Are Aquatic Invertebrates Retaining Microplastics in the Ottawa and Rideau Rivers?

by

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Abstract

Microplastics are microscopic sized plastics, 5mm or less in length. Microplastics are produced directly for cleaning and cosmetic products, and indirectly through the physical and chemical breakdown of larger plastic materials. These plastic particles can take thousands of years to fully decompose, and thus ultimately may end up in our waterways. Microplastics have been reported in the Ottawa and Rideau Rivers but little information exists on whether these microplastics are being ingested by animals in these rivers. One hundred and fifty invertebrates of classes *Malacostraca* and *Bivalvia* were collected from the Rideau River and 150 invertebrates of classes *Malacostraca* and *Insecta* from the Ottawa River. Nearly every single individual had microplastics in them and there was no significant difference (p-value = 0.26) in the number of invertebrates with microplastics in the Ottawa River versus the Rideau River. Microplastic concentrations were, however, significantly related to taxon (p-value = 2.67e-13) and weight (p-value < 2.2e-16).

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Chapter 1

Introduction

New studies are coming out documenting microplastic pollution in aquatic environments at an increasing rate, most however focus on the marine environment (Mora-Teddy and Matthei, 2020). Plastic comes in many varieties but almost all are liable to pollute waterways and potentially be consumed by animals, including humans. Plastic has many uses, yet is also readily discarded. Larger plastics can breakup in the environment by physical and chemical means (Covernton et al. 2019) into smaller and smaller fragments termed microplastics (plastic particles <5mm in size). Microbeads and single-use plastics have been of the greatest public concern of late due. Polystyrene beads are an environmental concern, but more recent literature is finding microfibres, presumably from clothes washed in the laundry, are often the dominant form of microplastics (Cole et al. 2013).

Plastic is a word derived from the Greek word “plastikos” meaning mouldable. Today’s usage generally refers to materials synthesized from the polymerization of organic molecules. The organic molecules themselves are usually derived from petroleum sources such as oil, coal and natural gas. Celluloid and Bakelite are some of the original plastic materials made, long before the rapidly increasing mass production boom of the late 1940’s and 1950’s known as the great acceleration (Steffan et al. 2015). Plastic production was very useful to humanity and allowed us to produce a myriad of new structures and technologies that made life easier. The durability, light weight and flame-resistant characteristics of plastic make it an ideal material for healthcare, construction, packaging, electronics, aerospace and automotive industries among many others. Globally, between 1950 and 2015, 8,300 million metric tons of plastic have been produced with nearly 60% of that being discarded in landfills, only 7% has been recycled, 10%

incinerated, (Geyer et al. 2017) and 10% ending up the environment where it accumulates. Current research projects a fivefold increase in plastic production within the following 30 years suggesting the threat of ever greater buildup of plastic waste in the environment (Mora-Teddy and Matthei, 2020).

Microplastics are frequently referred to plastic that is 5mm or less in length (Ivleva et al. 2017). Other research however has set the upper limit to 1mm (Browne et al. 2011; Dekiff et al. 2014; Cauwenberghe and Janssen, 2014), 2mm (Ryan et al. 2009) and even 10mm (Graham and Thompson, 2009). For the sake of this paper, plastics that are 5mm or less in length will be considered microplastics. Microplastics are not only produced and used in cosmetics and oral hygiene products, what are termed primary microplastics (Hoellein et al. 2017), but are also as a by-product of the breakdown of larger plastic pieces used in clothing and manufacturing among other industries (Vermaire et al. 2017). These microplastics from the breaksown of larger plastic material are called secondary microplastics (Hoellein et al. 2017). The breakup of plastics into smaller pieces and eventually small polymers may occur by chemical or physical processes such as solar radiation (Zhang et al. 2016). Once broken-down, plastics behave much like other sediment and are eventually transported to our waterways (Anderson et al. 2016). Plastics have been demonstrated to be endocrine disruptors, that is they throw the hormones of an organism out of their natural equilibrium (Sussarellu et al. 2016). In-vivo studies have also shown that chemicals in plastic are carcinogenic and may cause health or reproductive issues in high enough dosages (Nelms et al. 2017).

At the molecular scale, plastic can take a few hundred to a thousand years to biodegrade and it is apparent that our production of plastic has greatly exceeded its degradation (Singh and Sharma, 2008). Societies have implemented many ways to try to mitigate this, such as with

recycling programs and yet, as evidenced by phenomena such as the sea of plastic in the North Atlantic Gyre, more work needs to be done (Lavender Law et al. 2010). Previous literature has shown significant levels of microplastics in the bodies of worms, amphipods, decapods, barnacles, fish, birds and mussels (reviewed by Cole et al. 2013). Larger pieces of plastic may obstruct feeding and digestion apparati, making the animal sick or killing it. Smaller plastic pieces may get moved into the circulatory system where they would have direct contact with all of the organs, possibly leading to cancer or infertility (Cole et al. 2013). While the long-term effects of microplastic ingestion haven't been fully studied, it is important that the public know about what is in their food and water so they may make informed decisions for themselves. There is also a conservation component to consider as the organisms living in the freshwater are exposed to this microplastic pollution.

Commercially speaking, there are 5,300 different grades of plastic (Wagner and Lambert, 2018). As such, they all behave differently when they enter an ecosystem much like different types of sediment with different sizes, composition and buoyancy among other things. It has even been suggested that analyzing microplastics as a group is too homogenous and that different types of microplastic pollution require different types of diagnostics (Rochman et al. 2019).

Plastics are synthetic polymers, often derived from petroleum sources, that can be molded into solid objects. The most used plastics are composed of polypropylene, polyethylene, polyvinyl chloride, polystyrene and polyethylene terephthalate (Andrady and Neal, 2009). Polystyrene is one of the most ubiquitous polymers worldwide, both in production and as waste (ter Halle et al. 2016; Watts et al. 2015). There are other chemicals added to the plastic during production to enhance malleability or heat resistance, such as phthalates and polybrominated

diphenyl ethers (Cole et al. 2013). While natural plastic-like adhesives have been used for most of human history, the first synthetic plastics can be dated back to the 1600's when Mesoamerican people used rubber to make balls and figurines (Andrady and Neal, 2009). In the 1800's industrial chemical processes led to the industrial revolution when plastic production began to grow exponentially (Andrady and Neal, 2009). Currently, annual production of plastic is estimated at over 300 million tonnes (Galloway, 2015). It is thought that by the year 2050, 33 billion tonnes of plastic will have been created (Galloway, 2015). About 24.7 million tonnes of plastic enters wastewater streams every year and 14.5 million tonnes of plastic is used every year to package food, lending it direct contact to what humans ingest (Galloway, 2015). It is estimated that roughly 1 to 2.5 million tons of plastic flows from rivers into seas due to waste mismanagement (van Wijnen, Ragas and Kroeze, 2019). Many of these plastics begin as large, macro-sized plastics but eventually have broken up to microplastics from transport (van Wijnen, Ragas and Kroeze, 2019). While plastic has been used to create many ubiquitous modern-day inventions (Table 1, 2) that have enriched our lives and made them easier, that luxury has come at an environmental cost.

Table 1. Percentage plastic use by industry in Europe in 2012. Data obtained from Galloway, 2015.

Industry	Volume (millions of tons)	Percentage of Total
Packaging	18.1	39.4
Building and Construction	9.32	20.3
Automotive	3.76	8.2
Electronics and electrical	3.03	6.6
Agriculture	1.93	4.2
Other	10.3	22.4
Total	45.9	100

Table 2. Plastic demand in Europe by type in 2012. Data obtained from Galloway, 2015.

Type	Example	Demand	% Total Demand	% Recycled
PET Polyethylene terephthalate	Polyester fibre, soft drink bottle	2.98	6.5	20
PE-HD polyethylene high density	Plastic bag, plastic bottle, bottle cap	5.51	12.0	11
PVC Polyvinyl chloride	Window frame, waterproof boot, plumbing pipe	4.91	10.7	0
PE-LD polyethylene low density	Soap dispenser bottle, wire cable plastic, plastic knife, plastic bag	8.03	17.5	6
PP polypropylene	Plant pot, industrial fibre, stationary folder, bags	8.63	18.8	1
PS. PSE polystyrene	Plastic cup, food container, glasses frame	3.40	7.4	1
Other	Clothing, drink bottle, consumer item	9.82	19.8	0
Total		45.9	100.0	39

Depending on the chemical composition of microplastics, it may be found floating on the water surface, washed up on the shore, within the water column itself and even in sediments (Mora-Teddy and Matthei, 2020). Microplastic proliferation becomes evident in sedimentary records in the late 1950's (Turner et al. 2018). Microplastics account for 8% of the total ocean debris' mass but 94% of the pieces, which is thought to be roughly 2 to 4 trillion pieces of plastic (Lebreton et al 2018). Floats and buoys break down into smaller polystyrene particles, plastic bags break down into polyethylene fragments and many fabrics contain polyester fibres, even

before they are further broken down (Cole et al. 2013). Other sources of plastic pollution include synthetic fibres from clothing, tires from cars, and abrasive materials from cosmetics (van Wijnen, Ragas and Kroeze, 2019).

The release of plastic into the environment is problematic for many reasons. At a large scale, the rings of beer and soda cans have been detrimental to turtles as they can get caught in the rings and have impaired growth (Carr, 1987). Plastic bags are also eaten by some turtles that mistake them for jellyfish (Mascarenas, Santos and Zeppelini, 2004). If the thought of eating plastic concerns you, consider insidious 'microplastics', plastic particles so small that they get into our food and drinking water. Due to their size we are unable to see them, and therefore we accidentally ingest them (Cole et al 2013).

Consumption refers to the number of microplastics being directly ingested by each organism through processes such as feeding. This differs from retention which refers to the number of plastics entering the organism without being eliminated. Elimination is the egestion of microplastics which have left the body through processes such as defecation. Without elimination, microplastics subsequently build up in the organism in what is termed accumulation.

Biomagnification refers to the tendency of toxins to accumulate as they move along the trophic hierarchy. This occurs because organisms consume a lot of different toxins in their environment through ingestion, inhalation and retention of microplastics. Animals that eat the organisms get all the microplastics retained by their prey as well as the microplastics they have consumed directly (Kelly et al. 2007). A review of primary literature demonstrates that biomagnification of microplastics has occurred in humans (Kelly and Wright, 2017) through both ingestion of contaminated water and food, as well as simple inhalation.

Freshwater is one of the scarcest, yet most important natural resources to humans. Only 2.5% of all water on Earth is freshwater with the vast majority of that frozen in glaciers or groundwater with only approximately 0.007% of the earths water residing in freshwater rivers and lakes (Koehler, 2008). Aquatic ecosystems fall into one of two categories, lentic and lotic. Lentic environments have slow water movement and often occur in basins such as lakes and ponds. Lotic environments have faster water movement, as well, lotic environments are often long and connect to larger water bodies (Nel, Dalu and Wasserman, 2017). Lotic environments also have greater sedimentation due to erosive and weathering forces, which makes them responsible for transporting large numbers of microplastics into larger water bodies (Buffagni et al. 2009). There is less research done on microplastic pollution in lotic environments with most of the focus on lentic environments that transport microplastics to the ocean as a reservoir.

This thesis is divided into five chapters. The first chapter is the Introduction which provides basic information on plastic, plastic pollution and the environment. Chapter 2 is the Literature Review which provides information on the organisms being studied as well as microplastic pollution in organisms and where it has been found and in what organisms specifically. Chapter 3 covers the Materials and Methods section, details the physiographic and biotic regions of the rivers where the organisms were caught as well as the field, laboratory and statistical practises used to glean information about microplastic ingestion by these organisms. The fourth chapter is the Results and Discussion. of the thesis. The final chapter is the Conclusions, which details what was discovered in this thesis and recommendations about what future research needs around this topic.

1.1 Aims

The aim of my research was to examine how common microplastic retention is in invertebrate taxa of the two most prominent waterways in Ottawa. To do this, microplastic fragments in aquatic invertebrates in each of the two rivers were quantified. It is hypothesized that invertebrates in both rivers will have significantly greater levels of microplastics than blank samples, suggesting retention of microplastics by invertebrates. The taxa examined were from three different invertebrate classes; Insecta, Mollusca and Crustacea. The insects examined were stoneflies (Plecoptera) and the molluscs were mussels (Bivalvia). Both taxa are benthic, that is they live on substrates in and along the bottom and banks of the rivers (Howard and Cuffey, 2006). Both mussels and many insect larvae feed by a method known as filter feeding, where they passively wait, attached to substrate and filter out smaller food particles and plankton moving in the water current to obtain nutrients (Howard and Cuffey, 2006). Crayfish and amphipods were the chosen crustaceans to examine in the water ways. Crayfish also live and feed along the river substrate but are active feeders that graze on both animals and plants, such as chironomid larvae and bivalves (Simon and Garnier-Laplace, 2005), and thus are predicted to to have greater levels of microplastics, unlike the other organisms which are at the bottom of the trophic hierarchy (Gamradt and Kats, 1996). Given that crayfish occupy a higher trophic status than the other species, I aimed to find out if trophic status had a significant effect on microplastic abundance in tissue. This would be evidenced by significantly greater microplastic levels in crayfish viscera than the other taxa demonstrated by a One-Way ANOVA statistical test. Another aim of the research was to examine if larger organisms of the same taxa contain more microplastics, which would be predicted if organisms were retaining microplastics in the gastrointestinal tract as they grew bigger. It is hypothesized that larger organisms occupying

higher trophic statuses such as crayfish will show greater levels of microplastics due to a longer gastrointestinal tract and microplastics accumulation.

