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Factors influencing the variation of microplastic uptake in demersal fishes from the upper Thames River Ontario☆

Colleen M. Wardlaw [a,*], Patricia L. Corcoran [b], Bryan D. Neff [a]

[a] Department of Biology, University of Western Ontario, Canada
[b] Department of Earth Sciences, University of Western Ontario, Canada

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ABSTRACT

Microplastics (plastic particles <5 mm) are abundant in aquatic environments, particularly near urban areas. Little is known, however, about how variations in microplastic abundances within watersheds affect fishes. Microplastics were examined in demersal fishes—white sucker (*Catostomus commersonii*) and common carp (*Cyprinus carpio*)—across 11 sites in the Thames River, Ontario, Canada. Microplastics were found in 44% of white sucker, ranging from 0 to 14 particles per fish, and 31% of common carp, ranging from 0 to 128 particles per fish. Across both species, the number of microplastics was higher in urban sites than rural sites, and there was a positive relationship between the number of microplastics in the fish and the abundance of microplastics in the sediment. Body mass was also positively related to number of microplastics in fish. Together these results provide insight into environmental and biological factors that may be influencing microplastic ingestion in demersal fishes.

1. Introduction

Plastic pollution is one of the most persistent and abundant forms of environmental pollution (Moore, 2008; Ryan et al., 2009). Between 1 and 13 Mt of land-based plastic debris are estimated to reach the marine environment every year, and this amount is predicted to significantly increase should current trends in production, population and waste management continue (Jambeck et al., 2015; Lebreton et al., 2017). The mass production and mismanagement of plastic waste has led to the accumulation of plastic in the environment both in water and on land (Barnes et al., 2009). Microplastics (plastic particles $\leq$ 5 mm; Arthur et al., 2009) are problematic due to their ubiquity and small size, thereby enabling ingestion by biota, which may lead to physical and toxicological harm (Wright et al., 2013; Anbumani & Kakkar, 2018; Rist & Hartmann, 2018).

The majority of microplastic studies have been conducted in marine environments, but the number of freshwater studies has been steadily increasing. It has been found that microplastic abundances are greater near urban and industrial land use areas, and that rivers flowing through these regions both carry and disperse microplastics to the surrounding environment (Tibbetts et al., 2018). It has been estimated that 80% of plastic debris released from land into the marine environment is transported by rivers with approximately three quarters of this amount entering rivers from improper waste management and littering (Law & Thompson, 2014; Gallo et al., 2018). Rivers have been found to perform key roles in both retainment and transportation of microplastics to larger water bodies (Lebreton et al., 2017; Schmidt et al., 2017; Yan et al., 2021). In considering that population density, urban runoff and wastewater discharge have clear connections to other varieties of environmental pollution entering rivers, microplastics may follow similar dispersal routes (Taebi & Droste, 2004).

Microplastic ingestion has been observed in a variety of fish species across many habitats (Lusher et al., 2013; Neves et al., 2015; Rummel et al., 2016; Munno et al., 2021). The amount of particles fish ingest varies widely across species. The susceptibility to microplastic ingestion may differ among fish species based on the zone in which the fish resides (pelagic versus demersal) as well as the behaviour through which the fish feeds (Rummel et al., 2016; Güven et al., 2017; Jabeen et al., 2017; Murphy et al., 2017; Cera et al., 2022). Other investigations indicate that microplastic ingestion differs among feeding guilds (Sun et al., 2019; Dantas et al., 2020; Hurt et al., 2020). Ingestion of microplastics may also vary based on individual characteristics, such as body size (but see

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* Corresponding author.
E-mail address: cwardlaw@uwo.ca (C.M. Wardlaw).

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Foekema et al., 2013; Güven et al., 2017; Vendel et al., 2017; Chan et al., 2019; de Vries et al., 2020; Cimmaruta et al., 2022). Microplastic ingestion may also be linked to environmental factors. For example, population-dense and industrial areas have been reported to greatly contribute plastic debris to aquatic environments, and a correlation between abundance of microplastics and urban land usage has been reported (Yonkos et al., 2014; Baldwin et al., 2016; Chen et al., 2022). Due to higher availability of microplastics in sediment and waters surrounding urban areas, it may follow that fish from these locations ingest greater amounts of microplastics than fish from rural areas. As rivers flow directly through both urban and rural areas, they provide an opportunity to examine local variation of microplastic ingestion among fishes from the same watershed.

Limited data are available regarding microplastic ingestion in both freshwater and demersal fishes. To better identify the factors that influence microplastic ingestion in these fishes, a watershed with recently characterized microplastic levels in sediment was used. Corcoran et al. (2020) documented microplastic abundances in benthic sediment of the Thames River, Ontario, Canada. A range of 6–2444 particles/kg dry weight sediment was reported, with factors such as urban locations, fine-grained sediment and high organic matter associated with greater microplastic abundances. These abundances provide reference for background levels of microplastic that fish may ingest. This in turn allows investigation of the potential covariation between microplastic levels in sediment and amounts being ingested by bottom feeding fish.

This paper aims to: (1) present the morphology, abundance and types of microplastics found in the gastrointestinal tracts of two demersal fishes of the Thames River, (2) determine if body mass relates to the number of microplastics in each fish, (3) compare the number of ingested microplastics between two common demersal species from the same river, and (4) compare the number of ingested microplastics with land use and previously reported benthic sediment microplastic levels.

2. Materials and methods

2.1. Location of study

The Thames River is the second largest watershed in Ontario, extending 273 km through southwestern Ontario (UTRCA, 1998, Fig. 1). The watershed is divided into two regions denoted as the upper and lower Thames rivers. The upper Thames River is separated into three branches (north, middle and south), and the lower Thames River is composed of one main channel that flows southwest from the City of London into Lake St. Clair. The Thames River passes through both rural and urban areas with varying population densities (Table 1; Fig. 1). London is the largest urbanized area that the river crosses, with a population of 383,822 and covering approximately 420 km^2 (Statistics Canada, 2016). Overall, the Thames River watershed is home to approximately 800,000 people. Corcoran et al. (2020) provided evidence that microplastics are present in benthic sediment across a range of sites in the Thames River, with the highest reported abundances near urban centers and areas with high levels of organic debris (Table 1). In the current study, 11 locations in the upper Thames River with varying microplastic concentrations were selected from the Corcoran et al. (2020) study to collect fish (Fig. 1).

Fish were collected between July and October 2020. Sampling locations were classified as 'urban' or 'rural' land use using the 2006 definition of land classification from Statistics Canada. An urban area has a population of at least 1000 and a density of 400 or more people/km^2, whereas areas with lower population are considered rural.

