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Microplastics in White Sucker (Catostomus commersonii) and Common Carp (Cyprinus carpio) from the Upper Thames River, Ontario.

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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 variation in microplastic abundances within watersheds affects fishes. Microplastics were examined in demersal fishes—white sucker (*Catostomus commersonii*) and common carp (*Cyprinus carpio*)—across 11 sites in the Thames River, Ontario. Microplastics were found in 44% of white sucker, ranging from 0-14 particles per fish, and 31% of common carp, ranging from 0-128 particles per fish. Across both species, the number of microplastics in fish 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 the variation of microplastic ingestion in demersal river fishes.

Keywords

Microplastic, plastic pollution, Thames River, demersal fish, riverine fish, white sucker, common carp, FTIR

Summary for Lay Audience

Microplastics (plastic particles <5 mm) are a widespread form of pollution in the aquatic environment, and are of concern as they have been reported to be ingested by a number of organisms. Rivers often have high levels of microplastics, however few studies have been conducted in tributaries of the Great Lakes. In addition, limited information is available regarding factors that influence microplastic ingestion in bottom-dwelling fishes in rivers. Therefore, this study investigates a potential connection between sediment microplastic levels and ingestion by bottom feeding river fishes. This study also examines other factors that may influence ingestion of microplastics, such as differences among species, urban versus rural land use, and body size. Building on a previous study of microplastics in bottom sediment from the Thames River, Ontario, white sucker and common carp were collected from the upper Thames River. Overall, 44% of white sucker and 31% of common carp were found to contain at least one microplastic particle. Microplastics found in fish consisted of fragments, fibres and suspected tire wear particles, with the latter found in the greatest abundance. The number of microplastics in fish was found to be related to the body mass of individuals, with larger fish containing more microplastics. However, the number of microplastics did not differ between species, and this may be attributed to the similar way in which they feed. Land usage was related to number of suspected tire wear particles and fragments in fishes, but not fibres. Similarly, the number of fragments in fish were found to be related to abundance of fragments in sediment, but fibres lacked a relationship. Findings from this study show that individual factor of body size, as well as environmental factors such as land use and abundance of microplastics in sediment influence the number of microplastics that may be ingested by fishes. Overall, this study found evidence of microplastics in bottom-dwelling river fish in the Great Lakes system, and is the first study on biota of a proposed long-term investigation of microplastics in the Thames River.

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List of Abbreviations

Abbreviation	Meaning
FTIR	Fourier-transform infrared spectroscopy
HDPE	High density polyethylene
KOH	Potassium Hydroxide
LDPE	Low density polyethylene
PA	Polyamide
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride
WWTP	Wastewater treatment plant

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1 Introduction

1.1 Plastic Debris

1.1.1 Brief History of Plastic

Human influence on the environment has created a number of negative impacts including exploitation of natural resources and a variety of pollution forms. Plastic pollution has been noted as one of the most persistent and abundant forms of pollution to date (Moore, 2008; Ryan et al., 2009). Directly linked to anthropogenic activity, plastic pollution is considered as far-reaching, long-lasting and comparable in harm to climate change (Malizia & Monmany-Garzia, 2019). Plastic has been suggested as one of the markers of the currently proposed, human-induced epoch known as the Anthropocene, due to its ubiquity in the environment (Zalasiewicz et al., 2016).

The first use of synthetic plastics was in the form of Bakelite, which was created in 1907 to replace items that were expensive and becoming increasingly difficult to obtain, such as ivory and silk (Davis, 2015). Consumer plastic use fully emerged post World War II when it began to replace everyday items, but at a fraction of the cost and with rapid production. This resulted in ‘Throwaway living’, a term first coined in 1955 in Life magazine, describing the notion that disposable goods were more convenient and attractive as they cut down on household chores (LIFE, 1955). Modern consumerism has made the use of plastic into everyday goods and services unavoidable, including food and beverage packaging, fibres used to make clothing, construction and transportation materials, and technological enhancements. Consequently, mass exploitation and production of plastic goods can be viewed as problematic because plastic endures longer than the consumer service it provides. This is of particular concern with regards to the environment, as plastic debris has been reported to accumulate both on land and in aquatic ecosystems. Plastic remains widely used, as the attributes of being an inexpensive, adaptable material provides endless opportunities for application.

1.1.2 Plastic Types and Usage

Plastics are manufactured with different chemical properties for a wide range of functional uses. In general, plastics are relatively low density and mouldable resins that are unique in that they can be modified to produce desirable qualities for many different applications. Additives are often used to alter the properties of the plastic depending on the desired purpose (e.g., increased flexibility and hardness). Additives include pigments, foaming agents, plasticizers, fillers, flame retardants, antioxidants, lubricants, anti-microbials and heat stabilizers (ACC, 2005; Geyer, 2020). Some plastics have predominantly industrial applications, such as polyvinyl chloride (PVC), whereas others range in flexibility, such as polypropylene (PP) and polyethylene (PE), which have many everyday uses (Table 1.1). In Canada, the largest user of plastic materials is the packaging sector (ECCC 2020). This is in line with global plastic production, which estimates that 40% of plastics produced are being used for packaging, with a significant amount being used specifically for food and beverages (UNEP, 2016). Common types of packaging plastics are high density PE (HDPE) and low-density PE (LDPE) as films, however other plastics, such as polyethylene terephthalate (PET) and PP, are also used. Globally, PE and PP are the most produced plastics (Andrady & Neal, 2009). Textiles have also been noted to be a common source of fibre pollution to the environment, as they are typically composed of a blend of materials such as polyamide (PA), PET, acrylic and dyed cellulose-based fibres. Table 1.1 outlines different types of plastic, their applications, their approximate time to degrade and the amount of each type produced globally in 2017.

1.1.3 Production and Waste Management

The production of plastic has increased rapidly due to the combination of economic and population growth as well as technological advance. In 1950, the global production of plastic was estimated at 2 million metric tons (Mt), and in 2019, production was 368 Mt (Geyer et al., 2017; Geyer, 2020; PlasticsEurope, 2020). With the exponential rise in plastic production, waste management becomes increasingly important. Due to the durability of plastics, the ability to be effectively recycled or biodegrade varies;

Table 1.1 Main plastic types, common applications, time of degradation and production amounts. (1) Andrady & Neal, 2009, (2) PlasticsEurope, 2019, (3) Vieira et al., 2021, (4) Chamas et al., 2020, (5) Geyer, 2020.

Type of Plastic	**Acronym**	**Examples of Common Uses [1,2]**	**Approximate degradation time (years)[3,4]**	**Global Production (in 2017)[5]**
Polyethylene terephthalate	PET	Textiles (polyester), Soft drink & water bottles, Salad domes, Biscuit trays, Salad dressing containers	< 450	35.0 Mt
Polypropylene	PP	Packaging films, Bottles, Tubs, Potato chip bags, Straws, Microwave dishes, Kettles, Garden furniture, Lunch boxes, Packaging tape, Glass replacement, Pipes, Automotive parts	20-30	74.5 Mt
Low density polyethylene	LDPE	Plastic wrap, Garbage bags, Squeeze bottles, Sandwich bags, Trays and containers, Irrigation tubing, Mulch film	> 1000	70.1 Mt
High density polyethylene	HDPE	Shopping bags, Toys, Freezer bags, Milk and juice bottles, Ice cream containers, Shampoo bottles, Chemical & detergent bottles, Buckets, Rigid agricultural pipe, Crates		56.9 Mt
Polystyrene	PS	Food containers, Plastic cutlery, Packaging, CD and video cases, Building insulation , Imitation glassware, Low-cost brittle toys, Electrical/electronics	>500	26.3 Mt
Polyvinyl chloride	PVC	Window shutters, Furniture upholstery, Plumbing pipes and fitting, Cling film , Roof sheeting, Floor and wall covering, Garden hoses, Bottles, Automotive parts	> 100	39.4 Mt
Polyamide	PA	Textiles, Carpets, Automotive industry, Kitchen utensils, Sports wear	-	61.2 Mt (PP&PA)
Polyurethane	PUR	Building insulation, Pillows and mattresses, Insulating foams	-	30.7 Mt
Other Plastics				
Acrylonitrile butadiene styrene	ABS	Hub caps		
Polybutylene terephthalate	PBT	Optical fibres	-	17.5 Mt
Polycarbonate	PC	Eye glass lenses, roofing sheets		
Poly(methyl methacrylate)	PMMA	Touch screens		
Polytetrafluoroethylene	PTFE	Cable coating (telecommunication)		

none of the most commonly used varieties of plastic are biodegradable (Geyer et al., 2017). Efforts to recover plastic items are met with a number of additional challenges in recycling. Plastic types may be grouped into families of thermoplastics that may be heated and remoulded (e.g., PE, PP, PET and PVC), or thermosets, which are resistant to mechanical, chemical, and heat forces making them unable to be remoulded (e.g., unsaturated polyester resins, polyurethane (PUR), Epoxide) (Plastics Europe, 2019;

ECCC 2020). Furthermore, plastic waste is a heterogenous mixture that requires careful consideration when sorting. Plastics are often produced with a variety of additives and fillers that cannot be mixed when recycling, as the type and content of additives is regulated and may impact quality of later applications (Eriksen et al., 2018). Recycling also becomes difficult when the thermoplastics targeted for recycling have low melting points, and therefore may not completely destroy impurities such as food residue, labels and other contaminants that remain after cleaning (Schyns & Shaver, 2021).

The short-lived usage of single use plastics in combination with its durability introduces a disposal challenge, as the lifespan of the plastic greatly outlasts the application (Table 1.1). As of 2015, a total of 6300 Mt of plastic debris had been produced globally, and of this amount, 9% had been recycled, 12% incinerated and 79% left to accumulate in landfills or find its way into the environment (Geyer et al., 2017). Canada's waste management follows this trend. In 2016, of the 4667 kilotons of plastic brought to the Canadian market, 9% was recycled, 4% was incinerated for energy, 86% disposed of in landfill, and 1% released to the environment (ECCC, 2020). This in turn allows for greater proportions of plastic waste to accumulate in landfills and/or to leak into the environment. Between 1.15 and 12.7 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 quality of waste management continue (Jambeck et al., 2015; Lebreton et al., 2017). The mass production and mismanagement of plastic waste has ultimately led to the accumulation of plastic in the environment both in water and on land (Barnes et al., 2009). Once in the natural environment, plastic debris may pose a significant risk to organisms (See section 1.3).

1.2 Microplastics

'Microplastic' is a term that was first coined by Thompson et al. (2004) and was used to describe small particles of plastic found in marine water and sediment samples. The definition was later refined by Arthur et al. (2009) to describe plastic particles ≤5 mm in their largest dimension. Other size classifications of plastic debris include macroplastics (>25 mm) and mesoplastics (5-25 mm) (Lee et al., 2013). The term nanoplastic has also been used to capture the lower subsection of the microplastic size range, defined as plastic particles 1-100 nm in at least one dimension (Gigault et al., 2018). Following the microplastic size class as defined by the Government of Canada (ECCC, 2020), microplastics will be defined as plastic particles ≤5 mm for the purpose of this thesis.

Microplastics have been described based on origin, in which they are produced in a primary or secondary manner (Cole et al., 2011). Primary production occurs when plastics are purposefully manufactured in the micro (<5 mm) size range. Primary microplastics are used for industrial purposes, such as pre-production pellets, which are melted and poured into moulds to make plastic products, or as beads in consumer products, such as exfoliants for cosmetic cleansers. Microplastics are considered secondary if they result from degradation of larger plastic items. This breakdown is driven by environmental exposure, which damages the integrity and chemical properties of the plastic, causing it to become brittle. Environmental processes that weaken plastics include photodegradation, biodegradation, thermo-oxidative degradation, abrasion from weathering, and mechanical breakdown, such as wave action (Andrady, 2011; Corcoran, 2021). Examples of secondary microplastics are rubber particles from tire wear, and fragments from larger plastic items (also known as plastic 'confetti').

Microplastics are also categorized by morphology, with the main groupings being pellets, beads, fibres, fragments, foams, and films (Figure 1.1). Researchers use morphology as a way to identify the application associated with the plastic, such as fibres from textiles and pellets from industrial stock (Rochman et al., 2019). Fragments and fibres are the most common particles identified in environmental samples. Fibres may be of natural origin or plastic based. For example, natural fibre, such as cellulose, may come from animals or plants, whereas plastic fibres are often composed of PA, PP or PET. Rayon is an example of a fibre that is composed of plastic, but is produced from cellulose (Dris et al., 2018). It is therefore important to further categorize microplastics according to chemical composition in addition to morphology.

