microplastics

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Relationship of Microplastics to Body Size for Two Estuarine Fishes

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Abstract: In the northern Gulf of Mexico, microplastics are reported in very high concentrations, which are thought to be partly sourced from the Mississippi River. This study sought to quantify microplastics across body size in two fish species, the hardhead catfish (*Ariopsis felis*) and southern flounder (*Paralichthys lethostigma*), common to Gulf of Mexico estuaries. We hypothesized that counts of ingested microplastics would be higher in smaller fishes than larger fishes. Fish were sampled in 2018 and 2019 across coastal Louisiana and represented a balanced range of length classes. Both species in our study ingested microplastics—25% of southern flounder and 15% of hardhead catfish. There was a significant positive effect of total length on microplastic loads in hardhead catfish. Due to the biological importance and management relevance of fish length, the study of microplastic loads and effects on fish may need to move beyond aggregating a species to considerations of individual size.

Keywords: *Ariopsis felis*; *Paralichthys lethostigma*; estuary; total length

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1. Introduction

Microplastics are usually defined as plastics that are less than 5 mm in any dimension; however, there is no consensus lower size limit [1,2]. Microplastics can also be defined according to different characteristics such as their shape (e.g., fibers, foams, fragments, beads, or films) and their composition (usually referring to polymer type [3]). Microplastics are ubiquitous across most ecosystems and species, and estuaries are no exception. Multiple studies have documented microplastics in estuarine ecosystems. For example, microplastics were detected in the sediments of Vitória Bay, Brazil [4], and McEachern et al. [5] reported microplastics in both waters and sediments of Tampa Bay, Florida. Estuarine fish species have also been reported to contain microplastics. 38% of 120 fish sampled in the Mondego estuary in Portugal had ingested microplastics [6]. Kazour et al. [7] reported microplastics in juvenile European Flounder (*Platichthys flesus*); more specifically, 58% of wild European Flounder had microplastics in their digestive tract, and 75% of caged European Flounder had microplastics in their digestive tract. Microplastics have also been documented in numerous benthic fish species along the Texas Gulf coast [8]. Recent studies of estuarine fishes have reported fibers to be the most commonly detected shape (over 75% of the time) of microplastics [4–6,8]. Other studies provide specifics on microplastic polymers in estuarine systems; for example, Peters et al. [8] found that polyethylene terephthalate (PETE) and polyvinyl chloride (PVC) were the most detected polymers in estuarine fish, while Bessa et al. [6] found that polypropylene, polyester, and rayon were the most detected polymers in estuarine fish. Microplastics are of increasing concern in fishes due to their ability to harm fish physically (e.g., clogging digestive tracts [9,10]) as well as chemically from the persistent organic pollutants that are known to adhere to plastics and perhaps be transferred to fishes [11].

Recent work has reported high concentrations of microplastics in the northern Gulf of Mexico waters, especially close to the mouth of the Mississippi River [12]. About 40% of recreationally harvested fish species and 25% of the U.S. commercial fishery catches come from the Gulf of Mexico [13], which means that many people rely on (and consume) coastal fish species from Gulf of Mexico waters. Despite numerous studies of microplastics and fish, little information exists about the effect of fish size on microplastic load. Because body size is a very important variable of fish biology and important to anglers, any information on how microplastic loads relate to fish size could both improve our ecological understanding of microplastic loads and potentially inform any management that seeks to account for microplastics in fishes.

This study focuses on two fish species common to the northern Gulf of Mexico: hardhead catfish (*Ariopsis felis*) and southern flounder (*Paralichthys lethostigma*). Hardhead catfish are widely distributed in the Gulf of Mexico, southeastern U.S. Atlantic, and parts of the Caribbean [14] and live close to mud and submerged sand flats to opportunistically feed on algae, crustaceans, seagrasses, worms, and fish [15]. Stable isotope analyses of hardhead catfish in Louisiana support the idea that their diets remain constant across sizes and ages [16].

