and fragments (6%; Figure 2).

By far, foams were the numerically dominant (83.7%, n = 8,927 MP pieces) type of microplastics encountered, being approximately 10 times more numerous than the next closest MP type, fibers (9.3%, n = 990 MP pieces; Figure 3). However, high foam counts were concentrated in 31 individuals, each of which recorded > 100 MP particles, accounting for 79% of all foam particles. Films accounted for 6.5% (n = 695) of the microplastic pieces identified. Fragments (0.5%, n = 56) were a rare occurrence, with only 56 MP fragments observed across 38 individuals (6.2% of individuals) throughout the study (Figure 3).

<u>Species Specific</u>

Prevalence and Types

Microplastic prevalence varied by species, with 41% (n = 43), 84% (n = 98), 91% (n = 86), 73% (n = 95), and 84% (n = 139) of Atlantic Menhaden, Mummichog, Southern Flounder, Spot, and Striped Mullet containing MPs in their digestive tract, respectively. The most likely type of microplastic to be present in the digestive tracts of Southern Flounder, Spot and Striped Mullet were fibers, being present in greater than 50% of all individuals observed for each of these species (Figure 4). Films were also observed in >50% of all Mummichog, where they were the most commonly present type, and Southern Flounder investigated (Figure 4). Despite their numerical dominance (see above and below), foams were generally only found in between 17% (Atlantic Menhaden) and 31% (Mummichog)of individual fish, depending on the species (Figure 4). The least likely type of MP to observe in each species stomach were fragments, being found in only 2% (Atlantic Menhaden) to 11% (Southern Flounder) of stomachs (Figure 4). Further, even when microplastics were present, the absolute number of MPs fish^{-1} was generally low, with 77% (n = 33), 67% (n = 66), 43% (n = 37), 69% (n = 66), and 58% (n = 80) of Atlantic Menhaden, Mummichog, Southern Flounder, Spot, and Striped Mullet possessing 5 or fewer MPs when MPs were confirmed in the digestive tract. Overall, if you include fish where no microplastics were identified, 90% (n = 94), 73% (n = 85), 48% (n = 46), 78% (n = 101), and 64% (n = 106) of Atlantic Menhaden, Mummichog, Southern Flounder, Spot, and Striped Mullet possessed zero to five MPs in their digestive tract.

Cumulative proportions of different types of microplastics also varied amongst species, though foams were the most numerous type in all five species studied with at least 42% of all microplastics found in the digestive tracts being foams (Figure 5). This is despite foams only being observed in 17-31% of individual fish (Figure 4), and points to the idea that when foams occur they occur at generally high levels. That said, foams were relatively less common in Southern Flounder than observed in other species, with a greater proportion of MPs in flounder being fibers and films (Figure 5). Fragments made up less than 2% of microplastics in all species, with proportion composed of foams, fibers and films ranging from 5-31%, 3-26%, and 42-92%, respectively (Figure 5).

Microplastics Fish^{-1}

The microplastic concentration varied between species (Table 4), ranging from a low average value of 5.0 ± 2.7 MPs fish^{-1} (Atlantic Menhaden; median = 0) to a high average value of 35.5 ± 7.4 MPs fish^{-1} (Striped Mullet; median = 3). We detected differences among species for number of microplastics individual^{-1} (Kruskal-Wallis, χ^2 =89.2, df = 4, $p < 0.001$; Figure 6). Given the significant Kruskal-Wallis test, Dunn's test, post adjustment for multiple comparisons, suggests Atlantic Menhaden had fewer microplastics than the other five species (median = 0, $p < 0.0001$; Figure 6). Similarly, Southern Flounder (median = 6) possessed more microplastics than Mummichog (median = 3, $p < 0.0001$) and Spot (median = 2, $p = 0.0111$), though the concentration individual^{-1} was not greater than observed for Striped Mullet (median = 3, $p = 0.0564$; Figure 6). There was no difference ($p > 0.05$) in MPs individual^{-1} for Mummichog, Spot, and Striped Mullet (Figure 6).

Microplastics g^{-1} Wet Weight

To account for the high variation in fish sizes, we standardized microplastic counts by the whole weight (g) of individual fishes. Average MP concentrations g^{-1} wet weight ranged from 0.2 ± 0.05 MPs g^{-1} (Southern Flounder; median = 0.044) to 2.9 ± 0.63 MPs g^{-1} (Mummichog, median = 0.750; Table 4). We detected differences among species for MPs g^{-1} wet weight across species (Kruskal-Wallis, χ^2 =97.66, df = 4, $p < 0.001$), with Atlantic Menhaden possessing fewer (median = 0, $p < 0.05$) MPs g^{-1} wet weight than all other species (Figure 7). Similarly, Mummichog possessed greater ($p < 0.05$) MPs g^{-1} wet weight than other species (Figure 7). Dunn's test suggested no difference ($p > 0.05$) in MPs g^{-1} wet weight between Southern Flounder, Spot (median = 0.032), and Striped Mullet (median = 0.026; Figure 5).

Microplastics g^{-1} Digestive Tract

To account for the high variation in fish sizes and complexity of digestive systems based on feeding strategy, we standardized microplastic counts by the weight (g) of the digestive tracts of individual fishes. We completed this analysis on only four species, as we did not obtain digestive tract weights for Mummichog.

Average MP concentrations g^{-1} digestive tract ranged from 2.9 ± 1.8 MP g^{-1} (Atlantic Menhaden, median = 0.10) to 5.2 ± 1.7 MP g^{-1} (Spot, median = 0.38; Table 4), though, as with MPs individual^{-1} and MPs g^{-1} wet weight, the distribution within species was highly non-normal. As such, we continued to use the non-parametric Kruskal-Wallis test to identify differences in rank-sums amongst species, with once again differences being detected among species for MPs g^{-1} digestive tract weight (Kruskal-Wallis, χ^2 =23.93.66, df = 3, $p < 0.001$; Figure 8), with Southern Flounder possessing greater (median = 1.29, $p<0.05$) MPs g^{-1} digestive tract weight than all other species (Figure 8). Dunn's test suggested no difference ($p > 0.05$) in MPs g-1 digestive tract weight between Atlantic Menhaden, Spot, and Striped Mullet (median = 0.33; Figure 8)

QA/QC and Potential Laboratory Contamination

The number of microplastics found on procedural blanks was expressed as a percentage of total procedural blank microplastics divided by the total microplastic numbers for all species samples. This was used to compare contamination levels of procedural blanks to previous recommendations stating that for blanks counts < 10% of sample counts, contamination may be considered negligible (Gago et al. 2016, Provencher et al. 2017). We found a total of 430 microplastics across the procedural blanks (n = 60), suggesting a 4% contamination rate. Fibers accounted for 54% of microplastics found in the blanks, with films being 32.8%, and foams 13.3%. On average each blank contained 7.2 ± 0.9 MP. All microplastics identified in the blanks were scrutinized using the same criteria as samples and confirmed with the hot needle test.

