Impact of Fish Feeding Habitat and Diet on Microplastic Concentrations in Gastrointestinal Tracts of St. Lawrence River Fish

Impact of Fish Feeding Habitat and Diet on Microplastic Concentrations in Gastrointestinal Tracts of St. Lawrence River Fish

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Abstract

Approximately 370 million tons of plastic are being produced annually (Plastics Europe, 2020). Only a small portion is recycled due to poor waste management practices. An estimated eight million tons of plastic moves from the land to ocean every year (IUCN, 2018) while rivers transport between 1.15 and 2.41 million tons of debris into the oceans annually (Lebreton et al., 2017). The pervasiveness of microplastics (MP), plastic polymer debris less than 5mm in diameter, in aquatic environments, their ingestion by freshwater fish, and the accumulation of MP through trophic transfer in food webs raise concern for the sustainability of fisheries, food security, and public health (Campbell et al., 2017; Wagner et al., 2019). Fish are excellent indicators of aquatic ecosystem health since they integrate changes in their physical environment (Pinheiro et al., 2017). Assessing MP contamination in fish therefore provides valuable information about MP concentrations in freshwater systems and raises attention to potential risks.

My research investigated how fish feeding habitats, trophic position, body size (weight and length), and species variation influence MP ingestion and accumulation in St Lawrence River freshwater fish by collecting samples of pelagic, bentho-pelagic, and benthic fish species. I hypothesized that MP particles would be present in the GI tracts of most fish samples regardless of feeding habitat since MP can be found throughout the water column in most aquatic environments, and that the GI tracts of benthic fish would contain higher concentrations of MP beads and fragments while the GI tracts of pelagic fish would contain higher concentrations of MP fibers. I expected a greater concentration

of plastic particles would be found in benthic than pelagic fish because of the prevalence of MPs found in the St Lawrence River sediments (Crew et al., 2020; Castaneda et al., 2014), and the data modeling performed by Lebreton et al. (2018).

To address these questions, I collected 73 fish (seven species) from the Lake Saint Louis region of the St Lawrence River using traditional fishing lines and tackle. The fish samples were weighed and measured, gastrointestinal (GI) tracts removed, and contents chemically digested to eliminate organic matter. Once separated, MP particles were observed under a microscope and categorized according to physical characteristics (color, size) and morphology (fragment, bead, fiber), counted, and verified using a hot needle test.

All 73 fish contained MPs. MPs were higher in the GI tracts of these St. Lawrence fish (14.9 +/- 6.9; mean +/- SD) compared with other studies (0-10 MP/ fish; Gouin, 2020). I observed no significant relationship between body size, trophic level, or feeding habitat and MP load. Fibers were the most abundant MP morphology, consistent with other studies examining fish GI tracts (Jabeen et al., 2017; Horton et al., 2018; McNeish et al., 2018; Rochman et al., 2015). Feeding habitat may play a role in MP morphology abundance since a higher percentage of fibers were present in pelagic species, while fragments were more abundant in benthic species. The high mean abundance of MP/ fish demonstrated in this study suggest the pollution sources in the St Lawrence River are likely more numerous than those in other study areas. These findings highlight the need for greater understanding about the consequences and potential risks of plastic pollution in riverine environments and the need for more vigilant policy decisions regarding plastics production and waste management practices.

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Chapter I

Introduction

The pervasiveness of microplastics in marine and freshwater environments, their ingestion by freshwater fish, and the accumulation of microplastics through trophic transfer in food webs raise concern for the sustainability of fisheries, food security, and public health (Campbell et al., 2017; Wagner et al., 2019). Freshwater riverine systems play crucial roles in the collection and transport of plastic pollution. Their proximity to urban centers and the hydrology of a river basin enables the flushing of large quantities of debris in water to be moved great distances.

Microplastics (MP), plastic polymer debris less than 5mm in diameter, enter the environment through photodegradation or mechanical breakdown of larger plastic items, atmospheric deposition, industrial processes, agricultural runoff, and wastewater treatment plant (WWTP) effluent (Baldwin et. al., 2016; Wagner et al., 2014). Microbeads, considered primary microplastics, are tiny round manufactured MP used in industrial and hygiene products. Secondary microplastics, such as fibers, are degraded from larger items and originate from clothing, fishing nets, and plastic bags (Campbell et al., 2017). The sheer quantity of plastics produced, coupled with inappropriate waste management practices and the ability to linger for centuries, has resulted in the identification of MP in almost every terrestrial and aquatic environment in the world (Browne et al., 2011). Plastic debris, which accumulates contaminants such as organic pollutants (Ziccardi et al., 2016) and heavy metals (Holmes et al., 2012), threatens biodiversity by causing toxic and physical effects to biota (Silva-Cavalcanti et al., 2017).

Analysis of stomach contents from numerous fish taxa indicate that plastic debris is likely ingested both intentionally when mistaken for food, and unintentionally through trophic transfer (Silva-Cavalcanti et al., 2017). Several studies have shown the physical and physiological effects in fish, including reduced predatory performance, reproductive disruption, growth interruption, and mortality (Jaafar et al., 2020). Ingestion of plastic debris can cause internal injuries and blockage of the gastrointestinal tract, which can lead to starvation (Andrade et al., 2018; Possattro et al., 2011; Rummel et al., 2016). In addition to interfering with feeding or blocking the gastrointestinal tract, plastic debris poses chemical toxicological risks via food chain transfer and bioaccumulation (Andrade et al., 2018).

Fish are excellent indicators of aquatic ecosystem health since they integrate changes in their physical environment and are sensitive to environmental and anthropogenic pressures. Fish are an important biological element of freshwater ecosystems with significant economic and nutritional value worldwide (Pinheiro et al., 2017). Three billion people rely on seafood as their primary source of protein (WWF, 2018) and production is expected to reach over 200 million tons by 2030 (FOA, 2018). Despite its healthy image, microplastic ingestion results in the bioaccumulation of numerous pollutants in fish (Campanale et al., 2020) which raises concern for human health and food security (Barboza et al., 2018; Jaafar et al., 2020).

Fish serve as valuable samples for understanding MP concentrations in freshwater environments and therefore indicate potential risks to social and economic health (Gouvernement du Quebec, 2008). Since fish are recognized as effective bioindicators, quantification of microplastics in GI tracts can be employed for reliable monitoring of

microplastics pollution levels (Horton et al., 2018). Understanding how differences in fish feeding habits impact MP concentrations can help identify vulnerable species, potential trophic impacts, and help to clarify aquatic ecosystems most at risk but no studies have focused on this. Implementing plastic pollution mitigation strategies requires an understanding of not only the source of debris but also a quantification of its fate. “It is only once we can identify where MPs are, that we can establish harm to the environment and impart change” (National Geographic, 2015, para. 10).

As MP research in freshwater systems becomes more standardized and widespread, comparisons can be made between environmental variables, fish feeding habitats, diet, and MP prevalence in freshwater organisms. Increasing awareness about the environmental impacts of plastic pollution in freshwater ecosystems is necessary to initiate stronger environmental legislation that supports a more robust waste management and recycling infrastructure globally. Lacking the most obvious solution, to produce less plastic, it is essential to build a more comprehensive view of the environmental effects of plastic pollution by bridging knowledge and research between terrestrial, freshwater, and marine environments.

Previous research to examine microplastic concentrations in marine and freshwater fish have found inconsistent evidence of what drives microplastic ingestion. Projections of microplastic concentrations in different aquatic environments indicate that bottom sediments will exceed potentially harmful thresholds ahead of surface water (Everaert et al., 2018; Bosshard et al., 2020). Microplastic prevalence in samples of freshwater fish varies greatly across studies, ranging from 7.5 to 95.7% (Roch et al., 2019). Some of this variation is due to significant differences in research methods,

highlighting the need for more controlled investigations and research designed to better assess the impact of microplastic pollution (Bosshart et al., 2020).

Research Significance and Objectives

This research enhanced understanding of the consequences and potential risks of plastic pollution in riverine environments, adding to the body of evidence highlighting the ubiquity of anthropogenic debris in freshwater ecosystems. In particular, research by Crew et al. (2020) and Castaneda et al. (2014) revealed significant quantities of microplastics in St Lawrence River sediments. Therefore the proposed research investigated if these elevated microplastic sediment levels correlate with high microplastic concentrations in benthic fish living within this area.

The broader goal was address two gaps in research: the prevalence of microplastics in the gastrointestinal tracts of St Lawrence River freshwater fish, and the impact of fish feeding habitats and diet on these concentrations. Studying the incidence of microplastic ingestion in benthic fish in the St Lawrence River posed an opportunity to assess exposure risks in an area with high sediment pollution (Bosshart et al., 2020).

Another axis of study was the influence of food web position, comparing planktivorous, omnivorous, and carnivorous species to determine variation in microplastic concentrations due to diet. In addition, analysis of fish feeding habitat and diet-related particle type concentrations contributed to knowledge about impacts of pollution particle type. By examining variation in MP ingestion by feeding habits, the results can be used to help identify potential risks to vulnerable species and the impacts to ecosystems and human health. Determining which combinations of feeding habitat and diet attributes contribute to higher concentrations of anthropogenic debris in GI tracts can

be used to influence waste management policy decisions, and fish species can be better identified and categorized for monitoring.

My objectives in this study were therefore:

- To improve understanding about the location and potential risks of microplastic pollution in aquatic ecosystems
- To examine how fish feeding habitats and diet influence microplastic ingestion and relative mass of plastics in GI tracts of St Lawrence River fish
- To utilize the results for the identification of vulnerable fish species for monitoring
- To produce a data set which can be used for future comparisons of fish in freshwater systems
- To lead to more informed policy decisions about plastics production and waste management practices to curb microplastic pollution in aquatic ecosystems

Background

Approximately 370 million tons of plastic are being produced annually (Plastics Europe, 2020). Only a small portion is recycled due to poor waste management practices, while an estimated 8 million tons of plastic moves from the land to ocean every year (IUCN, 2018). Although much focus has been placed on highlighting the magnitude of plastic waste cluttering surface waters and beaches around the world, it is likely the larger threat lies in the darkness below. Lebreton et al. (2018) gathered data to model ocean plastic concentrations within and around the Great Pacific Garbage Patch (GPGP). Their results suggested the possibility that a larger quantity of plastic sinks to the sea floor than

previously believed; perhaps 50% sinks to the sea floor within a few miles of the coast (Lebreton et al., 2018).

Numerous studies have highlighted the ubiquity of MP in marine organisms while Rochman et al. (2015) made the connection to human consumption and potential risks to human health. Rochman et al. (2015) assessed the presence of anthropogenic debris in fish/shellfish being sold for human consumption through the collection of whole fish, GI tracts and whole bivalves from markets and fisherman in Makassar, Indonesia and from California, USA and found debris in more than 25% of cases. This emergence of marine microplastics as a global issue with a potential human health impact bolstered interest in microplastics as a freshwater contaminant.

Freshwater Research on Microplastic

For the past few decades research has been predominantly focused on the marine environment, with only 13% of all MP studies investigating freshwater systems (Wagner & Lambert, 2017). Among studies that investigated potential effects of plastic pollution on biota, only 21% of the effects were related to freshwater systems (Bucci et al., 2020; Bosshart et al., 2020). But overall, this problem has been neglected: "despite the large amounts of plastic debris input into seas and oceans by rivers, the interactions between this debris and the biota of these ecosystems are poorly studied" Collard et al. (2019), p. 12975.

The economic value of marine fisheries is a driving force behind the predominantly marine focused research; however, this demonstrates disregard for the economic value of freshwater resources and the ecosystem services they provide. Pollution is threatening the availability of freshwater, so it is increasingly important to

understand the sources and pathways of MP contamination in freshwater systems (Eerkes-Medrano et al., 2015). Researching MP pollution in freshwater fish can provide information about how MP are affecting freshwater ecosystems and put pressure on governments to hold pollution sources more accountable.

The popularity of marine microplastic research demonstrates a focus on the end-point pollution while there is a lack of concentration on upstream sources and impacts. Slowly bridging back connections to the sources through freshwater ecosystems research may establish economic, ecological, and human impacts and improve mitigation efforts.

Microplastic Concentrations in Freshwater Fish

Studies investigating the ingestion of MP by freshwater fish have been few and focused on limited regions worldwide. Sanchez et al. (2014) provided the first evidence that freshwater fish ingest MPs. They found MP in digestive tracts of 12% of the wild gudgeons (*Gobio gobio*) caught in 11 French streams. Phillips and Bonner (2015) documented the occurrence of MP ingestion by fishes in the freshwater drainages of the Gulf of Mexico and recorded an 8% occurrence. Much higher frequencies have since been recorded: Peter and Bratton (2016) found 45% of digestive tracts had MP in fish sampled in the Central Brazos River Basin, Texas. Jabeen et al. (2017) found MP in 95.7% of freshwater fish intestines and stomachs of fish collected from local fishermen and fishery markets in Shanghai, and Silva-Cavalcanti et al. (2017) found MP in 83% of the fish from an urban section of the Pajeu river in Northeast Brazil (*Hoplosternum littorale*). In total, these studies represent 34 fish species from around the world. More recent research by Andrade et al., (2019) found MP in 25% of specimens and 80% of species sampled in piranhas and other fish in a tributary of the Amazon River and

McNeish et al., (2018) found MP in 85% of fish species from three tributaries of Lake Michigan.

