FIRST-YEAR BIOLOGY STUDENTS INVESTIGATE MICROPLASTICS IN A UNIVERSITY ICHTHYOLOGICAL COLLECTION

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INTRODUCTION

Microplastics have been found across most environments and have become a focus of scientific research as their potential effects are just beginning to be described. Microplastics are extremely small pieces of broken-down plastic which the National Oceanic and Atmospheric Administration (NOAA) has defined as measuring < 5 mm. Microplastics are made of polymers such as polyethylene, polystyrene, polypropylene, rayon, nylon, polyester, cellophane, acrylonitrile, and natural fibers (e.g., Zimmermann et al., 2020). They can be found in almost all parts of the environment, including the land, air, and water, and have become one of the most important pollutants in some environments (Karami et al., 2017; Galafassi et al., 2021; Chang et al., 2022). Depending on characteristics of the microplastic particles, the environment, and ecosystem processes, plastic particles can disperse across aquatic systems or settle in substrates where microplastics have the potential to build up in river sediments. Additionally, microplastics may become momentarily or permanently confined by physical obstacles like dams or algal mats. Microplastics from these sediments can be released by disturbance events and begin their ecosystem cycle anew (Parker et al., 2021).

Most studies on aquatic microplastic pollution have been conducted in marine ecosystems but more diverse environments are being assessed (Eerkes-Medrano et al. 2015; Martin et al., 2019; Chang et al., 2022). There is evidence of ingestion of microplastics by both marine (Karami et al., 2017) and freshwater fishes (Galafassi et al., 2021); however, the majority of microplastic diet analysis research has been on freshwater fishes, particularly the Zebrafish *Danio rerio* (Galafassi et al., 2021). In the US, in the Muskegon, Milwaukee, and St. Joseph rivers, the concentration of microplastics varied significantly among fish species where microplastic concentration in Round Goby *Neogobius melanostomus* was substantially higher than that in Fathead Minnow *Pimephales promelas* and White Sucker *Catostomus commersonii* (McNeish et al., 2018). Historical studies on ingestion of microplastics by freshwater fishes have used museum specimens to demonstrate that fishes did not ingest microplastics prior to ~1950, and that ingestion rates have generally increased over time (Hou et al., 2021); however, other studies only found microplastics in rare instances or only in recent (< 30 years ago) samples (Toner and Midway, 2021).

Preliminary evidence of microplastic ingestion by fish emerged in 2010 after an analysis showed stomach contents of fish from the North Pacific Central Gyre contained microplastics (Galafassi et al., 2021). Many factors affect microplastic ingestion including fish species' traits, microplastic morphology, abiotic habitat factors, and time. Larger sizes of microplastics cannot be fully digested and thus contribute to the number of plastics found in the intestines of fish (Gamarro et al., 2020). Smaller plastics can be either retained, excreted, or incorporated into tissues. For example, retention times and rate of excretion for Mummichog *Fundulus heteroclitus* and Red Seabream *Pagrus major* were different depending on fish species as well as size and shape of microplastics (Ohkubo et al., 2020). Mummichogs excreted the plastics at a faster rate than Red Seabream, but both species had excreted > 95% of all ingested microplastics sized 250–850 µm after a 25-hour period. Another study assessed retention time of polyethylene microspheres of five different colors (red, blue, yellow, green, or gray) across four fish species (two freshwater [Japanese Medaka *Oryzias latipes* and Zebrafish] and two marine species [Indian Medaka *Oryzias melastigma* and Clown Anemonefish *Amphiprion ocellaris*], Okamoto et al., 2022). The study found that the color preference differed by fish species: Zebrafish and Clown

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Jayden Winsel is the lead author of this study and an undergraduate student at Emporia State University. Dr. Erika Martin is an Associate Professor of Aquatic Biology and Biology Education at Emporia State University. The rest of the authors are introductory biology students—primarily freshman biology majors—and their graduate student teacher. These experiments were integrated into the classroom. Through this project, students gained hands-on experience in scientific research.