To account for possible microplastic contamination in the air of the laboratory during sample processing, a time series of blanks were done (n = 30). Fibers accounted for 65% of microplastic contamination, films 39%, fragments 3%, and foams 2%. Peak MP contamination occurred at the fifteen-minute mark. On average, fish could be dissected in under two minutes.

We made no adjustment for potential laboratory contamination during the analyses for this report.

Significant Deviations
Despite attempts to obtain a minimum of 20 individuals from each of the five species of interest during each season, we were unable to obtain sufficient numbers of Atlantic Menhaden (n = 10) and Spot (n = 18) from the fall season and Atlantic Menhaden (n = 7) and Southern Flounder (n = 16) from the winter season. Despite this, overall sample sizes exceeded original project goals for all species (119-206%), all seasons (117-207%), and overall (153%; Table 1). The difficulty obtaining Atlantic Menhaden and Southern Flounder during the fall and/or winter months is explained by their migratory life history and general lower availability in South Carolina estuarine waters during these months.

Job 2
Evaluate within year temporal variability of microplastic concentrations within estuaries.

Methodology
To evaluate the effect a range of factors, including within year temporal period, had on predicted microplastics in the digestive tract, we employed a generalized linear model (GLM) framework. Considered covariates included species, wet weight (g), and one of two temporal variables (categorical season variable or continuous day of year variable). For the categorical variable seasons, we defined seasons as

- Winter: January – March collection dates,
- Spring: April – June collection dates,
- Summer: July – September collection dates, and
- Fall: October – December collection dates.

Day of year for individual collections ranged from 12-346, suggesting samples derived from mid-January through early-December. Year of collection was not included as a variable, which implicitly assumes that the within year temporal pattern was consistent across the years surveyed.

The full model contained the main effects of species, fish weight (g), one of the temporal variables (season or day of year) and all two-way and three-way interactions amongst terms. Stepwise regression, based on BIC, was used to identify the best fit negative binomial GLM. We also used BIC to select from the best fit model containing season versus the best fit model containing day of year.

Results

The results of the stepwise selection of the negative binomial generalized linear model indicated the fixed effects of species ($p < 0.0001$), fish weight (g, $p = 0.1063$) and season ($p < 0.0001$) were explanatory factors for the number of microplastics present in the gut of the individuals. In addition to these main effects, the final model retained the two-way interactions of species and fish weight (g, $p < 0.0001$) and species and season ($p < 0.0001$). The best fit model including the temporal effect of season was superior to the best fit model including the temporal effect of day of year.

In general, the best fit negative binomial GLM suggests no to a weak effect of fish weight (g) on microplastics predicted to occur in individual species, with typically much higher uncertainties at greater fish weights (Figure 9). A stronger effect is generally apparent for season, though the season of higher MP burden varies by species (Figure 9). For Atlantic Menhaden, higher MP abundances, regardless of weight, are predicted to occur in the summer, followed, by the winter with little to no MP observed in Atlantic Menhaden during the spring and fall (Figure 9). For Mummichog, though the overall predicted number of MPs individual^{-1} is generally low, higher concentrations can be expected in the spring and summer (Figure 9). Southern Flounder are the only species where one can expect higher concentrations of MPs in the winter, though there is a fairly consistent pattern of increasing MPs as weight increases across all seasons (Figure 9). Based on the data, Spot are predicted to have higher MP burdens during the summer and fall, with very little to no microplastics observed in spot digestive tracts during the winter and spring (Figure 9). Finally, Striped Mullet show a clear seasonal peak in MP concentrations in the summer relative to the other three seasons, with all of the highest individual fish burdens, including a single fish with 610 pieces of MP in its digestive tract, being collected during the summer months (Figure 9).

Significant Deviations

None

Job 3

Contrast results to a previously completed sister study conducted on a guild of fish species from the highly urbanized Charleston Harbor estuary published by Parker et al. (2020).

Results and Discussion

Parker et al. (2020) previously documented microplastics in the digestive tracts of Bay Anchovy, Atlantic Menhaden, Spotted Seatrout, Spot, and Striped Mullet collected from Charleston Harbor, with the concentration in Atlantic Menhaden g^{-1} of fish being the highest reported for any fish species at that time (24.8 ± MP g^{-1} fish). Their study was similar to the present study in that it collected similar fish species with differing feeding ecologies and utilized similar methodologies in sample processing and microplastic isolation. Across 284 individuals investigated in their study, 99% possessed at least one piece of MP in their digestive tract with an average ± standard error of 26.9 ± 4.7, 5.8 ± 1.6, and 30.7 ± 1.6 MP $fish^{-1}$, MP g^{-1} wet weight, and MP g^{-1} digestive tract weight, respectively. The median number of MP $fish^{-1}$, g^{-1} wet weight, and g^{-1} digestive tract weight was 3.0, 1.6, and 9.9, respectively. Each of these exceeds the values observed in the current study across 611 individuals, suggesting Charleston Harbor MP burdens for fish were higher than fish living in the less urbanized ACE Basin. The proportion of fish possessing at least one MP in their digestive tract was 24% lower, at only 75%, while the average and median MP fish-1 was 54% and 50% higher (see Table 4), respectively. The differences between MP g^{-1} wet weight and MP g^{-1} digestive tract weight (see Table 4) were even larger with Charleston Harbor values being at least 500% greater and median values be >1500% higher. While some of this could have been explained by different species studied, the same pattern was true for the three species included in both studies (Atlantic Menhaden, Spot, and Striped Mullet) with Charleston Harbor fish exhibiting greater MP $fish^{-1}$, MP g^{-1} wet weight, and MP g^{-1} digestive tract weight (see Table 2 Parker et al. 2020 & Table 4).

Differences between the two estuarine systems continue to manifest at the individual species level. In the Parker et al. (2020) study, the smallest species by weight, Bay Anchovy, reported the lowest average number of microplastics (1.9 MP $fish^{-1}$), while their largest species, Spotted Seatrout, reported the highest average microplastic concentration (82.6 MP $fish^{-1}$). This differed from the present study where Atlantic Menhaden had the lowest average MP concentration (5.0 ± 2.7 MP $fish^{-1}$) but was not the smallest sized fish encountered and Striped Mullet had the highest average MP concentration (35.50 ± 7.4 MP $fish^{-1}$) though it was not the largest fish encountered. Therefore, microplastic abundance was best compared to the Parker et al. (2020) study when expressed as MP g^{-1} wet weight. Mummichog, which was the smallest species encountered (average weight 4.5 ± SE 0.27 g), had the highest concentration of microplastics per gram (2.86 ± 0.62 MP g^{-1}). Southern Flounder, the largest species encountered in this study (average weight 318.04 ± SE 47.58 g), had the smallest concentration of microplastics per gram (0.19 ± 0.05 MP g^{-1}).