Southern flounder is a valuable recreational and commercial fish species throughout its range in the Gulf of Mexico and southeastern U.S. Atlantic Ocean. Southern flounder have an estuarine-dependent life cycle, as they spend their juvenile years in various parts of estuarine and coastal waters [17,18]. Southern flounder are also demersal (like hardhead catfish), but they are an ambush predator that camouflage on the benthos and feed mostly on crustaceans in the juvenile stage and fish as they grow [19]. Although no studies report on microplastics in hardhead catfish, Phillips and Bonner [20] documented microplastics in stomach contents of southern flounder (but the sample size was only eight fish, and they did not report how many fish ingested microplastics).

Though it is now widely documented that fish ingest microplastics, we do not always know the factors that contribute to individual fish microplastic loads. Therefore, to better understand microplastic loads in fishes, we attempted to quantify microplastic loads in two estuarine fishes and examine factors, such as body size, polymer types, shapes, and colors of microplastics, possibly related to their ingestion. Studying those factors may advance an understanding of the mechanism of microplastic ingestion by fishes.

2. Materials and Methods

2.1. Study Area and Sampling

All fish samples used in this study were collected from coastal Louisiana, USA. Hardhead catfish and southern flounder were selected because of their ubiquity across coastal Louisiana and our ability to sample them across a wide range of sizes. All hardhead catfish were collected in June and July 2018 by the Louisiana Department of Wildlife and Fisheries (LDWF) as part of their fishery-independent sampling program. Sampling was conducted out of the Bourg and Lacombe LDWF field offices and primarily took place in the Pontchartrain Basin and Timbalier and Terrebonne Basin according to LDWF sampling procedures using trammel nets, gill nets, trawls, and seines [21]. The total sample size for hardhead catfish was n = 40, which was equally balanced among four different total length (TL) categories (0–99 mm TL, 100–199 mm TL, 200–299 mm TL, and >300 mm TL) in order to analyze the effect of body length on microplastics.

Southern flounder were collected from October 2018 to April 2019 by the LDWF fishery-independent sampling program and also from fishery-dependent sampling. For fishery-independent samples, southern flounder were collected from across coastal Louisiana and not limited to specific basins. Fishery-dependent samples were collected from two seafood dealers in Louisiana and from recreational fishing activities. Approximately half of the southern flounder samples were collected with LDWF fish trawls, while the rest of the fish were collected with different methods including hook and line, electrofishing, seine, and trammel nets. The total sample size for southern flounder was n = 50, which was equally balanced among five different total length categories (0–99 mm TL, 100–199 mm TL, 200–299 mm TL, 300–399 mm TL, and >400 mm TL) in order to analyze the effect of body length on microplastics.

2.2. Lab Methods

Although frozen shortly after collection, all samples were defrosted before processing. Both hardhead catfish and southern flounder were measured for total length (mm TL), body weight (g), and gastrointestinal tract (GIT, for hardhead catfish) or stomach (for southern flounder) weight (g). Individual fish were dissected with scissors from the anus to the esophagus and the complete GIT or stomach was removed, weighed, and stored in glass jars. Hardhead catfish GITs and southern flounder stomachs were digested in glass beakers using a modified method of Foekema et al. [22]. Samples were placed in glass jars and fully submerged in a 10% filtered KOH (potassium hydroxide) solution for the KOH to dissolve the organic matter and leave the plastics unmodified. Glass jars with samples were kept in a water bath with a controlled temperature of 60 °C for 24 h and were manually stirred every few hours until the organic tissues were fully digested. After digestion, the slurry solution was vacuum filtered through a nylon net filter paper with 20 µm pore size, and our methods allowed for detection of microplastics ranging from 20 µm to 5000 µm. Each filter paper was stored in a labeled, covered petri dish and kept for 24 h in an incubator at 50 °C to ensure that samples were dry before any further processing. Macroscopic particles were removed with stainless-steel forceps and processed using an FTIR spectrometer (Thermo Fisher Scientific iS5, Waltham, MA, USA) in attenuated total reflectance (ATR) mode to determine if they were plastics. The other smaller, putative particles on filter papers were examined using an FTIR microscope (Thermo Fisher Scientific Nicolet iN10) in transmission and reflection modes. Plastic polymer libraries were installed on both instruments. We identified if each particle was plastic or not by matching the particle spectra with the libraries using Omnic Pecta software. After confirming the occurrence of plastic, the Omnic Pecta software along with the FTIR microscope (Thermo Fisher Scientific Nicolet iN10) were used to identify every microplastic found at the polymer level according to the libraries installed. We adopted a spectral match threshold of 70%; however, other spectra between 60–70% matches were confirmed or denied with further visual examination. Individual microplastic concentration was calculated as the number of plastic particles per fish.

