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Short Communication

Historic fish samples from the Southeast USA lack microplastics

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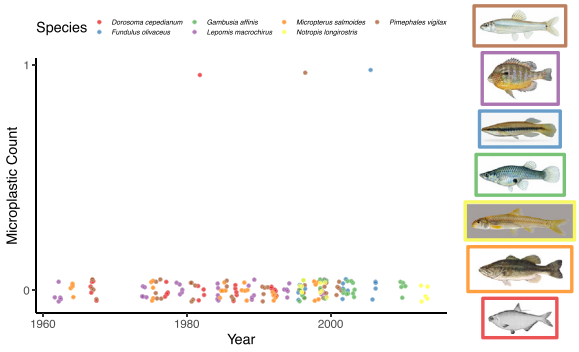
HIGHLIGHTS

- 185 historic fish samples were analyzed for microplastics.
- Seven species represented five different U.S. freshwater rivers.
- A total of three microplastic particles were found.
- Microplastics were likely uncommon in 20th century fish samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Most of earth's systems and the organisms that inhabit them are known to contain microplastics, which are well documented to have lethal and sublethal effects on living things. Due to their generally short timeframe and recent focus, contemporary studies of microplastics in fish are unable to inform past patterns of microplastic ingestion, and as such there is a knowledge gap regarding when microplastics began showing up in fishes. We examined $n = 185$ historical (museum) fish samples representing seven species from five freshwater systems across 51 years in order to look for microplastic samples over time. We found only three microplastic particles, two of which were in the more recent years of collection (1996 and 2006). Although our results are not conclusive toward understanding the true nature of microplastic occurrence over time in fishes, our findings present strong evidence that southeast U.S. stream fish likely did not ingest large numbers of microplastics during the 20th century.

1. Introduction

Dating back to 1950s, the plastics industry has been developing new plastic compounds and expanding production in an effort to create consumer convenience and demand (Clark et al., 2016), often for single-use plastic items. However, this convenience comes at a cost, as many plastics have a relatively short consumer lifespan and are discarded in garbage, recycling, or directly into the environment. The increasing volume of plastics in the environment, combined with long degradation time of the compounds that make up plastics, result in plastics persisting for long periods in many environments (Barnes et al., 2009; Li et al., 2018; Phillips and Bonner, 2015). Reports of plastics polluting marine ecosystems are common and date back to the early 1970s (Carpenter and Smith, 1972; Colton et al., 1974), although there has been a recent focus on freshwater systems and organismal (e.g., fish) ingestion studies now commonly reported (Cole et al., 2011; Eerkes-Medrano et al., 2015).

Microplastics are small plastic particles ≤5 mm in diameter (Moore, 2008; Neves et al., 2015), and similar in size to the natural food of several freshwater fish species. Upon entering aquatic ecosystems

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microplastics are often consumed by aquatic organisms; for example, directly through incorrect identification as small prey, or indirectly by consumption of another food item already containing microplastics. Such consumption can result in numerous negative and neutral effects (Foley et al., 2018). The primary types of negative effects include physical challenges and chemical exposures. Physical challenges occur when microplastic particles cause internal abrasions, blockages, and other harm (reviewed in Wright et al., 2013). Chemical exposures occur when plastic species themselves or the toxic substances adhered to the plastic are transferred to an organism (Batel et al., 2016). For example, studies have shown persistent organic pollutants (POPs) adhering to plastics, which may then be bioavailable to aquatic organisms that ingest the plastic (Jabeen et al., 2017; Moore, 2008; Rochman et al., 2013; Teuten et al., 2009).

