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#### CONTRIBUTED PAPER

## Conservation Biology 🗞

## Microplastic contamination in Great Lakes fish

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Article Impact Statement: Great Lakes fish are heavily contaminated with microplastics; actions are needed to mitigate contamination in freshwater ecosystems.

#### Abstract

Freshwater ecosystems, generally adjacent to human population and more contaminated relative to adjacent marine ecosystems, are vulnerable to microplastic contamination. We sampled 7 species of fish from Lake Ontario and Lake Superior and assessed their gastrointestinal (GI) tracts to quantify ingested microplastics and other anthropogenic particles. A subset of the microparticles were chemically analyzed to confirm polymer types and anthropogenic origins. We documented the highest concentration of microplastics and other anthropogenic microparticles ever reported in bony fish. We found 12,442 anthropogenic microparticles across 212 fish (8 species) from nearshore Lake Ontario, 943 across 50 fish (1 species) from Humber River, and 3094 across 119 fish (7 species) from Lake Superior. Fish from Lake Ontario had the greatest mean abundance of anthropogenic microparticles in their GI tracts (59 particles/fish [SD 104]), with up to 915 microparticles in a single fish. Fish from Lake Superior contained a mean [SD] of 26 [74] particles/fish, and fish from Humber River contained 19 [14] particles/fish. Most particles were microfibers. Overall, >90% of particles were anthropogenic, of which 35-59% were microplastics. Polyethylene (24%), polyethylene terephthalate (20%), and polypropylene (18%) were the most common microplastics. Ingestion of anthropogenic particles was significantly different among species within Lake Ontario (p < 0.05), and the abundance of anthropogenic particles increased as fish length increased in Lake Ontario ( $\rho = 0.62$ ). Although we cannot extrapolate the concentration of microplastics in the water and sediments of these fish, the relatively high abundance of microplastics in the GI tracts of fish suggests environmental exposure may be above threshold concentrations for risk.

#### **KEYWORDS**

anthropogenic particle, freshwater, Great Lake, ingestion, microplastics, plastics

#### Contaminación por Microplásticos en Peces de los Grandes Lagos

Resumen: Los ecosistemas de agua dulce, generalmente contiguos a poblaciones humanas y más contaminados en relación con los ecosistemas marinos adyacentes, son vulnerables a la contaminación por microplásticos. Muestreamos siete especies de peces del Lago Ontario y del Lago Superior y analizamos sus tractos gastrointestinales (GI) para cuantificar los microplásticos ingeridos, además de otras partículas antropogénicas. Un subconjunto de las micropartículas fue analizado químicamente para confirmar los tipos de polímero y los orígenes antropogénicos. Documentamos la concentración más alta de microplásticos y de otras micropartículas antropogénicas en 212 peces (ocho especies) del Lago Ontario, 943 en 50 peces (una especie) en el Río Humber y 30,094 en 119 peces (siete especies) del Lago Superior. Los peces del Lago Ontario tuvieron la mayor abundancia promedio de micropartículas antropogénicas en sus tractos GI (59 partículas/pez [DS 104]), con hasta 915 micropartículas en un solo pez. Los peces del Lago Superior tuvieron un promedio [DS] de 26 [74] partículas/pez y los peces del Río Humber tuvieron 19 [14] partículas/pez. La mayoría de las partículas eran microfibras. En general, ≥90% de las partículas

eran antropogénicas, de las cuales el 35–39% eran microplásticos. El polietileno (24%), el tereftalato de polietileno (20%) y el polipropileno (18%) fueron los microplásticos más comunes. La ingesta de partículas antropogénicas tuvo una diferencia significativa entre las especies del Lago Ontario (p < 0.05) y la abundancia de las partículas antropogénicas incrementó conforme aumentó la longitud de los peces en el Lago Ontario (p = 0.62). Aunque no podemos extrapolar la concentración de microplásticos en el agua y los sedimentos para estos peces, la abundancia relativamente alta de microplásticos en los tractos GI de los peces sugiere que la exposición ambiental puede estar por encima del umbral de concentraciones para el riesgo.