Microplastics outnumber the amount of larger fraction plastic debris in the environment, however they contribute only a small fraction to the total mass (Cózar et al., 2014). The abundance of microplastics is increasing because larger plastic articles break down

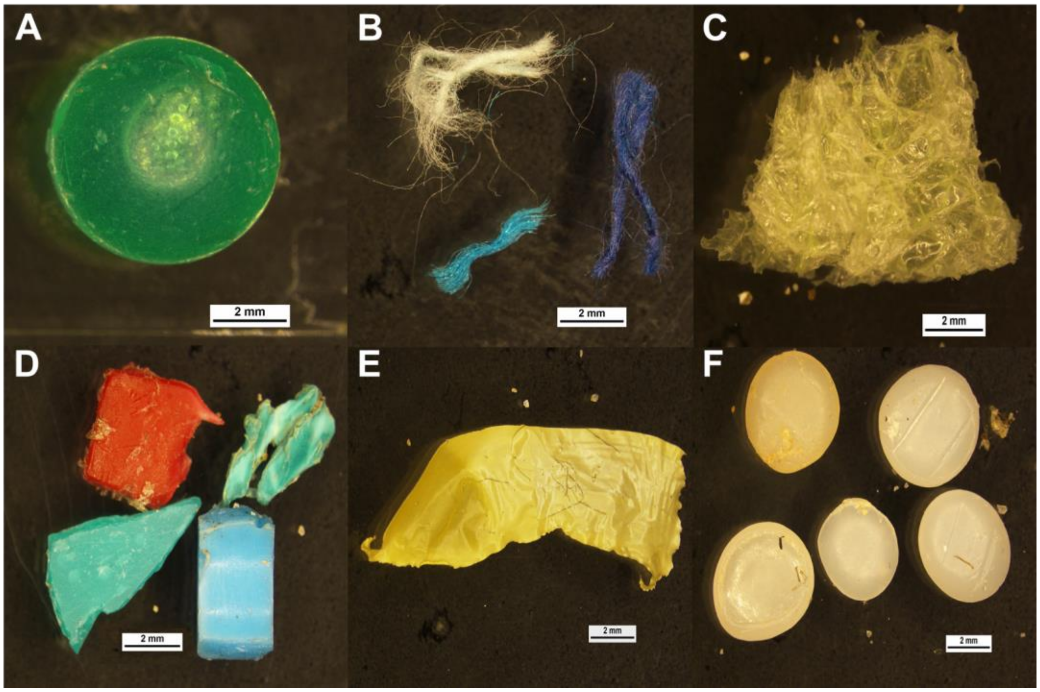

Figure 1.1. Examples of morphologies of plastic debris with associated description and example. (A) Bead: spherical in shape and smooth in texture, (B) Fibre: thread or filament-like structure; may be individual strand or bundled, (C) Foam: fragment of spongy material that may have pockets of trapped gas or be solid, (D) Fragment: irregular shaped, broken or separated from larger item; may be jagged, (E) Film: thin moderately flexible sheet-like structure, and (F) Pellet: generally elliptical, round, or cylindrical.

continually. A study by Efimova et al. (2018) simulated fragmentation of plastic items in a coarse bottom swash zone, and found that plastic items 2 cm in size generated 3.6×10^4 (LDPE), 1.1×10^6 (PS), 5.5×10^2 (PP) and 2.0×10^4 (PS foam) microplastics after 24 hours. Another issue with microplastics in the environment is that their small size and plastic properties contribute to high mobility. Most plastic items are low density and buoyant and therefore a large proportion of plastic debris items float, which facilitates long-range transport (Geyer et al., 2017). In combination with other mechanisms, such as atmospheric and hydrological transport, microplastics can be readily transferred away from source locations and be widely dispersed. In terms of spatial distribution, microplastics have been reported globally in both aquatic and terrestrial habitats, and have even been found in Arctic regions (Lusher et al., 2015a; Bergmann et al., 2017; Huntington et al., 2020).

1.2.1 Sources of Microplastics to Aquatic Environments

Microplastics have diverse sources and pathways, especially with respect to aquatic systems. Aquatic-based sources contribute 2% of microplastics to the environment and are mainly generated by shipping and fishing related activities (Boucher & Friot, 2017). Fisheries and aquaculture employ a variety of equipment made from synthetic materials such as nets, lines, and floats, and plastic materials are incorporated into boats, such as paint and anti-fouling coatings (Lusher et al., 2017). This gear generates secondary microplastics. Deshpande et al. (2020) reported that approximately 380 tons of plastic-based commercial fishing gear is lost each year in Norway alone, and over time, this gear will produce secondary microplastics.

The vast majority (98%) of plastic entering the aquatic environment originates from land-based sources. Major pathways from land to aquatic environments include wastewater effluent (25%), road run off (66%), and transport by wind (7%) (Boucher & Friot, 2017). A variety of factors control the abundance of microplastics in aquatic systems, including catchment size, location of wastewater treatment plants (WWTPs), hydrological dynamics (e.g., water flow, storm events), waste treatment (e.g., landfills), land use (e.g., urban, rural, forest, agricultural), and population size (Yonkos et al., 2014; Eerkes-Medrano et al., 2015). Plastic debris is more likely to be generated in areas with higher waste production, such as in centers with high population density and industrial activities (Andrady, 2017). For example, accidental spills of pre-production resin pellets within factories and during transportation results in pellets being deposited into water bodies (Mato et al., 2001; Corcoran et al., 2020a). In general, greater microplastic abundances in urbanized areas is a trend identified in a number of studies (Baldwin et al., 2016; Ballent et al., 2016; Dikareva & Simon, 2019; Townsend et al., 2019; Grbić et al., 2020). A substantial amount of microplastics emitted from urban areas are tire wear particles. These particles entering watersheds are correlated with vehicle traffic, which is common in population dense areas. Tire wear particles are responsible for 28% of secondarily produced microplastics entering oceans, with 0.23-4.7 kg generated per year (Boucher & Friot, 2017; Jan Kole et al., 2017).

Fibres are another common type of microplastic, representing 35% of secondarily produced microplastics globally, with an estimated 0.28 Mt entering aquatic environments annually (Boucher & Friot, 2017; Belzagui et al., 2020). Wastewater treatment plants have been noted as pathways for fibre transport (Browne et al., 2011; Dris et al., 2015). A major contributor of fibres to WWTPs is water from domestic washing machines (Napper & Thompson, 2016). A study of wastewater treatment in Glasgow on the River Clyde found that although 98% of microplastics were retained and removed, effluent still discharged 6.5 million microplastic particles daily (Murphy et al., 2016). A review by J.Sun et al. (2019) examined capture of microplastics in WWTPs, and found between 1 to 10,044 particles/L in influent and 0 to 447 particles/L in effluent. With the wide variety of sources of microplastics to the aquatic environment, microplastics have been found to accumulate in marine and freshwater environments globally.

1.2.2 Microplastics in Marine Environments

Plastic debris in the marine environment has been suggested to be one of the most significant forms of pollution (Barnes et al., 2009). Plastic debris was first recorded in marine surface waters in 1972 in the western north Atlantic Ocean, with an average of 3500 objects and 290 g/km^2 (Carpenter & Smith, 1972). Since this time, many more studies have gathered evidence on the abundance of plastic debris in the marine environment, offering a more comprehensive image of the prevalence and consequences of plastic pollution. Modeling of microplastic pollution has estimated that > 5.25 trillion microplastic particles are floating on the surface of the oceans globally, weighing approximately 270,00 tons (Eriksen et al., 2014; Van Sebille et al., 2015).

The physical characteristics of the plastic itself, such as density, buoyancy, size and shape, can play a role in the transportation and fate of microplastics (Horton & Dixon, 2018). For example, a low-density material such as polystyrene (PS; 0.045 g cm^3) floats and is therefore easily transported in surface waters. In contrast, PVC, with a higher density of 1.1-1.58 g cm^3 will more likely become deposited in sediment (Zhang, 2017). Studies of microplastics in surface water generally employ surface water trawls in transects to collect samples, whereas benthic sediment sampling involves sediment coring or grabs in order to determine the mass, concentration or general counts of microplastics in a given area. Both benthic sediment and surface water are important in determining microplastic concentrations in the environment because each matrix involves microplastic capture in different ways. For example, samples collected from the North Sea contained 2.8-1188.8 particles/kg sediment, and 0.1- 245.4 particles/m^3 in surface waters (Lorenz et al., 2019). Surface water samples differ from sediment based on factors that influence the movement and deposition of microplastics in marine environments, as well as freshwater.

Different marine settings may have different capacities to accumulate plastic debris. A study by Law et al. (2010) used plankton net tows in transects on the Caribbean Sea and North Atlantic Sea to map spatial patterns and concentrations of plastic debris between 1986 and 2008. The authors found that >60% of tows contained plastic, with the highest concentration of 20,300 pieces/km^2 in the North Atlantic Subtropical Gyre. Gyres are systems of rotating ocean currents, and these currents often carry and trap microplastics (Moore et al., 2001; Ryan et al., 2009; Lebreton et al., 2012; Eriksen et al., 2013a; Cózar et al., 2014). Estuaries and coastal settings have also been shown to contain high microplastic abundances because they receive plastic debris from both marine and inland sources; the latter include urban areas, and sites of river outflow (Ryan et al., 2009). For example, Claessens et al. (2011) found that the average concentration of microplastics in harbour sediment from the Belgian coast (166.7 particles/kg) was significantly higher than the continental shelf (97.2particles/kg) and beaches (92.8 particles/kg). This highlights that the large proportion of microplastics being accumulated in the marine environment is greatly attributed to areas of human activity.

1.2.3 Microplastics in Freshwater Environments

The majority of microplastic studies have been conducted in marine environments, but freshwater studies have been steadily increasing. Microplastics have been reported from freshwater lakes worldwide, including in Asia (Free et al., 2014; Wu et al., 2018), Africa (Egessa et al., 2020), North America (Eriksen et al., 2013b; Ballent et al., 2016; Dean et al., 2018; Anderson et al., 2017;) and Europe (Imhof et al., 2013; Fischer et al., 2016; Faure et al., 2017; Leslie et al., 2017). Lakes may function as collection sites for microplastics due to a variety of source waters entering a semi-closed basin. Distribution may depend on lake morphology such as lake size, shape and depth (Belontz et al., 2021), current circulation and weather events (Cable et al., 2017; Hoffman & Hittinger, 2017), proximity to high population areas and plastic industries and proximity to inflowing tributaries (Ballent et al., 2016; Corcoran et al., 2020a). A study of two lakes in Italy showed that surface water microplastic concentrations were 2.7- 3.4 particles/m^3 in Lake Chiusi and 0.8- 4.4 particles/m^3 in Lake Bolsena, with sediment concentrations of 234 and 112 particles/kg in lake Chiusi and Bolsena, respectively (Fischer et al., 2016). This study demonstrates that concentrations differ in relation to nearby land inputs and lake-related features such as catchment area, surface area, depth and wind pattern. In Taihu Lake, China, microplastic concentrations were reported at 3.4–25.8 particles/L in surface water and 11–235 particles/kg in sediment, and 0.2-12.5 particles/g reported in Asian clams (*Corbicula fluminea*) (Su et al., 2016). This lake is located proximal to one of the most populated areas in China, which is thought to contribute microplastic inputs through large amounts of effluent and waste from rivers and non-point sources. Similarly, microplastics in Lake Victoria in eastern Africa ranged from 0.02–2.19 particles/m^3, with areas of the lake containing greater abundances thought to be correlated with higher intensity human activity (Egessa et al., 2020).

It has been estimated that 9887 tons of plastic debris enter the freshwater Laurentian Great Lakes system per year (Hoffman & Hittinger, 2017), and studies focusing on this area have reported varying levels of microplastics. Eriksen et al. (2013b) reported an average of 43,000 particles/km^2 in surface waters of the Great Lakes, with an extrapolated >466,000 particles/m^2 as a maximum. This is comparable to the highest concentrations reported from the North Pacific Subtropical Gyre. Other studies have reported surface water microplastic concentrations of ~ 17,000 particles/km^2 from Lake Michigan (Mason et al., 2016), and 0.8 particles/L from surface waters of Lake Ontario (Grbić et al., 2020). Microplastics are also common in benthic sediment of the Great Lakes. Nearshore, tributary and beach sediment from Lake Ontario have been reported to contain average abundances of 980, 610 and 140 particles/kg of sediment, respectively, with the highest concentration reported at 28,000 particles/kg in Etobicoke Creek (Ballent et al., 2016). Sampling of 66 beaches along the Laurentian Great Lakes resulted in 12,595 pellets, for an average of 19.1 pellets/m^2; factors such as population density, presence of plastic industries, beach grain size and evidence of past spills were found to be related to pellet abundances on beaches (Corcoran et al., 2020a). For Lake Erie, Dean et al. (2018) found concentrations of 0-391 particles/kg sediment in nearshore samples, 50-146 particles/kg from beach samples and 10-462 particles/kg sediment from tributary samples. A general consensus found throughout freshwater studies is that microplastic abundances are greater proximal to urbanized and industrial land use areas, and rivers that pass through these hotspots distribute microplastics.