There are several limitations to the research. The taxa between the rivers differ due to physical and chemical factors, thus making it difficult to compare if the levels of microplastics between the rivers is significantly different. As well, no longitudinal data was collected to see if the invertebrates are retaining the microplastics in their guts for extended periods of time and making biomagnification difficult to quantify.

Chapter 2

Literature Review

Microplastics are small enough to be ingested by organisms and are even able to pass through biological membranes (Mora-Teddy and Matthei, 2020). In lotic habitats, microplastics themselves provide a habitat for unique microbes in water, particularly those with close proximity to wastewater effluent (Hoellein et al. 2017). These assemblages of bacteria differ between sediment, water, and seston and show a 'stream-like' succession pattern radiating from wastewater treatment plants in lotic systems (Hoellein et al. 2017). The bacteria that apparently thrive on microplastics typically include both plastic degrading bacteria such as *Pseudomonas*, as well as disease causing bacteria such as Campylobacteraceae (Hoellein et al. 2017). Microplastic studies from freshwater systems are rarer than marine systems, despite being the major input to oceans (Hoellein et al. 2017). That said, microplastics have still been found on every continent in the world at levels similar to marine systems (Mora-Teddy and Matthei, 2020). Sources of plastic pollution are termed either point sources or diffuse sources (van Wijnen, Ragas and Kroeze, 2019). The main source of microplastics in freshwater appears to be urban runoff particularly after heavy rainfall, as well as waste water effluent from treatment plants and grey water, which comes from urban areas (Mora-Teddy and Matthei, 2020). Many microplastics enter wastewater systems when clothing is washed, cosmetics are washed off, or from the breakdown of tires of cars driving on the road (van Wijnen, Ragas and Kroeze, 2019). Microplastics tend to accumulate more in the benthic zone than suspend in water, but that again, depends on the type, size and overall composition of the microplastics themselves (Hoellein et al. 2017). Overall, benthic zones have shown to be a sink for microplastics over a longer period of time, however

more research is needed to parse the different modes of transportation based on size and type (Hoellein et al. 2017). Microplastics have also been found in tap water and bottled water as well, with the greatest number coming from reusable plastic bottles (Eerkes-Madrano, Leslie and Quinn, 2019). Water from household taps in one study did not exceed 4 particles/m^3 (Eerkes-Medrano, Leslie and Quinn, 2019).

While microplastics often enter an aquatic system via storm surge overflows and proximity to wastewater treatment plants (Windsor et al. 2019), atmospheric microplastic deposition plays a significant role in microplastic pollution as well (Wright et al. 2020). The literature is just emerging but microplastics have been found in both outdoor as well as indoor air (Wright et al. 2020), particularly metropolises such as London, United Kingdom where 575 to 1008 microplastics/m^2/d in the air have been reported (Wright et al. 2020). The majority of these microplastics are fibres, which approximate 92% of microplastics found (Wright et al. 2020). Wind and air currents play a significant role in the movement of microplastics throughout the atmosphere, transporting them up to 95km (Wright et al. 2020). As one study notes, microplastics may be ingested by household dust, food and simple inhalation (Eerkes-Medrano, Leslie and Quinn, 2019).

2.1 Microplastics Consumption by Organisms

Despite the research focus on animals, plastic pollution poses a potential threat to aquatic life in general. Previous research has shown microplastic consumption by algae (Cole et al. 2013). This is particularly insidious as algae are not only very prevalent in aquatic systems but also form the basis for many food webs. Biomagnification refers to the tendency of toxins to accumulate at the top of trophic hierarchies while energy decreases. One level above phytoplankton in aquatic trophic hierarchies are zooplankton. Zooplankton include amphipods

and copepods. A review of the primary literature suggests that not only do copepods consume plastic, but it has suggested the plastic directly affects the organism by impairing feeding (Cole et al. 2013).

2.2 Microplastics Consumption by Invertebrates

Invertebrate animals include all protostomes, echinoderms and non-vertebrate chordates. Unlike other metazoans, invertebrates and vertebrates alike have bilateral symmetry, ancestrally. Bilateral animals begin development from a zygote forming a mouth and anus, with the mouth first in protostomes and second in deuterostomes (Kocot, 2016). In either case, the digestive tract is the first structure to be formed out of the negative space between cells. The digestive tract is therefore one of the most primitive features of vertebrates and invertebrates alike. Few organisms have modified the digestive tract extensively through evolutionary time (besides the addition of multiple organs) and all animals still possess digestive tracts in modern time.

The gastrointestinal (GI) tract is the first point of entry for water, food, and subsequently, plastic for most animals via mouth parts. The size of plastic is potentially infinite, with its cheap production and strong physical integrity, it can be molded to large sizes, and its strong resistance to degradation means it can become nanoscopic in size. There is no consensus on what defines a nanoplastic, but they are generally considered to be plastics that are less than 1 μm in size (Koelmans, 2019). Most animals thankfully have pre-adaptations (since no organism has adapted to a novelty such as plastic yet) to not ingest large plastic pieces that comes their way but again, the sizes are infinite and many aquatic invertebrates do not have sophisticated enough structures to account for much of what is consumed microscopically.

While the circulatory, nervous and endocrine systems of invertebrates and vertebrates

have evolved independently, they possess similar structures and physiologies by convergence. Invertebrates have an open circulatory system and their nerves formed by the fusion of ganglia. Vertebrates on the other hand have closed circulatory systems and their nerves were formed by the extension of the notochord, a synapomorphy of all vertebrates (Kocot, 2016). A synapomorphy is a shared derived characteristic, in two or more organisms. This differs from synplesiomorphies which are shared ancestral characters and autapomorphies which are unique derived characteristics (Scott, 1996). Despite this, hormone synthesis and sensitivity are very similar. Estradiol, one of the most potent estrogens, particularly for ovulation, has regulatory effects on almost all vertebrates and many invertebrates as well (McLachlan, 2001). Invertebrates such as crustaceans (Baldwin and LeBlanc, 1994; LeBlanc and McLachlan, 2000; Waddy, S, Aiken, D, deKlejin, D, 1995), echinoderms (LeBlanc, 1999), molluscs (Ferall and LeGall, 1983) and cnidarians (Tarrant, Atkinson and Atkinson, 1999; Atkinson and Atkinson 1992) have all demonstrated estrogen production. The fact that so many invertebrates produce and/or are sensitive to estrogens and estrogen-like chemicals despite reproductive systems evolving independently suggests a primitive and highly conserved evolutionary role for estrogens within the animal kingdom (McLachlan, 2001). Thus, the Endocrine Disrupting Chemicals (EDC) that affect vertebrates also often interact with invertebrates, either antagonistically or agonistically and either affecting hormonal release or second messengers downstream, depending on the hormone receptors present (Crews et al. 2000). Some research even suggests that almost all animals produce or are sensitive to certain estrogens (McLachlan, 2001)

More than 95% of animal taxa are invertebrates and despite their critical role within an ecosystem, the effects of new EDC's such as phthalates, nonylphenol (NP), nanoparticles,

biocides, UV filters, bisphenol A (BPA) and bisphenol S (BPS) are poorly understood. Nematode worms show significant behavioral and reproductive changes at very low levels of BPS (Chen et al. 2016). Yet, previous research suggests it is one of the most ubiquitous pollutants and can impact a variety of invertebrate taxa allowing it access to multiple ecosystems and trophic chains (Mersha et al. 2015). Since the surrounding waters in urban areas are generally where pollutants concentrate, aquatic taxa are the most at risk (Herrero et al 2018).

Preliminary research has shown that zooplankton are capable of consuming microplastics including bivalve and decapod larvae, and copepods. Many different copepod taxa ingest microplastics, particularly *Temora longicornis* and *Centhropages typicus* (Cole et al. 2013). Zooplankton such as copepods not only ingest microplastics, but many times plastic is found in the external and feeding appendages. Microplastic consumption is not restricted to any type of zooplankton and has been found in meroplankton, brachyura, holoplankton and microzooplankton. Filter feeders such as doliolids and euphausiids were also found to have consumed microplastics (Cole et al. 2013). Even phytoplankton such as dinoflagellates have been found to consume microplastic by engulfment via a cytostome (Cole et al. 2013). Multiple studies demonstrate microplastic consumption by zooplankton and describe egestion containing microplastics as well as trophic transfer up the hierarchy (Cole et al. 2013). Microplastics of a variety of shapes and sizes have been found to be consumed by other organisms and have led to gut blockage. Marine wildlife sometimes retain plastic in their gut for the rest of the individual's life (Cole et al. 2013). Below I review the literature on microplastic ingestion by the animal groups examined in this thesis.

2.3 Bivalvia

Molluscs are soft bodied invertebrates that include bivalves, cephalopods and gastropods, mussels are of the order Bivalvia (Jell, 1980). Bivalves include clams, scallops, mussels and oysters and are noted for being sessile filter feeders, composed of two hard shells along the outside, symmetrical on the hinge line (Morgan and Rhona, 1981). They lack a head and many other ancestral traits of molluscs such as the odontophore and radula. Bivalves make excellent microplastic indicators for a variety of reasons. They are numerous and occupy many different habitats (Shoults-Wilson et al. 2014). They are also sessile and tend to live close to shorelines making them easy to access. Their benthic, filter feeding lifestyle provides extra insight into the composition of waters that flow through and they are often the bottom of most trophic hierarchies and thus (Nelms et al. 2018), toxins such as microplastics are likely to bioaccumulate up the chain (Shoults-Wilson et al. 2014).

Bivalves feed via modified gills known as ctenidia (Gould and Calloway, 1980). Zebra mussels (*Dreissena polymorpha*) are a family of freshwater mussels indigenous to lakes in Russia and Ukraine but through anthropochory, travelling in ship ballasts, have spread throughout the world including Canadian waterways. As an invasive species, zebra mussels have dominated landscapes, outcompeting native fauna. Their success has been detrimental to human societies as well, as they clog pipes and other human infrastructure that allows us to connect with and use the water, as well as shorelines where they damage marine development, and harm and dissuade human usage (Mackie et al. 1989).

There are plenty of data regarding bivalve consumption of microplastics. Microplastics have been found in many bivalve taxa including oysters (*Saccostrea glomerata*) (Scanes et al. 2019), Asian green mussel (*Perna viridis*) (Rist et al. 2016), Manila clams (*Venerupis philippinarum*), Pacific oysters (*Crassostrea gigas*) (Covernton et al. 2019), sea snails

(*Cerithidea cingulate*, *Thais mutabilis*), clams (*Amiantis umbonella*, *Amiantis purpuratus*) (Naji et al. 2018), gooseneck barnacles (*Lepas spp*.) (Goldstein and Goodwin, 2013) and oysters (*Pinctada radiata*) (Naji et al. 2018), in places such as Australia (Scanes et al. 2019), the Persian Gulf, Germany (Naji et al. 2018), Tunisia (Abidli et al. 2019), Canada (Davidson and Dudas, 2016) and the North Pacific Subtropical Gyre (NPSG) (Goldstein and Goodwin, 2013). Zebra mussels (*Dreissena polymorpha*), are particularly explored likely due to their prevalence and consideration as a nuisance. There is evidence showing microplastics in the ctenidia of zebra mussels (Parolini et al. 2020). Microplastics, including polystyrene microbeads have been found in zebra mussels in Italy (Magni et al. 2019) and Lake Michigan (Shoults-Wilson et al. 2014),

2.4 Decapoda

Aside from barnacles, most crustaceans are Malacostraca (De Grave et al. 2009). Decapods are an order of Malacostraca, the name coming from Greek meaning "10 feet" and these include crustaceans that are a part of human diets including crabs, lobsters and shrimp (Gutierrez, 2012). There are also freshwater decapods such as copepods, amphipods and crayfish. Previous literature discusses how pesticide use such as cyclodiene, including heptachlor impairs the function of cytochrome P450 (Snyder and Mulder, 2000). Cytochrome P450 is found in many organisms across kingdoms, humans included (Lamb et al. 2009). Cytochrome P450 is responsible for refolding damaged proteins, thus its inhibition leads to accelerated cell death in the individual (Snyder and Mulder, 2000). Additionally, crayfish are highly sensitive to pollution and eutrophication, thus making them a good indicator species of anthropogenic impact on freshwater ecosystems. Indicator species are taxa that while numerous, have very specific environmental conditions with a wide spatial or temporal distribution (Bal et al. 2018).

Crayfish are freshwater decapods of the superfamily Astracoidea. Like other decapods, crayfish are vagile and benthic, they scavenge benthic environments for small plants and animals whether alive, dead or detritus (Schilderman et al. 1999). Crayfish are a dietary staple of Cajun people living in the American South (Gutierrez, 2012).

Amphipods are crustaceans. Due to their small size, amphipods are abundant in many marine systems and almost all freshwater systems around the globe and form the basis of numerous food webs (Wade et al. 2004). As a typical zooplankton, they spend most of their life floating but have limited motility and are frequently found in sediment (Barnard et al. 1980).