#### PALABRAS CLAVE:

agua dulce, Grandes Lagos, ingesta, microplásticos, partícula antropogénica, plásticos

## INTRODUCTION

Microplastics (MPs) have been observed in all ecosystems (Koelmans et al., 2019; Law, 2017; Wang et al., 2019), and contamination in fish, an indicator of ecosystem functioning (Foley et al., 2018; Wang et al., 2020; Wright et al., 2013), is widespread. Although most work has been done in marine systems, freshwater fishes alone represent over 14,000 described species (Tedesco et al., 2017). Moreover, contamination in freshwater systems is dilute relative to the oceans and often directly adjacent to human populations—the source of plastic pollution. This may lead to concentrations in freshwater systems being typically higher than in the oceans. As such, filling knowledge gaps related to the factors governing exposure and impact of MPs on fish, especially in freshwater, is warranted (Wang et al., 2020).

Globally, 19-21 million tonnes of plastic waste were estimated to enter aquatic ecosystems in 2016 (Borrelle et al., 2020). Even under current commitment limits set by governments around the world, this number is predicted to more than double by 2030 (Borrelle et al., 2020). Plastic waste in marine and freshwater ecosystems break down into MPs, generally classified as <5 mm (Arthur et al., 2009) or <1 mm (Hartmann et al., 2009) in size. MPs are a complex, persistent contaminant class consisting of an array of sizes, polymers, colors, and morphologies and may contain pigments or additives (e.g., plasticizers or flame retardants) (Rochman et al., 2019).

The Laurentian Great Lakes (hereafter Great Lakes) contain 21% of the world's available freshwater (US-EPA, 2019) and valuable fisheries (DFO, 2005). The Great Lakes and its fish are also of great cultural significance to indigenous communities. Microplastics have been found in all five Great Lakes (e.g., Eriksen et al., 2013; Mason et al., 2016, 2020; Hendrickson et al., 2018) and their tributaries (e.g., Baldwin et al., 2016; McNeish et al., 2018; Grbić et al., 2020). The greatest concentrations reported are in Lake Ontario (Ballent et al., 2016; Mason et al., 2020). Despite the ecological and economic importance of Great Lakes fish, very limited data on their MP burden are available.

To understand MP contamination in freshwater fish residing in aquatic ecosystems with known MP contamination, we sampled fish from Lake Ontario, 1 of its tributaries (Humber River), and Lake Superior. We quantified and characterized MPs and anthropogenic particles in gastrointestinal (GI) tracts of 13 fish species with different body sizes and habitats. We predicted a lower abundance of MPs and anthropogenic particles in fish from Lake Superior relative to Lake Ontario due to differences in human population density and industry within their watersheds.

## **MATERIALS & METHODS**

#### Sample collection and processing

Fishes were collected from Lake Ontario from May to September 2015 via electrofishing in Humber Bay, Toronto Harbour (assisted by Toronto and Region Conservation Authority), and Hamilton Harbour (assisted by Ontario Ministry of the Environment, Conservation and Parks [MECP]) (Supporting information Appendix S1). Fish collections that did not involve endangered species were approved by Ontario Ministry of Natural Resources and Forestry (MNRF) Southern Region (permit 1079447) and followed the animal use protocol of MNRF's Aquatic Research and Development Section Animal Care Committee.

Species collected included brown bullhead (Ameiurus nebulosus), white sucker (Catostomus commersonii), yellow perch (Perca flavescens), round goby (Neogobius melanostomus), emerald shiner (Notropis atherinoides), common shiner (Luxilus cornutus), and spottail shiner (Notropis hudsonius). Small fishes, including fathead minnow (Pimephales promelas), round goby, and emerald and common shiner, were also collected from the Humber Bay and River by seining. Sample sizes and collection locations are listed in Supporting information Appendix S2. All fishes were euthanized by a blow to the head and kept on ice during transportation to the MECP laboratory.

For all fish collected, wet weight (grams) and total length (centimeters) were recorded and the GI tracts were dissected from the top of the esophagus to the anal pore. External tissue or fat was removed, and the entire tracts were placed in polypropylene (PP) containers for chemical digestion. For some fish, GI tracts were emptied by massaging the contents into the container. Emptied GI tracts were examined under a microscope for large, visible MPs and anthropogenic particles (herein referred to collectively as anthropogenic particles) (e.g., Lusher et al., 2017) before digestion.

