Fall 12-2020

# The Occurrence of Microplastics Among Freshwater Fish Guilds in a South Carolina Reservoir

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# THE OCCURRENCE OF MICROPLASTICS AMONG FRESHWATER FISH GUILDS IN A SOUTH CAROLINA RESERVOIR

A Thesis

Presented to the Faculty

Of the

College of Arts and Sciences

In Partial Fulfillment

Of the Requirement for the Degree

Of

Master of Science

In the Department of Biology

Winthrop University

December 2020

By

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**Abstract**

Plastic pollution has become a global concern. Plastic pollution can be defined as macroplastic (> 2.5 cm), mesoplastic (2.5 cm to 5 mm), and microplastic (<5 mm). Microplastics are either manufactured for industrial use (primary microplastics) or are small degraded pieces of plastic from larger plastic items (secondary microplastics). Freshwater microplastics have been understudied compared to marine microplastics, but research has increased in recent years. Research has attempted to determine whether different feeding guilds of fishes were more prone to consume microplastics. In this study I examined if freshwater fishes (gizzard shad, blue catfish, white perch, and black crappie) from different feeding guilds accumulated microplastics in the gastrointestinal tract (GIT) and gill structures. I also wanted to determine if there was a relationship between microplastic concentrations among the different fishes. I found that microplastic concentrations differed among feeding guilds in GIT samples and gill samples, however weight had no relationship with microplastic concentrations except in gizzard shad gill samples. Mean microplastic concentrations in GIT samples ranged from 8.66 ± 3.63 SE microplastics per individual (white perch) to 52.30 ± 13.61 SE microplastics per individual (black crappie). Mean microplastic concentrations in gill samples ranged from 24.36 ± 6.82 SE microplastics per individual (blue catfish) to 69.66 ± 12.24 SE microplastics per individual (black crappie). A species had unexpectedly high numbers of microplastics in their gill structures. This suggests that further research needs to investigate whether morphological or physiological factors contribute to microplastic accumulation in freshwater fishes.

**Acknowledgments**

I would like to express my sincere and genuine gratitude to my thesis advisor, Dr. Cynthia Tant. Dr. Tant may not be aware of all the ways she has altered my life. She offered me a position in her lab as an undergraduate and gave me the opportunities to learn and grow under her advisement and mentorship throughout my undergraduate and my graduate experience. I would also like to thank my thesis committee members, Dr. Jennifer Schafer and Dr. Salvatore Blair. These two went above and beyond to help me with this thesis and their help is greatly appreciated. My fellow graduate classmates and officemates, Ebone' Simpson, Alexis Nystrom, and James Schlott, also need to be acknowledged for their willingness to help me dissect so many fish and clean up afterwards. Beyond just helping with my thesis, they gave me friendship while in graduate school. A special acknowledgment is owed to Preston Chrisman and Robert Stroud from SCDNR. Those two really went above their call of duty as state employees to help me with this thesis. Lastly, I would like to thank my family and my son. They have allowed me to pursue this educational journey and have all chipped in to help with my son on the long days and nights that are required of a graduate student.

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## Introduction

### *Plastic Pollution and Microplastics*

Plastic pollution has become a global concern since the 1940s. Before WWII, plastic was considered to be an environmentally friendly alternative to finite natural resources such as wood and ivory (Laufer 1947). After the war, plastic became a convenient, affordable, and trendy commodity (Brandon et al. 2019, Rocha-Santos et al. 2015). Plastic soon became readily available and so abundant that today an estimated 4,900 million metric tons of plastic have been discarded in natural areas and landfills (Geyer et al. 2017). Plastic pollution can enter the environment from multiple sources, including accidental spillage from industrial transport, mismanaged waste disposal, and output from wastewater treatment plants (Talvitie et al. 2014). Once in the environment, movement of plastics occurs via runoff, rain, winds, and/or tides (Jambeck et al. 2015, Rochman and Hoellein 2020).

Plastic pollution can be defined as macroplastic (> 2.5 cm), mesoplastic (2.5 cm to 5 mm), and microplastic (<5 mm). Microplastics are either manufactured for industrial use (primary microplastics) or small degraded pieces of plastic from larger plastic items (secondary microplastics) (Thompson et al. 2004). Microplastics are generally classified into groups such as fragment, fiber, sphere, film, foam, or bead (Baldwin et al. 2016, Lusher et al. 2017). Plastics slowly degrade from environmental elements (biological, chemical, and photolytic processes) to create secondary microplastics. For example, when polystyrene (PS), a very common plastic polymer used in packaging and single-use food-

grade products, has been exposed to UV light and heat from the sun, it turns yellow and slowly become brittle, leading to degradation of the polymer (Yousif and Haddad 2013).

*Microplastics in Marine Studies*

In the last decade, marine studies have dominated research on plastic pollution (e.g., Hildago-Ruz et al. 2012, Rocha-Santos et al. 2015, Van Cauwenberghe et al. 2015). Typical studies focus on sediment (Blettler et al. 2017, Claessens et al. 2011, Claessens et al. 2013, Thompson et al. 2004), surface water (Gray et al. 2018, Karlson et al. 2017, Wieczorek et al. 2018), and/or animals (Cole et al. 2014, Lopez-Lopez et al. 2018, Wieczorek et al. 2018). Macroplastic and mesoplastic pollution has been linked to fatalities in marine megafauna (Lusher 2015, Young and Elliott 2016). Most fatalities are from the ingestion of macro- and mesoplastics that block the gastrointestinal tract (GIT), resulting in starvation. Discarded and/or forgotten fishing nets also become a form of plastic pollution, and many organisms die from entanglement (Derraik 2002, Kuhn et al. 2015). However, it is not clear how microplastic pollution may affect organisms. Mortality from microplastic deaths is typically not reported or is unknown due to less research on the smaller organisms microplastics may affect (Kuhn et al. 2015). It is important to determine the occurrence of microplastics in smaller marine fauna because smaller organisms support the food web in aquatic ecosystems.

Several studies have examined the occurrence of microplastics in various marine organisms. Bivalves and other filter feeding organisms have higher concentrations of microplastics than mass feeding organisms (Davidson et al. 2016, Karlsson et al. 2017, Li et al. 2019). Microplastics accumulated in the gills of shore crabs (*Carcinus maenas)*

causing oxygen consumption levels to fluctuate due to changes in osmoregulation of ions ($Na^+$ and $Ca^{2+}$) within the gill filaments (Watts et al. 2016).

Sixty eight percent of sixty-two sea-run brown trout (*Salmo trutta)* individuals from the west coast of Sweden, had microplastics in their gastrointestinal tracts, hereafter referred to as GIT (Karlsson et al. 2017). A study examining multiple fish species in the Adriatic Sea found that 28% of sampled specimens contained microplastics in the GIT, with an average of one particle per individual in tub gurnard (*Chelidonichthys lucernus*) and 1.7 particles per individual in a species of sardine (*Sardina pilchardus*) (Avio et al. 2015). Jabeen et al. (2017) found that 100% of marine fish samples purchased from a market in Shanghai, China contained microplastics, with the most abundant microplastic being fibers. Furthermore, microplastic concentrations were higher in demersal (benthic or benthopelagic) fishes than in pelagic (open water) fishes. The average abundance of microplastics of two demersal fish species was highest in a species of file fish (*Thamnaconus septentrionalis*) with 7.2 particles per individual and by weight in a species of croaker (*Collichthys lucidus*) with 17.2 particles per gram (Jabeen et al. 2017).

*Microplastics in Freshwater Studies*

Freshwater microplastics have been understudied compared to marine microplastics, but research has increased in recent years (Blettler et al. 2018, Eerkes-Medrano et al. 2015, Horton et al. 2017, Li et al. 2018, Lusher et al. 2017). One of the first freshwater microplastic studies sought to quantify and identify microplastics in the Great Lakes (Eriksen et al. 2013). Another study looked at a remote lake in northern Mongolia

and found that the remote lake had more microplastics than Lake Huron and Superior of the Great Lakes (Free et al. 2014). A freshwater microplastic study using sediment from a river in Germany found microplastics of varying sizes and types in all sediment samples (Klein et al. 2015). Freshwater studies have documented microplastics in Asiatic clams (*Corbicula fluminea)* (Rochman et al. 2017, Su et al. 2016, Su et al. 2018) and several species of fish (McNeish et al. 2018, Pinheiro et al. 2017, Roch et al. 2019). It is not clear what impact microplastics have on freshwater organisms. Organisms with microplastics in their GIT taken from natural environments are thought to have indirectly ingested microplastics from normal feeding behaviors (Scherer et al. 2018). Several studies have tried to understand the impact in laboratory settings by feeding microplastics directly to organisms (Eerkes-Medrano et al. 2015, Horton et al. 2017, Li et al. 2018, Pinheiro et al. 2017). Rehse et al. (2016) found that the mobility of *Daphnia magna* was negatively impacted by ingestion of microplastic particles measuring 1 µm, which caused *D. magna* to cease swimming.