The literature itself on microplastic ingestion by crayfish is scant, as not a single source was found, however evidence of microplastic ingestion by amphipods is abundant. Microplastics have been found in various species of amphipods including *Gammarus pulex* (Weber et al. 2018), *Hyalella Azteca* (Au et al. 2015), *Gammarus setosus* (Iannilli et al. 2019), *Gammarus fossarum* (Blarer and Burkhardt-Holm, 2016) and *Talitrus saltator* (Iannilli et al. 2018) as well as other crustaceans such as copepods (*Centropages typicus* and *Temora longicornis*; Ivleva et al. 2016) and daphnia (*Daphnia magna*) in various water bodies including Germany (Weber et al. 2018), United States (Au et al. 2015), the Svalbard Archipelago (Iannilli et al. 2019), Switzerland (Blarer and Burkhardt-Holm, 2016) and Italy (Iannilli at al. 2018).

2.5 Plecoptera

Insects are some of the most diverse invertebrates and over a third of the animal kingdom is comprised of them (Chapman, 2006). There are numerous orders of insects, often dating back to the carboniferous (Engel and Grimaldi, 2004). Their diversity and sheer number make them an excellent proxy for toxicology and pollution research. Stoneflies are of the order Plecoptera

(Fochetti and Tierno de Figueroa, 2008). The moniker Plecoptera refers to the ability of the insect to fold its wings horizontally, which Diptera or true flies can not do (DeWalt, Kondratieff and Sandberg, 2015). Stoneflies are hemimetabolous insects, not having a pupal life stage, instead going straight from larva to adult; of the superorder Exopterygota which includes earwigs, webspinners, grasshoppers, locusts and crickets (Rebora et al. 2017). Thus far, almost 3500 different species of stonefly have been described. All North American taxa are of the suborder Arctoperlaria which is over 3000 of such species. Like odonates, stoneflies are carnivorous, winged insects.

Stoneflies are incredibly speciose and found everywhere except Antarctica. Additionally, like crayfish, stoneflies are very sensitive to human pollution, thus they make an excellent indicator species for environmental assessment (Schilderman et al. 1999). Stoneflies thrive in well-oxygenated waters rendering dry land and eutrophic water bodies hostile to their existence, particularly in juvenile stages (Fochetti and Tierno de Figuerosa, 2008). The juvenile stages of plecopterans are called nymphs. While nymphs are aquatic, they are terrestrial in adult form, living near the body of water they developed in (DeWalt, Kondratieff and Sandberg, 2015). Adult stoneflies usually live on land, but some taxa are aquatic their entire life cycle (Holst, 2000)

Plectoptera originated in the Carboniferous period but diversified in the Mesozoic era (Zwick, 2000). Ecologically speaking, stoneflies have a very important role. They are both primary and secondary consumers, depending on the species and like bivalves, are preyed upon by other, larger invertebrates, thus biomagnification potential is high. There is even some evidence that they have been used in human diet for some cultures (Fochetti and Tierno de Figuerosa, 2008). Although their sensitivity to pollution makes them a useful proxy for

environmental research, it also means that they are most jeopardized by factors such as climate change, urbanization and overpopulation, with some taxa going extinct in Europe (Fochetti and Tierno de Figuerosa, 2008).

The insect digestive tract can be divided into three sections including hind-, fore- and midgut. The midgut is where enzymes are most abundant thus digestion is greatest, but in some taxa digestion occurs in the foregut from saliva. Despite the wide variety of digestive enzymes in the alimentary canal; much like many vertebrates, all insect digestive enzymes are hydrolases and thus react similarly to that of mammals (Fochetti and Tierno de Figueroa, 2008). Not only are stoneflies an important part of streams and rivers but serve a number of other uses to the public. The fly-fishing industry uses them for bait, and they are a useful model in evolutionary science due to their diverse and global but partitioned biogeographic distribution, as well as indicators of water quality. While they do not damage crops or harm us in any way, they are very sensitive to anthropogenic effects (Fochetti and Tierno de Figueroa, 2008).

No data regarding microplastic consumption by stoneflies has been reported, however there are numerous studies about microplastic consumption by insects, including true flies. Microplastics have been found in taxa such as worms (*Plodia interpunctella*), beetles (*Tenebrio molitor*) (Oliveira, 2019), chironomids (*Chironomus riparius*) (Silva et al. 2019), mayflies (*Ephemeroptera*), caddisflies (*Trichoptera*) (Windsor et al. 2019) and mosquitoes (*Culicidae*) (Al-Jaibachi et al. 2018), and in countries such as Germany (Ehlers et al. 2019), Portugal (Silva et al. 2019) and the United Kingdom (Nelms et al. 2019). One study on microplastic consumption by mosquitos (*Culex*) details how insects are a significant vector in transporting microplastics into terrestrial and even aerial environments in the United Kingdom (Al-Jaibachi et al. 2018). Another study details the enhancement of oviposition certain insects are having due to

microplastic pollution. Research done in the North Pacific Subtropical Gyre suggests for pelagic insects, lightweight microplastics with high buoyancy provide a substrate for *Halobates sericeus* to lay eggs (Goldstein et a1. 2012)

Chapter 3

Study Region and Methods

3.1 St. Lawrence Lowlands

Ottawa is located within the St. Lawrence Lowlands physiographic region. Like other lowlands, the terrain is flat, fertile with abundant sedimentary bedrock relative to the surrounding Canadian shield, which is composed mostly of thick layers of harder igneous rock. The last glacial period in Canada was 20,000 years ago, the weight of the ice sheet had depressed the region (Faroud, 1974). Additionally, Ottawa is underlain by a fault line and adjacent graben, a large trench created by the diversion of two fault lines, making the land between effectively drop. The lower elevation already exacerbated by the weight of the extra ice, resulted in extensive and long-term flooding after the melt of the last glacier in the region. This flooding formed what is known as the Champlain Sea, the Ottawa river is a vestige of this sea, highlighting the depth of the river as well as how much has flooded into it (Ritter, 1995). The lowlands extend north eastward through the Ottawa valley, an area of extensive agriculture and the St. Lawrence sea way connecting to the Atlantic Ocean, historically having some of the highest economic value in Canada. (Chapman and Putnam, 1970).

3.2 Great Lakes – St. Lawrence Forest biome

In terms of biota, Ottawa is situated in the Great Lakes – St. Lawrence Forest biome. The Great Lakes – St. Lawrence Forest biome is characterized by mixed, deciduous and coniferous vegetation. Due to the region's southern location in Ontario and Canada, there is a greater diversity of temperate broadleaf trees and herps, which are the most threatened group of vertebrates (Payette et al. 2017). However, its position is northern enough within Canada that

wetlands such as bogs, fens, marshes and swamps are abundant and house their own unique biota. Some of the most common trees include oaks, maples, pines, Eastern Hemlock (*Tsuga canadensis*) and White Cedar (*Thuja occidentalis*).

Unlike the Great Lakes lowlands and the St. Lawrence lowlands which are separated by the Canadian shield and classified separately, physiographically speaking, they are classified together. The southeastern branch of the Great Lakes – St. Lawrence biome extends past Lake Ontario around Toronto and Hamilton towards Point Pelee. Unfortunately, while the biome has regions such as the Bruce Peninsula containing many endemic taxa and First Nations reserves, its proximity to large metropolises such as Ottawa (Figure 1) and Toronto make urbanization a severe and constant threat as population continues to grow within the region from high birth and immigration rates, and low death and emigration rates (Payette et al. 2017).

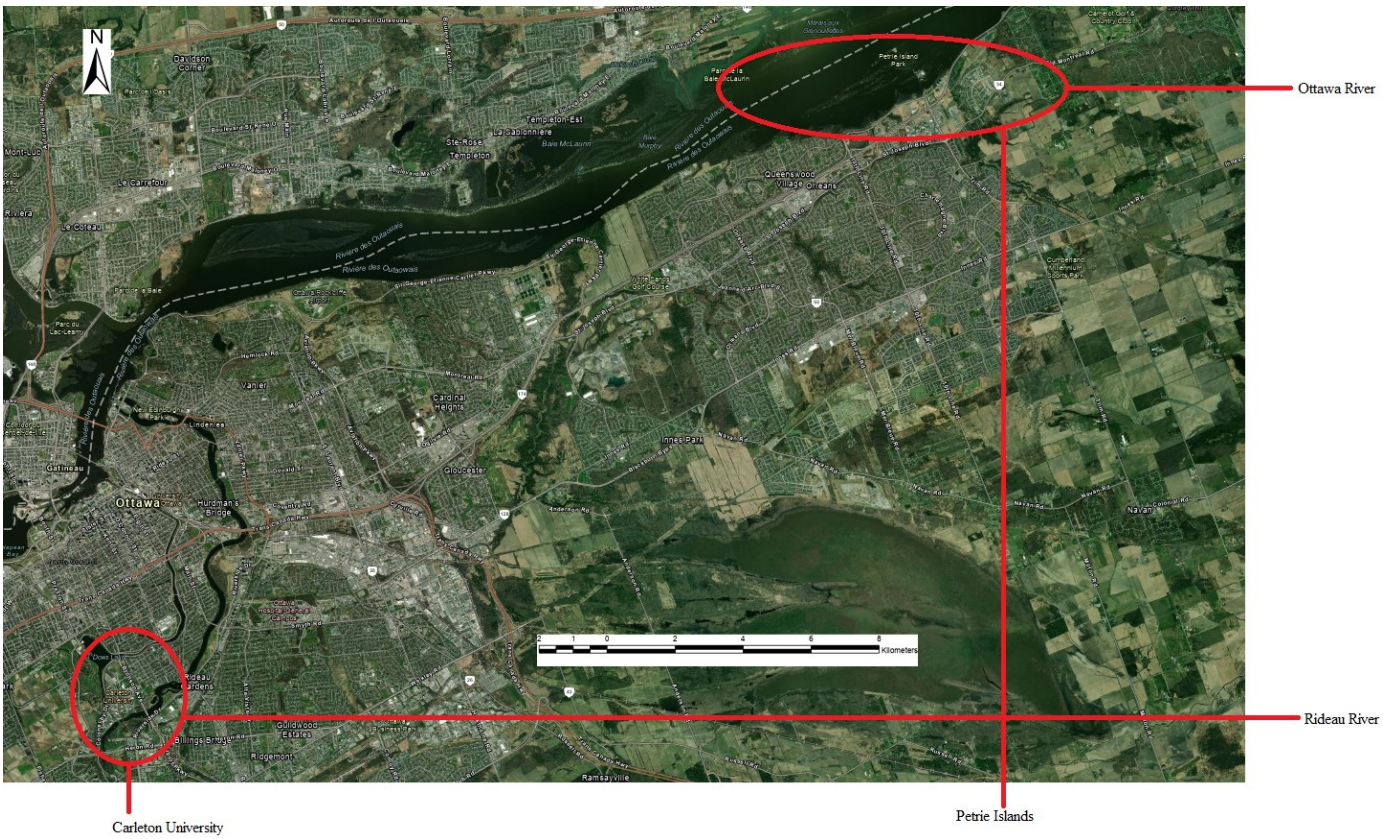


Figure 1. Aerial view of Ottawa, Ontario. Pictured are the two study sites, Petrie Islands in the Ottawa River and Carleton University by the Rideau River. Screenshot obtained from ArcGIS Pro and edited with Paint.

3.3 Field Methods

Sampling of the Ottawa and Rideau rivers occurred over the summer of 2018, researchers would go sampling at least once a week. In the Ottawa River (Figure 2) benthic samples were collected with a D-net and sieves. Sampling in the Ottawa River took place at Petrie Island, which is downstream from the Ottawa wastewater treatment plant. The site was accessed near a pipe that connected the river on both sides where access was easy and bivalve shells were abundant (Figure 3). Amphipod (Amphipoda), snail (Gastropoda) and stonefly (Plecoptera) taxa were plucked from the filter with metal forceps and individuals were transferred to WhirlPak bags.

Figure 2. Petrie Island. Photo at one of two study sites. Photo at one of two study sites where aquatic invertebrates were collected for dissection.

Figure 3. Petrie Island. Photo at one of two study sites where aquatic invertebrates were collected for dissection.

The Rideau River was accessed off Carleton University's Campus Ave near the Nesbitt building. Crayfish have evolved to hide during the day when the sun is out and predators such a gulls may try to eat them (Dikareva and Simon, 2019), thus at night, they were easier to catch. Crayfish and zebra mussels from the Rideau River were hand harvested. The researchers would climb down the rocks at the side of campus and catch the crayfish (Decapoda) by hand. Cobble sized rocks would also be overturned to collect zebra mussels (Mollusca) found alongside the crayfish. Once individuals had been collected, they were transported back to the lab in a bucket

and frozen, awaiting dissection (Dikareva and Simon, 2019). All samples were frozen in the laboratory to await further analyses. In total, 87 crayfish and 63 zebra mussels were collected from the Rideau River and 5 snails, 58 stoneflies and 87 amphipods were collected from the Ottawa River. These organisms were selected due to their abundances in the Ottawa and Rideau rivers. The taxa differ between the two rivers due to calcium levels which are higher in the Rideau River, thus larger organisms with shells can live there as shells require calcium to construct (Martel and Madill, 2018). The reason different organisms were chosen between the rivers is because they were abundant enough to provide a large sample size and represented different feeding strategies such grazers and filter feeders. This however limits the potential information gleaned between the two rivers than if the organisms were the same.