Longnose sucker (*Catostomus catostomus*), white sucker, lake whitefish (*Coregonus clupeaformis*), round whitefish (*Prosopium cylindraceum*), cisco (*Coregonus* spp.), lake trout (*Salvelinus namay-cush*), and yellow perch were collected in 2016 at 3 locations in northern Lake Superior as part of the Ministry of Natural Resources and Forestry Lake Superior Fish Community Index Netting program (Supporting information Appendices S1 and S2). Monofilament gillnets (305 m) composed of panels ranging in mesh size from 114 to 152 mm were used. The total length (centimeters) was recorded. The GI tracts were dissected as above and stored in sterile high-density polyethylene whirl-pak bags, frozen, and forwarded to the MECP laboratory.

All samples were processed using 4N potassium hydroxide digestions, followed by wet peroxide oxidation and density separation were needed. Supporting Information contains details on processing (Appendix S3), quality assurance, and quality control (Appendices S3 and S4) (e.g., steps to avoid contamination, blanks, spike recoveries).

# Microparticle morphology, quantification, and extraction

Detailed descriptions of the quantification and extraction protocols are provided in Supporting information Appendix S5. Briefly, the filters were examined using a dissecting microscope (10-80× magnification; Leica S8 APO Stereozoom; Leica Microsystems, Canada). Images were taken of anthropogenic particles, which were quantified and categorized according to color and morphology (fragment, fiber, film, foam, sphere, and pellet [Rochman et al., 2019]), and irregular microbead and commercial fragments (Helm, 2017). Particle counts included only those confidently resembling anthropogenic particles based on visual characteristics and texture.

#### Particle characterization

A representative subsample of microparticles underwent spectroscopic analysis (2.5%, 5%, and 4% of particles from Lake Ontario, Humber River, and Lake Superior, respectively; n = 462 microparticles). Five to 10 representative particles were analyzed from each of a subset of individual fish (n = 55 fish, representing at least 10% from each water body) with Fourier transform infrared (FTIR) and Raman spectroscopy. Additional details regarding subsampling and chemical analysis are in Supporting information Appendix S6. A description of how we categorized microparticles by material type is in Supporting information Appendix S7.

#### Data analyses

Statistical analyses were performed in R. Two separate 1-factor analysis of variances (ANOVA) based on a permutation test

 $(\alpha = 0.05)$ , with factor being species, were performed for Lake Ontario and Lake Superior to test for differences in abundance of anthropogenic particles among species. Permutation-based ANOVAs were performed using the ImPerm package. A Tukey honestly significant difference (HSD) pairwise post-hoc test was run on all samples within lakes when findings were significant. A 2-way ANOVA was performed to test for differences in abundance between the 2 species sampled in both Lake Ontario and Lake Superior (yellow perch and white sucker) and was followed by a Tukey-HSD post hoc test to test for differences in particle abundance between species and lakes. Spearman correlations ( $\rho$ ) were used to assess the relationship between particle abundance and total fish length for each lake. For all findings, effects sizes  $(\eta^2, r, \text{Cohen's } d)$  were calculated using the heplots, rstatix, and coin packages. Plots were generated using the ggplot2 package. We used nonmetric multidimensional scaling (NMDS) to examine patterns of assemblage structure of anthropogenic particles (by morphology) among habitats and locations within a lake with 2-dimensional ordinations based on Bray-Curtis distances calculated with the function metaMDS in the vegan package. Three outliers from Lake Ontario were removed prior to analysis (white sucker, Humber Bay; white sucker, Toronto Harbour; brown bullhead, Hamilton Harbour), and 1 outlier was removed from Lake Superior (longnose sucker, Mountain Bay).

## RESULTS

## Particle quantification and confirmation

Overall, blank contamination was relatively low (mean of 2 particles/blank [SD 2] for Lake Ontario and Humber River, 2 particles/blank [SD 2] for Lake Superior) (Supporting information Appendix S8), and anthropogenic particle counts were not corrected based on blank contamination. Particles were sorted into material type groups based on spectroscopy (Supporting information Appendix S9); 90% of particles from Lake Ontario, 98% from Humber River, and 93% from Lake Superior were confirmed anthropogenic. Of these anthropogenic particles, 59%, 54%, and 35% were MPs for Lake Ontario, Humber River, and Lake Superior, respectively. Only 1-3% of particles were natural, and 0-7% were categorized as unknown. Of the synthetic polymers detected, PE, PP, polyethylene terephthalate (PET), polyethylene vinyl acetate (PEVA), and acrylic were most common (Supporting information Appendix S10).