2.2. Study species: white sucker and common carp

This study examined two demersal (bottom dwelling) species of fish: white sucker (*Catostomus commersonii*) and common carp (*Cyprinus carpio*). White suckers are native to Ontario and can be found throughout the Great Lakes basin, residing in the riffles and pools of creeks and rivers, as well as lakes (Holm et al., 2009). White suckers typically feed

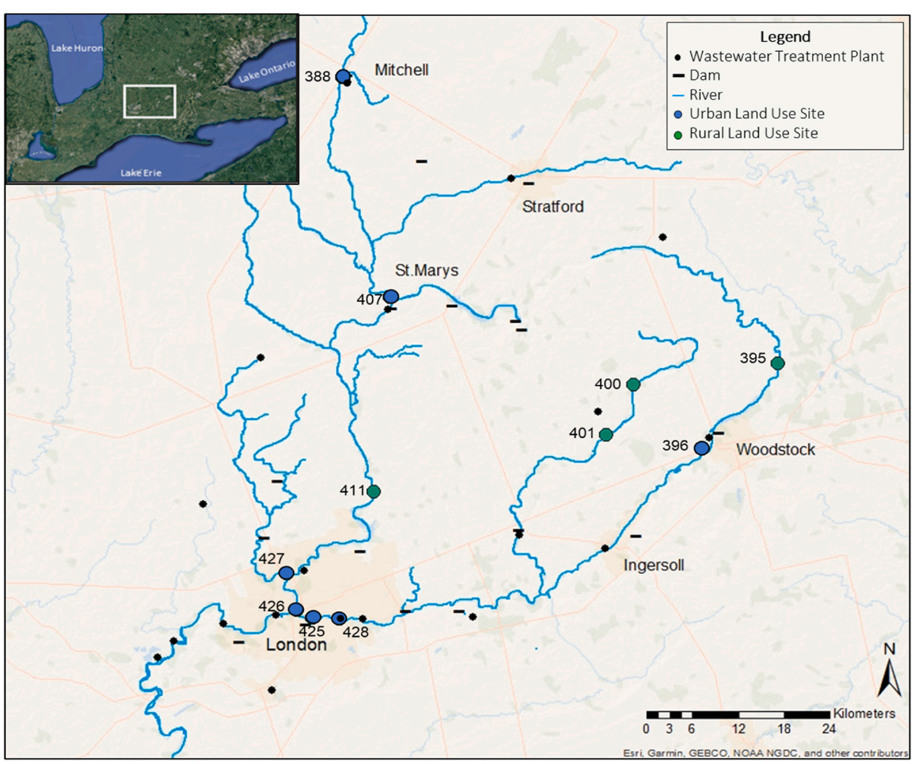

Fig. 1. Eleven sampling locations in the upper Thames River, Ontario. Colours of markers indicate sample locations as urban (blue) and rural (green). Map produced in ArcMap 10.4.1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Summary of common carp (*Cyprinus carpio*) and white sucker (*Catostomus commersonii*) collection sites in the upper Thames River, Ontario.

SITE:	388	396	407	425	426	427	428	395	400	401	411
Coordinates	43.4596, −81.2024	43.1267, −80.7794	43.2623, −81.1466	42.9742, −81.2390	42.9810, −81.2569	43.0134, −81.2688	42.9725, −81.2067	43.1911, −80.6907	43.1839, −80.8602	43.1387, −80.8928	43.0879, −81.1658
City/town	Mitchell	Woodstock	St. Marys	London	London	London	London	Innerkip	Braemar	Embro	Thorndale
Population density (per km^2)	951.3	835.3	582.5	913.1	913.1	913.1	913.1	29.4	29.4	15.4	30.4
Land Use	Urban	Urban	Urban	Urban	Urban	Urban	Urban	Rural	Rural	Rural	Rural
Substrate	silt	very fine sand	silt	fine sand	very fine sand	fine sand	fine sand	medium sand	fine sand	medium sand	fine sand
Organic Content	high	high	high	medium	high	medium	high	medium	medium	medium	high
Fragments (#/kg sediment)	470	182	31	150	1882	293	387	46	17	7	29
Fibres (#/kg sediment)	199	89	15	109	562	50	241	216	123	46	111

NB: Organic content, substrate and number of fragments/kg sediment and fibres/kg sediment as reported by Corcoran et al. (2020).

on aquatic insects, small crustaceans, molluscs, fish eggs, detritus, and plant material (Scott, 1967; Eder and Carlson, 1977). Common carp is an introduced species that exists in moderate abundance throughout southern Ontario, residing in shallow inland lakes, reservoirs, and rivers (Holm et al., 2009). Common carp exhibit opportunistic feeding behaviour, generally scavenging the substrate for aquatic vegetation, detritus and benthic macro invertebrates (e.g., larval insects, gastropods, crayfish) (Summerfelt et al., 1971; Eder and Carlson, 1977; Panek, 1987). As both white sucker and common carp exhibit feeding behaviours closely associated with sediment, they are good targets for examining the covariation between microplastic found in the sediment and those obtained from the gastrointestinal tracts of the fish.

2.3. Collection of fishes

Fish were collected using electrofishing and seine netting. Electrofishing was conducted using a HT-2000 Battery Backpack Electrofisher (Halltech Aquatic Research, Guelph, Ontario) with voltage settings of 150 v and a frequency of 80 Hz. Stunned fish were collected using a pole net. An alternative capture method used a minnow seine, (FIPEC industries, Grande-Rivière, Quebec) with specifications of a 15.2 m × 1.2 m net with a mesh size of 1.3 cm and a round central pocket. Fish capture by seine net involved two individuals holding the net with a weighted footrope across the bottom and headrope with floats at the water's surface in a 'U' shape. The net was dragged upstream with users wading against the current; fish were collected when the net was beached. All fish captured by either method were placed in a bucket containing oxygenated river water to prevent re-capture. Approximately 15 white suckers were collected at each of the 11 sites (n = 172 total) whereas common carp were captured at only 4 sites (n = 58 common carp total). Fish not matching target species were released. Following capture, fish were euthanized using a lethal dose of clove oil and measurements of total length (cm) and mass (g) were recorded (Table 2). Fish samples were transported on ice to Western University and stored at −20 °C until time of processing. All capture methods were carried out in accordance with Western University's animal care and use policies, Department of Fisheries and Ocean's Species at Risk act and Ministry of Natural Resources specimen collection guidelines.