3.4 Laboratory Methods

Following collection, frozen individuals were thawed in a fridge for 24 hours. After the 24 hours, the individual invertebrates were weighed and dissected. The individual was removed from the bag it was stored in and placed in a glass petri dish. Metal forceps were used to examine the specimen under a Leica stereomicroscope to determine if any microplastics were present on the outside of the individual. The individual was then dissected, its viscera transferred to the petri dish and exoskeleton discarded. The crayfish and zebra mussels could be opened by removing the carapace or snipping the adductor muscle respectively. Amphipods, stoneflies and snails were much harder to eviscerate so they were just crushed and digested whole (Carreras-Colom et al. 2018). Thirty percent hydrogen peroxide was added to the sample to oxidize organic material and then the sample was covered with aluminum foil. After preparing several vials, they were placed in a hot water bath at 25° Celsius for 48h to speed up digestion. Following digestion each tube was filtered through 100 μm Nitex mesh. Once the contents had been filtered, the residue

was backwashed into its corresponding beaker with distilled water to await counting of microplastics under at 40 times magnification under a Leica stereomicroscope. Microplastics have irregular shapes and unnatural colors like fluorescent pink or blue that are not common in nature, thus singling out individual pieces of plastic (Figure 4; 5). Though visual inspection was possible, there are however some limitations with visual identification. First, microplastics are smaller and therefore more difficult to detect and identify. The shapes they come in and polymers they are composed of are widely varied (Mora-Teddy and Matthei, 2020). Primary microplastics are typically shaped like pellets or beads, while secondary microplastics often take the form of foams and fibres (Mora-Teddy and Matthei, 2020). Clear plastics would be harder to detect. Some could be modified cotton or cellulose, not necessarily plastic, but still anthropogenic. Very transparent plastics might be easy to miss. Mechanized methods for identifying polymer types are with scanning electron microscopy (Eerkes-Medrano, Leslie and Quinn,), Raman Microspectroscopy and Fourier Transformed Infrared Spectroscopy (FTIR) but but access to this specialized equipment is limited. Only dyes can be identified, while most microplastics found were microfibres (Cabernard et al, 2017). Color breakdown was not directly studied, however there are standard ways of referencing color in microplastics according to a 120 Pantone color palette (Marti et al. 2020). Tandem and light microscopy, including dark- and bright-field spectroscopy may also be used, however, visual identification is sufficient in most cases, even if there is risk of overassessment (or underassessment) (Windsor et al 2019). This process was repeated for 15 blank samples only without any organic content. Blank samples were collected from tap water, with Carleton's drinking water being the baseline to compare from. Blank samples were collected and analyzed to estimate possible background level of microplastic contamination in the laboratory. It is important to note that plastic particles less than

100 µm in size were not evaluated as was 100 µm as that was the size of mesh that the samples were filtered through. Plastic particles were counted and recorded per taxon per sample (Haegerbaeumer et al. 2019).

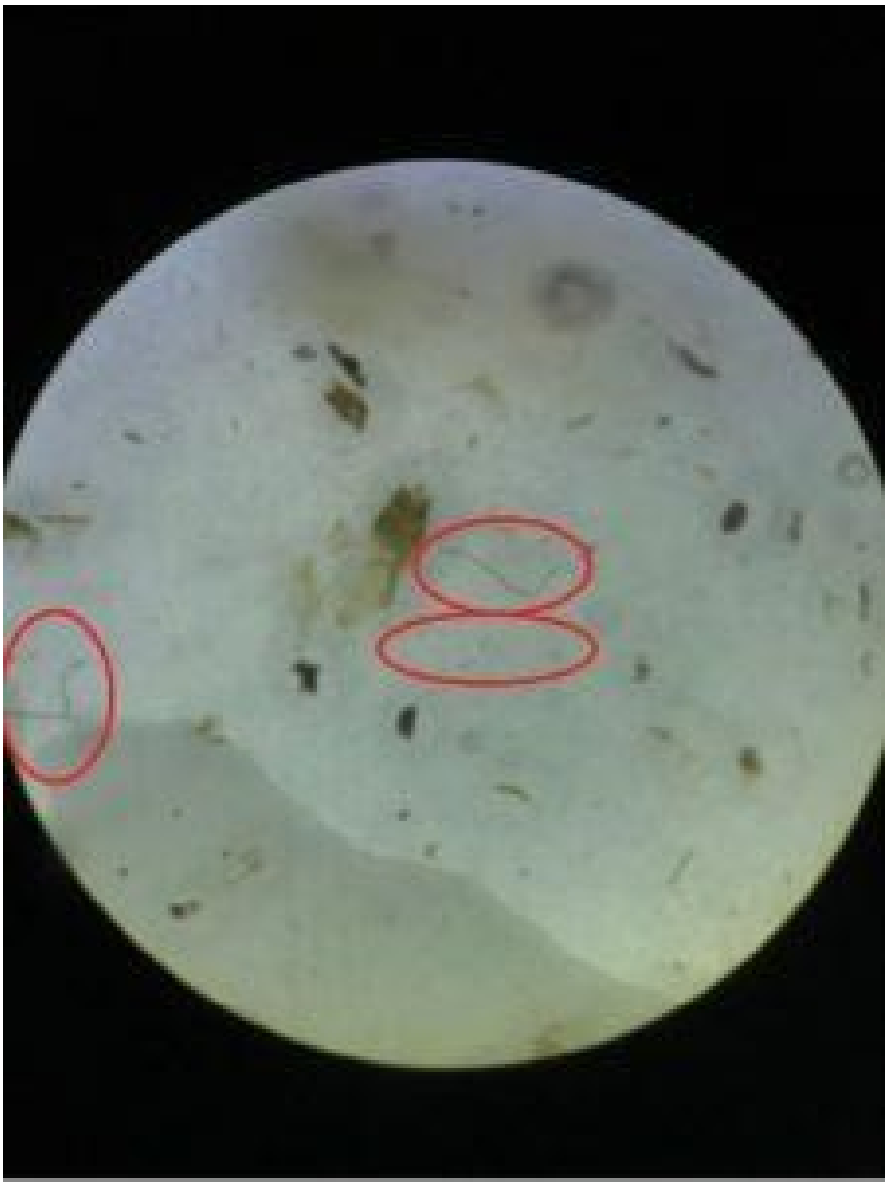


Figure 4. Microplastics in crayfish viscera.

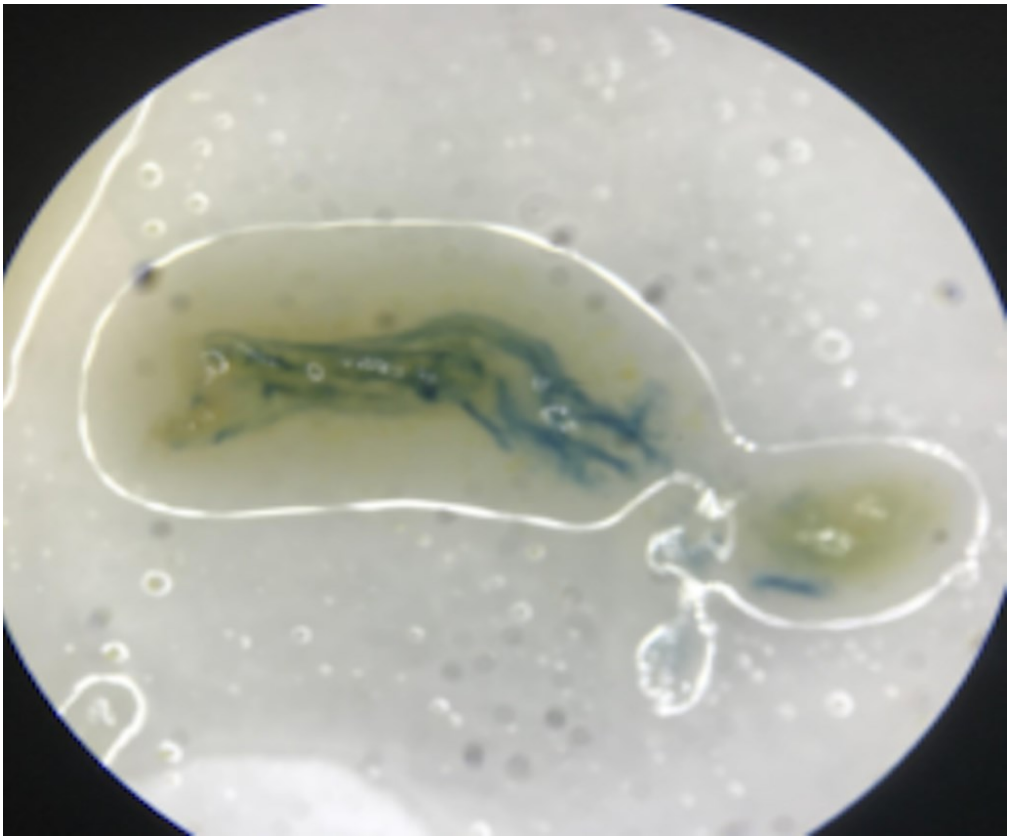


Figure 5. Photos of microplastics under a stereo microscope.

3.5 Statistical Methods

The total number of microplastics found in each organism were counted. Once the totality of the data were collected, the counts of microplastic per organism were tested for normality with the Shapiro-Wilk normality tests in R. Shapiro-Wilk tests are useful because they inform the user as to whether or not the data significantly differ from a normal distribution. Once normality was established, equality of variances was tested using Levene's and Bartlett's tests in R. These tests are conducted to better understand if the variances between groups are equal and therefore to get a clearer picture of the distribution of data. Due to multiple taxa, the degree of variance in microplastic count and individual weight were established using a One-Way ANOVA to compare the means of taxa count and weight (Khan et al. 2017). ANOVA tests are conducted to

see if the means between multiple groups are significantly different or not. Wilcoxon Rank Sum tests and a Spearman's Rank Correlation were carried out in R as well, with the same data to determine whether there were any differences in mean microplastic concentrations in the invertebrates between the Ottawa and Rideau Rivers. Afterwards, because animal weight and microplastic measurements are both continuous and hypothesized to be correlated, a linear regression was used to test whether wet animal weight was correlated with microplastic abundance in the animals. The tests were carried out using the R Statistical programming software (R Core Team, 2019).

Chapter 4

Results

In total, 10,033 microplastics were found in the animals collected from the Ottawa and Rideau rivers and 251 microplastics were counted in the 15 blank samples (Figure 6; Appendix 1). Microplastic count and organismal weight was also recorded for each sample (Amphipod, n = 87; Stonefly, n = 58; Snail, n = 5; Crayfish, n = 87; Zebra Mussel, n = 63). The results of the Shapiro-Wilk Tests indicated microplastic count between rivers ($W = 0.87458$, p-value < 0.001) were not normally distributed. Microplastic count within taxa however, were normally distributed ($W = 0.96557$, p-value = 0.1785) as was taxa weight ($W = 0.98545$, p-value = 0.4408). Barlett's (Bartlett's K-squared = 13.709, df = 4, p-value = 0.008) and Levene's Tests (Df F value Pr(>F) group 4 33.971 < 2.2e-16 ***) indicated significant equal variances except for Levene's Test of taxon versus count (Df F value Pr(>F) group 4 2.1215 0.07815). A One-Way ANOVA of count versus taxon and weight versus taxon revealed significant variance in count between taxa (Pr(>F) = 0.0249 *; Figure 7) but was only significant for stonefly and amphipod taxa weight. One-Way Analysis of variance tests showed significant variation in taxa weight (p < 2e-16 ***; Figure 8) but not microplastic count (p = 0.264) between taxa in rivers. Given that the data were not normally distributed, to compare microplastic count with organismal weight, a Spearman's Rank Correlation was performed and showed no significant relationship between the weight of the organism and the number of microplastics in the organism (S = 4459502, p-value = 0.8768; Figure 7, 8, 9, 10, 11).

Table 3. Statistical tests of microplastic count and weight by taxon and rivers

Test	Data	Result
Shapiro-Wilk	Microplastic Count between Rivers	W = 0.87458, p-value < 0.001
Shapiro-Wilk	Microplastic Count between Taxa	W = 0.96557, p-value = 0.1785
Shapiro-Wilk	Taxon Weight between Taxa	W = 0.98545, p-value = 0.4408
Bartlett's Test	Microplastic Count between Taxa	Bartlett's K-squared = 13.709, df = 4, p-value = 0.008
Levene's Test	Microplastic Count between Taxa	Df F value Pr(>F) group 4 33.971 < 2.2e-16 ***
One-Way ANOVA	Microplastic Count between Taxa	Pr(>F) = 0.0249 *
One-Way ANOVA	Microplastic Weight between Taxa	p < 2e-16 ***
Spearman's Rank Correlation	Microplastic Count and Taxon Weight	S = 4459502, p-value = 0.876

4.1 Microplastic Count

Results of a Shapiro-Wilk test showed Rideau River counts were not normally distributed (W = 0.95445, p-value = 0.00007817; Table 4), as were Ottawa River counts (W = 0.98043, p-value = 0.03105). A one-sample Wilcoxon Rank Sum was done on Rideau River samples which indicates microplastic levels were significantly greater than background rates found in blank samples (V = 11175, p-value < 2.2e-16). A one-sample Wilcoxon Rank Sum test was done on the Ottawa River counts as well and showed significantly greater levels of microplastics compared to the blank samples (V = 11325, p-value < 2.2e-16). The maximum number of microplastics found in a single organism was 101 (Table 5), which was found in a zebra mussel ($\bar{x}$ = 30.9, $\tilde{x}$ = 25, stdev = 19.4) from the Rideau river ($\bar{x}$ = 32.4, $\tilde{x}$ = 29, stdev = 18.0). The minimum number of microplastics found was in a crayfish ($\bar{x}$ = 33.4, $\tilde{x}$ = 32, stdev = 17.0) from the Rideau river which had 0 microplastics (Figure 9). All other organisms had at least one

microplastic present (n = 300). The largest number of microplastics found in an organism in the Ottawa River (x = 34.5, x = 33.5, stdev = 15.4) was 91 which was found in a stonefly ($\overline{x}$ = 39.7, $\tilde{x}$ = 43, stdev = 17.1), while the minimum number of microplastics found was 3 in an amphipod ($\overline{x}$ = 31.2, $\tilde{x}$ = 29, stdev = 13.6). The minimum number of microplastics found in stoneflies was 7 and 8 for zebra mussels (Figure 9). The maximum number of microplastics found in crayfish was 87 and 78 for amphipods. The minimum number of microplastics found in snails ($\overline{x}$ = 32.6, $\tilde{x}$ = 32, stdev = 6.1) was 8 and the maximum was 101.