Rivers have been found to perform key roles in both retainment and transportation of microplastics to larger water bodies. 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 estimate entering rivers from improper waste management and littering (Law & Thompson, 2014; Gallo et al., 2018). The quantity of plastic reported to enter oceans sourced from rivers has been estimated at between 1.15 and 2.41 Mt (Lebreton et al., 2017). Rivers hold higher microplastic concentrations than marine environments because they flow through inland microplastic sources and there is less water volume to assist in dilution (McCormick et al., 2016). Factors that influence the distribution of microplastics in rivers include land use, population density, catchment area, rainfall, channel morphology, and amount of organic debris (Ballent et al., 2016; Corcoran, et al., 2020b). In considering that population density, urban runoff and wastewater discharge have clear connections to other varieties of environmental pollution entering rivers, microplastics may be integrated in this or follow similar dispersal routes (Taebi & Droste, 2004).

Similar to other water bodies, microplastic concentrations vary both among and within watersheds. Baldwin et al. (2016) surveyed floating plastic debris in twenty-nine great lakes tributaries and found a maximum concentration of 0.03 particles/L with the majority (98%) of items found to be microplastics. From source waters into Lake Ontario, Grbić et al. (2020) found 15.4 particles/L in storm water, 13.3 particles/L in waste water, and 0.9 particles/L in agricultural runoff, which demonstrates the significance of urban areas as suppliers of microplastics. In comparing the abundance of microplastics in different water bodies in the Yangtze delta region, Luo et al. (2019a) noted abundances in the freshwater systems of city creeks and rivers (1.8–2.4 particles/L) to contain greater microplastic abundances than in estuary and coastal areas (0.9 particles/L). The difference was attributed to proximity to city centers. Microplastics have also been reported in bottom sediment of rivers from various countries. For example, the Ganga River in eastern India reported between 99 and 410 particles/kg (Sarkar et al., 2019), tributaries of Lake Michigan contained a range of 33 to 6229 particles/kg (Lenaker et al., 2019) and the Rhine River in Germany contained 228-3763 particles/kg in shoreline sediment and 786-1368 particles/kg from river sediment (Klein et al., 2015). Overall, rivers both retain and are a major transport pathway for microplastics from inland sources to lakes and oceans. And with far reaching a prevalent nature of microplastics, a number of risks can be presented where biota come into contact with microplastics.

1.3 Hazards of Microplastics to Aquatic Life

1.3.1 Range of Influence

Microplastics are widely dispersed and accessible to biota in aquatic environments. Evidence of microplastics in biota was first noted by Carpenter et al. (1972) in their study of plastic ingestion in fish from Niantic Bay, following the initial discovery of plastics in neuston net samples from Sargasso Sea by Carpenter & Smith (1972). Since that time, much more research has been conducted that investigates microplastic ingestion by organisms occupying different environments. To date, microplastics have been found in cetaceans (Besseling et al., 2015; Lusher et al., 2015b) , seabirds (Provencher et al., 2015; Hamilton et al., 2021), fishes (Boerger et al., 2010; Neves et al., 2015), decapods (Farrell & Nelson, 2013; Watts et al., 2014), bivalves (Van Cauwenberghe & Janssen, 2014; Li et al., 2019), zooplankton (Cole et al., 2013; Desforges et al., 2015), and corals (Hall et al., 2015; Hankins et al., 2021). Impacts to primary producers have also been identified (Besseling et al., 2014; Bergami et al., 2017).

Microplastic quantification in field collected organisms document real conditions under which ingestion occurs. Some of the quantities recorded from mussels include 1.1-4.4 particles/g (Courtene-Jones et al., 2017), and in fish, 2.1 ± 5.8 particles/fish (Boerger et al., 2010). Although a variety of organisms have been found to ingest microplastic in both laboratory studies and field collected organisms, the impacts that microplastic ingestion may cause at a population level remains unknown (Wright et al., 2013). The susceptibility for organisms to ingest microplastics may be related to both the medium in which they are exposed and the way in which the organism feeds. For example, indiscriminate feeders, such as mussels that sit in bottom substrate and filter water, or baleen whales that passively filter plankton, may contain large quantities of microplastic (Van Cauwenberghe et al., 2015; Fossi et al., 2016). Although predatory behaviour in some species may present greater ability to visually and selectively feed, such as in some fish (de Sá et al., 2015; Ferreira et al., 2018), plastics that mimic common prey items may be mistakenly ingested; the same notion may be applied to scavenger species such as seabirds (Caldwell et al., 2020). Overall, abundances and types of microplastics ingested greatly varies and may be dependant on the environment from which the organism is collected.

1.3.2 Physical Damage from Plastic Ingestion

Once ingested, the physical consequences that microplastics may have on an organism can vary. First, microplastics may not have any physical impacts, and be egested or harmlessly pass through the digestive tract and be eliminated. Alternatively, microplastics may also be retained in the guts of organisms, potentially causing blockage, and as a result of a false sense of satiety, starvation can occur (Wright et al., 2013). This is a concern for organisms such as juvenile and newly hatched sea turtles, as blockage and declining body condition from starvation is more likely to occur more and at a more rapid rate from microplastic ingestion (McCauley & Bjorndal, 1999; Nelms et al., 2016). Seabird chicks may also experience similar problems, as they may receive microplastics from parental feedings (Acampora et al., 2017). Plastic items dilute the diets of organisms, causing malnourishment, reduction in feeding rate and in turn, a deteriorating body condition from catabolism of stored lipids (Ryan, 1987; Welden & Cowie, 2016). Indeed, microplastic ingestion is associated with reduced feeding and reproductive success in marine copepods (Cole et al., 2015) and decreased body mass in Norway lobster (*Nephrops norvegicus*) (Welden & Cowie, 2016). In addition, reduced feeding may also have the ecological impact of affecting predator-prey interactions (Van Colen et al., 2020), such as an overall reduction in predatory performance, as noted in the common goby (*Pomatoschistus microps*) (de Sá et al., 2015). The reduction in feeding after ingestion of microplastic has been found across a range of organisms, implying that this adverse effect may have the potential to impact aquatic communities.

Internal damages may occur from sharp-edged microplastics lacerating or being lodged in the digestive tract (Laist, 1987; Wright et al., 2013; Rochman et al., 2016). Lei et al. (2018) observed intestinal damage in zebra fish exposed to microplastics. Inflammation of the digestive tract has also been found as a result of ingestion. Ahrendt et al. (2020) also noted severity of lesions in gastrointestinal tracts of fish with increasing exposure to microplastic. Physiological consequences may also occur when ingested through respiratory organs such as gills. Shore crab (*Carcinus maenas*) displayed acute but non adverse change in respiratory function following microplastics inhalation (Watts et al., 2016). In field caught fish, Barboza et al. (2020) noted that 36% were found to have microplastic in their gills, and these fish had higher gill lipid peroxidation that can compromise functioning of the gills. Other damages to gills may include breakage of filaments, increased susceptibility to infection and reduced respiratory efficiency, which may ultimately lead to hypoxia and death (Jabeen et al., 2018).

1.3.3 Toxicity and Adverse Effects Caused by Microplastics

Ecotoxicological research has given further insight into potential adverse effects to organisms. Although the physical plastic may be non-toxic, leachate from the plastics may accumulate in organisms as a result of water or foodborne exposure (Teuten et al., 2009; Cole et al., 2011). For example, polybrominated diphenyl ethers are an endocrine disrupting chemical used as a flame-retardant in plastic-based textiles. Anderson & MacRae (2006) reported this additive to bioaccumulate in fish tissue, finding 5.8–29 μg/g lipid downstream from a WWTP in the Penobscot River, Maine. Toxicity to organisms has been linked to additives in plastics, with adverse effects including disruption in skeletal development in zebra fish caused by phthalate ester plasticizers (Pu et al., 2020), inhibition of photosynthesis in microalgal cells from leaching of fluorescent additives (Luo et al., 2019b), impairment in embryo development of mussels in leachate from both beached and virgin polypropylene pellets (Gandara e Silva et al., 2016), and immobility in daphnia exposed to PVC leachate (Lithner et al., 2012). Known adverse effects from plastic leachates include liver toxicity, cellular death, oxidative stress, impaired development and reproduction, reduced growth, tissue damage, impaired mobility, tumor production, endocrine disruption and mortality in organisms including zooplankton, fish and seabirds (Gore et al., 2015; Anbumani & Kakkar, 2018; Rist & Hartmann, 2018). With the range of adverse effects noted, the chemical components leaching from plastic add another layer to the complex threats already posed by microplastics.

The non-polar, porous and high surface area to volume ratio of plastics creates potential for them to accumulate various contaminants from the surrounding environment (Rochman, 2013; Rochman et al., 2014). Examples of these contaminants include polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pesticides, fertilizer and heavy metals such as cadmium or lead (Mato et al., 2001; Ashton et al., 2010; Frias et al., 2010; Lee et al., 2014). These contaminants have the potential to concentrate to a magnitude of 10^6 and if ingested, may be released into and accumulate in the tissues of organisms (Mato et al., 2001; Bakir et al., 2014; Rochman, 2015). Therefore, plastic debris in aquatic environments has the potential to act both as a source of, and as a transport medium for contaminants, which may negatively impact biota. For example, Rochman et al. (2013) showed that laboratory raised fish adult medaka (*Oryzias latipes*) display signs of hepatic stress after ingesting PE with sorbed chemical pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and polybrominated diphenyl ethers. Parra et al. (2021) found oxidative stress by lipid peroxidation, causing neurotoxicity and damage to the gill, digestive gland and gonad in Asian clam (*Corbicula fluminea)* after exposure to microplastics containing cadmium. These studies provide evidence that contaminants sorbed to microplastics are bioavailable and transferring to organisms.

1.3.4 Trophic Transfer of Microplastics

Ingestion of microplastics in lower trophic levels may result in plastics accumulation throughout the food chain. This has been demonstrated in both laboratory and field-collected organisms (Cedervall et al., 2012; Farrell & Nelson, 2013; Setälä et al., 2014; Santana et al., 2017; Nelms et al., 2018; Elizalde-Velázquez et al., 2020). Cedervall et al. (2012) also demonstrated the transfer of polystyrene nanoparticles from algae to zooplankton to fish and reported altered feeding behaviour in the fish as a result. Although trophic transfer may be observed, organisms may also egest or eliminate microplastic particles, limiting the ability to accurately extrapolate amounts of microplastics ingested and transferred to higher trophic levels. Farrell & Nelson (2013) showed that the small amount of microplastics transferred from prey, blue mussels (*Mytilus edulis*), to predator crabs (*Carcinus maenas*) declined over the trial period (21 days). Similarly, in considering trophic transfer of microplastics in hemolymph mussel (*perna perna)*, Santana et al. (2017) observed microplastic being transferred to predator crab (*Callinectes ornatus)* and the puffer fish (*Spheoeroides greeleyi)*, but noted a lack of evidence of particles remaining in predator tissues past 10 days.

Despite laboratory studies showing the ability of microplastics to transfer to upper trophic levels, it is largely unknown how microplastic may actually migrate up through food webs in a natural setting. Lusher et al. (2016) found 11% of mesopelagic fish collected from the Northeast Atlantic to contain microplastic in their digestive tracts with an average of 1.2 particles/fish. In considering mesopelagic fish accounting for 39-65% of striped dolphin (*Stenella coeruleoalba*) diet, the authors extrapolated that an individual dolphin may be ingesting roughly 463 million microplastics as a result of exposure to contaminated prey fish. Studies have also suggested the potential for trophic transfer to humans to occur. This is not improbable, as microplastics have also been found in many animals that humans eat, including Atlantic cod (*Gadus morhua*), tilapia (*Oreochromis niloticus*), skipjack tuna (*Katsuwonus pelamis),* and bivalves such as blue mussel (*Mytilus edulis*) (Browne et al., 2008; Lusher et al., 2013; Van Cauwenberghe & Janssen, 2014; Rochman et al., 2015; Bråte et al., 2016). Overall, many groups of aquatic organisms are susceptible to the hazard posed by microplastic exposure. Further insight is needed regarding how different groups may be interacting with microplastics in their environment.

1.4 Microplastics and Fish

1.4.1 Frequency of Microplastic Ingestion by Fishes Globally

Microplastic ingestion in fish has been observed in a variety of fish species across many habitats. In terms of frequency of plastic ingestion, described as percent of individuals containing at least one plastic item, studies from marine environments have reported 58% of individuals from 28 species sampled from the Mediterranean Sea (Güven et al., 2017), 36.5% in 10 species sampled from the English Channel (Lusher et al., 2013), 5.5% in 5 species collected from the North and Baltic Seas (Rummel et al., 2016), 18.9% in 26 species from the Portuguese coast (Neves et al., 2015), and 2.6% in 7 sampled from the North Sea (Foekema et al., 2013). Comparatively, freshwater studies have reported higher incidences of plastic ingestion in fish, such as 83% in 1 species from a river in northeast Brazil (Silva-Cavalcanti et al., 2017), 73% from 5 species from prairie creeks in Alberta (Campbell et al., 2017), 45% in 2 species from a river in Texas (Peters & Bratton, 2016), and 8.2% in 44 species from tributaries flowing into the Gulf of Mexico (Phillips & Bonner, 2015). Also among the few freshwater studies, fish from the Great Lakes basin have been reported with high frequency of ingestion. McNeish et al. (2018) reported that 85% of individuals in 11 fish species from tributaries flowing into Lake Michigan have ingested plastic. And recently, Munno et al. (2021) found 12,442 anthropogenic particles in fish from 8 species in Lake Ontario, 3094 from 7 species in Lake Superior and 943 from 1 species collected from the Humber River. These reported ranges may indicate that microplastic ingestion varies across species and habitats and therefore warrants further investigation into potential influences.