Microplastics can accumulate in the gill structures of aquatic organisms during normal respiration. A study examining see-through medaka fish (*Oryzias latipes*) found that latex nanoparticles, which are comparable in size to microplastics, can accumulate in the gills and GIT of adult fish (Kashiwada 2006). Gray and Weinstein (2017) found that freshwater grass shrimp (*Palaemonetes pugio*) ingested multiple sizes of microplastic particles that blocked the GIT and caused death. The most detrimental microplastics to cause mortality in the grass shrimp were small fibers (34 µm) and sphere particles (75 µm).

All the grass shrimp that died during this study had small fibers and spheres in their GIT as well as their gills (Gray and Weinstein 2017).

*Microplastics and Fish Feeding Guilds*

To a lesser degree, research has attempted to determine whether certain feeding guilds of fishes are more prone to consume microplastics (Lusher et al. 2013, McNeish et al. 2018, Roch et al. 2019). Guilds describe a group of organisms that exploit resources in a similar manner, such as feeding habits or the habitat zones the group of organisms occupy. Feeding guilds of fishes can be difficult to categorize and are often dependent on the researcher's preferences for classification. Typical fish feeding guilds consist of carnivores, herbivores, piscivores, planktivores, and omnivores but can vary from study to study (Westneat 2001). Fish feeding guilds may also include the ecological zone (benthic, benthopelagic, demersal, and pelagic) that fishes inhabit and often are included in the classification of the guild (Figure 1). The ecological zone a fish inhabits is usually indicative of the types of food or prey the fish exploits.

McNeish et al. (2018) found that round goby (*Neogobius melanostomu*), a benthic carnivorous species, had higher concentrations (19 particles per individual) of microplastics in their GIT than omnivore and detritivore taxa; however, the sample size was relatively small (n=14). Another study examining microplastic concentration of several species of fishes found that piscivorous fishes had lower microplastic concentrations than other feeding guilds such as omnivorous and planktivorous species

(Roch et al. 2019). In contrast, Lusher et al. (2013) reported no difference in microplastic concentrations in the GIT of fishes from different feeding guilds.

*Microplastics and Organism Size*

There is also conflicting evidence about whether the size of an organism is correlated with the microplastic load in the GIT. McNeish et al. (2018) found that round goby contained more microplastics in their GIT as their body size increased. A study using a species of zooplankton (*D. magna*) and a macroinvertebrate species of chironomid larvae (*Chironomus riparius*) also found that as the organism's body size increased, the microplastic load increased (Scherer et al. 2017). Whereas, Hurt et al. (2020) found that gizzard shad (*Dorosoma cepedianum*) microplastic concentrations significantly decreased as fish size increased. Conversely, studies investigating the occurrence of microplastics in marine fishes found that the relationship of microplastic concentration present in the GIT relative to the size (weight or length) was very weak and warranted further investigation (Bessa et al. 2018, Possatto et al. 2011).

*Research Objectives*

The overall goal of this study was to investigate microplastic accumulation in freshwater fishes in a variety of feeding guilds. The following questions were the focus of this study: 1) Does microplastic concentration differ among feeding guilds of freshwater fishes? 2) Does microplastic concentration increase as fish size increase in different

freshwater fishes? and 3) Do freshwater fish incidentally capture microplastic particles within their gill structures?

I predicted that microplastic concentrations would differ among feeding guilds of freshwater fishes and that higher weight fishes would have higher concentrations of microplastics. I also predicted that gizzard shad would accumulate more microplastics within their gill structures because of the presence of gill rakers used for filter feeding.

## Materials and Methods

There are currently no standard methods for sampling, processing, counting, or reporting microplastics (Eerkes-Medrano et al. 2015, Li et al. 2018, Pinheiro et al. 2017). The methods for this investigation combined modified versions of methods from several different studies. From the literature, the most common practices were selected that utilized environmentally safe chemicals, were cost-effective, and practical for this study.

### *Field Collection*

#### *Sampling Location*

The sampling location was determined by South Carolina Department of Natural Resources (SCDNR) based on their annual fish sampling schedule. The samples were collected from Lake Wateree on February 4$^{th}$ and 5$^{th}$, 2020. Lake Wateree is one of the largest reservoirs in the Catawba-Wateree River basin at 13,025 surface water acres with 348 km of shoreline. The average depth is 2.1 meters and maximum depth is 19.5 meters. Lake Wateree is located south (112 km) of Charlotte, NC, a highly urbanized city within

the watershed, and northeast (48 km) of another urbanized city, Columbia, SC (SCDNR 2019, Figure 2). The reservoir is used for hydroelectric power and drinking water. It is also a major recreational attraction, particularly for fishing. Although Lake Wateree is surrounded by urban development and housing, the Catawba River, which feeds the reservoir, flows through several rural areas. From the river and surrounding watershed there are several points of entry for microplastics, such as wastewater treatment plants, litter from the many recreational areas, and runoff.

*Study Species*

The targeted species, determined by SCDNR, were white perch (*Morone americana*), blue catfish (*Ictalurus furcatus*), and black crappie (*Pomoxis nigromaculatus*). The feeding categories for this study was modeled after SCDNR's classification system and may vary compared to other classification systems (SCDNR 2019). The blue catfish is an omnivore, white perch is a carnivore, and black crappie is a piscivore (Table 1). Gizzard shad (*Dorosoma cepedianum*) was not a targeted species by SCDNR but were incidentally captured while trapping the other species and were retained for this study. Gizzard shad were of interest because they are pelagic filter feeding fish that filter plankton from the water through gill rakers and may be one of the first species to come in contact with microplastics in the water column.

Blue catfish and white perch are demersal fishes, which tend to feed at or near the bottom but often move up and down the water column to feed. White perch are not true perch, but are part of the temperate bass family, Moronidae. Black crappie are

benthopelagic fish that often inhabit vegetative areas and will move from those areas to open waters to feed, while gizzard shad are pelagic fish that tend to stay in open waters while they feed on zooplankton and algae (SCDNR 2019, Figure 1).

*Fish Trapping and Dissection*

The fish were collected using two experimental gill nets, one floating 1.8 meters down below the surface and one floating 1.8 meters up from the bottom. The gill net had varying mesh sizes (25 mm to 102 mm) to select for the targeted species determined by SCDNR. The fish were sampled lethally, and those that survived were euthanized by SCDNR. Euthanized fish were received from SCDNR after age (removal of the otolith for a subset), sex, weight, and length had been recorded. There were 20 individuals of four species collected from two days of sampling by SCDNR. Most of the fish were dissected on site. Dissection removed the entirety of the GIT from the esophagus to the anal pore. The gill basket was also removed at this time. The organs were bagged separately, labeled, put on ice, and transported back to the lab to be frozen. SCDNR disposed of all unused fish parts. Fish samples were assigned a species-number code established by SCDNR. Any fish that were not dissected in the field were transported on ice back to the lab and dissected in the Ecology lab of Dalton Hall at Winthrop University.

*Water Conditions*

During fish sample collection, the Aqua TROLL 600 multiparameter sonde was used to measure temperature, pH, turbidity, conductivity, pressure, and dissolved oxygen

at the surface where surface water was collected, as well as location and time of sampling (In-Situ 2020, Table 2).

*Surface Water Samples*

Surface water samples (n=5) were collected via one-liter grab samples during the sampling event in the same water where the fish were collected. Surface water samples give approximate microplastic concentration within the water where the fish were caught. Grab samples give a better estimate of microplastics present in water samples than net or sieved samples (Barrows et al. 2017). Typically, neuston nets or plankton nets and sieves (333 µm) are used to collect microplastics from surface waters. However, many microplastics are smaller than the pore size on the nets and sieves and may underestimate the actual concentration of microplastics present (Barrows et al. 2017).

***Lab Processing***

*Surface Water Samples*

Surface water samples were measured for exact volume and filtered through a 250 µm sieve. The sieve was then rinsed into a filter tower onto a plain white 0.45 µm filter (Millipore MF nitrocellulose membrane filters). Once the sample had been filtered, the filter was removed, placed into a 100 mL beaker, and rinsed with 20 mL of 30% $H_2O_2$ to remove any organic material present in the sample (McNeish et al. 2018). $H_2O_2$ is used because it is cost-effective, environmentally safe, and gives relatively high recovery rates in microplastic samples without compromising the integrity of the microplastics (Karlson

et al. 2017, Li et al. 2018). The beaker was covered with aluminum foil to prevent any atmospheric contamination and placed in an incubator at 50°C for at least 24 hours. Lusher et al. (2017) reported that $H_2O_2$ treatments have been incubated between 20°C and 65°C for 2 hours to 3 weeks to increase the digestion rate of the organic material present in the sample. The time may vary depending on the amount of organic material in the sample. Once removed from the incubator, the $H_2O_2$ treated water sample was filtered again onto a black gridded 0.45 µm filter (Millipore MF nitrocellulose membrane filters) (Shim et al. 2016, Staton et al. 2019). The filter was removed from the filter tower and placed inside a clean petri dish until microplastics were counted.

