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A fish tale: a century of museum specimens reveal increasing microplastic concentrations in freshwater fish

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Abstract. Plastic is pervasive in modern economies and ecosystems. Freshwater fish ingest microplastics (i.e., particles <5 mm), but no studies have examined historical patterns of their microplastic consumption. Measuring the patterns of microplastic pollution in the past is critical for predicting future trends and for understanding the relationship between plastics in fish and the environment. We measured microplastics in digestive tissues of specimens collected from the years 1900–2017 and preserved in museum collections. We collected new fish specimens in 2018, along with water and sediment samples. We selected four species: *Micropterus salmoides* (largemouth bass), *Notropis stramineus* (sand shiner), *Ictalurus punctatus* (channel catfish), and *Neogobius melanostomus* (round goby) because each was well represented in museum collections, are locally abundant, and collected from urban habitats. For each individual, we dissected the digestive tissue from esophagus to anus, subjected tissue to peroxide oxidation, examined particles under a dissecting microscope, and used Raman spectroscopy to characterize the particles' chemical composition. No microplastics were detected in any fish prior to 1950. From mid-century to 2018, microplastic concentrations showed a significant increase when data from all fish were considered together. All detected particles were fibers, and represented plastic polymers (e.g., polyester) along with mixtures of natural and synthetic textiles. For the specimens collected in 2018, microplastics in fish and sediment showed similar patterns across study sites, while water column microplastics showed no differences among locations. Overall, plastic pollution in common freshwater fish species is increasing and pervasive across individuals and species, and is likely related to changes in environmental concentrations. Museum specimens are an overlooked source for assessing historical patterns of microplastic pollution, and for predicting future trends in freshwater fish, thereby helping to sustain the health of commercial and recreational fisheries worldwide.

Key words: *freshwater; long-term study; plastic pollution; time series.*

Introduction

Plastic production has been underway for over 100 yr. Advancements in the chemical engineering of plastic polymers began in the early 20th century (e.g., Bakelite), and the production was industrialized in the 1950s (Jambeck et al. 2015, Geyer et al. 2017). Mass production of plastic facilitated a shift from reusable materials to single-use products in the global economy (Worm et al. 2017). The most commonly produced plastic polymers are polyethylene (PE), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyvinyl chloride (PVC), polyurethane (PU), polyethylene terephthalate (PET), and polystyrene (Rochman et al. 2019). The wide diversity of plastic polymers are manufactured for different uses. For example, PET is commonly used for water bottles, and LDPE is abundant in single-use plastic bags (Rochman et al. 2019). Given acceleration of plastic production since the mid-1900s, the generation of plastic waste has also increased. The amount of plastic waste created from 1950 to 2015 was approximately 6,300 million metric tons (Mt), which is expected to increase to 34,000 Mt by 2050. Of the 6,300 Mt of plastic waste produced from 1950 to 2015, about 78% (4,900 Mt) was discarded in landfills and the environment (Geyer et al. 2017).

Plastic pollution is pervasive to all parts of the world and is found in a diversity of forms (Rochman et al. 2019). Plastic litter is ubiquitous, having been documented in oceans (Eriksen et al. 2014, Gewert et al. 2017), freshwaters (Rech et al. 2015, McCormick and

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Hoellein 2016), terrestrial environments (Rillig 2012), the atmosphere (Dris et al. 2018), and urban and remote locations (Barnes et al. 2018, Dris et al. 2018). Plastic litter in the environment is often classified by size class, including macroplastics (>200 mm), mesoplastics (5–200 mm), and microplastics (<5 mm) (Eriksen et al. 2014), however, size class delineations are still under discussion in the literature (Hartmann et al. 2019). Major point sources of plastic to aquatic environments include wastewater treatment plant (WWTP) effluent, street runoff, and dumping (Jambeck et al. 2015, McCormick et al. 2016, Horton and Dixon 2018). In addition, plastic can fragment into smaller pieces in situ via abiotic and biotic degradation (Gewert et al. 2015), and is moved by water and atmospheric currents (Dris et al. 2018, Hoellein et al. 2019).

Plastic debris, especially in the form of microplastics, are found in consumer goods such as seafood, sparking concerns about the effects of microplastic ingestion on aquatic organisms and humans (Rochman 2015, Wright and Kelly 2017). Microplastics are common in gastrointestinal tracts of wild and farm-raised fish, as well as aquaculture mussels (Wright et al. 2013). Ingestion of microplastics can cause hepatic stress and loss of digestive function in fish (Rochman 2013, Pedà et al. 2016), and histological and inflammatory responses in mussels (Von Moos et al. 2012). However, microplastic exposure may not have an effect on the physiology and overall health of some aquatic organisms (Critchell and Hoogenboom 2018, Lo and Chan 2018). More research is needed to understand the physiological effects of microplastics, including studies that examine a broad variety of organisms and compares the rates of microplastic ingestion relative to microplastic concentrations in the environment (Foley et al. 2018, Lo and Chan 2018).

Research on the physiological outcomes of microplastic exposure has been underway for approximately 10–15 yr, but microplastics have most likely been an increasing pollutant in aquatic ecosystems since the mid-1900s. Therefore, organisms have probably been consuming microplastics for many decades, but the magnitude of exposure over that time is not well understood. A few studies have examined historical specimens for microplastics, but have not shown a temporal trend in concentration. For example, Courtene-Jones et al. (2019) found that microplastic concentrations in deep-sea invertebrates did not increase significantly from the 1970s to 2015. Beer et al. (2018) studied marine fish collected from 1980s to 2010s and found there were no significant differences in microplastic concentration over time. However, no previous studies have examined historical patterns of microplastic ingestion in freshwater fish, or included specimens from decades before and after the industrialization of plastic. It is important to understand how the patterns of microplastic concentration has changed in freshwater fish because they are critical for healthy food webs, are an important food source for commercial and recreational fisheries at a global scale (McIntyre et al. 2016), and measuring historical patterns will be critical for predicting future trends.

For this study, we measured microplastic concentrations in four common freshwater fish species collected from 1900 to 2018 in urban aquatic ecosystems in Chicago, Illinois, USA. We expected microplastic concentrations in fishes to increase after 1950, following the industrialization of plastic production. We expected similar patterns among all species, and that the diversity of polymer types would increase over time. Finally, in 2018 we collected water and sediment concurrent with fish specimens. We predicted that microplastics in sediment and water would be positively correlated with the amount in fish.

Methods

Historical specimen selection

We selected study organisms from historical fish specimens collected during 1900–2017 and archived at the Field Museum of Natural History in Chicago, Illinois, USA, with additional specimens from the Illinois Natural History Survey (INHS) and University of Tennessee (UT; Appendix S1: Table S1). We first searched for fish species from the region that met the following criteria: (1) at least five individual specimens available for dissection for each species from most decades in the record (1900–2010) and (2) all specimens of the same species were collected in the same or closely adjacent water bodies, situated in Chicago's urban and suburban areas. Relatively few species met these selection filters. In fact, no species had specimens from every decade since 1900, so we selected for those with the oldest and most complete record over the entire study period. Our study species were sand shiner (*Notropis stramineus*) collected at Hickory Creek (n = 23), largemouth bass (*Micropterus salmoides*) collected at the confluence of Brewster Creek and the Fox River (n = 34), and channel catfish (*Ictalurus punctatus*) collected at two locations in the Illinois River (n = 18; Table 1). In addition, we added round goby (*Neogobius melanostomus*) collected from Lake Michigan, at Calumet Park in Chicago (n = 15). Round gobies are invasive in Lake Michigan, and first appeared in the local collections in 1994. We included this fish because it is well represented in museum collections and is an ecologically significant local taxon. The study species vary in diet and habitat. Bass, catfish, and gobies are predators (trophic level 3.3–4.4), where gobies are primarily benthic invertivores, catfish are piscivores and invertivores, and adult bass are largely piscivores. Sand shiners are classified as omnivores (trophic level 2.2; Table 1; Froese and Pauly 2017). Finally, we acknowledge the data set is unbalanced among species, sites, and time periods. This design represents the best achievable data given the significant restrictions in specimen collection regimes and availability for dissection across >100 yr long duration of the study.