2.4. Sample processing

Fish were removed from the freezer and allowed to thaw prior to dissection. A horizontal incision was made along the ventral side of the fish from the anal pore to below the pectoral fin. The gastrointestinal tract from each fish from the esophagus to the anal pore was extracted from each fish and the mass (g) was recorded using an aluminum dish. The gastrointestinal tracts from the fish underwent tissue digestion following a protocol adapted from Foekema et al. (2013) and Rochman et al. (2015), which has previously been used for microplastic retrieval in mussels and fish (Dehaut et al., 2016; Foekema et al., 2013; Lusher

Table 2
Summary of common carp (*Cyprinus carpio*) and white sucker (*Catostomus commersonii*) collected from 11 sites in the upper Thames River, Ontario. Body mass, total length and gastrointestinal tract (GI) mass are presented as the mean followed by the range in parentheses.

	URBAN							RURAL			
SITE:	388	396	407	425	426	427	428	395	400	401	411
White sucker											
Sample size (n)	15	14	15	15	16	16	15	15	15	21	15
Body mass (g)	12.2	27.8	28.1	11.2	20.9	33.8	11.7	36.8	2.2	26.6	15.4
	(3.9–30.1)	(3.1–53.4)	(4.7–151)	(5.7–19.4)	(2.3–119)	(3.1–363)	(5.1–43.2)	(7.2–117)	(1.8–13.8)	(3.5–142)	(4.1–59.7)
Total length (cm)	9.7	13.3	12.6	9.7	10.9	11.9	9.7	14.4	6.6	11.3	10.4
	(7.0–14.8)	(6.6–17.2)	(6.9–25.2)	(7.9–11.6)	(6.0–21.7)	(6.7–42.0)	(7.0–16.1)	(8.3–22.4)	(5.4–10.8)	(6.3–22.4)	(7.0–17.9)
GI mass (g)	0.86	1.94	1.98	0.80	1.51	2.74	0.78	2.55	0.23	2.24	1.08
	(0.2–2.1)	(0.2–3.3)	(0.3–9.8)	(0.4–1.4)	(0.2–8.2)	(0.2–32.0)	(0.3–2.5)	(0.6–6.8)	(0.1–1.4)	(0.2–16.7)	(0.2–4.7)
Common Carp											
Sample size (n)			1		8			22			27
Body mass (g)			70.1		692			489			218
			Na		(8.9–5443)			(18.3–5670)			(5.3–4899)
Total length (cm)			16.6					18.6			14.9
			Na		(7.8–71.2)			(9.4–71.0)			(71.1–80.0)
GI mass (g)			5.76		37.89			30.25			20.65
			Na		(0.5–71.2)			(1.4–300)			(0.4–477)

et al., 2017). Each gastrointestinal tract was submerged in a 20% KOH solution in a glass vessel and incubated in a drying oven at 45 °C for 48 h or until fully digested. The digested samples were filtered over a 10 μm polycarbonate membrane filter using a Nalgene vacuum filtration system. Samples containing large amounts of material after digestion were first size fractioned in 300 μm and 100 μm sieves and then were vacuum filtered. Both the material from the sieves and the filter papers were stored in glass petri dishes covered with aluminum foil until time of visual identification.

2.5. Visual identification

The material collected from the sieves and filters was visually examined using a Nikon SMZ 1500 stereomicroscope (Melville, NY) with a magnification range of 0.75–12x. Suspected microplastic particles were measured using NIS Elements (v 4.30) imaging software, counted and visually categorized based on colour and shape, and then placed on double sided tape inside a glass Petri dish. Two shapes of microplastics were found in this study and categorized based on being a fragment (irregular shaped, broken or separated from larger item; may be jagged) or a fibre (thread or filament-like structure; may be individual strand or bundled).

2.6. Material analysis

Material analysis was conducted to verify the composition of the particles obtained from the fish. From the collected particles, ~10% was subsampled and analyzed using FTIR spectroscopy at the Surface Science Western facility at the University of Western Ontario. Selection of particles was done using a random number generator, across the entire pool of particles sampled, resulting in the characterization of 32 particles from white sucker, and 19 particles from common carp. The selected samples were transferred to a diamond compression cell and were analyzed under a Hyperion 2000 microscope of a Bruker Tensor II instrument in transmission mode. The spectra were collected from 4000 to 600 cm^{-1}, with 32 scans and a resolution of 4 cm^{-1}. Composition of particles was determined qualitatively based on a match between the locations of the sample and reference peaks from the spectral library by a technician, rather than using % similarity to the library, to ensure the plastic type was appropriately identified from the environmentally exposed samples. A sample of double sided tape was also analyzed along with the particles to account for potential interference produced by the glue adhesive.

2.7. Quality control and contamination

As sample processing may introduce contamination (e.g., from equipment or airborne sources), measures for quality assurance and control were taken. Samples were prepared in laboratories with restricted access and low traffic and were processed in either a fume hood or under laboratory settings with filters fitted over air vents to limit airborne contamination. All samples were handled wearing nitrile gloves and a 100% cotton laboratory coat. Workstations were wiped down with Kimberly-Clark WypAll waterless cleaning wipes prior to working on samples. Equipment such as dissection tools and petri dishes were rinsed three times with reverse osmosis water prior to use and tools were cleaned between samples to prevent cross contamination. Visual identification of microplastics was performed on a stereomicroscope under a metal enclosure to further protect the sample from airborne contamination. All samples were kept covered with clean aluminum foil at all stages of processing.

Furthermore, procedural blanks ($n = 17$) containing 20% KOH were employed to act as negative controls for each sample batch (a batch consisted of between 12 and 20 fish samples) following the digestion and filtering methods. During each batch of dissections, a glass Petri dish filled with reverse osmosis water was left open during sample processing (~3 h) to serve as an air blank to document airborne contamination ($n = 12$). Microscope blanks ($n = 4$) in the form of double-sided tape on a microscope slide were also placed on the microscope stand during manual sorting of microplastics (~3 h) as another measure of airborne contamination. The procedural, air and microscope blanks were inspected under the stereomicroscope and particles resembling microplastics were counted and recorded. Both air blanks and microscope blanks contained fibres at much greater frequencies than observed for the fish samples or the procedural blanks, indicating that these latter methods capture fibre contamination at greater rates than the samples of interest. Therefore, correction of microplastic abundances based on blanks was accounted for using only the procedural blank. Particles found in procedural blanks on average amounted to 1 white fibre (range = 0–3, $n = 17$); therefore 1 white fibre was subtracted from each count from the fish when white fibres were detected. In addition, based on FTIR results, counts from fish were "normalized" by subtracting the proportion of non-plastic cellulose fibres identified in FTIR from numbers found in fish samples based on similarity in colour and shape. For example, if 2 of 3 black fibres were found to be cellulose, the number of black fibres would be corrected to a third of its original proportions in fish.

