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Microplastic Accumulation in the Digestive Tract of Young-Of-Year Atlantic Sharpnose Sharks (Rhizoprionodon terraenovae) in the Grand Strand, SC

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Microplastic Accumulation in the Digestive Tract of Young-Of-Year Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*) in the Grand Strand, SC

By

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Abstract

This study focused on the presence and accumulation of microplastic fibers in the digestive tract and livers of young-of-year Atlantic Sharpnose Sharks (*Rhizopriondon terraenovae*) from two sampling locations along the Grand Strand of South Carolina. *R. terraenovae* is a small, mesopredatory elasmobranch found abundantly along northwestern Atlantic Ocean coastlines. Thirty specimens of *R. terraenovae* were collected from May through August of 2020. Microplastics were found in all specimens. A total of 672 plastic particles were identified over the course of the study, with an average of 22.4 ± 10.5 (SD) plastics per specimen. The majority of the plastics were classified as fibers (91.4% of total), followed by films (4.3%), fragments (3.7%), pellets (0.6%), and were clear in color (47%). This study did not find evidence to support monthly microplastic accumulation during the four-month sampling period. Moreover, the potential for prenatal transfer in *R. terraenovae* remains uncertain. However, this project was the first to survey microplastic counts over a four-month timeframe in sharks and reports some of the highest reported microplastic levels in sharks.

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Introduction

The transmission of plastic waste into the environment is a mounting issue; researchers estimate 10% of all plastics produced, or between 4.8 to 12.7 million tons, enters the ocean each year (Avio *et al*. 2017; Cole *et al*. 2013; Jambeck *et al*. 2015). Plastic contamination is not confined to heavily polluted waters or coastal development; plastics have been reported as distant as Antarctica (Sfriso *et al*. 2020) and as deep as the hadopelagic zone (6,000 to 11,000 m deep) (Courtene-Jones *et al.* 2017; Jamieson *et al.* 2017). Microplastics (MPs), defined as plastic debris less than 5 mm in diameter (Arthur *et al*. 2009; Cole *et al*. 2011), pose a potentially serious and understudied danger to the biota of the ocean. These particles can travel great distances due to their low density and can remain in the ocean for hundreds of years (Klein *et al*. 2017; Barboza *et al*. 2020). Plastic concentrations vary throughout oceanic regions, influenced by anthropogenic and environmental factors, such as wind, waves, and currents (Desforges *et al*. 2014).

Classification of Microplastics

MPs can be classified as primary or secondary based on their source and means of disposal. Primary microplastics are directly deposited as pellets or powders from land-based sources, while secondary microplastics are produced by the degradation of large plastics from interactions with environmental influences (Alimba & Faggio 2019; Cole *et al*. 2011; Thompson 2015). MPs can be categorized into four different shape classes: fibers, films, fragments, and pellets (Figure 1). Fibrous MPs are the prevalent form of plastic in the marine environment, where in some studies they can account for up to 80-90% of MPs recorded (Suaria *et al.* 2020; Wu *et al.* 2020). Fibers make up 14.5% of global plastic production and are essential components of clothing, furnishings, construction, and automotive products (Suaria *et al.* 2020). MP fragments and films are byproducts of environmental pressures on larger plastics, such as weathering through sand abrasion, friction with hard substrate, wave impact, and occasional animal interactions (Andrady 2017; Oliveira *et al*. 2020; So *et al.* 2022). Other forms of marine plastics, such as pellets, or 'nurdles', are primary building blocks in the manufacturing of large-scale plastic products. These plastics are occasionally released into the environment as a loss of industrial process production or as a result of spills during transportation (Jiang *et al.* 2021; Pozo *et al*. 2020). MPs can be further divided into six major color groupings, white/clear, blue, black, clear, red, and 'other'. Color is an important factor in classifying plastics, as color often plays a significant role in identifying production source and composition.

Ingestion of Microplastics

A growing threat to marine organisms is MP ingestion, where plastic particles are ingested either directly or indirectly (Li *et al*. 2021). Direct plastic ingestion occurs when MP are mistakenly consumed as prey (Sfriso *et al*. 2020) or are passively ingested during respiration (Lusher 2015). Indirect ingestion describes the intake of plastics through the consumption of prey species, where organisms contaminated with previously ingested MPs can be transferred to the gastrointestinal tract of predator species (Athey *et al.* 2020; Miller *et al.* 2020; Nelms *et al*. 2018; Wright 2013). Factors, such as plastic shape, composition, and residence time in the consumed organism can affect which MPs are retained in the predator species versus excreted (Au *et al*. 2017). Once ingested, these plastics can impair movement by disrupting buoyancy and leaving these organisms more vulnerable to predation (Ryan 2016). Further, MP presence in the gastrointestinal tract can negatively affect energy accumulation (Bhuyan 2022) by obstructing feeding apparatuses (Alimba & Faggio, 2019) or inducing a false sensation of satiation (Ryan 2016), and in extreme cases, starvation (Jovanovic 2017). MPs are found in all trophic levels of marine organisms, with plastic presence being reported in plankton (Cole *et al*. 2013), crustaceans (Potocka *et al*. 2018; Villagran *et al*. 2010), fishes (Andreas *et al.* 2021; Parker *et al.* 2020), and cetaceans (Fossi *et al*. 2012).

The direct influence of these foreign particles is not the only concern. Plastics also act as a carrier for harmful chemicals such as persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), and dichloro-diphenyl-trichloroethane (DDT) (Bakir *et al*. 2014). As plastics fragment over time, these chemicals are released into the environment or tissue in which they reside (Hirai *et al*. 2011; Neves *et al*. 2015). POPs have been linked to endocrine disruption, leading to hormone imbalance, as well as fertility alterations in teleosts (Abel & Grubbs, 2020; Rochman *et al*. 2014; Smith *et al*. 2018). For instance, in Japanese Medaka (*Oryzias latipes*), chronic exposure to POPs increased egg production as a result of disrupted endocrine function (Hu *et al*. 2020). PCBs are known to cause liver cancer, mutations, and even fatalities in animals (Abel & Grubbs, 2020). DDT is able to accumulate in fatty tissue and create hormonal imbalances in males (Rabitto *et al*. 2011). In addition, translocation of plastic fibers through the stomach lining and digestive tract has been observed (Pullen 2019). These foreign particles can cause inflammatory damage and harbor the aforementioned chemicals (Li *et al*. 2021). Sussarellu *et al*. (2016) showed that Pacific oysters (*Crassostrea gigas*) experienced a significant reduction in the number of ovulated eggs, as well as the level of sperm motility. Plastics can also negatively affect autotrophic animals, such as phytoplankton, by reducing chlorophyll absorption due to the presence of plastic fragments in their tissue (Laist 1987).

Plastics in Elasmobranchs

MP studies are an emerging field of research, with at least fifteen published studies concerning their presence in sharks from 2013 - 2022, covering a variety of species (Table 1). The methods and subsequent results used to document and quantify MP vastly differ across studies, due to elasmobranch species inhabiting diverse environments and occupying different trophic levels. Avio *et al*. (2015), for example, reported MP presence in 44% of the stomachs of Spiny Dogfish (*Squalus acanthias*) (n = 9), while Parton *et al.* (2020) found MPs in 58% of the stomachs of the same species (n = 12). Even in studies with a similar location, differing methodology can produce notably different results.

Two studies on the Blackmouth Catshark (*Galeus melastomus*) in the Mediterranean Sea highlight this contrast. Valente *et al*. (2019) reported MPs in 78.1% (n = 32) of *G. melastomus* specimens using chemical digestion and filtration of the digestive tract, whereas Alomar & Deudero (2017) reported MPs in 16.8% (n = 125) of *G. melastomus* specimens through dissection and stomach analysis. In comparing elasmobranch species of different trophic levels, current research has suggested that higher trophic species are more suspectable to MP influences due to the trophic transfer of MP-associated persistent pollutants (Maes *et al*. 2020; Whitacre 2008).

While the methodology and extraction rates vary, the majority of studies confirm that clear and blue fibrous plastics are the most prevalent types of MPs in elasmobranchs (Alomar & Deudero, 2017; Bellas *et al*. 2016; Kooi & Koelmans, 2019; Martí *et al*. 2020; Neves *et al*. 2015). These studies have shown that MP ingestion occurs in several elasmobranch species and are associated with increased levels of PBTs and PCBs (Fossi *et al.* 2014; Yong *et al.* 2021). The long-term effects of MPs in most elasmobranch species are largely unknown (Parton *et al*. 2020), though long-lived species and filter feeders are believed to be at greatest risk (Corsolini *et al*. 2014; Fossi *et al*. 2014; Germanov *et al*. 2019).