Table 1. Summary of information about study specimens and locations.

Fish species	Common name	Trophic level	Mass range (g)	Number of fish	Water body	Latitude, Longitude	Decades
Micropterus salmoides	Largemouth bass	3.8–4.4	0.3–136.0	40	Brewster Creek	41°58'17.0" N 88°16'44.5" W	1900, 1940,1950, 1960, 1980, 1990, 2000, 2010, 2018
					Fox River	41°58'32.6" N 88°17'32.4" W	1900, 1940, 1950, 1960, 1980, 1990, 2000, 2010, 2018
Notropis stramineus	Sand shiner	2.4	0.1–10.1	29	Hickory Creek	41°30'58.1" N 87°52'37.4" W	1900, 1950, 1960, 1970, 1980, 2000, 2018
Ictalurus punctatus	Channel catfish	3.4–4.2	0.8–128.4	18	Illinois River	40°17'51.2" N 90°04'03.5" W	1900, 1920, 1950, 1960, 1970, 1990, 2000, 2010
					Illinois River	39°50'00.5" N 90°33'59.0" W	1900, 1920, 1950, 1960, 1970, 1990, 2000, 2010
Neogobius melanostomus	Round goby	3.3	0.1–39.7	32	Calumet Pike	41°43'19.9" N 87°31'28.3" W	1990, 2000, 2010, 2018

Contemporary fish, water, and sediment collection

In 2018, we collected contemporary samples for microplastics that matched the historical collection sites for focal species: Brewster Creek (Brewster), Hickory Creek (Hickory), and the shoreline of Lake Michigan at Calumet Park (Calumet; Table 1). We did not collect channel catfish in 2018 because the museum samples included catfish from the 2010 decade.

We collected fish, water, and sediment samples at all three sites in late summer and early fall 2018. Sand shiner ($n = 6$) and largemouth bass ($n = 6$) were collected from Hickory and Brewster Creeks, respectively, using wading seine nets (McNeish et al. 2018). Round gobies ($n = 17$) were collected from Calumet Park using fishing rods and earthworms (*Lumbricus terrestris*). All collected fish were identified on site, and the study species were euthanized (MS-222; Tricaine-S; 0.25 g/L) and preserved in 70% ethanol (McNeish et al. 2018). Protocols were in accordance with ethical guidelines and regulations and approved by Loyola University Chicago's Institutional Animal Care and Use Committee.

Water and sediment samples were collected from Hickory, Brewster, and Calumet, upstream from where the fish were collected. Hickory and Brewster Creeks were wadeable streams (depth = approximately 0.3 m), which made water and sediment collection relatively straightforward. Water column samples from Hickory and Brewster were collected directly in 2-L glass bottles ($n = 2$ bottles per site) that were acid washed and thoroughly rinsed with denionized (DI) water that we filtered through 0.363-μm mesh in the laboratory (Barrows et al. 2018, McNeish et al. 2018). While standing downstream of the sampling site, bottles were uncapped, rinsed three times with sample water, and filled, while completely submerged under water, to avoid air bubbles and atmospheric microplastic contamination. Sediment samples, consisting of mainly fine organic material were collected ($n = 2$ containers per site) from the left and right side of the streambed using 100-mL specimen containers rinsed with filtered DI water before use (Hoellein et al. 2017). Specimen containers were also uncapped and recapped under water. The collection site at Calumet Park was a sea wall adjacent to Lake Michigan (depth = approximately 3.3 m) and required additional equipment for sampling. Water samples ($n = 2$ bottles) from Calumet were collected using a carefully cleaned 2.2-L horizontal Van Dorn Sampler (Wildco, Alpha, Yulee, Florid, USA), and stored in 2-L glass bottles until analysis. We used a standard $15 \times 15 \times 15$ cm Ekman Grab (Wildco, Model 196 B12) to collect sediment samples ($n = 2$ containers). The majority of sediment from Calumet Park consisted of gravel and fine particles. All materials in the Ekman Grab were transferred into 160-mL sterile specimen containers. To prevent sample loss, we rinsed the inside of the bottom of the Ekman Grab with filtered DI water into the 160-mL pre-cleaned specimen containers. Immediately after collection, all samples were taken to the laboratory for storage until microplastic processing.

Sample preparation and microplastic quantification

Museum specimens and fish collected in 2018 were processed in an identical fashion for microplastic analyses (McNeish et al. 2018). We recorded maximum total fish length (cm), as measured from the mouth to the end of the caudal fin, and fish wet mass (g) for each individual (Lusher et al. 2013). We rinsed tools, containers, work surfaces, and the external fish surfaces with filtered DI water, before and after each dissection. A ventral cut was made from the urogenital opening to the esophagus to expose and remove the entire digestive tract. Digestive tissues were placed into acid-washed and DI-rinsed glass jars, and covered with foil.

Sediment samples from specimen containers (50 mL of sample per container) were transferred onto a stacked sieves system (0.3-, 1.0-, and 4.75-mm sieves) using clean forceps and filtered DI water. Large particles from the top and middle sieves were rinsed with filtered DI water three times to make sure any microplastics adhering to the surface would be accounted for. We discarded sediment particles (i.e., small rocks) that remained in middle and top sieve after rinsing. Material in the bottom sieve

was collected and transferred into acid-washed glass jars using pre-cleaned forceps, and rinsing contents into jars with filtered DI water. Glass jars were immediately covered with foil.

All fish digestive tissue and sediment samples followed the same procedure of drying, digestion, and filtration. Sediment and fish tissues were dried at 75°C for 24–48 h (1320 Economy Oven, VWR, Radnor, Pennsylvania, USA). Oxidation of organic material occurred with the addition of 20 mL of iron sulfate catalyst (0.05 M Fe (II)) and 20 mL of 30% H_2O_2 at 70°C. To agitate the contents for digestion, a stir-bar (rinsed with filtered DI water) was placed in the jar. Throughout each digestion, we added additional 30% H_2O_2 in increments of 20 mL until the solution was clear of particulates and the reaction no longer occurred, or the solution reached a maximum volume of 200 mL (i.e., sediment samples). Wet peroxide oxidation is effective in removing organic material and does not affect most microplastics (Lusher et al. 2017, Munno et al. 2018). Digested solutions were vacuum filtered onto gridded filters (0.45 μm pore size; Whatman, Pittsburgh, Pennsylvania, USA). Finally, we agitated river water samples to suspend particles, and filtered 1 L onto gridded filters, with no digestion or sieving (McNeish et al. 2018). All filters were immediately placed into 20-mL aluminum weighing dishes, covered with foil, and dried at 30°C for 4–24 h (Thermo Fisher Scientific Incubator, Marietta, Ohio, USA). We did not observe any differences in the digestion reaction for museum specimens (first preserved in formalin, then transferred to ethanol), relative to the 2018 fish tissues (preserved in ethanol).

Microplastic particles on all filters were visually identified using a dissecting microscope (25–30× magnification; Model ASZ30L3, Bausch & Lomb, Rochester, New York, USA). Common microplastic categories by morphotype included fragment, fiber, pellet, bead, and foam (Lusher et al. 2017). Samples were assessed by two independent researchers. If counts did not agree, a third independent assessment was completed. The only microplastic morphotype in all fish, water, and sediment samples were fibers.