Anemonefish preferred any color, but Japanese Medaka preferred blue or green, and Indian Medaka preferred red or green. Okamoto and colleagues (2022) also found that excretion rate differed among species, and after 24 hours, most individuals had excreted > 90%; however, one Zebrafish only excreted 10%, demonstrating that some individuals might be more likely to retain microplastics. While evidence is mounting to understand the extent and effects of microplastic pollution, predictable mechanisms will remain unclear until substantial data are available. This report is a small addition in the pursuit of the accumulation of data.

The destination of microplastics when ingested by fish is highly variable. Post-ingestion, microplastics can be excreted, as described above. However, microplastics that are retained have been found in the gastro-intestinal (GI) system and gills, and there is evidence that microplastics can accumulate in fish body tissues (Gamarro et al., 2020; Galafassi et al., 2021), including stomach, muscle, liver (Collard et al., 2018), or skin (Abbasi et al. 2018). Retention of microplastics in fish tissue is of particular importance for human consumption of fishes. Although there are limited studies on microplastics in species that are often canned or consumed whole, 14%-15% of European Pilchards *Sardina pilchardus* and European Anchovies *Engraulis encrasicolus* caught along the Mediterranean coast had microplastics in their GI tract (Fossi et al., 2018). An investigation on the presence of microplastics in 20 different canned sardine and sprat products found that 16 brands contained no microplastics, but microplastics were detected in the four remaining brands (Karami et al., 2018). Microplastics in canned products can either come from contamination of the fish or from contamination during the canning process (Gamarro et al., 2020). Other organisms are affected by microplastic ingestion. In fact, an investigation of Norway Lobsters *Nephrops norvegicus* revealed that 83% contained microplastics, mostly filaments, in the stomach. It has already been demonstrated that microplastics occur in human blood (Leslie et al., 2022). Given that microplastics are found in the body of organisms, it is of interest to understand the effects the presence of these particles might have on organisms that ingest them. In 2013, Galafassi and colleagues (2021) conducted a literature review over the toxicological effects on fishes exerted by chemically absorbed microplastics. They found effects that range from no effect up to physical problems related to ingestion/excretion due to blockage, changes in feeding behavior, inflammation, alteration of metabolism, altered immune system function, and growth. Likelihood of microplastic ingestion affecting fish is multifaceted and depends on type of plastic, fish species, amount ingested, and other factors like age and size of the plastic and the fish.

The most common type of microplastic ingested is dependent on factors including species' traits and habitat type or location; however, the top three plastic types are generally considered to be polyethylene, polypropylene, and polystyrene (de Haan et al., 2019). Similarly, the most abundant type of microplastic present in the gut of four species of fish (Indian Mackerel *Rastrelliger kanagurta*, Spotty-face Anchovy *Stolephorus waitei*, Greenback Mullet *Liza subviridis*, and Belanger's Croaker *Johnius belangerii*) was polypropylene (47.2% of particles), and the second most common was polyethylene (41.6%) (Karami et al., 2017). These microplastic types were the most common because industries use those types of plastic polymers in their productions, and plastics with a

Figure 1: Students dissecting museum samples of Kansas fishes in the laboratory room.

Figure 2: Students dissecting museum samples of Kansas fishes.

lower density than seawater can float on the surface, which may be a factor dictating which organisms are affected (Karami et al., 2017). The broad goal for this study was to determine if fish in Kansas have ingested microplastics. This was done by analyzing the gut contents of museum specimens of fish collected in Kansas to determine the presence of microplastics.

METHODS

Museum specimens were acquired from Emporia State University. Fish species were selected if there were at least three individuals of the same species available for dissection. After initial selection, three species were identified that had enough individuals: Bluntnose Minnow *Pimephales notatus*, Largemouth Bass *Micropterus nigricans*, and Longear Sunfish *Lepomis megalotis*. All fish were collected between 1970–2020; however, due to insufficient museum labeling, e.g., missing collection date and/or precise location, we could only assess differences in microplastic levels among species identity and not over time or by location (see Hou et al., 2021 for a more comprehensive museum study). Individuals were cut open from the anus to the bottom of the jaw, taking care not to cut into the fish's internal organs (Figure 1). The stomach and intestines were then removed (Figures 2 and 3). Gut contents were

