Evidence of Microplastic Translocation in Wild-Caught Fish and Implications for Microplastic Accumulation Dynamics in Food Webs

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ABSTRACT: The presence of microplastics within the gut of animals is well documented. Whether microplastics bioaccumulate in organisms and biomagnify in food webs remains unclear and relies on the ability of microplastics to translocate to other tissues. Here, we demonstrate the widespread presence of microplastics and other anthropogenic microparticles in the gastrointestinal tract, fillet, and livers of seven species of sportfish from Lake Simcoe, Ontario, Canada. Larger fish had a higher microplastic load compared to smaller fish, but the opposite trend was observed with translocated microplastics standardized by fish mass (i.e., smaller fish contained more translocated particles per gram wet weight than larger fish). Moreover, we observed no evidence of biomagnification as there was no significant relationship between the trophic level and total or translocated microplastics per individual. Overall, this suggests that microplastics are translocating, but that excretion of translocated particles or growth dilution may be occurring rather than bioaccumulation and biomagnification. Moreover, the assemblages of shapes and material types varied among tissues, suggesting that particle characteristics may predict biological fate. Our findings highlight the need for further work to understand the mechanisms of microplastic translocation and excretion and the implications for the dynamics of microplastics accumulation in food webs and human exposure.

KEYWORDS: plastic, translocation, bioaccumulation, growth dilution, biomagnification, freshwater fish

INTRODUCTION

Our plastic waste is accumulating in marine, freshwater, and terrestrial ecosystems. Due to its durability, plastic persists in the environment, ultimately fragmenting into microplastics. This has led to the widespread accumulation of environmental microplastics. The long-range transport, persistence, and ubiquity of microplastics globally increases the likelihood of ingestion and intake, which poses a risk to many organisms including humans.

Direct ingestion of microplastics has been observed in hundreds of species and across many trophic levels. Other routes of entry include via the gills or indirect ingestion through the consumption of prey. Trophic transfer from prey to predator has been demonstrated in laboratory experiments, including in a planktonic food web and from mussels (Mytilus edulis) to crabs (Carcinus maenas). These observations are not limited to the laboratory and have been observed in the field as well. Still, evidence for bioaccumulation and/or biomagnification of microplastics within a food web has not yet been observed. For bioaccumulation to occur, microplastics must be retained in the tissues, increasing in concentration over the life of the organism. For biomagnification, microplastics in tissues must increase with trophic position.

There is evidence of microplastic translocation to tissues, suggesting bioaccumulation is possible. Laboratory studies show translocation of microplastics less than 5 μm to the liver, fillet, and brain of fish, but some studies suggest otherwise. Our previous laboratory study did not detect translocation of 10–300 μm microplastics in the livers, gonads or fillets of Rainbow Trout (Oncorhynchus mykiss). Moreover, translocation of microplastics was not observed with Pacific Oyster (Crassostrea gigas), Goldfish (Carassius auratus), or Shore Crabs (C. maenas). In general, laboratory studies observing the translocation of particles >20 μm are uncommon. In contrast, field studies report larger particles beyond the gut of wild-caught animals. For instance, microplastics 124–438 μm in size have been detected in the livers of fish. This conflicting evidence suggests that further work is needed to understand the fate of microplastics in organisms and the mechanism(s) that lead to translocation. This is fundamental to the larger question...
of whether concentrations in tissues would be of concern and increase over time and up food chains, i.e., bioaccumulation and biomagnification.

In addition to concerns about the fate in food webs, evidence suggesting that microplastics can translocate has led to concern about a direct route of exposure to humans when we eat seafood. There are three general categories for concern, all relevant to food security. First, people are concerned about food safety. Because the gut and stomach contents of fish are not typically eaten by humans, the risk of consuming plastic particles is assumed to be low.31 However, this risk changes with the translocation of microplastics to the fillet, and an accumulation of microplastics over time or up food chains can result in higher concentrations in our food supply. Specifically, since humans are apex predators, biomagnification may result in our consumption of the most microplastic-contaminated fish.32,33 Second, people are concerned with food quality. For instance, the potential reduction in food consumption and growth, as well as a loss of ingesting microplastics.26 These effects of microplastic ingestion on organisms include reduction in food consumption and growth, as well as a loss of lipids, the latter of which reduces nutritional value.34,35 Third, people have expressed concern with food availability.36,37 There is evidence of reduced fecundity and growth in animals that ingest microplastics.38 These effects have implications for fish population growth and could impact fish stocks.31,33,38