2.8. Statistical analysis

Data were checked for normality using a Shapiro-Wilk test. A general linear mixed effects model (lmer) was used to examine the relationship between body mass and the other study variables. Body mass was transformed using log10 to provide a normal distribution and compared with fixed factors of land use (with levels urban and rural) and species (with levels white sucker and common carp) and site included as a random factor. The number of fragments, fibres and suspected tire wear particles ingested by fish were examined using a generalized linear mixed effects model (glmm) with a poisson distribution that included species (with levels white sucker and common carp), body mass of fish and land use (with levels of urban and rural) as fixed factors, and collection site as a random factor. Spearman's rho was used to measure the relationship between the abundances of fragments and fibres previously found in sediment against the counts of fragments and fibres collected from fish. All statistical analyses were carried out using packages *dplyr* and *glmmTMB* in RStudio (version 4.0.2) and all figures were produced using package *ggplot2*. Results were considered statistically significant at $\alpha = 0.05$.

3. Results

3.1. Fish collections

A total of 230 fish were collected for this study, with 172 white sucker collected across the 11 sampling locations, and 58 common carp collected from four locations (Table 2). Body mass differed significantly between species (lmer; $F_{1,221.19} = 18.85$, $p < 0.001$) with common carp having larger mean body mass (70.1 g–691.9 g) than white sucker (2.2 g–36.8 g) (Table 2). The body mass of collected fish did not differ significantly between urban and rural sites (lmer; $F_{1,8.59} = 0.25$, $p = 0.63$). Similar patterns were observed for both body length and the mass of the gastrointestinal tract (Table 2).

3.2. Collected particles from fish

Overall, 485 particles were visually identified from the gastrointestinal tracts and categorized based on morphology as either fibres or fragments (Fig. 2). Fragments were the dominant particle type observed in the fish samples, comprising about 2/3 of the total particles. For procedural blanks used to document potential contamination of samples, all of the observed particles were fibres (Fig. S1).

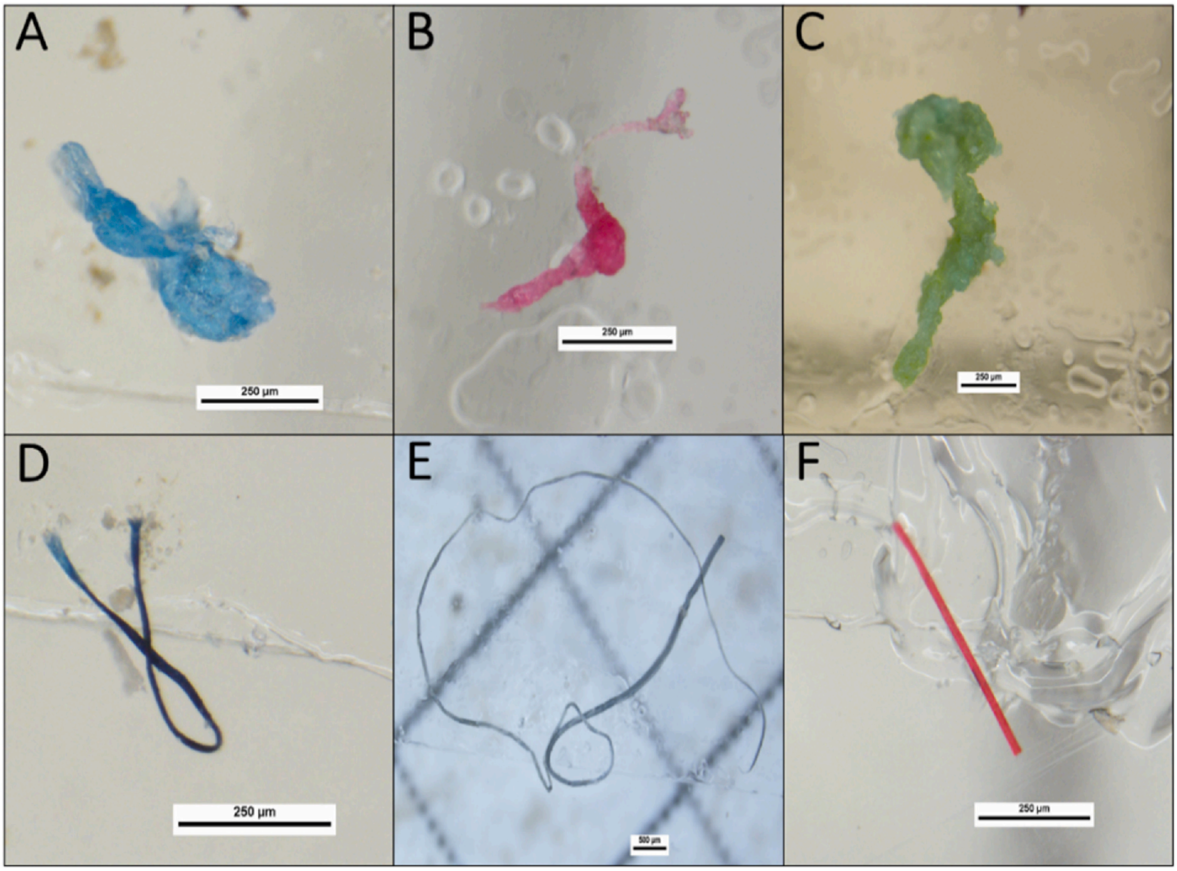

Fig. 2. Examples of microplastics collected from demersal fish in the upper Thames River, Ontario. Images show fragments (A–C) and fibres (D–F).

3.3. Identification of microplastics

A total of 25 fragments and 26 fibres collected from fish, and 9 fibres from blanks were analyzed for chemical composition using FTIR. Of the 25 analyzed fragments, the majority were black (79%), followed by blue and green (8%) and red, pink and yellow (4%). Colours of analyzed fibres were blue (36%), red (28%), white (16%), black (12%), clear (4%), and grey (4%); fibre colours found in low abundance in fish such as pink, purple and green were not analyzed. Fibres analyzed from blanks were mainly white (55%), followed by blue (22%), red (11%) and black (11%). Analyzed fragments were identified as polyvinyl chloride (PVC; 4%), polypropylene (PP; 4%), polyethylene (PE; 4%), acrylic paint (16%), possible industrial coating identified as a plasticizer (alkyd) + sodium carbonate (4%), a possible paint chip identified as red pigment + aluminosilicate (4%), and the majority of the fragments were unknown black particles (64%); these black fragments were the most common particles found in fish (Fig. 3; Fig. S2). The black fragments were not FTIR active and therefore produced weak spectra, with possible identifications as potential rubber with stearate, hydrocarbon, hydrocarbon ester, metal carboxylate components, carbon black, calcium carbonate and potassium bicarbonate. From the fibres, the majority were identified as cellulose (58%), followed by polyethylene terephalate (PET; 19%), acrylonitrile (12%), proteinaceous polyamide (PA; 4%), aramid fibre (4%), and Nylon (4%) (Fig. S2). Of the 9 particles analyzed from the blanks all were identified as cellulose.