The present study found a smaller concentration of microplastics in Atlantic Menhaden (0.27 ± 0.08 MP g^{-1}), while Atlantic Menhaden in Charleston Harbor were found to have a much higher microplastic concentration by weight (24.8 ± 12.5 MP g^{-1}; Parker et al. 2020). Atlantic Menhaden are filter feeders with functional gill raker opening ranging from 12 to 37 µm (Friedland et al. 2006) and have been shown to retain particles as small as 7 µm (Friedland et al. 1984). That is much smaller than the filter size used in the present study (63 µm) and could suggest that Atlantic Menhaden are retaining smaller microplastics than were found with our methodologies. Payton et al. (2020) estimated that adult Atlantic Menhaden in Charleston Harbor could consume thousands of microplastics smaller than were analyzed here by consuming microplastic-contaminated zooplankton. Atlantic Menhaden are obligate filter feeders and have been shown to have a diet comprised of 81% marine snow (Lewis and Peters, 1994). Marine snow has been shown to be important for redistribution of microplastic particles in marine and estuarine systems and could contribute to the higher levels of microplastics observed in Charleston Harbor. The lower levels observed in Atlantic Menhaden captured in the ACE Basin could reflect fewer point sources to the ACE Basin, differences in spatial location of fish capture leading to lower reliance on marine snow, or potentially other unmeasured effects.

Mummichog had significantly higher average concentrations of microplastics per gram fish (2.86 ± 0.62 MP g^{-1}) than Atlantic Menhaden, Spot, Striped Mullet, and Southern Flounder. Mummichog are an omnivorous species of killifish known for its hardiness due to its ability to tolerate highly variable salinity, temperature fluctuations, low oxygen levels, and heavily polluted ecosystems (Burnett et al. 2007; Page and Barr, 2011). Omnivorous fish that consume a wide variety of resources have an increased chance of intaking microplastics in an active or accidental way (Mizraji et al. 2017). Active ingestion can be due to their foraging behavior of a wide variety of resources and throughout the water column (Romeo et al. 2015; Mizraji et al. 2017). Garcia et al. (2020) also found high numbers of microplastics in omnivorous fishes in freshwater systems in Brazil. None of the fish species sampled in Parker et al. (2020) exhibited an omnivorous feeding style.

Both Spot and Striped Mullet microplastic concentrations (0.72 ± 0.15 MP g^{-1} fish; 0.59 ± 0.20 MP g^{-1} fish, respectively) were lower in ACE basin than Charleston Harbor (2.5 ± 0.8 MP g^{-1} fish; 2.5 ± 0.9 MP g^{-1} fish, respectively; Parker et al. 2020). Spot and Striped Mullet both serve as important benthivores in estuarine systems commonly consuming detritus. As secondary consumers, the possibility of trophic transfer is a factor in microplastic occurrence within both species. Southern Flounder microplastic concentrations (0.19 ± 0.05 MP g^{-1} fish) were lower than Atlantic Menhaden (0.27 ± 0.08 MP g^{-1} fish). Southern Flounder, as predatory carnivores, could also experience trophic transfer of microplastics.

Within the present study, foams accounted for the highest percentage of microplastics encountered (83.7%), differing from fibers being the highest microplastic encountered in Charleston Harbor (77.4%; Parker et al. 2020). Fibers had the highest frequency of occurrence throughout all species in Charleston and all but Mummichog in ACE Basin (Parker et al. 2020). Importantly, no tire wear particles were observed in ACE Basin. However, as expected in an urbanized estuary, tire wear particles accounted for 1.2% of microplastics encountered in Charleston Harbor (Parker et al. 2020). This study chose to account for any films present as well, where Parker et al. (2020) chose to not report presence of films due to interference by crustacean carapaces in the sample matrices. This did not appear to be a problem encountered within the ACE Basin study.

Given the limited geographic and temporal scope of the Parker et al. (2020) study, it is not known whether the concentrations of microplastics observed in a suite of fish species collected from Charleston Harbor are representative of the larger coastal South Carolina coastline. The present study aimed to gain a baseline of potential within year temporal variations of microplastic occurrence as well as to continue to investigate the relationship between fish wet weight and microplastics. As reported above, the best fit negative binomial GLM suggests no to a weak effect of fish weight (g) on microplastics predicted to occur in individual species, with typically much higher uncertainties at greater fish weights (Figure 9). A stronger effect is generally apparent for season, though the season of higher MP burden varies by species (Figure 9). Interestingly, Parker et al. (2020) suggested a much stronger relationship between the number of microplastics and digestive tract weight (g; see Figure 4 Parker et al. 2020), including for Spot and Striped Mullet, though a negative correlation between digestive tract weight and number of MPs was observed for Atlantic Menhaden. While the weight metric was not the same (wet weight current study vs. digestive tract weight Parke et al. 2020), it is important to note the lack of a within, temporal metric in Parker et al. (2020) that was the dominant effect in the current study. Parker et al's (2020) samples were limited to a narrow temporal window (May-September

2008) with an overall much smaller sample size. Perhaps if a temporal component was included in the Parker et al. (2020) study, a much weaker relationship between digestive tract weight and microplastics would have bene observed. This would seem to be supported by the observation in the ACE Basin that Atlantic Menhaden, a species with extremely high MP burdens in the previous study, possessed higher MP burdens during the summer in ACE Basin (Figure 9). This was the period when most Atlantic Menhaden were captured in Charleston Harbor. Spot, Mummichog and Striped Mullet also had higher MP during the summer than other months (Figure 9). The higher prevalence of microplastics in Southern Flounder during the winter was of note, given this is a temporal period when most mature individuals migrate offshore for spawning.

It is important to note the present study had a higher sample size which could explain discrepancies of average microplastic counts between the two studies. Further, while Menhaden showed significant vulnerability in their feeding style within Charleston Harbor, our study found that Mummichog's omnivorous feeding style pointed to a vulnerable feeding habit within ACE Basin. Since Parker et al. (2020) had no species representative of an omnivorous feeding style, it is difficult to discern if the difference was due to estuary, urbanization, or other factors. Both studies showed the significance feeding ecology can play on microplastic exposure to estuarine finfish and provided two comparable baseline studies for urbanized and non-urbanized estuaries in South Carolina. Consumers across the trophic levels are vulnerable against microplastic occurrence and ingestion. The present study highlighted the importance of conservation needs, even in our least developed estuaries along the coast, to help curb the future contamination of microplastics.

Significant Deviations
None

Presentations

Benson, C., J. Ballenger, B. Beckingham, G. Sancho. Microplastics in estuarine finfish in ACE Basin. Estuarine Finfish Research (EFR) Science Discussion, South Carolina Department of Natural Resources, Charleston, SC, August 2022

Benson, C., J. Ballenger, B. Beckingham, G. Sancho. Microplastic Exposure for Key Ecological Species in Coastal South Carolina. SC American Fisheries Society Conference, McCormick, SC, March 2023

Sancho, G., B. Parker, M. Lattomus, B. Ingram, C. Benson, A. Galloway, B. Frazier, J. Ballenger, B. Beckingham. Ingestion of microplastics by fishes in estuarine habitats: how different feeding strategies and trophic pathways influence exposure. ASLO Aquatic Sciences Meeting, Palma De Mallorca, Spain, June 2023

Budget Report

A separate financial report will be submitted by the grant's administrator.