Ingestion of microplastic particles was first observed in a study by Carpenter et al. (1972), which reported 8 out of 14 species of coastal marine fishes had ingested opaque plastic spherules. In the time since Carpenter et al. (1972) reported this, studies of microplastics in fishes has greatly increased (as reviewed in Foley et al., 2018; Wang et al., 2020); however, to our knowledge all studies examine contemporary fish samples and are unable to inform past patterns of microplastic ingestion. Although a rough temporal estimate of microplastics in fishes could be pieced together based on published literature, this approach would be limited to the dates (years) in which sampling was conducted, and very biased toward recent years, when the vast majority of studies have been conducted. This study was designed to explicitly deal with the effect of time related to microplastic ingestion by fishes. Through the control of sample species and location, we examined seven freshwater fish species dating back as far as the 1960s, in order to estimate historical microplastic loads in common freshwater fish species.

2. Methods

2.1. Fish sampling

Seven freshwater species of fish were chosen for this study based on their availability and abundance in the Louisiana State University (LSU) and Tulane Universities (TU) fish collections. All species were selected based on the criteria that 1) samples were available over multiple years (ranging from 10 to 37 years; Table 1), 2) samples were available at approximately 5-year intervals, 3) within a year ≥5 samples were available (both to increase our sample size and to ensure we were not destructively sampling all the samples from a given year and location, and 4) all samples for a given species were taken from the same location (or within 10 km). The species meeting the above criteria in LSU's collection were sampled from the late 1990s to the early 2010s and included, *Notropis longirostris* (longnose shiner), *Gambusia affinis* (mosquitofish), and *Fundulus olivaceus* (blackspotted topminnow). The species meeting the above criteria from TU's collections were sampled from the early 1960s to the early 2000s and included, *Dorosoma cepedianum* (American gizzard shad), *Lepomis macrochirus* (bluegill), *Micropterus salmoides* (largemouth bass), and *Pimephales vigilax* (bullhead minnow). Fish samples were collected from rivers and tributaries located in Alabama and Louisiana (Fig. 1), typically close to road crossings which permitted access to the habitat and is common for ichthyological collections.

2.2. Sample processing

Each individual fish was removed from the jar in which it was being preserved, assigned a sample ID, and measured for standard length (mm). Fish samples were originally preserved in 10% formalin for at least one week, then in two rinses of water for a few days, then into 50% ethanol, and permanently into 70% ethanol. Individual fish were dissected by removing their stomach and intestines. Once removed, stomachs and intestines were placed together in a scintillation vial and labeled with the fish's sample ID. Contents of the vial were then broken down using a method by Foekema et al. (2013). The vials were filled with a 10% potassium hydroxide (KOH) solution and sealed. After a minimum of 24 h (or until digestive tracts were completely liquified), contents of the sample were filtered through a 20 μm nylon net filter paper using a vacuum filtration system. Excess contents of the jar were rinsed using filter deionized (DI) water into the filtration system. Filter paper was placed directly into a petri dish, sealed, and labeled. The petri dishes were then placed in a drying oven set at 60 °C for 24 h.

The laboratory facility in which all the work was done took steps to minimize airborne contamination. Air filters were used in the lab, and lab personnel were only permitted to wear 100% cotton outer garments while doing any work. The 10% KOH solution was filtered using the same 20-μm filter paper before being put in the vial to digest the stomachs, as was the water used to rinse the vials or for any other purpose. Additionally, the whole process took place over the shortest time possible so that any unaccounted for air exposure was minimized. The laboratory facility in which the work was done has, for years, implemented best practices that have been used to process dozens of microplastics sampling events.

2.3. Polymer analysis

Individual plastic particles found within each fish sample were further analyzed using Fourier transform infrared (FTIR) spectroscopy. FTIR analysis characterizes microplastics at the molecular level using infrared radiation (Chen et al., 2020). Each filter paper was first examined visually under a dissecting microscope. If a putative plastic was visually identified it was then removed using stainless steel forceps and processed through the FTIR spectrometer. A ThermoScientific Nicolet iN10 spectrometer was used to identify the plastic particles. To determine polymer composition of plastic particles, spectra taken from each particle were compared to a library of polymer spectra from the OMNIC (ThermoFisher) software library. Particles were considered to be plastic if their spectrum had over a 80% match (Digka et al., 2018; Lefebvre et al., 2019) with a polymer spectrum from the pre-existing OMNIC spectral library.