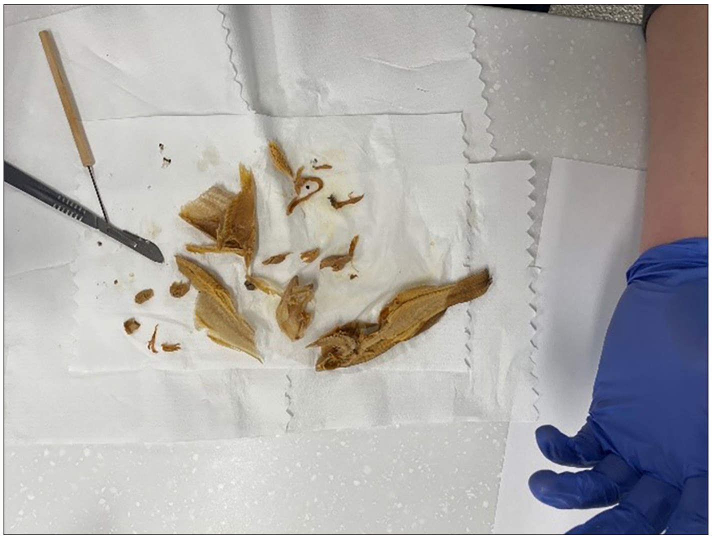

Figure 3: An Emporia State University student's sample of a dissected Largemouth Bass.

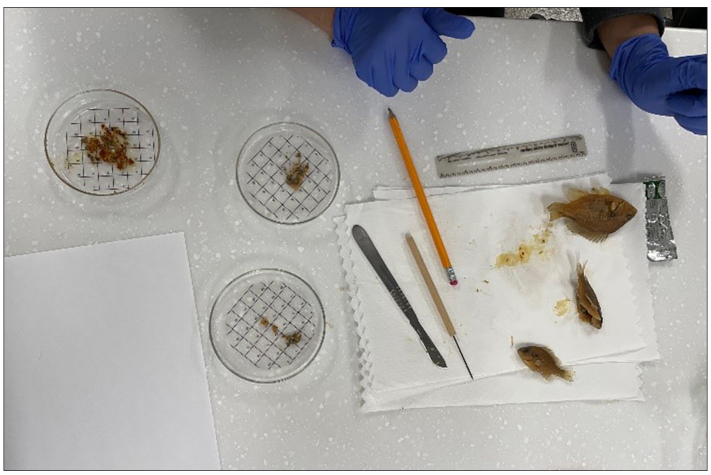

Figure 4: Several samples of students' dissected fishes, and the gut contents from each fish in its own petri dish.

removed and placed on a gridded petri dish (Figure 4) and were spread as thinly as possible on the petri dish, by either using a pick or gloved fingers.

A petri dish was then placed under a microscope at either 100 or 400 magnification (Figure 5). The microplastics within the gut contents were counted across the grid. Data were analyzed using a chi-square test on the average count of microplastics among species.

RESULTS

A total of eight Bluntnose Minnow, three Largemouth Bass, and 16 Longear Sunfish were analyzed. We found differences among the groups, where Longear Sunfish had more microplastic particles than the other two species. Mean counts of microplastics in Bluntnose Minnow was 2.5 (standard deviation [SD] + 2.56), Largemouth Bass was 2 (+ 1.73), and Longear Sunfish was 25.75 (+ 40.24) (Figure 6). Data were non-normal and skewed, and median counts found Bluntnose Minnow at 1.5, Largemouth Bass at 3, and Longear Sunfish at 13 microplastic particles per individual. The range of data for species was as follows: 0 to 7 particles for Bluntnose Minnow, 0 to 3 for Largemouth Bass, and 0 to 142 for Longear Sunfish.