1.4.2 Ecological Variation in Microplastic Ingestion in Fishes

Some fish species may be susceptible to ingest microplastic based on the zone in which the fish resides as well as the behaviour in which the fish feeds. Studies have reported demersal feeding fish to ingest higher amounts of plastic (Jabeen et al., 2017; Murphy et al., 2017), whereas others report pelagic fish to contain higher microplastic abundances (Güven et al., 2017; Rummel et al., 2016). Sediment has been found to retain microplastics and consequently organisms associated with generalist bottom feeding activity could face greater exposure (Rummel et al., 2016). Conversely, positively buoyant plastics will more commonly be reported in pelagic fish as they mistake them for prey (Choy & Drazen, 2013). In comparing feeding guilds of fish with ingested microplastic, it has been found that omnivorous fish ingest a much higher amount of fibres than herbivores and carnivores in intertidal fish (Mizraji et al., 2017), that there is no difference in feeding guilds of zoobenthivores and omnivores in coastal fish (Dantas et al., 2020), that predatory species had ingested more microplastics than the filter feeding species in a freshwater reservoir (Hurt et al., 2020), and no difference in feeding guilds between omnivores, zooplanktivores, benthivores, and nektivores from the Yellow Sea (X. Sun et al., 2019). With much variability in findings, there remains a question as to how the foraging strategy of species influences the degree to which organisms are ingesting microplastics.

1.4.3 Body Size of Individual Fishes

Ingestion of microplastic may vary on the scale of individuals, such as based on body size. Studies have found microplastic ingestion in fishes to occur independently of size variables (Foekema et al., 2013; Güven et al., 2017; Vendel et al., 2017; Chan et al., 2019; de Vries et al., 2020). Given that body size was not observed to be a significant influence of microplastic abundance among pooled estuarine species, Vendel et al. (2017) suggest that acquired microplastic ingestion may be more linked to environmental factors. Studies that have identified size of fishes as a factor related to microplastic ingestion suggest additional reasoning, such as sometimes being species dependent (McNeish et al., 2018), some being dependent on water body (Munno et al., 2021) or finding one size variable such as length or gastrointestinal mass to be of more significance than total mass (Peters & Bratton, 2016). Studies that have indicated positive size relationships with microplastic ingestion have also speculated the cause with other ecological factors, such as that larger fish are required to ingest more food material due to higher energy demand, and therefore have higher likelihood of ingesting microplastics in this process (Horton et al., 2018). Or, that larger fishes are often associated with being older, and therefore have had longer times to accumulate microplastic in the gut. This however follows the logic that not all microplastics will be excreted and some are being retained in the gut (Munno, 2017; Roch et al., 2021). In general, reports of microplastic abundances in fish being related to body size vary across studies. Therefore, relationships between microplastic numbers and body size of individuals in conjunction with other factors such as habitat warrants further investigation.

1.4.4 Habitat Influence on Microplastic Ingestion in Fish

Population-dense and industrial areas have been reported to greatly contribute plastic debris in aquatic environments, and a correlation between abundance of microplastics and urban land usage is often noted (Yonkos et al., 2014; Baldwin et al., 2016). Therefore, due to higher availability of microplastics in sediment and waters surrounding urban areas, it may follow that fish from these locations are ingesting higher amounts of microplastic than fish from rural, or offshore areas. For example, Peters & Bratton (2016) found sunfish collected from urban areas contained the highest abundances of microplastic, followed by those collected from downstream of urban locations and sunfish from upstream of urban areas contained the lowest abundances of microplastic. Similarly, studies considering coastal fishes as well as fishes from other urbanized watersheds have reported higher numbers of microplastic in fish, indicating microplastic ingestion may be greatly related to the proximity to pollution source (Phillips & Bonner, 2015; McNeish et al., 2018; Parker et al., 2020; Garcia et al., 2020). As rivers pass directly through areas of both urban and rural land usage, they make for ideal setting to observe potential local variation of microplastic ingestion among fish from the same watershed.

Taking into account that sediment has been reported to retain microplastic, it follows that fish that feed close to sediment may also be ingesting microplastic. However, very few studies contrast the relationship between microplastic numbers in sediment in relation to fish. In the Fengshan river in Taiwan, amount of microplastics in sediment was reported at 508-3987 particles/kg and in demersal and benthopelagic fish, 14–94 particles/fish. Significant trends were found when considering the amounts of fibres present in the sediment with amounts ingested by fish, as well as amount of fragments present in water correlated to abundances ingested by fish, suggesting they could be obtaining different particle shapes from different sources (Tien et al., 2020). Likewise, the sizes, shapes and colours of microplastics reported in sediment and ingested by four species of fish in Lake Ziway in Africa were found to be similar, suggesting that the ingestion of microplastics by the fish could be potentially coupled with sediment debris (Merga et al., 2020). In order to understand the potential impacts of microplastics, there is a need to establish if a relationship exists between the amount of microplastic ingested in demersal fish and the existing load of microplastic in sediment.

1.5 Rationale and Objectives

Currently, limited data are available regarding microplastic ingestion in both freshwater and demersal fishes. In order to better identify factors that influence microplastic ingestion in these fish, considering a watershed with recently characterized microplastic levels in sediment is required. Corcoran et al. (2020b) documented microplastic abundance in benthic sediment of the Thames River, Ontario. A range of 6-2444 particles/kg dry weight sediment was reported with urban locations, fine-grained sediment and high organic matter containing the greatest microplastic abundances. These findings suggest that high population and urban land use are contributing factors to high abundances of microplastic in sediment. The microplastic abundances previously reported from sediment of the Thames River provide references for background levels of microplastic that may be available for fish to ingest. This will allow for investigation into the potential covariation between microplastic levels in sediment and amounts being ingested by bottom feeding fish.

Overall, there is a need to better understand the factors that control the variation of microplastic uptake across different species and habitats, especially in freshwater environments where much information is lacking. Therefore, the goal of this thesis is to address microplastic ingestion in demersal fish of the upper Thames River, Ontario. Associated with this goal are the following objectives: (1) to collect information regarding the morphology, abundance and type of microplastics collected from the gastrointestinal tracts of demersal fish, (2) to determine if body mass relates to the number of microplastics in fish, (3) to compare the number of ingested microplastics between two common demersal species from the same river, and (4) to compare the number of ingested microplastics with land use and previously reported benthic sediment microplastic levels. These objectives will provide broader insight into microplastic ingestion by demersal feeding fish, thereby contributing information to the relatively small pool of freshwater fish studies. Overall, findings from this study will reveal the susceptibility of riverine demersal fish to ingest plastic debris within an urbanized watershed and provide environmentally relevant monitoring data, which may benefit policy development surrounding risks and impacts of microplastics entering freshwater environments.

2 Methods

2.1 Location of Study

The Thames River is the second largest watershed in Ontario, extending 273 km through southwestern Ontario (UTRCA, 1998; Figure 2.1). The watershed is divided into two regions denoted as the upper and lower Thames River. 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 north branch of the upper Thames River starts near Mitchell, Ontario, flows through St. Marys and then meets the south branch of the Thames River in London. The south branch of the river begins in Tavistock, flows through Woodstock and Ingersoll, and then flows into London. The middle branch of the river links into the south branch near Dorchester. The Thames River passes through both rural and urban areas with varying population densities (Table 2.1; Figure 2.1). London is the largest urbanized area that the river crosses, populated at 383,822 and covering approximately 420 km^2 (Statistics Canada, 2016). Overall, the Thames River watershed is home to approximately 800,000 people.

A number of established First Nation communities including Chippewas of the Thames First Nation, Oneida Nation of the Thames, Munsee Delaware Nation and Delaware Nation at Moraviantown reside in the Thames River watershed for generations. This study acknowledges the traditional territory of the Attawandaron, Anishinaabeg, Haudenosaunee, and Lunaapeewak peoples and the waters of the Thames River, known in the Ojibwe and Anishnaabemowin language as Deshkan Ziibi (“Antler River”) in which the study was conducted (UTRCA, 2021).

Corcoran et al. (2020b) have recently provided evidence that microplastics are present in benthic sediment across a range of sites in the Thames River, Ontario with the highest reported abundances of microplastic near urban centers and areas with high levels of organic debris (Table 2.1). Eleven locations in the upper Thames River with varying

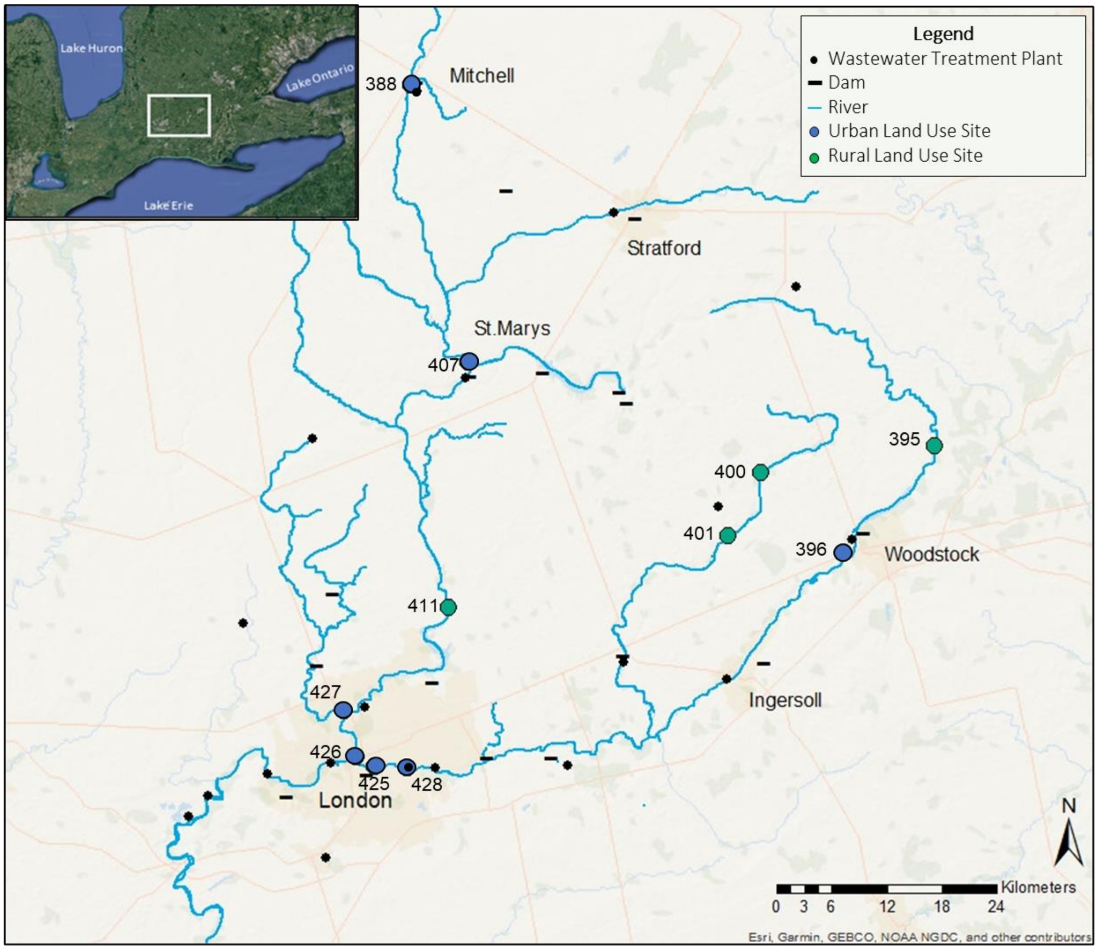

Figure 2.1 Eleven sampling locations located in the upper Thames River, Ontario. Colours of markers indicate sample locations as urban (blue) and rural (green). Map produced in ArcMap 10.4.1

microplastic concentrations were selected from the Corcoran et al. (2020b) study to collect fish (Figure 2.1). These locations were selected in part based on similar features such as high organic content in sediment and similar grain size of sediment to reduce confounding attributes that might influence plastic abundance in sediment (Table 2.1).

Locations were selected to reflect both urban and rural land uses surrounding the river. Although sediment in both the upper and lower Thames river watersheds was sampled by Corcoran et al. (2020b), the lower Thames river presented challenges such as high water depth and high velocity flow that made for unfavourable sampling conditions for the fish

Table 2.1 Summary of sampling locations 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	42.97417, -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

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

collection methods laid out in Section 2.3. Therefore, this study focuses on microplastic ingestion in fish in the upper Thames River.