*Gastrointestinal Tract and Gill Processing*

Fish samples were retrieved from the freezer and allowed to thaw for at least two hours. Once the GIT or gill basket was thawed, the wet weight was obtained, and the sample was placed in an appropriately sized beaker for peroxide digestion of organic material. The volume of 30% $H_2O_2$ used for each sample was determined based on the size and weight of the GIT or gill basket (Lv et al. 2019). Following Jabeen et al. (2016), I used no more than 50% of the total volume of the container for the digestion step. Each sample was covered with aluminum foil to prevent any atmospheric contamination of microplastics and placed in an incubator at 50°C for at least three days but up to 14 days (Karami et al. 2017). Following digestion, a saturated saline solution (1.2 g/ml) was added to each sample for density separation of the microplastics from any remaining organic material. The sample was left overnight to separate in the incubator (Claessens et al. 2011, Jabeen et al.

2016, Li et al. 2015, Thompson et al. 2004). This step allowed any organic particulates and fish tissues or bones that did not digest to separate and settle in the solution. This saline separation step was carried out twice to improve recovery of microplastics (Jaafar et al. 2020). The microplastic particles floated on the top of the saturated saline solution and were decanted into a filter tower and filtered through a black gridded 0.45 µm filter. Once samples were filtered, the filter was removed from the filter tower and placed inside a clean petri dish until microplastics were counted.

*Quantification of Microplastics*

There are several methods reported for quantifying and determining the polymer type of microplastics. Some of the methods used are Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and simple visual counting with a dissecting microscope (Li et al. 2018). These methods have varying degrees of results. FTIR and SEM can be very costly and time consuming but can potentially identify the polymer type of the microplastics found. Visual counting with a dissecting microscope is easy and cost-effective, but also time consuming. There is also more room for error in simple visual counting. Many microplastics are either too small to detect by simple visual counting or can be misidentified (Roch et al. 2019).

The filters from the surface water samples and the fish GIT and gill samples were observed under a stereo microscope (Olympus SZX12) with a fluorescent adapter. To detect microplastics with more accuracy and ease, the fluorescent properties of microplastics were utilized (Said and Heard 2020). A Royal Blue fluorescent light in the

440-460 nm wavelength and a 500 nm longpass emission filter, stereo microscope adapter was used to detect and quantify microplastics (NightSea, USA). The fluorescent adapter caused the microplastics to fluoresce bright orange or green, and the use of black gridded nitrocellulose filters reduced background fluorescence. Quantification was accomplished by observing the gridded filter beginning at the top of the filter and moving over and down on the grids of the filter, counting each fluorescing microplastic at 125X magnification. The hot needle test was used on any particle that could not easily be identified as plastic. Counts were recorded by type of microplastic found (fragment, fiber, sphere, film, and foam).

*Contamination Mitigation Protocols*

Several steps were taken to reduce or eliminate any potential source of microplastic contamination during this study. The area in the lab was cleaned before and after any sample processing with 70% ethanol and no-shed cloths. Procedural blanks and environmental blanks were taken from the lab throughout the course of the study to consistently monitor for any water or airborne sources of contamination. Procedural blanks were processed alongside samples following the same protocols but only contained the $H_2O_2$ treatment and saturated saline solution. Final microplastic counts were corrected by subtracting the average microplastics found in the procedural lab blanks (6.125 microplastics) from each individual fish's total microplastic count for the GIT and gill samples (Dioses-Salinas et al. 2020, Karlson et al. 2017). Environmental blanks consisted of leaving filters out in the work area for 24 hours. The contamination for these blanks was

minimal (average 5 microplastics per filter) and were not included in any correction because samples were consistently covered with aluminum foil to prevent any airborne contamination (Dioses-Salinas et al. 2020). Cotton lab coats and nitrile gloves were worn when processing samples. The use of any excess plastic material was eliminated where possible. Glassware or metal was used wherever possible. Ultrapure water (Elga Purelab Flex3 with POU 0.2 µm filter) was used to make the saturated salt solution. The saturated salt solution and hydrogen peroxide were filtered using a 0.45 µm filter before being used in any sample. All glassware and equipment were washed with commercial detergent and triple rinsed with Ultrapure water to prevent any cross contamination. To reduce contamination in the field, stainless steel pans and dissecting tools were used. Environmental blanks were taken from the water hose used to rinse all equipment and pink fishing gloves were used to help identify any fiber contamination. The water hose blanks were not used in any corrections because the samples did not come into direct contact with the water from the water hose, but samples were taken to help identify if any source of contamination was present in the samples (Dioses-Salinas et al. 2020).

*Statistical Analyses*

Analysis of covariance (ANCOVA) was used to determine if the concentration of microplastics differed among species and across fish weights (the covariate). GIT and gill samples were analyzed separately. A Bonferroni *post-hoc* analysis was used to determine pairwise differences in microplastic concentration among the four species. The gill samples did not meet all the assumptions of ANCOVA and were also analyzed using a 1-

way analysis of variance (ANOVA) and simple regression analyses was used to compare microplastic concentrations in gills to weight in each species. A Tukey's *post-hoc* analysis was used to determine the differences in microplastic concentrations among the four species. Results were recorded as microplastics per individual fish for the ANCOVA and ANOVA and microplastics per gram for the regression analyses. Kolmogorov–Smirnov tests were used to determine if the data were normally distributed. Number of samples were adjusted for two of the species because of missing weights for an individual from blue catfish and black crappie (n=19); and gizzard shad and white perch (n=20). Data that were not normally distributed were transformed using a $\log_{10}+1$ correction. Levene's tests were used to determine if variances were equal (Dytham 2011, Rothwell 2020). Analyses were conducted using SPSS version 25.

**Results**

Microplastic concentrations in GIT samples were significantly different among the four species ($F_{3,73}=7.457$, $p\leq0.000$), but weight had no effect on microplastic concentration in the GIT samples (ANCOVA, $F_{1,73}=2.574$, $p=0.113$). Gizzard shad had significantly greater concentrations of microplastics than white perch ($p=0.033$), while blue catfish ($p=0.015$) and white perch ($p=0.001$) had a significantly lower microplastic concentration than black crappie (Figure 3). Mean microplastic concentrations in GIT samples ranged from 8.66 ± 3.63 SE microplastics per individual (white perch) to 52.30 ± 13.61 SE microplastics per individual (black crappie).

There were significant differences in the microplastic concentrations in gills among the four species (ANOVA, $F_{3,73}$=3.141, p=0.030, Figure 4). Mean microplastic concentrations in gill samples ranged from 24.36 ± 6.82 SE microplastics per individual (blue catfish) to 69.66 ± 12.24 SE microplastics per individual (black crappie). Simple regression analyses showed a relationship between microplastic concentrations and weight only in gizzard shad (gizzard shad: $r^2$=0.198, $F_{1,18}$=4.453, p=0.049; blue catfish: $r^2$=0.007, $F_{1,16}$=0.113, p=0.741; white perch: $r^2$=0.177, $F_{1,18}$=3.881, p=0.064; black crappie: $r^2$=0.175, $F_{1,17}$=3.609, p=0.075, Figure 5).

One-liter surface water samples (n=5) were collected during fish collection and contained an average of 47.7 ± 3.94 SE microplastics per liter.

The most common types of microplastics found in this study were fragments and fibers. Less than 2% of the microplastics present in the GIT or gill samples consisted of spheres, films, and foams. Gizzard shad had the highest percentage of fibers in the GIT (60%); fibers contributed to only 20% of the microplastic concentration present in the other three species. Blue catfish, white perch, and black crappie had greater than 79% microplastic fragments present in their GITs (Figure 6). Gizzard shad also had the highest percentage of fibers present in the gill samples (47%); less than 10 % of the microplastics present in the other species were fibers. Ninety percent of the microplastics present in blue catfish, white perch, and black crappie gill samples were fragments (Figure 7).

## Discussion

There were significant differences in microplastic concentrations in the GIT samples among the four species. Black crappie ingested significantly more microplastics than white perch and blue catfish and gizzard shad ingested significantly more microplastics than white perch. There were also significant differences in microplastic concentrations in gill samples among the four species. There were differences among blue catfish, gizzard shad and white perch and also among gizzard shad, white perch, and black crappie. This would indicate that microplastics are omnipresent in all habitats that the fish inhabit and available to all feeding guilds.

There were no significant relationships between microplastic concentrations and weight in the GIT samples. There was a small relationship between gizzard shad gill microplastic concentrations and weight. The simple regression analysis determined that weight only accounted for about 20 % of the microplastic concentrations present in the gizzard shad and indicated that there are other factors contributing to microplastic concentrations.

Although the weight of the fish did not contribute to microplastic concentration present in the fish GIT and the relationship was minimal in the gills, other factors should be considered in future studies such as ontogeny, diet and feeding habits at different life cycle stages, and morphological or physiological differences among species (Drenner 1977, Keast 1968, Schaus et al. 2002, Schmitt et al. 2019). Microplastic research in freshwater fishes and feeding guild should be expanded to include all major species found in a location. There are many factors that can contribute to microplastic accumulation

found in fish that may contribute to one species accumulating microplastics more than another species.