Laboratory controls

To account for laboratory microplastic contamination relevant to fish and sediment samples, we performed digestion and filter controls ($n = 12$). Empty, acid-washed glass jars underwent the same wet peroxide oxidation procedures as other samples. We placed the liquid through a gridded filter, rinsed the filtration apparatus with filtered DI water, and examined the filter under a dissecting microscope (McNeish et al. 2018). Microplastics on control filters were identified and processed as described in *Sample preparation and microplastic quantification*. Microplastic contamination (mean ± SE; number of particles/filter) was 2.75 ± 0.7 fibers for digestion and filter controls, similar to previous work (McCormick et al. 2016, Hoellein et al. 2017, McNeish et al. 2018). We did not collect field blanks, as previous projects in the region found no contamination from opening and closing sample containers in the field (McCormick et al. 2016, Hoellein et al. 2017, McNeish et al. 2018). For conservative estimates, we corrected the number of fibers found on each fish and sediment filter by removing a count of three fibers from the total, independent of color. The water column samples required different control samples, as these were not digested, but rather directly filtered and then counted. For the controls, we placed a filter on the filtration system, rinsed it with filtered DI water (the same amount we would use if a sample was present), and then placed the filter in a weigh boat, covered with it foil, and enumerated particles as described above. Our average contamination from this process was 0.85 particles/filter ($n = 9$), so we subtracted 1 particle/filter from the in situ water samples.

Particle chemical characterization

We selected a subsample of fibers to measure length and chemical composition. For fish, water, and sediment samples, we randomly selected and removed one to four fibers from each sample filter where the total fiber count was not equal to zero after microplastic count correction (based on laboratory controls). In addition, we randomly selected one to three fibers from control filters. The fibers were removed from the filters using pre-cleaned forceps and transferred onto a clear, double-sided tape. Using a permanent marker, we drew a circle around each fiber, and the sample ID number. The tape was placed in clean glass petri dishes (Brookson et al. 2019). A total of 479 fibers were counted for the entire project, and we randomly collected 269 (56%). Using an ocular micrometer, we measured the length (mm) of all 269 selected fibers. From these, we selected 96 fibers spanning dates and sample types for polymer analysis (20% of all particles).

Particles were analyzed using Raman spectroscopy (Horiba Raman XploRA PLUS confocal microscope, Piscataway, New Jersey, USA) operating with LabSpec6 software (version 6.5.1.24, Horiba, Piscataway, New Jersey, USA) and equipped with a charge coupled device detector (−60°C, 1024 × 256 pixels). Spectra were acquired a 100× LWD objective (NA = 0.8) resulting in laser powers of 11.2 mW (532 nm) and 20.2 mW (785 nm) at 100% filter. Spectral resolution ranged from 1.3 cm^{-1} (785 nm excitation laser, 600 grooves/mm) to 3.3 cm^{-1} (532 nm excitation laser, 1,200 grooves/mm). Various parameters for spectral acquisition (e.g., hole diameter, slit width, filter, acquisition time, delay and number of accumulations) were selected and adjusted based on recommendations from the application-based library (Munno et al. 2020). Baselines of the resulting spectra were corrected manually in LabSpec6, and additional automatic correction may have been applied by the Bio-Rad KnowItAll (Hercules, California, USA)

and ID expert spectral matching software (baseline, vertical clipping, intensity distortion, horizontal offset, vertical offset, Raman intensity distortion). Acquired spectra were matched using the KnowItAll and ID Expert software to reference spectra from the KnowItAll Raman Spectral Library and the Spectral Library of Plastic Particles (SLoPP and SLoPP-E; Munno et al. 2020). Peaks that corresponded to various functional groups in the spectra were compared to peaks in the reference spectra and assessed based on position on the Raman shift and intensity of the peaks, allowing for the identification of polymer types. Particles where polymer types were not detected, but dyes or resins were detected, were categorized as anthropogenic materials.

Historical patterns in urbanization, plastic production, and pollution

We provide historical context for the pattern of fish microplastic in this study by generating a composite graph that compiled our results (mean ± SE; number of particles·fish^{-1}·decade^{-1}) directly with data on regional population growth, global plastic production, and microplastic accumulation in a coastal environment over the same time period. Historical patterns for human population growth of the Chicago metropolitan region (as "combined statistical area," which includes the sampling sites), from 1900 to 2010 (the most recent census) were obtained from the US Census Bureau (data *available online*).[6] Geyer et al. (2017) published values for global plastic production rates from 1950 to 2015 (Appendix S1: Table S1 in that study). Brandon et al. (2019) documented an exponential increase in accumulation rates of marine microplastics from 1945 to 2009 in coastal California, summarized with an exponential equation ($y = 1.385 \times 10^{-40} e^{0.0475x}$), which we also plotted on our composite figure.

Data analyses

Generalized linear models (GLM) were conducted to determine if microplastic abundance (number of microplastic particles/yr) was impacted by time (year sampled), fish mass (g), and fish length (cm) similar to statistical methods in Hall et al. (2018) and Nix et al. (2018). Due to unequal sample size of fish across years sampled, the number of fish sampled in each year (fish count) was also included in GLMs. Our data was zero-inflated, as historical fish did not contain microplastics in digestive tissues prior to mid-century, as such, these data were not easily normalized with transformations. Data from three fish species (bass, catfish, and shiner) spanned from 1900 to 2018 with data from round goby included from 1994 to 2018. Therefore, GLM analyses were conducted with microplastic abundance data that included all fish species (pooled data set) and on a second data set that excluded the round goby data to determine if the addition of the round goby data impacted temporal patterns for the most recent years sampled. We identified the most appropriate statistical distribution (Poisson, Gaussian, negative binomial [NB], zero-inflated Poisson [ZIP], and zero-inflated negative binomial [ZINB]) for microplastic abundance for both data sets. The best statistical distribution was negative binomial for both data sets and was determined by using model selection (model.sel (), MuMIn package; Barton 2020) and Akaike's information criterion corrected for sample size (AIC_c) for each distribution (Appendix S1: Table S2). A series of GLMs (glm.nb() max. iteration = 50, MASS package; Venables and Ripley 2002) were conducted for both data sets with a combination of time, fish mass, fish length, and fish count. Models were ranked based on AIC_c and model weights (w_i) to identify the best model for both data sets and 95% confidence intervals (confint(), stats package; R Core Team 2019) were calculated for the best fitting model. Variables were checked for multicollinearity (vif(), car package; Fox and Weisberg 2019) for top models and all model variables had a VIF < 5 and were considered not collinear. Models were considered competing if the models were within an AIC_c difference (ΔAIC_c) of 2 of the top-performing model.

Similar statistical analyses were conducted at the fish species level to determine if time, fish mass, fish length, and fish count explained microplastic abundance patterns observed for each species. Largemouth bass (*Micropterus salmoides*) was the only species with a large enough sample size for GLMs to converge (n = 12 yr), as catfish, round goby, and sand shiner data sets failed to converge due to low samples sizes (n = 9 yr, n = 7 yr, and n = 9 yr, respectively). There were three competing statistical distributions (Appendix S1: Table S2). The bass data set was analyzed using a negative binomial distribution (the second ranked distribution) due to failed model convergence using the zero-inflated negative binomial distribution (top ranked distribution). A series of GLMs (glm.nb(), maximum iteration = 100, MASS package]) were conducted with the bass data set with a combination of time, fish mass, fish length, and fish count using identical statistical methods as presented for pooled and goby excluded data sets above. All GLM analyses were conducted in R version 3.6.1.

For fish, sediment, and water samples collected in 2018, we conducted several additional analyses. We used a one-way ANOVA to compare the microplastic concentration among the three sites for the water, sediment, and fish. Following a significant ANOVA, we used Tukey's multiple comparisons test to detect differences among sites. A two-way ANOVA was used to compare fiber length (mm) among the three sites collected in 2018 with sample type (i.e., fish, water, or sediment) and site as main effects.

For two analyses we compared proportions (i.e., polymer type and particle size), so we used a Chi-square test

[6] https://www.census.gov/programs-surveys/metro-micro/data.html

of independence after converting data to relative abundance. Polymer type and particle size distributions were compared over time and among sample types. Both metrics were measured on a subset of particles (20–56%) and data were grouped by decade to examine temporal trends. We included results for controls in each analysis to determine if patterns in the environmental samples were distinct. Finally, we used linear regression to determine if polymer richness (i.e. the number of materials detected) and diversity (i.e., Shannon-Weiner index), changed among dates. For all regressions and ANOVA, we checked if data sets met the normality and homoscedasticity assumptions of ANOVA and regression using K-S Lilliefor's test and Levene's test, respectively. No transformations were needed. ANOVA and regression statistics were completed using SYSTAT (SYSTAT, Chicago, Illinois, USA), and chi square analyses (chisq.test(), stats package; R Core Team 2019) were conducted in R version 3.6.1.