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 people, 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 species of fish: white sucker (*Catostomus commersonii*) and common carp (*Cyprinus carpio*). White sucker is a member of the family Catostomidae and is one of the most common fishes in North America. Native to Ontario, this species can be found throughout the Great Lakes basin, residing in a variety of habitats, such as in the riffles and pools of creeks and rivers, as well as in lakes (Holm et al., 2009). White sucker is a pollution tolerant species. White suckers are demersal (bottom-dwelling) fish that typically feed on aquatic insects, small crustaceans, molluscs, fish eggs, detritus, and plant material (Scott, 1967; Eder and Carlson, 1977). White suckers are an important prey species for predatory fishes such as muskellunge (*Esox masquinongy*), northern pike (*Esox lucius*), and walleye (*Sander vitreus*) (Scott, 1967). Common carp is a member of the Cyprinidae family. Often mistaken as an invasive Asian carp variety, common carp is an introduced, non-invasive member of the carp family that exist in moderate abundance throughout southern Ontario (Holm et al., 2009). Common carp are widespread due to tolerance to a wide range of habitat conditions that include shallow inland lakes, reservoirs, and rivers with a variety of bottom substrates, in both clear and turbid waters (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). The presence of common carp may negatively impact other species through habitat destruction as well as resource competition. As both white sucker and common carp exhibit feeding behaviours closely associated with sediment, they may be good targets for determining the covariation between microplastic found in the sediment and those obtained from the gastrointestinal tract of the fish.

2.3 Collection of Fish

White sucker was targeted for capture, with common carp gathered opportunistically. Fish were collected using electrofishing and seine netting. Electrofishing was conducted using a HT-2000 Battery Backpack Electrofisher with voltage settings of 150 v and a frequency of 80 Hz. Fish were temporarily stunned by the electrical current running through the water and were collected using a pole net. An alternative capture method used a minnow seine constructed by FIPEC industries (Grande-Rivière 45, rue du Parc, Grande-Rivière, Quebec) with specifications of a 50 ft x 4ft net with a mesh size of ½ inch, 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 both methods were placed in a bucket containing oxygenated river water to prevent re-capture. The aim was to collect ~15 white suckers at each site (n=172 white suckers total) and common carp were collected opportunistically from 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 2.2). 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 for 30 minutes 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 and the mass (g) was recorded in an aluminum dish. Fin clips from each fish were also taken at time of dissection and stored in 95% ethanol to serve as specimen vouchers. The gastrointestinal tracts from the fish underwent tissue digestion following a protocol adapted from Foekema et al. (2013) and Rochman et al. (2015). The use of 20% potassium hydroxide (KOH) has been found to sufficiently degrade fish tissues for the recovery of microplastic. Although 10% KOH is commonly used, 20% was found to be more efficient for the digestions. The increased concentration of KOH has been noted to still produce accurate spectra when identifying plastic type of microplastics using Fourier transform infrared spectroscopy (FTIR) (Munno et al., 2018). The efficacy of KOH to degrade tissue has been validated by Rochman et al. (2015) and has been employed by a variety of other studies for microplastic retrieval from organisms such as mussels and fish (Dehaut et al., 2016; Foekema et al., 2013; Lusher et al., 2017). In brief, the KOH solution was prepared by dissolving KOH pellets (Fisher Scientific) in reverse osmosis water to produce a 20% w/v solution. Each gastrointestinal tract was digested in a glass vessel using 20% KOH and incubated in a drying oven at 45℃ for 48 hours or until fully digested. The KOH was used in enough volume to submerge the tissue. The digested fish samples were filtered over a 10 µm polycarbonate membrane filter using a Nalgene vacuum filtration system. Samples containing large amounts of undigested material were first size fractioned in 300 µm and 100 µm sieves and then were vacuum filtered. Both the digested material from the sieves and the filter papers were stored in glass petri dishes covered with aluminum foil until time of visual identification.

Table 2.2 Summary of common carp (*Cyprinus carpio*) and white sucker (*Catostomus commersonii*) collected across the 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		16.7			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)

2.5 Visual Identification

The material remaining from the sieves and filters was visually examined using a Nikon SMZ 1500 stereomicroscope 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. Manually sorted items were numbered based on site, specimen number and item number and characterized based on shape and colour.

2.6 Material Analysis

Material analysis was conducted to verify the composition of the particles obtained from the fish. A subsample of 10% of the particles collected from the fish were selected using a random number generator on Microsoft Excel to be analyzed using FTIR spectroscopy at the Surface Science Western facility at the University of Western Ontario. 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 – 600 cm^{-1}, with 32 scans and a resolution of 4 cm^{-1}.

2.7 Quality Control and Contamination

As sample processing may introduce potential 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 cotton laboratory coat (100%). 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 3x 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.

Procedural blanks (n=17) containing 20% KOH were employed to act as negative controls for each sample batch (a batch consisted of between 12-20 fish samples) following the digestion and filtering methods. Additionally, during each batch of dissections, a glass petri dish filled with reverse osmosis water to serve as an air blank (n=12) was left open during sample processing (~ 3 hours) to document airborne contamination. 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 hours) to observe 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 and were not normally distributed. A general linear mixed effects model (lmer) was used to check the relationship of body mass with other study variables. Body mass was transformed using log10 to follow 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. To consider the impact of multiple influencing variables that potentially influence the number of fragments, fibres and suspected tire wear particles ingested by fish, a generalized linear mixed effects model (glmm) with a poisson distribution was used, with variables considered in the model including fixed factors of species (with levels white sucker and common carp), body mass of fish and land use (with levels of urban and rural), and collection sites as a random factor. To address the research objective regarding the potential covariation of fish ingesting microplastic based sediment level microplastic, Spearman's rho was used to measure the correlation 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 α=0.05.

3 Results

3.1 Fish Collections

A total of 230 fish were collected for this study, with 172 white suckers collected across the eleven sampling locations, and 58 common carp collected from four locations (Table 2.2). Body mass differed significantly between species (lmer; $F_{1,221}=18.85$, $p<0.001$) with common carp having larger body mass than white sucker. Mean body mass of white sucker ranged from 2.2 g to 36.8 g, and common carp ranged from 70.1 to 691.9 (Table 2.2). The body mass of collected fish did not differ significantly between urban and rural sites (lmer; $F_{1,9}=0.25$, $p=0.63$). Similar patterns were observed for both body length and the mass of the gastrointestinal tract (Table 2.2).

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 (Figure 3.1). Fragments were the dominant particle type observed in 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 (Figure 3.2).

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%). Fibres analyzed from blanks were mainly white (55%), followed by blue (22%), red (11%) and black (11%). Analyzed fragments were identified as PVC (4%), PP (4%), PE (4%), acrylic paint (16%), possible industrial coating identified as a plasticizer (alkyd) and sodium carbonate (4%), a possible paint chip

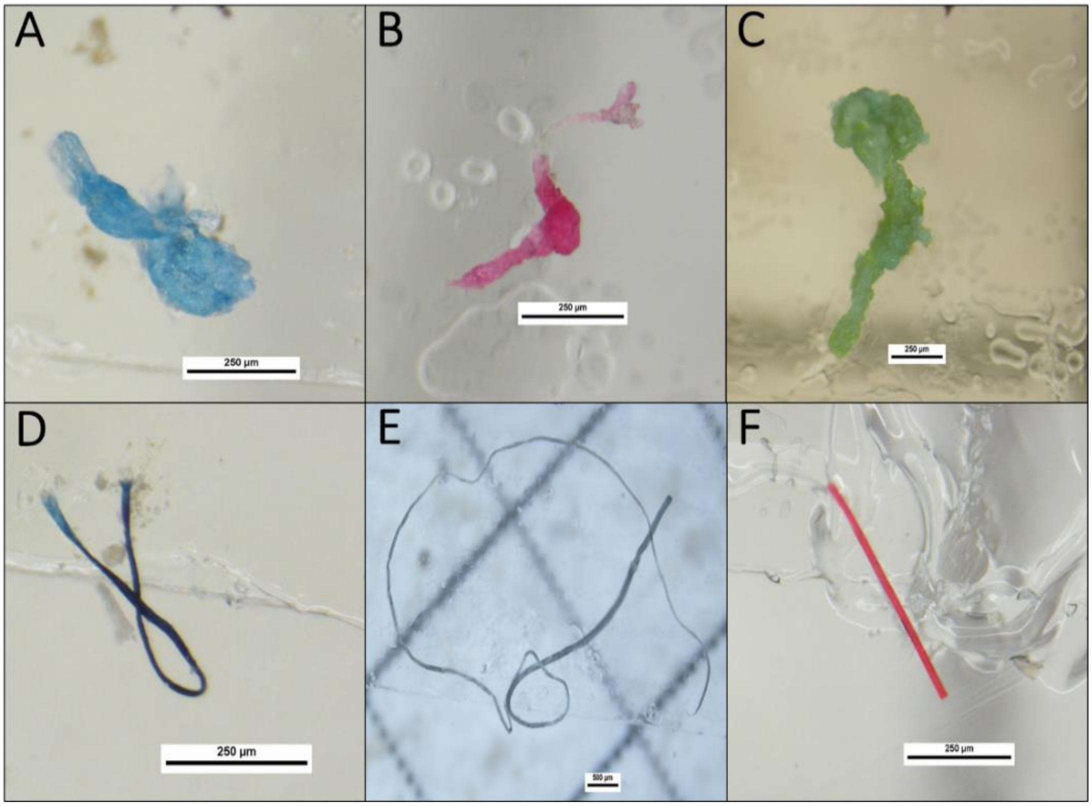

Figure 3.1 Examples of microplastics collected from demersal fish in the upper Thames River, Ontario. Images show fragments (A-C) and fibres (D-F).

identified as red pigment and aluminosilicate (4%), and the majority of fragments were unknown black particles (64%); these black fragments were the most common particles found in fish (Figure 3.3; Figure 3.4). 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 PET (19%), acrylonitrile (12%), proteinaceous PA (4%), aramid fibre (4%), and nylon (4%) (Figure 3.4). Of the 9 particles analyzed from the blanks all were identified as cellulose.

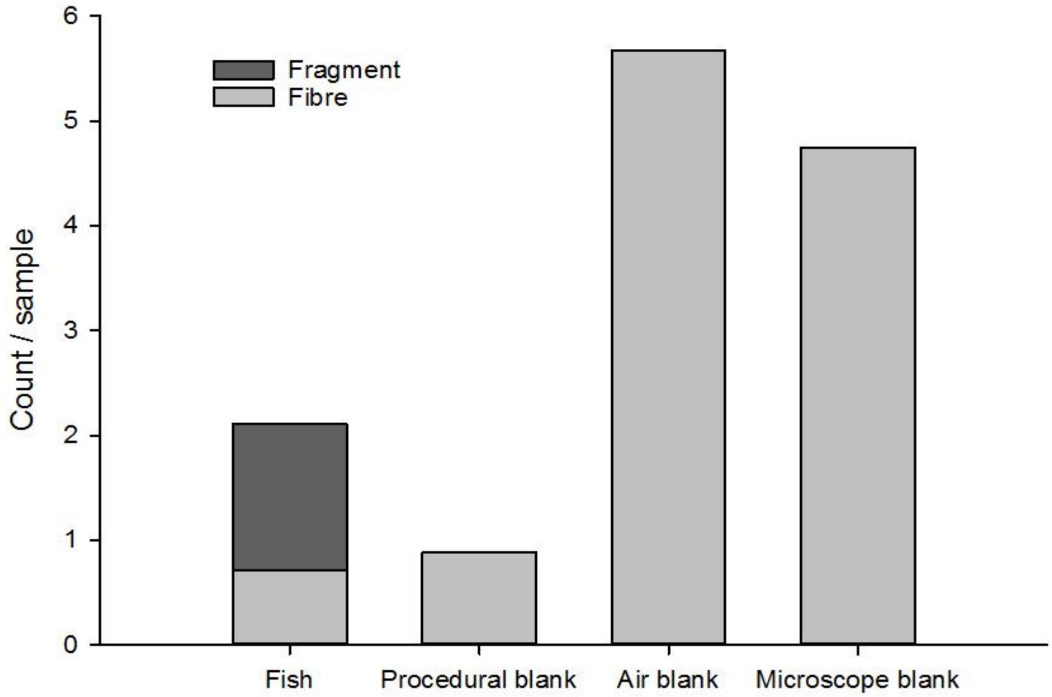

Figure 3.2 Comparison of the number of fibres and fragments collected from the gastrointestinal tract of fishes from the Thames River, Ontario and negative controls. Procedural blanks were processed with fish samples containing only KOH; air blanks were an open petri dish during fish dissection; microscope blanks were taken under the microscope while characterizing samples.