In this study, we quantified and characterized microplastics and other anthropogenic microparticles in the gastrointestinal (GI) tracts, fillets, and livers of seven species of freshwater fish from Lake Simcoe (Ontario, Canada). We aimed to answer the following questions: is translocation to different tissues occurring? do we observe patterns relevant to biological/ecological traits? and is bioaccumulation and/or biomagnification occurring? Local and indigenous communities around Lake Simcoe have expressed concerns regarding microplastics in fish caught for human consumption. The Lake Simcoe system, home to recreational and subsistence fishing, provides a case study to investigate these fundamental questions to better understand the accumulation of microplastics in aquatic food webs.

## MATERIALS AND METHODS

### Fish Sampling

Fish were collected by the Fish Contaminant Monitoring Program of Ontario Ministry of the Environment, Conservation and Parks in partnership with Ontario Ministry of Natural Resources and Forestry using 6 foot trap nets or gill nets from three specified locations across Lake Simcoe (Kempenfelt Bay, near Orillia, and Georgina Island). Fish were sacrificed with a blow to the head as per the Ontario Ministry of Natural Resources Aquatic Research and Development Section Animal Care Committee Class Protocol. During transport, fish were kept in insulated coolers containing ice packs and were immediately frozen and kept at −20 °C until further analysis. A total of 69 fish from seven different species were collected, and their GI tract, fillet, and liver were analyzed for microplastics. Collected species included Micropterus dolomieu (Smallmouth Bass), Micropterus salmoides (Largemouth Bass), Esox lucius (Northern Pike), Perca flavescens (Yellow Perch), Amieturus nebulosus (Brown Bullhead), Catostomus commersonii (White Sucker), and Coregonus clupeaformis (Lake Whitefish). Each species was classified by habitat and trophic level according to the data available from a global database on fish species (FishBase; Table S1).39,40

### Fish Dissections

Prior to dissections, fish were left at room temperature to defrost. The exterior of each fish was rinsed with tap water to remove any potential contamination from the plastic freezer bags in which fish were kept. Each fish was weighed using a hanging hook scale, and the total length was measured with a ruler. For dissections, fillet, liver, and GI tract were individually sampled and stored in Whirl-Pak bags at −20 °C until further analysis. To avoid cross-contamination, the GI tract was removed last. Only one skinless fillet per fish was used for analysis. We multiplied the abundance of microplastics found in one fillet by two when reporting our results to account for a total number of microplastics per individual. Open Whirl-Pak bags were used as blanks and underwent the same procedural steps as samples. Some samples were not processed as they were too difficult to dissect (1 gut sample and 12 liver samples (including all White Sucker livers)). Thus, the sample sizes for GI tract, fillet, and liver were 68, 69, and 57, respectively.

### Sample Digestions

GI tracts, fillet, and livers were individually transferred to 1 L polyethylene containers rinsed with tap water. Samples were then submerged in a 10% KOH (w/v) solution for 24 h in a 60 °C oven, a “benchmark protocol” for the extraction and characterization of microplastics from seafood tissues.40 For samples that were not fully digested or had excessive fatty residues, samples underwent either one or both of two additional treatments (detergent soak and wet peroxide oxidation) to minimize organic debris and residues. After treatment, fillet and liver samples were rinsed through 125 μm (top) and 63 μm (bottom) metal sieves, while GI tract samples were rinsed through a 125 μm sieve. For further details about the extraction, see the Supporting Information (SI).

### Plastic Sorting and Quantification

Samples were examined under a Leica S8 APO microscope at 10−45X, and suspected microplastics were picked with fine tweezers. All suspected microplastics were picked and mounted on double-sided adhesive tape for images, measurements, and Raman analysis, and their color and category recorded. The categories included were fibers, films, foams, spheres, pellets, and fragments, which are typical classifications for microplastics.41 Pictures of each suspected microplastic were taken, and OMAX Toupview software (version 3.7; TouTek) was used to measure and record the length of the plastic in the longest dimension.