A two-sample Wilcoxon Rank Sum Test did reveal a significant difference in microplastic count between the rivers and the blanks (W = 742.5, p-value = 0.00001192). In fact, the mean microplastic concentration found in the rivers ($\overline{x}$ = 33.4) are over double that of blanks ($\overline{x}$ = 16.7)

Table 4. Statistical tests of microplastic count by rivers and blank samples

Test	Data	Results
Shapiro-Wilk	Rideau River Microplastic Counts	W = 0.95445, p-value = 0.0000781
Shapiro-Wilk	Ottawa River Microplastic Counts	W = 0.98043, p-value = 0.03105
Wilcoxon Rank Sum	Rideau River and Blank Samples	V = 11175, p-value < 2.2e-16
Wilcoxon Rank Sum	Ottawa River and Blank Samples	V = 11325, p-value < 2.2e-16

Table 5. Results of microplastic count and weight by taxon and river.

			Count			
Taxon/River	Mean	Median	Stdev	Max	Min	Sample Size (n)
Crayfish	33.411	32	17.022	87	0	87
Amphipod	31.195	29	13.614	78	3	87
Stonefly	39.687	43	17.096	91	7	58
Snail	32.6	32	6.1486	39	24	5
Mussel	30.904	25	19.420	101	8	63
Rideau	32.36	29	18.048	101	0	150
Ottawa	34.526	33.5	15.387	91	3	150
Total	33.443	32	16.777	101	0	300
			Weight (g)			
Taxon/River	Mean	Median	Stdev	Max	Min	
Crayfish	6.833	5.17	4.153	19.9461	2.114	
Amphipod	0.018	0.010	0.0448	0.412	0.001	
Stonefly	0.018	0.018	0.009	0.0365	0.002	
Snail	1.462	0.577	2.214	5.4172	0.317	
Mussel	0.402	0.342	0.293	1.7657	0.105	
Rideau	4.1327	3.619	4.487	19.9461	0.105	
Ottawa	0.0663	0.013	0.4472	5.4172	0.002	
Total	2.099	0.168	3.778	19.9461	0.002	

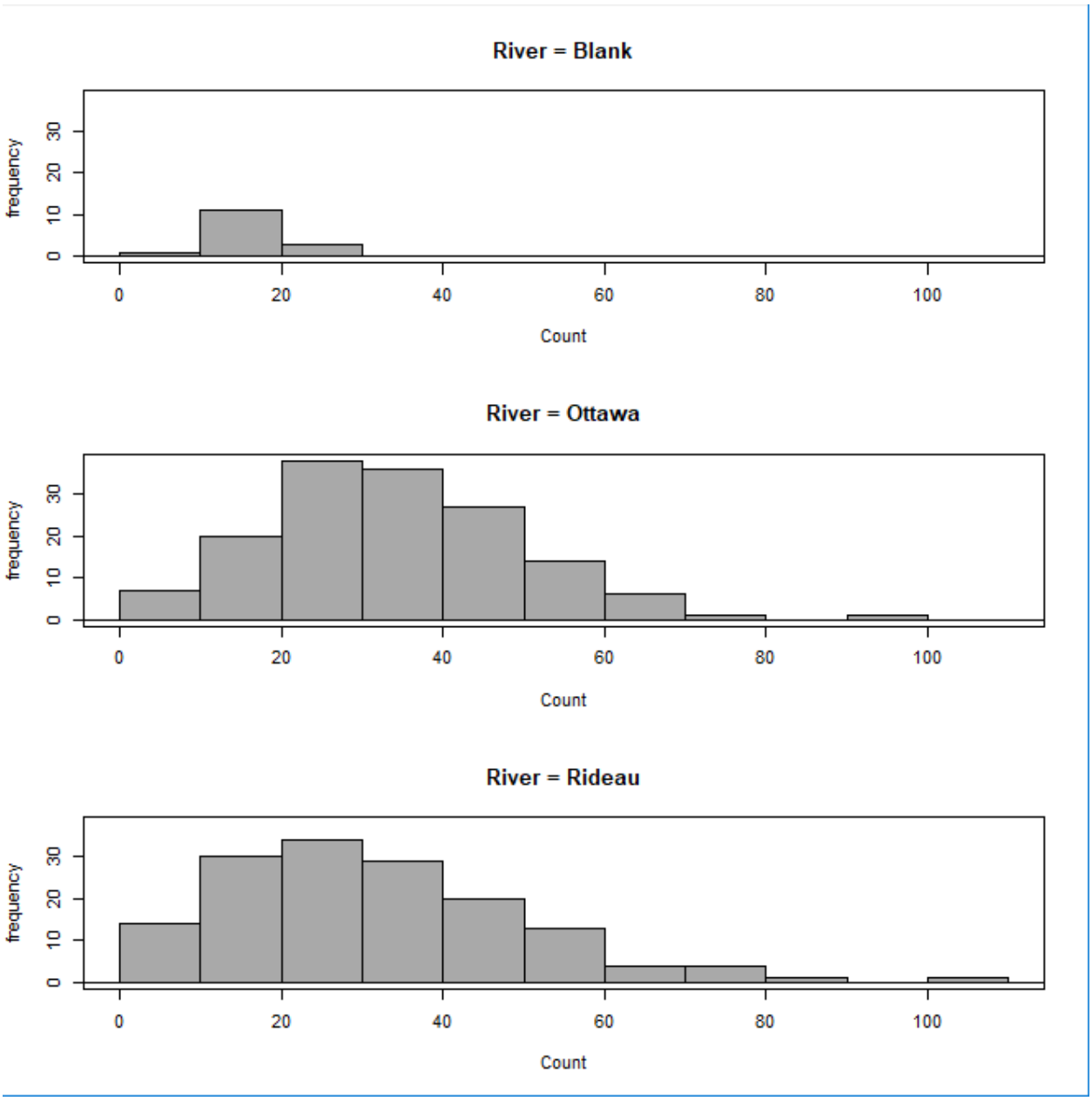
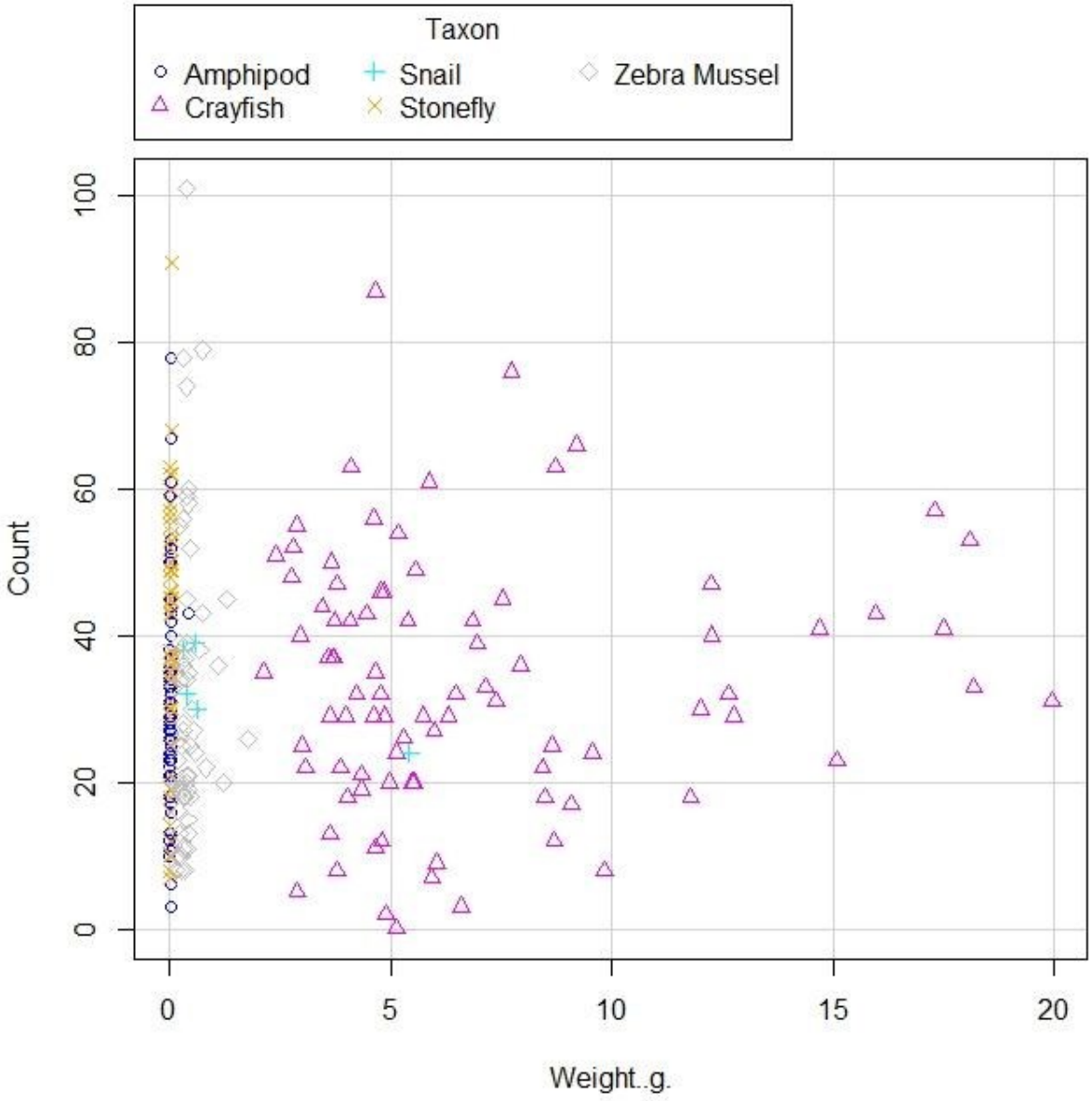


Figure 6. Histogram of microplastic counts in blank vs River groups**.**


Taxon
Amphipod
Crayfish
Snail
Stonefly
Zebra Mussel
Count
0
20
40
60
80
100
0
5
10
15
20
Weight..g.