The variation within and among species makes the differences interesting. For instance, black crappie had more microplastics in their GITs and gills than the other three species. White perch had more microplastics in their gills than in their GITs. Blue catfish was the only species that had any gill samples without microplastics, and they had less microplastics in their gills overall. Gizzard shad, white perch, and black crappie have gill rakers and filter feed for plankton during juvenile stages, even though white perch and black crappie are considered piscivores or carnivores as adults. These observations indicate that microplastic concentrations in fish feeding guilds are complex, multifaceted, and warrant further investigation.

In my study, gizzard shad had more microplastics present in their gills than in their GITs. This study found many more microplastics than the previously mentioned studies. The use of the fluorescent microscope adapter may have allowed for detection of smaller microplastics particles that would otherwise go undetected. A study in a freshwater reservoir looking at gizzard shad found a range of microplastics in both GIT and gills. GIT samples ranged from zero to 28 microplastics per individual and gill samples ranged from one to 30 microplastics per individual. gizzard shad (Hurt et al. 2020).

Gill physiology and morphology traits may be important to understand when conducting microplastic research in fishes, especially in filter feeding species. The production of mucus or the induction of mucous cells is a common physiological response when contaminants are present in the gills (Harper and Wolf 2009). It may be crucial to

know if there are any specialized cells or mucous cells that produce extra mucus that may contribute to an abundance of microplastics found in one species compared to other fishes. Most studies have been focused on heavy metals, suspended solids, or ions but some smaller microplastics could be comparable in size to these known contaminants that induce such a physiological response (Blair et al. 2017, Harper and Wolf 2009). One study examining the effects of microfiber exposure on Japanese medaka fish found inflammation in the gill structures and increased mucus production as well as several other physiological and morphological responses in the gills (Hu et al. 2020). The presence or absence of gill rakers may also be important to consider when studying microplastics in fishes.

*Gizzard Shad*

Gizzard shad are pelagic planktivorous fish that inhabit the open waters and feed on zooplankton and algae. Gizzard shad are an important species within the food web of a lake or reservoir as they are important prey items for larger fish species (SCDNR 2019, Iowa Department of Natural Resources 2020). Gizzard shad are not typically sought after for game fishing, but they are used for bait fish (SCDNR 2019).

Gizzard shad have many gill rakers and the morphological shape of their gills are different that the other species in this study (Figure 8). Their gill arches are closer together, forming two limb-like structures and contain approximately 190 gill rakers on the lower limb of the first gill arch (Iowa Department of Natural Resources 2020). One study reported that gizzard shad gill rakers could filter food particle sizes from one to 70 microns, where the gill rakers captured 100% of the larger food particles (Drenner 1977). Another study

determined that the spaces between gizzard shad gill rakers could vary between water body locations and populations and suggested that gill structure may be phenotypically plastic depending on ontogeny and other fishes present (Wallesar et al. 2014). These findings could suggest that microplastic accumulation in gizzard shad may be more complicated than just the presence of gill rakers and may need to account for other factors present in gizzard shad habitats.

*Blue Catfish*

Blue catfish are demersal and opportunistic generalist omnivorous fish. Studies have found catfish species to be generalist feeders, and food items only vary due to location or habitat (SCDNR 2019, Schmitt et al. 2019). Blue catfish have also been known to ingest many anthropogenic items such as condoms, chicken bones, and plastic (Possatto et al. 2011, Schmitt et al. 2019). In this study, blue catfish were the only species to have larger plastic items found in their GITs, which is in line with previous studies. One individual had large pieces of plastic film in their GIT that resembled degraded plastic bag (Figure 9).

Blue catfish were also the only species to have individuals that did not have microplastics present in their gills. Although the microplastic concentrations varied from zero to 107 microplastics per individual and averaged 24.36 ± 6.82 SE microplastics per individual, blue catfish had the lowest microplastics present in their gills compared to the other species. This could be due to any number of factors, but blue catfish have few gill rakers and are opportunistic feeders throughout their life cycle (Ross 2001, Schmitt et al. 2019). Schmitt et al. (2019) conducted a large study on catfish diet and concluded that blue

catfish are primarily herbivores until they reach larger sizes and then begin to consume other fish as part of their diet. Blue catfish being classified as herbivores until they reach a certain size could suggest why the blue catfish in this study had less microplastics in their gills and relatively lower microplastics in their GITs compared to two of the other three species. Blue catfish simply do not eat where microplastics are found in the water column and are not ingesting prey items that may contribute to their microplastic load until they are much larger. The blue catfish are eating in benthic areas and feeding on vegetation. This study used blue catfish that ranged from 349 grams and 266 mm long to 10,160 grams and 958 mm long. Further investigations could reveal if microplastic concentrations vary according to size in blue catfish.

*White Perch*

White perch are demersal carnivorous fish. White perch are semi-anadromous and typically migrate from estuarine areas, upriver to freshwater areas to spawn. However, in impounded water bodies they will migrate within the lake or reservoir to spawn (SCDNR 2019). Habitat and migration within the lake may need to be considered when studying microplastic concentration in this species.

One study found that white perch remained planktivores, feeding on zooplankton throughout their lives and only consumed other fish and larger invertebrates after reaching a length of 100 mm, and even then preferred zooplankton (St-Hilaire et al. 2002). Another study found that white perch adults retained 17-21 gill rakers on the first gill arch which attributed to their preference of zooplankton as their primary food item (Fritzsche 1980).

In this study, white perch had 8.66 ± 3.63 SE microplastics per individual in their GITs and 50.88 ± 10.78 SE microplastics per individual in their gills. All the fish in this study were over 100 mm in length (average length in this study was 219.8 mm). If the larger fish in this study also preferred zooplankton as in the previous study, this could explain the higher number of microplastics in the gills as compared to the GIT in this species. The presence of gill rakers may contribute to higher concentrations of microplastics in the gills of this species. It may also be beneficial to determine if physiological gill differences in semi-anadromous fishes contribute to increased microplastic concentrations in gills.

*Black Crappie*

Black crappie adults are classified as benthopelagic piscivores or carnivores with a diet consisting of fish, mollusks, aquatic insects, and small invertebrates, whereas juveniles are considered planktivores (SCDNR 2019). However, an extensive study reported this species to be more of a planktivore throughout their life and only consume larger prey items as they reach larger sizes, between 125 mm to 300 mm in length (Keast 1968). Keast proposed that black crappie only then consume larger prey items and other fish to metabolically maintain a larger body and are incidentally piscivorous. He reported that black crappie's diet, even as adults who consumed larger prey items, consisted mostly of zooplankton across multiple sites and studies. In the same study, Keast examined the gills of several black crappie and determined that even as adults, the fish still filter feed, and their gills retain multiple fine gill rakers into adulthood. Black crappie have the largest and most abundant gill rakers (ranging from 25-29 rakers on the first arch to 14-15 on the fourth arch) compared to other centrarchids in the same family (Keast 1968).

In the current study, it was found that black crappie had more microplastics found in the gills compared to the other three species (69.66 ± 12.24 SE microplastics per fish). The size and number of gill rakers the black crappie retain into adulthood could contribute to this abundant accumulation of microplastics in the gill structures. Microplastics are in the size range of the zooplankton black crappie have been found to consume the most, specifically species of Diptera larvae (Keast 1968). These larvae are typically 8 mm in length or smaller and can be comparable to microplastics 5 mm or smaller (Soil & Water Conservation Society of Metro Halifax 2020). If black crappie gills have evolved to retain the multitude of gill rakers and allow them to filter feed throughout their adult life, it would be possible to conclude that the morphology of the gills also allow for microplastics to accumulate more easily in black crappie than other fish found in the same body of water. Gizzard shad and white perch also have gill rakers and are relatively comparable to the size of the black crappie used for this study, however gizzard shad only had 35.6 ± 11.85 SE microplastics per fish in the gill structures and white perch had 50.88 ± 10.78 SE microplastics per fish compared to 69.66 ± 12.24 SE microplastics per fish found in black crappie. Future research should investigate the possible size of the interraker spaces and if that contributes to microplastic accumulation.

*Future Focus of this Study and Beyond*

For the future focus of this study, the aim is to quantify microplastics and make observations about two other species from Lake Wateree; striped bass (*Morone saxatile*) and white bass (*Morone chrysops*). Gill physiology and morphology will also be examined more closely to determine if there are any underlying traits that may contribute to

microplastic concentration differences found. Identification of different types of microplastics found may also be attempted.

Beyond this study, microplastic research and knowledge is growing at a steady rate and seems to change each time a new study is published. Most of the studies call for standardization for conducting and reporting microplastic research (Hidalgo-Ruz et al. 2012, Akdogan and Guven 2019). Many detection and extraction methods have been reported for microplastic research. Polymer identification methods have also been heavily explored (Fu et al. 2020). Polymer identification methods are costly, time consuming, and not always accurate (Dioses-Salinas et al. 2020). Although FTIR and SEM have been touted as the most widely used means to identify polymers, they are not available to all researchers and frequently require samples to be sent off for identification purposes which may lead to lengthy waiting times for results or contamination issues (Dioses-Salinas et al. 2020, Fu et al. 2020).