Recommendations

Close the grant.

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Tables

Table 1: Species collected as part of the ACE Basin microplastics project.

Species	Common Name	Family	Feeding Type	Main Food
Brevoortia tyrannus	Atlantic Menhaden	Alosidae	Planktivorous	Phytoplankton, Zooplankton, Detritus
Fundulus heteroclitus	Mummichog	Fundulidae	Omnivorous	Detritus, Diatoms, Copepods
Paralichthys lethostigma	Southern Flounder	Paralichthyidae	Predatory	Bony Fish, Crustacea
Leiostomus xanthurus	Spot	Sciaenidae	Zoobenthic generalist	Zoobenthos, Nekton, Detritus
Mugil cephalus	Striped Mullet	Mugilidae	Benthic herbivore/ detritivore	Benthic Algae, Detritus, Diatoms

Table 2: Specimens collected, by species and season, for microplastic analysis. Shown is the original proposed target number of specimens as well as the number of specimens collected (Realized). Seasons are based on annual quarters, with Spring, Summer, Fall and Winter representing the months of April-June, July-September, October-November, and January-December, respectively.

	Season									
	Spring		Summer		Fall		Winter		Total	
Species	Target	Realized	Target	Realized	Target	Realized	Target	Realized	Target	Realized
Atlantic Menhaden	20	53	20	34	20	10	20	7	**80**	**104**
Mummichog	20	37	20	25	20	21	20	34	**80**	**117**
Striped Mullet	20	42	20	42	20	41	20	40	**80**	**165**
Spot	20	53	20	29	20	18	20	30	**80**	**130**
Southern Flounder	20	22	20	30	20	27	20	16	**80**	**95**
Total	**100**	**207**	**100**	**160**	**100**	**117**	**100**	**127**	**400**	**611**

Table 3: Standard length (mm), total length (mm), whole weight (mm), and digestive tract weight (g) descriptive statistics by species and across all samples. Proved are sample sizes (n), averages ± SE and ranges for each continuous variable.

	Standard Length (mm)			Total Length (mm)			Whole Weight (g)			Digestive Tract Weight (g)		
Species	n	Avg ± SE	Range	n	Avg ± SE	Range	n	Avg ± SE	Range	n	Avg ± SE	Range
Atlantic Menhaden	104	91.52 ± 2.47	35-176	92	118.77 ± 3.25	45-219	104	20.78 ± 26	1-116	26	4.85 ± 0.58	2-12
Mummichog	117	53.43 ± 1.21	22-82	96	65.39 ± 1.49	24-96	117	4.5 ± 0	1-13	0		
Southern Flounder	95	203.84 ± 10.46	50-481	92	253.79 ± 12.71	81-575	95	318.04 ± 87	2-2200	87	9.39 ± 1.26	1-52
Spot	130	120.9 ± 4.12	30-180	107	162.4 ± 5.79	37-227	130	71.65 ± 89	1-202	89	3.76 ± 0.18	1-9
Striped Mullet	165	174.63 ± 4.54	71-310	151	227.3 ± 6.05	99-400	165	156.44 ± 151	8-632	151	13.54 ± 0.9	1-80
Total	**611**	**130.38 ± 3.11**	**22-481**	**538**	**171.47 ± 4.20**	**24-575**	**611**	**111.34 ± 9.02**	**1-2200**	**353**	**9.41 ± 0.54**	**1-80**

Table 4: Microplastics fish^{-1}, MPs g^{-1} of fish, and MPs g^{-1} digestive tract descriptive statistics by species and across all samples. Proved are sample sizes (n), ranges, average ± SE, median, and interquartile ranges (IQR) for each variable.

	MPs Fish^{-1}					MPs g^{-1} Fish					MPs g^{-1} Digestive Tract				
Species	**n**	**Range**	**Avg ± SE**	**Median**	**IQR**	**n**	**Range**	**Avg ± SE**	**Median**	**IQR**	**n**	**Range**	**Avg ± SE**	**Median**	**IQR**
Atlantic Menhaden	104	0-271	5.00 ± 2.70	0	0.00-1.25	104	0.0-6.0	0.271 ± 0.0815	0.000	0.000-0.100	26	0.0-45.2	2.89 ± 1.75	0.10	0.00-1.17
Mummichog	117	0-178	9.21 ± 2.08	3	1.00-6.00	117	0.0-57.5	2.864 ± 0.6269	0.750	0.200-2.000					
Southern Flounder	95	0-104	11.53 ± 1.86	6	2.00-12.00	95	0.0-3.0	0.194 ± 0.0469	0.044	0.012-0.170	87	0.0-52.0	3.63 ± 0.83	1.29	0.37-3.36
Spot	130	0-289	16.28 ± 4.24	2	0.00-5.00	130	0.0-11.6	0.723 ± 0.1473	0.032	0.000-0.671	89	0.0-95.0	5.17 ± 1.74	0.38	0.00-1.50
Striped Mullet	165	0-610	35.52 ± 7.41	3	1.00-9.00	165	0.0-29.0	0.592 ± 0.2046	0.026	0.006-0.129	151	0.0.84.3	4.20 ± 0.99	0.33	0.06-1.13
Total	**611**	**0-610**	**17.46 ± 2.34**	**2**	**1.00-7.00**	**611**	**0.0-57.5**	**0.939 ± 0.1417**	**0.051**	**0.003-0.500**	**353**	**0.0-95.0**	**4.21 ± 0.66**	**0.50**	**0.06-1.86**