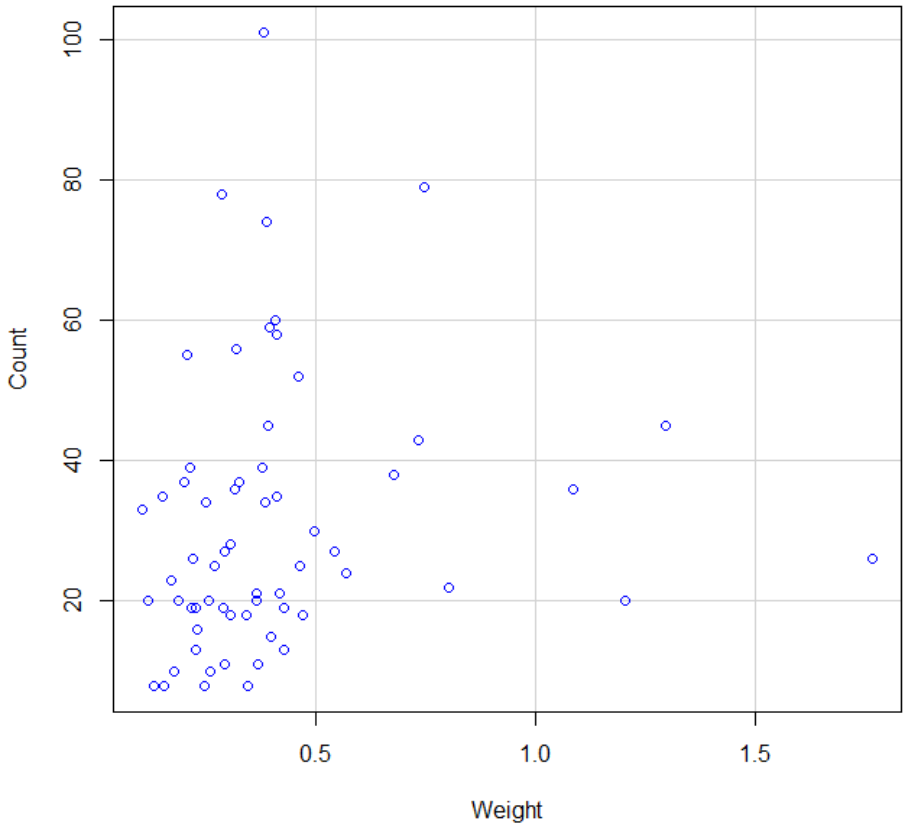


Figure 7. Mussel weight (g) vs microplastic count

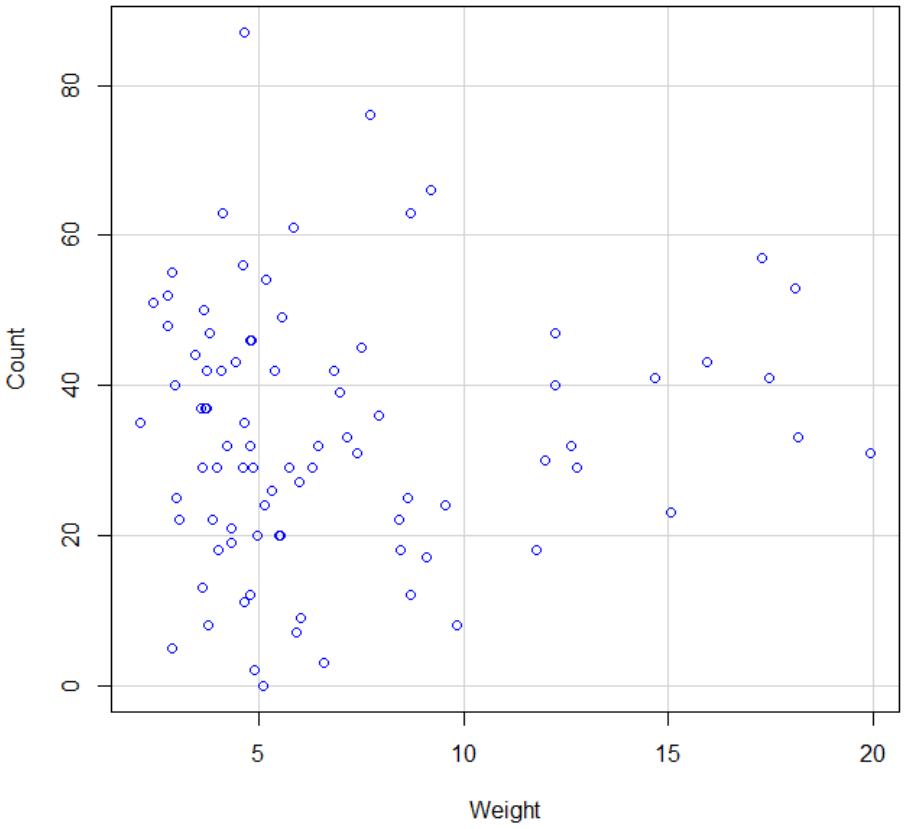


Figure 8. Crayfish weight (g) vs microplastic count

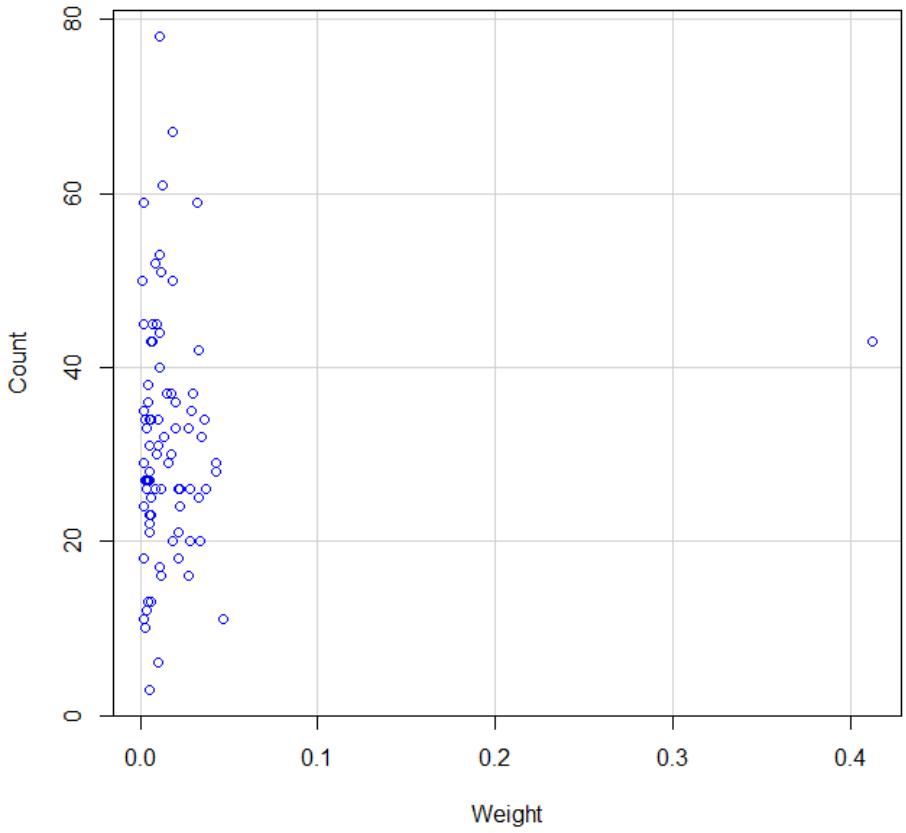


Figure 9. Amphipod weight (g) vs microplastic count

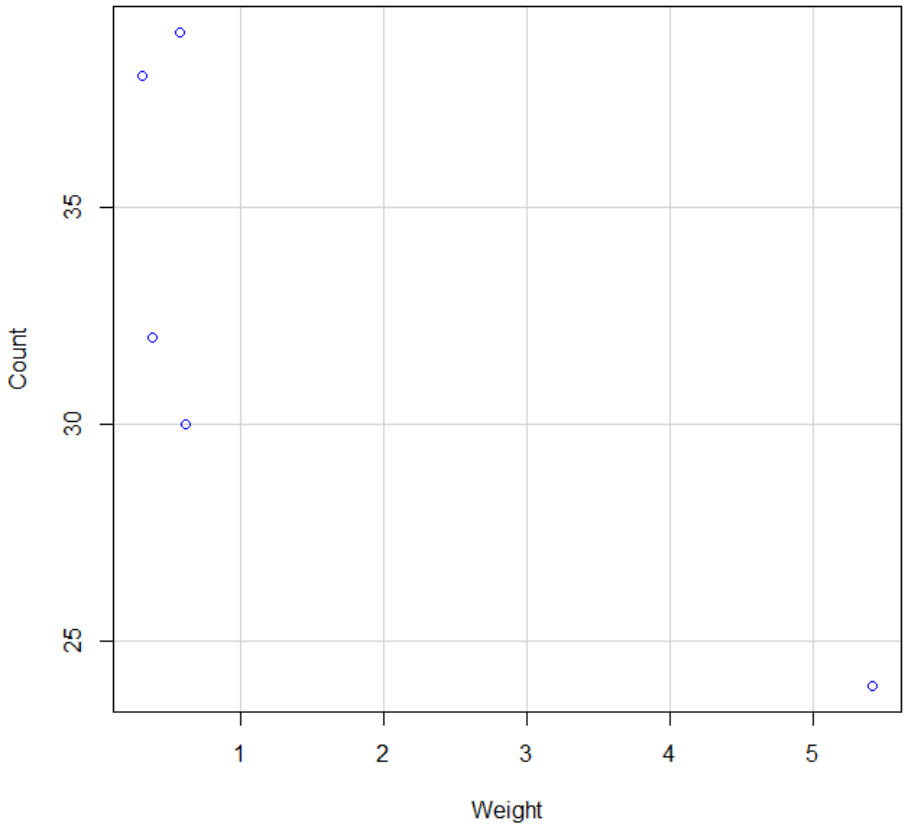


Figure 10. Snail weight (g) vs microplastic count

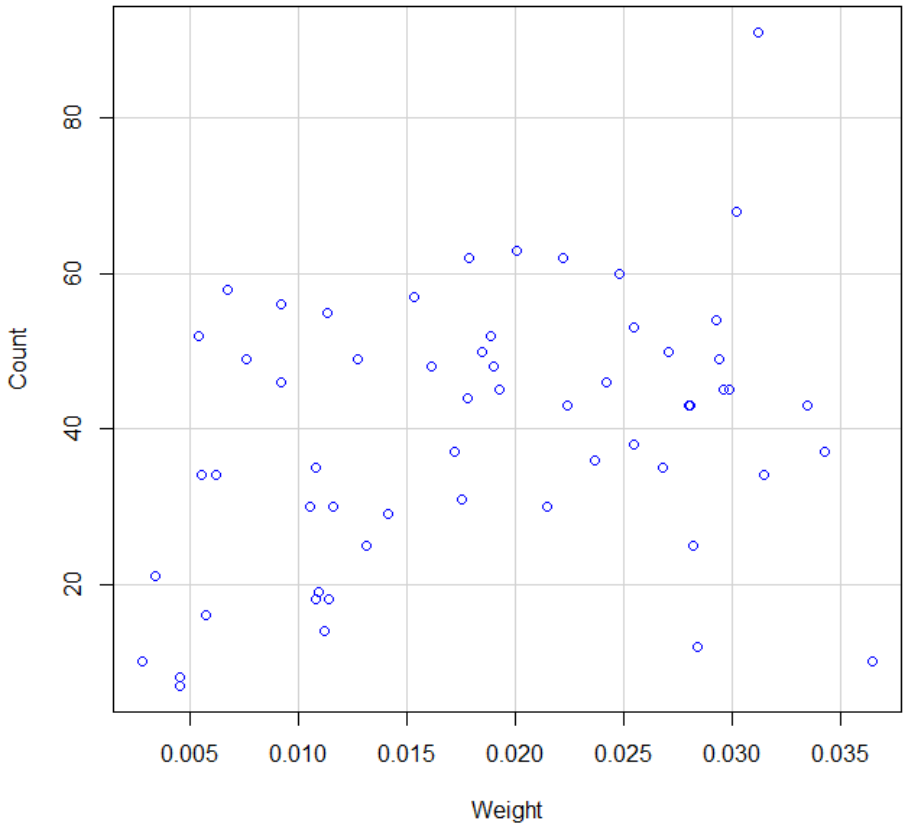


Figure 11. Stonefly weight (g) vs microplastic count

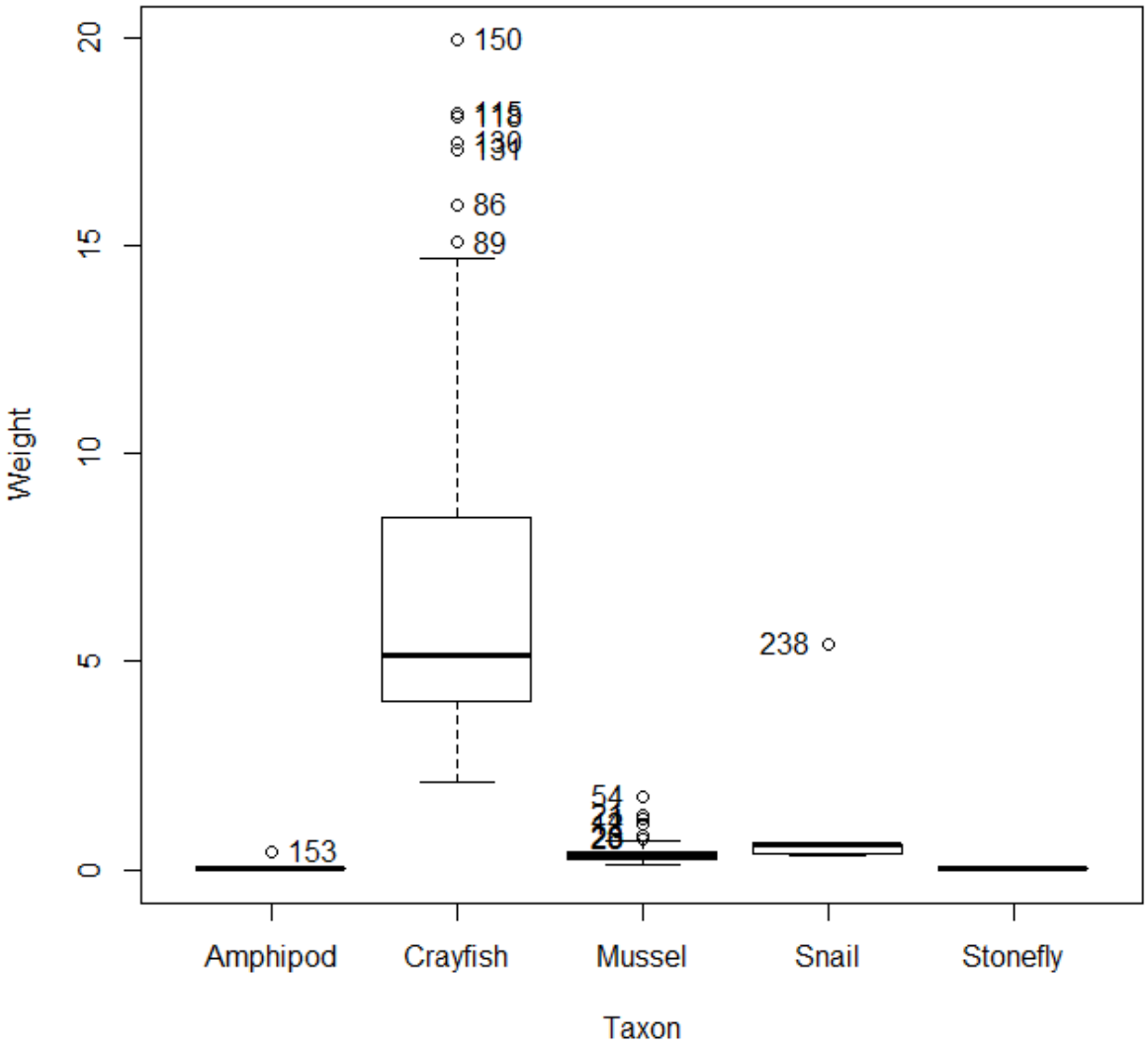


Figure 12. Boxplot of organismal weight (g) per taxon.