Fish Feeding Behavior and Microplastic Ingestion

The pattern of microplastic ingestion between pelagic and benthic fish remains unclear. The significance of foraging preferences in the accumulation of microplastics in different fishes is debatable since existing research is scattered, inconsistent, and lacks comparability. This variation is due to significant differences and shortcomings in research design, as well as uncertainty about fish physiology and ecosystem interactions. Contaminated laboratory conditions, insufficient sample collection, ineffective digestion treatments, lack of polymer type identification, and varied filter size selection, highlight the need for more thorough research design (Bosshart et al., 2020).

The extreme range of microplastic prevalence in freshwater fish, ~ 7.5 to 95.7% (Roch et al., 2019), demonstrates the influence of many known and unknown variables impacting the results. The focus of research examining MP concentrations has varied between the proximity to anthropogenic pollutants, feeding habitat differences, diet preferences, and the impact of environmental load (either in sediment or water column) on accumulation in biota. Currently, there are no clear and repeatable inferences that can be made. This is because microplastic particles can accumulate within fish species in multiple ways including direct ingestion, indirect ingestion through trophic transfer, and absorption through gills or other organs (Watts et al., 2014; Gundgodu et al., 2020). Species differences, growth stage within a specific species, migratory pattern, food availability, or perhaps even fish size may influence the ingestion pathway.

Trophic Position: Diet Preferences

The degree to which each uptake method influences MP contamination remains unknown. Ingestion of plastic differs among organisms and is dependent on many factors including the size and abundance of particles and presence of natural prey. MP are similar in size to plankton. In planktonic food webs, zooplankton ingest and retain microplastics, and when they are consumed by larval fish (Steer et al., 2017), this material is passed to higher trophic levels (Setälä et al., 2014). Planktivores may encounter MP at similar frequencies to their natural food source and are indiscriminate feeders that capture any particles within an appropriate size range. MP are not digested or absorbed following ingestion since there are no enzymatic pathways available for the breakdown of synthetic polymers. Instead, they are egested, or they pass through cell membranes and accumulate within muscle and organ tissues (Khan et al., 2017; Andrade et al., 2019). Little is known about the residence time of ingested particles and the factors which influence accumulation (particle type and size?) therefore MP contamination in GI tracts likely only reflect a snapshot of the recent feeding activities.

In the Baltic Sea, Setälä et al. (2014) examined microplastic transfer in planktonic food webs and found that organisms ingesting MP debris can act as vectors for litter transfer to higher trophic level organisms. Farrell and Nelson (2013) building on work by Browne et al. (2008), demonstrated that blue mussels could be vectors for microplastics in the benthic environment. In freshwater, Campbell et al. (2017) observed significantly more MP in northern pike, and hypothesized that, as apex predators, pike may have increased concentrations due to trophic transfer from smaller species. McNeish et al. (2018) measured the abundance of MP in fish and surface waters from tributaries of Lake

Michigan, USA and found a relationship between fish size and number of MP particles, and a significant effect of feeding group on MP concentration in fish, suggesting predator-oriented fish may obtain MP via trophic transfer from prey items. In contrast, Pazos et al. (2017) found no relationship between MP abundance in fish and fish trophic group in the Rio de la Plata estuary, Argentina and Dantas et al. (2020) found no significant relationship between MP and trophic guilds in fish from Fortaleza coastal zone, Brazil. These findings suggest trophic position can play a role in MP abundance but may be species specific.

Habitat Preferences

Numerous benthic, and pelagic fish species around the world have been documented to have ingested MPs (Rochman et al., 2015; Tanaka & Takada, 2016; Güven et al., 2017; Ory et al., 2017, 2018; Azevedo-Santos et al., 2019; Walkinshaw et al., 2020). Although these species occupy different aquatic compartments, most fish, regardless of habitat differences, were exposed to MPs.

MPs vary in density and can therefore settle throughout the water column (Gundgodu et al., 2020). Previous studies have suggested that there is a relationship between habitat use and MP contamination with conflicting results. For instance, Rummel et al. (2016) and Güven et al. (2017) suggested that pelagic species contained more MPs when compared to benthic species, while Lusher et al. (2013), Neves et al. (2015), Markic et al. (2018), and Klangnurak and Chunniyom (2020) found that pelagic and demersal species did not differ in MP content (Gundgodu et al., 2020). On the other hand, Jabeen et al. (2017) investigated plastic pollution in 21 species of sea fish and six species of freshwater fish from China and found that benthic species ingested

significantly more plastic particles than pelagic species. Similar results were found by McGoran et al. (2017), who found benthic species had ingested far more MPs in the River Thames than a pelagic species. In addition, Koongolla et al. (2020) noticed a significant difference between demersal and pelagic species in the Beibu Gulf, South China Sea, with MP abundance higher in benthic fishes than pelagic species.

Environmental Load

The environmental load of MP in surface waters and sediments has been a significant area of research. Although numerous hypotheses predict that polymer density determines MP distribution in the water column, in fact, many additional factors are at play including biofouling, aggregation and zooplankton uptake, as well as the unique hydrodynamics of each aquatic system. There has been much speculation about the degree surface water or sediment pollution can serve as a proxy for MP ingestion in fish (Bosshart et al., 2020). Bosshart et al. (2020) investigated the impact of environmental load in sediment on microplastic accumulation in benthic round goby located in the Rhine River. Although bottom sediments contained high microplastic concentrations (1.4 x 105 particles m3), they found negligible MP ingestion rates (one particle in 417 fish) and therefore concluded that higher environmental microplastic concentrations are not necessarily mirrored by higher ingestion rates in fish sampled from such areas. In contrast, Horton et al. (2018) showed a significant relationship between MP ingestion and proximity to the River Thames source. Although the abundance of MP in surface waters of the River Thames has not been determined, the results reflect the fact that the number of MP inputs to the river increase with distance from the source due to increasing urbanization as the Thames flows towards London.

Fish Size

The increase of MP with increased fish size may be due to the increased volume of food required to meet energy demands of larger fish. Parker et al. (2020) examined MP occurrence in fish with different feeding characteristics and detected significant differences in MP abundance between multiple species based on their size. The smallest species had the lowest number of MP (1.9 MP per fish) while the largest fish had the highest (82.6 MP per fish). Gundgodu et al. (2020) examined the relationship between fish length, mass, and MP abundance. MPs per gram of fish mass were calculated for each species and a statistically significant negative relationship was detected between fish mass and the quantity of MPs (Pearson $r = –0.48$; $P < 0.05$), but fish size (cm length) did not predict the quantity of MPs (Pearson $r = 0.15$; $P > 0.05$). In addition, Horton et al. (2018) found that size of fish was correlated with the quantity of MP in the gut. Larger mainly female fish were more likely to ingest the max possible number of particles than smaller mainly male fish. Gender and length of the fish were not related ($p > 0.05$, interaction effect of two-way ANOVA) indicating gender and length influenced ingestion independently.

This evidence indicates that MP abundance is best compared between species when expressed as concentrations per units of fish size, fish weight or gut weight. Few other studies report MP in fish by concentration. Further study is required to understand to what degree fish size and gender specific difference may lead to increased energy demands, increasing volume of food consumed and possible MP ingestion.

Comparing Fish MP Results

There is currently no standardized methodology for measuring the quantity of MP in the guts of marine and freshwater organisms. Not having a common set of repeatable, reliable, and targeted metrics for quantifying MP in freshwater organisms limits reliable comparisons between studies (Collard et al., 2019):

- In many studies low sample size prohibits reliable statistical analyses and trend definition. A sample size of 50 individuals per fish species has been defined as sufficiently reliable to achieve statistical power when testing for percentage of occurrence between species (Collard et al., 2019).
- Several chemical digestion methods have been used for the extraction of microplastics from fish guts (oxidizers, hydroxides, or enzymes) with varying effectiveness, yielding different densities of microplastics (Friesen et al., 2019).
- Lack of target particle size and detailed measurements of particles hinders accurate comparisons between study results.
- Varying methods used for particle identification (visual sorting or the use of spectroscopy (Fournier or Raman), yield very different results since visual observations tend to underestimate the numbers of microplastic fragments and, overestimate microplastic fibers compared to spectroscopic analyses (Song et al., 2015).
- The unit of measure used to express study results vary between percentage of contaminated individuals, number of particles per sample, and number of particles per gram of gastrointestinal tract contents (Collard et al., 2019). Although percentage is used most frequently, the unit gives little valuable quantitative

information for comparison since a fish with only one microplastic particle should not be considered the same as one which contained twenty.

- There remain many unknowns in this field of study, and it is only when there is a high enough volume of consistent and comparable results that solutions can be found.

The St. Lawrence Watershed and Microplastics

The Laurentian Great Lakes watershed is the largest source of freshwater in the world. The St. Lawrence River flows 700 miles from Lake Ontario to the Atlantic Ocean. This complex ecosystem is comprised of calm lake expanses and narrow stretches with fast moving currents. The variability of this system greatly influences the river's habitats and fish communities. Many small- and large-scale anthropogenic disturbances such as effluents discharge, and an artificial split by a shipping channel that restricts the river flow, contribute to the wide contrasts among the fish communities of the different sections of the river (Gouvernement du Quebec, 2008). This region experiences a continental climate with warm summers, strong seasonality, and cold winters. Daily mean temperature ranges from -5.8°C in January, to 25.8°C in July. Mean precipitation ranges from 75 mm in January to 95 mm in July (Environment Canada, 2021).

Microplastic Pollution in Surface Water and Sediment

Eriksen et al. (2013) were first to report on an open-water survey for plastic pollution within the Laurentian Great Lakes system. Of the 21 net tows performed in Lake Superior, Huron and Erie, all samples except one contained plastic. This 2013

discovery revealed an important upstream source of plastic pollution into the North Atlantic Ocean using the St. Lawrence River as the pathway.

In 2014 a team from McGill University discovered microplastic beads in the St. Lawrence River sediments (Castaneda et al., 2014). Previously detected in surface waters of lakes and rivers, this was the first microplastics discovery in freshwater sediments. The team sampled sediments from 25 locations along a 320 km stretch of the St. Lawrence River and measured over 1000 microbeads per litre of sediment, a quantity which rivals the most polluted ocean sediments (Castaneda et al., 2014). Motivated by these findings, a different team from McGill quantified the abundance of different types of microplastics in sediments and surface water samples and related these quantitative differences to environmental variables such as different land use and WWTF effluent outflow points along the St. Lawrence River (Crew et al., 2020). Mean concentrations of microplastics in sediments were among the highest recorded for the world's freshwater and marine systems (832 +/-150 SE plastic particles per kg) (Crew et al., 2020).

The prevalence of MP found in the St. Lawrence River sediments raises the question of whether fish and other organisms are ingesting this debris and what the consequences of this may be for human and ecosystem health.

Possible Patterns of MPs in St. Lawrence River Fish

Although previous studies of MPs in freshwater fish have produced varying results, when coupled with the prevalence of MP found in the St. Lawrence River sediments (Crew et al., 2020; Castaneda et al., 2014), and the data modeling performed by Lebreton et al. (2018), we might expect that feeding habitat and diet impact the type

and quantity of microplastics ingested by freshwater and marine fish: fibers are light weight and stay suspended in the water column longer, whereas fragments and beads sink and get sequestered into the sediments. Therefore, benthivores are likely exposed to microplastics that have settled on to the sediments, while pelagic species are more likely to consume microplastics suspended in the water column (Campbell et al., 2017).

Research Question, Hypotheses and Specific Aims

My research focused on addressing the following question: How do fish feeding habitats and diet preference influence microplastic ingestion and accumulation in St Lawrence River freshwater fish? I proposed the following hypotheses:

- H1: The gastrointestinal tracts of most benthivorous and pelagic fish species contain microplastic particles.
- H2: The gastrointestinal tracts of benthic fish contain higher concentrations of microplastic beads and fragments than the gastrointestinal tracts of pelagic fish, while the gastrointestinal tracts of pelagic fish contain higher concentrations of microplastic fibers than the gastrointestinal tracts of benthivorous fish.
- H3: A greater concentration of plastic particles would be found in benthic than pelagic fish.
- H4: There would be a greater frequency of occurrence of plastic particles in omnivorous and carnivorous than in herbivorous fish species.