3.4. Data correction

Based on the quantity of fibres identified as natural cellulose (natural composition), microplastic counts were corrected by subtracting the proportion of cellulose based on colour from each sample (i.e. each fish).

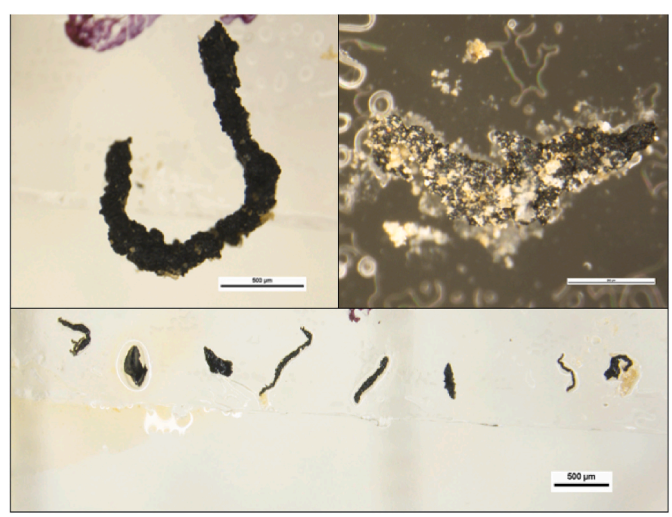

Fig. 3. Examples of unknown black particles collected from demersal fish from the upper Thames River, Ontario.

Cellulose was identified as 5/9 blue fibres, 3/7 red fibres, 3/4 white fibres, 2/3 black fibres and 1/1 gray fibre. Fibres of remaining colours (purple, pink, green) were uncommon in the fish (Table 3) and were not represented in the FTIR analysis, and so were not corrected. In addition to correcting data based on FTIR results, white fibres wherever present were assumed to be contamination and were removed from all samples given the proportions observed in blank samples. Following correction of the data, 375 microplastic particles remained. A new subcategory was made based on the number of black unknown fragments, which were

Table 3
Microplastic counts based on qualities of shape and colour from each of the 11 sites in common carp (*Cyprinus carpio*) and white sucker (*Catostomus commersonii*). Data presented in table has been blank corrected.

		Common Carp				White Sucker								
		Rural		Urban		Rural			Urban					
Colour	Shape	395	411	426	395	400	411	388	396	407	425	426	427	428
black	**fibre**	0	2	5	1	0	0	1	0	0	0	0	1	1
blue	**fibre**	2	2	4	0	3	2	1	0	0	1	2	2	1
clear	**fibre**	0	0	2	0	0	0	0	0	0	0	0	0	0
green	**fibre**	0	1	0	0	0	0	0	0	0	0	0	0	0
pink	**fibre**	0	0	0	1	0	0	0	0	0	0	0	1	0
purple	**fibre**	0	0	0	0	0	0	0	0	0	0	1	0	1
red	**fibre**	2	2	4	0	2	1	0	1	1	0	1	0	1
black	**fragment**	2	0	113	1	0	1	8	1	2	44	38	35	23
blue	**fragment**	0	0	4	0	3	0	3	0	0	1	2	4	1
clear	**fragment**	0	0	0	0	0	0	0	0	0	0	0	1	0
green	**fragment**	1	0	2	0	0	0	1	0	0	0	0	0	0
orange	**fragment**	0	0	0	0	0	0	0	0	0	0	2	0	0
pink	**fragment**	0	1	1	0	0	0	0	0	0	0	0	0	0
red	**fragment**	0	1	0	0	0	0	0	0	0	4	3	3	1
white	**fragment**	1	0	1	0	0	0	0	1	0	0	0	0	0
yellow	**fragment**	0	0	2	0	0	0	0	0	0	0	0	2	1
Total	TWP	2	0	113	1	0	1	8	1	2	44	38	35	23
	Fibre	4	7	15	2	5	3	2	1	1	1	4	4	4
	Fragment	2	2	10	0	3	0	4	1	0	5	7	10	3
	All	8	9	138	3	8	4	14	3	3	50	49	49	30

NB: All black fragments were classified as tire wear particles (TWP), so the fragment total does not include black fragments.

suspected to be tire wear particles on the criteria that the article was able to return back to original shape after compression, and there was no crumbling or breaking when compressed (Knight et al., 2020). Following corrections, the abundance of particles in fish was 15.2% fibres, 13.3% fragments and 71.5% suspected tire wear particles. Table 3 outlines the count data on microplastic particles collected from each site in each species following data correction. Most microplastics collected from the fish were in a size range between 200 and 800 μm (Table S1). Hereafter we consider only the corrected data.

3.5. Microplastics in fish following correction

Overall, 44% of white suckers (n = 76) and 31% of common carp (n = 18) contained at least one particle suspected to be microplastic in the gastrointestinal tract following blank- and FTIR-normalization of data. White suckers contained between 0 and 14 particles per individual, with an average of 1.27 (±2.25 SD), and common carp contained between 0 and 128 particles per individual with an average of 2.69 (±16.62 SD).

The number of microplastic particles identified from the gastrointestinal tracts did not differ between species for fragments (glmm; $X^2 = 0.43$, $p = 0.51$), fibres (glmm; $X^2 = 0.04$, $p = 0.83$) and suspected tire wear particles (glmm; $X^2 = 1.42$, $p = 0.23$).

Land use was significantly related to the number of fragments (glmm; $X^2 = 5.83$, $p = 0.01$) and suspected tire wear particles (glmm; $X^2 = 18.02$, $p < 0.001$), but was not related to number of fibres in fish (glmm; $X^2 = 0.0009$, $p = 0.97$; Fig. 4). In general, the fish collected from the locations around London (sites 425, 426, 427, 428) had a higher proportion of individuals with microplastic particles and those individuals had more particles (Fig. 4).

Body mass showed a significant positive relationship with the number of fibres (glmm; $X^2 = 59.28$, $p < 0.001$), the number of suspected tire wear particles (glmm; $X^2 = 25.90$ $p < 0.001$) and the number of fragments (glmm; $X^2 = 24.11$, $p < 0.001$). Thus, larger fish tended to have more particles in their gastrointestinal tracts.