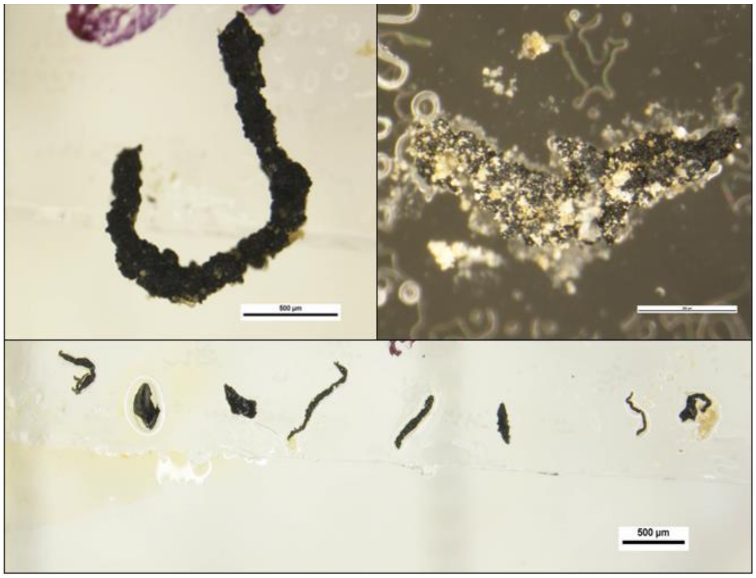

Figure 3.3 Examples of unknown black particles suspected to be tire wear collected from demersal fish from the upper Thames River, Ontario.

3.4 Data Correction

Based on the quantity of fibres identified as cellulose (natural composition), microplastic counts were corrected by subtracting the proportion of cellulose based on colour from each sample (i.e., each fish). 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 (i.e., purple, pink, green) were found in low abundance in fish (Table 3.1) and were not represented by FTIR, and therefore 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 data 375 microplastic particles remained. A new subcategory was made based on the number of black unknown fragments which are suspected to be tire wear particles (Figure 3.3). Figure 3.5 shows the total number of microplastics before and after correction of cellulose fibres. Following corrections, the abundances of particles in fish were 15.2% fibres, 13.3% fragments and 71.5% suspected tire wear particles. Table 3.1 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 3.2). Hereafter only the corrected data are analyzed.

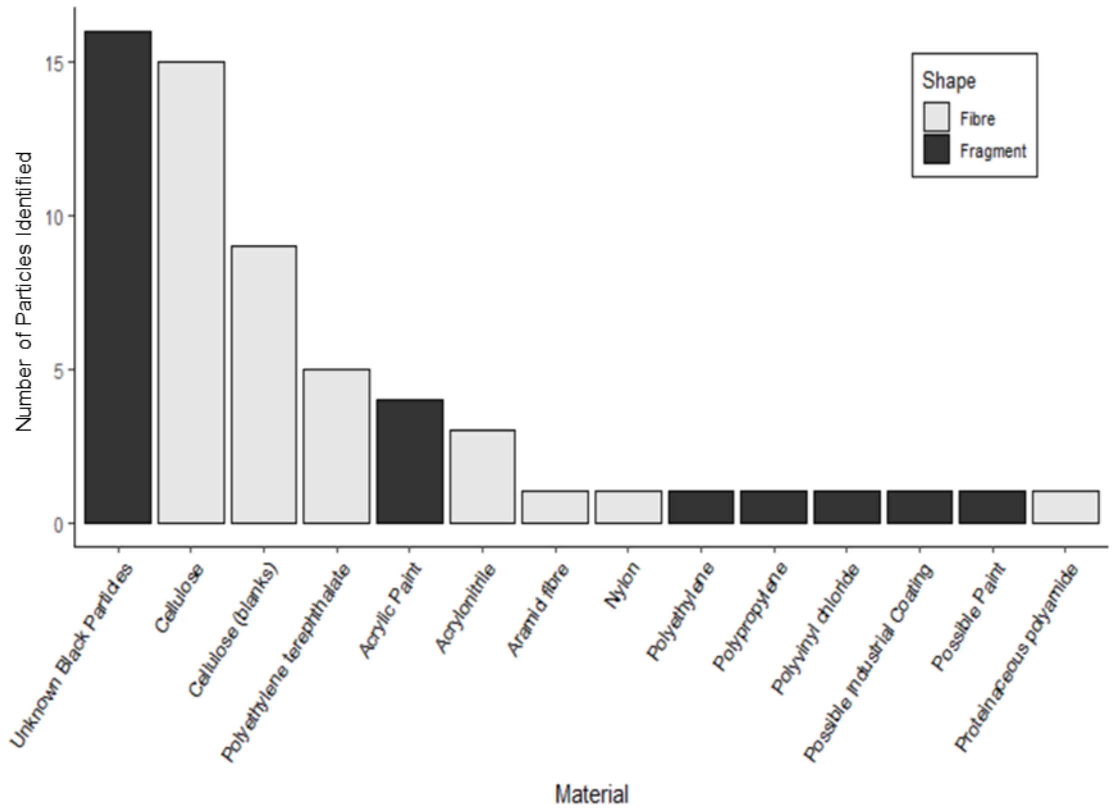

Figure 3.4 Composition of particles retrieved from gastrointestinal tracts of fish from the upper Thames River, Ontario as determined by Fourier Transform Infrared Spectroscopy (FTIR). Unknown black particles were composed of: possible rubber (stearate or metal carboxylate), calcium carbonate, carbon black, potassium bicarbonate, and hydrocarbon. Possible industrial coating was composed of plasticizer (alkyd) and sodium carbonate. Possible paint was composed of red pigment and aluminosilicate.

Table 3.1 Microplastic counts based on qualities of shape and colour from each of the 11 sites and two species.

		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: For the totals, all black fragments are classed as tire wear particles (TWP), therefore the total for fragments does not include black fragments.

Table 3.2 Summary of microplastic size collected from both common carp (*Cyprinus carpio*) and white sucker (*Catostomus commersonii*).

Size (µm)	Count
0-100	16
100-200	71
200-400	123
400-800	114
800-1000	19
1000-5000	27
5000+	5

3.5 Microplastics in Fish After 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 observed in the gastrointestinal tract 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 (glmm; X^2=0.0009, p=0.97; Figure 3.6). 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 contained more particles (Figure 3.6).

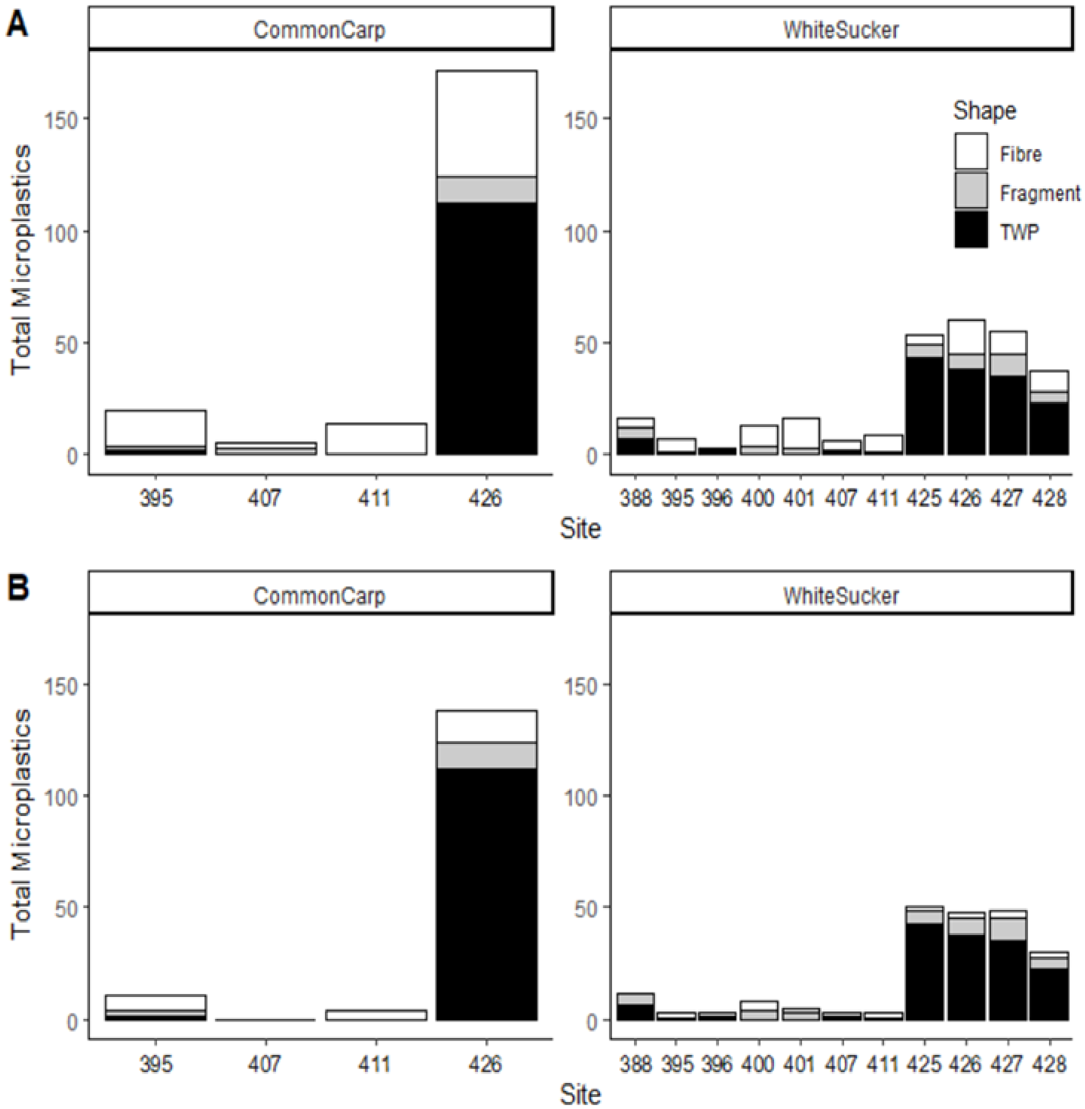

Figure 3.5 Fragment, fibre and tire wear particle (TWP) abundances in common carp (*Cyprinus carpio*) and white sucker (*Catostomus commersonii*) at each sampling location in the Thames River, Ontario. (A) shows pre-normalized data, (B) shows data post FTIR and blank normalization.

With regards to total body mass of fish, a positive significant relationship was found for the number of fibres (glmm; X^2= 59.28, p<0.001) and the number of suspected tire wear particles (glmm; X^2= 25.90 p<0.001) and for fragments (glmm; X^2=24.11, p<0.001). Thus, larger fish tended to have more particles in their gastrointestinal tracts.

A positive correlation 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; Figure 3.7 A). 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; Figure 3.7B). A correlation with the suspected tire wear particles was not examined because there were no tire wear particles reported in the sediment samples (Corcoran et al., 2020b).