Biology of Atlantic Sharpnose Sharks

The Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae)* is a small requiem shark species that inhabits the east coast of the United States, the Caribbean Ocean, and the Gulf of Mexico (Carlson & Brusher, 1999; Castro 1993; Ehnert-Russo & Gelsleichter 2019). *R. terraenovae* can reach a TL of 110 cm and live up to a maximum of ten years (Köhlmann 1987). At birth, *R. terraenovae* pups are born at a total length (TL) of 29-37 cm and grow at rate of 15-23 cm per year until marturity (Compagno 1984). Female *R. terraenovae* reach sexual maturity around two to three years old at a TL of 85-90 cm (Parsons 1985). *R. terraenovae* are viviparous and reproduce yearly, with litter sizes ranging from four to seven pups (Carlson & Baremore, 2003).

R. terraenovae females typically reach their reproductive peak in April, with a gestation period ranging from 11-12 months (Hoffmayer *et al.* 2013; Drymon *et al*. 2020). As winter approaches later in the year, the pregnant females will travel to deeper waters in large sexually segregated schools. As spring nears, *R. terraenovae* then return to the shallow coastal waters to give birth (Carlson & Baremore, 2003). Shortly after parturition, the females will continue to mate, and the process begins once again. Unlike other coastal shark species, juvenile *R. terraenovae* display multiple forms of residency, where instead of conventional long-term residence in a nursery or protected area, *R. terraenovae* utilize wide ranging movements across a combination of coastal bays and estuarine habitat.

Young-of-year *R. terraenovae* feed primarily on arthropods, mollusks, and small teleosts (Bethea *et al*. 2006; Gelsleichter *et al*. 1999; Hoffmayer & Parsons, 2003), before switching to larger teleost prey later in maturity. *R. terraenovae* are opportunistic hunters and have a greater range of prey items than other mesopredatory sharks of similar size and habitat (Hoffmayer & Parsons, 2008). *R. terraenovae* also display an ontogenetic shift in trophic position creating difficulties in describing exact prey and habitat preferences (Altobelli &. Szedlmayer, 2020; Carlson *et al*. 2008; Delorenzo *et al*. 2014; Drymon *et al*. 2011; Morgan *et al*. 2020).

R. terraenovae was chosen as the focus of this study due to its regional ubiquity, trophic position within the coastal ecosystem (Drymon *et al*. 2011), and previous MP-focused research in this species (Pullen 2019). *R. terraenovae*'s role as a mesopredator illustrates a link between top predators and lower trophic levels, and thus serves as a model for understanding MP in a coastal marine community. Lastly, *R. terraenovae* is listed by the IUCN as a species of least concern so collecting specimens would not unduly impact the population nor require special collection permits. In sampling an abundant and healthy stock, insights may be gained for threatened species, such as juvenile Sandbar Sharks (*Carcharhinus. plumbeus)* (Collatos *et al.* 2020; Cortez *et al*. 1999). By focusing on young-of-year *R. terraenovae*, knowledge can be gained into the potential effects of MP presence on growth rate and size.

The presence of MPs in *R. terraenovae* was first described by Pullen (2019), who investigated MP presence in adult *R. terraenovae.* In Pullen's study, 16 mature male *R. terraenovae* were caught during May and July 2018 from experimental longlines in Winyah Bay, SC. Winyah Bay is the fourth largest estuary on the east coast of the United States in terms of discharge rate, with an estuarine drainage area of 24,633 km^2 (Gray *et al*. 2018; Kim & Voulgaris, 2008). Winyah Bay is believed to be a nursery ground of several shark species, including *R. terraenovae* (Abel *et al.* 2007; Bethea *et al.* 2006, Bruce 2014; Callatos *et al*. 2020; Pullen 2019*)*.

Pullen dissected her specimens and identified MP presence, where they were then categorized by shape, size, and color. MPs were found in all specimens (n = 16), with a mean of 57.93 ± 11.71 (SD) MPs per individual, then the highest reported average in the literature in sharks. A total of 927 plastic particles were identified across all specimens with the frequency ranging from 34 to 75 MPs per individual. The prevalent shape of recorded MPs were fibers (93.6%), then fragments (5.7%), films (0.5%), and pellets (0.1%). The prevalent size class of MPs was < 1 mm (54.8%), followed by 1 to 2 mm (25.1%), then 2 to 3 mm (11.7%), 3 to 4 mm (4.2%), 4 to 5 mm (2.2%), and > 5 mm (2.0%). The prevalent MP color recorded was blue (41%), then clear (22%), black (15%), and gray (9%). Further, Pullen assessed the stomach lining for MP presence, where three particles were identified as a result of translocation. Lastly, Pullen found no correlation between MP counts and body length per individual, hepasomatic indices (HIS), or condition factor (CF).

The objective of the current study was to expand on the research of Pullen (2019) by identifying and quantifying plastic presence in the digestive tract and livers of young-of-year *R. terraenovae* in coastal South Carolina. Initially, Winyah Bay was the desired sampling location for this study, however access to research boats were restricted during the onset of COVID-19, so two piers along coastal South Carolina were selected as an alternative solution. Additionally, shark abundance is typically high at piers, where activities such as fish cleaning and discard influence shark behavior and provide a more reliable catch, as opposed to fishing efforts in Winyah Bay (Isner 2021; Martin *et al*. 2019).

R. terraenovae specimens were collected from two pier locations, in order to better sample the local population and see if there was a difference in plastic concentration per specimen by location. After the *R. terraenovae* specimens were captured, the digestive tract and liver were removed, chemically digested, and examined, as previous studies have documented plastic bioaccumulation in both organs (Barboza *et al*. 2018; Collard *et al*. 2017). The identified plastics were then categorized by shape, size, and color. A sub-objective of this study was to investigate prenatal transfer of plastics by comparing MP presence at an early developmental stage to previously recorded MP counts in adult *R. terraenovae*. Establishing the occurrence of MP transfer in *R. terraenovae* in utero should allow more research into understanding developmental effects due to MP presence.

Methods

Sampling

A total of thirty young-of-year *R. terraenovae* were caught at monthly intervals at two locations from May to August 2020. Cherry Grove (33.83° N, 78.63° W) and Garden City (33.58° N, 79.00° W) are beach communities 43.6 km apart, that were selected for their location at opposing ends of the Grand Strand (Figure 2), and presence of nearby piers that encourage teleost and elasmobranch activity (Martin *et al.* 2019).

Sampling was performed via hook-and-line from the beach, using locally caught shrimp as bait. Sampling began an hour before predicted high tide, a period where fish activity is heightened and more susceptible to ambush from predatory fish (Able *et al*. 2013). Once captured, the *R. terraenovae* specimens were examined for the presence of an umbilical scar, indicating their status as a neonatal/young-of-year shark (Duncan & Holland 2006; Olin *et al*. 2011; Parsons 1985). If a visible ubilical scar was present, the *R. terraenovae* was then encased in aluminum foil to prevent plastic contamination through the gills. The wrapped specimens were placed on ice until deceased and subsequently frozen at -23° C until initial assessment and dissection. Animal collection and processing procedures performed in the field were conducted under the Institutional Animal Care and Use Committee (IACUC) research permit #2015.05.

Contamination Measures

In order to minimize contamination from non-project related MPs, several safeguards were used in the lab area. All activities related to dissecting, filtration, and observation were performed while wearing 100% cotton lab coats and masks, and latex gloves. All glassware and filtration apparatus were cleansed with alcohol and dried in the vacuum hood for each specimen. Initially, samples were processed in a lab equipped with a fume hood, where much of the examination and filtration was performed.

As the project progressed, concerns arose about airborne MPs and a separate analysis area was enclosed by 100% cotton sheets. This allowed the researcher to view and identify MPs in a controlled setting without the heightened risk of contamination from foot traffic. In both the dissection lab (Lab 1) and MP analysis lab (Lab 2), a control petri dish was set aside five separate times to establish a baseline of atmospheric MP contamination in both locations. The quality control samples were then averaged to establish the number of foreign MPs in order to redact them from the final counts. To limit water MP contamination, the Milli-Q® Reference Water Purification System was used to filter all water used in filtration and decanting. Additionally, five control tests of the amount of water used per individual assessment was used in order to establish an aquatic MP contaminant baseline.