A positive relationship was found between the number of fragments found in sediment and the number of fragments collected from the fish (Spearmans Rho; rho = 0.166 p = 0.01; Fig. 5A). However, no relationship was found between the number of fibres reported in sediment and number of fibres collected from the fish (Spearman's Rho; rho = −0.016 p = 0.80; Fig. 5B). A relationship with the suspected tire wear particles was not examined because there were no tire wear particles reported from the sediment samples (Corcoran et al., 2020).

4. Discussion

The compositions of microplastics identified from environmental samples varies from study to study, but often includes common types of plastic. Of the fibres analyzed by FTIR in the present study, 15 of 26 (58%) were identified as cellulose, and the remaining 11of 26 (42%) as consisting of plastic. These proportions are similar to those found in the sediment of the Thames River, wherein 67% of the fibres analyzed were cellulose and 33% were plastic (Corcoran et al., 2020). Large amounts of cellulose fibres are common in similar studies of rivers where natural based fibres have been found to outnumber plastic fibres (Stanton et al., 2019). The compositions of the plastic-based fibres collected from white sucker and common carp were also similar to those in sediment, with PET, acrylonitrile and Nylon, although fibres identified as PA and aramid (a type of PA) were found in the fishes but were not reported in the sediment. This could be a function of the small percentage of particles analyzed from each study, or that the PA and aramid particles in the sediment study were grouped with Nylon (a commercial name often used interchangeably with PA). The fragments analyzed by FTIR consisted of a variety of materials including PVC, PE, PP, acrylic paint, and possible matches to industrial coating and another variety of paint. These types of fragments were previously reported from the sediment (Corcoran et al., 2020) and are among the more common types of plastic used in society (PlasticsEurope, 2017). A review suggests that the most common types of plastics ingested by fish include PE, PP, polystyrene (PS), PA and PET (Sequeira et al., 2020). With the exception of PS, these types of plastic were observed in the samples of white sucker and common carp. Overall, the compositions of microplastics identified in this study align with those found in the sediment and are consistent with studies of other rivers and fishes.

Interestingly, the most common particles observed in the present study were black fragments that produced ambiguous FTIR characterizations due to unsaturated spectra. These fragments were composed of possible rubber (stearate or metal carboxylate), calcium carbonate, carbon black, potassium bicarbonate, and hydrocarbon. Based on these

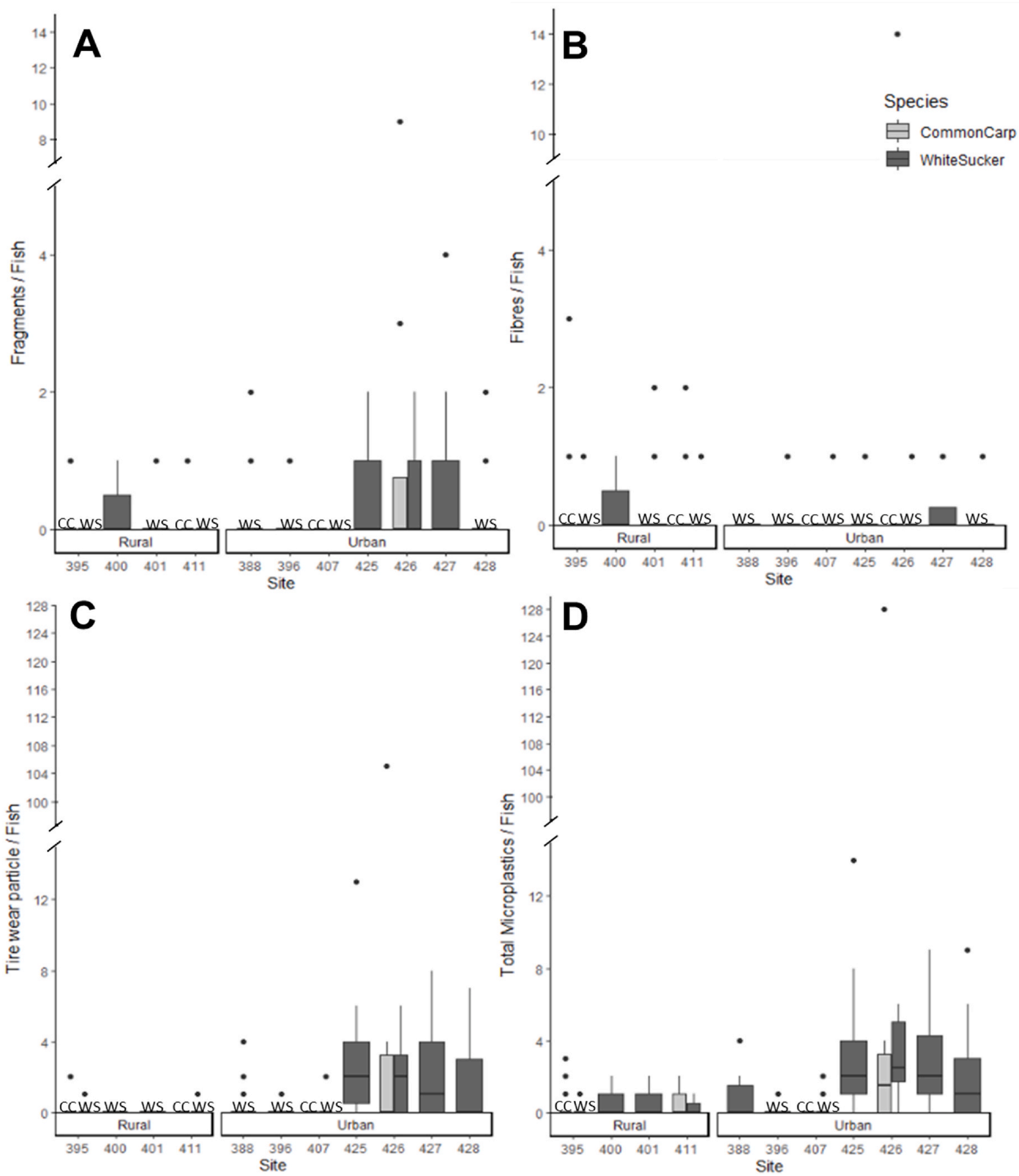

Fig. 4. Abundances of microplastic per fish collected from the 11 sampling sites. Panels display microplastic groupings as (A) fragments, (B) fibres, (C) suspected tire particles and (D) total microplastics. Common carp (*Cyprinus carpio*) is represented by light shade or 'CC' where box is not present, and white sucker (*Catostomus commersonii*) is represented by dark shading or 'WS'. The box shows the 25th and 75th percentiles, the whiskers show the 10th and 90th percentiles, and individual points show data that fall outside that range.