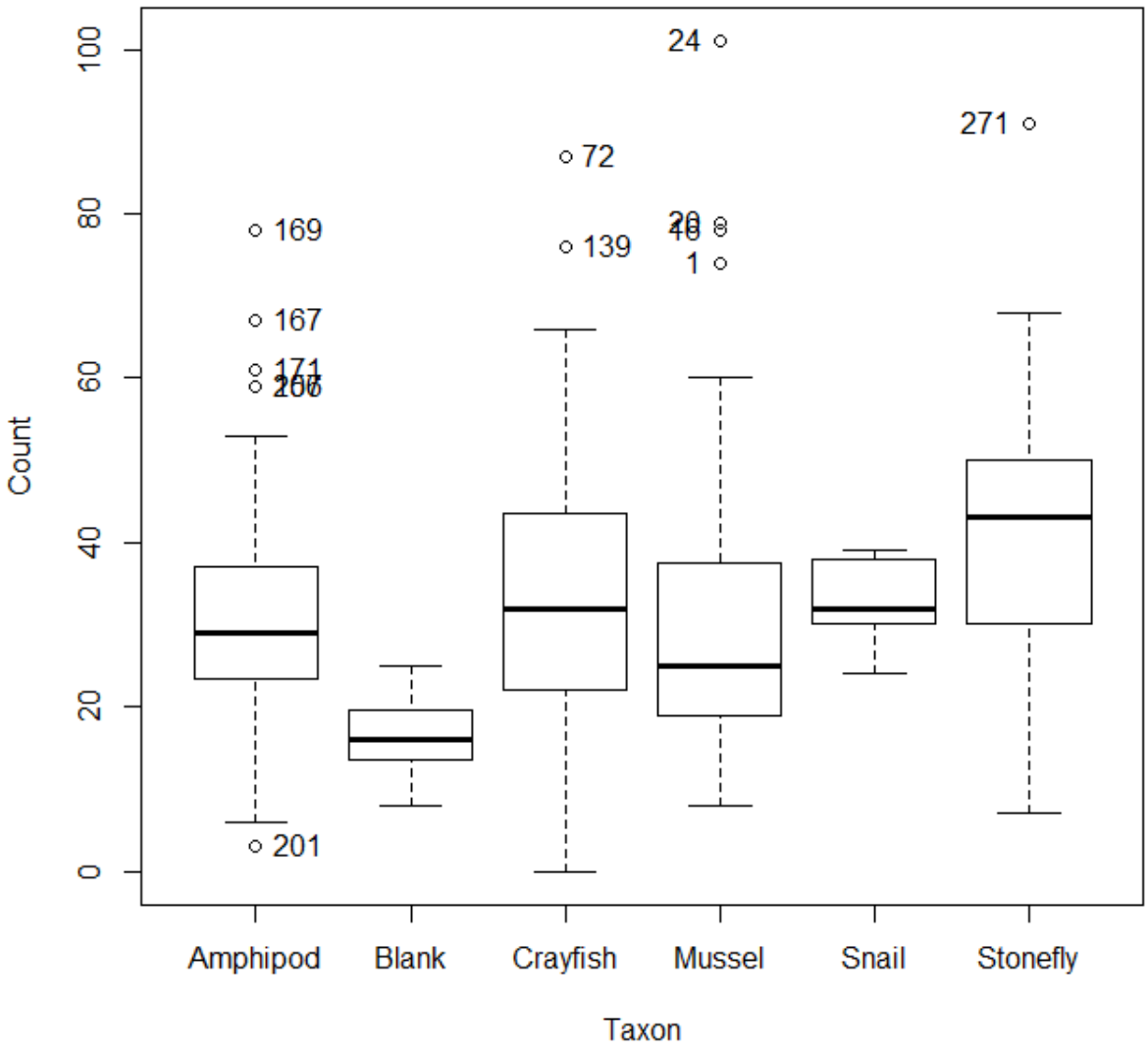


Figure 13. Boxplot of microplastic count between taxa and blank**.**

Discussion

In this study, nearly all samples, including the blanks, had microplastic present. However, microplastic concentrations in the blank samples were significantly lower than in Rideau and Ottawa river samples. These results are consistent with a growing body of literature that indicates microplastics are ubiquitous including in remote environments such as the Arctic (Trevail et al. 2015), the Bering Sea (Thompson et al. 2004), Svalbard (Lusher et al. 2015), the Greenland Sea (Amelineau et al. 2016) and in the Atlantic waters around Scotland (Lusher et al. 2014). While the microplastics vary in shape, size and color, all were microfibres, consistent with previous findings (Gallagher et al. 2016).

While there was variation in microplastic abundance between organisms, there was no significant increase in microplastic contamination with increasing weight of the organism or trophic status of the organism as predicted. Previous research showed microplastic ingestion up to 0.14 MP mg tissue−1 in macroinvertebrates across feeding guilds independent of habitat or ecological niche space in freshwater environments (Windsor et al. 2019). This suggests that microplastics are ubiquitous, especially in urban areas, and that they are not being retained and transferred up the trophic hierarchy, in fact detritivores and filter feeders are most at risk of consuming microplastics (Windsor et al. 2019). The literature is scant as microplastics have only been topical in recent years but there is some evidence of plastic being retained and magnified in individuals such as the Norway Lobster (*Nephrops norvegicus*, Welden and Cowie, 2016). There is also evidence of bioaccumulation of microplastics in blue mussels off the coast of Belgium (Leslie et al. 2017) and in the East China Sea (Kolandhasamy et al. 2018). Therefore, there is some evidence of microplastics being retained in the guts of invertebrates regardless of the findings here. Possible reasons for this include different organisms and study sites.

Almost all studies have found ubiquitous numbers of microplastics. One study describes microplastics being found in deep sea sediments in the Porcupine Abyssal Plain, Congo Canyon and the Nile Deep Sea Fan (Cauwenberghe et al. 2013). Microplastics are often found in river sediments in high concentrations but are cleared out by floods (Windsor et al. 2019). Another study describes microplastics found in an Indian shipyard (Reddy et al. 2006), as transportation is one of the most pollutive industries (Galloway, 2015).

No significant difference in microplastic levels were found in the invertebrates between the two rivers, despite them being from different families. Previous studies haver suggested that microplastic concentrations are significantly greater in marine systems in northern latitudes than expected compared to southern latitudes, most likely due to wind and water currents (Trevail et al. 2015). It stands to reason that microplastic levels should have some effect on their concentrations in organisms, but invertebrate physiology and anatomy likely allow microplastics to pass through. In contrast, previous literature suggests that invertebrates such as the Norway Lobster (*Nephrops norvegicus*) retain microplastics in their foregut for extended periods of time (Welden and Cowie, 2016). Research on fish found microplastics were able to pass through the gut but acted as a vector for absorption of other pollutants (Khan et al. 2017). Previous research in marine waters of East Greenland showed an abundance of microfibres as well. In fact, 97.2% of microplastics found were filamentous (Amelineau et al. 2016), which were the types of microplastics found in this study. In another study which focused on little auks (*Alle alle*), 100% of all individuals contained microplastics, despite being remote from urban activities (Amelineau et al. 2016).

Further evidence that microplastics are not being retained in the gut of the different invertebrates is the lack of association between organismal weight and microplastic count. This

again suggests microplastics are passing through the guts of different invertebrates instead of accumulating. One study showed 24.1% of plastic debris came from the gular pouches of the little auks themselves compared to 16.7% from plankton (Amelineau et al. 2016). This may seem like some evidence of trophic transfer of microplastics, however when the sea surface itself was studied, 68% of sea surface litter were microplastics (Zhao et al. 2016) and terrestrial bird gut contents had 54.9% microplastics (Lenz et al. 2015). In the case of the Norway lobster (Murray and Cowie, 2011), the authors noted that some microfilaments are unable to pass through the gastric mill and are hence retained in the foregut (Murray and Cowie, 2011). This is likely because gastric mills in crustaceans have evolved to breakdown softer organic materials than plastic. The gastric mills of crayfish were not looked at specifically in this study, but they would be much smaller (Chisaka and Kozawa, 2003) and harder to identify (Murray and Cowie, 2011)

Further evidence that microplastics are not accumulating in organisms is the lack of a significant relationship between trophic state and microplastic concentrations in this study. Despite occupying a higher trophic state (Simon and Garnier-Laplace, 2005), crayfish did not have significantly greater microplastic levels compared to the other invertebrates sampled as was predicted, in fact, the only invertebrate that did not have a single microplastic was an individual crayfish. Recent evidence has also suggested that microplastics do not bioaccumulate down the trophic hierarchy (Walkinshaw et al. 2020).

With regards to trophic state, most other studies, however, have found all levels of trophic state were susceptible to microplastic ingestion, but not necessarily one more than another (Trevail et al. 2015). Only one study was found that documents microplastic transfer from blue mussel (*Mytilus edulis*) to the European green crab (*Carcinus maenas*) (Farrell and Nelson, 2013). In this study, 0.5 μm polystyrene beads were exposed to blue mussels before

being fed to crabs. Within 48 hours microplastics had moved from the mussel's gut into its circulatory system (Farrell and Nelson, 2013). The haemolymph, characteristic of invertebrate circulatory systems, was then measured. After 24 hours, the blood level of microspheres reached 15033 ml^{-1} and dropped to 267 ml^{-1} after 21 days. Plastics were even found in the gills, stomach and ovaries among other body parts (Farrell and Nelson, 2013). Much of this can be ascribed to the laboratory setting which this experiment took place, whereas the research here was conducted in natural systems and no organisms were purposefully fed microplastics.

My results indicate that organisms in the Ottawa and Rideau Rivers are ingesting microplastics but there was no evidence of significantly greater concentrations in the Ottawa River versus the Rideau River or microplastic concentrations being affected by trophic status or weight of the organism. The median level of microplastics found in invertebrates in the Rideau river was 29 while the median number of microplastics found in invertebrates in the Ottawa River was 33.5. Regardless of the study, all find ubiquitous levels of microplastics in aquatic environments but there is little evidence of accumulation in the guts of invertebrates.

While the long-term effects of microplastic ingestion have not been fully studied yet, it is important that the public know about what is in their food and water so they may make informed decisions for themselves and what they consume. If there are substantial levels of microplastics in food that is eaten, or in the food their food eats, people may be less inclined to eat said foods. There is also a conservation component to consider as the organisms living in the freshwater are exposed to this plastic pollution. Possible solutions include finding ways to ensure against plastic contamination such as researching plastic substitutes or imposing bans. Other solutions include directing more funding towards waste management programs and hiring more people, with better equipment to clean microplastics out of our water (Harrison et al. 2011). Tighter rules,

regulations and sanctions in waste management is another viable option such as with plastic bags. Analysis looking at 201 stores in California from 2008 – 2015 reveals stores that banned plastic bags found a reduction in plastic use by 40.3 million pounds (Van Doren, 2017). What is known is that microplastics can be found in habitats all around the world, with most organisms, often almost every individual having consumed them (Thompson, 2015). The fact is, improper waste management, littering and breakdown of larger plastics leaves microscopic residues in almost all aquatic invertebrates, benthic and pelagic, neritic and planktonic alike (Thompson, 2015).

Chapter 5

Conclusions

This study has shown that Ottawa's main waterways not only contain microplastic, but that these microplastics are being ingested by aquatic invertebrates. All organisms living in the waterways were found to be ingesting microplastics regardless of weight, taxon or trophic level. This suggests the ingestion of microplastics by invertebrates in both the Ottawa and Rideau River is common. Interestingly, the amount of microplastics ingested by invertebrates was not significantly different between the two rivers despite different types of invertebrates being sampled in the two rivers.

The fact that trophic state, weight and taxon were not significantly related to microplastic concentration has strong implication with regards to gut retention of microplastics. This study, as well as previous studies suggest that bigger organisms occupying a higher trophic state, in water richer in microplastics will not necessarily retain more microplastics in their gastrointestinal tract. In fact, the only hypothesis that was supported by the results was that microplastics were higher in organisms than in the blank samples. High plastic exposure has been linked to a variety of health issues in animals including a false sense of satiety, cellular damage, gastrointestinal blockage and thrombosis (Farrell and Nelson, 2013).