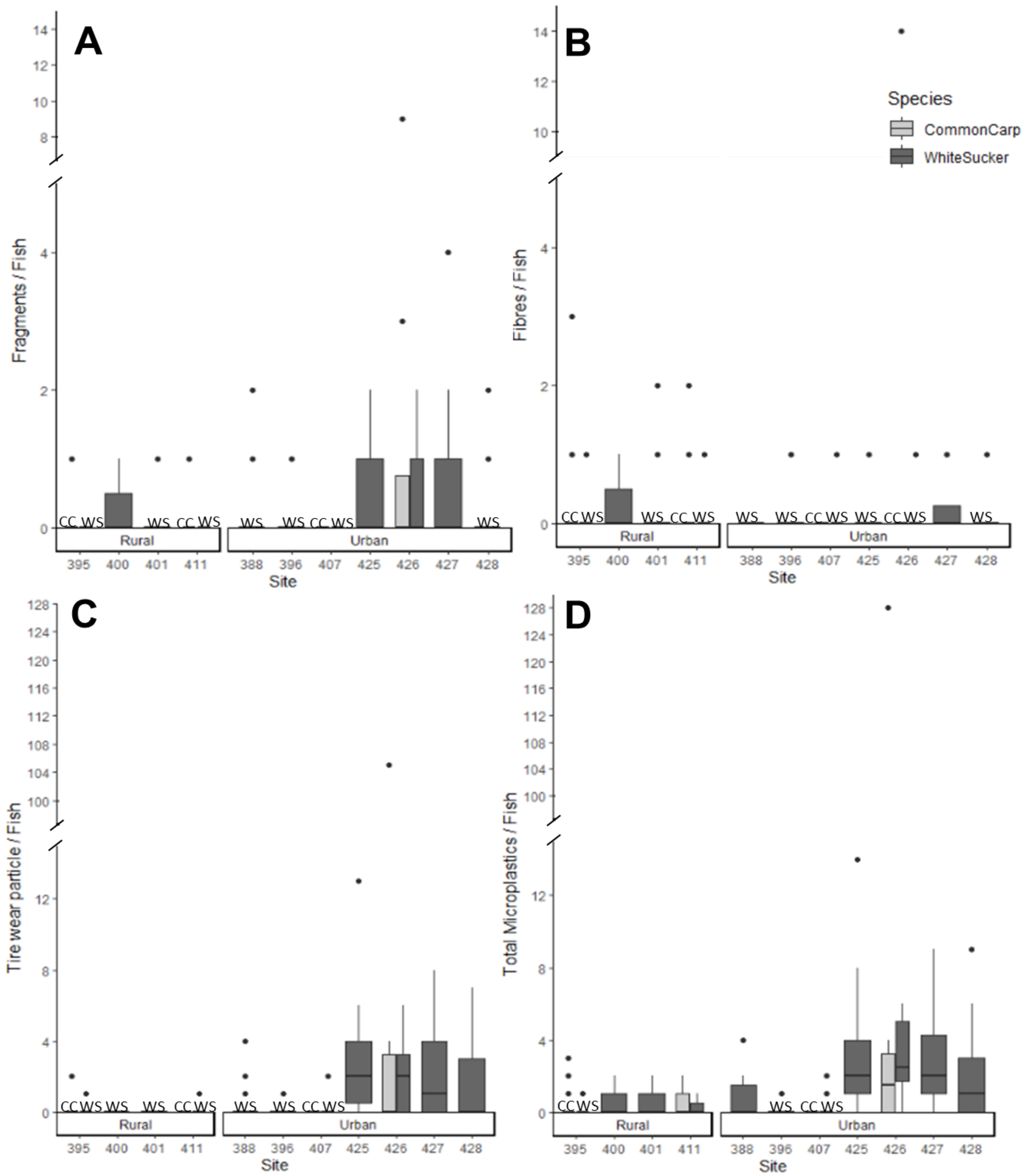

Figure 3.6 Abundances of microplastic per fish collected from the 11 sampling sites. Panels display microplastic groupings as (A) fragments, (B) fibres, (C) suspected tire particles and (D) total microplastics. Common carp (*Cyprinus carpio*) is represented by light shade or 'CC' where box is not present, 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.

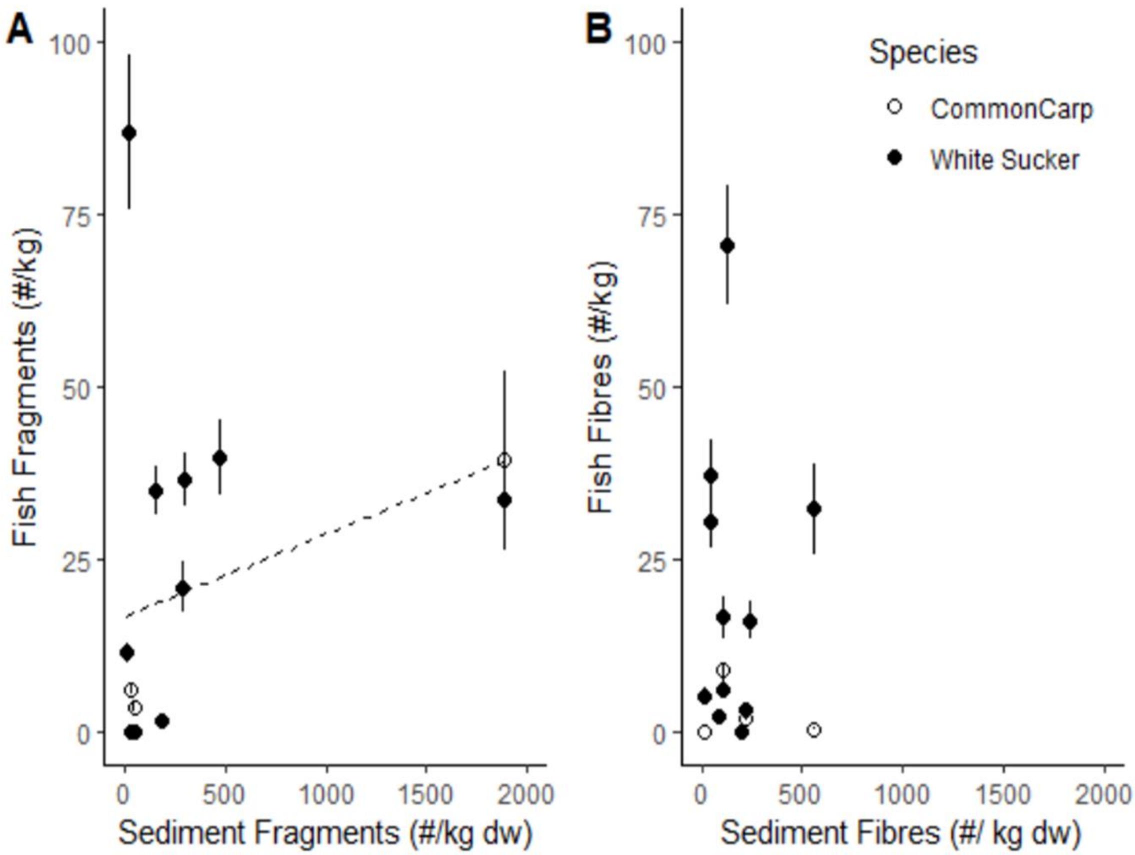

Figure 3.7 Microplastic abundances present in sediment and fish shown by (A) fragments and (B) fibres. Sediment microplastics presented as microplastic /kg dry weight sediment and number of microplastics in fish presented as mean microplastics /kg fish± SE. Colour of point represents species of fish: common carp (*Cyprinus carpio*) (open) and white sucker (*Catostomus commersonii*) (solid).

4 Discussion

4.1 Composition of Microplastics from Fish

The composition of microplastics identified from environmental samples varies from study to study, but often include common types of plastic. Of the fibres analyzed by FTIR in the present study, 15 of 26 (58%) were identified as cellulose based, and the remaining 11 of 26 (42%) as consisting of plastic. These proportions are similar to those found in the sediment of the Thames River, wherein 67% of the microplastics analyzed were cellulose and 33% were plastic (Corcoran et al., 2020b). 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 composition 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; this is 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 in the sediment (Corcoran et al., 2020b) and are among the more common types of plastic used in society (Plastics Europe, 2017). A review suggests the most common types of plastics ingested by fish include PE, PP, 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 composition of microplastics identified in this study align with those found in the sediment and are consistent with studies of other rivers and fishes.

4.2 Black Fragments and Relations to Tire Wear

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 this composition, in addition to morphological similarities (e.g., elongated or cylindrical in shape, coated with minerals, size range of 5 to 220 µm, see Kreider et al., 2010; Sommer et al., 2018), it is suspected that these black fragments are tire wear particles. Other criteria used to identify tire wear particles in the Thames River fishes include the particle being able to return back to original shape after compression and lack of crumbling or breaking when compressed (Knight et al., 2020). 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.

4.3 Comparison of Microplastics in White Sucker and Common Carp

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 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.

4.4 Land Use in Relation to Microplastics in Fish

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 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). Previous studies that have found higher abundances of fibres in fish from urbanized rivers have typically included 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.

4.5 Microplastics in Sediment and Fish

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 correlation 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 at 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., 2020b). Alternatively, the lack of tire wear in sediment may suggest it may not be the primary source of microplastic to the demersal fish, and that they are obtaining tire wear particles from other resources such as other substrates (e.g., algae, periphyton, decomposing organisms). Overall, based on the correlation 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 microplastics.

4.6 Microplastic and Fish Size

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), 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). 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 eleven 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, show 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) was 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 tract.

4.7 Variation of Microplastic Abundances Among Species

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. 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). Although more research is needed, growing evidence suggests feeding and habitat use may be a factor determining ingestion rates of microplastic in fishes.

4.8 Limitations and Future Directions

There remain a number of important questions about microplastic uptake that were beyond the scope of this thesis. First, there is some question about the repeatability of microplastic measures across seasons and across years. Feeding rates are known to differ throughout the year, being highest in the summer and lowest in the winter (Kestemont & Baras, 2007). This would be predicted to influence the rate of microplastic ingestion, and thus microplastic abundance might be higher in fish collected in the summer. Few studies have tested this relationship, and the Thames River fish data were collected during a 3-month period of a single year, with fish from most sampling sites collected on a single day. These data thus have limited capacity to speak to the question of microplastic ingestion across time. Studies are needed that consider temporal trends of microplastic abundances in the same habitats over time. A study of this design for benthic sediment has been proposed by Corcoran et al. (2020b) and is currently under way.

One challenge of studying microplastic ingestion in fish is teasing apart species-level variation in microplastic levels from microhabitat-level effects. In the present study, this limitation can be noted in the low capture success for common carp at many sites. This in turn may affect the statistical power of the model due to uneven sample size between white sucker and common carp. In addition, this allows less comparisons to be made for the variables in the model related to size, land use and sediment levels of microplastic compared to number ingested for common carp. A lower frequency of ingestion for common carp than white sucker was observed, with most common carp obtained from rural locations. If at least 15 common carp had been able to have been collected per site, the study design would have been better balanced and may have reflect different outcomes. Although others have conducted similar studies investigating microplastic ingestion by fishes with highly variable sample sizes for each species, fish capture is limited to a generalized location (Neves et al., 2015; Murphy et al., 2017; Chan et al., 2019). In general, balanced study designs are important for better controlling variance and making stronger statistical power. Therefore, when possible, future studies may wish to keep the sample size of species across multiple sites closer in number to better be able to address small scale variation of microplastic ingestion by fishes.

Another challenge in understanding microplastic abundance is the relative accessibility of different sites to sample. Shallow streams offer more favourable conditions for sampling river fishes because most capture methods require the water to be wadable. In the original study design, the plan was to collect fish from below London and southwest towards Chatham-Kent where the Thames River flows into Lake St. Clair in order to better capture the Thames River watershed as a whole. This additional data would have allowed for more comparisons with land use and more data on number of microplastics in the sediment, as well as better mirror the parent sediment study by Corcoran et al. (2020b). Unfortunately, upon surveying sampling sites in the lower Thames River it became evident that these locations provided challenges, such as high water levels, and high rate of flow that made them unsafe for sampling using the available collection methods. Although the present study was able to capture microplastic ingestion in fish of the upper Thames River, future studies may wish to further investigate expanded ranges of watershed to investigate additional variation of landscape scales in the Thames River, such as upstream and downstream, or land use such as forest and sub-urbanized areas.

The toxicological consequences of microplastic ingestion are also poorly understood in field-collected organisms. Many have considered the potential adverse effects as a result of microplastic ingestion with a wide range in reported effects (See section on Hazards to Aquatic Life). Whereas laboratory-based studies may control the exposure concentration and track residency time of microplastics, field-based studies are limited to a single time point (i.e., time of capture) and cannot extrapolate much beyond this. While this study is still valuable wherein it provides environmentally relevant levels of microplastic ingestion by white sucker and common carp that are comparable to other studies, the underlying implications from ingesting microplastic cannot be addressed. Some studies have considered body condition (Fultons condition factor (K)) by using a ratio length and mass variables (Compa et al., 2018; de Vries et al., 2020; Filgueiras et al., 2020; Foekema et al., 2013; Garcia-Garin et al., 2019). Effectiveness of comparison is questionable, as many factors besides microplastic ingestion, such as resource availability, may influence this metric. Alternative methods that directly compare an individual's health to abundance of microplastic in field collected fish (i.e., blood, gut biome) may be useful to consider adverse effects related to microplastic ingestion.

4.9 Conclusion

With the prevalence of microplastics in the environment, monitoring the ingestion of microplastic by biota becomes increasingly important to better understand the potential implications to organisms, and further to the ecosystems that are being contaminated by microplastic. This study provides the first examination of microplastic abundances in fishes of the Thames River, ON. This study shows that land usage and microplastic abundances in sediment are 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. White sucker and common carp were found to contain similar numbers of microplastics, but different from other populations discussed in previous studies, suggesting that other factors, such as number of microplastics in the local environment of these fish, may play a role in their ingestion. 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 across the world.

The variation of microplastic ingestion by fish appears to be related to human activity as well as environmental availability. Studies may wish to work towards identifying robust indicators that may be used to predict trends in microplastic ingestion, such as the way in which the present study directly compares levels of microplastic in sediment to the numbers in fish. In addition, more work on how spatial and temporal variations of microplastics across watersheds impacts ingestion by fishes is also needed. This study, along with the recent survey of microplastics in sediment (Corcoran et al., 2020b), are the first investigations to be part of a proposed long-term study of microplastics in the Thames River, Ontario that seek to further address these points.

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Appendices

Appendix A Summary of studies concerning microplastic ingestion in fish collected in the field. Frequency describes proportion of individuals containing greater than one microplastic.