### Identification and Characterization Using Micro-Raman Spectroscopy

After blank correction (see QA/QC), a subset of particles was selected for chemical identification by micro-Raman spectroscopy. All particles in the blanks were analyzed (N = 45). From each sample, at least 10% of particles in each color/category combination (e.g., blue fiber, black fragment) from each size fraction were analyzed, which led to a total of 39% of all particles (N = 359). Chemical identification with micro-Raman (HORIBA Raman XploRA Plus) was done in LabSpec6 software using a 785 nm (range 50−2000 cm−1) or 532 nm (range 50−4000 cm−1) laser with a 100× long working distance microscope objective. Reported particle numbers were not adjusted according to spectroscopy. Therefore, from here forward, the terms suspected microplastics, microplastics, and particles are used interchangeably and refer to any particles that were suspected to be microplastics. For further details about spectroscopy and how we assigned material IDs, see the SI.

### QA/QC

All work surfaces and tools were rinsed with tap or RO water before, during, and after handling samples. RO water was used for all processing except dissections. An air purifier ( Fellowes AeraMax 290, Itasca, Illinois) was always present, and all work surfaces were wiped down to minimize dust. Cotton laboratory coats were worn whenever possible, and a lint roller
was used to remove fibers from clothing. Laboratory blanks (n = 10) were run each day of dissections and carried through sample processing (i.e., open Whirl-Pak bags were rinsed out and the contents followed the same digestion and extraction steps as tissue samples). The average number of particles for each color/category (e.g., blue fiber, black fragment) found per size fraction across all blanks were subtracted from each sample. To report conservative numbers, the average number of microplastics from each color and category in blanks was rounded up and subtracted from sample values (e.g., 0.4 red fibers rounded up to 1 red fiber). After blank subtraction, a total of 887 out of 1406 microplastics were included in the results. Finally, we performed spike recovery tests in the laboratory and had recoveries >85% (see the SI and Table S2 for details).

### Data Analysis

The Shapiro–Wilks test was used to assess the normality of the data and the Bartlett’s test to assess the homogeneity of variances. Because these assumptions were not met, the nonparametric Kruskal–Wallis test was conducted to determine whether differences exist in microplastic concentrations among species (for total concentration and for concentration in the fillet and liver), followed by a post-hoc Dunn’s test with Bonferroni correction. To assess differences in habitat type, the nonparametric Mann–Whitney test was performed.

The relationship between the number of microplastics in GI tracts and the number of microplastics in other tissues was assessed using Spearman’s rank correlation. The aim of this analysis was to determine whether the GI tract is a relevant proxy for microplastic accumulation in other tissues. We used the same correlation analysis to investigate whether concentrations in fillet increase with those in liver, as would be expected provided that the liver acts as the first processing site for foreign materials. Additionally, simple linear regression was conducted to determine whether a relationship exists between (1) fish mass, (2) fish length, (3) trophic level, and (4) body condition (K) and the total quantity of microplastics in sportfish (N\text{Total}). To account for differences in fish size and investigate evidence of bioaccumulation, we did the same linear regression analyses using standardized microplastic counts. Since the wet weight of the fillets was not recorded, the wet weight of the whole fish (gram w. w.) was used to standardize suspected microplastic numbers in fillets (C\text{Fillet}) and for fillets and livers combined (C\text{Fillet+Liver}). The wet weight of liver tissues (gram w. w.) was recorded; thus, the liver wet weight was used to standardize the number of suspected microplastics in livers (C\text{Liver}). The number of suspected microplastics present in all analyzed tissues, GI tract, fillet and liver, fillet only, and liver only will be referred to as N\text{Total}, N\text{GI tract}, N\text{Fillet-Liver}, N\text{Fillet}, and N\text{Liver} respectively, while concentrations standardized by weight will be represented as C\text{Fillet-Liver}, C\text{Fillet}, and C\text{Liver}. Body condition was calculated from fish weight (W, g), and body length (L, cm) using Fulton’s Condition Factor formula: K = 100 × W/L². It should be noted that when assessing model assumptions, there were three outliers for fish mass and fish length that skewed the residual normality. However, when these points were excluded, there was no change in significance; thus, these data points were kept. A significance level of α = 0.05 was considered for all analyses. Statistical analysis was performed using R statistical software (version 3.6.1).