Figures

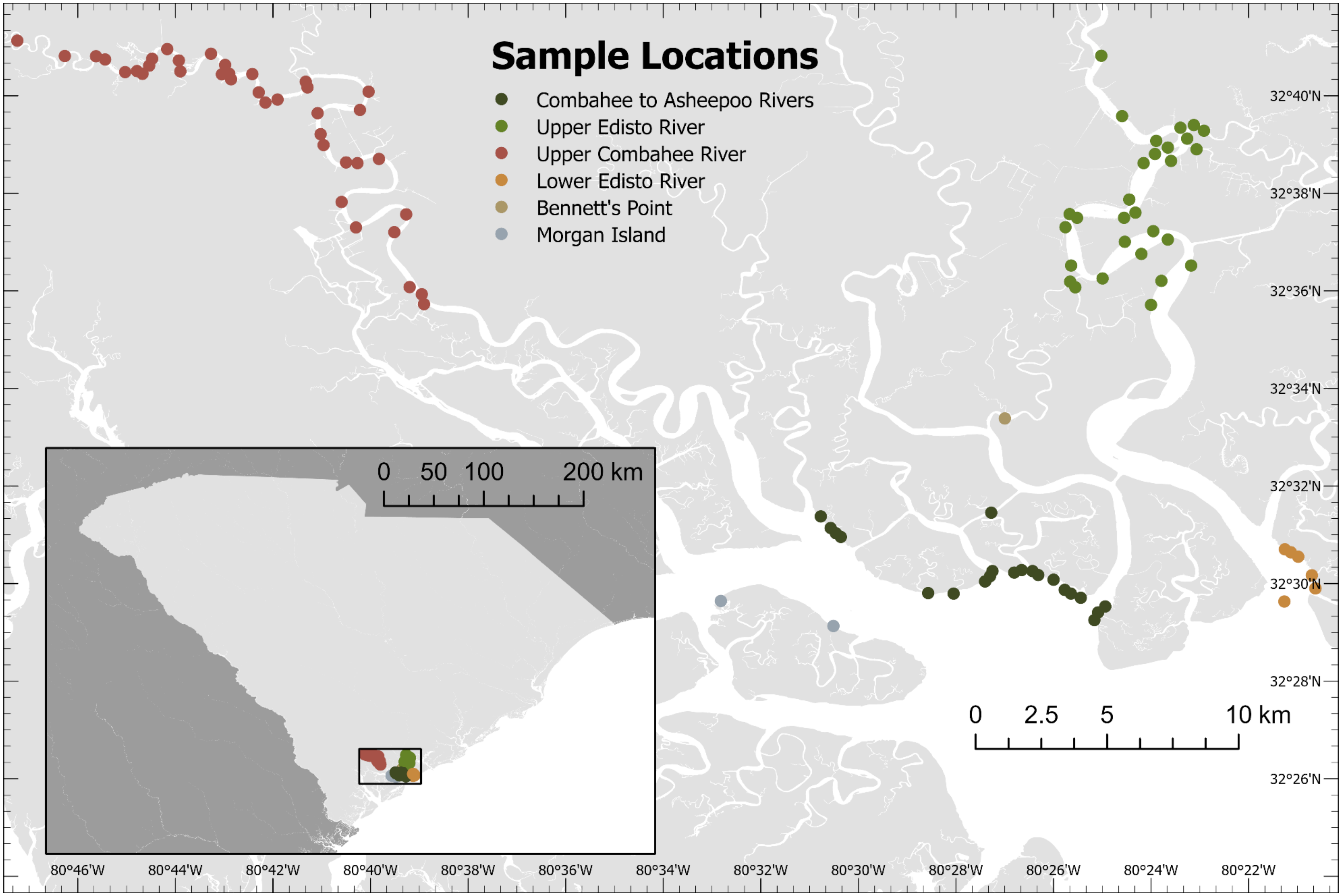

Figure 1: Location of study in South Carolina. Sampling sites (n = 98) indicated by stars are located throughout the mouth of the ACE Basin up into the Combahee and Edisto Rivers.

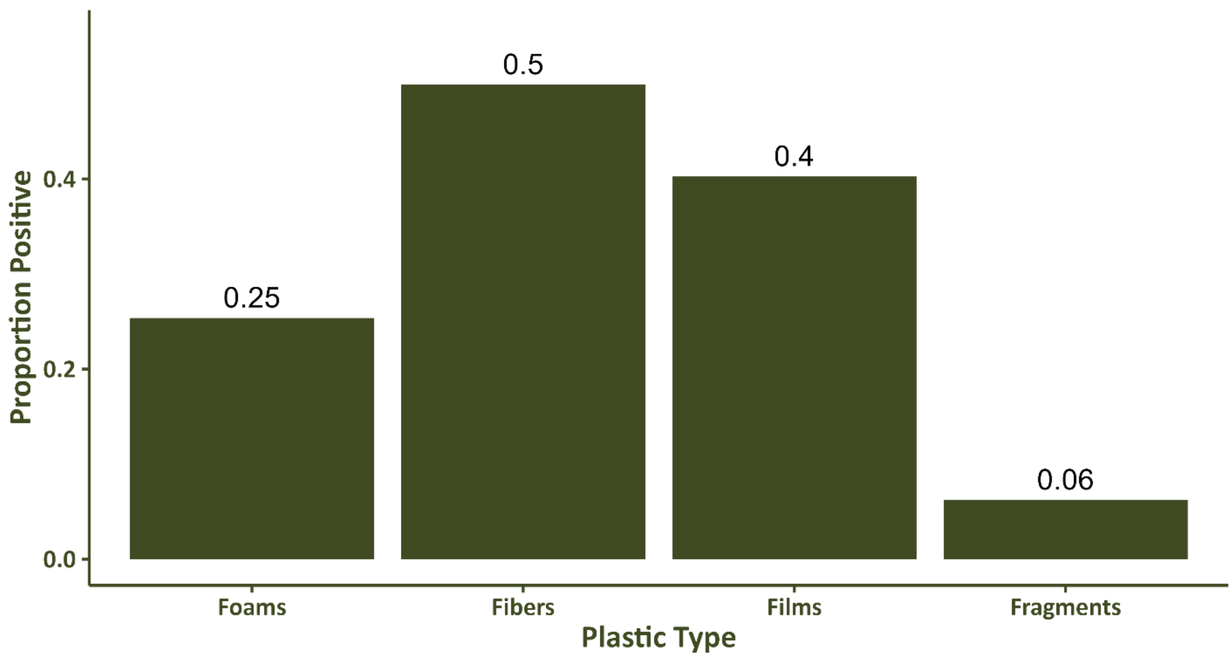

Figure 2: Proportion of digestive tracts positive for a given MP type across all five species encountered in the study.