Continuing research on levels and effects of microplastics in the environment is of paramount importance. Quantifying and identifying microplastics in local basins, such as the St. Lawrence River will illuminate new levels of microplastics for comparative studies and allow researchers to target site specific ways of mitigating plastic pollution. An increase in awareness

of microplastics may help increase funding and citizen participation in research, as well as improving mitigation and response measures (Trevail et al. 2015). More research needs to be done on freshwater systems and in particular on streams, as they are the primary connection between the water and the land (Mora-Teddy and Matthei, 2020). In fact, a crucial component of marine plastic pollution is that which is transported by rivers and streams (van Wijnen, Ragas and Kroeze, 2019).

The study of the ingestion of microplastics by organisms such as invertebrates, could increase monitoring of plastic pollution (Trevail et al. 2015). More research is needed to monitor the behavior of the environment as well as keep tabs on the levels of toxins in ecosystems (de Souza Machado et al. 2018). This will help better inform strategic management and policy of microplastic pollution (de Souza Machado et al. 2018). By studying the chemical effects of plastic pollution, research can further test how ingredients such as bisphenol-A affect animal and even human physiology, through drinking or consumption of seafood (Trevail et al. 2015). While larger plastic pieces can cause entanglement for some organisms, smaller plastic pieces threaten the health of an organism due to a variety of polymer types and other biopersistant and xenobiotic additives (de Souza Machado et al. 2018). Other effects of microplastic ingestion include abrasion of mucosa and false sense of satiation (de Souza Machado et al. 2018).

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Appendix 1 – Raw Data

Ottawa River – Summer 2018				
#	Taxon	Count	Length	Mass per Animal (g)
1	Amphipod	30		0.0097
2	Stonefly	16		0.0057
3	Stonefly	18		0.0108
4	Stonefly	12		0.0284
5	Stonefly	34		0.0062
6	Stonefly	8		0.0045
7	Stonefly	19		0.0109
8	Stonefly	7		0.0045
9	Stonefly	10		0.0028
10	Stonefly	10		0.0365
11	Stonefly	25		0.0131
12	Stonefly	14		0.0112
13	Snail	24		5.4172
14	Snail	30		0.6189
15	Snail	39		0.5776
16	Snail	38		0.3177
17	Snail	32		0.3833
18	Stonefly	50		0.0185
19	Amphipod	52		0.0082
20	Stonefly	25		0.0282
21	Amphipod	43		0.412
22	Amphipod	43		0.0061
23	Stonefly	62		0.0179
24	Amphipod	40		0.011
25	Amphipod	59		0.0323
26	Amphipod	29		0.0161
27	Amphipod	42		0.0325
28	Amphipod	36		0.0199
29	Amphipod	44		0.0109
30	Amphipod	45		0.0093
31	Amphipod	34		0.01
32	Amphipod	34		0.0359
33	Amphipod	37		0.0149
34	Amphipod	26		0.0277
35	Amphipod	25		0.0061
36	Amphipod	67		0.0183
37	Amphipod	51		0.012
38	Amphipod	78		0.0106

39	Amphipod	53		0.0109
40	Amphipod	61		0.0127
41	Amphipod	18		0.0214
42	Amphipod	28		0.0423
43	Amphipod	21		0.0217
44	Amphipod	26		0.0223
45	Amphipod	25		0.0332
46	Amphipod	26		0.037
47	Amphipod	20		0.0339
48	Amphipod	33		0.0271
49	Amphipod	11		0.047
50	Amphipod	33		0.0275
51	Amphipod	35		0.029
52	Amphipod	24		0.0225
53	Amphipod	29		0.0428
54	Amphipod	31		0.0105
55	Amphipod	32		0.0343
56	Amphipod	37		0.0172
57	Amphipod	37		0.0295
58	Amphipod	26		0.0118
59	Amphipod	32		0.013
60	Amphipod	50		0.0179
61	Stonefly	44		0.0178
62	Stonefly	37		0.0343
63	Stonefly	35		0.0268
64	Stonefly	34		0.0055
65	Stonefly	54		0.0293
66	Stonefly	30		0.0116
67	Stonefly	49		0.0127
68	Stonefly	43		0.0335
69	Stonefly	21		0.0034
70	Stonefly	31		0.0175
71	Stonefly	52		0.0189
72	Stonefly	29		0.0141
73	Stonefly	45		0.0296
74	Stonefly	63		0.0201
75	Stonefly	91		0.0312
76	Stonefly	68		0.0302
77	Stonefly	60		0.0248
78	Stonefly	37		0.0172
79	Stonefly	55		0.0113
80	Stonefly	43		0.028
81	Amphipod	30		0.0173
82	Amphipod	23		0.0061
83	Amphipod	23		0.0052
84	Amphipod	34		0.005
85	Amphipod	28		0.0053
86	Amphipod	43		0.0067
87	Amphipod	36		0.0042
88	Amphipod	17		0.0111

89	Amphipod	27		0.0025
90	Amphipod	3		0.0055
91	Amphipod	12		0.0037
92	Amphipod	10		0.003
93	Amphipod	16		0.0121
94	Amphipod	13		0.0061
95	Amphipod	21		0.0051
96	Amphipod	59		0.002
97	Amphipod	26		0.0035
98	Amphipod	27		0.0033
99	Amphipod	27		0.0055
100	Amphipod	29		0.0021
101	Amphipod	27		0.0041
102	Amphipod	33		0.0039
103	Amphipod	45		0.0071
104	Amphipod	34		0.0024
105	Amphipod	38		0.0044
106	Amphipod	35		0.0019
107	Amphipod	45		0.002
108	Amphipod	50		0.0015
109	Amphipod	26		0.0219
110	Amphipod	6		0.0105
111	Amphipod	34		0.0059
112	Amphipod	31		0.0051
113	Amphipod	34		0.0031
114	Amphipod	22		0.0052
115	Amphipod	13		0.0044
116	Amphipod	11		0.0021
117	Amphipod	13		0.0043
118	Amphipod	18		0.0023
119	Amphipod	27		0.0027
120	Amphipod	24		0.0021
121	Stonefly	49		0.0294
122	Stonefly	38		0.0255
123	Stonefly	45		0.0299
124	Stonefly	50		0.0271
125	Stonefly	56		0.0092
126	Stonefly	57		0.0153
127	Stonefly	48		0.019
128	Stonefly	49		0.0076
129	Stonefly	35		0.0108
130	Stonefly	46		0.0092
131	Stonefly	52		0.0054
132	Stonefly	58		0.0067
133	Stonefly	18		0.0114
134	Stonefly	46		0.0242
135	Stonefly	43		0.0224
136	Stonefly	36		0.0237
137	Stonefly	48		0.0161
138	Stonefly	45		0.0193

139	Stonefly	30		0.0215
140	Stonefly	43		0.0281
141	Stonefly	53		0.0255
142	Stonefly	30		0.0105
143	Stonefly	62		0.0222
144	Stonefly	34		0.0315
145	Amphipod	33		0.0198
146	Amphipod	20		0.0279
147	Amphipod	26		0.0216
148	Amphipod	26		0.0085
149	Amphipod	20		0.0186
150	Amphipod	16		0.027
Rideau River – Summer 2018				
#	Taxon	Count	Length	Mass per Aniaml (g)
1	Zebra Mussel	74		0.3873
2	Zebra Mussel	39		0.3782
3	Zebra Mussel	43		0.7326
4	Zebra Mussel	20		1.2013
5	Zebra Mussel	30		0.4956
6	Zebra Mussel	24		0.5669
7	Zebra Mussel	11		0.366
8	Zebra Mussel	8		0.343
9	Zebra Mussel	18		0.342
10	Zebra Mussel	8		0.2444
11	Zebra Mussel	35		0.1511
12	Zebra Mussel	36		1.0857
13	Zebra Mussel	33		0.105
14	Zebra Mussel	60		0.4071
15	Zebra Mussel	59		0.3937
16	Zebra Mussel	78		0.2862
17	Zebra Mussel	20		0.363
18	Zebra Mussel	34		0.3838
19	Zebra Mussel	58		0.4092
20	Zebra Mussel	79		0.7458
21	Zebra Mussel	45		1.2953
22	Zebra Mussel	56		0.3161
23	Zebra Mussel	45		0.39
24	Zebra Mussel	101		0.3794
25	Zebra Mussel	55		0.2042
26	Zebra Mussel	20		0.1846
27	Zebra Mussel	23		0.1679
28	Zebra Mussel	22		0.8002
29	Zebra Mussel	21		0.3636

30	Zebra Mussel	10		0.257
31	Zebra Mussel	25		0.462
32	Zebra Mussel	8		0.13
33	Zebra Mussel	13		0.2238
34	Zebra Mussel	10		0.1768
35	Zebra Mussel	8		0.1543
36	Zebra Mussel	18		0.4682
37	Zebra Mussel	11		0.2901
38	Zebra Mussel	18		0.3056
39	Zebra Mussel	19		0.2157
40	Zebra Mussel	37		0.2003
41	Zebra Mussel	19		0.288
42	Zebra Mussel	23		0.1686
43	Zebra Mussel	37		0.3238
44	Zebra Mussel	39		0.2122
45	Zebra Mussel	20		0.1172
46	Zebra Mussel	16		0.2296
47	Zebra Mussel	19		0.2254
48	Zebra Mussel	20		0.2541
49	Crayfish	52		2.78
50	Crayfish	22		3.07
51	Crayfish	12		4.79
52	Crayfish	20		5.48
53	Crayfish	0		5.12
54	Crayfish	8		3.77
55	Crayfish	5		2.89
56	Crayfish	2		4.88
57	Crayfish	87		4.64
58	Crayfish	35		4.66
59	Crayfish	37		3.57
60	Crayfish	3		6.6
61	Crayfish	40		2.95
62	Crayfish	8		9.83
63	Crayfish	29		4.62
64	Crayfish	49		5.57
65	Crayfish	22		8.43
66	Crayfish	29		6.29
67	Crayfish	7		5.93
68	Crayfish	9		6.04
69	Crayfish	32		4.23
70	Crayfish	11		4.65
71	Crayfish	43		15.97
72	Crayfish	18		11.78
73	Crayfish	19		4.33
74	Crayfish	23		15.08
75	Crayfish	17		9.08
76	Crayfish	45		7.52
77	Crayfish	20		5.52
78	Crayfish	36		7.93
79	Crayfish	33		7.14

80	Crayfish	39		6.96
81	Crayfish	56		4.61
82	Crayfish	44		3.45
83	Crayfish	63		8.72
84	Crayfish	61		5.86
85	Crayfish	54		5.17
86	Crayfish	63		4.1
87	Crayfish	32		4.77
88	Crayfish	29		4.6
89	Crayfish	29		3.99
90	Crayfish	26		5.3
91	Crayfish	25		2.98
92	Crayfish	22		3.87
93	Crayfish	18		4.02
94	Crayfish	13		3.61
95	Crayfish	51		2.41
96	Zebra Mussel	52		0.46
97	Zebra Mussel	27		0.5403
98	Zebra Mussel	27		0.2907
99	Zebra Mussel	34		0.2487
100	Zebra Mussel	38		0.6758
101	Zebra Mussel	26		1.7657
102	Zebra Mussel	13		0.426
103	Zebra Mussel	28		0.3035
104	Zebra Mussel	21		0.4175
105	Zebra Mussel	19		0.4261
106	Zebra Mussel	15		0.3973
107	Zebra Mussel	26		0.2195
108	Zebra Mussel	36		0.3138
109	Zebra Mussel	35		0.4109
110	Zebra Mussel	25		0.2678
111	Crayfish	42		6.8446
112	Crayfish	29		12.755
113	Crayfish	41		14.6898
114	Crayfish	66		9.2016
115	Crayfish	33		18.1827
116	Crayfish	12		8.6999
117	Crayfish	29		5.7345
118	Crayfish	53		18.0976
119	Crayfish	24		9.5447
120	Crayfish	32		6.4602
121	Crayfish	40		12.2543
122	Crayfish	46		4.8295
123	Crayfish	47		3.7877
124	Crayfish	42		4.0811
125	Crayfish	46		4.7783
126	Crayfish	50		3.6449
127	Crayfish	29		3.6297
128	Crayfish	25		8.6388
129	Crayfish	42		5.385

130	Crayfish	41		17.49
131	Crayfish	57		17.2941
132	Crayfish	20		4.9606
133	Crayfish	35		2.114
134	Crayfish	18		8.47
135	Crayfish	32		12.6306
136	Crayfish	48		2.7577
137	Crayfish	29		4.8665
138	Crayfish	55		2.8752
139	Crayfish	76		7.7138
140	Crayfish	30		11.9907
141	Crayfish	47		12.2271
142	Crayfish	27		5.9783
143	Crayfish	43		4.4401
144	Crayfish	42		3.7216
145	Crayfish	21		4.3236
146	Crayfish	24		5.1334
147	Crayfish	31		7.3883
148	Crayfish	37		3.6836
149	Crayfish	37		3.7183
150	Crayfish	31		19.9461
		Blank Samples		
#	Taxon	Count	Length	Weight (g)
	Blank	13		
	Blank	16		
	Blank	15		
	Blank	18		
	Blank	14		
	Blank	13		
	Blank	18		
	Blank	21		
	Blank	20		
	Blank	19		
	Blank	25		
	Blank	13		
	Blank	14		
	Blank	24		
	Blank	8		