3. Results

We sampled a total of $n = 185$ historical fish samples for microplastics (Table 1) and found a total of three microplastic particles

Table 1
Description of fish samples analyzed for microplastics. No refers to the species number in Fig. 1. SL is the standard length measure of the individuals.

No	Species	Collection	Location	Years	SL (mm)	*n*
1	*Dorosoma cepedianum*	Tulane	Red River, LA	1967–1992	24–80	25
2	*Lepomis macrochirus*	Tulane	Alabama River, AL	1962–1999	20–56	35
3	*Micropterus salmoides*	Tulane	Alabama River, AL	1964–1998	18–73	30
4	*Pimephales vigilax*	Tulane	Red River, LA	1967–2001	26–54	40
5	*Fundulus olivaceus*	LSU	Comite River, LA	1996–2006	29–56	15
6	*Gambusia affinis*	LSU	Atchafalaya basin, LA	1996–2010	14–36	25
7	*Notropis longirostris*	LSU	Tickfaw River, LA	1999–2013	30–51	15

Fig. 1. Map of approximate sampling locations for species in this study. The numbers refer to the species collections and can be referenced in Table 1.

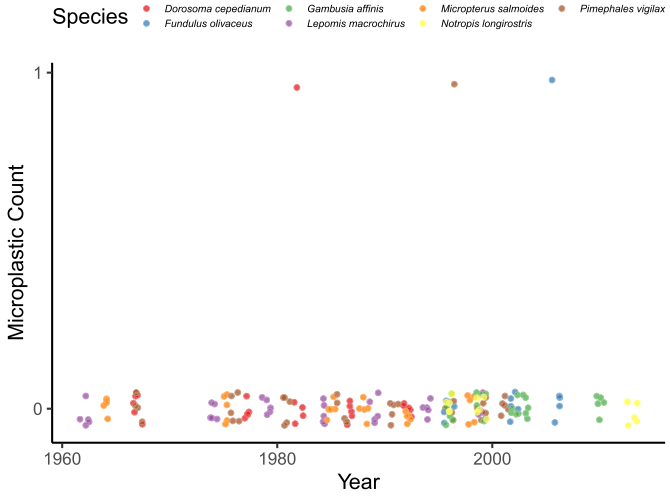

Fig. 2. Counts of microplastics for $n = 185$ fish samples taken from 1962 to 2013. Each dot represents a single fish sample, all of which contained either 0 or 1 microplastic. Dot colors correspond to fish species and dots are jittered for visual purposes.

(Fig. 2). Each of the three microplastics was found in a different species of fish sampled in Louisiana. Although one of the three microplastic observations came from a fish collected in 1982, the other two microplastics observations came in 1996 and 2006. All three of the microplastic particles were fibers, one black, and two blue in color, and they ranged between 0.5 mm to 4 mm. One of the microplastic particles was HD Polyethylene and two were polypropylene, and all plastic species were matched to >80%. Although we were prepared to use statistical models to draw inferences on our results, the extreme rarity of positive microplastic observations yielded a data set that was not suited for most statistical models and whose results did not require the inferences provided by statistical approaches.

4. Discussion

Dozens of microplastics studies are published every year, and despite a potential publication bias for positive results, microplastics are commonly reported in fish species that span habitats from marine to freshwater, flowing to still waters, and urban to rural settings. And while it may be assumed that fish sampled from a time period with lower microplastic loads in the environment, this study is among the first to support the claim that microplastics in fishes is a relatively recent phenomenon. In fact, several studies have reported microplastics in the