To evaluate patterns of microplastic composition between blank samples and fish tissue samples, and among tissue types, species, habitats, size fractions, and sites, nonmetric multidimensional scaling (nMDS) was used. We made separate two-dimensional ordinations for particle morphology and chemical ID, using Euclidean distance (Vegan package; version 2.5-7) in R. When nMDS showed distinct patterns, PERMANOVAs were run to determine whether there were significant differences between the assemblage structures of microplastic by morphology and chemical ID. Using the function ‘adonis2’ (also from Vegan package), we ran PERMANOVAs for sample type, tissue type, and size fraction. Differences were considered significant when p < 0.05.

To assess the level of human exposure as a result of consumption, the measured number of microplastics in fillet of each fish sample was converted into a 227 g (8 oz or half a pound) meal equivalent. Since weights of the extracted fillets were not measured, one meal equivalent number of fillets were estimated according to the type and size of fish. Yearly intake of microplastics was then calculated by assuming consumption of the fish on a two meals per week basis. The fish consumption rate of 65 g/day used here can be considered above average based on global per capita consumption of 55 g/day, 90th percentile U.S. freshwater fish consumption of 22 g/day, and fish intake recommended by various health agencies (e.g., at least 20 g/day by Health Canada, 30 g/day by American Heart Association, 30 g/day by USDA).

### RESULTS AND DISCUSSION

**QA/QC.** Two size fractions (>125 and 63–125 μm) were collected for blanks (n = 10). The smaller fraction contained no observable microplastics, while the >125 μm fraction had an average (±standard deviation, s.d.) of 4.5 ± 3.9 suspected microplastics. The morphology of the particles in the blanks consisted of 99% fibers and 1% fragments (Figure S1a) and were comprised of, on average, 0.8 black fibers, 1.3 blue fibers, 1.7 clear fibers, 0.1 green fibers, 0.4 red fibers, 0.1 purple fibers, and 0.1 black fragments (Table S3). Chemical identification of particles in the blanks revealed that 2% of particles were natural, 42% had an unknown origin, while 56% had anthropogenic origins (Figure S1b). Overall, 7% of suspected microplastics were confirmed plastic. There were two polyethylene imine fibers and one polypropylene fiber (Figure S1b). The nMDS plots for particle morphology and chemical ID revealed distinct groups for blank and fish tissue samples (Figure S1c,d). A PERMANOVA test showed that the differences were statistically significant for chemical ID (p < 0.001; Table S8) but not morphology (p = 0.6; Table S8). Moreover, suspected microplastic sizes in blanks ranged from 143 to >5000 μm, with the majority of particles larger than 1000 μm. In comparison, the majority of particles in the fillet and liver were smaller than 1000 μm (Figure S6). The differences observed between the blank and tissue samples suggest that blank-corrected microplastic levels presented in this study cannot be attributed to laboratory contamination.

**Microplastics Are Present in Fish Tissues.** Suspected microplastics were observed in all tissues and all species. Total particle amounts per fish (i.e., the sum of suspected microplastics across all studied tissues, N\text{Total}) ranged from 0 (a Yellow Perch from Kempenfelt Bay) to 84 (a Largemouth Bass from Orillia). The N\text{Total} average (±s.d.) was 17.0 ± 15.5 particles per fish with 99% of fish containing at least one particle (see Table S4 for sample data). Typically, microplastic averages range from 0 to 10 particles/individual according to a review of over 800 species. Thus, Lake Simcoe fish are on the higher end of contamination compared to other species, but this is partly due to including the fillet and liver in addition to the GI tract, which...
is uncommon in most studies. The average value for Lake Simcoe fish falls within the typical range at 7.6 particles/individual with 90% of fish containing at least one particle when only GI tracts are included ($N_{\text{GI tract}}$).