To prevent contamination, nitrile gloves were used. We ran the gloves through the FTIR which reported that they were made of polybutadiene (21 percent acrylonitrile). All equipment and work surfaces were washed with distilled water before use, and GITs and stomachs were placed immediately in the clean glass jars. New petri dishes were closed immediately after putting the filter paper in each of them to prevent any aerial contamination. Control samples were processed regularly to test the possible contamination from the liquid potassium hydroxide, air, and equipment. Glass jars with filtered KOH but without stomachs were used as blanks, filtered, and scanned. No microplastics were identified in the control samples. Contamination practices were the same as those reported and used in Toner and Midway [23].

2.3. Data Analysis

Because microplastic data were counts and many fishes had zero counts, a Zero Inflated Poisson (ZIP) model was used to test the relationship between body size (TL) and microplastic ingestion. The use of a ZIP model allowed for the accommodation of zero inflation while still performing the Poisson regression (even if not truly zero-inflated). The ZIP model is composed of two linked models. The first model is a logistic regression that models suitability using a Bernoulli distribution, which effectively estimates whether an observation is a value (i.e., a zero) that should be included in the Poisson model, or whether it is a zero that should be further excluded. The model is written as:

$$\omega_i \sim Bernoulli(\varphi_i)$$

where ω is the logistic model output, *i* represents the observational units, and φ is the probability. The second model is a modified Poisson dealing with the counts:

$$C_i \sim Poisson(\omega_i \times \lambda_i)$$

$$\lambda = \alpha + \beta_1 \times x_1 + \beta_2 \times x_2$$

where C_i is the observed counts and λ is the Poisson parameter for counts. The zero-inflated model structure was then used with a linear predictor to model our hypothesis about fish total length. The intercept is α, β_1 is the coefficient (slope) of total length, x_1 is the observed total length, β_2 is the coefficient (slope) of GIT or stomach weight, and x_2 is the observed GIT or stomach weight. We were primarily interested in the effect of total length but included GIT or stomach weight as a covariate that may influence microplastic counts. Each species was modeled separately.

This model was designed to address the hypothesis that total length is significantly positively related to microplastic counts in hardhead catfish and southern flounder. No covariates were added to the binomial component of the model. Although the model allows for this possibility, we had no hypotheses that could be represented by a covariate on the binomial response, in addition to the fact that the unconditional binomial model is recommended in situations when unknown environmental covariates determine suitability [24].

We were also interested in descriptive analyses—descriptions of our data that may be relevant or of interest but for which we had no hypotheses. (For example, we had no hypotheses about color of microplastic particles ingested, yet any results may be of interest and provoke new ideas.) We evaluated the microplastic sizes, polymer types, shapes, and colors that were found in fish GITs and stomachs. All analysis was performed using R [25].

3. Results

3.1. Microplastics and Total Length

A total of 15% of hardhead catfish had ingested microplastics. A total of eight microplastic particles were identified in six individual fish GIT; four fish each ingested only one microplastic particle and two fish each ingested two microplastics particles. 25% of southern flounder ingested microplastics; a total of 16 microplastic particles were identified in 13 fish stomachs. Ten fish each ingested only one microplastic particle, and three fish each ingested two microplastics particles.

The ZIP models found no effect of the GIT weight covariate in hardhead catfish (β_2 = 0.07, SE = 0.05, p-value = 0.18) or the stomach weight covariate in southern flounder (β_2 = 0.10, SE = 0.05, p-value = 0.06; Figure 1). The proportion of true zeros (φ) estimated by the binomial part of the ZIP models was relatively high; φs for hardhead catfish and southern flounder were estimated to be p = 0.67 and 0.99, respectively. The ZIP for southern flounder estimated no effect of total length on microplastic counts ($\beta_1 = -0.002$, SE = 0.003, p-value = 0.35), although total length was reported as a significant positive effect on microplastic counts in hardhead catfish (β_1 = 0.02, SE = 0.01, p-value = 0.03).