Specific Aims

Completing this research required that I:

1. Collect independent samples of pelagic and benthic fish species with different diet preferences (herbivore, omnivore, carnivore).
2. Euthanize, measure, weigh, and freeze whole fish until dissection.
3. Dissect and weigh whole gastrointestinal tracts from all fish samples.
4. Perform chemical digestion of gastrointestinal tract organic material using pancreatic enzymes.
5. Filter debris for analyses.
6. Observe debris using a microscope to categorize particles according to physical characteristics (color, size) and type (fragment, bead, fiber).
7. Count and weigh microplastic particles found in each gastrointestinal tract.
8. Randomly select particles from each gastrointestinal tract sample to determine chemical composition using spectroscopic analysis (Raman or Fournier).
9. Analyze the relationship between fish feeding habitats and diet and microplastic particle concentration using a cross referenced 2 factor analysis of variance (feeding habitat = pelagic, benthic. diet= herbivorous, omnivorous, carnivorous).
10. Analyze the relationship between fish feeding habitats and diet and microplastic particle type (fragment, fiber, bead).
11. On the basis of these results, recommend more informed policy decisions about microplastic production and waste management practices to curb microplastic pollution in aquatic ecosystems.

Chapter II

Methods

The below sections detail the study area, sample collection, extraction methods, identification, statistical analysis, and high-level workflow used for this research.

Study Area Description and Selection

Sampling sites were selected along the St Lawrence River (Figure 1) from an area southeast of Montreal around Grosbois Island to the northwestern shoreline of Lake Saint Louis as far as Sainte-Anne-de-Bellevue. This region of the river (Figure 2) is a diverse ecosystem with different flow patterns including narrow fast flowing areas and calm waters which form lakes. Lake Saint Louis is located at approximately 45°23'59.99" N - 73°48'59.99" W, 25 km from downtown Montreal, the largest city in the province of Quebec.

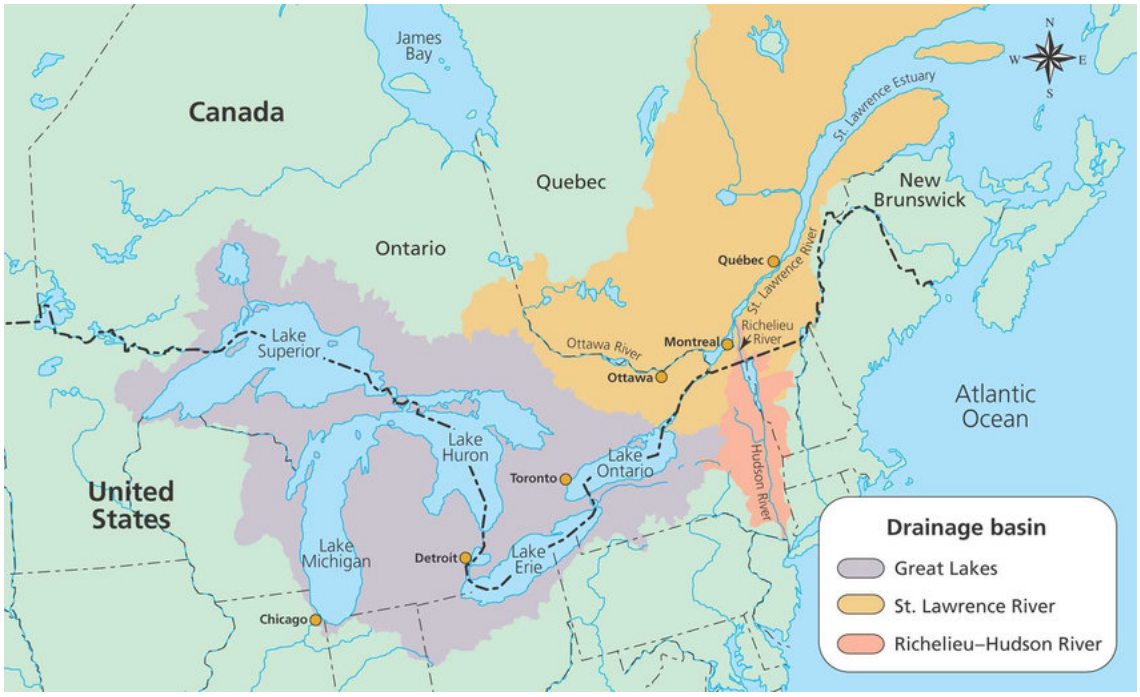

Figure 1. Map of the Great Lakes-St. Lawrence River drainage basin.

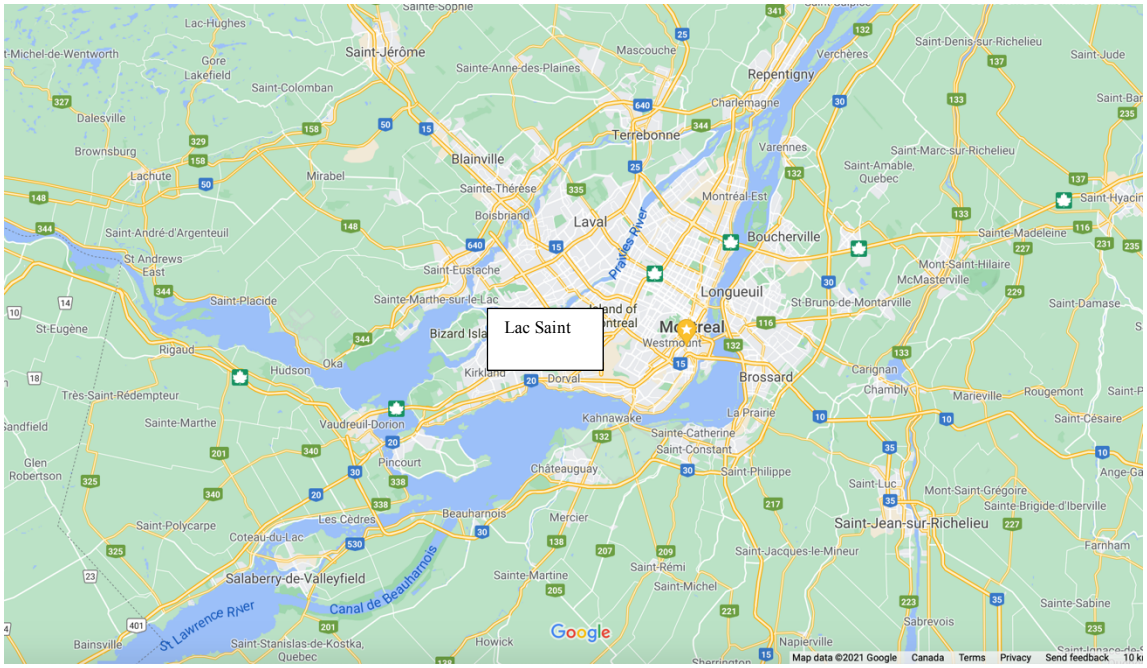

Figure 2. Map of Lac Saint-Louis and the surrounding area.

Sample Collection

All fishes were euthanized by a blow to the head, wrapped in aluminum foil and kept on ice in insulated coolers during transport. Fish were immediately frozen and kept at −10 °C until further analysis. A total of 73 fish from seven different species were collected, and their GI tract analyzed for microplastics. Collected species include *Micropterus dolomieu* (Smallmouth Bass), *Lepomis macrochirus* (Bluegill), *Esox lucius* (Northern Pike), *Perca flavescens* (Yellow Perch), *Sander vitreus* (Walleye), *Neogobius melanostomus* (Round Goby), *Ambloplites rupestris* (Rock Bass) and *Lepomis gibbosus* (Pumpkinseed). Each species was classified by feeding habitat and trophic level according to the data available from Fishbase, a global database on fish species (McIlwaith et al., 2021).

Table 1. Fish species information.

Fish Species	**Milieu**	**Food**	**Depth range**	**Length**	**Trophic level**
Bluegill	Bentho-pelagic	Carnivore	0 - 20 m	~ 19 cm	3.2
Walleye	Bentho-pelagic	Carnivore	0 - 27 m	~ 54 cm	4.5
Pumpkinseed	Bentho-pelagic	Carnivore	0 - 41 m	~ 10 cm	3.3
Smallmouth Bass	Bentho-pelagic	Carnivore	1 - 7 m	22 -? cm	3.6
Northern Pike	Pelagic	Carnivore	0 - 30 m	25 - 63 cm	4.1
Perch	Bentho-pelagic	Carnivore	0 - 56 m	~ 19 cm	3.7
Round Goby	Benthic	Carnivore	0-30 m	4 - 30 cm	3.3
Rock bass	Bentho-pelagic	Carnivore	0 - 21 m	~ 15 cm	3.4

Target fish species were collected using conventional fishing tackle, casting along the shoreline, or from motorized fishing boat. An effort was made to collect fish samples from each feeding habitat (pelagic, benthic) and diet group (omnivore, herbivore, carnivore) combination (Table 2); however, fish species within the collection area were found to be exclusively carnivorous. Collected fish species included round goby (benthic), perch, rock bass, smallmouth bass, pumpkinseed, bluegill, walleye (bentho-pelagic) and northern pike (pelagic).

Table 2. Fish habitat and diet preference category distribution.

	Carnivorous
Benthic	Benthic- Carnivore (Round goby)
Bentho-pelagic	Bentho-pelagic- Carnivore (Perch, Rock bass, Smallmouth bass, Pumpkinseed, Bluegill, Walleye)
Pelagic	Pelagic- Carnivore (Northern pike)

Fish Dissections

All steps were performed under a laminar flow hood with synthetic-free clothing, with rubber gloves worn at all times. All labware was rinsed three times with filtered deionized water (20 μm pore size, Polycarbonate (PCTE) Membrane Filters, Sterlitech). Prior to dissections, fish were left at room temperature to defrost, removed from aluminum foil wrapping, and rinsed with deionized water. Whole fish were weighed using an analytical electronic balance (ZQ-563, Baoshishan) to the nearest 0.01g and total length measured to the nearest 0.1 cm using a ruler.

GI tracts were removed from each fish by creating an incision from the anal opening down the ventral side of the fish to below the throat, and then upward past the pectoral fin and back toward the anal opening at an angle to remove outer flesh (Campbell et al., 2017). Once exposed, gastrointestinal tracts were carefully extracted using scissors and forceps, placed in a 250 ml (pre-weighed) glass beaker, and weighed to the nearest 0.01g. Prior to each use, glass beakers were washed with soap and water and then rinsed three times with filtered deionized water to avoid contamination. To mitigate possible contamination of samples, each step was performed under a laminar flow hood (Collard et al., 2015; Campbell et al., 2017).

Digestion and Extraction of MP from GI Tracts

Chemical digestion was used to separate MP particles from the organic matter of each GI tract. All samples were processed using 4N (g/L) potassium hydroxide (KOH) solution prepared by dissolving potassium hydroxide flakes into filtered deionized water in a glass beaker covered with aluminum foil. Following a modified method from Foekema et al. (2013) to extract anthropogenic debris from the gut content of fish, each sample beaker containing the GI tract was filled to ~ three times the volume of sample tissue with KOH solution in deionized water (Munno et al. 2018, adapted from Foekema et al. 2013). Each beaker was covered with aluminum foil and set aside to incubate for 24-48 hours at room temperature until organic matter was dissolved. If necessary, a magnetic stir bar was added to the beaker and placed on a magnetic stirrer (MS-500, Intellab) for one hour until organic matter was more completely digested. Potassium hydroxide (KOH) was chosen as the digestion agent since it offers the best balance of efficiently eliminating biological material while being inert to most plastic polymers

(Karami et al., 2016). Incubating fish tissues in a KOH solution at room temperature has demonstrated a ~97.7 % digestion efficiency after one night (Friesen et al., 2019; Collard et al., 2019).

Filtration

Although many factors have contributed to the varied findings in MP pollution between studies, filter selection has led to significant inconsistency in the estimated levels of MP contamination. The minimum pore size used for filtering during analysis dictates the smallest size of MP particles that can be detected, directly affecting the number of MPs identified (Gundogdu et al., 2020). In previous studies, pore size has ranged from 20 micrometers (Gundogdu et al., 2020) to 120 micrometers (Lusher et al., 2013) to 300 micrometers (Bosshart et al., 2020) and as high as 500 micrometers (Rummel et al., 2016). Since the aim of this study was to examine all possible MP particles contained in fish samples, and to identify whether there is a dominant size range where MP pollution occurs, 300-micrometer, 125-micrometer, and 20-micrometer pore size sieves were used to target three different particle size ranges (Munno et al., 2021; Collard et al., 2019). Following chemical digestion, the dissolved solution of GI tract contents (digestate) was poured through the stainless-steel sieve stack containing the 300 um, 125 um and liquid collection tray. Once emptied, the beaker was rinsed three times using a squirt bottle filled with deionized water to ensure all particles were removed from the beaker and captured into the sieve stack. The 300um sieve was removed from the stack and held at an angle over the 125um sieve while deionized water was used to rinse the contents of the sieve three times so all particles were pushed to one edge of the sieve. Particles were then carefully rinsed into a 75mm glass petri dish with cover and labeled

for further analysis. All petri dishes were rinsed with deionized water and inspected under microscope for possible contamination prior to use. These steps were repeated for the 125um sieve. All liquid collected in the sieve tray, which contained digestate and deionized water collected during rinsing the 300 um and 125 um sieves, was poured into a 1000 ml glass beaker. The final filtration step was achieved by pouring all liquid contained in the 1000 ml beaker through a four-inch stainless steel 20um sieve over the collection tray. Particles were then carefully rinsed into a 75mm glass petri dish with cover and labeled for further analysis. All residual mixture of digestate and deionized water remaining after each filtration step was set aside in sealed glass jars and discarded as hazardous waste. The filtration process resulted in three 75 mm glass petri dishes per fish, each containing the following particle ranges: >300um, 300um- 125um, 125um-20um.