Results

Microplastic concentration in all fish

A total of 119 fish, across four species, collected from the years 1900–2018 were examined (Table 1). We observed no microplastics in the digestive tracts of fish prior to the 1950s (i.e., only contamination; Fig. 1). Beginning in the 1950s, sand shiner and largemouth bass showed mean (±SD) microplastic concentration of 0.20 ± 0.45 and 1.40 ± 1.67 particles/fish, respectively. The first observation of microplastics in channel catfish was in samples collected in the 1960s with a mean of 0.40 ± 0.55 particles/fish. Round gobies invaded the Great Lakes during the 1990s, and the mean microplastic concentration of round goby from the 1990s was 1.20 ± 1.79 particles/fish. Contemporary fish samples collected in 2018 showed mean microplastic concentrations for sand shiner, largemouth bass, and round goby were 5.17 ± 2.40, 2.50 ± 1.87, and 2.06 ± 2.44 microplastic particles/fish, respectively (Fig. 1).

Generalized linear models that contained time and fish count were the best models for pooled data sets with and without round goby, with 54.6% and 87.6% of the model weight explained for pooled and round goby excluded models, respectively (Table 2). However, time + fish length was a competing model for the pooled data set (Table 2). These models revealed that time was a significant positive coefficient for both data sets (Table 3), indicating that microplastic abundance in fish increased through time independent of the number of fish sampled (fish count). In addition, time was the most important factor explaining microplastic abundance across all models as the sum of model weights with the time variable was 99.93% and 99.94% for the pooled and round goby excluded data sets, respectively (Table 2). Models with only fish mass, length, and count variables were found to have minimally contribute to model weight (Table 2). For bass alone, microplastic abundance patterns were best explained by time + fish length with 91.73% of the model weight explained by these variables (Table 2). Time was a significant positive coefficient (Table 3), suggesting the microplastic abundance in bass increased through time independent of fish size. Time was the most important variable explaining microplastic abundance in bass with the sum of model weights that included time was 97.56% (Table 2).

Microplastics comparison between fish and environmental samples

Fish, water, and sediment samples were collected in 2018 at Hickory, Brewster, and Calumet sites. Fish collected from Hickory Creek had significantly higher mean (±SE) microplastic concentration (5.17 ± 0.98 particles/fish), than fish from Calumet (2.06 ± 0.52 microplastic particles/fish), while fish in Brewster Creek were intermediate (ANOVA, $F = 3.624$, df = 2, 26,

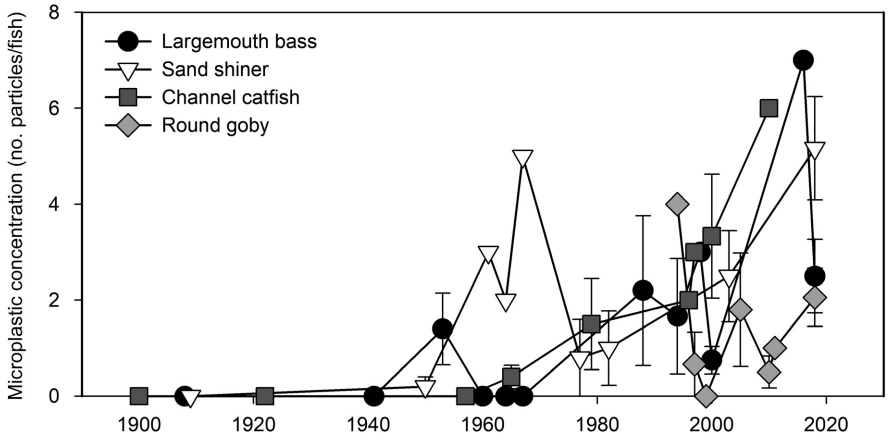

Fig. 1. Microplastic concentration (mean ± SE) as number of particles per individual by year from 1900 to 2018 for each fish species.

TABLE 2. Model selection results evaluating fish microplastic abundance for three permutations of the data set: all fish species pooled, the pooled data set with the round goby (*Neogobius melanostomus*) excluded, and for largemouth bass (*Micropterus salmoides*) alone.

Model	df	LL	AIC_c	ΔAIC_c	w_i
Pooled					
Time + Count	4	−62.38	134.42	0	0.5457
Time + Length	4	−62.64	134.94	0.52	0.4216
Time + Mass	4	−65.28	140.23	5.81	0.0299
Time	3	−69.30	145.57	11.15	0.0021
Length	3	−71.01	148.97	14.55	0.0004
Count	3	−71.36	149.68	15.25	0.0003
Mass	3	−72.56	152.08	17.66	<0.0001
Null	2	−81.01	166.48	32.06	<0.0001
Goby excluded					
Time + Count	4	−56.95	123.57	0	0.8760
Time + Length	4	−58.92	127.50	3.94	0.1224
Time	3	−65.50	137.97	14.40	0.0007
Time + Mass	4	−64.83	139.34	15.77	0.0003
Count	3	−66.23	139.42	15.86	0.0003
Length	3	−66.63	140.21	16.65	0.0002
Mass	3	−71.74	150.44	26.87	<0.0001
Null	2	−73.04	150.53	26.97	<0.0001
Bass alone					
Time + Length	4	−20.82	55.35	0	0.9173
Time + Count	4	−24.03	61.77	6.42	0.0371
Time	3	−27.08	63.16	7.81	0.0185
Null	2	−29.44	64.22	8.87	0.0109
Length	3	−27.98	64.96	9.61	0.0075
Count	3	−28.70	66.40	11.05	0.0037
Time + Mass	4	−26.66	67.03	11.68	0.0027
Mass	3	−29.14	67.28	11.92	0.0024

Notes: AIC_c is Akaike's information criterion corrected for sample sizes; ΔAIC_c is the difference from the best model; w_i is the AIC_c weight.

$P = 0.041$; Fig. 2a). Similarly, sediment collected from Hickory Creek had significantly higher microplastic concentration (830 ± 150 microplastic particles/L), compared to Brewster (330 ± 170 microplastic particles/L) and Calumet (307 ± 56 microplastic particles/L; ANOVA, $F = 7.692$, df = 2, 7, $P = 0.017$; Fig. 2b). In contrast, there was no difference in microplastic concentration in water among the three sites (ANOVA, $F = 4.066$, df = 2, 4, $P = 0.109$; Fig. 2c).

Particle composition varied among sample types: size and chemical composition

We examined patterns in particle size and chemical composition over time and among sample types. Considered by relative abundance of size categories, the distribution of fiber size showed marginal differences among decades without controls included ($\chi^2 = 86.5$, df = 70, $P = 0.088$) and no difference with controls included ($\chi^2 = 91.6$, df = 80, $P = 0.176$) (Appendix S1: Fig. S1). For samples collected only in 2018, the average fiber size in fish collected in 2018 was 1–2 mm (Appendix S1: Fig. S2), with a similar distribution in size categories to samples from 2010 and 2000 (Appendix S1: Fig. S1). There was no significant difference in fiber size when comparing among fish, sediment, and water samples (ANOVA, $F = 0.085$, df = 2, 124, $P = 0.432$), or among the three sites (ANOVA, $F = 1.320$, df = 2, 124, $P = 0.271$; Appendix S1: Fig. S2).