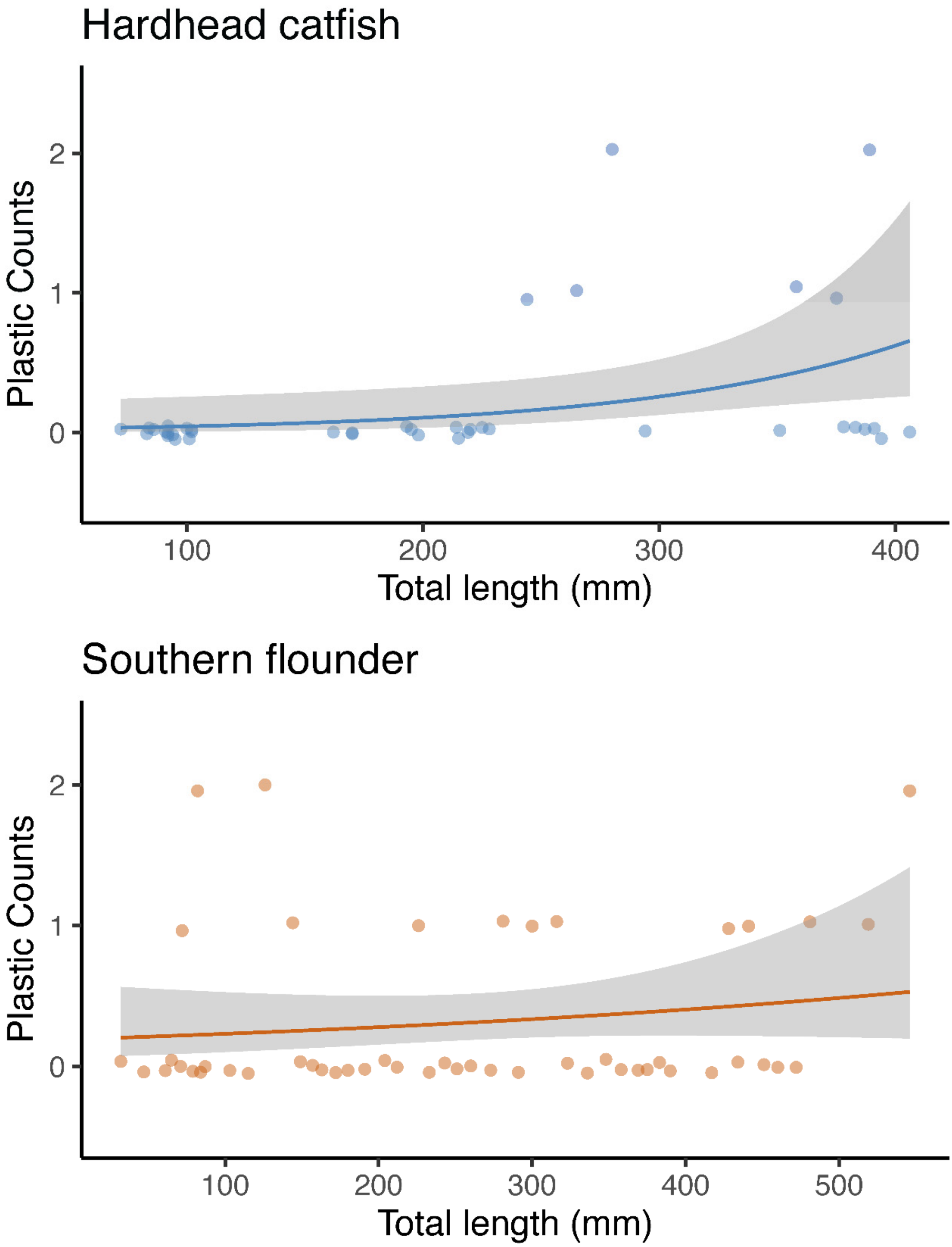

Figure 1. Relationship between microplastic counts and fish total length for hardhead catfish and southern flounder captured in coastal Louisiana. In each panel, the dots represent data, the solid line represents a Poisson model fit, and the gray polygon represents uncertainty around the model.