Large variability in microplastic contamination of freshwater fish has been reported. For example, fish from Lake Ontario (Canada) contained an average of 58.7 particles/individual and 100% of individuals sampled contained at least one particle, while fish from Taihu Lake (China) contained an average of 2.4 particles/individual with 95.7% of samples contaminated with at least one particle. Some of the variability may be explained by the level of contamination in these systems. Lake Simcoe surface water concentrations range from 0 to 0.7 particles/L, whereas Lake Ontario concentrations range from 0 to 2.4 particles/L in surface water. However, the surface water concentrations for Lake Taihu range from 3.4 to 25.8 particles/L. For marine systems, the percentage of contaminated fish seems to be lower. For instance, only 2.6% of fish from the North Sea and 28% of fish from the Adriatic Sea contained microplastics, with both studies reporting 1–2 particles/individual. In addition to differing levels of contamination, the large variability across systems may be attributed to feeding strategy, habitat, water volume to surface area ratio, lack of standardization among study methods, and proximity to microplastic sources.

Microplastic Abundance Varies between Tissues. There have only been a few field studies that have investigated the contamination of other tissues by microplastics. In this study, the particle amount per tissue per fish ranged from 0 to 40 in the GI tract ($N_{\text{GI tract}}$: mean $7.6 \pm 7.5$), 0 to 70 in the fillets ($N_{\text{fillet}}$: mean $8.2 \pm 1.1$ when including both fillets), and 0 to 9 in the livers ($N_{\text{liver}}$: mean $1.5 \pm 2.0$; Figure 1; Tables S4–S7). In general, the percentage of individuals with microplastics in these tissues and the amounts observed were much higher than expected. Collard et al. found that 5% of livers from European Chub in Parisian rivers contained at least one microplastic particle, but none were observed in fillet samples. However, Collard et al. only took a 2–4 g subsample of fillet, whereas we used a whole fillet. In marine fish, there have been observations of microplastics in 8 out of 10 livers of European Anchovy, while there were no microplastics observed in the liver or muscle of Asian Seabass from the Yangtze Estuary in China. However, these studies corrected for confirmed plastic, whereas we did not use spectroscopy correct. Still, concentrations generally increase with decreasing particle size, and the present study was limited by the smallest size fraction of 63 μm, suggesting that our data is conservative. Overall, the relatively higher levels of contamination observed here are unique and suggest that future studies should include tissues other than the GI tract in freshwater species, particularly in systems with relatively high contamination.

Microplastic Composition Varies between Tissues. Across all samples, the majority of microplastics were fibers (72%). The remaining consisted of films (16%), fragments (11%), and foam (1%; Figure S1a). Significant differences in morphology were evident among tissue types ($p < 0.001$; Figure S3a and Table S8). The assemblage composition of morphologies was different in GI tract samples compared to fillet and liver (Figures S3a and S4b). GI tracts had a high proportion of fibers (94%) and few films (5%) and fragments (1%). In contrast, fillets contained similar proportions of fibers
The ability for microplastics to translocate to other organs is an emerging area of research, and there is still much to understand about the extent and mechanisms of transport for small particles within the body. Conflicting evidence about particle translocation has led to doubt about whether microplastics can accumulate in other tissues.66 Here, we report the presence of a considerable number of particles in a large proportion of the fillet and livers of surveyed fish.

Overall, fibers were the most abundant shape in all samples, but there were unique microplastic compositions in different tissues. This suggests that shape may be another important factor relevant to the mechanism that leads to translocation. This is similar to what has been demonstrated for nanoparticles.67 Interestingly, foam particles were observed in the fillet and liver but not in GI tract. Although reasons behind these patterns are unclear, one possibility is that having a more compact shape (i.e., a fragment or a folded film) facilitates the passage through cells by endocytotic processes.68,69

The sizes of particles observed in the fillet and liver tissues were much larger than what has been observed in laboratory-based translocation studies. The most common size observed in the fillets and livers was approximately 100−400 μm in length. In laboratory studies, there have been observations of microplastics between 1 and 10 μm in size within the liver, fillet, spleen, and kidney of various fish species, as well as in the hemolymph and hemocytes of mussels and crabs.14,21,22,70 There are also numerous studies that demonstrate the translocation of particles in the nanometer range.20,74−80 Experimental evidence supporting translocation of larger-sized microplastics is limited. Still, there are laboratory observations of microplastics of approximately 200−600 μm in fish livers, and microplastics 180−250 μm in the hepatopancreas of fiddler crabs.57,81,82 Moreover, the particle sizes observed in the present study are similar to observations in wild-caught fish.29,30 However, we did not quantify or characterize particles smaller than 63 μm, limiting our ability to compare to laboratory studies using smaller-sized particles.