A total of 12,442 anthropogenic particles were extracted from 212 fish collected across 3 locations in Lake Ontario (Supporting information Appendix S11a,b). Particle abundances ranged from 3 to 915 particles/fish (mean = 59 [SD 104] particles/fish (median = 26). Considerable variation existed among species, among locations (Figure 1a), and within habitats (Figure 1b). Three demersal fish were the most contaminated, including a brown bullhead from Hamilton Harbour and white sucker from Humber Bay and Toronto Harbour (915, 519, and 510 particles/fish, respectively). The permutation-based ANOVA indicated a significant difference in contamination among species for Lake Ontario (pooling location of



**FIGURE 1** (A) Number of anthropogenic particles per fish in Lake Ontario grouped by location (n = 211 fish) (1 outlier removed, brown bullhead [BB] with 915 particles), (B) mean number of anthropogenic particles for fish in 2 areas in Lake Ontario, (C) number of anthropogenic particles per fish in Lake Superior grouped by location (n = 119 fish) (1 outlier removed, longnose sucker [LS] with 790 particles), and (D) mean number of anthropogenic particles for fish in 3 areas in Lake Superior (horizontal line in bars, mean; whiskers or error bars, SD; bar ends in [A] and [C], range; CS, common shiner; ES, emerald shiner; FM, fathead minnow; SS, spottail shiner; WS, white sucker; YP, yellow perch; C, cisco; LT, lake trout; LW, lake whitefish; RW, round whitefish). Sample sizes are provided in Supporting information Appendix S2. Sample locations in lakes (A, C) were pooled for statistical analyses due to small and uneven sample sizes. Analyses were performed on the whole lake (Supporting information Appendix S13)

collection within the lake due to unequal sample sizes of species at each location) (p < 0.05,  $\eta^2 = 0.5$ ) (Supporting information Appendix S12). The effect size showed a moderate difference in contamination among species in Lake Ontario. White sucker and brown bullhead had significantly more particles than all other species (p < 0.05) and were not significantly different from one another (p > 0.05). (Supporting information Appendix S13a). Summary statistics for each species, location, and habitat are in Supporting information Appendix S14. Differences in contamination among fish residing in benthopelagic and demersal habitats (as described in Supporting information Appendix S2) were not significant within the lake; a result that was confirmed by a small effect size (Mann-Whitney U test; p > 0.05, r = 0.05). Benthopelagic species had a mean (SD) of 35 particles/fish (22) (median = 27 particles) compared with a mean of 62 particles/fish (110) for demersal species (median = 25 particles). Although the mean for demersal fish was nearly double that of benthopelagic, the variation among individuals within each habitat was high. A total of 943 anthropogenic particles were extracted from 50 common shiner collected

in Humber River (Supporting information Appendix S15). The mean (SD) number of anthropogenic particles was 19 particles/fish (14) (median = 15 particles). Abundances ranged from 2 to 68 particles/fish. A *t* test determined that there was no significant difference in mean abundance between common shiner in Humber River and Lake Ontario (p > 0.05, Cohen's d = 0.5) (Appendix S16); abundances were similar among locations and the effect size was moderate.

A total of 3094 anthropogenic particles were extracted from 119 Lake Superior fish (Supporting information Appendix S11c,d) (mean = 26 particles/fish [SD 74], median = 9, range 1-790) (Figure 1c). The most contaminated fish was a Mountain Bay longnose sucker (790 particles). The permutation ANOVA indicated no significant difference among species (pooling location of collection) (p > 0.05,  $\eta^2 = 0.03$ ) (Supporting information Appendix S17); the small effect size and pattern showed no trend (Supporting information Appendix S13b). Summary statistics for all species, locations, and habitats (as described in Supporting information Appendix S2) are provided in Supporting information Appendix S14. Mean (SD) anthropogenic



**FIGURE 2** Comparison of number of anthropogenic particles in white sucker (WS) (23 fish) and yellow perch (YP) (14 fish) from Lake Ontario (23 and 14 fish, respectively) and Lake Superior (8 fish for both species) (whiskers, range; box ends, quartiles; middle horizontal lines, medians; differing letters, groups are statistically different from each other)

particles was 21 particles/fish (18) (median = 12 particles) in benthopelagic species, 33 particles/fish (94) (median = 19 particles) in demersal species, and 36 particles/fish (26) (median = 11 particles) in pelagic-neritic species (Figure 1d). A Kruskal– Wallis nonparametric test determined no significant differences among habitats for the whole lake (p > 0.05,  $\eta^2 = -0.005$ ). Similar to Lake Ontario, variability among individuals was high (Figure 1d) and effect size was very small.