We documented a wide range of materials in environmental samples and controls, including anthropogenic (cellulosic, unknown), cellulosic, cellulose acetate, acrylic, polyamide, polyethylene, polyester/PET, polyurethane, and "unknown" (Fig. 3). Comparing among fish samples from 1950 to 2018, we observed no significant difference in the relative abundance of material types ($\chi^2 = 63.2$, df = 54, $P = 0.184$; Fig. 3), and no difference when control samples were included in the comparison ($\chi^2 = 74.8$, df = 63, $P = 0.147$; Fig. 3). The relative abundance of polymers over time was variable, with no clear temporal direction. Contrary to our prediction, there was not a significant pattern for the number of polymer types detected over time (adjusted $r^2 = 0.122$, $P = 0.234$) and no pattern for diversity of types over time (adjusted $r^2 < 0.001$, $P = 0.415$; Appendix S1 Fig. S3). Chi-square test of independence showed a marginal difference in the relative proportion of polymer types in fish, sediment, and water collected in 2018

TABLE 3. Model coefficients, associated statistical results, and 95% confidence intervals from the top models evaluating the effects of time and fish count on microplastic abundance in fish across three permutations of the data set: all fish species pooled, the pooled data set with the round goby (*Neogobius melanostomus*) excluded, and largemouth bass (*Micropterus salmoides*) alone.

Coefficient	Estimate	SE	*Z*	*P*	95% CI	
					Lower	Upper
Pooled						
Intercept	−50.7328	11.214	−4.524	<0.0001	−74.56	−29.11
No. fish	0.0996	0.020	5.099	<0.0001	0.06	0.15
Time	0.0261	0.006	4.607	<0.0001	0.02	0.04
Goby excluded						
Intercept	−50.8487	10.379	−4.899	<0.0001	−72.59	−31.02
Fish count	0.2398	0.037	6.416	<0.0001	0.16	0.32
Time	0.0259	0.005	4.941	<0.0001	0.02	0.04
Bass						
Intercept	−65.0368	13.768	−4.724	<0.0001	−95.39	−38.38
Length	0.0638	0.016	4.107	<0.0001	0.03	0.10
Time	0.0325	0.007	4.731	<0.0001	0.02	0.05

Note: Fish count is number of fish sampled in each year.

(χ^2 = 53.9, df = 40, P = 0.070; Appendix S1 Fig. S4), with no difference when considering the control samples in the comparison (χ^2 = 56.0, df = 45, P = 0.127). Overall, sediment contained more particles identified as synthetic fibers compared to water samples, while water samples had more fibers made of cellulosic material (Appendix S1: Fig. S4). Finally, the pattern for polymer types in controls was distinct from environmental samples considered together. We did not find fibers made of cellulose acetate, polyamide, polyethylene, polyurethane, or unknown polymer types in our control samples, which were found in environmental samples (Fig 3, Appendix S1: Fig. S4).

Fish microplastic over time: comparison to urbanization, plastic production, and pollution

We compared the pattern of increasing microplastics in fish directly to trends in regional population growth, plastic production, and marine sediment microplastics over the same time period (1900–2020; Fig. 4). The population of the Chicago region grew from 2.8 million in 1900 to 9.8 million in 2010, in a linear fashion, although with some tapering during 2000–2010. Calculations by Geyer et al. (2017) showed acceleration in plastic production rates at a global scale, starting in 1950, and with some variation from the trend according to global economic changes (e.g., recession in 2008; Fig. 4). Brandon et al. (2019) documented an exponential increase in accumulation rates of marine microplastics from 1945 to 2009 in coastal California (Fig. 4).

DISCUSSION

Examining temporal patterns of microplastic concentration in organisms is critical to understand historical trends in plastic litter and make predictions about future levels of contamination. To our knowledge, this is the first examination of historical microplastic patterns in freshwater organisms. Prior to this study, most research on historical patterns of microplastics was focused on marine environments, so results from this research are critical to inform conservation of freshwater resources and to understand the movement of plastics from terrestrial sources to the oceans.

Microplastic concentration in freshwater fish (1900 – 2018)

The historical patterns of microplastic abundance in freshwater fish from 1900 to 2018 showed two clear stages: prior to 1950, there were no microplastics in fish specimens, and after 1950 there was a significant increase in the number of microplastic particles per fish over time. The results were consistent with our expectations. That is, widespread plastic production was absent prior to 1950 before the pace of invention and manufacturing of plastic polymers accelerated, which was reflected in the lack of microplastics presence in historical fish specimens prior to the 1950s. Plastic production became industrialized in the 1950s (Geyer et al. 2017, Worm et al. 2017), which is when plastic pollution would also be expected to increase in the environment.

The overall pattern of an increase in microplastics in fish specimens after 1950 could be attributed to (1) increasing population in the region, and (2) increasing use and disposal of plastic products. These changes occurred simultaneously and over many decades, so it is not within the scope of this study to examine the role of these individual factors. However, we provide context for the pattern of increasing microplastics in fish by comparing our results to regional population growth, as well as plastic production and pollution trends over the same time period. Broadly speaking, our data aligns

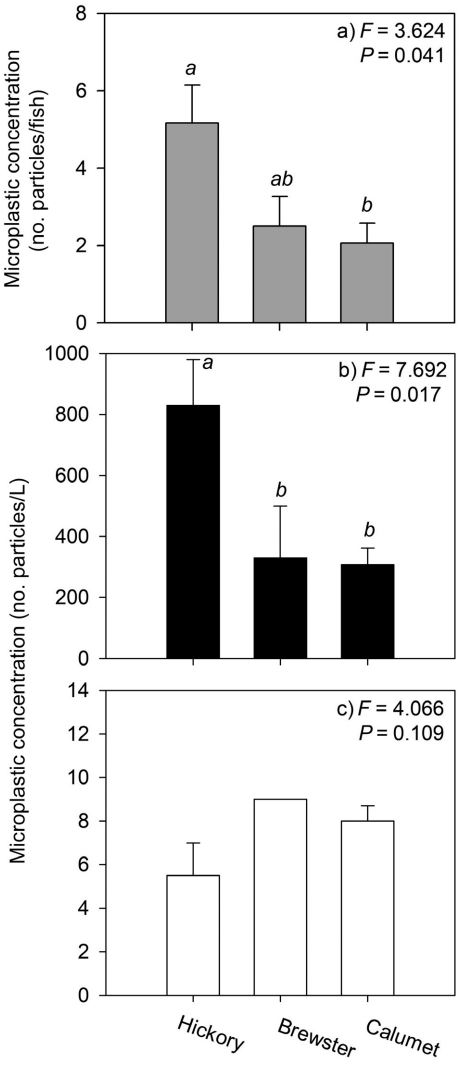

FIG. 2. Microplastic concentration (mean ± SE) in (a) fish, (b) sediment, and (c) water, collected in 2018 from Hickory Creek, Brewster Creek, and Calumet Park. Results from one-way ANOVA comparing across sites are in each panel (df = 2,26 in (a) 2, 7 in (b) and 2, 4 in (c). Different lowercase letters indicate significant difference among sites as shown by Tukey's test at a significance of $P < 0.05$.

with patterns in both plastic production and pollution rates, which were both exponential (Geyer et al. 2017, Brandon et al. 2019). The pattern is aligned with linear increases in population for the region as well (Fig. 4). However, regional population growth has slowed in the last decade while global plastic production has not. Overall, growing human populations and accelerating access to plastic products, and therefore an increase in plastic waste, are reflected as microplastic contaminants in historical and contemporary fish specimens.

Despite the relatively robust historical trends, we note that variation among individuals, species, and dates was high. We suggest the high variability is important to consider as a result itself, with important implications for future studies. Many factors affect abundance of microplastics in fish, including environmental concentrations, habitat preference, size, developmental stage, trophic level, feeding style, and gut retention time (Pedà et al. 2016, Lusher et al. 2017, McNeish et al. 2018). All factors vary among individuals of the same species in the same site, as well as among individuals in different sites and times. In addition, in this analysis, different species were collected at different sites. Thus, this work cannot distinguish the role of individual factors such as trophic level on microplastic concentration. More research is needed to quantify factors that simultaneously determine microplastic abundance within organisms for historical and modern samples. Future analysis will need larger sample sizes for each date and taxon, and experiments with live fish.