Dissection and Observation

The sharks were then processed in the same manner as Pullen (2019), in accordance with the methods provided in Avio *et al*. (2015). Out of the MP examination methods described by Avio *et al*. (2015), protocol six was the preferred method for this study due to the high extraction rate (90%) of plastic particles and reduced cost of chemicals. Before dissection, each *R. terraenovae* specimen was thawed to room temperature, sexed, weighed, and measured by fork length (FL), precaudal length (PCL), and total length (TL). An incision was made along the length of the *R. terraenovae* specimen to expose the abdominal cavity. The digestive tract and liver were then removed from the *R. terraenovae* specimen and the organs were examined for any deformities. The organs were then added to a 250 ml NaCl hypersaline solution (1.2 g/cm^3) in an aluminum tin, while stirring and decanting for ten minutes. Once completed, the stirring and decanting step was repeated with a second 250 ml NaCL solution, to further break down the organic matter.

The remaining solution used to break down the organic matter was then vacuum-filtered using six 47 mm gridded cellulose-nitrate filters with a 0.45 µm pore size (GF/B, Whatman, USA). The remaining organic matter was transferred to borosilicate petri dishes with a 15% H_2O_2 solution, before being dried in an oven for eight hours (50 ˚C). Upon completion, the six spent gridded cellulose-nitrate filters and petri dishes containing dried organic matter were examined for MPs under a binocular dissecting microscope (40x), while noting particle size shape, and color. When a particle came into question regarding composition, the hot needle test was used to differentiate between organic matter and plastic. FIJI Image J software was used to digitally measure and compare the MPs through photographs taken of the petri dishes.

Statistical Analysis

Using R-studio, one way ANOVA tests were performed to determined differences to test for significant differences within the five different color groupings, within the four different shapes, and among the four different size classes. When *P*-values were significant, a Tukey post-hoc test was applied to determine which groups differed significantly. In comparing the distribution of MPs between sites (shape categories, color, and class sizes), two-way ANOVA tests were performed to examine interactions between the factors. To assess accumulation, linear regression was used to assess if there were any trends related to PCL and MP abundance over the four-month sampling period.

COVID-19 Limitations

The original design for this study was to catch neonate Atlantic Sharpnose Sharks via longline in Winyah Bay beginning in March, in order to potentially observe prenatal transfer of MPs. Unfortunately, due to the COVID-19 pandemic, this was not possible. Access to equipment to perform longline sampling, student help, YSI meter, university labs, freezer space, and beach access was restricted, as well as an institutional moratorium on boat use.

Additionally, equipment necessary to measure water samples at each sampling site was unavailable, so MP concentrations were used from previous research in nearby Winyah Bay. MP particles were found in all samples taken from the surface microlayer of Winyah Bay, with an average concentration of 30.8 ± 12.1 particles/L across the bay (Gray *et al.* 2018).

Results

MPs were recorded in all specimens (n = 30), with the frequency ranging from 4 to 51 MPs per individual. During the entire four-month sampling, six males and nine female sharks were caught at Cherry Grove, and ten males and five females at Garden City. Of the 30 *R. terraenovae* collected, 53.3% were male (n = 16). A total of 672 plastics particles (corrected for contamination) were identified, with an average of 22.4 ± 10.5 (SD) particles per shark specimen.

Microplastic Shape

The predominant shape of MPs identified were fibers (91.4%), followed by films (4.3%), fragments (3.7%), and pellets (0.6%), with fibers being the dominant shape for all individuals (Figures 3 & 4). There were significant differences in MP shape (ANOVA; $R^2 = 0.892$; df = 3, 116; F = 113.87 ; $P < 0.00001$), as well as differences between the two subgroups, one composed of fragments, films, and pellets, and the other composed of fibers (Tukey post-hoc test; Table 3) There was no significant difference in the distribution of MP shapes between the two locations (Two-way ANOVA; P = 0.217; $F_A = 1.494$; $\eta^2 = 0.02$).

Microplastic Size

MPs ranged in length from 0.01 to 4.36 mm, with an mean of 0.47 ± 0.43 mm (SD). The dominant size class among the identified plastics was the less than 0.5 mm class (66.1%), followed by 0.5 to 0.99 mm (23.3%), 1 to 1.5 mm (7.6%), and greater than 1.5 mm (3%) (Figure 5). There were significant differences between MP size classes with subgroups of less than 0.5 mm, subgroup of 0.5 to 0.99 mm, and a subgroup of 1 to 1.5 mm and greater than 1.5 mm (Tukey post-hoc test; Table 4). There was no significant difference in the distribution of MP sizes between the two locations (Two-way ANOVA; $P = 0.134$; $F_A= 0.92$; $\eta^2 = 0.03$).

Microplastic Color

The primary color of identified plastics was white/translucent (47.0%), followed by black (24.9%), blue (20.1%), and red (7.3%). The remaining uncategorized colors (0.7%) made up the rest of the plastics (Figure 6). There were significant differences in abundance of colored MPs (ANOVA; R2 = 0.755; df = 4, 174; F = 35.62; $P < 0.00001$), as well as differences between color groups (Table 5) . There were significant differences between the subgroup of MP colors of black and blue, and subgroup of red and other, and subgroup of clear MPs. There was no significant difference in the distribution of MP colors between the two locations (Two-way ANOVA; $P = 0.352$; $F_A= 1.05$; $\eta^2 = 0.01$).

Precaudal Length

The mean PCL in sampled *R. terraenovae* was 25.1 ± 3.0 (SD) centimeters. There was a weak direct relationship between PCL and MP length ($R^2 = .025$; F = 0.72; P = .405) and a weak inverse relationship occurred between PCL and MP abundance ($R^2 = .001$; F = 0.029; P = .866) Finally, there was a weak direct relationship between PCL and MP size ($R^2 = .025$; F = 0.72; P = .405).

Sampling Sites

Garden City (28.1 ± 8.8 (SD) particles) had significantly more MPs per specimen than Cherry Grove (16.7 ± 9.2 (SD) particles) (ANOVA; $R^2 = 0.798$; df = 29,195; F = 11.96; P < 0.01). (Tables 6 & 7) (Figures 3 & 4). Additionally, no significant relationships were recorded for either Cherry Grove or Garden City between mean MP monthly differences across the four month sampling interval (Linear regression: Garden City (P = 0.4881) and Cherry Grove (P = 0.1473). There were also no significant differences between the precaudal lengths in Cherry Grove and Garden City specimens (P = 0.437). As mentioned in the previous sections, there were no significant differences in the MP distribution between Cherry Grove and Garden City for MP shape, size, or color.

Control

Analysis of the control samples recorded 14 contaminant plastics per specimen examined, with the majority of foreign MPs classified as fibers (97.8%). Plastics were corrected for color and shape class, with clear fibers as the dominant MP identified, with an average of 10.1 MP per specimen examined, followed by blue fibers (1.9), black fibers (0.8), red fibers (0.8), and other miscellaneous colors and shapes (0.4). Controls in Labs 1 and 2 yielded airborne contaminant averages equaling 3.2 and 2 MP per specimen examined, respectively. Samples taken from the Milli-Q water filtration system averaged 8.8 MP per specimen examined, with clear fibers (80%) as the prevalent MP identified. For each of the MP counts in each specimen, ten clear fibers were redacted, as were two blue fibers, one black fiber, and one red fiber. Regardless of data corrections, the same significant differences existed in color and shape. Due to COVID-19 protocols, masks were always required in all lab areas. Disposable face masks similar to those we used, were found to be a major source of plastic shedding and likely a source of contamination in this study (Chen *et al.* 2021).

Discussion

This study is the first to determine monthly MP counts in sharks, and possibly marine organisms as a group, outside of a controlled laboratory environment. The results from this study demonstrate that MP counts in young-of-year *R. terraenovae* specimen are comparable to those found in adult *R. terraenovae* (Pullen 2019). Although there was not sufficient evidence to demonstrate plastic accumulation across a four-month interval, the high counts of MP corroborate the ubiquity of plastics in the coastal ecosystem. Major findings include the quantification and categorization of MPs by shape, length, and color. This study also provides a baseline for future research on plastic occurrence in the digestive tract of *R. terraenovae*, and may suggest prenatal transfer of plastics in utero.