One thing lacks in many microplastic studies; what to do once microplastics have been detected or identified. There are very few practical and feasible suggestions on how to remedy microplastic pollution in freshwater. The most commonly suggested solutions are wastewater treatment plant modifications that will capture microplastics (Schmaltz et al. 2020, Talvitie et al. 2017). These types of modifications range from membrane bioreactors, sand filters, and dissolved air flotation. These modifications are typically costly, easily fouled, and clog the treatment process. One researcher suggested it takes more of a broad approach to reduce microplastic pollution by using an upstream/downstream method. This method couples the removal of microplastic

contamination at the wastewater treatment plant while also removing as many sources of microplastic contamination from the surrounding watershed and environment (Wong et al. 2020).

Other solutions include replacing traditional plastics with bioplastics made from organic polymers such as algae or fungi (Wong et al. 2020). However, these types of plastics are still very costly and time-consuming to produce and are not feasible to replace all the types of plastic used in today's industries. The use of biological agents to reduce or degrade plastics has been explored, such as using "plastic-eating" bacteria or fungi (Wong et al. 2020). These types of biological means are not ideal because of the unknown implications to the ecosystems that could potentially occur from introducing these organisms in large quantities into environments where they are not typically found (Solomon and Palanisami 2016, Wong et al. 2020).

Another solution is to list microplastics as a Persistent Organic Pollutant (POP) (Lohmann 2017). There are four criteria that a pollutant needs to meet to be listed as a POP; it needs to be persistent in the environment, bioaccumulate in organisms, have long-range transport in the environment, and have adverse effects on humans or animals. Microplastics have been reported in multiple studies that meet all four of these criteria (Lusher 2015, Rochman and Hoellein 2020). Listing microplastics as a POP would allow legislations to require manufacturing operations to put measures in place that would reduce further microplastic pollution from occurring. Such measures could include making it mandatory to include microfiber filters on the manufacturing of new washing machines and dryers or requiring industrial laundering services to have filters on any discharge from

their facilities to capture microfibers at the source (Schmaltz et al. 2020). This would benefit both freshwater and marine ecosystems.

Future research should focus more on the toxicological implications of microplastics in organisms and removal strategies. Although detection and identification methods are important in discovering microplastic pollution, without any standardization, many of the studies implementing new methods for detection and identification are moot. It is well known that microplastics are in almost every environment from sea to air, and the types of polymers that constitute microplastics vary just as much as the places you can find them; however there is little being done to combat the issue of removal and prevention of more microplastics entering the environment every day. There is still too little known about what microplastic pollution is capable of when it comes to living organisms ingesting microplastics.

Table 1. Target species and their feeding guilds, habitat preferences, and food habits (FishBase 2019, SCDNR 2019).

| Species | Feeding Guild | Habitat Preference | Food Habits |
|---|---|---|---|
| Gizzard Shad (*Dorosoma cepedianum*) | Adult: Planktivore | Large rivers, reservoirs, lakes, ponds, pools, and sluggish backwaters; Pelagic | Eats microscopic plants and animals by filter feeding with their gill rakers |
| Blue Catfish (*Ictalurus furcatus*) | Adult: Omnivore | Prefer rivers and large creeks with moderate to swift current but also do well in large impoundments; Demersal | Eats clams, snails, insects, mussels, fish, and plant material |
| White Perch (*Morone americana*) | Adult: Carnivore | Found in most SC waters; Demersal | Very diverse diet; will eat worms, crabs, insects, and small fishes |
| Black Crappie (*Pomoxis nigromaculatus*) | Adult: Piscivore | Found in vegetated areas; prefer cool, clear waters; Benthopelagic | Eat small fish but also bivalves, snails, crayfish, and aquatic insects |

Table 2. Environmental observations and data recorded with Aqua Troll 600 sonde at time of fish collection in Lake Wateree.

| **Measurements** | **Data** |
|---|---|
| Time and Date | February 5, 2020 @ 10:44 |
| Temperature | 12.52 ºC |
| Conductivity | 0.055 µs/cm |
| Turbidity | 24.2 NTU |
| Dissolved Oxygen | 10.78 mg/L |
| pH | 6.63 |
| Latitude | 34.36615536 |
| Longitude | -80.728761 |
| Observations | Weather: cloudy/overcast 18.8 ºC, light south west wind @ 2.5 mph, water clear and calm |

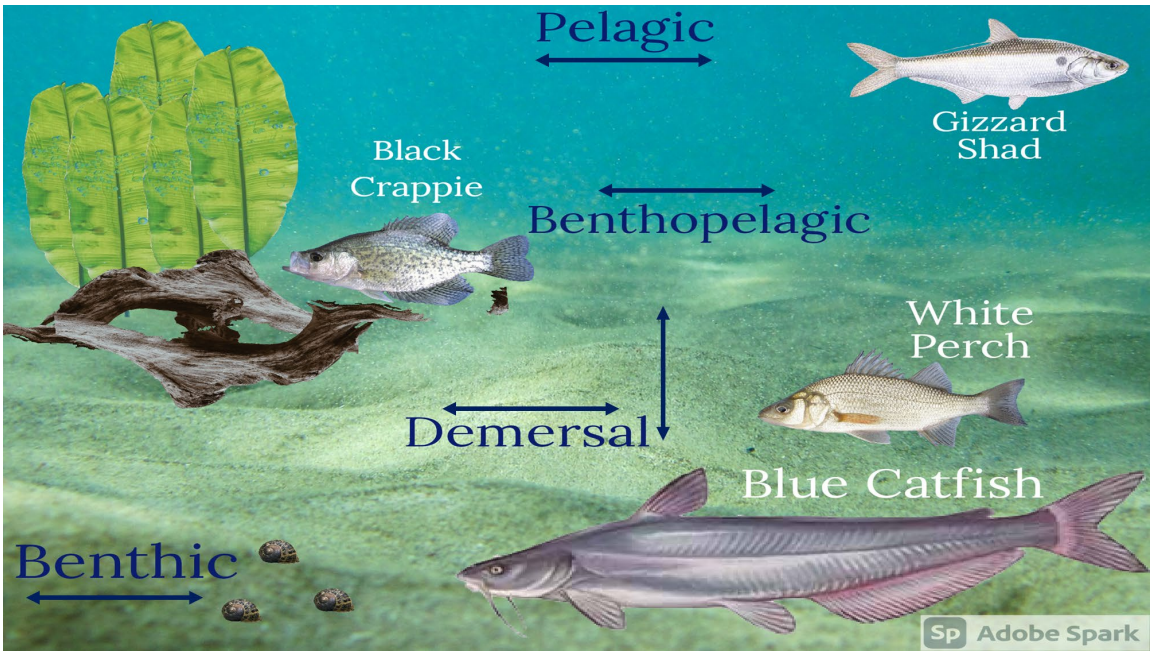


Figure 1. Visual of the ecological zones the targeted species occupy. Gizzard shad are pelagic planktivores, black crappie are benthopelagic piscivores, white perch are demersal carnivores, and blue catfish are demersal omnivores. Benthic ecological zone is included to show the distinction between the zones.

Figure 2. Map delineating the watershed (yellow area) that contributes to Lake Wateree (blue pin) (USGS StreamStats 2020).

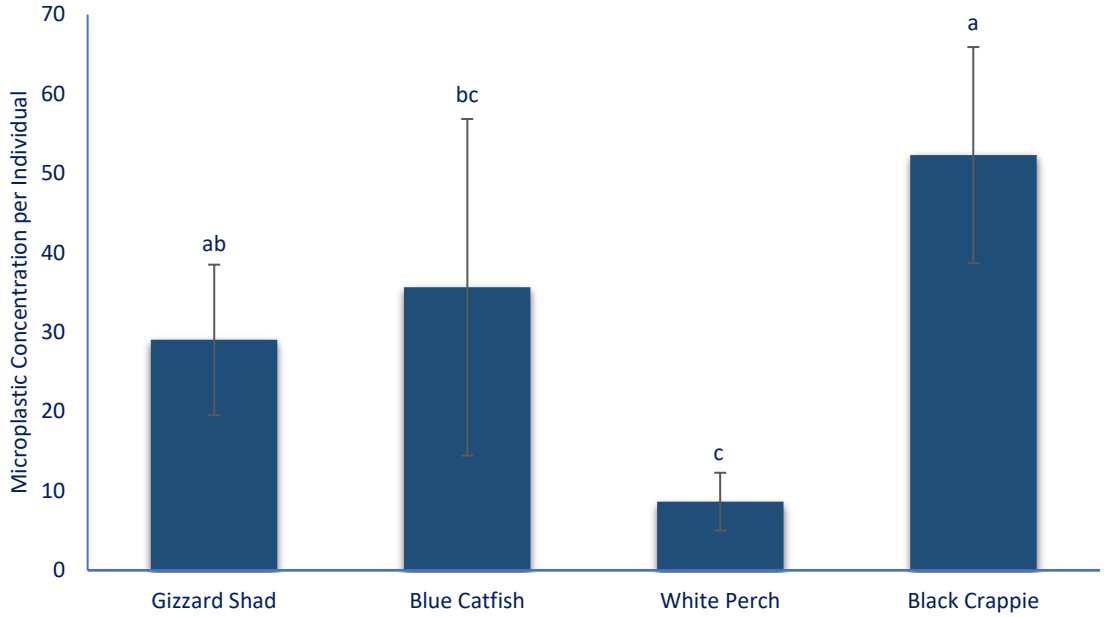


Figure 3. Mean microplastic concentrations in the GIT of the four species (ANCOVA: $F_{3,73}$=7.457, p≤0.001). Letters represent significant differences among species. Error bars represent ± 1 SE.