MP Particle Sorting and Quantification

Samples were assessed visually and categorized according to their physical characteristics. Suspected microplastics were categorized according to color and morphology (fiber, fragment, bead) (Rochman et al., 2019). Particle counts included only those confidently resembling MP particles based on visual characteristics using the Hidalgo-Ruz Rules (Hidalgo-Ruz et al., 2014). Each petri dish was examined under a AmScope zoom stereo microscope at 7- 45 x, and suspected microplastics were categorized according to color, size fraction (>300 um, 300-125 um or 125-20 um), and morphology, which are typical classifications in microplastics. Pictures of selected suspected MP particles were taken using a AmScope 3MP digital camera.

Examination and Identification

Purely visual examination cannot be used to reliably distinguish between plastic polymers and other natural particles; therefore, a representative subsample of microparticles underwent further testing. 10% of suspected particles were randomly selected and tested using a hot-needle test (De Witte et al., 2014). To increase accuracy and reduce the potential for particle moisture to compromise results, all petri dishes were placed in an oven at 300 degrees C to ensure samples were adequately dry prior to hot needle testing. The test was performed under a AmScope zoom stereo microscope at 7-45 x using a small flame and 8cm metal needle. When in the presence of a very hot needle, plastic particles melt or curl. The needle was held as close as possible to the suspected particle without touching to allow a clear view of the response. Suspected MP particles were verified when movement, curling and melting occurred in the presence of the needle.

QA/ QC

Due to the occurrence of MP particles in air and water, a variety of steps were taken to mitigate possible contamination of samples. All MP processing steps were performed under a laminar flow hood. All work surfaces and tools were rinsed with tap water and then with deionized water between each fish sample. Only deionized water was used during MP processing, including throughout the rinsing and filtering steps. Deionized water used during MP processing, was filtered through a 20um polycarbonate (PCTE) membrane filter using a glass filtration apparatus, prior to use. All glassware including beakers and petri dishes were washed with tap water, baked at 450 degrees C for four hours, and rinsed with deionized water prior to use. A 100% cotton lab coat was

worn during MP processing. Only glass and stainless-steel equipment was used, and all samples, sieves, dissection tools, and beakers were covered with aluminum foil between use. To quantify potential contamination of samples from MP present in the lab, procedural blanks were run in parallel to fish samples and underwent full laboratory procedures. One blank was run for every ten fish samples 10% (n= 7) to account for potential cross contamination during the extraction procedure and provided a measure of any contamination from solutions and equipment used during fish processing. The contamination blanks, which quantified environmental MP present within the lab, were obtained by placing a beaker containing deionized water on the work surface during the dissection, GI tract removal, and chemical digestion steps of a fish sample. The blanks followed the same processing steps as fish samples and were filtered through 300um, 125um and 20um sieves, rinsed into petri dishes and labeled for further analysis. Suspected MP particles were found in four of the seven samples and in each of the three size fractions. The majority of suspected particles were > 300 um. The morphology of the particles in the blanks consisted of 100% fibers (two black, two grey, one red, and one blue). Hot needle test verification confirmed all suspected particles as plastic. The large difference observed between the blanks and fish samples indicated that the microplastic concentrations revealed in this study cannot be attributed to laboratory contamination; therefore, microplastic particle counts were not corrected based on blank contamination (Rochman et al., 2019).

Chapter III

Results

A total of 1088 suspected microplastic particles were extracted from 73 fish collected from the Lake St Louis region of the St Lawrence River. Microplastic particles were observed in all fish samples from all species. The number of particles found in an individual fish ranged from four in a bluegill to 33 in a perch. The mean (+/- SD) across all fish was 14.9 +/- 6.9 MP per fish with 100% of fish containing at least one MP particle (Appendix 1).

The most contaminated were bass, goby and perch containing a mean of 16-19 MP/ fish and the least contaminated were pumpkinseed and bluegill which contained only nine per fish on average (Figure 3).

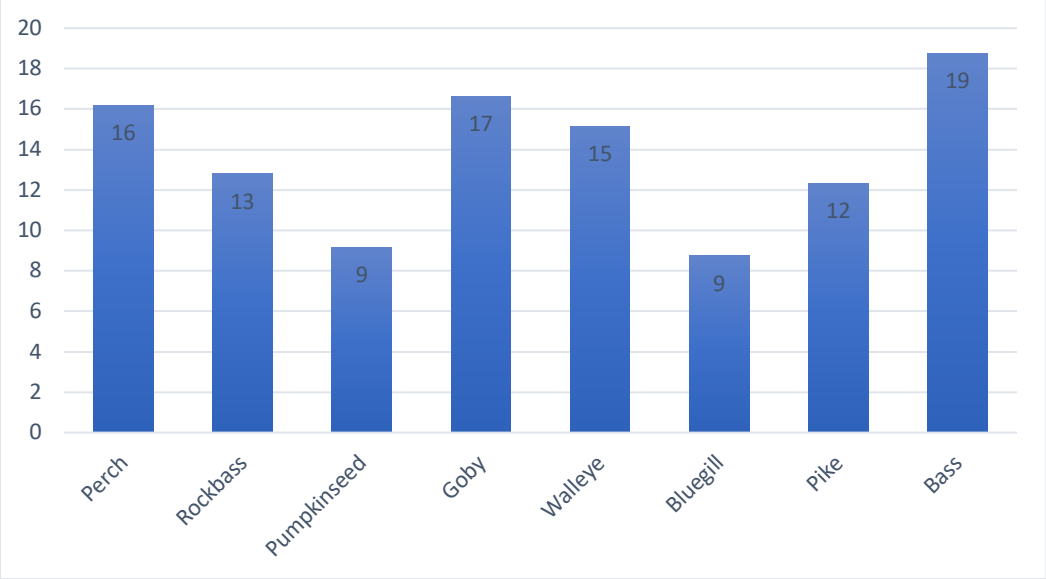

Figure 3. Mean number of MP particles per fish for all species collected.

Particle Morphology and Color

Fibers, fragments, and beads were found in the fish from this region. Fibers were predominant (85%), followed by fragments (7%), fiber bundles (5%), and beads (2%) (Figure 4).

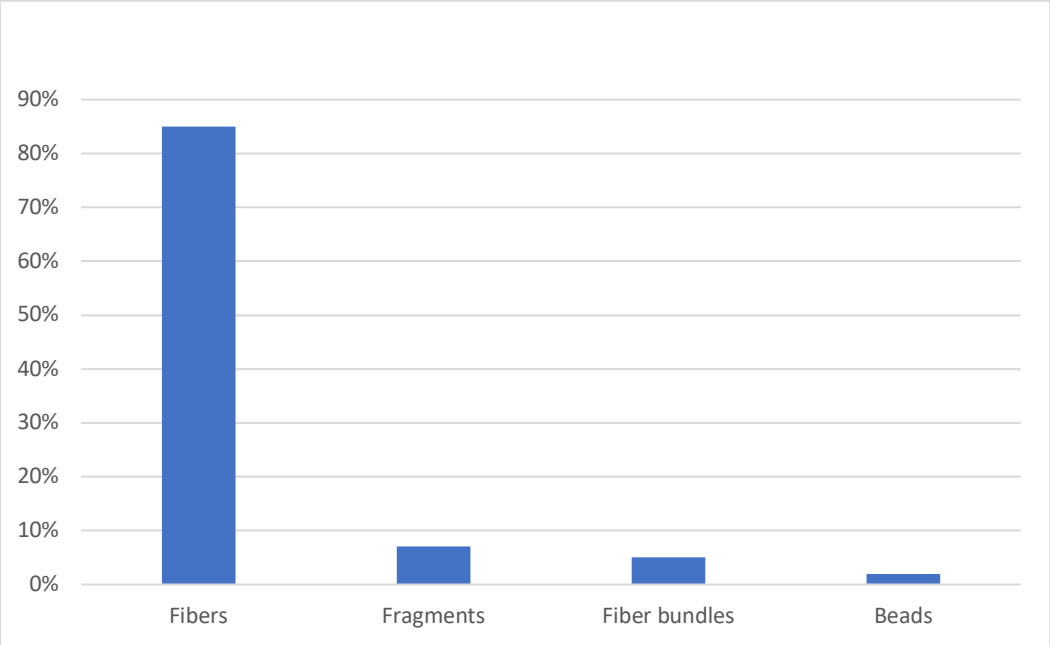

Figure 4. MP morphology.

The predominant particle colors were black, blue, red, and grey (Figure 5). Although most species consistently displayed a dominant distribution of black MP, followed by blue and red, rock bass contained an almost equal percentage of black, blue, and red MPs (Figure 6).

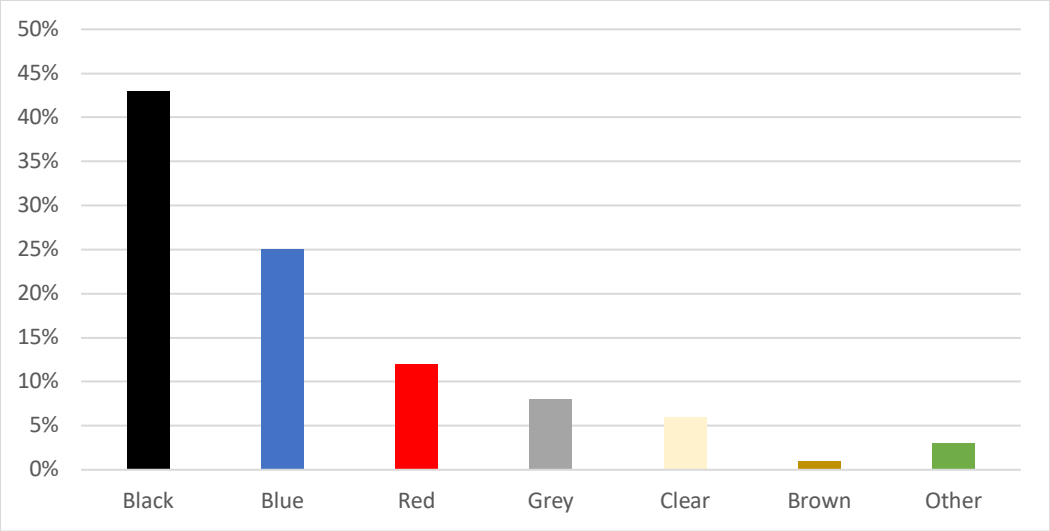

Figure 5. MP color distribution from all species collected.

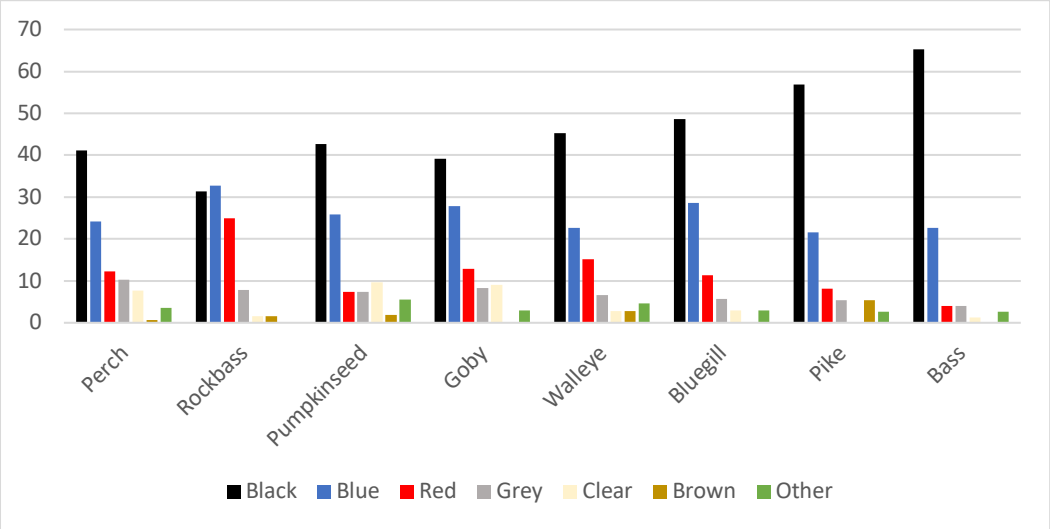

Figure 6. MP color distribution for each species.

When comparing the three size fractions of MP particles found in St Lawrence River fish, the majority of MP contained in the GI tracts of rock bass, pumpkinseed and goby were 300-125 um (followed by 125-20 um and >300 um). Most MP contained in

the GI tracts of walleye, pike, perch, bass, and bluegill were the smallest 125-20 um (Figure 7).