Microplastic Presence

MPs were ubiquitous in *R. terraenovae*. MPs were found in all 30 individuals, with an average abundance of 22.4 ± 10.5 (SD) per specimen. This is consistent with Pullen (2019), who reported MPs in all specimens examined (n = 16). However, Pullen (2019) recorded more MPs per adult *R. terraenovae* (57.93 ± 11.7) than in the young-of-year *R. terraenovae* specimens in the current study. This could be attributed to the age and size of the individuals, where the average PCL of adult specimens examined was 71.1 ± 5.4 cm compared to 25.7 ± 3.0 cm in the young-of-year specimens. Although, when standardizing the results of the two studies between MP presence and PCL, Pullen (2019) (0.81 MP per PCL cm) and the current study (0.87 MP per PCL cm) recorded similar MP to body length ratios. Parton *et al*. (2020) examined MP presence in four demersal shark species: *S. canicula*, *M. asterias*, *S. acanthias*, and *S. stellaris* where MP presence occurred in only 67% of specimens (n = 46) at a density of 0.74 particles per individual, emphasizing the high abundance of MPs in this current study.

An example of MPs in juvenile elasmobranchs was investigated by Bernardini *et al*. (2018), who recorded plastics in 34.9% of juvenile Blue Sharks (*Prionace glauca*). While both species vary greatly in size, habitat, and prey preference, this remains the only published study to examine MPs in a juvenile elasmobranch species. The difference in plastic abundance in the sampled *P. glauca* (Bernardini *et al*. 2018) compared to abundance in *R. terraenovae* in the present study (100%) could be attributed to the extraction methodology and MP shape parameters used in each study. In Bernardini *et al*. (2018), fibers were excluded in the analysis, whereas the dominant MP shape recorded in the current study were fibers (91.3%). Additionally, in the results provided by Bernardini *et al*. (2018), only the visual identification of *P. glauca* stomach contents were performed as opposed to chemical digestion of the digestive tract of *R. terraenovae* in the current study.

Prey frequency and foraging preference differ between juvenile and adult *R. terraenovae*. As *R. terraenovae* mature, prey variety decreases, implying dietary refinement with age (Bethea *et al*. 2007, Harrington *et al*. 2016; Plumlee & Wells 2016). Whereas juvenile *R. terraenovae* target mollusks, crabs and small teleosts, mature sharks shift towards a mostly piscivorous diet. The dietary shift could potentially affect the number of MPs encountered, with crustaceans and mollusks having previously been reported with higher numbers of MPs than teleost species (Danopoulos *et al*. 2020; Smith *et al*. 2018; Waddell *et al*. 2020).

The high MP abundance reported by this study may be largely attributed to the inclusion of MP fibers, that the samples were captured in a highly commercialized region, as well as the inclusion of the liver in the analysis of the *R. terraenovae* specimens. The liver was included in this study, as MP occurrence in hepasomatic tissue is hypothesized to be the result of MP translocation from the intestinal barrier, therefore plastics found in the liver may contribute to a more comprehensive count of MPs across the digestive tract (Collard *et al*. 2018).

Due to time constraints, MPs from the liver and digestive tract were not recorded separately and instead the MP abundance was derived from the entirety of the combined organic matter and the solution used to break the organs down, thereby it is unknown what percentage of MPs were attributed to each respective organ.

Comparisons to Related Studies

MP fibers were the dominant plastic type collected from *R. terraenovae*. As *R. terraenovae* are highly migratory, it unknown whether the MPs identified in this study originated from local sources or from more distant locations. Fibers are likely derived from fishing gear, rope, various land-based sources such as packaging and plastic bags, as well as ingested plastics in prey items (Alomar & Deudero, 2017; Bernardini *et al*. 2018; Huang *et al*. 2020; Isaac & Kandasubramanian 2021; Martí *et al.* 2020). Another major source of fibers is sewage effluent from washing machines and textile manufacturers, where polyester fabrics shed particles that are then deposited into the environment (Browne *et al*. 2011). Clear fibers are also created by the photo-oxidation of colored fibers in the surface layer of the ocean, where the MP ink absorbs the UV light, and the effected plastic loses color (Espinosa *et al*. 2016; Isaac & Kandasubramanian 2021).

The high percentage of fibrous plastics is consistent with a related study of MPs in Winyah Bay, SC, where 90% of MP samples from surface water were identified as fibers (Ladewig, 2018). In that study, however, blue was the dominant MP color (32%), which is in agreement with the Pullen (2019), who reported high blue MP occurrence (41% of MPs identified) in specimens of *R. terraenovae* in Winyah Bay. Both studies examined estuarine environments, where MP color may vary due to increased fishing activity and maritime equipment, whereas the results of the current study (47% white/translucent) are closely aligned with the dominant clear MPs reported across the global ocean surface (Alomar & Deudero, 2017; Bernardini *et al*. 2018; Huang *et al*. 2020; Martí *et al*. 2020). Estuarine environments have been reported to contain fishes with higher levels of plastic ingestion as opposed to coastal regions (Harris 2020; Savoca *et al*. 2021). Furthermore, increased MP counts could be attributed to the low wave energy of Winyah Bay concentrating the MP's distribution in the water column (Ladewig 2018).

The dominant size class of identified MPs in *R. terraenovae* was less than 0.5 mm (66.1%), with a mean of 0.47 ± 0.43 mm (SD). This is in agreement with Pullen's (2019) study, where most MPs were in the smallest class size, less than 1 mm (55%). Previous research has highlighted the significance of MP size in the marine environment, as Lehtiniemi *et al*. (2018) suggest that MP size, rather than shape, has a greater influence on ingestion rates. Lehtiniemi *et al*. (2018) investigated MP ingestion in fishes and mysid shrimp and postulates that even in high MP concentration regions, the ingestion of an environmentally relevant shape of MP is inconsequential, rather, the size of the MP corresponds to which MPs are ingested by marine organisms.

Microplastic Accumulation

The lack of significant plastic buildup over the four-month sampling period in the digestive tract and liver of *R. terraenovae* suggests that there was no additional accumulation of MPs. The presence of MPs in the digestive tract and liver of *R. terraenovae* are presumably the result of the ingestion of MPs in the water column and contaminated prey items in the Grand Strand region, not specific to either site.

Currently, there are no studies that extend beyond enumerating MP accumulation in elasmobranch species, although, such studies have been conducted in other marine organisms. In comparison to similarly sized cetaceans, sharks may be less vulnerable to the potential effects of MPs due to the lesser amount of adipose tissue in elasmobranchs compared to cetaceans and are therefore less likely to bioaccumulate MPs and associated toxins in their body fat (Fossi *et al*. 2014). Another potential mechanism of MP rejection is intestinal eversion, a mechanism used by several elasmobranch species to rinse the stomach of mucous and indigestible material (Christie 2012). In studies concerning teleost species, Huang *et al.* (2019) described a positive relationship between fish body length and MP abundance across 30 fish species (n = 120), indicating that MP accumulation is likely occurring. According to the results of the study, carnivorous fishes showed higher MP abundance as opposed to omnivorous species, indicating that plastics similar in appearance to prey species may be a pathway to greater MP intake (Huang *et al*. 2019). While several studies have reported a positive relationship between body length and MPs in fish species (Boerger *et al*. 2010; Ferreria *et al*. 2019; Santos *et al*. 2016; Ugwu & Gómez, 2021; Wright 2013; Xiong *et al*. 2019), habitat type and health are more reliable correlates of MP abundance than diet (Li *et al*. 2021; Parks *et al*. 2020; Valente *et al*. 2019).

While the rate of plastic ingestion in elasmobranch species is understudied, it seems probable that a variable plastic load exists in *R. terraenovae*, where plastics are ubiquitous in both the water column and prey items but are not retained in the digestive tract. Based on the low variation between means over the four-month sampling period, it is unlikely that MPs are accumulating in the individuals in this study. Additionally, there is no evidence of MP abundance increasing with body length in *R. terraenovae*, suggesting that most MPs have a short residence time within the digestive tract and are not retained. The high MP counts reported in Pullen (2019) and this current study can be attributed to the ubiquity of MPs in the environment, which are ingested during respiration or through prey species and then excreted.

Comparison of Sampling Locations

There was a significant difference in MP abundance between specimens collected in Cherry Grove and Garden City. Specimens captured in Garden City (28.1 ± 8.8) had greater abundance per specimen as opposed to Cherry Grove (16.7 ± 9.2). Because this study did not measure MP from the water samples, we can only speculate on the causes of these differences. Cherry Grove and Garden City are part of a developed coastal region known as the Grand Strand, which is home to major tourist destination, Myrtle Beach, which was visited by 12.8 million tourists in 2020 (Shifflet 2021). Cherry Grove, located at the northern end of Grand Strand, is a neighborhood within the city of North Myrtle Beach (17,000 residents), while on the opposing end, Garden City has a population of 10,000 residents. During the summer months, both communities see a surge in tourism and resulting coastal pollution from fishing and recreational activities. While Cherry Grove and Garden City are subjected to similar populations and fishing pressures, Cherry Grove is adjacent to an undeveloped barrier island, Waites Island, a region that is nearly absent of land-based pollution.