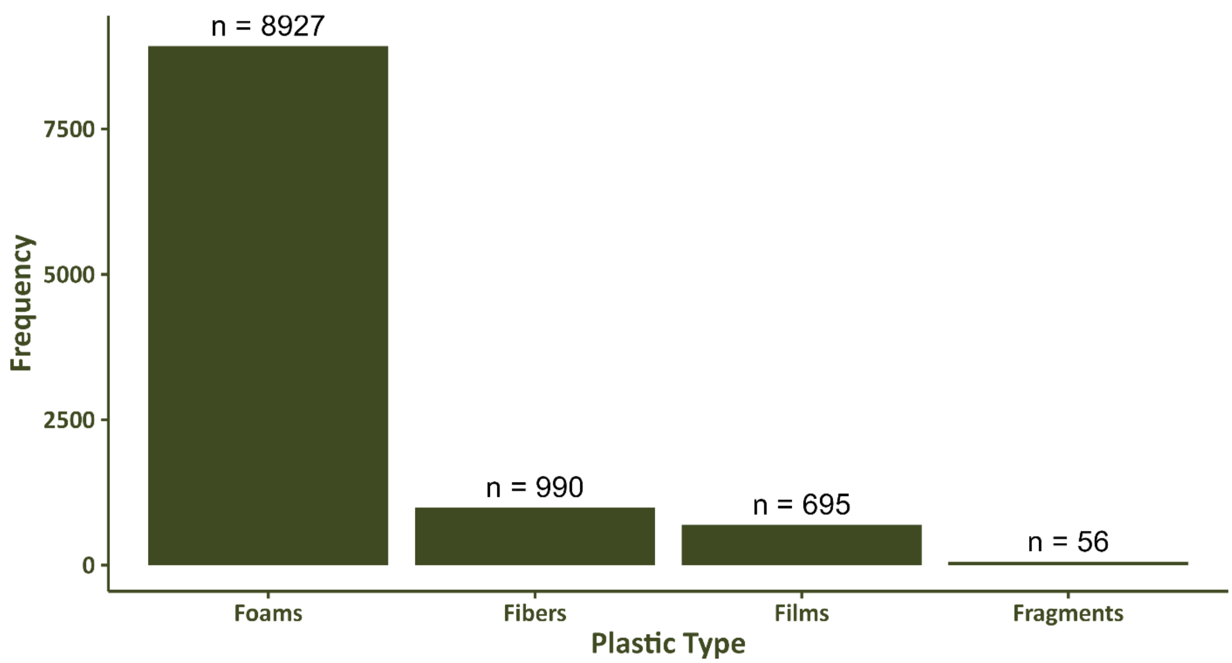

Figure 3: Microplastic frequency by type across all five species encountered in the study.

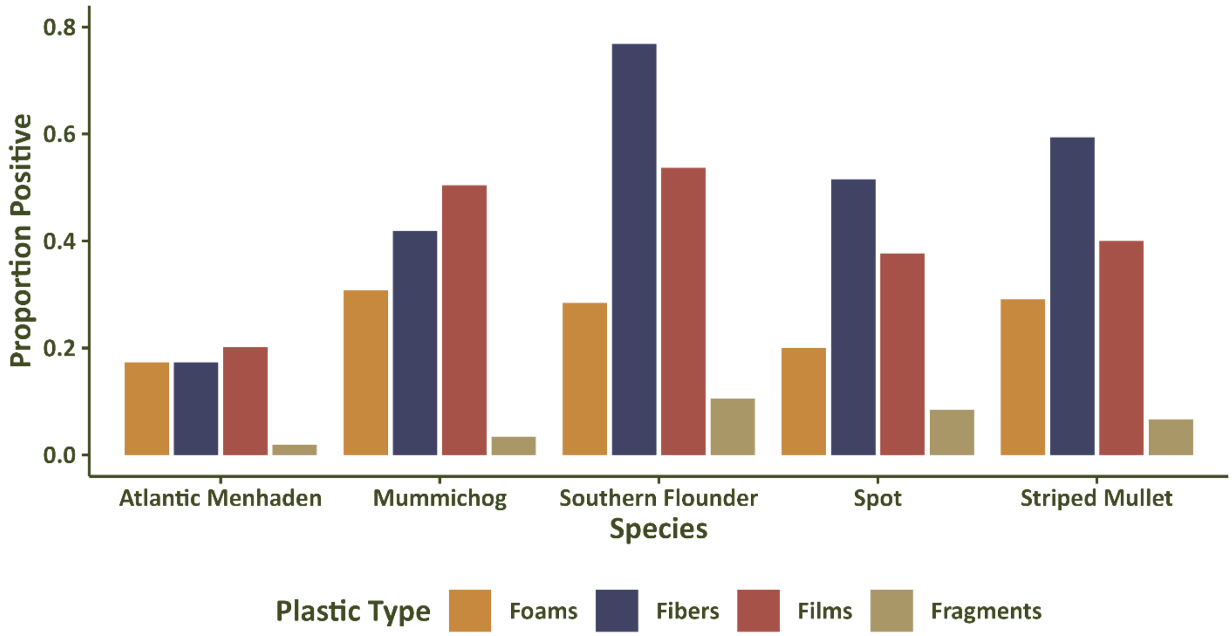

Figure 4: Proportion of digestive tracts positive for a given MP type by species.

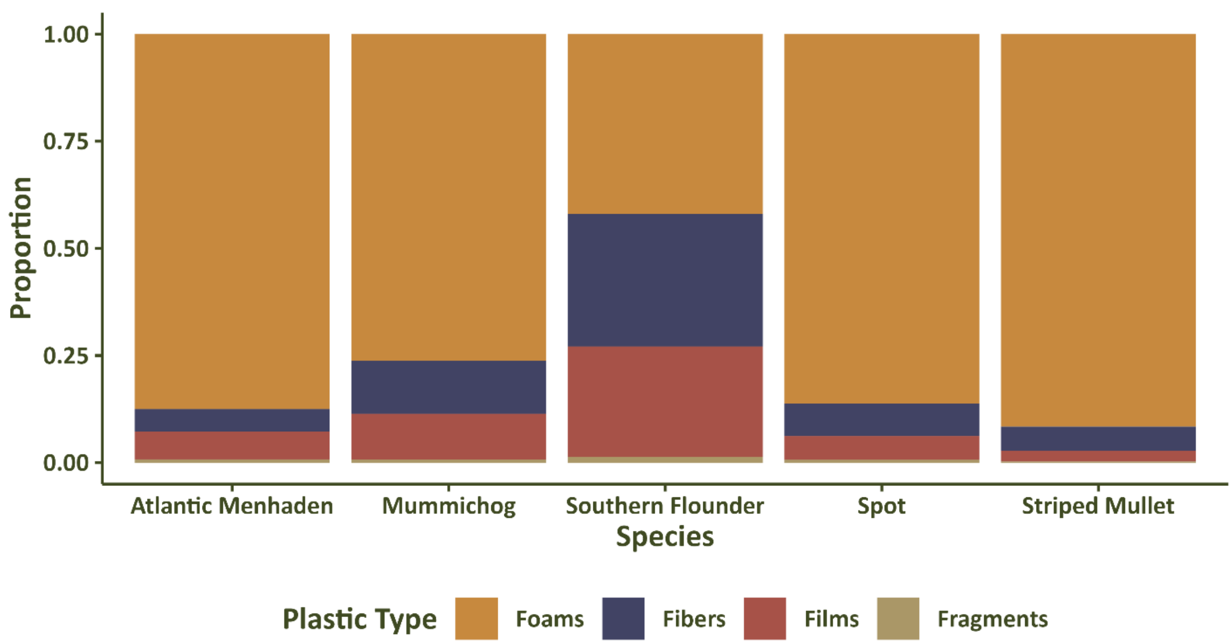

Figure 5: Proportion of total MPs represented by different plastic types by species.