3.2. Microplastics Descriptions

The spectra of the nitrile gloves that were used during fish processing did not match any of the microplastics polymer spectra that were found in the fish samples. 75% of microplastics ingested by hardhead catfish were blue and 25% were white, while southern flounder ingested an equal portion of blue and black colored particles (Figure 2). 75% of the microplastics that hardhead catfish ingested were fragment shaped, while 93% of the microplastics in southern flounder stomachs were fibers (Figure 3). We detected a range of polymer types, with polypropylene being the most common in hardhead catfish and polystyrene and polyacrylonitrile being the most common in southern flounder.

Figure 2. Tree plots quantifying microplastic colors, shapes, polymers, and sizes found in hardhead catfish and southern flounder captured in coastal Louisiana. In each of the four panels, fish species are separated by the vertical white line, and a breakdown of the microplastic attributes is quantified by species. Microplastic sizes are defined as small (20–1000 µm), medium (1000–2500 µm), and largest (>2500 µm). For color areas in which there was not enough room to include the label, FR = fragment, PP = polypropylene, and PI = polyisoprene.

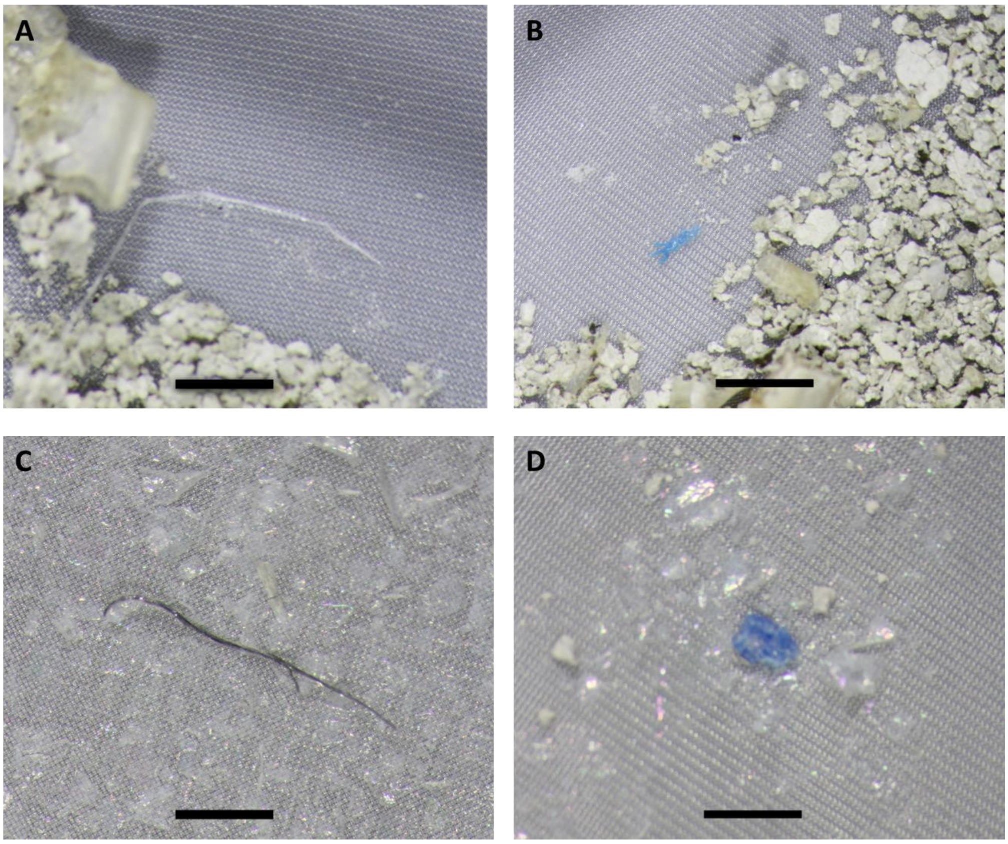

Figure 3. Microplastics found in fish from coastal Louisiana on filter paper. White fiber (**A**) and blue fragment (**B**) found within hardhead catfish; black bar = 1 mm for scale. Black fiber (**C**) and blue fragment (**D**) found within southern flounder; black bar = 1 mm for scale. The other fragmented particles on the filter paper are fish tissue residues from the digestion process.