compositions, the ability to be compressed, in addition to morphological similarities to other studies that have identified tire wear in environmental samples (e.g., dark in colour, elongated or cylindrical in shape, coated with minerals, size range of 5–220 μm, see Kreider et al., 2010; Sommer et al., 2018), it is suspected that these black fragments found in the Thames River fish are tire wear particles. A total of 72% of all microplastics collected from the fish are suspected to be tire wear particles, with about one quarter of white suckers containing at least one tire wear particle, whereas fewer than 10% of the common carp contained a tire wear particle. Parker et al. (2020) reported 14% of individuals considered across five fish species to have ingested tire wear from an urbanized estuary of the Charleston Harbor, USA. There are few other studies, however, reporting suspected tire wear particles in fishes. Alternative sources of these black fragments may be asphalt, rubber playground turf, mulch, and crumb rubber (Gugliemotti et al., 2012). More research is needed to better understand the source of the black fragments in the samples and their prevalence in rivers and fishes more generally.

Microplastic levels show substantial variation among studies, even for studies considering the same species. A total of 44% of white suckers contained at least one microplastic particle, with a range of 0–14 particles per fish. A study of white suckers from creeks in Saskatchewan

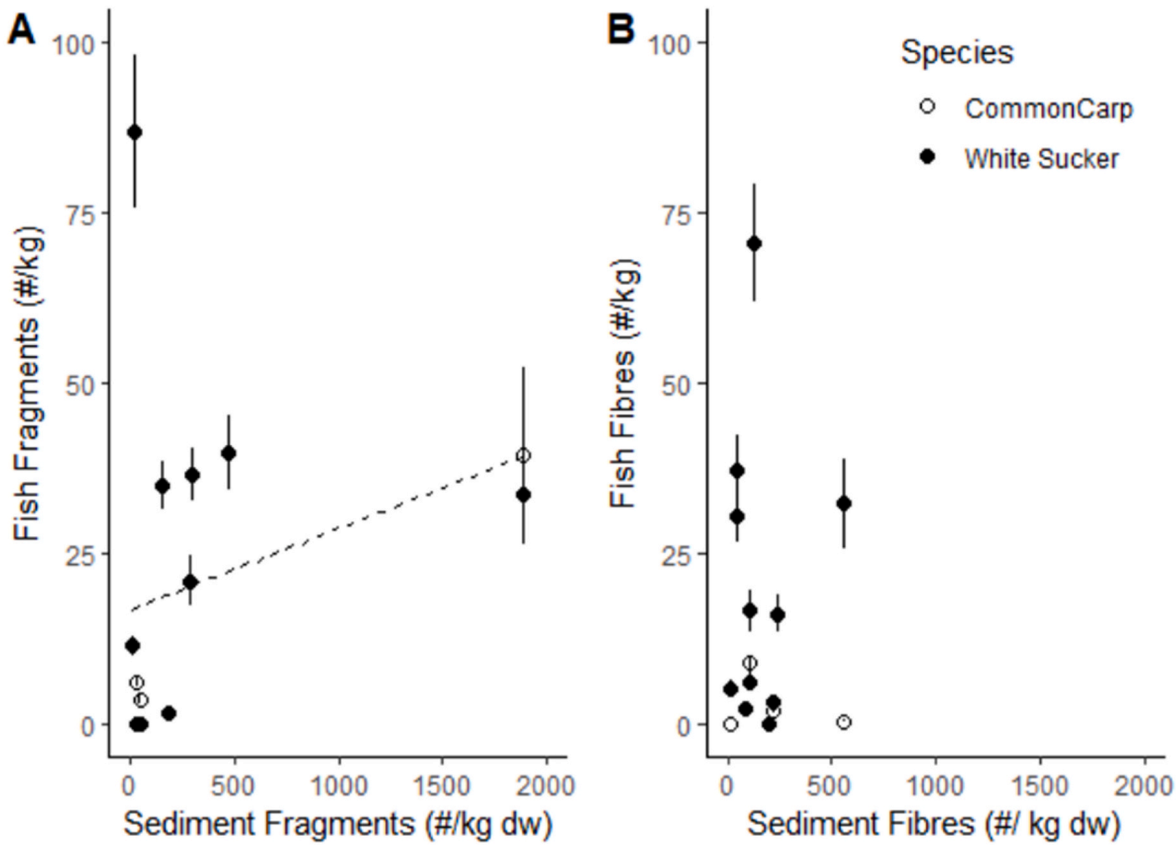

Fig. 5. Microplastic abundances present in sediment and fish shown by (A) fragments and (B) fibres. Sediment microplastics presented as a count of the total number of microplastic/kg dry weight sediment and number of microplastics in fish presented as mean microplastics/kg fish± SE. Common carp (*Cyprinus carpio*) are shown as open circles and white sucker (*Catostomus commersonii*) as filled circles, with the dotted line representing the regression line.

reported that 72% of 32 fish contained at least one particle (Campbell et al., 2017). Munno et al. (2021) reported white suckers from Lake Huron and Lake Ontario to contain a range of 0–510 particles per fish, whereas McNeish et al. (2018) reported white suckers in tributaries of Lake Michigan to contain a range of only 0–35 particles per fish. In the present study 31% of common carp contained at least one microplastic particle, with a range of 0–128 particles per fish. Another study of common carp from Lake Ziway in Ethiopia reported that 39% of 45 fish contained at least one microplastic particle (Merga et al., 2020). Baldwin et al. (2020) reported a range of 0–17 microplastic particles per fish in common carp from Lake Mead, USA, whereas Zheng et al. (2019) reported a smaller range of only 0–1 particles per common carp from the Pearl River, China. This variation in microplastic abundance across studies may reflect differences among sites in which white sucker and common carp were collected. For example, previous reports of microplastic abundances in the sediment of Lake Ontario are much higher than at the Thames River sites examined (Ballent et al., 2016; Munno et al., 2021), potentially explaining why white suckers collected from Lake Ontario contained higher numbers of microplastics than the Thames River. As number of microplastics in fishes differ across populations of the same species, considering additional factors related to land use and the presence of microplastics in sediment may help to understand variation.