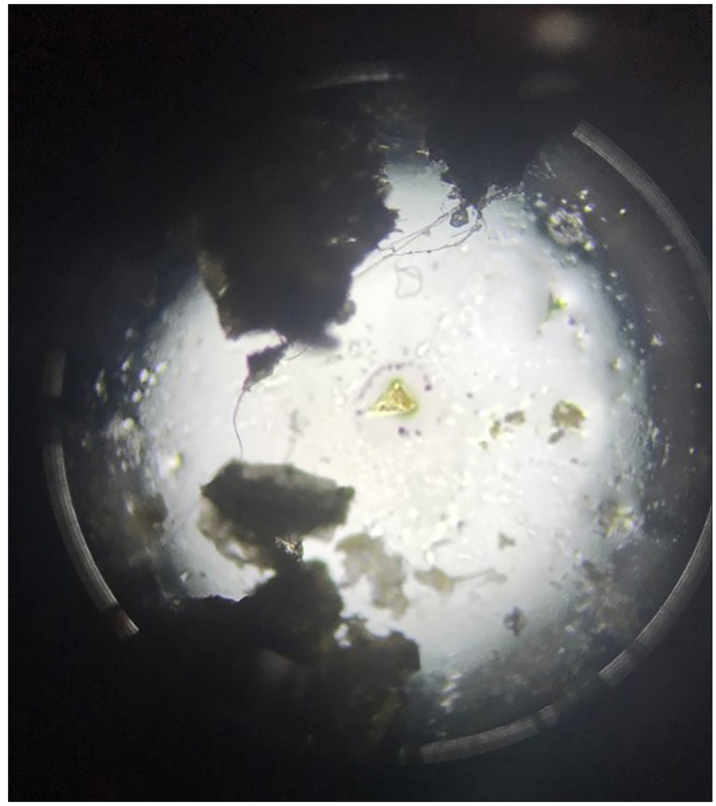

Figure 5: Fish gut contents seen through a microscope at 100 X.

DISCUSSION

We found that microplastic density in gut contents varies by species. Here, we find that the leuciscid/cyprinid (Bluntnose Minnow) and centrarchid (Largemouth Bass) had fewer microplastics particles than the centrarchid Longear Sunfish. Due to restrictions inherent from our data, we are unable to state with certainty why these differences occur; however, we will discuss potential key explanatory factors of abiotic habitat and species traits.

It has been suggested that the number of microplastics is positively associated with urbanization (Peters and Bratton 2016), where more people equal more plastics. This relationship, however, does not always occur (Dikareva and Simon 2019). The number of plastic particles ingested by two species of sunfish (Bluegill *Lepomis macrochirus* and Longear Sunfish) from a Texas river basin were correlated with urbanization, where the number of plastic particles in the gut of fish increased as urbanization increased (Peters and Bratton 2016). Conversely, across three rivers feeding into Lake Michigan with different dominant land-use (forest, urban, agriculture), the total concentration of microplastics across rivers was similar, but the concentration within fish differed significantly (McNeish et al., 2018), thus demonstrating that the total amount of plastics in the environment might not be a good indicator of ingested microplastics. Instead, species' traits, like feeding ecology or habitat preferences, are important. Other key traits might be fish size, as researchers have demonstrated that the number of ingested plastic particles can be positively correlated with individual fish length (Peters and Bratton 2016). In this same study, the number of plastics ingested was also positively correlated with ingestion of other food items, suggesting that ingestion of microplastics is incidental (Peters and Bratton 2016). All individuals assessed in our study were small enough to fit in-

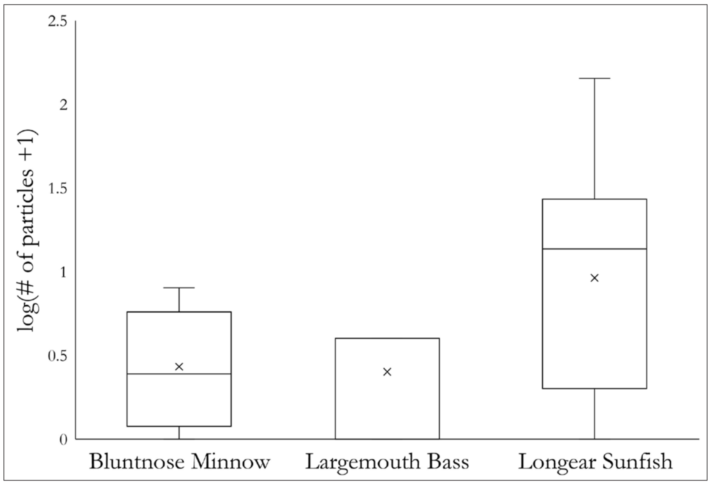

Figure 6: Log+1 mean (x) and median (line) amount of microplastics found in three different species of Kansas fish with standard deviation.

