Microplastic prevalence in two fish species in two U.S. reservoirs

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Scientific Significance Statement

The emergence of microplastics as a potential contaminant requires understanding the distribution and potential consumption of microplastics across ecosystems and species. Freshwater environments are known to have high concentrations of microplastics, but very little is known about the concentrations of microplastics within lacustrine fish. The very high prevalence and numbers of microplastics in two fish species in agricultural reservoirs in this study indicates that lacustrine fish may be at high risk of microplastic contamination.

Abstract

Microplastics in the environment can impact feeding and physiological functions of organisms. Most studies have focused on marine systems, and studies of lacustrine organisms are less common. We examined microplastic concentration in 72 gizzard shad and 24 largemouth bass from two agricultural reservoirs in the midwestern U.S.A. with differing shoreline development. Microplastics were found in 100% of the fish, with 1–49 No. Fish⁻¹, independent of shoreline development. Bass had higher concentrations overall than shad. For bass, microplastics were more concentrated in the guts rather than gills, but the opposite was the case for shad, suggesting that feeding guild may influence where microplastics accumulate in fishes. The prevalence and concentrations reported here are greater than reported in marine systems and higher than reported in riverine studies. Our results underscore the ubiquitous distribution of microplastics and suggest that lake ecosystems in agricultural landscapes may be particularly susceptible to microplastic contamination.

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Author Contribution Statement: R.H. and W.L.P. designed the research question and study approach. R.H. designed field sampling scheme, collected samples, and performed laboratory analyses. W.L.P. performed data analysis and created graphs and tables. R.H., W.L.P., and C.M.O. wrote and edited the paper.

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Microplastics can be found in nearly every environment, from marine, freshwater, terrestrial, and even atmospheric. These small (< 5 mm) plastics are derived through the degradation of larger plastics into smaller fragments or fibers, as well as fibers that are released from fabrics and beads that have been used in industry or personal care products. One of the main sources of microplastics for freshwater ecosystems appears to be wastewater treatment plants (WWTPs), and urbanization has been associated with higher levels of microplastic concentration in rivers (Peters and Bratton 2016). However, even in remote locations, the breakdown of plastic waste and subsequent distribution of particles by wind can lead to high levels of contamination, such as in Lake Hovsgol, Mongolia (Free et al. 2014). Microplastic concentrations in freshwater ecosystems can be orders of magnitude higher than in marine environments (Mani et al. 2015), leading to high potential for consequences for aquatic food webs.

Ingestion of microplastics appears to be widespread and can have a range of physiological effects (Cole et al. 2011, Rochman et al. 2015). Microplastic concentrations have been reported from over 100 species of fish, invertebrates, and marine mammals (Cole et al. 2011; Fossi et al. 2014; Rochman et al. 2015). Microplastics are composed of synthetic organic polymers that can contain possibly hazardous chemical additives and can also adsorb harmful chemicals from the environment and serve as vectors for persistent organic pollutants (Rochman et al. 2013). As plastic degrades, particle size decreases, allowing ingestion by a larger range of organisms, and the larger surface area to volume ratio can increase the potential for leaching of harmful chemicals in plastics including cadmium, lead, and phthalates (Shim and Thompson 2015). Chemicals leached from ingested microplastics can be absorbed directly into the consumer (Rochman et al. 2013; Ding et al. 2018) leading to physiological impacts on reproductive success, behavior, and growth (Rochman et al. 2014).

Studies of microplastic concentrations in fish have been conducted primarily in marine systems (de Sá et al. 2018), and far less is understood about fish microplastic concentrations in freshwater systems, particularly lacustrine systems. Trophic level differences have been noted in marine (e.g., Nelms et al. 2018) and estuarine (e.g., Ferreira et al. 2019) systems, with varying amounts of microplastics in different fish tissues (Su et al. 2019). In a study of freshwater fish in three tributaries to Lake Michigan, ecological and morphological traits corresponded with microplastic abundance in gastrointestinal tissues, indicating that functional feeding group might influence microplastic consumption (McNeiush et al. 2018). Thus, species traits and trophic level may be important factors related to fish microplastic concentrations and could influence whether microplastics accumulate in the gills or gastrointestinal tissues. Furthermore, several riverine studies have found higher fish microplastic concentrations in urban landscapes (Peters and Bratton 2016; Silva-Cavalcanti et al. 2017). However, explicit relationships across trophic levels and the role of development have not been specifically investigated for lacustrine systems.

In this study, we examined microplastic concentrations in two fish species at different trophic levels in two drinking water reservoirs in an agricultural landscape. Gizzard shad (Dorosoma cepedianum) are filter feeders (Heidman et al. 2012) and are a food source to a variety of sportfish, including predatory largemouth bass (Micropterus salmoides), for whom gizzard shad are a common diet item (Garcia-Berthou 2002). Our questions were (1) Does the concentration of microplastics differ between fish of different trophic levels and between different tissue types? (2) Does shoreline development influence fish microplastic concentration?