Our study is the first to examine the change in microplastic concentration in freshwater fish and to include data from a century of specimen collection, however, others have reported historical patterns in different ecosystems and habitats. Matsugama et al. (2017) used sediment cores (1880s–2000s) from marine environments in Japan, Thailand, Malaysia, and South Africa to show that microplastic concentration has increased significantly since the 1950s. A similar trend was shown for microplastic accumulation rates in off the coast of Southern California (Brandon et al. 2019). Finally, Turner et al. (2019) found increased abundance in microplastics from more recent sediment layers (1960s–2018) from cores collected in a London lake. These trends align with our results and cover pre- and post-plastic industrialization time periods that span many decades.

To our knowledge, only two published studies have measured the change in microplastic concentration within organisms over time, and those results do not show the same clarity as the patterns reported in this study or the results from sediments described above. Courtene-Jones et al. (2019) found no significant differences in microplastic concentration in echinoderms (*Ophiomusium lymani* and *Hymenaster pellucidus*) collected in the North East Atlantic Ocean between 1976–1994 and 2014–2015. The authors reported mostly fragments in the echinoderms, and the most common polymers were polyamide and polyester. Beer et al. (2018) analyzed *Clupea harengus* (Atlantic herring) and *Sprattus sprattus* (European sprat) from collected in the Baltic Sea (Bornholm Basin) from 1987 to 2015, and found no significant differences in microplastics over time. Fibers dominated the particles found in marine fish.

The reasons that we noted a change in microplastics in freshwater fish over time, but the same trend has not yet been reported for marine organisms, could be attributed to the long duration of this study, which was well matched to the time scale of increasing urbanization and population for the region. This study includes dates prior to mid-1950s, earlier than studies with marine

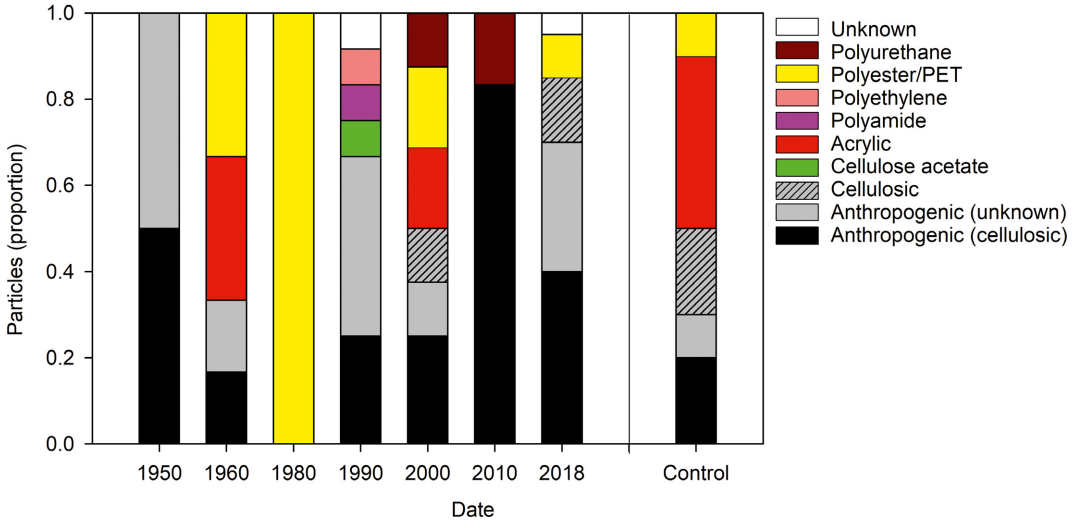

FIG. 3. Polymer relative abundance in fish samples from 1950 to 2018, as well as laboratory digestion controls.

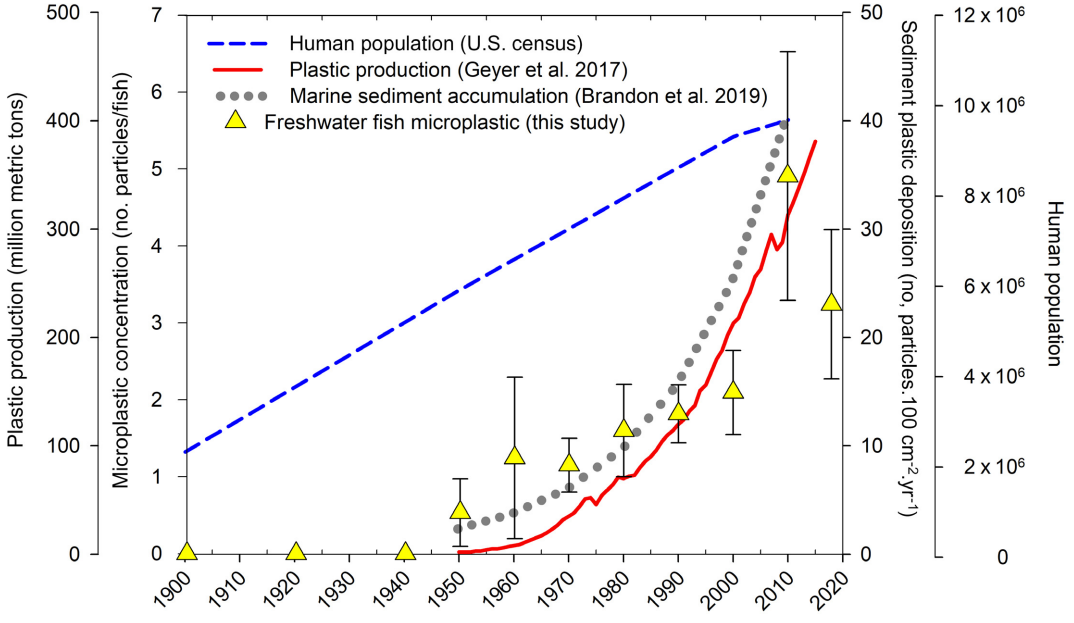

FIG. 4. Temporal patterns in microplastic concentration in fish (mean ± SE; data from this study averaged among individuals per decade; 1900–2018), global plastic production rates (Geyer et al. 2017; 1950–2015), marine sediment microplastic accumulation (Brandon et al. 2019; 1950–2010) and population of the Chicago Metropolitan Region, combined statistical area (U.S. census; 1900–2010).

organisms, increasing the likelihood of capturing a temporal pattern. In addition, the rapid urbanization in the Chicago area over the 1900s captured major changes in pollution that were encompassed by the dates of this analysis. Freshwaters also have much lower water volume than marine ecosystems, so temporal changes in microplastics might be more apparent in freshwater taxa. Testing this hypothesis will require more analyses of historical specimens from a greater diversity of marine and freshwater ecosystems.

Microplastics in contemporary fish, water, and sediment relative to published values

The measurements of microplastic concentration in fish and water samples we completed in 2018 were lower than recent studies from urban areas in the same region. Mean microplastic concentrations in our contemporary fish samples ranged from 2 to 5 particles/fish and water samples from 4 to 9 particles/L. At the nearby, urban Milwaukee River (Wisconsin, USA), McNeish et al.

(2018) found a mean of 10 particles/fish and 30 particles/L in the water column. We can attribute differences to species and site characteristics, as McNeish et al. (2018) showed results from 11 species, spanned a larger gradient of trophic levels, and was conducted in large rivers. However, there are few other studies that have compared microplastics in freshwater fish across environmental gradients, such as proximity to point sources, seasonality, and watershed position (Pazos et al. 2017, Perry et al. 2020). We suggest season-specific, longitudinal assessments of microplastics in water, sediment, and organisms along the river continuum (i.e., from headwaters to estuaries) will help resolve environmental drivers of microplastic dynamics in lotic ecosystems (McCormick et al. 2016, Barrows et al. 2018, Rodrigues et al. 2018).

In contrast to microplastic concentrations in the water column, microplastic abundance in the sediment of our study sites were similar to other regional studies. Microplastic concentrations in our sediment samples ranged from 120 to 980 microplastic particles/L, in the same range as the North Shore Channel in Skokie, Illinois, at 36–1,613 particles/L (Hoellein et al. 2017). It is possible that sediment may represent a record of inputs over a longer time period compared to microplastics in fish or water, but this has not been examined. More studies that compare microplastic density, deposition, and movement among habitats in stream ecosystems are needed (Horton and Dixon 2018, Hoellein et al. 2019, Windsor et al. 2019).