Number of Species	Number of individuals used (total)	Location of Study	Environment	Mean ± SD	Frequency	Authors
11	74	Michigan, USA	Freshwater	10 ±2.3 to 13 ±1.6	85.0%	(McNeish et al., 2018)
44	418	Gulf of Mexico (tributary)	Freshwater	NA	8.2%	(Phillips & Bonner, 2015)
13	294	Southern Brazil	Freshwater	NA	21.4%	(Garcia et al., 2020)
2	426	Brazos river basin, Texas	Freshwater	NA	45.0%	(Peters & Bratton, 2016)
8	212	Lake Ontario, Canada		59 ±104		
7	119	Lake Superior, Canada		26 ±74		
1	50	Humber River, Canada	Freshwater	19 ±14	NA	(Munno et al., 2021)
6	6	Han River, south Korea	Freshwater	22.0 ± 16.0	100.0%	(Park et al., 2020)
5	181	Wascana Creek, Saskatchewan	Freshwater	NA	73.5%	(Campbell et al., 2017)
1	186	French rivers, France	Freshwater	NA	12.0%	(Sanchez et al., 2014)
1	48	Pajeú River, Brazil	Freshwater	3.6 ± NA	83.0%	(Silva-Cavalcanti et al., 2017)
1	64	Thames River, UK	Freshwater	0.69 ± 1.25	33.0%	(Horton et al., 2018)
2	96	Illinois, USA	Freshwater	24.7 ± 2.5, 5.2 ± 0.4	100.0%	(Hurt et al., 2020)
16	172	Xingu River basin, Amazon	Freshwater	NA	26.7%	(Andrade et al., 2019)
1	10	Great Lakes, Canada	Freshwater	10 ± 14	65.0%	(Athey et al., 2020)
2	40	Lake Victoria, Africa	Freshwater	NA	55.0% and 33%	(Biginagwa et al., 2016)
22	1167	Southwest Germany	Freshwater	0.2 ± 0.5	18.8%	(Roch et al., 2019)
10	504	English Channel	Marine	1.90 ± 0.10	36.5%	(Lusher et al., 2013)
26	263	Portugal coast	Marine	0.27 ± 0.63	19.8%	(Neves et al., 2015)
6	670	North Pacific Gyre	Marine	2.10 ± 5.78	35.0%	(Boerger et al., 2010)
7	1203	North Sea	Marine	NA	2.6%	(Foekema et al., 2013)
27	141	North Pacific subtropical gyre	Marine	NA	9.2%	(Davison & Asch, 2011)
5	290	North and Baltic Sea	Marine	1.44 ± NA	5.5%	(Rummel et al., 2016)
2	406	North and Baltic Sea	Marine	0.24 ± NA	23.0%	(Lenz et al., 2016)
21	342	Southern Ocean and Australia	Marine	2 ± NA	0.3%	(Cannon et al., 2016)
8	116	Gulf of Mexico	Marine	NA	10.4%	(Phillips & Bonner, 2015)
1	70	South Africa harbor	Marine	3.8 ± 4.7	72.8%	(Naidoo et al., 2016)
1	64	Tokyo Bay, Japan	Marine	2.34 ± 2.5	77.0%	(Tanaka & Takada, 2016)

10	716	North Atlantic Ocean	Marine	1.2 ± 0.54	11.0%	(Lusher et al., 2016)
1	302	Norwegian coast	Marine	1.77 ± NA	3.0%	(Bråte et al., 2016)
5	125	Adriatic Sea	Marine	1.39± NA	28.0%	(Avio et al., 2015)
1	337	Mediterranean Sea	Marine	3.75± 0.25	58.0%	(Nadal et al., 2016)
4	212	Spain, Atlantic, Mediterranean coasts	Marine	1.56± 0.5	17.5%	(Bellas et al., 2016)
3	121	Mediterranean Sea	Marine	1.21± NA	18.2%	(Romeo et al., 2015)
28	1337	Mediterranean Sea	Marine	2.36± NA	58.0%	(Güven et al., 2017)
5	147	Hongkong coast, China	Marine	2.4± 2.3	54.0%	(Chan et al., 2019)
10	595	North Pacific subtropical gyre	Marine	NA	19.0%	(Choy & Drazen, 2013)
11	76	Fish market, Indonesia	Marine	NA	28.0%	(Rochman et al., 2015)
12	64	Fish market, California	Marine	NA	25.0%	(Rochman et al., 2015)
1	115	Mediterranean Sea	Marine	NA	24.3%	(Battaglia et al., 2016)
9	84	Northeast Atlantic, Scotland	Marine	1.8 ± 1.7	47.7%	(Murphy et al., 2017)
4	133	Moorea Island, French Polynesia	Marine	1.25 ± 0.13	21.0%	(Garnier et al., 2019)
7	292	Southeastern Pacific Ocean	Marine	NA	2.1%	(Ory et al., 2018)
1	205	Newfoundland, Canada	Marine	NA	2.4%	(Liboiron et al., 2016)
26	1504	Ionian Sea	Marine	1.3±0.2	1.9%	(Anastasopoulou et al., 2013)
1	192	North Pacific Ocean	Marine	NA	24.4%	(Jantz et al., 2013)
3	120	Mondego estuary, Portugal	Marine	1.67 ± 0.27	38.0%	(Bessa et al., 2018)
7	105	Agulhas Bank, South Africa	Marine	3.72 ± 2.73	87.0%	(Sparks & Immelman, 2020)
19	1320	Yellow Sea	Marine	0.41±NA	34.0%	(Sun et al., 2019b)
7	214	Northeast Brazil	Marine	NA	55.0%	(Dantas et al., 2020)
7	233	Northern Atlantic crossing	Marine	1.1 ± NA	73.0%	(Wieczorek et al., 2018)
3	93	Sydney Harbour, Australia	Marine	1.8 ± NA	37.0%	(Halstead et al., 2018)
46	189	Amazon River estuary	Marine	1.2 ±5.0	13.7%	(Pegado et al., 2018)
13	35	South Sea, China	Marine	1.96 ± 1.12	100.0%	(Zhu et al., 2019)
1	74	Vancouver Island, Canada	Marine	1.2 ± 1.4	59.0%	(Collicutt et al., 2019)
4	174	KwaZulu-Natal, South Africa	Marine	0.79 ± 1.00	52.0%	(Naidoo et al., 2020)
21 and 6	NA; 20-40 per spp	Yangtze estuary and Taihu Lake, China	Marine, Freshwater	NA	100.0% and 95.7%	(Jabeen et al., 2017)
69	2333	Northeast Brazil	Marine	1.06 ± 0.30	9.0%	(Vendel et al., 2017)

Appendix B Samples analyzed for composition using Fourier-transform infrared spectroscopy. Sample names listed as Site-Species-Individual- Particle. (WS= white sucker, CC= common carp).

Sample Name	Colour	Shape	FTIR Result
411-CC-10-2	blue	fibre	PET
426-CC-4-13	green	fragment	PVC
426-CC-4-4	black	fragment	Possible rubber, stearate or metal carboxylate, calcium carbonate
426-CC-4-9	black	fragment	Possible rubber, similar to 426 CC 4-4
426-CC-8-1	blue	fibre	PET
411-CC-16-1	pink	fragment	PP
427-WS-2-2	black	fragment	Not a common plastic; possible carbon black, small amount of calcium carbonate
427-WS-1-1	blue	fibre	Cellulose
388-WS-11-2	green	fragment	Acrylic paint
395-WS-7-1	black	fibre	Cellulose
427-WS-5-1	red	fibre	Proteinaceous polyamide
426-WS-14-5	black	fragment	Possible rubber, similar to 426 CC 4-4
426-WS-6-2	black	fragment	Acrylic paint
426-WS-5-1	red	fibre	PET
426-CC-4-16	black	fibre	Acrylonitrile
400-WS-11-1	blue	fragment	PE
426-CC-4-92	black	fragment	Not a common plastic; possible carbon black
401-WS-17-2	red	fibre	Cellulose
425-WS-10-2	red	fragment	Possible paint, Red pigment + aluminosilicate
425-WS-8-4	black	fragment	Not a common plastic; possible carbon black
425-WS-1-3	black	fragment	Not a common plastic; possible carbon black
425-WS-10-1	black	fragment	Not a common plastic, inorganic, potassium bicarbonate
395-WS-5-2	blue	fibre	Cellulose
428-WS-11-5	black	fragment	Industrial coating: possible plasticizer (alkyd) + sodium carbonate
426-CC-4-87	black	fragment	Possible rubber, hydrocarbon + calcium carbonate
425-WS-7-1	white	fibre	Cellulose
428-WS-2-2	black	fragment	Possible rubber, metal carboxylate +calcium carbonate
425-WS-8-3	black	fragment	Not a common plastic; possible carbon black
426-CC-4-120	black	fragment	Acrylic paint
428-WS-5-2	black	fragment	Possible carbon black mostly
411-CC-14-2	blue	fibre	Cellulose
425-WS-9-1	blue	fibre	Cellulose
400-WS-5-1	red	fibre	Cellulose
427-WS-8-1	black	fragment	Possible carbon black mostly
427-WS-1-1	blue	fibre	Cellulose
427-WS-5-1	yellow	fragment	Paint chip, acrylic + calcium carbonate
428-WS-10-1	white	fibre	Aramid fibre
388-WS-1-3	grey	fibre	Cellulose
426-CC-4-151	red	fibre	Acrylonitrile
426-CC-4-68	clear	fibre	Nylon
426-CC-4-33	red	fibre	Cellulose
395-WS-3-2	black	fibre	Cellulose
426-CC-4-8	black	fragment	Mostly calcium carbonate
411-CC-13-3	blue	fibre	PET
400-WS-4-2	white	fibre	Cellulose
426-CC-4-103	black	fragment	Possible rubber, hydrocarbon ester + calcium carbonate
427-WS-10-2	white	fibre	Cellulose
426-CC-4-128	black	fragment	Possible rubber, similar to 426CC4-103
411-WS-9-1	blue	fibre	Acrylonitrile
426-CC-4-56	red	fibre	PET
401-WS-5-1	red	fibre	Cellulose
PROBLANK-4-2	white	fibre	Cellulose
SEPT_23_AIR_6	white	fibre	Cellulose
OCT14AIR-6	red	fibre	Cellulose
NOV6-AIR-2	white	fibre	Cellulose
DEC2-AIR-5	blue	fibre	Cellulose
NOV16-AIR-1	white	fibre	Cellulose
NOV-23-AIR_3	blue	fibre	Cellulose
PROBLANK-9-1	white	fibre	Cellulose
OCT28-AIR-4	black	fibre	Cellulose

Appendix C Summary of fibre abundance and colour from three blanks methods.

	Procedural (n=17)	**Air (n=12)**	**Microscope (n=4)**
white fibre	14	58	14
black fibre	0	2	2
blue fibre	0	4	1
red fibre	0	2	0
purple fibre	0	0	1
pink fibre	0	1	1
yellow fibre	0	1	0
gray fibre	1	0	1
total fibre	**15**	**68**	**20**

*Blanks (i.e., samples not containing fish tissue) were taken to document potential airborne contamination while processing samples. Procedural blanks refer to blanks that were processed with fish samples containing only KOH, Air blanks refer to the open petri dish during fish dissection, and Microscope refers to blanks taken under the microscope while characterizing samples.

Appendix D Ethics approval from Western University's Animal Care Committee for use of fishes.

eSirius3G

Western

PI :	Neff, Bryan
Protocol #	2018-084
Status :	Approved (w/o Stipulation)
Approved :	07/01/2018
Expires :	07/01/2022
Title :	Behavioural and molecular ecology of fishes

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Curriculum Vitae

Name:	Colleen Wardlaw
Post-secondary Education and Degrees:	University of Guelph Guelph, Ontario, Canada 2015-2019 BSc in Environmental Sciences.
	University of Western Ontario London, Ontario, Canada 2019-2021 MSc in Biology
Honours and Awards:	Queen Elizabeth II Graduate Scholarship in Science and Technology 2020-2021
	Dean’s Honor List, University of Guelph 2017-2019
	Entrance Scholarship, University of Guelph 2015
Related Work Experience	Research Assistant University of Guelph 2016-2019
	Teaching Assistant The University of Western Ontario 2019-2020

Publications and Conferences:

Wardlaw, C., & Prosser, R. S. (2020). Investigation of Microplastics in Freshwater Mussels (*Lasmigona costata*) From the Grand River Watershed in Ontario, Canada. Water, Air, and Soil Pollution. https://doi.org/10.1007/s11270-020-04741-5

Poster: Wardlaw, C., Prosser, R.S. 2021. Filtering out the Truth: Investigation of Microplastics in Freshwater Mussels (*Lasmigona costata*) in the Grand River watershed. Society of Environmental Toxicology and Chemistry North America 40th annual meeting, Toronto, Canada, November 3-7, 2019.

Presentation: Wardlaw, C., Corcoran, P.L, Neff, B.D. 2021. Microplastics in White Sucker (*Catostomus commersonii*) and Common Carp (*Cyprinus carpio*) from the Upper Thames River, Ontario. International Association for Great Lakes Research 64th annual meeting, Online, May 17th-21st 2021.