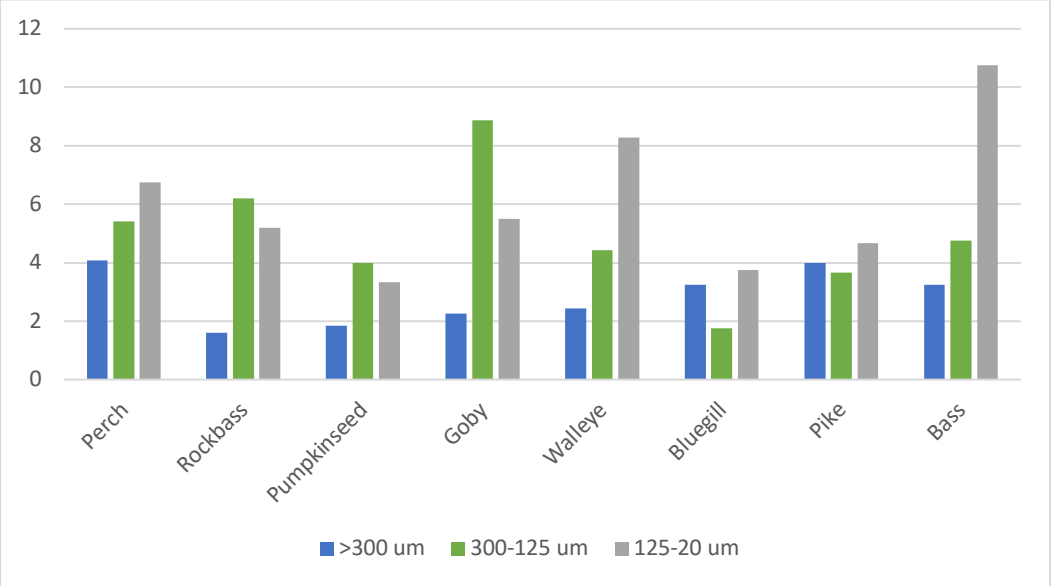

Figure 7. MP size fraction distribution.

Body Size

There was no relationship between MP abundance and fish length when regressed across all fish (r2= 0.002, p= 0.72, n= 73) however, significant variation existed between species. Bluegill exhibited a strong negative correlation (r= -0.94, r2= 0.88, p= 0.06, n= 4) whereas Bass (r= 0.87, r2= 0.76, p= 0.13, n= 4) and Goby (r= 0.83, r2= 0.69, p= 0.01, n= 8) exhibited a positive correlation. All other species showed little correlation (Figure 8 a and b).

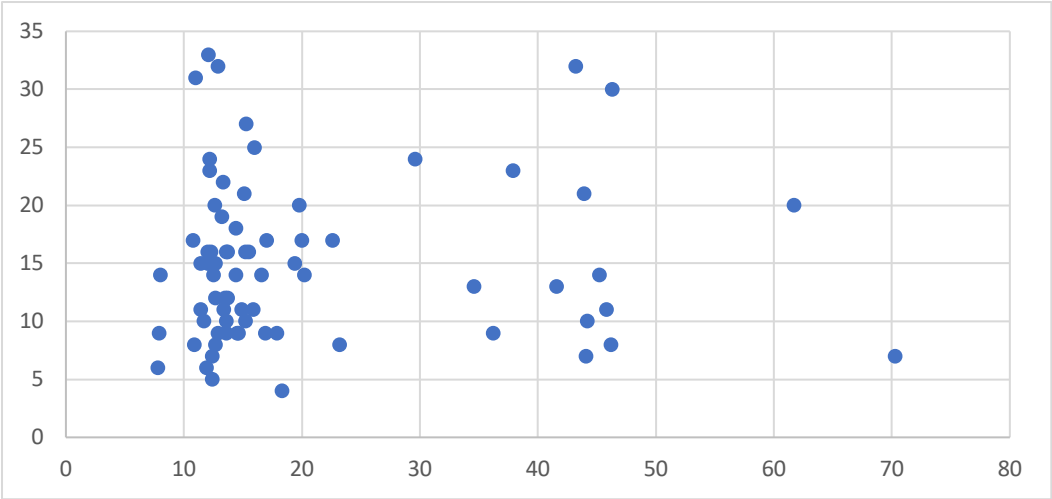

Figure 8a. Number of MP per fish compared to length for all species collected.

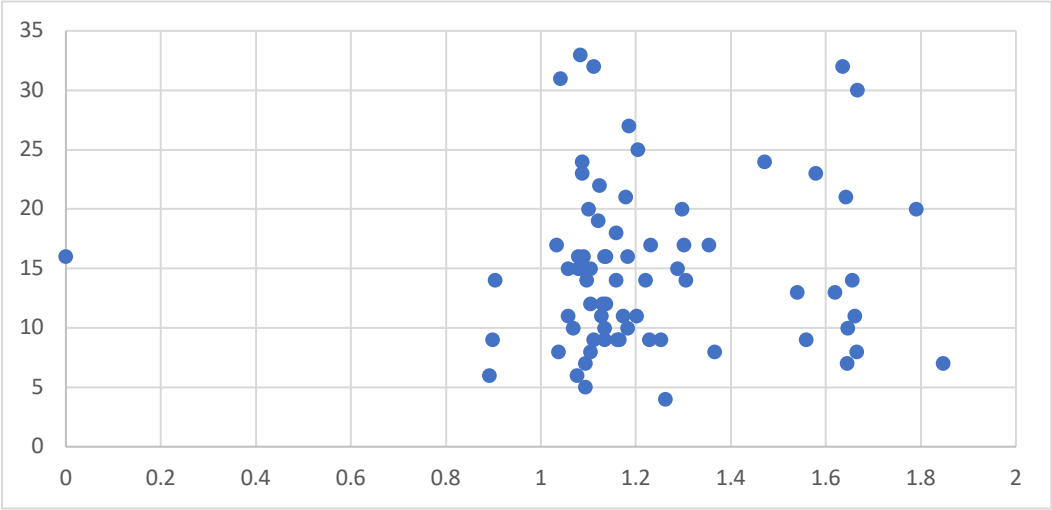

Figure 8b. Number of MP per fish compared to log length for all species collected.

MP abundance showed a poor, non-significant correlation with fish weight (r= 0.20, r2= 0.04, p= 0.12) (Figure 9 a and b) and fish gut weight was not correlated with MP abundance (r= 0.04, r2= 0.002, p= 0.72) (Figure 10 a and b). A great variation existed between species. While most species showed little correlation between fish

weight and MP abundance, Goby (r= 0.83, r2= 0.69, p= 0.01) and Bass (r= 0.87, r2= 0.76, p= 0.13) showed a positive correlation and Bluegill showed a negative correlation (r= -0.94, r2= 0.88, p= 0.06).

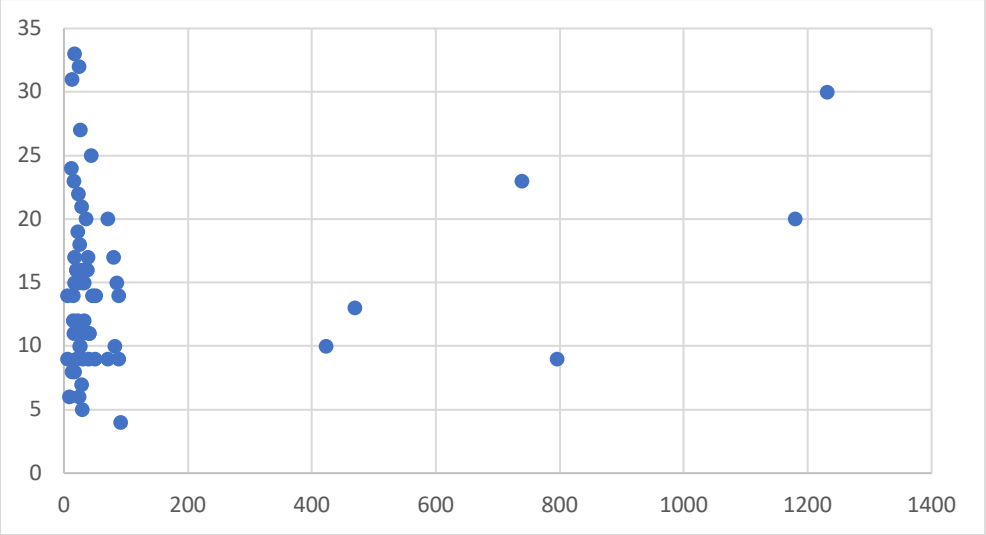

Figure 9a. Fish weight and number of MP per fish for all species collected.

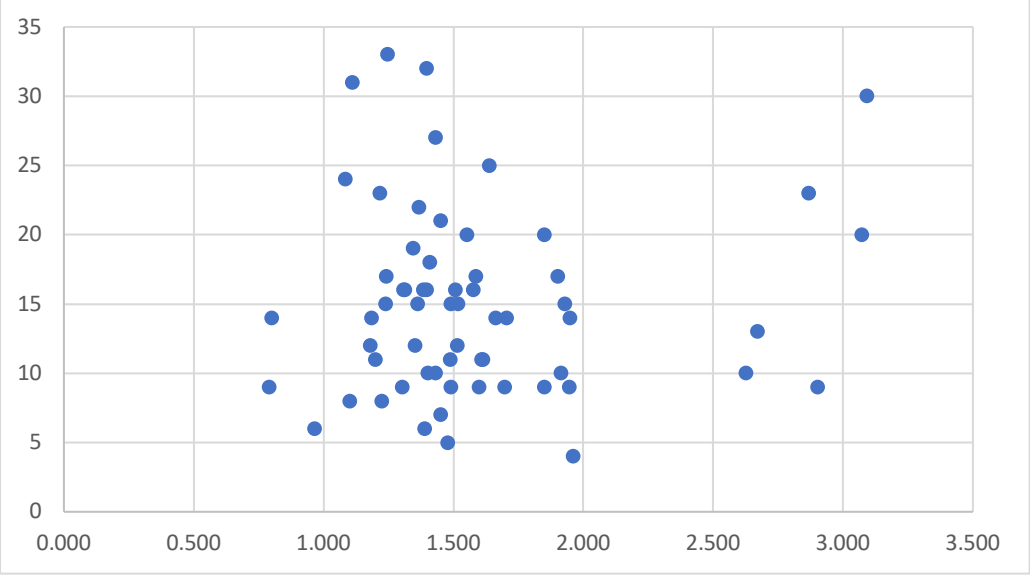

Figure 9b. Fish log weight and number of MP per fish for all species collected.

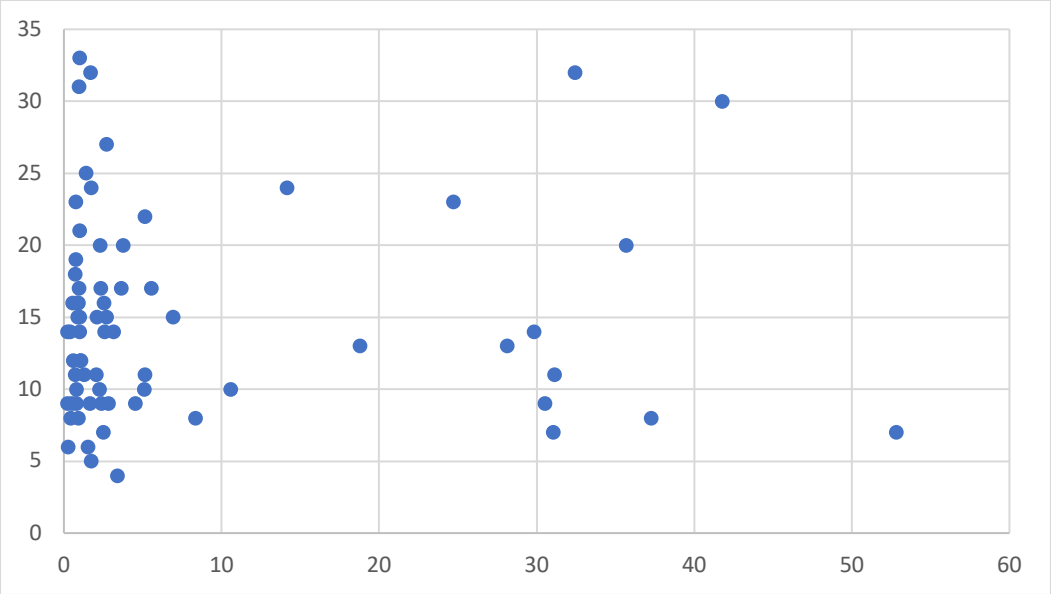

Figure 10a. Mean number of MP per fish compared to gut weight for all collected fish.