A 2-way ANOVA showed a significant interaction between species and lake (p < 0.05,  $\eta^2 = 0.13$ ) (Figure 2). The abundance of anthropogenic particles in fish was significantly different among lakes (p < 0.05,  $\eta^2 = 0.49$ ) and species (p < 0.05,  $\eta^2 = 0.22$ ). Anthropogenic particle abundances were higher for Lake Ontario (Figure 2), although this pattern was significant only for white sucker; effect size was relatively small for species and moderate for lakes. A Tukey HSD post-hoc test confirmed white sucker was significantly different from yellow perch in Lake Ontario (p < 0.05) and that white sucker was significantly different among lakes (p < 0.05). Yellow perch in Lake Superior was also significantly different from white sucker in Lake Ontario (p < 0.05); however, yellow perch was not significantly different among lakes (p > 0.05).

### Particle morphology and color

All morphologies were found in fish from Lake Ontario, except pellets. Fibers were predominant (62%), followed by fragments (36%) (Supporting information Appendix S11a,b). Most fragments (84%) did not possess physical features to indicate commercial or cosmetic product origin (irregular beads) (Supporting information Appendix S18a); 6% may have commercial origin and 10% cosmetic origin. The predominant particle colors were black, clear, blue, and white (Supporting information Appendix S19a). An NMDS plot showed no clear distinction among habitats or locations (Figure 3a,b), suggesting that the suite of MPs found in fish is not driven by location or

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preferred habitat. Still, there was greater variability among demersal species and in Humber Bay. Demersal species contained a larger proportion of the rarer morphologies (i.e., spheres, films, and foams) (Supporting information Appendix S11a).

Anthropogenic particles of all morphologies, except spheres and pellets, were found in Humber River. Fibers were most predominant (77%) followed by fragments (18%) (Supporting information Appendix S15). Of the fragments, many (87%) did not possess features to indicate commercial or cosmetic origin (irregular beads); 13% may have been commercially derived (Supporting information Appendix S18b). The predominant particle colors are black, clear, blue, and white (Supporting information Appendix S19b).

Anthropogenic particles of all morphologies were found in fish from Lake Superior, except pellets. Fibers were predominant (81%), followed by fragments (15%) (Supporting information Appendix S11c,d). Most fragments (92%) did not indicate commercial or cosmetic origin (Supporting information Appendix S18c); 8% may be of commercial and cosmetic origin. The predominant colors were black, clear, blue, and white (Supporting information Appendix S19c), and there was a relatively high number of pink and yellow films. No clear distinction existed for anthropogenic particle morphologies among habitats or locations within the lake (Figure 3b,d); however, Mountain Bay and Thunder Bay may have had slightly more variability (Figure 3d). Stacked bar charts appeared similar, except that the proportion of fragments in benthopelagic species was slightly higher (Supporting information Appendix S11c).

### **Body size**

When log total length of all species collected in Lake Ontario (n = 211 fish, excludes fish with incomplete data) was compared with total abundance of anthropogenic microparticles, there was a strong Spearman's rank correlation ( $\rho = 0.62$ , p < 0.05) (Figure 4a). There did not appear to be a single species driving the trend. While some species tended to be larger than others and there appeared to be groups of observations within the plot, variation within species existed for log total length and log microparticle abundance (Figure 4a). When the log total length of all species collected in Lake Superior (n = 79 fish, fish with incomplete data excluded) was compared with the log total abundance of particles (Figure 4b), a Spearman's rank correlation showed little correlation ( $\rho = 0.17$ , p > 0.05). No clear trend appeared within or among species (Figure 4b).