We compared the microplastic concentration in fish, water, and sediment collected at three study sites in 2018 to investigate whether the microplastics found in fish were related more strongly to the concentration in water or the sediment. Hickory Creek had significantly higher microplastics for fish and sediment (Fig. 3, Table 2), but there was no significant difference in the microplastic concentration in the water column among sites. We attributed this result to the capacity for sediment to act as a sink for microplastics in freshwaters (Vianello et al. 2013, Hurley et al. 2018, Hoellein et al. 2019). The similarity in the spatial variation of microplastics for sediment and fish was intriguing, and could be related to fish life history and microplastic uptake. The study species are each at some stage invertivores and may have incidentally consumed microplastics via their benthic prey, thereby reflecting the ultimate source in sediments. Thus, microplastics in fish might be developed as an indicator of microplastics in sediment (or vice versa). This finding is useful for ecosystem monitoring as quantifying microplastics in sediment presents more challenging technical obstacles than fish tissue. In addition, fish are routinely collected for ecosystem assessment or fisheries and digestive tissue is typically discarded. However, we acknowledge our analysis was conducted across a low number of sites and taxa, and the pattern merits more robust assessment over a larger range of conditions. In addition, by measuring only digestive tracts, our data may more directly reflect fish foraging behavior in the benthos than if other tissues were included (e.g., gills). Future studies could document comparisons among the water column, sediment, and food web concentrations among a wider array of fish feeding guilds, species, and tissues, and thereby reveal which pool of microplastics might be the best "indicator" of microplastic levels in aquatic ecosystems. This analysis will inform management and policy, as there is a growing interest in refining assessment strategies for microplastic pollution.

Particle characterization

All of the particles found in our fish, water, sediment, and laboratory control samples were fibers. This aligns with previous studies where fibers are the dominant microplastic morphotype found in fish, surface water, benthic sediment, and control samples (Hoellein et al. 2017, Barrows et al. 2018, McNeish et al. 2018). We expected to find mostly fibers in our samples as other studies have noted streams are commonly contaminated with microplastic fibers (Baldwin et al. 2016, Miller et al. 2017, Barrows et al. 2018). Using the same methods, McNeish et al. (2018) found fibers represented 97–100% of fish collected in 3 tributaries of Lake Michigan. We do not have a hypothesis regarding the lack of other particle shapes. However, future studies may benefit from comparing the assemblage of particle shapes among fish species, size, and location. Mean fibers length in our samples was 1–2 mm, which is consistent with previous work from rivers in the region (McNeish et al. 2018). The major sources of microplastic fibers include wastewater treatment plant effluent contaminated with fibers from textiles (Browne et al. 2011), as well as atmospheric deposition (Dris et al. 2018). The relative composition of particles is relevant to biological interactions. A meta-analysis by Foley et al. (2018) suggested fibers and fragments generated negative effects on survival of aquatic organisms, whereas round microplastic particles were more likely to have neutral or no effect on aquatic organisms.

A wide variety of materials were observed in our fish samples. From 1950 to 1980, we found four materials: anthropogenic (unknown), anthropogenic (cellulosic), acrylic, and polyester/PET. From 1990 to 2018, we found six additional types: cellulosic, cellulose acetate, polyamide, polyethylene, polyurethane, and "unknown." Overall, the temporal patterns in materials are consistent with diversity of plastic polymers common in consumer products and in plastic litter (Zalasiewicz et al. 2019). Of the identifiable polymers, polyester/PET occurred most consistently in our data set (Fig. 3) and is one of the most widespread microplastic pollutants (Courtene-Jones et al. 2019).

Fibers consisted of a variety of materials, which reflects the complexity of anthropogenic pollution and an important challenge for this field of study. Some of the fibers represent mixtures of natural and synthetic

materials, some could not be conclusively identified (e.g., unknown), and some may not contain plastic polymers (e.g., cellulosic). In the latter case, however, those fibers have a wide variety of anthropogenic modifications, including processing (i.e., viscose) and additions of dyes and chemicals (e.g., flame retardants), which can interfere with successful polymer identification, and may preclude their categorization as "natural" rather than synthetic. The suite of variable compounds included as microplastics will likely have a corresponding variation in the physiological impacts. We apply the term microplastics here but acknowledge the inherent complexity in the wide array of chemical compounds and anthropogenic modification that it incorporates (Rochman et al. 2019).

Conclusion and future studies

Microplastic concentration in freshwater fish from the Chicago region increased significantly from 1950 to 2018, which aligns with rates of plastic production, patterns of plastic pollution documented in other ecosystems, and population growth. Microplastics in fish from urban freshwater ecosystems may be a proxy for estimates of environmental pollution in historical and contemporary conditions, and used to predict future patterns of microplastics. More research is needed to investigate the relationship between environmental concentrations and microplastic concentrations in fish, ecological and physiological factors that affect microplastic exposure and retention within fish, the capacity for freshwater fish to serve as an indicator of plastic pollution relative to marine organisms, and to quantify how fish and plastic that moves between freshwater and marine ecosystems affect global plastic budgets. This analysis informs a greater understanding of the historical context for plastic pollution, and thereby support management and prevention offers to reduce microplastics in the environment.

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Literature Cited

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

Barnes, D. K., S. A. Morley, J. Bell, P. Brewin, K. Brigden, M. Collins, T. Glass, W. P. Goodall-Copestake, L. Henry, and V. Laptikhovsky. 2018. Marine plastics threaten giant Atlantic Marine Protected Areas. Current Biology 28:R1137–R1138.

Barrows, A. P., K. S. Christiansen, E. T. Bode, and T. J. Hoellein. 2018. A watershed-scale, citizen science approach to quantifying microplastic concentration in a mixed land-use river. Water Research 147:382–392.

Barton, K. 2020. MuMIn: Multi-Model Inference. R. package, editor. https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf

Beer, S., A. Garm, B. Huwer, J. Dierking, and T. G. Nielsen. 2018. No increase in marine microplastic concentration over the last three decades—a case study from the Baltic Sea. Science of the Total Environment 621:1272–1279.

Brandon, J. A., W. Jones, and M. D. Ohman. 2019. Multidecadal increase in plastic particles in coastal ocean sediments. Science Advances 5:eaax0587.

Brookson, C. B., S. de Solla, K. J. Fernie, M. F. Cepeda, and C. M. Rochman. 2019. Microplastics in the diet of nestling double-crested cormorants (*Phalacrocorax auratus*), an obligate piscivore in a freshwater ecosystem. Canadian Journal of Fisheries and Aquatic Sciences 76:2156–2163.

Browne, M. A., P. Crump, S. J. Niven, E. Teuten, A. Tonkin, T. Galloway, and R. Thompson. 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environmental Science & Technology 45:9175–9179.

Courtene-Jones, W., B. Quinn, C. Ewins, S. F. Gary, and B. E. Narayanaswamy. 2019. Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976–2015), a study from the North East Atlantic. Environmental Pollution 244:503–512.

Critchell, K., and M. O. Hoogenboom. 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (*Acanthochromis polyacanthus*). PLoS ONE 13:e0193308.

Dris, R., J. Gasperi, and B. Tassin. 2018. Sources and fate of microplastics in urban areas: a focus on Paris Megacity. Pages 69–83 *in* M. Wagner and S. Lambert, editors. Freshwater microplastics. Springer, Cham, Switzerland.

Eriksen, M., L. C. Lebreton, H. S. Carson, M. Thiel, C. J. Moore, J. C. Borerro, F. Galgani, P. G. Ryan, and J. Reisser. 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS ONE 9:e111913.

Foley, C. J., Z. S. Feiner, T. D. Malinich, and T. O. Höök. 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Science of the Total Environment 631:550–559.

Fox, J., and S. Weisberg 2019. An R companion to applied regression. Third edition. Sage, Thousand Oaks California, USA.

Froese, R., and D. Pauly. 2017. FishBase. www.fishbase.org

Gewert, B., M. Ogonowski, A. Barth, and M. MacLeod. 2017. Abundance and composition of near surface microplastics and plastic debris in the Stockholm Archipelago, Baltic Sea. Marine Pollution Bulletin 120:292–302.