Site differences in coastal MP abundance have been suggested to be linked to high-density populations in several aquatic environments (Desforges *et al.* 2014; Yonkos *et al*. 2014). However, recent work has indicated that population density may not be the best predictor for coastal or nearshore MP concentrations. Factors such as land development, regional ecology, and geographic features were more strongly correlated with MP presence (Schuyler *et al.* 2021). For example, Gray *et al*. (2018), reported greater MP concentrations in Winyah Bay, a small town (< 10,000 residents) in a rural area, versus Charleston Harbor, a heavily industrialized and population dense region (> 138,000 residents). Between inshore (within 9 miles of land) and nearshore (adjacent to the shoreline) locations, the greatest densities of MPs, are consistently reported in nearshore coastal regions as the environment is in close proximity to a combination of land-based MP outfalls, such as coastal cities, rivers, and runoff zones (Tudor & Williams 2019). If inshore rather than nearshore sampling was utlilized in this study, it is possible that *R. terraenovae* MP concentrations may have differed compared to the concentrations recorded in Pullen (2019) and the current study, due to lack of consistent MP sources away from land.

In studies concerning MP sampling location and elasmobranch species, Alomar and Deudero (2017) reported MP presence in the Blackmouth Catshark (*Galeus melastomus*) which were sampled from two locations with similar habitat type characteristics and anthropogenic effect. The project found no significant differences in MP concentration in *G. melastomas* between the two locations, further highlighting the ubiquity of MPs and a proposed lack of MP concentration trend based upon location. Further, MP concentrations are difficult to ascertain across different oceanic regions, as MPs are vulnerable to vertical mixing through wave, wind, and tide effects (Hidalgo-Ruz *et al*. 2012; Isobe *et al.* 2017; Yu *et al*. 2019). Regardless of the cause of differing MP concentrations, these combined results indicate that MPs can vary regionally, thus it is important that future studies on MP abundance include multiple study sites in order to provide a more robust estimate of the average MP density in the population.

One of the issues in determining the source of ingested-MPs in marine organisms is the assumption that the species of interest, *R. terraenovae,* has a fixed home range and does not move between sampling sites or beyond the range of the study. Accordingly, juvenile *R. terraenovae* in coastal South Carolina are known to frequently travel between estuarine environments and nearshore regions, and do not adhere to a discrete habitat (Carlson *et al*. 2008; Maxwell 2015). While it has been noted that the home range for juvenile *R. terraenovae* is typically small (average = 1.29 km^2), this species also exhibits multiple forms of residency, where time spent in a region varies on the individual for reasons not understood (Carlson *et al*. 2008). Heupel *et al*. (2007) and Carlson *et al*. (2008) suggest that species, such as juvenile *R. terraenovae*, may have an advantage over strongly philopatric species, due to the trade-offs associated with increased foraging opportunities and quality of prey. As *R. terraenovae* are a highly productive species, the benefits of a set nursey habitat may be limited, whereas constant travel for high-quality prey items may be more advantageous to promote the increased growth rate observed in juveniles of this species.

As such, due to the extent and frequency with which young-of-year *R. terraenovae* immigrate across various coastal areas, in order to determine if the identified plastics were ingested in the regions sampled, telemetric data of captured specimen would be necessary to indicate the recent movements before capture. Therefore, the recorded plastic counts of this current study possess greater validity in describing the MP concentration within a species rather than a specific location.

Another potential issue in the study design could be sampling from locations associated heavy fishing activity, as there is a potential bias for sampling *R. terraenovae* specimen that may be influenced by anthropogenic factors associated with pier activity. This phenomen is described by Martin *et al*. (2019), where the behavior of Blacktip Sharks (*Carcharhinus limbatus*) tagged at several piers along the Grand Strand of South Carolina were affected by increased tidal height, barometric pressure, and fishermen present. *C. limbatus* was influenced by the increased availability of food associated with fishing activity (bait, gutting/cleaning, and other fishes attracted to the food) and spent time at the same piers that they were originally tagged at. In the same manner, it is possible that the *R. terraenovae* specimens captured at Cherry Grove and Garden City were frequently visiting the same pier, as opposed to the natural tendency for *R. terraenovae* to constantly immigrate across multiple sites.

Prenatal Transfer

The potential transfer of plastics during fetal development poses serious health ramifications in both animals and humans. Recent studies have discovered MPs in all placental regions in humans and rats (Fournier *et al.* 2020; Ragusa *et al.* 2021). While no published studies to date have explored prenatal MP transfer in elasmobranchs, *R. terraenovae* could be susceptible to this form of transfer. *R. terraenovae* are viviparous, they possess a similar gestation period length to the aforementioned prenatal MP-transmitted subjects, and nurture their unborn pups via placental sac (Castro & Wourms, 1993), which indicates that unborn *R. terraenovae* are subjected to conditions similar to species with published evidence of prenatal transfer. Considering the age (<1 yr. old), size, and plastic presence in the *R. terraenovae* specimens in this study relative to those described in Pullen (2019), the significant plastic presence at an early age could suggest that MP retention begins during prenatal development. Research focusing on MP presence in pregnant *R. terraenovae* would help to further this question and develop a baseline of transfer rates and timeline.

Significance

To what extent MP ingestion influences the overall health of *R. terraenovae* remains unclear, as the current study did not assess negative effects of MP counts. However, due to the size of these plastics, past research has confirmed MPs can infiltrate and reside in tissue and organs, where the organism is further exposed to concentrated MP-associated organic chemicals. Additionally, plastic particles can inflict physical damage to surrounding tissue, and some cases restrict bodily functions, such as digestion and respiration. Further, the risk of constant exposure to organic chemicals may inflict negative developmental effects, immune deficiencies, hormonal disruption, and increased rate of cell mutation and death. Regarding the species of interest, *R. terraenovae* is an important species in the marine ecosystem, where in their role as a mesopredator, they both regulate prey species too small for larger predators and provide an important food source to apex predators. Additionally, *R. terraenovae* are heavily utilized by fishermen as bait, as well as being consumed directly, thereby identifying a direct pathway for exposure to MPs and potentially harmful chemicals in humans and other predator organisms. As past research has described the deleterious effects of organic chemicals linked to MPs, it can be assumed that the MPs described in this study have a similar capacity to adversely affect the health of an organism over time.

Limitations and Future Directions

The intent of this study was to create a baseline examination of MP abundance and accumulation in young-of-year *R. terraenovae*. Nonetheless, due to experimental constraints during COVID-19 and the scope of MPs examined (0.1 – 5.0 mm), a large number of plastic particles, such as nanoplastics (0.001–0.1 μm), were excluded from the analysis. Previous research has shown that the majority of plastic particles are smaller than 0.5 mm in surface water samples (Medina *et al*. 2021), meaning that many MP studies are not describing the total plastic population. Moreover, studies such as De Sales-Ribeiro *et al*. (2020), have addressed the issue of misinterpretation of MP presence and impact in the digestive tract of fish species and its delusive effect on future studies. In their research, De Sales-Ribeiro *et al*. (2020) chronically exposed MPs to a group of

Zebrafish (*Danio rerio*) in a controlled lab environment. *D. rerio* did not display histopathological changes in either the digestive tract or liver, contradicting the reported physical effects of MPs in teleosts in the majority of existing literature.

The indeterminate parameters surrounding quality control remains a divisive topic for many plastic-related studies, as contamination safeguards and analysis vary between studies. While foreign plastic introduction is unavoidable in dissection and assessment of species, further precautions must be followed in order to decrease the exposure. Filtering all water sources before introduction, working in airflow restricted spaces, and distinctive clothing color are examples of methodology that past studies have used to improve the yield and quality of the results.

Future research should address the gap of information in quantifying the MP rate of weathering and fragmentation. Currently, identification techniques such as Fourier-transform infrared spectroscopy (FTIR) can be used to describe the extent of weathering, but weathering varies due to polymer type, additive makeup, and environmental influences (GESAMP, 2015). In determining plastic concentrations in the marine environment, future studies should also include inland sources of primary MP production, as spillage and runoff rates in coastal regions will help to describe MP introduction and movement (Schuyler *et al*. 2021). Additionally, further examination of accumulation in elasmobranch species over a larger timeframe will improve the current knowledge of plastic retention and potential health effects.