**Methods**

**Study sites**

We sampled gizzard shad and largemouth bass from two hypereutrophic drinking water reservoirs for the City of Bloomington, McLean County, Illinois. Evergreen Lake (maximum depth 17 m) has a watershed of 107 km² with 19% developed landcover overall but with parkland along the shoreline and in the area immediately around the lake. Lake Bloomington (maximum depth 11 m) has a 180 km² watershed with a shoreline that is highly developed with permanent homes on septic systems, although the watershed is only 8% developed landcover. The land cover of both reservoirs is dominated by row crop agriculture (Evergreen Lake 82%; Lake Bloomington 90%).

**Fish collection**

In total, we collected 72 gizzard shad and 24 largemouth bass. Fish were collected from Lake Bloomington and Evergreen Lake within a 1-month time from late July through early August 2018. Fish were collected using boat electrofishing from six different randomly selected locations in each lake (Supporting Information Fig. S1). From each location, we collected three juvenile (< 1 yr old) and three adult gizzard shad (> 1 yr old) and two largemouth bass (> 30 cm length). Fish were individually labeled and wrapped in aluminum foil and immediately put in a cooler and transported to the laboratory where they were frozen (−20°C) for further processing.

**Microplastic quantification**

Each fish was thawed at room temperature, measured, weighed, and dissected. The fork length and mass of all fish were recorded. All further steps were performed under the fume hood, and all glassware and laboratory tools were rinsed three times with distilled water before being used. Fish were dissected to remove the entire gastrointestinal tract (hereafter referred to as the gut) and both gills. Tissue was placed in a glass container and frozen at −20°C.
We quantified the microplastics for the gut and gills of the fish from each reservoir (Perry et al. 2019). To assess microplastic number in each sample, referred to as microplastic concentration and using units No. Fish$^{-1}$, we used a modified potassium hydroxide and hydrogen peroxide digestion, which we used because the alkali saponifies fats and breaks down tissues (e.g., Rochman et al. 2015). We added 10 mL of 1 mol L$^{-1}$ KOH and 5 mL of sodium dodecyl sulfate (0.5% w/v [ca. 5 g L$^{-1}$]) per gram of fish tissue, in a 150 mL beaker covered with aluminum foil. The beaker was placed in a water bath for a minimum of 24 h at 50°C, during which time the beakers were gently shaken multiple times. After the 24-h incubation, the contents were filtered through 0.8 μm cellulose membrane filters. Filters were then placed back into their original beaker for a wet peroxide oxidation using standard procedures (Masura et al. 2015) to digest any remaining organic material. The filter was rinsed and removed and examined under a microscope for any microplastics. The liquid had approximately 6 g sodium chloride per 20 mL of sample added to it and was transferred to a separatory funnel to separate out the inorganic material (Masura et al. 2015). The upper solution from the density separation was filtered through a 0.8 μm cellulose membrane filter which was placed into a covered petri dish (Masura et al. 2015). The filters were examined under a light microscope and microplastic particles (MPs) were counted and categorized into two main microplastic types—fibers and fragments (no beads were present). For 10% of the samples, we used a heated needle touched to all MPs in samples to positively confirm identification of microplastics. All of the MPs in these samples were positively identified as microplastics showing melting when touched to the hot needle. Because the hot needle method was only used on 10% of the samples, it is possible some nonplastic items may have been misidentified as plastic in other samples. Since almost all of our microplastics were colored fibers, the number of misidentified particles is likely low.

**Quantification of laboratory microplastic contamination**

We carried out environmental and digestion controls of samples to account for water, reagent, and atmospheric contamination. We exposed three separate petri dishes with two pieces of tape on each dish for 30 min (the amount of time a sample is exposed during digestion) under the fume hood; only two microplastic fibers were found in total. We filtered 10 L of the nanopore water used in the making of reagents; 15 microplastic fibers were found (1.5 fibers L$^{-1}$, and about 600 mL of reagent is used for each digestion).

We placed 100 mL of 1 mol L$^{-1}$ KOH and 50 mL of sodium dodecyl sulfate (0.5% w/v [ca. 5 g L$^{-1}$]) in a foil-covered beaker in the hood along with actual digestions. We then added 60 mL 30% hydrogen peroxide, and 60 mL of 0.05 mol L$^{-1}$ Fe(II) solution to the beaker and then filtered the solution through a 0.8 μm cellulose membrane filters. Microplastic contamination was 2.2 (± 0.75 SE) fibers per control; this level of contamination is at the lower end of the range found in McNeish et al. (2018), who examined fish in midwestern streams. Based on these controls, we corrected our determination of MPs in samples by removing two microplastics per sample.