The disconnect between lab studies and field observations may be due to the differences in exposure time. For instance, De Sales-Ribeiro et al.83 observed translocation to the liver of zebrafish during a subchronic exposure and not during an acute exposure. Also, there may be multiple factors in the environment that may affect the likelihood of translocation. Damaged tissues

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**Figure 2.** Correlation between the number of suspected microplastics in different tissues, using Spearman’s rank correlation. (a) Correlation between the number of suspected microplastics in the gut and the number of suspected microplastics in the fillet and liver, using all individuals. One Lake Whitefish (LW) was excluded as it did not have gut data; (b) correlation between the number of suspected microplastics in the liver and in the fillet, using all individuals. Note that White Sucker does not have liver data. For all, regression line, ρ (R), and p-value are indicated.
(inflammation, lesions, etc.) make it easier for particles to get across the epithelial lining. This may also explain why we observed natural particles, like minerals, in the fillet samples (Figure S4d). While we typically avoid picking natural particles from samples, some were identified due to the similarity between colorful mineral fragments and plastic fragments. The evidence of natural minerals in the fillet suggest that physical particles are able to translocate, despite physiological mechanisms to prevent this. Additionally, there are many factors that affect the ability of a particle to translocate across tissues, such as surface charge, weathering, shape, particle rigidity, GI tract motor activity, and exposure in the environment. For instance, the formation of an eco-corona on microplastic spheres after environmental exposure resulted in a higher likelihood of internalization into macrophage cells compared to pristine microplastics with no eco-corona. In mammals, this form of transcellular uptake occurs through M cells in Peyer’s patches surrounding the intestinal mucosa, and only recently have cells with M cell-like activity been observed in salmonids and teleost fish. A more likely route for larger particles to translocate is through paracellular diffusion (or persorption). This process can accommodate particles of up to 130 μm and involves particles moving through the tight junctions between cells, but it is a rare process. Still, the most common particle size observed in the present study was >200 μm and is similar to the sizes seen in other field observations. None of these proposed mechanisms fully explain the presence of the larger particles, and more studies on aquatic organisms, specifically larger organisms like fish, are required.

**Patterns Relevant to Fate and Effects of Microplastic Translocation.** Across all species, there was a positive correlation between N_GI tract and N_Fillet+Liver (R²(66) = 0.29, p = 0.015; Figure 2a). However, for individual species, only White Sucker showed a significant correlation (no liver data for White Sucker). There have been a few proposed mechanisms for particle internalization. One is endocytosis, the internalization of particles through cells and into the circulatory fluid, which has been observed in mussels with 80 μm particles. In mammals, this form of transcellular uptake occurs through M cells in Peyer’s patches surrounding the intestinal mucosa, and only recently have cells with M cell-like activity been observed in salmonids and teleost fish. A more likely route for larger particles to translocate is through paracellular diffusion (or persorption). This process can accommodate particles of up to 130 μm and involves particles moving through the tight junctions between cells, but it is a rare process. Still, the most common particle size observed in the present study was >200 μm and is similar to the sizes seen in other field observations. None of these proposed mechanisms fully explain the presence of the larger particles, and more studies on aquatic organisms, specifically larger organisms like fish, are required.
Sucker; Figure S8a). Northern Pike and Lake Whitefish showed opposite trends, while the rest did not show any trend. Moreover, there was no correlation between \( N_{\text{Liver}} \) and \( N_{\text{Fillet}} \) (\( R_{adj}^2 = 0.21, p = 0.11; \) Figures 2b and S8b). These unclear trends suggest that short-term concentrations of microplastics in the GI tract are not correlated to what is in the tissues. This may represent the potential for bioaccumulation or growth dilution, with the concentrations of particles in the tissues likely representing chronic exposure. Bioaccumulation is the net result of all competing processes of uptake (both dietary and respiratory) and elimination, whereby the concentration of microplastics in the tissues exceeds the concentrations in the surrounding environment. \(^{51,52}\) Growth dilution occurs when the rapid growth of an organism results in a greater than proportional gain in biomass relative to the accumulation of particles in the tissues. \(^{91}\) Microplastics have been observed to be eggested relatively efficiently from the GI tract, \(^{24,92,93}\) but the rate of particle excretion from other tissues is less understood. To obtain a clear understanding of the kinetics of microplastics in organisms, future studies should investigate tissue-specific microplastic uptake, accumulation, and/or excretion over time.