Conclusion

The study of MP consumption and contamination will advance current knowledge of how MPs move through trophic levels and their ontogenetic effects on size, fecundity and health indicators in sharks and other marine animals. The absence of significant MP increase over the course of this study suggests MPs are not accumulating in the digestive tract and livers of young-of-year *R. terraenovae*. While the rate of MPs excreted is unknown, the absence of a trend indicates a variable amount of plastic in the digestive tract that constantly fluctuates due to a combination of feeding, respiration, and expulsion. The high counts of MPs at an early age may indicate potential prenatal transfer of plastic, but more work is necessary to confirm this. Furthermore, future research is necessary to identify health risks and complications posed by MP absorption and accumulation in the marine organisms.

Tables

Table 1. Current literature on elasmobranch species examined for MP presence from 2013 to 2022. Sample size (N), plastic occurrence, filtration analysis, and fiber classification are noted. Adapted from Bernardini *et al*. 2018 and Pullen 2019.

Species	N	Plastic Occurrence	Filtration Analysis	Fibers Included	Author
Centroscymnus coelolepis	11	9%	No	Yes	Cartes *et al.* 2016
Centrophorus granulosus	5	0%	No	Yes	Anastasopoulou *et al*. 2013
Etmopterus spinax	16	6%	No	Yes	Anastasopoulou *et al*. 2013
Etmopterus spinax	323	6%	No	Yes	Deudero & Alomar, 2015
Etmopterus spinax	9	11%	No	Yes	Cartes *et al.* 2016
Etmopterus spinax	34	61.8%	Yes	Yes	Valente *et al*. 2019
Galeus melastomus	741	3%	No	Yes	Anastasopoulou *et al.* 2013

Galeus melastomus	125	16%	No	Yes	Alomar & Deudero, 2017
Galeus melastomus	125	16%	No	Yes	Cartes *et al.* 2016
Galeus melastomus	32	78.1%	Yes	Yes	Valente *et al*. 2019
Lamna nasus	13	100%	Yes	Yes	Maes *et al*. 2020
Mustelus asterias	12	66.6%	Yes	Yes	Parton *et al*. 2020
Prionace glacua	95	25%	Yes	Yes	Bernardini *et al*. 2018
Rhizoprionodon lalandii	6	33%	No	No	Miranda *et al*. 2016
Rhizoprionodon terraenovae	16	100%	Yes	Yes	Pullen, 2019
Rhizoprionodon terraenovae	**30**	**100%**	**Yes**	**Yes**	**Present Study**
Scyliorhinus canicula	1	0%	No	Yes	Anastasopoulou *et al.* 2013
Scyliorhinus canicula	72	15.3%	Yes	Yes	Bellas *et al*. 2016
Scyliorhinus canicula	12	75%	Yes	Yes	Parton *et al*. 2020
Scyliorhinus canicula	30	66.7%	Yes	Yes	Valente *et al*. 2019

Scyliorhinus canicula	20	5%	No	No	Smith, 2018
Scyliorhinus stellaris	10	70%	Yes	Yes	Parton *et al*. 2020
Squalus acanthias	16	6%	Yes	No	Avio *et al*. 2015
Squalus acanthias	323	6%	No	Yes	Anastasopoulou *et al*. 2013
Squalus acanthias	12	58%	Yes	Yes	Parton *et al*. 2020
Squalus blainville	9	11%	No	Yes	Anastasopoulou *et al*. 2013
Squalus blainville	17	12%	Yes	Yes	Neves *et al.* 2015

Table 2. Contamination averages for foreign plastics encountered during dissection and visual analysis per individual specimen. Plastics recorded in both the Milli-Q water filtration system and airborne contaminants were combined to determine the averages. Additionally, only fibers were included, as all the other combined shape classes made up 2.2% of all contaminate plastics recorded. Each average was redacted from the total count of plastics per shark specimen, for a total of 14 plastics removed per individual.

	Clear	**Blue**	**Black**	**Red**	**Other**	**Total**
Average Contaminant Microplastics	10.1	1.9	0.8	0.8	0.4	14

Table 3. Post-hoc analysis (Tukey test) comparing microplastic shape present among individuals. (α-level = 0.05). Fibers were significantly different than the subgroup of films, fragments, and pellets.

Shape	N	Mean (SD)	Frequency	Treatment
Fiber	614	20.47 (10.05)	30	a
Film	29	0.97 (1.10)	17	b
Fragment	25	0.83 (1.12)	14	b
Pellet	4	0.13 (0.35)	4	b

Table 4. Post-hoc analysis (Tukey test) comparing microplastic size present among individuals. (α-level = 0.05). The size class, less than 0.5 mm, was significantly difference than the class, 0.5 – 0.99 mm, and the subgroup of 1.1 – 1.5 mm and greater than 1.5 mm.

Size Class (mm)	N	Mean (SD)	Frequency	Treatment
0.5<	444	14.80 (7.76)	30	a
0.5-0.99	157	5.23 (4.35)	30	b
1-1.5	51	1.70 (1.15)	27	c
>1.5	20	0.67 (0.88)	14	c

Table 5. Post-hoc analysis (Tukey test) comparing microplastic color present among individuals. (α-level = 0.05). The color classification, clear, was significantly different than the subgroup of blue and black, as well as the subgroup of red and other colors.

Color	N	Mean (SD)	Frequency	Treatment
Clear	316	10.53 (6.96)	30	a
Black	167	5.57 (2.39)	29	b
Blue	135	4.50 (2.74)	27	b
Red	49	1.63 (1.13)	26	c
Other	5	0.17 (0.38)	5	c

Table 6. Microplastic abundance in *R. terraenovae* at Cherry Grove, SC.

ID	Month	Sex	Precaudal Length (cm)	Number of MPs (corrected for contamination)	Mean MP Length (mm) (SD)
CG1	May	F	21.5	4	0.33 (0.36)
CG2	May	F	23.6	8	0.48 (0.41)
CG3	May	F	23.1	27	0.46 (0.41)
CG4	May	M	24.9	22	1.00 (0.45)
CG5	May	F	23.6	14	0.40 (0.11)
CG6	June	M	23.1	22	0.57 (0.33)
CG7	June	F	22.8	4	0.43 (0.35)
CG8	June	M	24.1	11	0.42 (0.31)
CG9	July	M	22.7	22	0.42 (0.27)
CG10	July	M	26.2	32	0.53 (0.39)
CG11	July	M	28.7	10	0.38 (0.35)
CG12	August	M	29.8	6	0.49 (0.34)
CG13	August	F	27.3	21	0.46 (0.58)
CG14	August	F	26.1	28	0.47 (0.45)
CG15	August	F	31.1	20	0.53 (0.52)
Total				**251**	**0.49**

Table 7. Microplastic abundance in *R. terraenovae* at Garden City, SC.

ID	Month	Sex	Precaudal Length (cm)	Number of MPs (corrected for contamination)	Mean MP Length (mm) (SD)
GC1	May	M	24.2	38	0.45 (0.34)
GC2	May	M	23.8	27	0.51 (0.35)
GC3	May	F	23.9	51	0.49 (0.39)
GC4	May	M	24.5	36	0.35 (0.24)
GC5	May	M	22.7	24	0.44 (0.39)
GC6	June	F	22.9	25	0.32 (0.35)
GC7	June	F	22.6	20	0.47 (0.33)
GC8	June	M	25.7	26	0.39 (0.43)
GC9	July	M	30.6	14	0.41 (0.38)
GC10	July	M	25.0	28	0.46 (0.42)
GC11	July	F	26.4	32	0.40 (0.35)
GC12	August	F	28.6	26	0.31 (0.20)
GC13	August	F	32.4	25	0.47 (0.55)
GC14	August	F	30.4	20	0.62 (0.74)
GC15	August	F	29.0	29	0.71 (0.76)
Total				**421**	**0.45**