**Data analysis**

All statistics and data manipulation were conducted using R, version 3.5.2 and RStudio, version 1.2.1356 with the tidyverse package, version 1.2.1. To test for differences in microplastic concentrations in largemouth bass vs. gizzard shad in Lakes Bloomington and Evergreen, we used a two-way ANOVA with lake and fish species as fixed factors using the R package car version 3.0-2 (Fox and Weisberg 2011). To test for differences in the relationship between fish microplastic concentrations and size between the lakes, we used an analysis of covariance testing using the R package car version 3.0-2 (Fox and Weisberg 2011). We used a log$_2$ transformation of length (cm) and number of microplastics for both fishes to meet assumptions of normality. To test for differences in microplastic concentrations in the gills and gut in gizzard shad in Lakes Bloomington and Evergreen, we used a three-way ANOVA with species, lake, and location of microplastic as fixed factors. We used a Bonferroni-corrected post F-test to compare all pairwise combinations using the R packages emmeans, version 1.3.3. For Lake Bloomington, we quantified shoreline development by calculating the density of houses along the 100 m length of shoreline where the fish were collected. We then regressed microplastics concentration in each fish species vs. this house density using a linear regression. We did not conduct this test for Evergreen Lake as it has no shoreline development.

![Fig. 1. Mean microplastic concentration (No. Fish$^{-1}$) in gizzard shad and largemouth bass in Bloomington and Evergreen. Letters indicate significant differences between all pairwise comparisons ($p < 0.05$). Error bars represent ± 1 SE.](image)
Results

We detected microplastics in 100% of the fish we sampled, with a total range of 1–49 No. Fish$^{-1}$. In gills, the concentration of MPs ranged from 1 to 30 No. Fish$^{-1}$ and in the gut the numbers ranged from 0 to 28 No. Fish$^{-1}$ (Supporting Information Table S1). Largemouth bass had significantly higher microplastic concentrations than gizzard shad (24.7/2.5 No. Fish$^{-1}$ compared to 5.2/0.4 No. Fish$^{-1}$; $F_{1,92} = 106$, $p < 0.0001$, Fig. 1). However, there were no differences between the lakes (Fig. 1).

Microplastic concentrations in gizzard shad declined significantly with size ($r^2 = 0.14$, $p = 0.001$, Fig. 2). There were no significant differences between lakes in the relationship between microplastic concentrations and gizzard shad length (Analysis of Covariance (ANCOVA), $F_{1,68} = 1.24$, $p = 0.27$, Fig. 2). The relationship between microplastic concentrations and largemouth bass size was significant ($r^2 = 0.17$, $p < 0.046$), but the relationship was highly leveraged by two points from within a small size range (Fig. 2b). The ANCOVA testing for differences between the two lakes for largemouth bass was not significant ($F_{1,20} = 0.31$, $p = 0.58$, Fig. 2b).

There was a significant interaction between fish and microplastics from either gill or gut ($F_{1,184} = 31.68$, $p < 0.0001$). For gizzard shad, there were significantly higher microplastic concentrations in the gills than in the gut (Fig. 3). In contrast, for largemouth bass, there were significantly higher microplastic concentrations in the guts than in the gills (Fig. 3). Fish microplastic concentrations were not related to housing density for gizzard shad (Fig. 4a) nor largemouth bass (Fig. 4b).

Discussion

We found high prevalence and abundance of microplastics in two fish species in these reservoirs. Microplastics were present in 100% of the fish. Comparably high prevalence of microplastics in freshwater fish has been found in several rivers in the midwestern U.S.A. (85%; McNeish et al. 2018), Canadian prairie creeks (73%; Campbell et al. 2017), an urbanized river in Texas (45%; Peters and Bratton 2016), as well as an urban river in South America (83%; Silva-Cavalcanti et al. 2017). These results are in contrast to studies in Europe, where...
only 9% of gudgeon in Belgian rivers had microplastics (Slootmaekers et al. 2019), 25% of gudgeon in French rivers (Sanchez et al. 2014), 25% of chub (Squalius cephalus) in a river near Paris (Collard et al. 2018), and 33% of roach in the River Thames, UK (Horton et al. 2018). It is unclear whether the wide range reported for the prevalence of microplastics is due to ecological (riverine vs. lacustrine), watershed land-use, or cultural differences. Fish microplastic concentrations were relatively high in our study (1–49 No. Fish$^{-1}$) compared to those reported in other freshwater research (e.g., up to 20 No. Fish$^{-1}$) in McNeish et al. 2018, which did not include predators).