Larger fish generally contained a higher abundance of microplastics. There was a positive relationship between \( N_{\text{Total}} \) and fish mass and length (mass: \( R_{adj}^2 = 0.15, p < 0.001, \) Figure 3a; length: \( R_{adj}^2 = 0.20, p < 0.001, \) Figure S9a). This trend was consistent across all species but significant for only Largemouth Bass (Figure S10). The trend based on GI tract likely relates to the increase in food volume required for larger organisms. \(^{94}\) To account for differences in fish size, we standardized the quantity of translocated microplastics by fish mass (\( C_{\text{Fillet+Liver}}; C_{\text{Fillet}} \)) in the case of liver particles, we standardized by liver mass (\( C_{\text{Liver}} \); see the Materials and Methods section). When \( C_{\text{Fillet+Liver}} \) were compared to fish size, there was a significant negative trend (mass: \( R_{adj}^2 = 0.078, p = 0.011, \) Figure 4a; length: \( R_{adj}^2 = 0.10, p = 0.0041, \) Figure S12a). When species were considered individually, statistically significance diminished (Figures 4b and S12b). This negative trend was also evident when \( C_{\text{Fillet}} \) (mass: \( R_{adj}^2 = 0.05, p = 0.036, \) Figure S11a,b; length: \( R_{adj}^2 = 0.066, p = 0.018; \) Figure S12c,d) and \( C_{\text{Liver}} \) (mass: \( R_{adj}^2 = 0.063, p = 0.034, \) Figure S11c,d; length: \( R_{adj}^2 = 0.078, p = 0.020; \) Figure S12e,f) were examined. Largemouth Bass and Smallmouth Bass showed a significant negative relationship of \( C_{\text{Liver}} \) with fish length but not mass (LB: \( R_{adj}^2 = 0.63, p = 0.021; \) SB: \( R_{adj}^2 = 0.69, p = 0.025; \) Figure S12f). However, sample sizes per species were low, and hence the results should be viewed with caution.

Still, these observations suggest the potential for either the excretion of translocated particles, as has been demonstrated with nanoparticles, \(^{67}\) or growth dilution. Growth dilution of contaminants is a known phenomenon, demonstrated with mercury in salmonid \(^{95}\) and organic ultraviolet absorbers in Barbell chub and Redbelly tilapia. \(^{96}\) Laboratory studies that increase our understanding of particle uptake and excretion, including the consideration of species-specific differences, are critical to confirm our results. Investigations must ask questions about whether microparticles are able to be excreted from the tissues and/or whether faster growth rates result in lower particle concentrations (i.e., growth dilution effect). Future field studies interested in bioaccumulation versus growth dilution should sample a wider variation of body sizes from within one species.

Though we observed a positive trendline, there was a statistically nonsignificant relationship between trophic level and \( N_{\text{Total}} \) (\( R_{adj}^2 = 0.02, p = 0.13, \) Figure 3b), \( C_{\text{Fillet+Liver}} \) (\( R_{adj}^2 = 0.013, p = 0.17; \) Figure S13a), \( C_{\text{Fillet}} \) (\( R_{adj}^2 = 0.0047, p = 0.25; \) Figure S13c), and \( C_{\text{Liver}} \) (\( R_{adj}^2 = -0.017, p = 0.80; \) Figure S13e). Other studies have also observed no increase in microplastic load in higher trophic levels, though these studies only used gut concentrations. \(^{51,97,98}\) Diepens and Koelmans \(^{99}\) theoretical MICROWEB model predicts trophic dilution rather than biomagnification. A more thorough study using organisms with a clearer food web structure is needed to test this hypothesis in the field.