same species we investigated. Recent work by Hurt et al. (2020) reported that for both *Dorosoma cepedianum* and *Micropterus salmoides*, 100% of all samples had microplastics despite local land development patterns. Peters and Bratton (2016) looked at sunfish across an urban gradient and found that 45% (144 of 318) of *Lepomis macrochirus* contained microplastics in their stomachs. A large multi-species study by Phillips and Bonner (2015) reported microplastics in samples of *Pimephales vigilax* ($n = 3$) and *Gambusia affinis* ($n = 5$). Although sample sizes were low due to the nature of the study, their work confirms that contemporary samples of these species consume microplastics in environments where microplastics are present. Although we are not aware of any studies that have examined *Fundulus olivaceus* or *Notropis longirostris* for microplastics, this may be attributable to the perceived lack of importance of the species and not because they do not consume microplastics. In fact, Phillips and Bonner (2015) reported four *Notropis* congeners and one *Fundulus* congener, all five of which were reported to ingest microplastics. Despite our historical samples not producing many microplastics, it is clear that the species we studied readily consume microplastics, suggesting that historic microplastic loads were either non-existent or too low for fish to consume them.

Negative results are part of science (Weintraub, 2016) and despite potential publication biases for positive results, there are many instances where the lack of a presence or effect can be very informative to a study system. However, it remains important to critically consider study factors that could also lead to the lack of an effect. To this end, we have considered several aspects of the study design that could affect the results, but also include reasons why they might not have an effect. First, we don't know microplastic loads (historic or current) in the waterbodies from which our study fish came. This is a limitation, but one experienced by numerous similar studies of fish. We cannot know the historical microplastic load in the rivers in our study; however, we can know that US rivers are well documented in carrying microplastics (Scircle et al., 2020; Toner, 2020), and that the rivers we studied run through urban and developed areas, which only increases the likelihood of microplastic loads. Second, fish size is a documented factor found to effect microplastic ingestion (e.g., Su et al., 2019). Fish size in our study was limited to small fishes (for logistical and availability reasons); however, studies have found microplastics more prevalent in lower trophic organisms (Walkinshaw et al., 2020) and smaller-sized fishes to have higher microplastic loads than larger fish of the same species (Hurt et al., 2020). This could be for a number of reasons, but possibly due to the planktivorous nature of small fish that might directly consume microplastic particles (compared to larger, piscivorous fish that would only indirectly consume microplastics). Although our study was not able to explicitly account for size, the fact that all our samples were approximately from the same range does afford us some control over the potential effect of size. A third consideration was that we limited our filtration to detect only microplastic particles greater than 20 μm. In doing so, we may have missed some number of smaller microplastics; however, detection and identification of microplastics smaller than 20 μm is problematic (given our equipment), suggesting that even if we did use a smaller filter, we would not have much confidence in what we detected. A final consideration was the effect chemical preservative on microplastic particles. Any microplastics we sampled were necessarily in chemical solutions for years to decades. However, many microplastics are not affected by chemical preservative as degradation of plastic will be minimum in either formalin or alcohol. Specifically, we detected polyethylene and polypropylene, both of which are resistant to ethanol and formalin (Lusher et al., 2017). Most of consumer plastics are long chain polymers, and therefore have almost no reaction with formalin and very limited solubility with alcohol. Further, polymer spectra should be mostly unaltered in FTIR, and in fact we did see relatively high percent matches that improve our confidence in polymer identification.

Microplastics in physical environments and the organisms that inhabit those environments are a relatively new ecological phenomenon, yet little attention has been paid to the history of the problem. A rapidly growing literature has emerged to document the current state of the problem, but almost no studies have sought to go back in time and investigate and confirm the lack of microplastics in historical samples (with the exception of Modica et al., 2020). Although we were limited by the collections made by individuals up to 50 years ago, these same collections made this study possible. And by controlling for fish species, size, and location, we were able to isolate the effect of time and provide strong evidence for a clear lack of microplastics in multiple fish species in the 20th century.

CRediT authorship contribution statement

Kerrin Toner: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Stephen R. Midway:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare no conflict of interest.

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