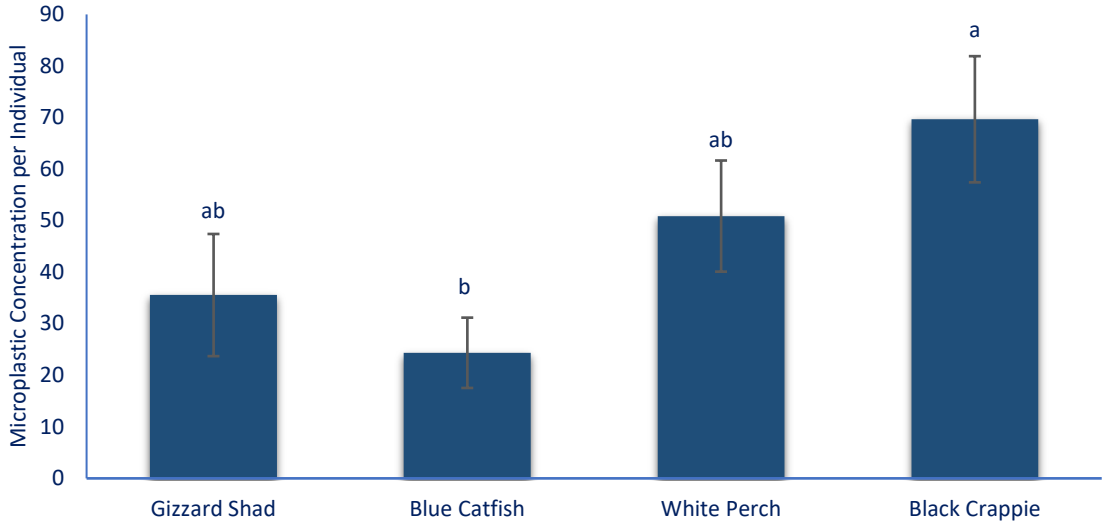


Figure 4. Mean microplastic concentrations in the gills of the four species (ANOVA: $F_{3,73}$=3.141, p=0.030). Letters represent significant differences among species. Error bars represent ± 1 SE.

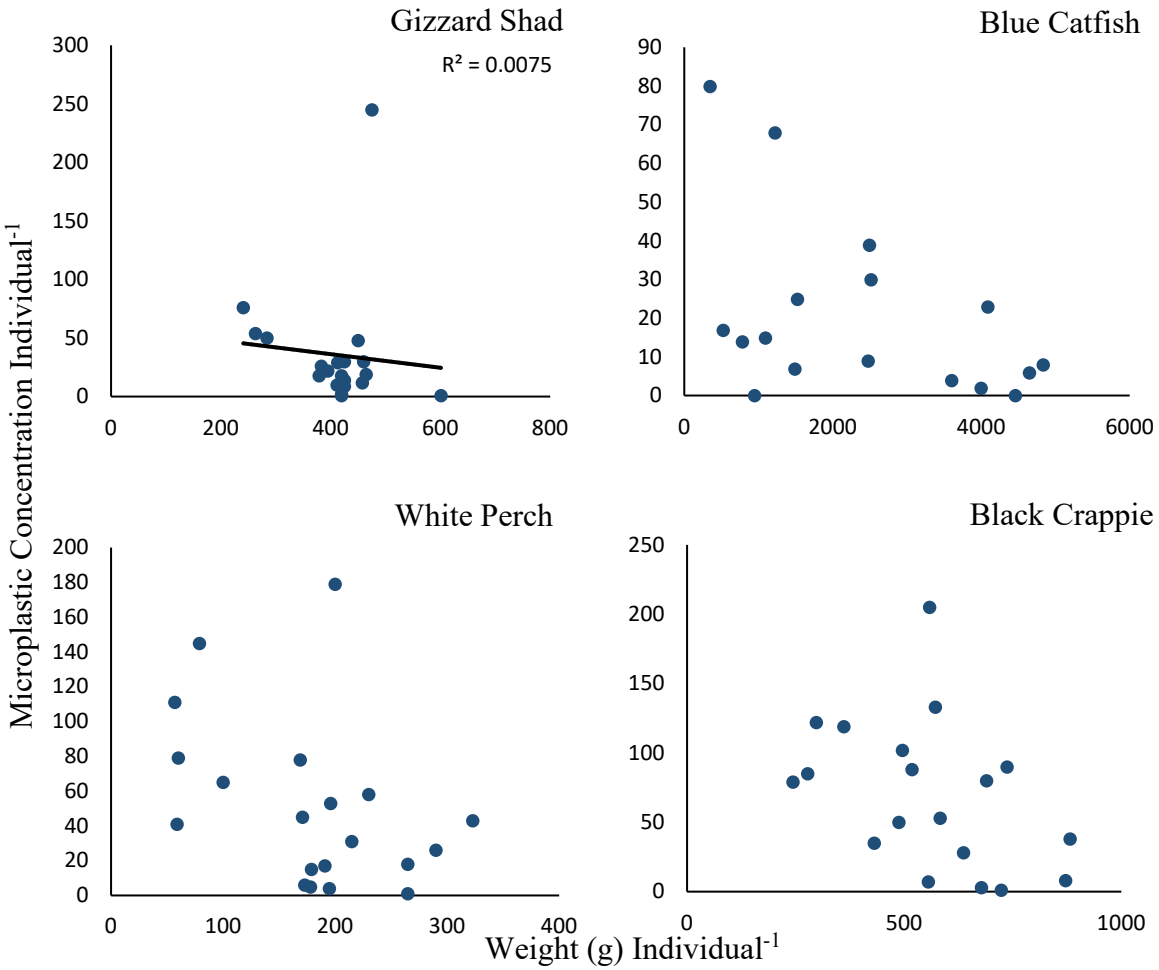


Figure 5. Regression analyses comparing weight of fish to microplastic concentrations present in the gills in the four target species.

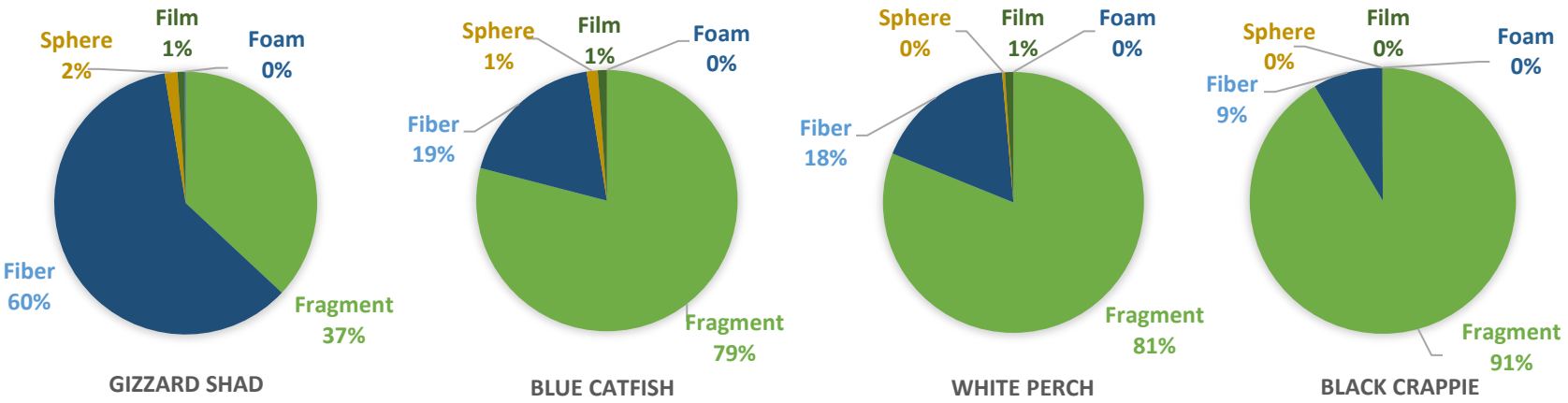


Figure 6. Percentages of microplastic types in GIT samples for the four species.