Figures

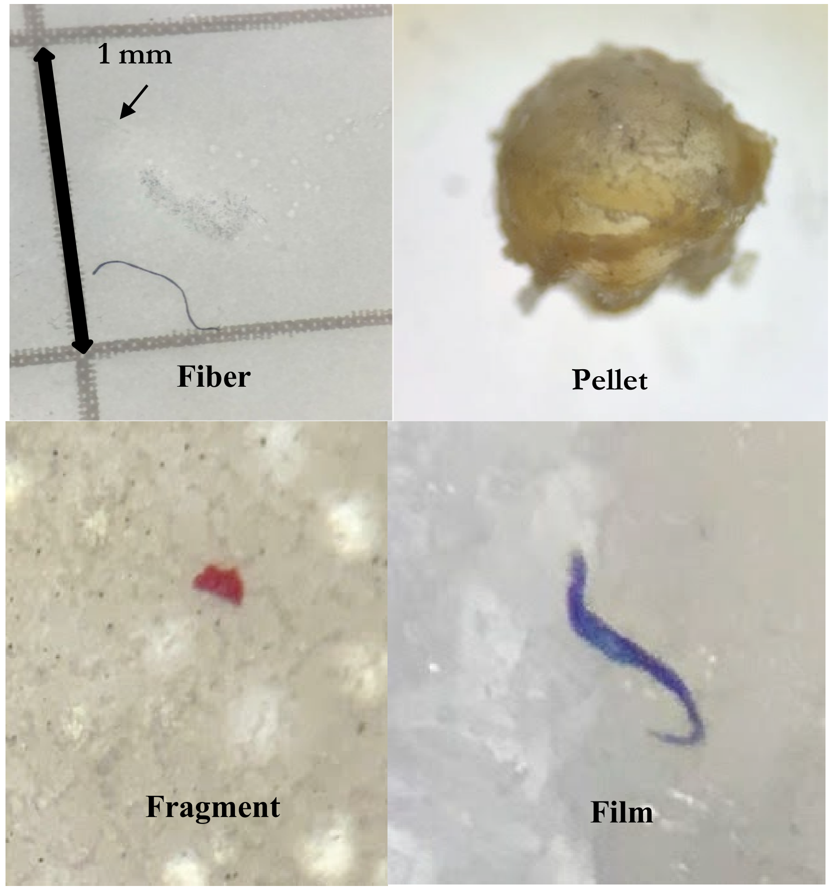
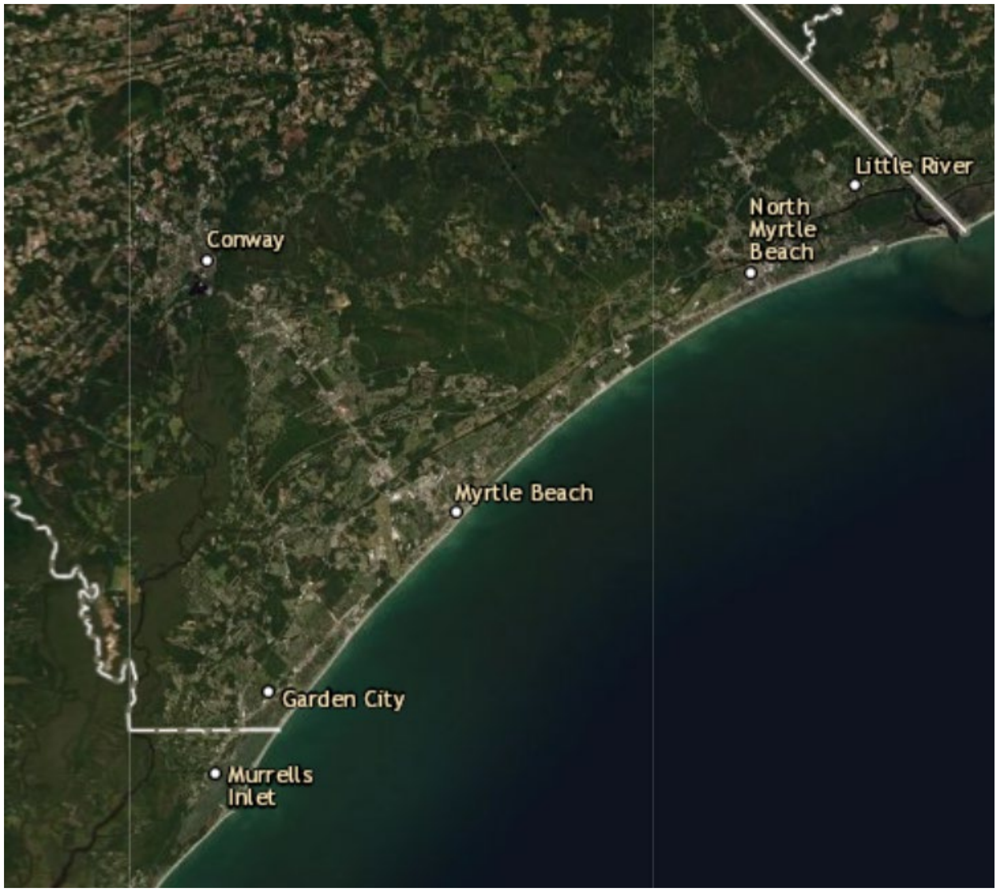


Figure 1. The four common microplastic shape classes. Scale bar represents 1 mm.



USGS. (n.d.). Satellite Image of Coastal South Carolina. EarthExplorer. Retrieved June 9, 2022, from https://earthexplorer.usgs.gov/.

Figure 2. Aerial view of the Grand Strand, SC including sampling sites, Garden City and Cherry Grove (North Myrtle Beach).

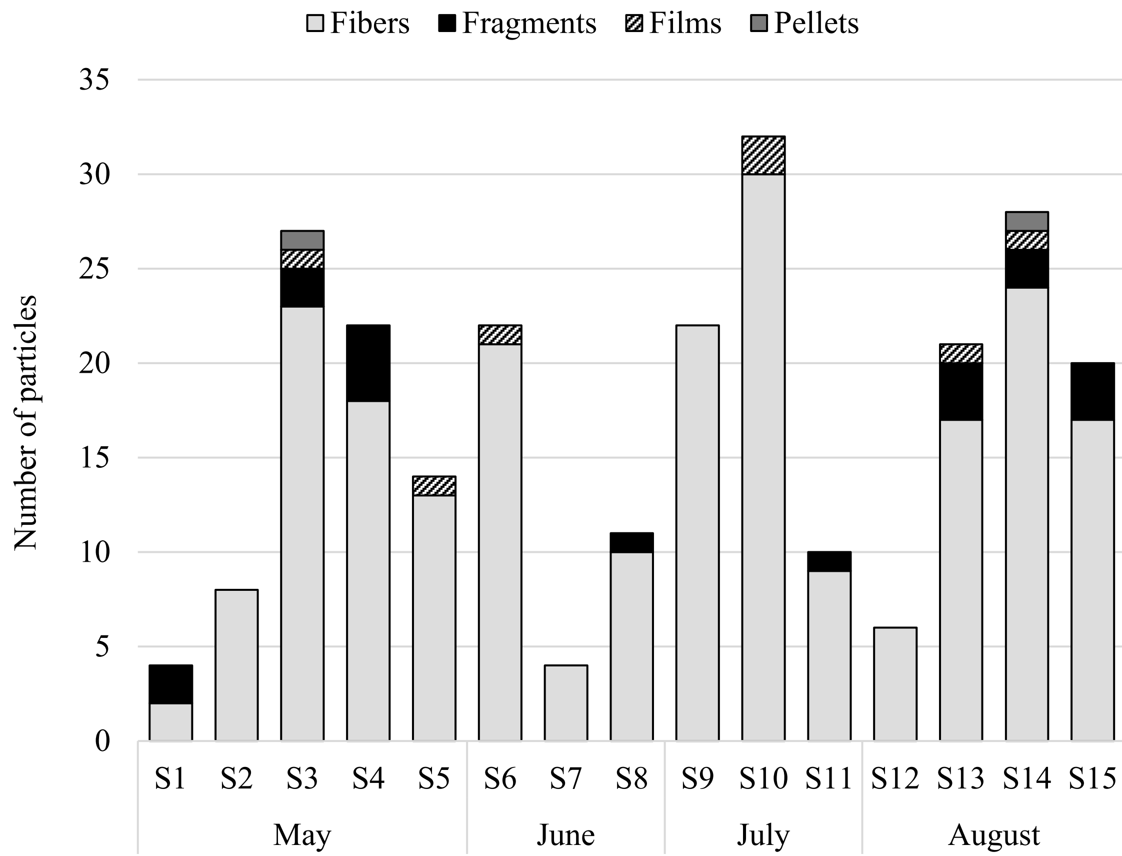


Figure 3. Distribution of shape categories of microplastics in *R. terraenovae* at Cherry Grove, SC over a four-month interval. Specimens were caught in May (S1-S5), June (S6-S8), July (S9-S11), and August (S12-S15).