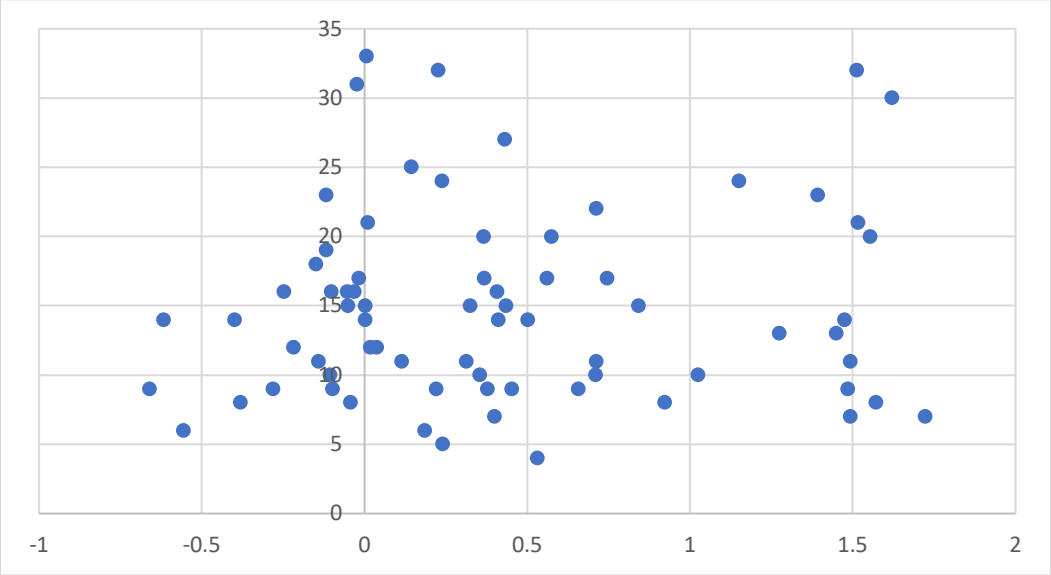

Figure 10b. Mean number of MP per fish compared to log gut weight for all fish of all species collected.

Most fish species showed no correlation between gut weight and MP abundance except for Bluegill ($r= -0.89$, $r2= 0.80$, $p< 0.05$) and Goby ($r= 0.91$, $r2= 0.83$, $p= 0.002$).

Since data points for comparisons of all fish were heavily clustered, log data was also displayed (Figure 11 a-h).

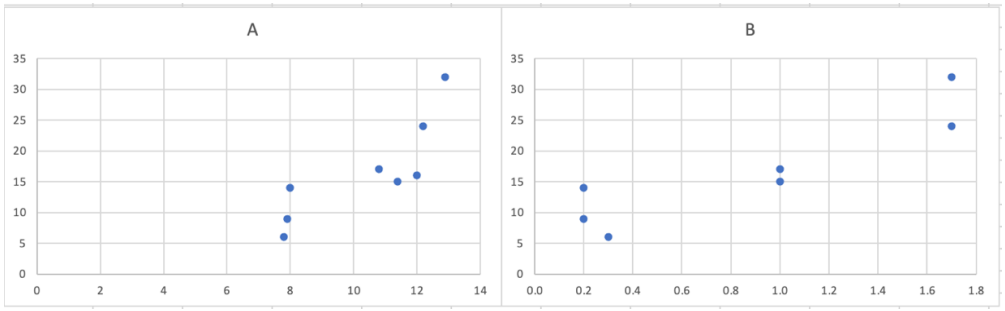

Figure 11a. Number of MP vs. goby weight (A) and goby gut weight (B).

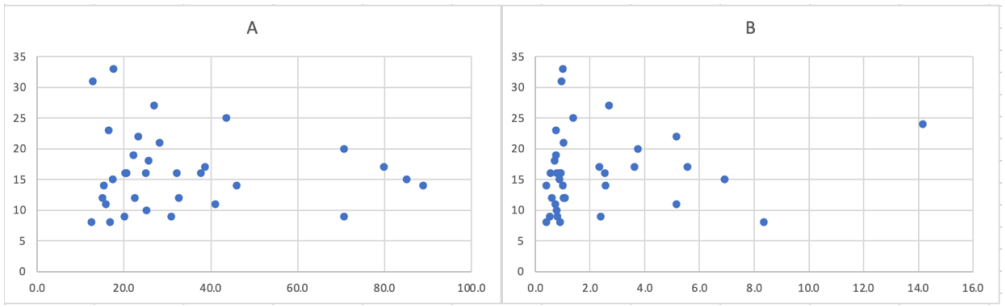

Figure 11b. Number of MP vs. perch weight (A) and perch gut weight (B).

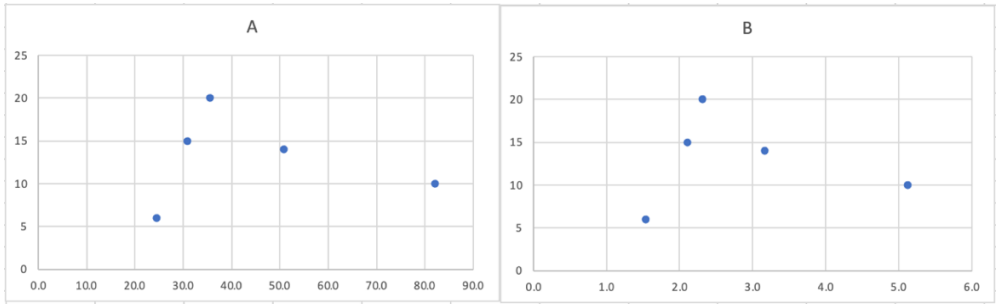

Figure 11c. Number of MP vs. rock bass weight (A) and rock bass gut weight (B).

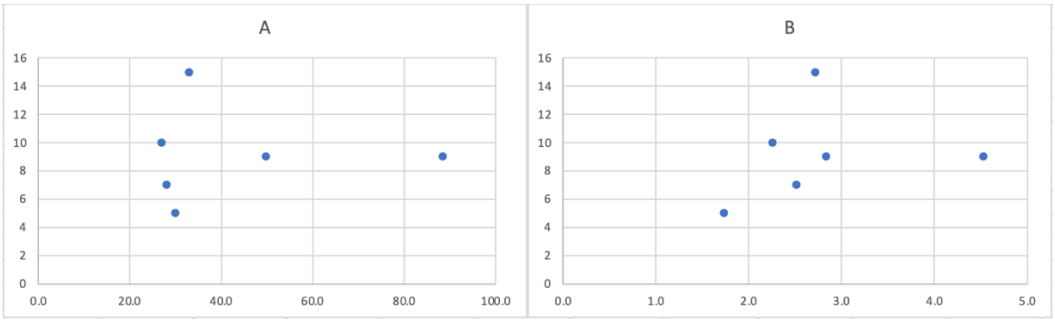

Figure 11d. Number of MP vs. pumpkinseed weight (A) and pumpkinseed gut weight (B).

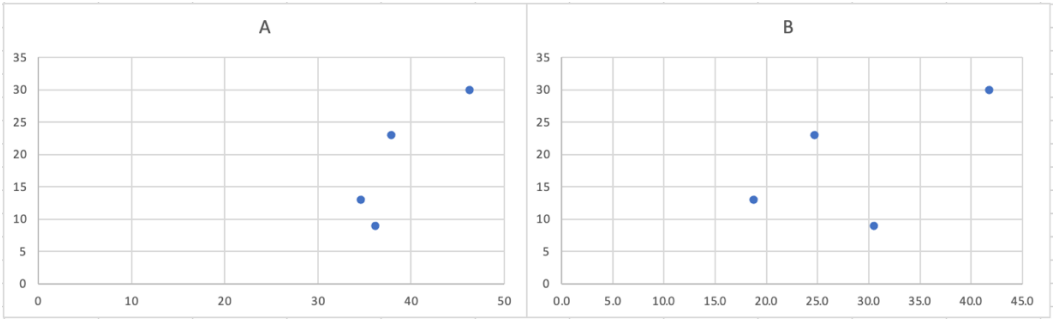

Figure 11e. Number of MP vs. bass weight (A) and bass gut weight (B).

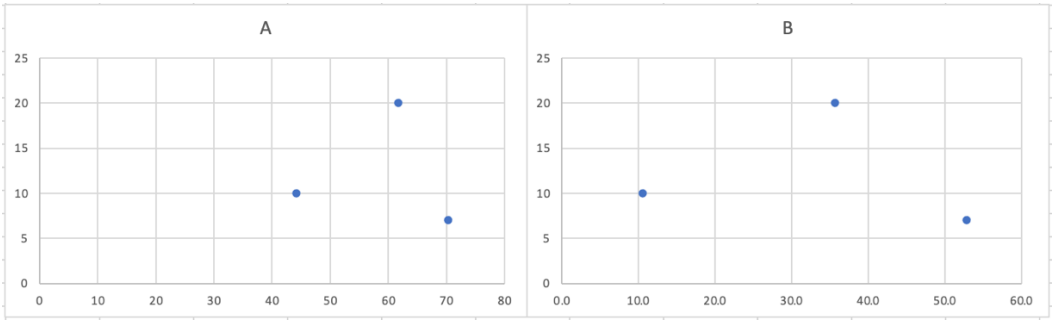

Figure 11f. Number of MP vs. pike weight (A) and pike gut weight (B).

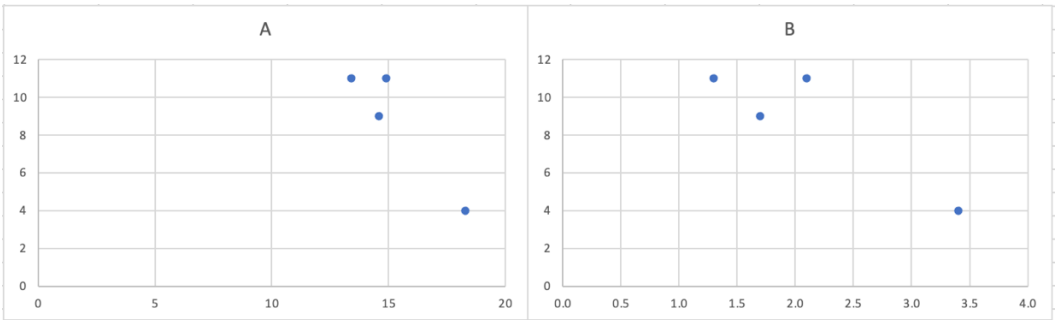

Figure 11g. Number of MP vs. bluegill weight (A) and bluegill gut weight (B).

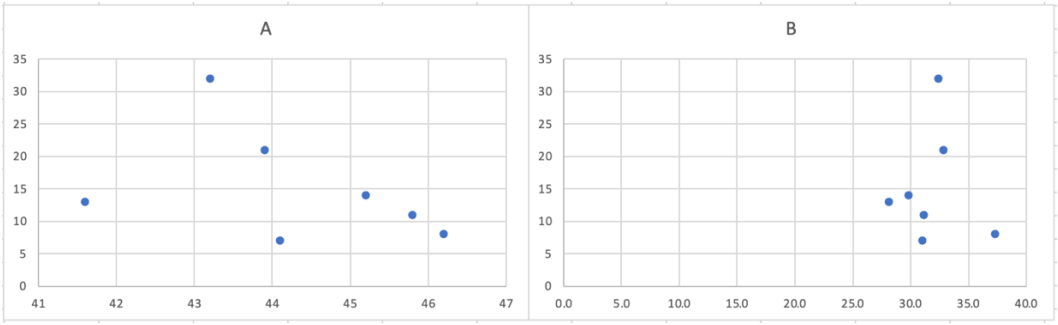

Figure 11h. Number of MP vs. walleye weight (A) and walleye gut weight (B).

Differences in MP in fish residing in benthic, benthopelagic and pelagic habitats were not significant (Figure 12). Benthic species had a mean of 16.6 +/- 8.2 MP/ fish (median= 11.5 MP) compared with a mean of 14.9 +/- 6.8 MP/ fish (median= 12.5 MP) for benthopelagic fish and mean of 12.3 +/- 6.6 MP/ fish (median= 20 MP) for pelagic fish (Figure 20). However, pelagic fish showed a significantly higher median number of MP per fish (20 MP) when compared with other feeding habitats (11.5 MP and 12.5 MP).