side glass jars to be preserved, so while Largemouth Bass in nature would have the potential to be larger than the Bluntnose Minnow, all individuals analyzed here are fairly small (~2 to 5 inches total length). While the two centrarchids were larger, the size differences and number of individuals do not provide enough information to make definitive conclusions whether individual size influences microplastic ingestion. Similarly, without consistent location information, we are unable to know which individuals might have been captured at sites with more or less microplastic pollution. One characteristic we can discuss to some is species traits. Largemouth Bass can be found in diverse habitat types from ponds to rivers but prefer vegetation and are opportunistic obligate carnivores (Kansas Fishes Committee, 2014). Longear Sunfish are generally found in small to medium-sized rivers and are opportunistic invertivores but also feed on small fish and fish eggs (Kansas Fishes Committee, 2014). Finally, Bluntnose Minnow prefer low-flow pools and backwaters in large rivers and are herbivores/detritivores, feeding on organic matter like algae. It is possible that the feeding preferences and strategy are determining factors in microplastic ingestion. It is interesting that the Longear Sunfish, a species that prefers items like fish eggs, also has the highest microplastic count in the gut. Though again, with such a small sample size, strong conclusions are not possible.

While we demonstrated that three species of Kansas fishes ingest microplastics at varying amounts, we are limited in scope due to lack of information provided on museum labels and sample size. Information provided on labels varied substantially, from as little information as just a common name, to complete information of common and scientific name, location, date, and collector name. Should readers be interested in pursuing this work with students or citizen science projects, it is worth noting that some students had difficulty getting the gut contents to be spread thin enough to analyze for microplastics using the microscope. This difficulty suggests that estimates of microplastics here are likely conservative. For comparison of microplastic counting methods, see work by Wagner et al. (2017), which includes use of standard light microscopy as well as other methods: scanning electron microscopy plus energy-dispersive X-ray spectroscopy, Fourier transform infrared micro-spectroscopy, and Raman micro-spectroscopy. Regardless of the limited scope of this study, it is clear that microplastics are in Kansas and are being ingested by our native fishes. Future work will assess the composition, source, and ecological impacts of microplastics in the Great Plains.

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FishMap.org combines numerous data sources to provide a better view and more complete understanding of fish species distribution. It uses data from NatureServe, the National Atlas, the USGS water resources and Nonindigenous Aquatic Species programs, FishNet2, iNaturalist.org, GBIF, and iDigBio.

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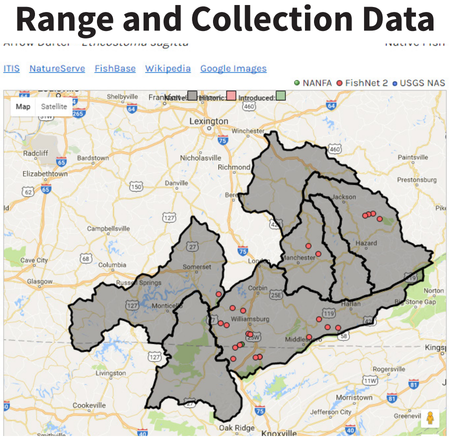
Range and Collection Data



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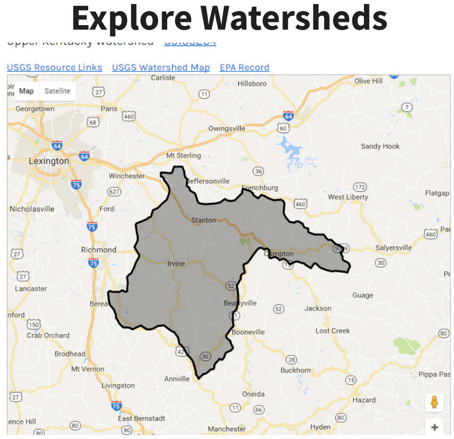

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