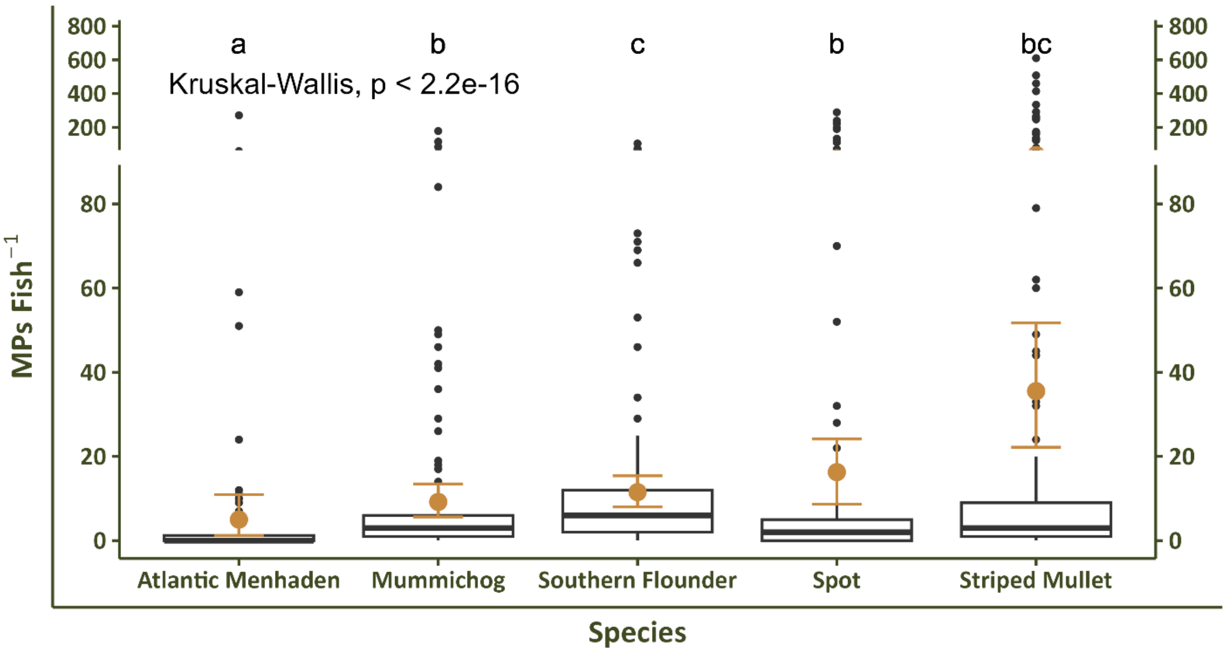

Figure 6: Box plot of number of MP particles present in the gut of individuals by species. Also provided is the mean ± 95% CI number of MPs per individual (yellow dots & yellow error bars) and the p-value of the non-parametric Kruskal-Wallis rank sum test. Finally, the letters represent significant differences, as determined using the non-parametric Dunn's test, after correcting for multiple comparison using Holm's method. Note the break in the y-axis scale used to show the long-right tail of MP fish^{-1}.

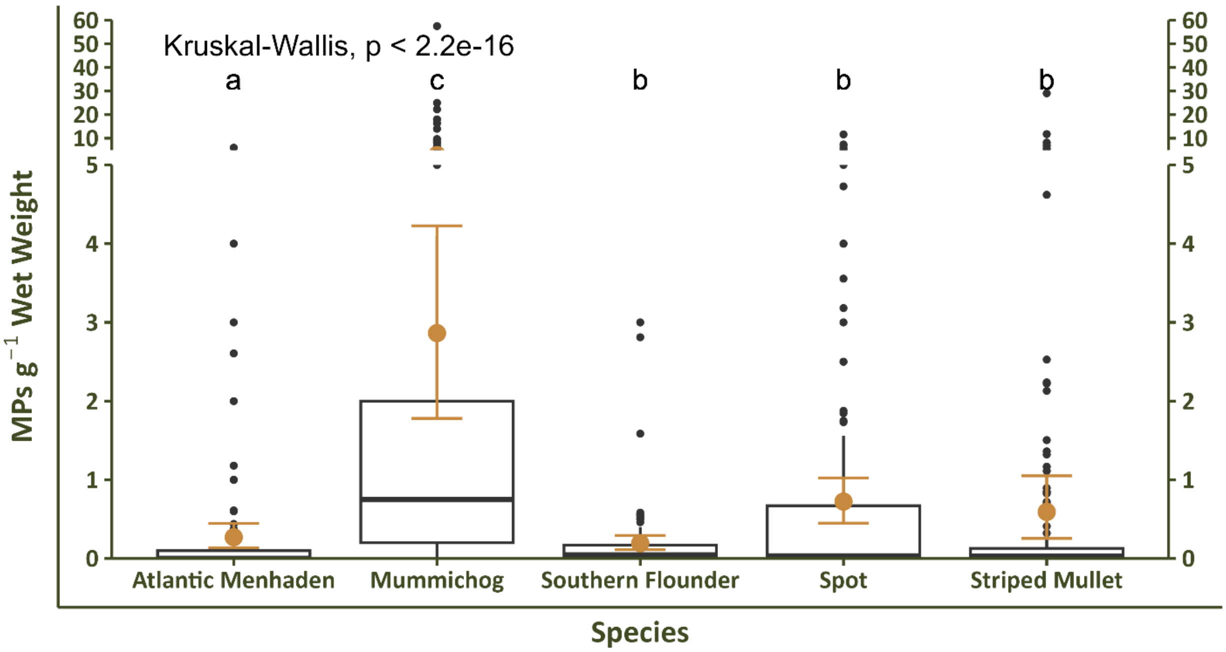

Figure 7: Box plot of number of MP particles present g^{-1} wet weight in the gut of individuals by species. Also provided is the mean ± 95% CI number of MPs g^{-1} wet weight (yellow dots & yellow error bars) and the p-value of the non-parametric Kruskal-Wallis rank sum test. Finally, the letters represent significant differences, as determined using the non-parametric Dunn's test, after correcting for multiple comparison using Holm's method. Note the break in the y-axis scale used to show the long-right tail of MP g^{-1} wet weight.