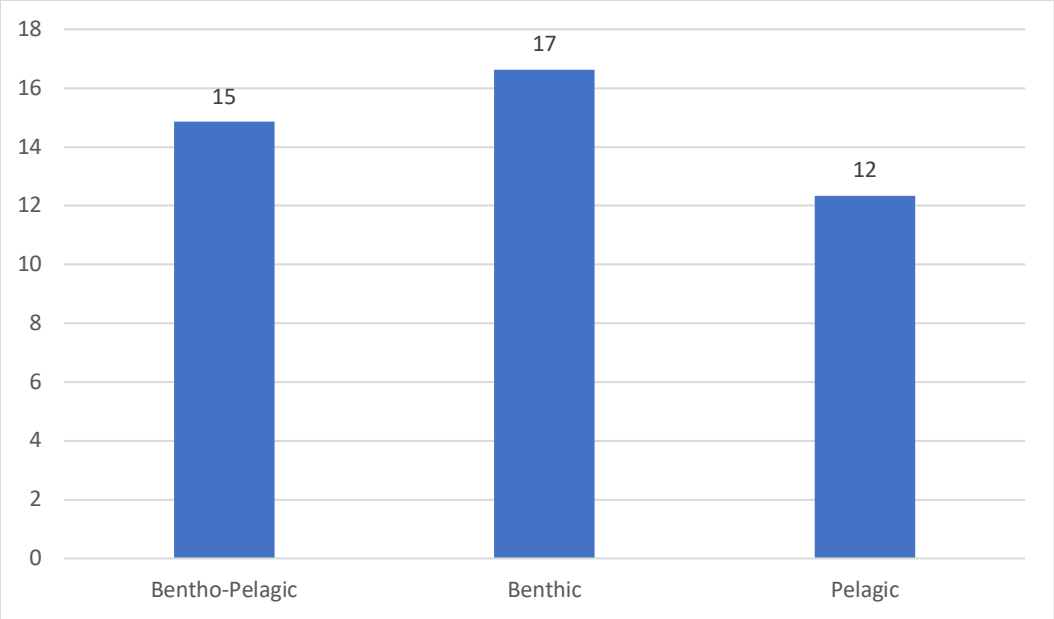

Figure 12. Mean number of MP per fish by feeding habitat.

When the number of MPs per fish was categorized by feeding habitat (benthic, bentho-pelagic, pelagic) and compared with the number of fibers per fish, pelagic species contained the most fibers (92%) followed by bentho-pelagic species (85%) and benthic species with the fewest fibers (35%) (Figure 13).

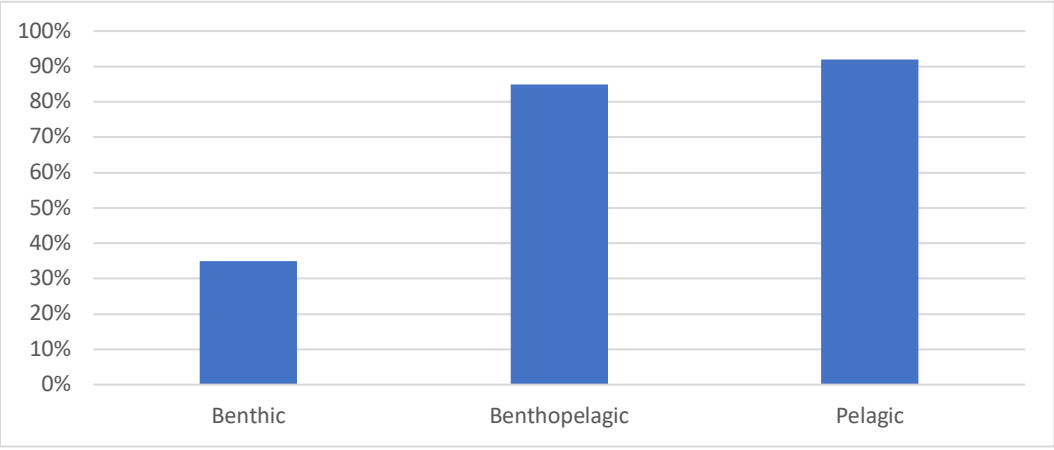

Figure 13. Percentage of fibers found in fish from different feeding groups.

When the number of MPs per fish was categorized by feeding habitat and compared with the number of fragments per fish, bentho-pelagic species contained the most fragments (10%) followed by benthic species (6%) and pelagic species with no fragments (0%) (Figure 14).

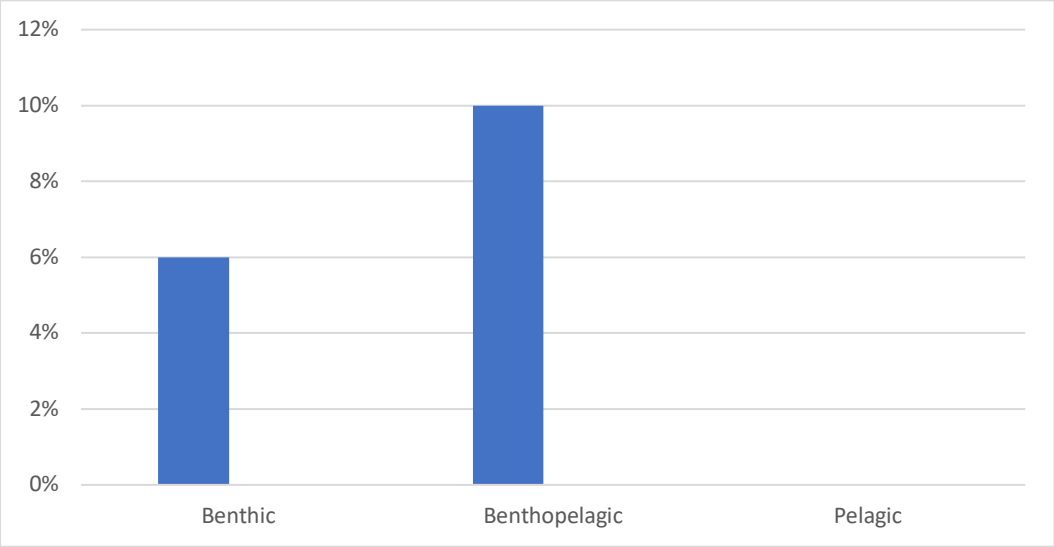

Figure 14. Percentage of fragments found in fish from different feeding groups

All fish species ranged in trophic position from 3.2 to 4.5, indicating their degree of carnivory in the food web (Table 3). When the average of all fish from the third trophic position were compared with fish from the fourth trophic position there was no difference in the concentration of MPs (mean = 14 per fish for both) (Figure 15).

Table 3. Trophic position of fish species.

	Trophic	Particles
Bluegill	3.2	9
Walleye	4.5	15
Pumpkinseed	3.3	9
Bass	3.6	19
Pike	4.1	12
Perch	3.7	16
Goby	3.3	17
Rock bass	3.4	13

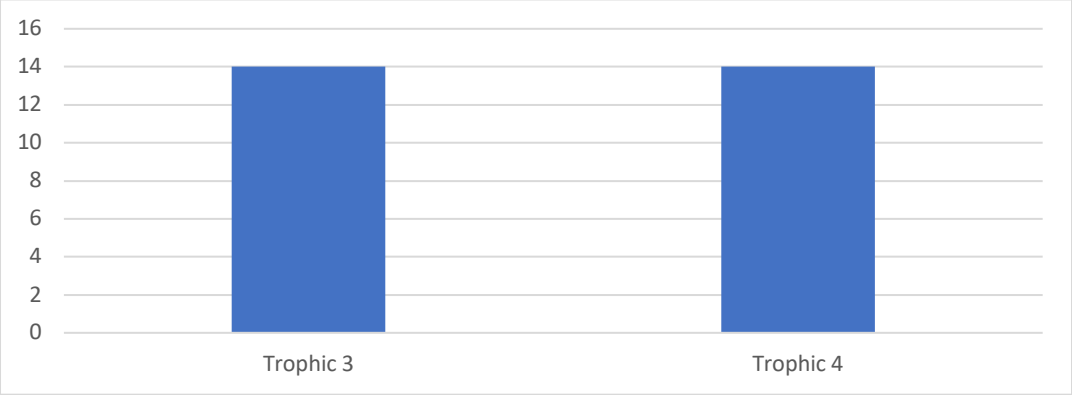

Figure 15. Trophic position and MP concentration.

When the color distribution of MP per fish was categorized by feeding habitat (Figure 16 a-c), each of the feeding habitats resulted in a similar MP color distribution, although pelagic fish contained the highest percentage of black MP and contained no clear MP.

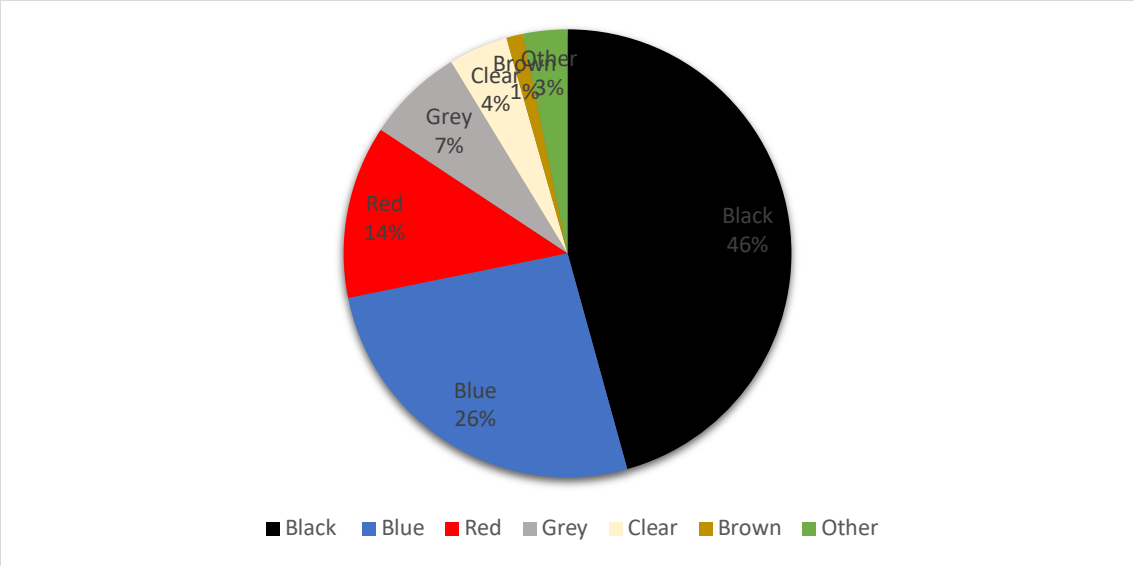

Figure 16a. Color distribution of MP found in Bentho-pelagic fish.

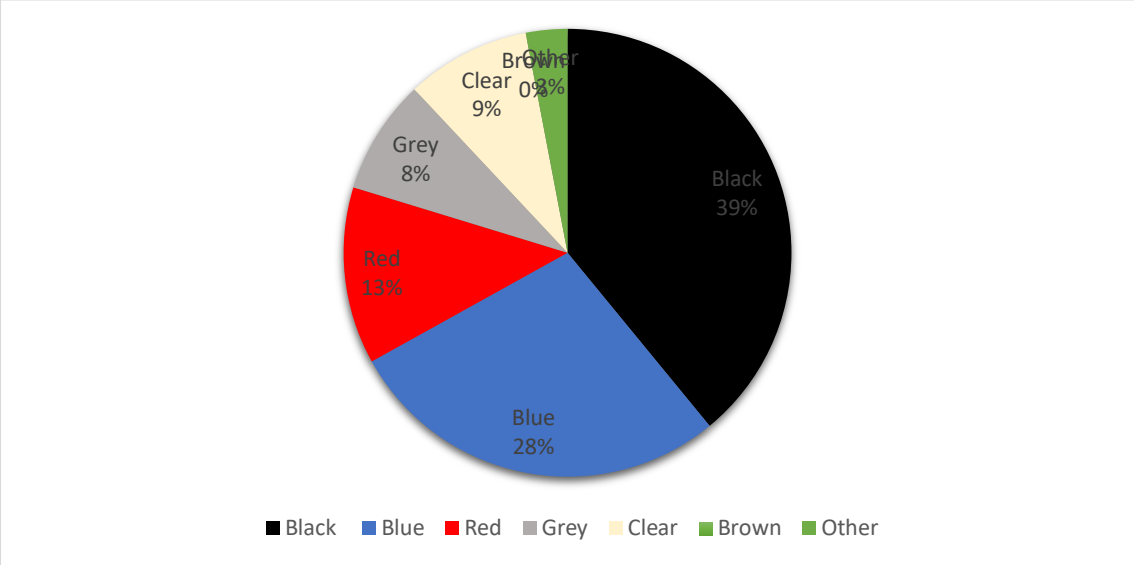

Figure 16b. Color distribution of MP found in Benthic fish.

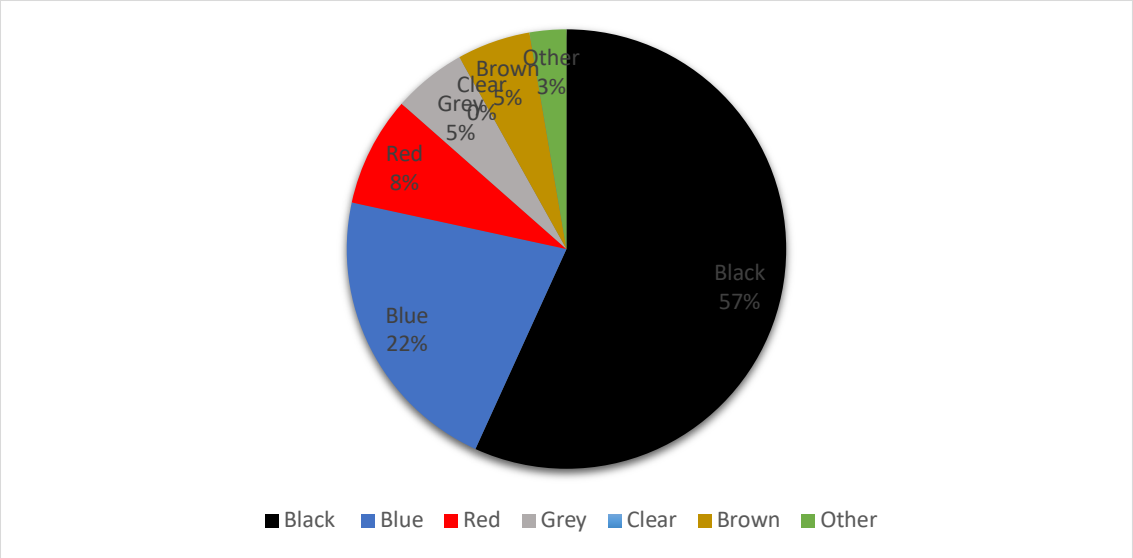

Figure 16c. Color distribution of MP found in Pelagic fish.