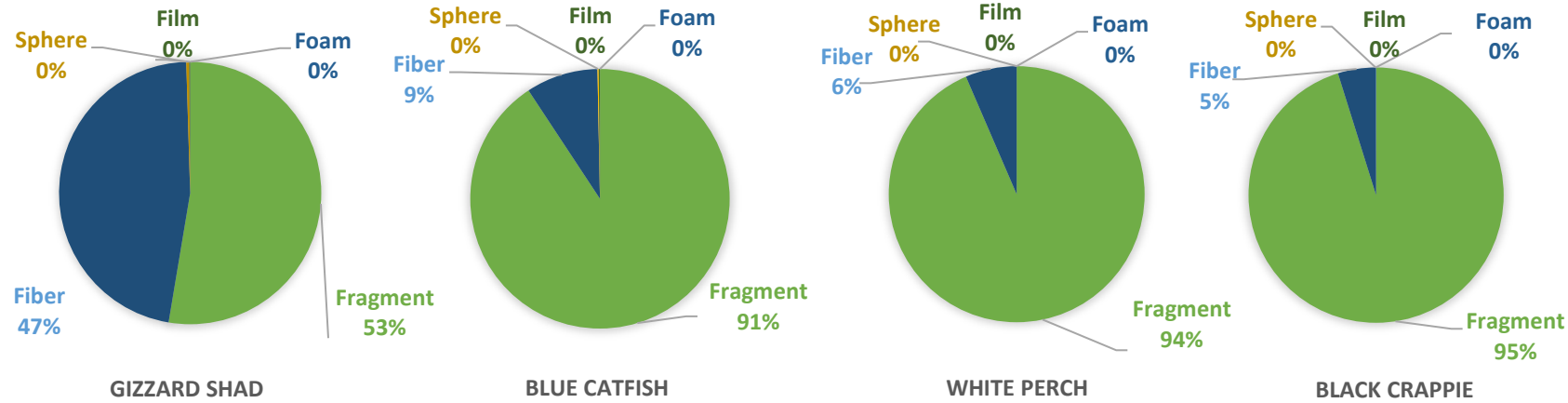


Figure 7. Percentages of microplastic types in gill samples for the four species.


Figure 8. Comparison of morphological shape and presence of gill rakers in the different species gills: A) gizzard shad B) blue catfish C) white perch D) black crappie.

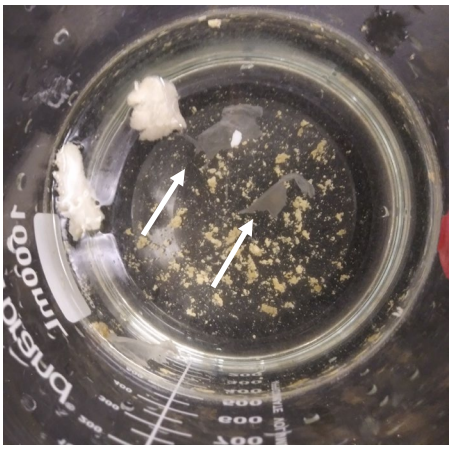

Figure 9. Large plastic film type pieces and other large anthropogenic items found the GIT of a blue catfish.

## Literature Cited


Akdogan Z and Guven B. 2019. Microplastics in the environment: a critical review of current understanding and identification of future research needs. Environmental Pollution. 254: 1-24.

Baldwin A, Corsi S, and Mason S. 2016. Plastic debris in 29 Great Lakes tributaries: relations to watershed attributes and hydrology. Environmental Science and Technology. 50: 10377-10385.

Barrows A, Neumann C, Berger M, and Shaw S. 2017. Grab vs. neuston tow net: a microplastic sampling performance comparison and possible advances in the field. Analytical Methods. 9: 1446-1453.

Bessa F, Barria P, Neto J, Frias J, Otero V, Sobral P, and Marques J. 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Marine Pollution Bulletin. 128: 575-584.

Blair S, Matheson D, and Goss G. 2017. Physiological and morphological investigation of Arctic grayling (*Thymallus arcticus*) gill filaments with high salinity exposure and recovery. Conservation Physiology. 5(1).

Blettler M, Abrial E, Khan F, Sivri N, and Espinola L. 2018. Freshwater plastic pollution: recognizing research biases and identifying knowledge gaps. Water Research. 143: 416-424.

Brandon J, Jones W, and Oham M. 2019. Multidecadal increase in plastic particles in coastal ocean sediments. Science Advances. 5: 1-6.

Claessens M, De Meester S, Van Landuyt L, De Clerck K, and Janssen C. 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Marine Pollution Bulletin. 62: 2199-2204.

Claessens M, Van Cauwenberghe L, Vandegehuchte M, and Janssen C. 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Marine Pollution Bulletin. 70: 227-233.

Cole M, Webb H, Lindeque P, Fileman E, Halsband C, and Galloway T. 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Scientific Reports. 4(4528): 1-8.

Davidson K and Dudas S. 2016. Microplastic ingestion by wild and cultured manila clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. Archives of Environmental Contamination and Toxicology. 71: 147-156.

Derriak J. 2002. The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 44: 842-852.

Dioses-Salinas D, Pizarro-Ortega C, and De-la-Torre G. 2020. A methodological approach of the current literature on microplastic contamination in terrestrial environments: Current knowledge and baseline considerations. 730: 1-13.

Drenner R. 1977. The feeding mechanics of the gizzard shad. [dissertation]. [Lawrence, Kansas]. University of Kansas.

Dytham C. 2011. Choosing and Using Statistics: A Biologist's Guide. Pondicherry, India. Wiley-Blackwell.

Eerkes-Medrano D, Thompson R, and Aldridge D. 2015. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritization of research needs. Water Research. 75: 63-85.

Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H, and Amato S. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Marine Pollution Bulletin. 77(1-2): 177-182.

FishBase. [Internet]. [cited 12 December 2019]. Froese, R. and Pauly, D. Editors. FishBase.World Wide Web electronic publication. Available at www.fishbase.org.

Free C, Jensen O, Mason S, Eriksen M, Williamson N, and Boldgiv B. 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Marine Pollution Bulletin. 85: 156-163.

Fritzsche R. 1980. Early osteological development of white perch and striped bass with emphasis on identification of their larvae. Transactions of the American Fisheries Society. 109: 387-406.

Fu W, Min J, Jiang W, Li Y, and Zhang W. 2020. Separation, characterization and identification of microplastics and nanoplastics in the environment. Science of the Total Environment. 721: 1-26.

Geyer R, Jambeck J, and Law K. 2017. Production, use, and fate of all plastics every made. Science Advances. 3(1700782): 1-5.

Gray A and Weinstein J. 2017. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (*Palaemonetes pugio*). Environmental Toxicology and Chemistry. 36(11): 3074-3080.

Gray A, Wertz H, Leads R, and Weinstein J. 2018. Microplastics in two South Carolina estuaries: occurrences, distribution, and composition. Marine Pollution Bulletin. 128: 223-233.

Harper C and Wolf J. 2009. Morphological effects of the stress response in fish. ILAR Journal. 50(4): 387-396.

Hildalgo-Ruz V, Gutow L, Thompson R, and Thiel M. 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environmental Science and Technology. 46: 3060-3075.

Horton A, Walton A, Spurgeon D, Lahive E, and Svendsen C. 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of the Total Environment. 586: 127-141.

Hu L, Chernick M, Lewis A, Ferguson P, and Hinton D. 2020. Chronic microfiber in adult Japanese medaka (*Oryzias latipes*). PloS ONE. 15(3).

Hurt R, O'Reilly C, and Perry W. 2020. Microplastic prevalence in two fish species in two U.S. reservoirs. Limnology and Oceanography Letters. 5: 147-153.

In-Situ. [Internet]. [cited 8 November 2019]. Aqua TROLL 600 Multiparameter Sonde. Available at https://in-situ.com/products/water-quality-testing-equipment/aqua-troll-600-multiparameter-sonde/.

Iowa Department of Natural Resources. [Internet]. [cited 10 October 2020]. Details: Gizzard Shad. Available at iowadnr.gov.

Jabeen K, Su L, Li J, Yang D, Tong C, Mu J, and Shi H. 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environmental Pollution. 221: 141-149.

Jaafar N, Musa S, Azfaralariff A, Mohamed M, Yusoff A, and Lazim A. 2020. Improving the efficiency of post-digestion method in extracting microplastics from gastrointestinal tract and gills of fish. Chemosphere. 260: 1-15.

Jambeck J, Geyer R, Wilcox Ch, Siegler T, Perryman M, Andrady A, Narayan R, and Law K. 2015. Plastic waste inputs from land into the ocean. Science. 347(6223): 768-771.

Karami A, Golieskardi A, Choo C, Romano N, Ho Y, and Salamatinia B. 2017. A high-performance protocol for extraction of microplastics in fish. Science of the Total Environment. 578: 485-494.

Karlsson T, Vethaak A, Almroth B, Ariese F, van Velzen M, Hassellov M, and Leslie H. 2017. Screening for microplastics in sediment water, marine invertebrates, and fish: Method development and microplastic accumulation. Marine Pollution Bulletin. 122: 403-408.

Kashiwada S. 2006. Distribution of Nanoparticles in the See-through Medaka (*Oryzias latipes*). Environmental Health Perspectives. 114(11): 1697-1702.

Keast A. 1968. Feeding biology of the black crappie, Pomoxis nigromaculatus. Journal of the Fisheries Research Board of Canada. 25(2): 282-297.