Gewert, B., M. M. Plassmann, and M. MacLeod. 2015. Pathways for degradation of plastic polymers floating in the marine environment. Environmental Science: Processes & Impacts 17:1513–1521.

Geyer, R., J. R. Jambeck, and K. L. Law. 2017. Production, use, and fate of all plastics ever made. Science Advances 3: e1700782.

Hall, L. K., R. T. Larsen, R. N. Knight, and B. R. McMillan. 2018. Feral horses influence both spatial and temporal patterns of water use by native ungulates in a semi-arid environment. Ecosphere 9:e02096.

Hartmann, N., T. Hüffer, R. C. Thompson, M. Hassellöv, A. Verschoor, A. E. Daugaard, S. Rist, T. M. Karlsson, N. Brennholt, and M. Cole. 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environmental Science & Technology 53:1039–1047.

Hoellein, T. 2020. Dataset. Hou et al. A fish tale A century of museum specimens reveal increasing microplastic concentrations in freshwater fish. Mendeley Data, V1, https://doi.org/10.17632/xfgycsgxt4.1

Hoellein, T. J., A. R. McCormick, J. Hittie, M. G. London, J. W. Scott, and J. J. Kelly. 2017. Longitudinal patterns of microplastic concentration and bacterial assemblages in surface and benthic habitats of an urban river. Freshwater Science 36:491–507.

Hoellein, T. J., A. J. Shogren, J. L. Tank, P. Risteca, and J. J. Kelly. 2019. Microplastic deposition velocity in streams follows patterns for naturally occurring allochthonous particles. Scientific Reports 9:3740.

Horton, A. A., and S. J. Dixon. 2018. Microplastics: an introduction to environmental transport processes. Wiley Interdisciplinary Reviews: Water 5:e1268.

Hurley, R., J. Woodward, and J. J. Rothwell. 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. Nature Geoscience 11:251–257.

Jambeck, J. R., R. Geyer, C. Wilcox, T. R. Siegler, M. Perryman, A. Andrady, R. Narayan, and K. L. Law. 2015. Plastic waste inputs from land into the ocean. Science 347:768–771.

Lo, H. K. A., and K. Y. K. Chan. 2018. Negative effects of microplastic exposure on growth and development of *Crepidula onyx*. Environmental Pollution 233:588–595.

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

Matsuguma, Y., H. Takada, H. Kumata, H. Kanke, S. Sakurai, T. Suzuki, M. Itoh, Y. Okazaki, R. Boonyatumanond, and M. P. Zakaria. 2017. Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. Archives of Environmental Contamination and Toxicology 73:230–239.

McCormick, A. R., and T. J. Hoellein. 2016. Anthropogenic litter is abundant, diverse, and mobile in urban rivers: Insights from cross-ecosystem analyses using ecosystem and community ecology tools. Limnology and Oceanography 61:1718–1734.

McCormick, A. R., T. J. Hoellein, M. G. London, J. Hittie, J. W. Scott, and J. J. Kelly. 2016. Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. Ecosphere 7:e01556.

McIntyre, P. B., C. A. R. Liermann, and C. Revenga. 2016. Linking freshwater fishery management to global food security and biodiversity conservation. Proceedings of the National Academy of Sciences USA 113:12880–12885.

McNeish, R. E., L. H. Kim, H. A. Barrett, S. A. Mason, J. J. Kelly, and T. J. Hoellein. 2018. Microplastic in riverine fish is linked to species traits. Scientific Reports 8:11639.

Miller, R. Z., A. J. Watts, B. O. Winslow, T. S. Galloway, and A. P. Barrows. 2017. Mountains to the sea: river study of plastic and non-plastic microfiber pollution in the northeast USA. Marine Pollution Bulletin 124:245–251.

Munno, K., H. De Frond, B. O'Donnell, and C. M. Rochman. 2020. Increasing the accessibility for characterizing microplastics: introducing new application-based and spectral libraries of plastic particles (SLoPP and SLoPP-E). Analytical Chemistry 92:2443–2451.

Munno, K., P. A. Helm, D. A. Jackson, C. Rochman, and A. Sims. 2018. Impacts of temperature and selected chemical digestion methods on microplastic particles. Environmental Toxicology and Chemistry 37:91–98.

Nix, J. H., R. G. Howell, L. K. Hall, and B. R. McMillan. 2018. The influence of periodic increases of human activity on crepuscular and nocturnal mammals: testing the weekend effect. Behavioural Processes 146:16–21.

Pazos, R. S., T. Maiztegui, D. C. Colautti, A. H. Paracampo, and N. Gómez. 2017. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. Marine Pollution Bulletin 122:85–90.

Pedà, C., L. Caccamo, M. C. Fossi, F. Gai, F. Andaloro, L. Genovese, A. Perdichizzi, T. Romeo, and G. Maricchiolo. 2016. Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: preliminary results. Environmental Pollution 212:251–256.

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

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Rech, S., V. Macaya-Caquilpán, J. Pantoja, M. Rivadeneira, C. K. Campodónico, and M. Thiel. 2015. Sampling of riverine litter with citizen scientists—findings and recommendations. Environmental Monitoring and Assessment 187:335.

Rillig, M. C. 2012. Microplastic in terrestrial ecosystems and the soil? Environmental Science & Technology 46:6453–6454.

Rochman, C. M. 2013. Plastics and priority pollutants: a multiple stressor in aquatic habitats. Environmental Science & Technology 47:2439–2440.

Rochman, C. M. 2015. The complex mixture, fate and toxicity of chemicals associated with plastic debris in the marine environment. Pages 117–140 *in* M. Bergmann, L. Gutow, and M. Klages, editors. Marine anthropogenic litter. Springer, Cham, Switzerland.

Rochman, C. M., C. Brookson, J. Bikker, N. Djuric, A. Earn, K. Bucci, S. Athey, A. Huntington, H. McIlwraith, and K. Munno. 2019. Rethinking microplastics as a diverse contaminant suite. Environmental Toxicology and Chemistry 38:703–711.

Rodrigues, M., N. Abrantes, F. Gonçalves, H. Nogueira, J. Marques, and A. Gonçalves. 2018. Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). Science of the Total Environment 633:1549–1559.

Turner, S., A. A. Horton, N. L. Rose, and C. Hall. 2019. A temporal sediment record of microplastics in an urban lake, London, UK. Journal of Paleolimnology 61:449–462.

Venables, W. N., and B. D. Ripley 2002. Modern applied statistics with S. Fourth edition. Springer, New York, New York, USA.

Vianello, A., A. Boldrin, P. Guerriero, V. Moschino, R. Rella, A. Sturaro, and L. Da Ros. 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal and Shelf Science 130:54–61.

Von Moos, N., P. Burkhardt-Holm, and A. Köhler. 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environmental Science & Technology 46:11327–11335.

Windsor, F. M., I. Durance, A. A. Horton, R. C. Thompson, C. R. Tyler, and S. J. Ormerod. 2019. A catchment-scale

perspective of plastic pollution. Global Change Biology 25:1207–1221.
Worm, B., H. K. Lotze, I. Jubinville, C. Wilcox, and J. Jambeck. 2017. Plastic as a persistent marine pollutant. Annual Review of Environment and Resources 42:1–26.
Wright, S. L., and F. J. Kelly. 2017. Plastic and human health: a micro issue? Environmental Science & Technology 51:6634–6647.
Wright, S. L., R. C. Thompson, and T. S. Galloway. 2013. The physical impacts of microplastics on marine organisms: a review. Environmental Pollution 178:483–492.
Zalasiewicz, J., C. N. Waters, M. Williams, and C. Summerhayes. 2019. The Anthropocene as a geological time unit: a guide to the scientific evidence and current debate. Cambridge University Press, Cambridge, UK.

Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2320/full

Data Availability

Data are available (Hoellein 2020) in Mendeley Data: http://dx.doi.org/10.17632/xfgycsgxt4.1.