We saw significant shifts in microplastic concentrations within gizzard shad depending upon size. This decline in microplastic concentrations with size could be due to ontogenetic diet shifts, as gizzard shad shift from zooplankton to detritus (Yako et al. 1996). This shift typically occurs around 30 mm in length but can be later if plankton populations are high (Yako et al. 1996), as would be the case in these hypereutrophic agricultural reservoirs. In this case, our results suggest the shift could be occurring at 90 mm in length (Fig. 2a). Ontogenic diet shifts have not been substantially explored in fishes but have been found to influence fish microplastic concentrations in estuaries (e.g., Ferreira et al. 2019).

Our results support the potential for feeding guild and trophic transfer to influence fish microplastic concentrations. Largemouth bass in our reservoirs would be primarily consuming smaller gizzard shad; largemouth bass had on average 3.2-fold higher concentrations of microplastics compared to gizzard shad, signifying the possibility of trophic transfer in these two reservoirs. Differences in fish microplastic concentrations across feeding guilds have been found in some marine studies, where omnivorous fish had microfiber concentrations an order of magnitude greater than herbivores and carnivores (Mizraji et al. 2017). However, in other cases, riverine (Andrade et al. 2019) and estuarine (Vendel et al. 2017) fish had no differences among functional groups. Whether or not feeding guild appears to impact fish microplastic concentrations may depend on prevalence of microplastic concentration among individuals. Differences among feeding guilds was indicated in cases when prevalence of microplastics was high (such as with 100% in our study and 99% in Mizraji et al. 2017). In contrast, when prevalence was low (e.g., 25% in Andrade et al. 2019 and 9% in Vendel et al. 2017), differences among functional groups were not identified. In ecosystems where there is more movement across habitats, such as within an estuary, variation in exposure and consumption may obscure the potential role of feeding guild on microplastic ingestion (Ferreira et al. 2019).

Microplastic concentrations differed between fish tissue types, potentially influenced by feeding guild and trophic level. Gizzard shad, a filter-feeding fish, had significantly higher microplastic concentrations in their gills relative to their gut (Fig. 3). In contrast, largemouth bass, a predatory species, had significantly higher microplastic concentrations in their gut relative to their gills (Fig. 3). For gizzard shad, we also found a significant negative relationship between microplastic concentrations in the gills and fish size for larger gizzard shad, which would be consistent with ontogenetic diet shifts. Compared to other studies, our fish microplastic concentrations were substantially greater (three to four times) than those found in guts and gills of marine fish (Su et al. 2019). For these marine species, microplastic concentrations were generally higher in the gut compared to the gills (Su et al. 2019). Laboratory experiments have also found differences in MP contamination across fish tissue types (e.g., gills, gut, liver, brain), which has been related to particle size (Lu et al. 2016) and length of exposure (Ding et al. 2018).

The fact that fish in both reservoirs had microplastics in their tissues regardless of shoreline development underscores the widespread, ubiquitous nature of microplastic pollution in freshwater ecosystems. WWTPs are thought to be key contributors to MP contamination in freshwater systems. However, no WWTP effluent sources are in the watershed of LakeEvergreen Lake or Lake Bloomington. Other sources of water similar to what is found in WWTPs are storm-water runoff from villages within each of the lake’s watersheds (Hudson for Evergreen Lake; Towanda for Lake Bloomington) and septic tank leakage, but these are still minor aspects of land use. Thus, our results suggest that the source of microplastics may be associated with agricultural activities, which dominate these watersheds. Agricultural watersheds have previously been associated with high prevalence of microplastics in fishes (Campbell et al. 2017; McNeish et al. 2018) and agricultural soils have the potential to be a source of microplastics (Nizzetto et al. 2016). Additionally, automotive tire wear, and atmospheric deposition may be nonpoint sources of microplastics (Gray et al. 2018). Our findings suggest that agricultural land use may also increase microplastic abundance, although more work is necessary to determine the sources, pathways, and fates. The combination of high plastic production, insufficient waste management, and the impervious physical nature of plastic (chemical inertness and slow biodegradation) has ultimately resulted in a large accumulation of plastic debris in aquatic ecosystems.

**Conclusion**

Compared to other studies of both marine and freshwater fishes, the gizzard shad and largemouth bass in these agricultural reservoirs had high prevalence and concentrations of microplastics. Although we identified significant differences in the amounts and location of microplastics between tissue types for each species, the small sample size of this study limited our ability to determine the role of ontogenetic diet shifts and trophic transfer of microplastics. The source of these high levels of microplastics may be linked to agricultural activity in the watershed and warrants further investigation. Surveying freshwater microplastic concentrations in
fishes in lakes and rivers across a wider range of watershed land use types is necessary to improve our understanding of which freshwater ecosystems are most at risk for high microplastics concentrations.

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