Urban areas are known to be a major source of microplastics to rivers (Law, 2017), which may lead to greater microplastic levels in fishes from urbanized watersheds. Within the present study, fishes from urban sites had significantly more fragments and suspected tire wear particles in their gastrointestinal tracts than fishes from rural sites. In particular, fragments and suspected tire wear particles were most abundant at the four sites in London, the largest urban area included in the study. Indeed, Munno et al. (2021) found that within Lake Ontario, white suckers collected near the cities of Toronto and Etobicoke contained much higher abundances of microplastic than individuals collected offshore, suggesting that urban areas can influence microplastic numbers in fish (also see Peters & Bratton, 2016; McNeish et al., 2018; Garcia et al., 2020). Interestingly, there was no difference in the number of microplastic fibres between urban and rural fishes, whereas previous studies have shown fibres as the dominant particle type in fishes from urbanized watersheds (Peters & Bratton, 2016; Campbell et al., 2017; Silva-Cavalcanti et al., 2017; Bessa et al., 2018). However, it is important to note that some studies do not use FTIR or Raman spectroscopy for material analysis to distinguish natural and plastic materials, and therefore may overestimate the presence of plastic fibres in fishes. The lack of a relationship in the present study between land use and number of fibres may reflect the overall low abundance of fibres found in the fishes. Alternatively, the lack of relationship with land use may occur because fibres are more likely to remain suspended in the water column in rivers and carried downstream, thereby making them less likely to be ingested by the white sucker and common carp (see Lenaker et al., 2019). Indeed, previous studies that have found higher abundances of fibres in fish from urbanized rivers have typically examined non-demersal fishes (e.g., Peters & Bratton, 2016; McNeish et al., 2018). Regardless of microplastic particle type, this study adds to the growing evidence that urbanized areas are associated with greater microplastic uptake by fishes.

Sediment has been recognized as a sink for microplastics in aquatic environments (Browne et al., 2011; Woodall et al., 2014; Corcoran, 2015) and therefore sediment microplastic levels may affect the microplastic amounts found in fishes. There was a positive relationship noted between the number of fragments found in sediment and the number found in fish, but no relationship for fibres. Some studies have shown that microplastics have similar size, shape, colour and abundance in sediment and fishes, suggesting that fish may be picking up microplastics directly from sediment (Wang et al., 2019; Merga et al., 2020; Tien et al., 2020). In the present study, the most frequently observed microplastic particles in the fishes was tire wear, which was not observed in the sediment from these sites, suggesting that the source of these microplastic particles was not the sediment. However, tire wear particles have previously been reported in river sediment at 50–4400 mg/kg sediment in the Chesapeake watershed in USA, 26–4600 mg/kg sediment in Yodo watershed in Japan and 62–11600 mg/kg sediment in the Sein watershed in France (Unice et al., 2013), suggesting that tire wear may have been present in the Thames sediment, but sampling or processing methodology may have limited observations of it (see Corcoran et al., 2020). Alternatively, the lack of tire wear in sediment may suggest that it may not be the primary source of exposure of these microplastics, and that the fishes are ingesting tire wear particles from other substrates (e.g., algae, periphyton, decomposing organisms). Overall, based on the positive relationship with fragments, it appears that sediment levels of microplastic may be useful to predict individual abundance of fragments in demersal fishes, although this does not preclude other sources of exposure and ingestion of microplastics.

There is considerable uncertainty about the importance of body mass as a determinant of microplastic load in fishes. In the present study, there was a positive relationship between body mass and the number of fragments, fibres and suspected tire wear particles found in the gastrointestinal tracts of the white sucker and common carp. A relationship between body size and microplastic numbers has similarly been reported in a number of other studies of fishes (Boerger et al., 2010; Peters & Bratton, 2016; Horton et al., 2018; Huang et al., 2020; Parker et al., 2020; Cimmaruta et al., 2022), but not in all studies (Foekema et al., 2013; Güven et al., 2017; Vendel et al., 2017; Chan et al., 2019; de Vries et al., 2020; Atamanalp et al., 2022). This inconsistency across investigations may reflect both statistical and biological factors. For example, studies that include only a narrow range of body size may be less likely to produce a significant relationship than those that include a greater range of body sizes. McNeish et al. (2018) considered 11 species of river fish ranging from 4 to 12 cm and found only one species, which had one of the largest ranges in body size, showed a relationship between body size and the number of microplastics. Many studies that lack any relationship compare across pooled species which could mask species-specific effects of mass (Neves et al., 2015; Phillips & Bonner, 2015; Huang et al., 2020). However, even studies with larger sample sizes have reported a lack of any relationship (Chan et al., 2019; de Vries et al., 2020), suggesting alternative influences. The observed relationship between body mass and number of microplastics in fish may have also been the result of the model used, as variation across sampling sites, as well as potential exposure level to microplastic in the local environment (i.e., land use) were considered. Further research is also needed to disentangle microplastic retention from the actual amounts of gut contents, as larger fish tend to have greater amounts of gut content. Regardless, it isn't yet clear if, all else being equal, larger fish have more microplastics in their gastrointestinal tracts.

Biological variation among species may also be a source of variation in the number of microplastics found in the gastrointestinal tracts. In the present study there was no significant difference in the number of microplastic fibres, fragments or suspected tire wear particles in the gastrointestinal tracts of white sucker and common carp. This lack of difference may reflect the fact that these species exhibit similar foraging niches (Eder and Carlson, 1977) and may ingest microplastics at similar rates. Alternatively, a study of planktivorous fish by Lopes et al. (2020) indicated that the abundance of fibres ingested by fishes was species dependent, suggesting that different species may show differing patterns of microplastic ingestion despite similar foraging habits. Other studies of demersal feeding fishes have not found significant differences in the number of microplastics across such species from the same collection sites (Bellas et al., 2016; Chan et al., 2019). Instead, investigations that have shown differences in microplastic numbers among species have typically included both demersal and pelagic fishes or fishes from different feeding guilds (Mizraji et al., 2017; McNeish et al., 2018; Hurt et al., 2020; Koraltan et al., 2022). Although more research is needed, growing evidence suggests feeding and habitat use may be a factor determining ingestion rates of microplastic in fishes.

5. Conclusion

In conclusion, this study provides the first examination of microplastic abundances in fishes of the Thames River, ON. This study shows that depending on the type of microplastics, land usage and microplastic abundances in sediment can be key variables of interest that influence the number of microplastics in fishes. In addition, the number of microplastics in fishes may vary based on the body size of an individual. These results have provided new insight about specific factors that influence microplastic abundance in fishes, while being broadly consistent with previous studies that have shown that microplastics are abundant in fishes around the world.

Author statement

C.M. Wardlaw: Study conceptualisation and design, Methodology, Data collection, Formal analysis, Writing of original draft, reviewing and editing. P.L. Corcoran: Study conceptualisation and design, Review and editing manuscript, Supervision. B.D. Neff: Study conceptualisation and design, Review and editing manuscript, Supervision. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120095.

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