The relationship of \( N_{\text{Total}} \) with body condition was examined as a proxy for fish health; no relationship was found (\( R_{adj}^2 = -0.0013, p = 0.34; \) Figure S9b). When comparing \( C_{\text{Fillet+Liver}} \) there was a significant positive relationship with body condition but with a low adjusted \( R^2 \) (\( R_{adj}^2 = 0.046, p = 0.042; \) Figure S13b), and when \( C_{\text{Fillet}} \) and \( C_{\text{Liver}} \) were examined, there was no significant relationship (\( R_{adj}^2 = 0.027, p = 0.093; \) \( R_{adj}^2 = -0.018, p = 0.87; \) Figure S13d,f). For discussion of these results, see the SI.

**Implications for Human Exposure.** Overall, the yearly intake of microplastics based on observations in this study ranged from 0 to 14 800 particles; however, this maximum is much higher than any other point and can be considered an outlier (Figure S14). If excluded, the maximum yearly intake of microplastics is approximately 5000 particles (Figure S14). Yellow Perch had the greatest variability, while Lake Whitefish had the lowest. Overall, if consumed, the majority of studied species (excluding Yellow Perch and Largemouth Bass) results in a mean yearly intake of <1000 microplastics (Figure S14). In comparison, Akhbarizadeh et al. \(^{28}\) investigated fish from the Persian Gulf and reported a mean yearly intake of 8788–28 860 microplastics based on a consumption of 300 g of fish per week. Using a consumption rate of 65 g/day (or 454 g/week) assumed here, this mean yearly intake becomes 13 315–43 727 microplastics. It is clear that while we used an above-average consumption rate, the microplastics observed in fillets of Lake Simcoe fish are still considerably less than that observed in the Persian Gulf. However, as noted previously, our results are constrained by a 63 μm mesh and may not reflect the whole burden. In contrast, Akhbarizadeh et al. \(^{28}\) had a size range as small as 2 μm. Still, the consumption of fish from Lake Simcoe may not be the significant pathway for microplastic exposure to humans. One analysis estimates that microplastic inhalation contributes 35 000–62 000 microplastic particles annually (considering particles >50 μm only). \(^{100}\)

In conclusion, we observed a high proportion of fish with microplastics in the three different tissue types examined—GI tract, fillet, and liver. The assemblages of shapes and material types within the tissues varied, suggesting that particle characteristics, other than size, are relevant to translocation. Moreover, body size, but not the trophic level, was a significant predictor of the total amount of microplastic in a fish. There was no correlation between the number of microplastics and body condition, which may be expected in Lake Simcoe where prey fish is plentiful for predatory fish. \(^{101,102}\) For translocated particles standardized by fish mass, there was a negative relationship with body size, but no relationship with trophic position.

The ability of microplastics to bioaccumulate and biomagnify is relevant to the question about its status as a persistent organic pollutant (POP). \(^{103}\) There are four characteristics that define a POP: persistence, long-range transport, bioaccumulation and biomagnification, and harmful effects to humans and wildlife (UNEP, 2001). While microplastics are known to have some of
the traits listed, including long-range transport, persistence, and harmful effects.4,105,106 The ability to accumulate up the food chain and over time requires more evidence. Here, our results suggest potential evidence of excretion or growth dilution rather than bioaccumulation in nature and no evidence for or against biomagnification across trophic levels. Further work is needed to confirm the patterns we observed and better understand the mechanism of translocation.

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c02922.

Additional information on the methodology of sample extraction and spectroscopy; further discussion on species and habitat differences and effects on body condition (Figures S1–14; Tables S1–10) (PDF)

Raw data of sampled fish (species, location, body size, trophic level, tissue weight, etc.) and microplastic characteristics (color, category, chemical ID, size, etc.) for all samples (XLSX)

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