## DISCUSSION

## Anthropogenic particle contamination in freshwater fish

All fish sampled from Lake Ontario and Lake Superior were contaminated with anthropogenic particles, ranging in abundance from 2 to 915 particles/fish. The highest mean was 59 particles/fish (SD 104) (median = 26) in Lake Ontario, and



**FIGURE 3** Nonmetric multidimensional scaling (NMDS) results for morphology of particles in (A) benthic and demersal species from Lake Ontario (n = 209 fish, stress value = 0.09) and (C) benthic, demersal, and pelagic-neritic species in Lake Superior (n = 119 fish, stress value = 0.08). The NMDS results for morphology of particles in fish from 3 locations in (B) Lake Ontario and (D) Lake Superior. Three outliers were removed for Lake Ontario and 1 was removed for Lake Superior to the NMDS analysis

the maximum abundance of anthropogenic particles in bony fish globally is 915 particles/fish. Mean abundances reported in marine environments are typically 0-2 particles/fish (e.g., Boerger et al., 2010; Lusher et al., 2013; Güven et al., 2017). Freshwater fish tend to have a higher relative abundance of MPs in their GI tracts, ranging from <1 (Horton et al., 2018) to 13 particles/fish (McNeish et al., 2018), although data are limited. Our results also showed contamination may be more frequent in Lake Ontario nearshore fish than other study locations. In tributaries to Lake Michigan, 85% of fish GI tracts contain MPs (McNeish et al., 2018). In freshwater studies, occurrences range from 30 to 96% (e.g., Horton et al., 2017; Jabeen et al., 2017; Silva-Cavalcanti et al., 2017), whereas in marine studies, typical occurrences range from 20 to 40% of the fish sampled (e.g., Boerger et al., 2010; Lusher et al., 2013; Güven et al., 2017), although higher (Güven et al., 2017; Jabeen et al., 2017) and lower (Foekema et al., 2013) percentages have been reported. These results are consistent with results of other studies that show freshwater fish are exposed to relatively high concentrations of MPs and other anthropogenic particles. While individual variability is large, our results suggest there is potential for impact to populations and communities.



**FIGURE 4** Correlation among log total length of fish and log number of anthropogenic particles for several species in (A) Lake Ontario (n = 212 fish,  $\rho = 0.63$ , p < 0.05) and (B) Lake Superior (n = 79 fish,  $\rho = 0.17$ , p > 0.05)

Though logistically difficult, studies assessing population or community-level impacts in real-world situations may be critical to conservation of freshwater fish.

Although we cannot extrapolate the concentration of MPs in the water and sediments of these to fish, the relatively high abundance of MPs and other anthropogenic particles we observed in the GI tracts of fish, which is a snapshot in time, suggests environmental exposure may be above threshold concentrations for risk (Koelmans et al., 2020). Koelmans et al. (2020) report thresholds for risk at 11-521 particles/L. Although we examined fish and not water, the range overlaps with the number of microparticles per individual fish that we observed. As countries around the world work to build a risk-assessment framework for MPs, locations like the Great Lakes, where contamination is relatively high (e.g., Eriksen et al., 2013; Mason et al., 2016, 2020; Hendrickson et al., 2018), should be prioritized. This will inform conservation of fish relevant to MPs but should also be considered in the context of multiple stressors, recognizing that fish in the Great Lakes face

climate warming (Magnuson et al., 1997), invasive species (Mills et al., 1993), and other chemical pollutants (ECCC & US-EPA, 2017). For example, there is evidence that warming can increase MP ingestion (Ferreira et al., 2016), and that interactions with ambient aquatic pollutants can make MPs more toxic (Rochman et al., 2013b).