Klein S, Worch E, and Knepper T. 2015. Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany. Environmental Science and Technology. 49: 6070-6076.

Kuhn S, Rebolledo E, and van Franeker J. 2015. Deleterious effects of litter on marine life. In: Marine Anthropogenic Litter. (Bergmann M, Gutow L, Klages M, eds). Springer, Cham, pp 75-116.

Laufer E. 1947. Plastics come of age. The Analysts Journal. 3(1): 32-42.

Li J, Liu H, and Chen J. 2018. Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. Water Research. 137: 362-374.

Li J, Lusher Am, Rotchell J, Deudero S, Turra A, Brate I, Sun C, Hossain M, Li Q, Kolandhasamy P, and Shi H. 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environmental Pollution. 244: 522-533.

Li J, Yang D, Li L, Jabeen K, and Shi H. 2015. Microplastics in commercial bivalves from China. Environmental Pollution. 207: 190-195.

Lohmann R. 2017. Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans-but should microplastics be considered POPs themselves? Integrated Environmental Assessment and management. 13(3): 460-465.

Lopez-Lopez L, Preciado I, Gonzalez-Irusta J, Arroyo N, Munoz I, Punzon A, and Serrano A. 2018. Incidental ingestion of meso- and macro-plastic debris by benthic and demersal fish. Food Webs. 14:1-4.

Lusher A. 2015. Microplastics in the marine environment: distribution, interactions and effects. In: Marine Anthropogenic Litter. (Bergmann M, Gutow L, Klages M, eds). Springer, Cham, pp 245-307.

Lusher A, McHugh M, and Thompson R. 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Marine Pollution Bulletin. 67: 94-99.

Lusher A, Welden N, Sobral P, and Cole M. 2017. Sampling, isolating, and identifying microplastics ingested by fish and invertebrates. Analytical Methods. 9: 1346-1360.

Lv W, Zhou W, Lu S, Huang W, Yuan Q, Tian M, Lv W, and He D. 2019. Microplastic pollution in rice-fish co-culture system: A report of three farmland stations in Shanghai, China. Science of the Total Environment. 652: 1209-1218.

McNeish R, Kim L, Barrett H, Mason S, Kelly J, and Hoellein T. 2018. Microplastic in riverine fish is connected to species traits. Scientific Reports. 8: 1-11. doi.org/10.1038/s41598-018-29980-9.

Possatto F, Barletta M, Costa M, Ivar do Sul J, and Dantas D. 2011. Plastic debris ingestion by marine catfish: an unexpected fisheries impact. Marine Pollution Bulletin. 62(5): 1098-1102.

Pinheiro C, Oliveira U, and Viera M. 2017. Occurrence and Impacts of Microplastics in Freshwater Fish. Journal of Aquaculture and Marine Biology. 5(6): 1-5.

Rehse S, Kloas W, and Zarfl C. 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilization of Daphnia magna. Chemosphere. 153: 91-99.

Roch S, Walter T, Ittner L, Friedrich C, and Brinker A. 2019. A systematic study of the microplastic burden in freshwater fishes of south-western Germany-Are we searching at the right scale? Science of the Total Environment. 689: 1001-1011.

Rocha-Santos T and Duarte A. 2015. A critical overview of the analytical approaches to the occurrence, the fate, and the behavior or microplastics in the environment. Trends in Analytical Chemistry. 65: 47-53.

Rochman C and Hoellein T. 2020. The global odyssey of plastic pollution. Science. 368(6496): 1184-1185.

Rochman C, Parnis J, Browne M, Serrato S, Reiner E, Robson M, Young T, Diamond M and Teh S. 2017. Direct and indirect effects of different types of microplastics in freshwater prey (*Corbicula fluminea*) and their predator (*Acipenser transmontanus*). Plos ONE. 12(11): 1-18.

Ross S. 2001. The Inland Fishes of Mississippi. Mississippi, USA. University Press of Mississippi. Jackson.

Rothwell J. 2020. ANCOVA in SPSS. Stats Tutor. University of Sheffield.

Said L and Heard M. 2020. Variation in the presence and abundance of anthropogenic microfibers in the Cumberland River in Nashville, TN, USA. Environmental Science and Pollution Research. 1-5.

[SCDNR] South Carolina Department of Natural Resources. [Internet]. [cited 8 November 2019]. South Carolina's Guide to Freshwater Fishes. Available at http://www.dnr.sc.gov/aquaticed.

Schaus M, Vanni M, and Wissing T. 2002. Biomass-dependent diet shifts in omnivorous gizzard shad: implications for growth, food web, and ecosystem effects. Transactions of the American Fisheries Society. 131: 40-54.

Scherer C, Brennholt N, Reifferscheid G, and Wagner M. 2017. Feeding type and development drive the investigation of microplastics by freshwater invertebrates. Scientific Reports. 7: 1-9.

Scherer C, Weber A, Lambert S, and Wagner M. 2018. Interactions of Microplastics with Freshwater Biota. Freshwater Microplastics. The Handbook of Environmental Chemistry. 58: 153-180.

Schmaltz E, Melvin E, Biana Z, Gunady E, Rittschof D, Somarelli J, Virdin J, and Dunphy-Daly M. 2020. Plastic pollution solutions: emerging technologies to prevent and collect marine plastic pollution. Environment International. 144: 1-17.

Schmitt J, Peoples B, Castello L, and Orth D. 2019. Feeding ecology of generalist consumers: a case study of invasive blue catfish *Ictalurus furcatus* in Chesapeake Bay, Virginia, USA. Environmental Biology of Fish. 102: 443-465.

Shim W, Song Y, Hong S, and Jang M. 2016. Identification and quantification of microplastics using Nile Red staining. Marine Pollution Bulletin. 113: 469-476.

Solomon O and Palanisami T. 2016. Microplastics in the marine environment: current status, assessment methodologies, impacts and solutions. Journal of Pollution Effects and Control. 4(2): 1-13.

Soil and Water Conservation Society of Metro Halifax. [Internet]. [cited 15 November 2020]. Zooplankton of Freshwaters. Available at lakes.chebucto.org.

Staton T, Johnson M, Nathanail P, Gomes R, Needham T, and Burson A. 2019. Exploring the efficacy of Nile Red in microplastic quantification: a costaining approach. Environmental Science Technology Letters. 6: 606-611.

St-Hilaire A, Courtenay S, Dupont F, and Boghen A. 2002. Diet of white perch (*Morone americana*) in the Richibucto Estuary, New Brunswick. Northeastern Naturalist. 9(3): 303-316.

Su L, Xue Y, Li L, Yang D, Kolandhasamy P, Li D, and Shi H. 2016. Microplastics in Taihu Lake, China. Environmental Pollution. 216: 711-719.

Su L, Cai H, Kolandhasamy P, Wu C, Rochman C, and Shi H. 2018. Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. Environmental Pollution. 234: 347-355.

Talvitie J, Mikola A, Koistinen A, and Setala O. 2017. Solutions to microplastic pollution-removal of microplastics from wastewater effluent and advanced wastewater treatment technologies. 123: 401-407.

Thompson R, Olsen Y, Mitchell R, Davis A, Rowland, John A, McGonigle D, and Russell A. 2004. Lost at sea: where is all the plastic? Science. 304: 838.

[USGS] United States Geological Survey. [Internet]. [cited 1 November 2020]. Stream Stats. Available at https://streamstats.usgs.gov.

Van Cauwenberghe L, Devriese L, Galgani F, Robbens J, and Janssen C. 2015. Microplastics in sediments: A review of techniques, occurrence, and effects. Marine Environmental Research. 111: 5-17.

Walleser L, Sandheinrich M, Howard D, Gaikowski M, and Amberg J. 2014. Spatial and temporal variation of the gill rakers of gizzard shad and silver carp in three midwestern rivers. North American Journal of Fisheries Management. 34: 875-884.

Watts A, Urbina M, Goodhead R, Moger J, Lewis C, and Galoway T. 2016. Effect of microplastic on the gills of the shore crab *Carcinus maenas*. Environmental Science and Technology. 50: 5364-5369.

Westneat M. 2001. Ingestion in Fish. Encyclopedia of Life Sciences. 1-6.

Wieczorek A, Morrison L, Croot P, Allcock A, MacLoughlin E, Savard O, Brownlow H, and Doyle T. 2018. Frequency of microplastics in mesopelagic fishes from the northwest Atlantic. Frontiers in Marine Science. 5(39): 1-9.

Wong J, Lee K, Tang K, and Yap P. 2020. Microplastics in the freshwater and terrestrial environments: prevalence, fates, impacts and sustainable solutions. Science of the Total Environment. 719: 1-15.

Young A and Elliott J. 2016. Characterization of microplastic and mesoplastic debris in sediment from Kamilo Beach and Kahuku Beach, Hawaii. Marine Pollution Bulletin. 113: 477-482.

Yousif E and Haddad R. 2013. Photodegradation and photostabilization of polymers, especially polystyrene: review. SpringerPlus. 2(398): 1-32.