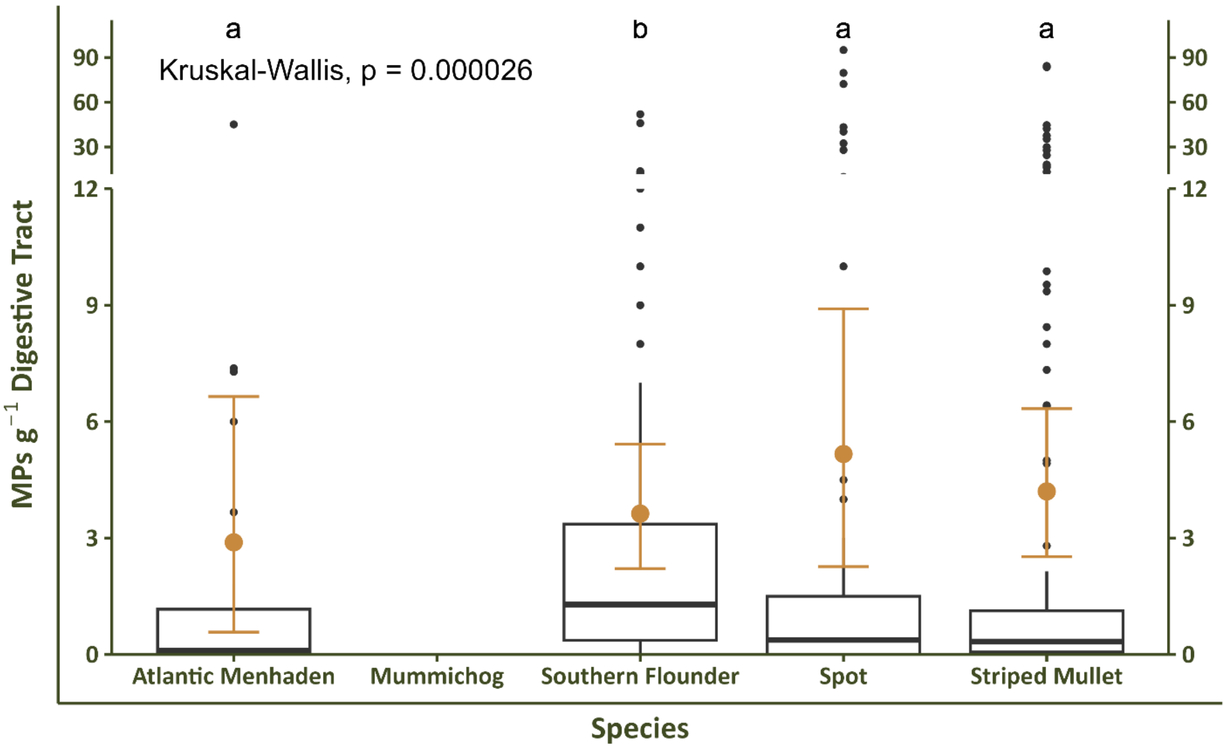

Figure 8: Box plot of number of MP particles present g^{-1} digestive tract weight in the gut of individuals by species. Also provided is the mean ± 95% CI number of MPs g^{-1} digestive tract weight (yellow dots & yellow error bars) and the p-value of the non-parametric Kruskal-Wallis rank sum test. Finally, the letters represent significant differences, as determined using the non-parametric Dunn's test, after correcting for multiple comparison using Holm's method. Note the break in the y-axis scale used to show the long-right tail of MP g^{-1} digestive tract weight.

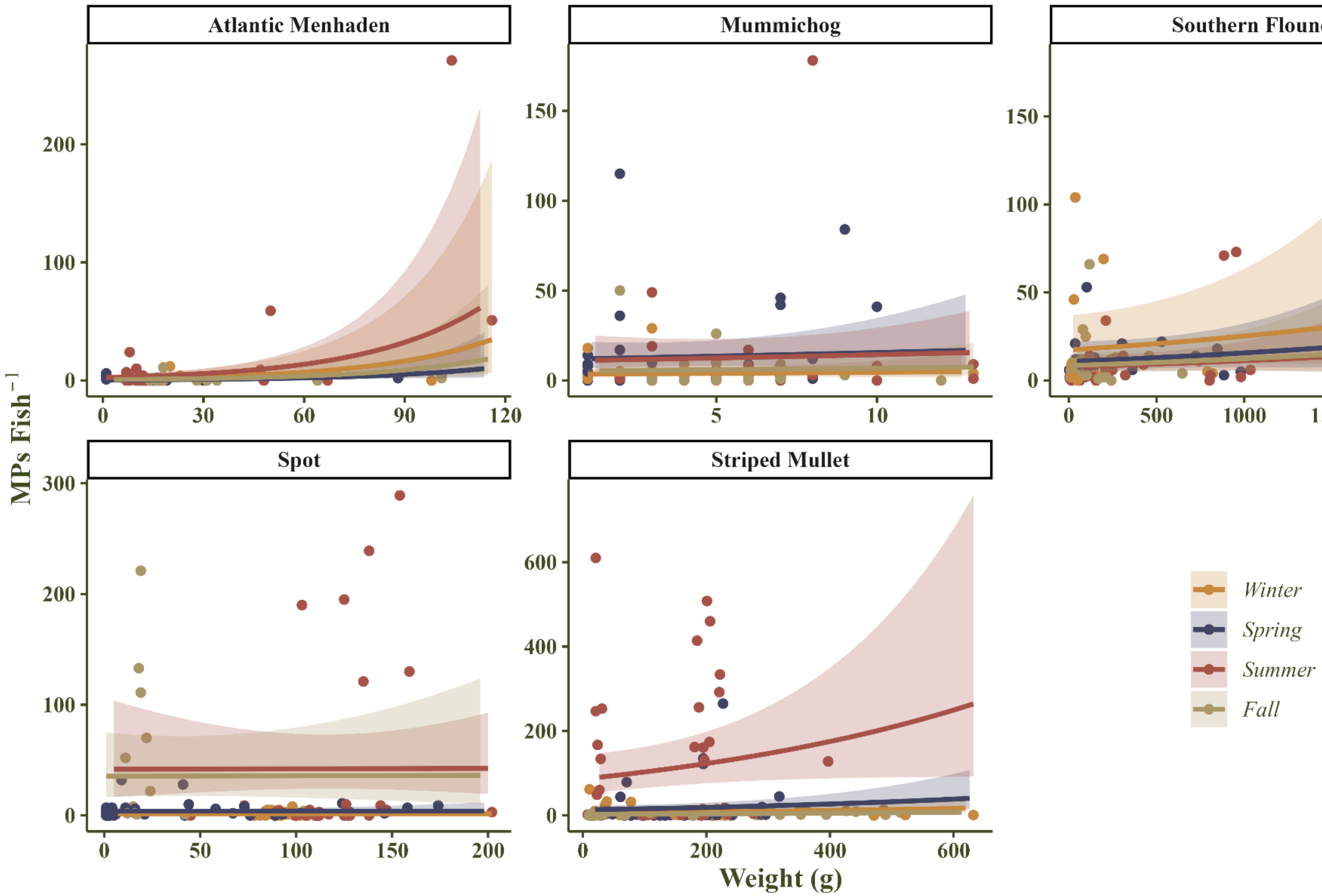

Figure 9: Negative binomial GLM model depicting the relation between fish wet weight (g), season (colored lines and shaded regions), and number of MPs in the gut of each species (panels). Points represent observed MP concentrations, lines represent best fit negative binomial GLM models, and shaded regions represent 95% confidence intervals. Different seasons are represented by unique colors: Winter (Orange), Spring (Blue), Summer (Red), Fall (Khaki).