Fish from Lake Ontario had more than double the level of contamination of Lake Superior fish (mean = 26 particles/fish [SD 74], median = 9), though the Lake Superior value was influenced by 1 individual containing 790 particles from Mountain Bay (Figure 2). Fish from the Humber River had a mean (SD) abundance of anthropogenic particles equal to 19 particles/fish (14) (median = 15 particles), similar to global freshwater abundances, although only 1 species was collected. Fish-collection methods differed among lakes. Although sampling in Lake Superior may have allowed for digestion and egestion during sampling, the large differences in contamination among lakes suggest this does not explain the variation entirely. Higher concentrations in Lake Ontario are likely attributed to land use and the positioning of the lake in the watershed. Urbanization differs drastically among Lake Ontario and Lake Superior. The sampling locations in Lake Ontario were near Toronto (and Greater Toronto Area; population 5.9 million) and Hamilton (population 750,000), 2 major metropolitan centres (Statistics Canada, 2018). Sampling in Lake Superior was near the city of Thunder Bay (population 120,000) and in Nipigon Bay east of the smaller communities of Nipigon (population 1600) and Red Rock (population 900) (Statistics Canada, 2018). Microplastics have been found repeatedly at higher abundances, including in fish, near large population centers (e.g., Baldwin et al., 2016; Dris et al., 2016; Mani et al., 2015), and their presence is linked to proximity to wastewater treatment plants, storm drains, and roadways (e.g., Mani et al., 2015; Peters & Bratton, 2016; Sutton et al., 2019). Plastic-based industries are also suggested sources in Toronto area streams (Grbić et al., 2020) and adjacent shorelines of Lake Ontario (Ballent et al., 2016). A portion of the particles from major urban centres (e.g., Detroit, Buffalo, and Cleveland on Lake Erie) may flow into Lake Ontario as the last lake in the chain. However, based on modeled inputs (Hoffman & Hittinger, 2017) and the location and species of fishes sampled, local inputs are likely to dominate exposures. To inform future conservation efforts, additional research that examines the relative importance of point source contamination will be critical to mitigate effects of MPs and other anthropogenic particles.

We found a significant difference with a moderate effect size in particle abundance among species in Lake Ontario (Supporting information Appendix S13a). One potential cause is the complexity of each species' GI tracts. Jabeen et al. (2017) observed more MPs in fish intestines than stomachs, suggesting the particles were trapped by the coiled structures in the intestines or that particles have a longer transit time in the intestine relative to the stomach. The species collected in Lake Ontario have varying anatomical structures, perhaps contributing to varying abundances of MPs. Brown bullhead and round goby have a segmented GI tract, whereas white sucker does not. Moreover, demersal species tended to have higher anthropogenic particle abundance. Demersal species reside near or in contact with sediments, and Lake Ontario nearshore sediments are more contaminated than sediments collected offshore and from less urbanized locations (Ballent et al., 2016). The relatively high variation in particle morphologies observed in demersal fish and in Humber Bay (Figure 3a,b) is consistent with the range of morphologies observed in sediment from this location (Ballent et al., 2016). Although a higher abundance in demersal species is consistent with other locations (e.g., Jabeen et al., 2017; McGoran et al., 2017; Phillips & Bonner, 2015), some studies report higher abundance in pelagic species (Güven et al., 2017; Bessa et al., 2018) or no relationship (Lusher et al., 2013; Neves et al., 2015).

Differing species composition among studies and variation among individuals within studies, as is common for all feeding studies in the Great Lakes, makes it challenging to predict how habitat informs contamination and may contribute to the absence of statistical significance or effect. Overlap in habitat usage by benthopelagic and demersal fishes may also make predictions challenging. Patterns vary by location. In regions where sediment concentrations are high, demersal species are likely to be disproportionately affected. Larger fish may also be disproportionately contaminated, so we suggest collecting a range of body sizes. Thus, local monitoring is necessary to understand contamination in local populations and inform conservation priorities. We also recommend larger sample sizes be collected in future studies to further examine the magnitude of the effect of species on anthropogenic particle abundance, which can inform conservation decision-making.

The positive correlation between fish body size and particle abundance in Lake Ontario may be related to species characteristics, because some tend to be larger than others (Figure 4a). This relationship should be explored further to assess whether it may be related to trophic transfer or feeding preferences. We did not see a significant relationship between body size and anthropogenic particle abundance in Lake Superior. This variability is consistent with the literature (e.g., Güven et al., 2017; Horton et al., 2018; McNeish et al., 2018). Thus, this relationship should be explored further to determine the source of differences among species.