4. Discussion

This study is the first record of microplastics in hardhead catfish and, to our knowledge, the first study specifically designed to examine the effect of fish total length on microplastic loads. We found that microplastics concentrations in hardhead catfish GIT significantly increased with increasing body length. For southern flounder, we recorded higher concentrations of microplastics in southern flounder stomachs comparing to the only published study [20] in which the sample size was only eight southern flounder and it was not reported how many fish ingested microplastics. However, Phillips and Bonner [20] found microplastics in 10.4% of 116 fish samples of different marine fish species that they studied. Southern Flounder body length was not found to have any significant effect on microplastic loads.

The goal of our study was to examine for microplastics in two estuarine fish species in the Gulf of Mexico and evaluate any relationship of microplastic concentrations to fish total length. Although we are not comparing the two fish species per se, it was interesting to see that 13 of our 50 southern flounder ingested microplastics, while only 6 out of 40 hardhead catfish ingested microplastics. However, we looked at microplastics in hardhead catfish in their entire digestive tracts while looking only in the stomachs in southern flounder. The main foraging difference between hardhead catfish and southern flounder is that hardhead catfish are benthic omnivores that have varied diets, while southern flounder are ambush predators, the adults of which mostly feed on fish. An ambush feeding behavior might contribute to the apparent random nature of microplastics of different sizes as microplastics might come indirectly from their prey or accidentally from the water column. In addition to foraging guild, at least one study has reported higher amounts of microplastics in higher tropic levels [26], which is consistent with our findings.

In addition to total length, we recognize that other variables may play a role in determining microplastic loads. Unfortunately, these variables are beyond our control to account for. First, we do not know the background levels of microplastics in the estuarine environments. Having some measure of available microplastics would provide helpful context for what we observed in the fish we sampled. Although environmentally available microplastics are not known, we can safely operate with the assumption that microplastics are present, if not abundant, in the northern Gulf of Mexico [12,27,28]. A second factor is the temporal change in microplastics. Microplastic loads in the environment and in fishes may change over several time scales, and evidence has already found some differences because of seasonal effects [29]. Coastal Louisiana is subject to freshwater input from the Mississippi River and other sources, and as such, there could be a seasonal effect that we were not able to capture. It was not logistically possible to collect all of the samples (especially with our design of length intervals) at the same time or same place, yet we did limit samples to coastal Louisiana.

The primary plastic polymers that were found were polypropylene and polystyrene, which represented about half of the polymers found in fish species. Wessel et al. [27] studied microplastics in the beach sediment of the Gulf of Mexico and they found that polypropylene and polyethylene were the most available microplastics polymers, followed by polystyrene, polyamide, and polyester. Polypropylene and polystyrene were among the five most common plastic polymers produced globally in 2012 [30]. Polypropylene represented 19% of global plastic production (54 million tons), while polystyrene represented 7% of the global plastic production (21 million tons). Unfortunately, we do not have water samples associated with the fish samples we studied, and, as a result, we are unable to conclude much about the random or non-random nature of microplastics in fish compared to their presence in fish environments.

Previous studies reported that fibers represented the majority of microplastics shapes that were found in fish [31,32]. We also found fibers to be the most common shape in the southern flounder we studied. However, hardhead catfish mainly ingested fragments. Less is known about distributions of microplastic colors, although microplastics in Mexican beach sediments were most often white, followed by blue and green [33]. In our study, hardhead catfish mostly ingested blue colored microplastics and some white ones, while southern flounder ingested an equal amount of blue- and black-colored microplastics. It is still unclear if fish non-randomly select shape or color of microplastics. More available reports and studies might contribute to useful information about microplastic ingestion by fish, or it may be that fish randomly ingest whatever microplastics they encounter. Fish body length is very important for several reasons. To individual fish, body length is an important biological trait that is correlated with natural mortality, foraging success, and other behaviors that can shape populations. Length also forms the basis of the most common fisheries' management tools—size regulations. As we now accept that most fish species ingest microplastics, we need to start considering factors within species, such as length, that are related to microplastic loads and how such factors may be considered for natural and managed populations.

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