Chapter IV

Discussion

All fish sampled from Lac Saint-Louis were contaminated with MP, ranging in abundance from four to 33 MP/ fish. The mean (+/- SD) across all fish was 14.9 +/- 6.9 MP per fish. While mean abundances of MPs reported in marine environments are typically 0-2 MP/ fish (Lusher et al., 2013; Gundogdu et al., 2020), freshwater fish typically have a higher relative abundance of MPs in their GI tracts ranging from less than 1 MP (Bosshart et al., 2020; Horton et al., 2018) to 13 MP/ fish (McNeish et al., 2018). A critical review of over 800 species by Gouin (2020) revealed microplastic averages ranging from 0-10 MP/ fish. The percentage of fish containing at least one MP typically range from 30-96% in freshwater studies (Andrade et al., 2019; Jabeen et al., 2017; Silva-Cavalcanti et al., 2017; Munno et al., 2018). Therefore, St. Lawrence River fish are more contaminated compared to other species and study areas, as 100% of fish sampled contained MPs in this study .

Fibers were the most abundant MP morphology found in St. Lawrence River fish followed by fragments (Figure 4 & Appendix 2). This is consistent with most studies examining fish GI tracts (Jabeen et al., 2017; Horton et al., 2018; McNeish et al., 2018; Rochman et al., 2015). Fibers made up approximately 85% of all MP particles found in the St Lawrence River fish, which is consistent when compared with results of other freshwater studies that report 75-100% fibers (Peter and Bratton, 2016; Pazos et al., 2017; Horton et al., 2018; McNeish et al., 2018). The results from this study suggest the

pollution sources in the Lake Saint Louis area of the St Lawrence River are likely more numerous than those in other study areas.

MP morphology can reveal information about the source of the MP contamination in a river system. The predominant MP found in St Lawrence River/ Lake Saint Louis fish were black fibers from the smallest size fraction (125-20 um). This is consistent with knowledge that fibers released from washing machine wastewater into river outflows (McIlwraith et al., 2019), and by long-range atmospheric deposition of fibers in water systems, are major contributors to MP pollution (Dris et al., 2016; Munno et al., 2021).

Across all species, there was no correlation between fish body length or weight and MP concentration; however, some individual species did demonstrate positive correlations. Although there did not appear to be any clear difference in mean MP concentrations between feeding habitats, pelagic species did demonstrate a significantly higher median concentration. As expected, pelagic species contained a higher percentage of MP fibers while benthic species contained the highest percentage of fragments. These results are consistent with my hypothesis that a species' feeding habitat affects the dominant MP morphology present in fish GI tracts. Since fibers are buoyant, they are more likely to be consumed by pelagic fish who feed at the top of the water column, while fragments which tend to consist of heavier plastic polymers, sink to the bottom and are fed on my benthic species. It is important to note that sample sizes for each species in this study were low, and hence the results should be viewed with caution.

Research Limitations

This research was limited because the quantity of fish obtained was affected by Quebec fishing limits and exceptions, the time frame allocated for the catch phase of this

research (late September 2021- November 2021), the change in season, and the level of fishing skill required to successfully catch a high volume of fish. The diversity of fish obtained was affected by the available species in the study area, and therefore a comparison of herbivorous, carnivorous, and omnivorous species was not possible. Most of the fish caught were smaller in size since they were caught by casting near the shoreline due to limited fishing boat access. Without adequate numbers of fish and size variability from each species, assessing the relationship between fish size and MP abundance was limited. Although fish were caught from each of the three feeding habitats (benthic, bentho-pelagic, and pelagic) the number of fish in each category was very unequal, limiting comparability and the reliability of results.

Access to laboratory equipment necessary for the dissection, extraction, filtration, observation, and verification was limited due to Covid restrictions. Although every effort was made to follow the highest quality procedures, more robust plastic verification techniques such as micro-spectroscopy was not available and was replaced with a hot needle test. Without spectroscopy MP polymer identification was not possible and therefore, analysis of the potential origins of MPs was outside the scope of this research. In addition, filtering the digestate through three different sieves (300 um, 125 um, and 20 um) may have resulted in the loss of MP particles and therefore undercounting, since MP can remain attached to the sieve while rinsing each sieve into the next. Lastly, despite thorough cleaning of all equipment, work area, and fume hood ventilation, potential sample contamination could have overestimated results, although the control blanks indicated this would have been slight.

Conclusions

The presence of MP in freshwater fish symbolizes the impact of plastic production and careless handling of plastic waste on the global crises of climate change, nature loss, and pollution as an increasing dire threat to humanity. This research serves as an example, as it demonstrates the ubiquity of MP in a local freshwater riverine environment and shows that MP are ubiquitous in the St Lawrence River ecosystem. These results identify patterns of MP particle morphology in fishes with different feeding habitats. Although some of these results are limited, they nonetheless add to the body of knowledge that improves understanding about the consequences and potential risks of plastic pollution in riverine environments, and can be used to guide policy decisions about plastics production and waste management practices.

The high MP concentrations identified in St Lawrence River fish species magnifies the need for stricter policy guiding municipal stormwater collection and wastewater treatment outflow, and water quality monitoring and standards for this area. High MP fiber concentrations in these fish indicates the need for a more holistic view of how the textile and clothing manufacturing industries, residential laundering practices and equipment, and wastewater drainage and treatment facilities are connected. The presence of MP concentrations in local freshwater fish demonstrates a pollution problem.

Only 9% of the nine billion metric tons of plastic ever produced has been recycled, the remainder ends its life in landfills, dumps, and the environment. (Geyer et al., 2017). Greater actions need to be taken from a regional to a global level to adopt policies and legislation to reduce or phase out plastic products, change waste management protocols, address stormwater runoff pathways, wastewater treatment

policy, and develop policies to limit fiber shed rates in the textile industries. Without these changes, there is expected to be approximately 12 billion metric tons of plastic litter in landfills and the environment by 2050 (UNEP, 2019). The ripple of consequences is expected to include a loss of resources, economic value, and numerous effects to the environment.

Appendix 1

Full Results of Fish Sampling Data

Table 4. Fish characteristics and MP abundance.

Individual #	Species	Habitat	Length	Weight	Gut weight	>300 um	300-125 um	125-20 um	total
1	Perch	BP	15.5	37.765	2.547	0	2	14	16
2	Perch	BP	12.1	17.651	1.012	22	6	5	33
3	Perch	BP	11	12.864	0.946	6	10	15	31
4	Perch	BP	14.5	30.855	0.798	4	1	4	9
5	Perch	BP	12.2	16.503	0.763	6	8	9	23
6	Perch	BP	12	17.368	0.889	1	3	11	15
7	Perch	BP	17.9	70.585	2.379	2	4	3	9
8	Perch	BP	11.4	15.817	0.72	2	2	7	11
9	Perch	BP	16.6	45.898	1.003	3	3	8	14
10	Perch	BP	15.2	32.146	0.884	3	10	3	16
11	Perch	BP	13.3	23.322	5.151	5	9	8	22
12	Perch	BP	13.7	32.623	1.088	2	7	3	12
13	Perch	BP	13.7	24.964	0.565	3	4	9	16
14	Perch	BP	12.3	20.521	0.789	3	1	12	16

15	Perch	BP	12.9	20.117	0.524	2	3	4	9
16	Perch	BP	13.2	22.139	0.762	8	8	3	19
17	Perch	BP	15.9	40.99	5.148	1	5	5	11
18	Perch	BP	16	43.557	1.391	11	10	4	25
19	Perch	BP	13.6	25.157	0.784	0	5	5	10
20	Perch	BP	10.9	12.579	0.416	3	3	2	8
21	Perch	BP	20.2	88.77	2.579	2	5	7	14
22	Perch	BP	13.5	22.43	0.605	7	1	4	12
23	Perch	BP	15.3	26.907	2.698	3	10	14	27
24	Perch	BP	20	79.779	3.628	5	4	8	17
25	Perch	BP	13.6	20.329	0.931	4	8	4	16
26	Perch	BP	14.4	25.689	0.708	2	12	4	18
27	Perch	BP	15.1	28.169	1.021	5	8	8	21
28	Perch	BP	17	38.639	2.333	1	4	12	17
29	Perch	BP	19.4	85	6.928	3	3	9	15
30	Perch	BP	23.2		8.364	4	2	2	8
31	Perch	BP	22.6		5.561	3	10	4	17
32	Perch	BP	12.7	16.789	0.904	2	2	4	8
33	Perch	BP	29.6		14.152	5	5	14	24
34	Perch	BP	12.7	15.123	1.04	5	3	4	12
35	Perch	BP	19.8	70.582	3.751	7	10	3	20
36	Perch	BP	12.5	15.317	0.398	2	4	8	14
37	Rock bass	BP	12.7	30.903	2.107	0	10	5	15
38	Rock bass	BP	12.6	35.515	2.315	2	5	13	20
39	Rock bass	BP	11.9	24.428	1.533	1	2	3	6
40	Rock bass	BP	14.4	50.755	3.167	1	9	4	14

41	Rock bass	BP	15.2	82.109	5.123	4	5	1	10
42	Pumpkinseed	BP	11.7	26.908	2.259	0	6	4	10
43	Pumpkinseed	BP	16.9	88.423	4.525	3	4	2	9
44	Pumpkinseed	BP	12.4	29.965	1.735	0	5	0	5
45	Pumpkinseed	BP	13.6	49.757	2.834	1	1	7	9
46	Pumpkinseed	BP	12.4	28.149	2.511	1	4	2	7
47	Pumpkinseed	BP	12.2	32.914	2.718	6	4	5	15
48	Goby	B	12.2	12.126	1.725	7	17	0	24
49	Goby	B	11.4	22.981	1.002	0	3	12	15
50	Goby	B	12	24.207		2	10	4	16
51	Goby	B	10.8	17.388	0.96	1	11	5	17
52	Goby	B	7.8	9.24	0.278	1		5	6
53	Goby	B	7.9	6.167	0.218	2	5	2	9
54	Goby	B	8	6.32	0.241	2	3	9	14
55	Goby	B	12.9	24.865	1.684	3	22	7	32
56	Walleye	BP	41.6		28.134	0	4	9	13
57	Walleye	BP	43.2		32.442	8	3	21	32
58	Walleye	BP	46.2		37.264	1	5	2	8
59	Walleye	BP	45.8		31.119	2	2	7	11
60	Walleye	BP	45.2		29.816	0	3	11	14
61	Walleye	BP	43.9			5	10	6	21
62	Walleye	BP	44.1		31.04	1	4	2	7
63	Bluegill	BP	13.4	30.672	1.299	2	3	6	11
64	Bluegill	BP	18.3	91.194	3.399	1	2	1	4
65	Bluegill	BP	14.9	40.495	2.052	7		4	11
66	Bluegill	BP	14.6	39.637	1.657	3	2	4	9

67	Pike	P	70.3		52.806	0	3	4	7
68	Pike	P	61.7	1180	35.689	10	3	7	20
69	Pike	P	44.2	423	10.582	2	5	3	10
70	Bass	BP	37.9	739	24.708	9	4	10	23
71	Bass	BP	36.2	796	30.512	4	4	1	9
72	Bass	BP	34.6	469	18.772	0	5	8	13
73	Bass	BP	46.3	1232	41.77	0	6	24	30

Appendix 2

Microplastic Morphology

Table 5. Distribution of microplastic morphology by fish species

Species	Distribution	# Particles	# Particle/ fish	# Fibers	# Fib / fish	# Fragments	# Frag / fish	# Beads	# Beads/ fish
Perch	36	583	16	535	15	38	1	10	0
Rock bass	5	64	13	59	12	4	1	1	0
Pumpkinseed	6	55	9	42	7	11	2	1	0
Goby	8	133	17	125	16	6	1	0	0
Walleye	7	106	15	100	14	3	0	3	0
Bluegill	4	35	9	29	7	4	1	2	1
Pike	3	37	12	36	12	1	0	0	0
Bass	4	75	19	60	15	11	3	4	1

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