## Particle characteristics

Fish in the Great Lakes we examined contained a broad range of particle morphologies; diversity was greatest in fish from Lake Ontario. Fibers were most abundant, followed by fragments (Supporting information Appendix S11). This is consistent with most studies examining fish GI tracts (e.g., Jabeen et al., 2017; Horton et al., 2018; McNeish et al., 2018), as well as water in the Great Lakes and tributaries (Baldwin et al., 2016; Hendrickson et al., 2018). Fibers made up approximately 65% of all anthropogenic particles in Lake Ontario fish, which is relatively low compared with results of other freshwater and estuarine studies that report 75-100% fibers (Horton et al., 2018; McNeish et al., 2018; Pazos et al. 2017). This suggests that point and nonpoint sources are more numerous and diverse in Lake Ontario, particularly near urban centers. Initiatives to reduce MP contamination could include filters on washing machines (McIlwraith et al., 2019), stormwater treatment measures such as bioretention cells (Gilbraeth et al., 2019), litter-reduction initiatives, and ensuring best practices are in place at industrial facilities to contain plastics (e.g., Tsui et al., 2020). For Lake Superior, fibers accounted for approximately 80% of anthropogenic particles. Fibers are subject to long-range transport to remote regions (Allen et al., 2019; Bergmann et al., 2019) and are subject to atmospheric deposition (Dris et al., 2016), which may contribute to our finding of no clear patterns among species and habitats in the more remote Lake Superior. For further discussion about particle morphologies and potential source apportionment, see Supporting information Appendix S5.

# Effects of MPs and considerations for conservation

The severity of effects for MPs on biota varies in the literature from nominal to severe. Effects range from neutral to negative changes in growth and reproduction and reduced survival (Foley et al., 2018; Bucci et al., 2019). For fish effects vary depending on species and life stage and the size, morphology, and polymeric composition of MPs. Round, fragmented, and fibrous MPs typically have negative impacts on survival (Foley et al., 2018). Some MPs have been observed to elicit an immune response that may interfere with disease resistance (Greven et al., 2016), cause histological changes to the gills and blood (Karami et al., 2016), or cause inflammation and lipid accumulation in the liver (Lu et al., 2016). Translocation of MPs outside the GI tract has also been observed. Further studies are needed to assess MP accumulation, translocation, and subsequent effects.

In the Great Lakes, like other freshwater systems, fishes are responding to multiple stressors from climate change (Magnuson et al., 1997), invasive species (Kao et al., 2014; DeRoy & MacIsaac, 2020), nutrients (Kao et al., 2014), and other pollutants (ECCC & US-EPA, 2017). Microplastics may lead to increased vulnerability. For example, MPs may reduce available energy needed to respond to other stressors because reduced feeding has been observed in larval and juvenile fish exposed to MPs (Folev et al., 2018). Moreover, organic contaminants are present in the Great Lakes, sometimes at concentrations above thresholds for toxicity (ECCC & US-EPA, 2017). Microplastics accumulate many chemical pollutants, including organics and metals (Rochman et al., 2013a; Munier & Bendell, 2018; Zhang et al., 2020). Greater endocrine disruption and liver toxicity occurs when fish are exposed to MPs that have accumulated environmental contaminants versus virgin MPs (Rochman et al., 2013b; Rochman et al., 2014).

Although we cannot infer environmental exposure from the number of MPs in the GI tract of a fish sampled from nature, hundreds of particles inside the body of several fish suggest concentrations are relatively high. These concentrations in the Lake Ontario fish are striking and likely not unique to the Greater Toronto Area. This result suggests that freshwater fish living near densely populated cities may be exposed to high

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concentrations of MPs, with potential implications for freshwater biodiversity across the world. As such, we recommend a combination of future monitoring to better understand exposure and laboratory experiments to understand risk. For monitoring, we recommend a range of species that reside in different habitats, especially in lakes with relatively high contamination (e.g., later in lake order, surrounding urbanization, high levels of other contaminants). We stress the value of including sediment-dwelling organisms in monitoring programs because demersal species are often particularly likely to ingest anthropogenic particles, though collection of sediment-dwelling organisms is sometimes challenging with conventional technologies. We also recommend sampling water and sediments to quantify exposure, characterizing particles by size, material type, and morphology because all are important for toxicity. Laboratory exposures should be environmentally relevant including relevant concentrations and exposure scenarios. Experiments should be designed to inform potential population-level effects relevant for fish conservation. Combined, such future work could be used in a risk assessment framework.

Our findings are important for the conservation of freshwater ecosystems. Most MP studies focus on marine ecosystems, but our findings greatly exceed levels reported in marine studies. Freshwater ecosystems are critical for maintaining biodiversity, containing approximately 6% of all described species, including fish, in only 0.02% of global waters (Dudgeon et al., 2006; Tedesco et al., 2017). Monitoring across habitats, species, and locations is necessary to understand the fate of MPs. Species with relatively high contamination can be identified via local monitoring, which can inform conservation goals.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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