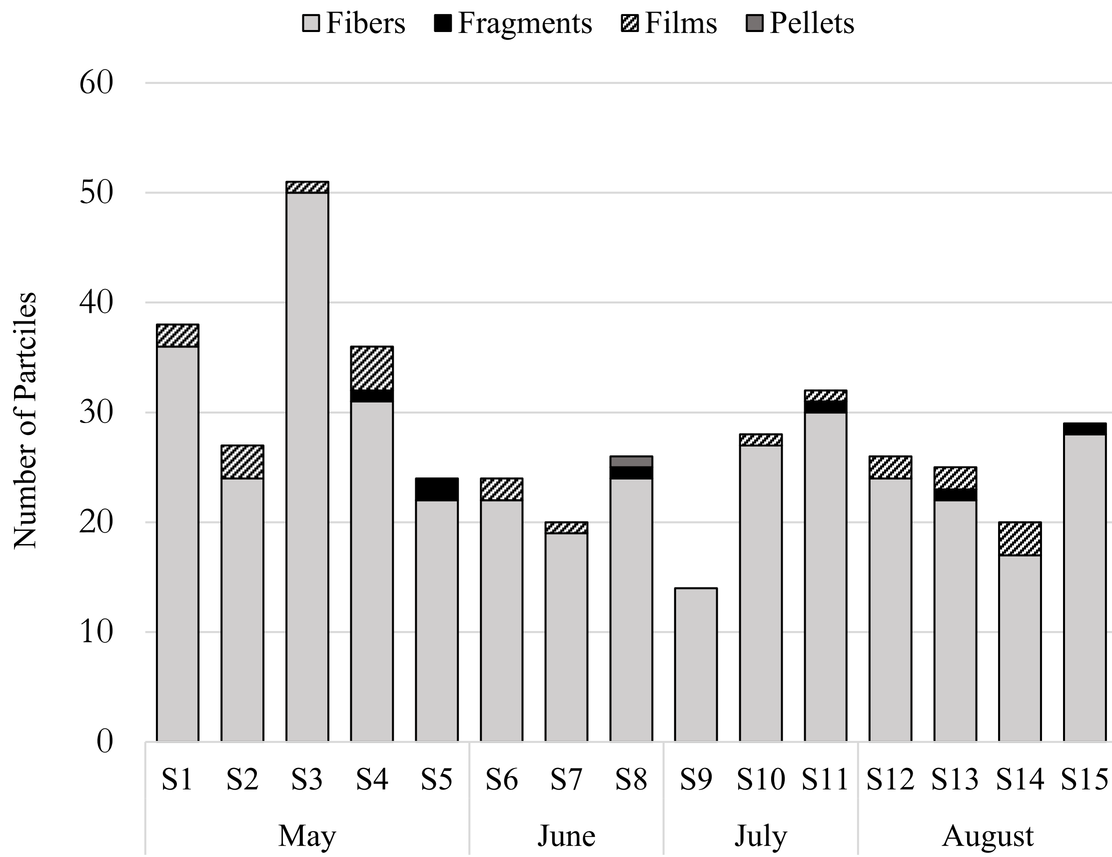


Figure 4. Distribution of shape categories of microplastics in *R. terraenovae* at Garden City, SC over a four-month interval. Specimens were caught in May (S1-S5), June (S6-S8), July (S9-S11), and August (S12-S15).

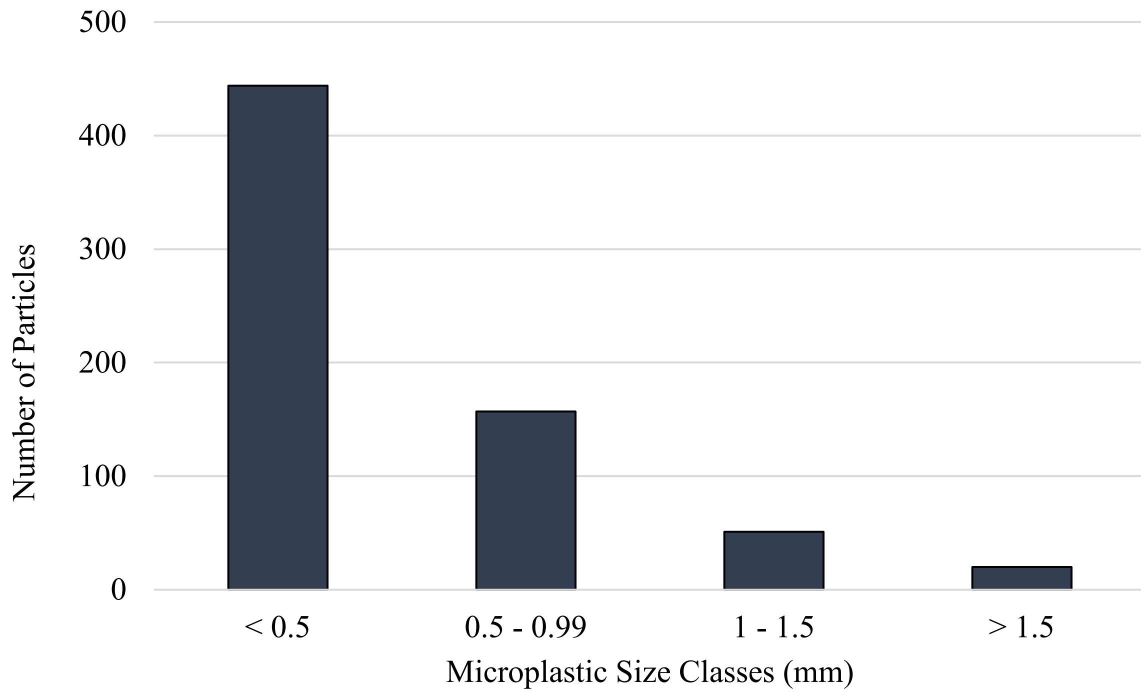


Figure 5. Distribution of size categories of microplastics in *R. terraenovae* at Cherry Grove and Garden City, SC over a four-month interval.

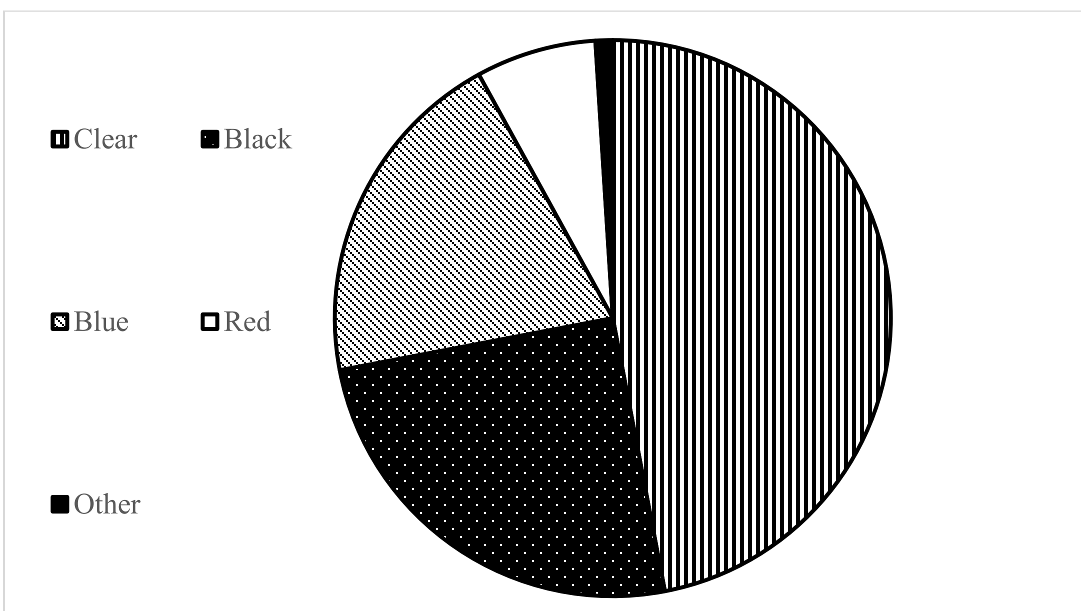


Figure 6. Distribution of color categories of microplastics in *R. terraenovae* combined for Cherry Grove and Garden City, SC over the four-month sampling interval.

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