INVESTIGATING THE GUT MICROBIOMES OF AQUATIC BENTHIC MACROINVERTEBRATES IN THE SAINT JOHN RIVER (NEW BRUNSWICK, CANADA)

by

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ABSTRACT

Aquatic invertebrates are key members of aquatic food webs; however, little is known about their gut microbiomes. Understanding how environment, taxonomy, dietary habits, and time alter gut bacteria is important in addressing current gaps in microbiome research. The objectives of this study were to characterize the gut microbiomes of aquatic invertebrates and to assess whether the composition of gut bacteria differs temporally and across taxa with different habitats and feeding habits. Invertebrates from 16 orders were collected from 8 sites within a 20 km reach of the Saint John River (New Brunswick, Canada) in fall 2016 and 2017, with these individuals containing nearly 20,000 operational taxonomic units. Gut microbiomes of invertebrates differed significantly among invertebrate taxa and temporally; however, few significant within-taxa differences were found among habitat types and no significant differences were observed among functional feeding groups. This study is the first to describe the gut microbiomes of aquatic invertebrates in the Saint John River. This information establishes a baseline of natural variability and diversity of aquatic invertebrate gut microbiomes, providing a foundation for future work.

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1. Investigating the gut microbiomes of aquatic benthic macroinvertebrates in the Saint John River (New Brunswick, Canada)

1.1 Introduction

1.1.1 General concepts of microbiomes

Most living animals possess an ecological community of bacterial microorganisms that inhabit their internal and external body spaces; this is referred to as an organism's microbiome (Lederberg and McCray, 2001; Thursby and Juge, 2017). Of the many bacterial communities inhabiting an organism's body, the largest by mass is within the digestive tract – a community of bacteria referred to as the gut microbiome (McFall-Ngai *et al*., 2013). These bacteria, which may exist commensally, symbiotically, or pathogenically in the guts of their hosts, often provide beneficial services including increasing the rate at which nutrients are metabolized, maintaining structural features of gut membranes, developing immune responses, and providing defense from pathogenic attack (Jandhyala *et al*., 2015). Gut bacteria are also able to break down food components that would otherwise be indigestible by a host organism (Jones *et al.*, 2013).

The ability of an organism to maintain a taxonomically diverse and functionally balanced gut microbiome is important for ensuring that the aforementioned benefits are realized. When the composition of an organism's gut microbiome becomes altered – often due to internal or external stressors – a phenomenon known as dysbiosis may be experienced by the host in which the imbalance reduces the benefits otherwise provided (Hamdi *et al*., 2011). In humans, digestive dysbiosis has been studied in great depth and

has been linked to multiple illnesses and diseases, such as obesity and inflammatory bowel diseases (Turnbaugh *et al*., 2006; Seksik, 2010). Dysbiosis in humans is often characterized by a reduction in bacterial diversity, particularly among the commensal bacteria that normally provide beneficial services to the host; this then allows opportunistic and pathogenic species to more easily colonize the gut, resulting in an increased risk of enteric infection (Prakash *et al.*, 2011). Several contributing factors are associated with the onset of dysbiosis in humans, including antibiotic use, the experience of psychological and/or physical stresses, and dietary elements (Hawrelak and Myers, 2004). Given the importance of the microbiome to human health, most of the literature focuses on understanding human microbiomes and there is a lack of information on other organisms.

1.1.2 Current gut microbiome research on terrestrial invertebrates

Previous studies of invertebrate gut microbiomes have mainly focused on a few terrestrial invertebrate taxa, especially those having important roles in pollinating crops, spreading disease, plant herbivory, and altering soil fertility (Jones *et al.*, 2013). For example, studies have investigated the gut microbiomes of honey bees and bumble bees due to the ecological and economical roles that they have in the pollination of crops (Martinson *et al.*, 2011; Engel *et al.*, 2012; Martinson *et al.*, 2012). Additionally, Taglavia *et al.* (2014) have investigated the gut microbiomes of the red palm weevil, a major pest of palm trees, while a few studies have investigated a broad range of terrestrial invertebrate taxa (7 orders – Colman *et al.*, 2012; 8 orders – Jones *et al.*, 2013; 21 orders – Yun *et al.*, 2014). Given the terrestrial origins of aquatic invertebrates (Bradley *et al.*,

2009), and because there are so few studies on aquatic species, I will review the state of science of terrestrial invertebrate gut microbiomes to provide a context for the present study.

There are several bacterial phyla and classes commonly found within the gut microbiomes of terrestrial invertebrates. The phylum, Proteobacteria, is generally dominant within the gut, with the relative abundance of bacterial sequences varying between 48% – 81% (Colman *et al.*, 2012: Jones *et al.*, 2013; Yun *et al.*, 2014; Mikaelyan *et al.*, 2015; Pérez-Cobas *et al.*, 2015; Kim *et al.*, 2017; Muturi *et al.*, 2017). Other common bacterial phyla include Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria (Colman *et al.*, 2012; Jones *et al.*, 2013; Yun *et al.*, 2014; Mikaelyan *et al.*, 2015; Pérez-Cobas *et al.*, 2015). At the bacterial class level, Alphaproteobacteria, Gammaproteobacteria, and Betaproteobacteria – all within the phylum Proteobacteria – commonly have high abundances, making up between 2.6% and 52.4% of the total gut bacterial sequences (Colman *et al.*, 2012; Jones *et al.*, 2013; Yun *et al.*, 2014). Bacterial orders such as Enterobacteriales, Bacillales, Lactobacillales, Actinomycetales and Burkholderiales are also common in the gut microbiome and include several opportunistic pathogens and mutualists, though their exact roles in the gut microbiome have yet to be established (Colman *et al.*, 2012; Singhal *et al.*, 2017). The relative abundances of bacterial families within the gut microbiomes of invertebrates are also variable. Some common gut bacterial families include Enterobacteriaceae, Lactobacillaceae, and Acetobacteriacae – all of which have been shown previously in studies of *Drosophila* and Dipterans to aid in protecting female individuals from sterility induced by nematodes, regulating larval development through insulin signaling, and

modulating signaling pathways that influence overall growth and metabolism (Singhal *et al.*, 2017).

The process through which invertebrate gut microbiomes are colonized is currently unknown. Several mechanisms by which this process may occur have been proposed, including the horizontal and vertical transmission of gut bacteria between individuals (Nalepa *et al.* 2001; Kitade, 2004; Martinson *et al.*, 2012), exchange between invertebrates and their environment (Nalepa *et al.*, 2001), exposure through diet (Nalepa *et al.*, 2001), changes throughout developmental life stages (Delalibera *et al.*, 2007; Vasanthakumar *et al.*, 2008; Colman *et al.*, 2012), and evolutionary histories between gut bacteria and their hosts (Pérez-Cobas *et al.*, 2015). The horizontal transmission of gut bacteria between individuals of the same generation has been hypothesized. Highly social invertebrates such as wasps partake in trophallaxis in which nestmates exchange regurgitated liquids between one another and, in the process, likely exchange gut bacteria (Nalepa *et al.*, 2001). Termites, another group of social invertebrates, also transfer bacterial symbionts during territorial defense, leading to similarities in the bacterial taxa observed between non-related individuals (Kitade, 2004). Similarly, honey bees, which are also highly social, possess bacterial symbionts within their gut microbiomes that are acquired during the first few days of their adult life stage through social interactions with other adult workers in their colony (Martinson *et al.*, 2012). In addition to social invertebrates, several taxa are known to partake in coprophagy – the ingestion of feces – which could lead directly to the introduction of specific fecal and environmental bacteria into their gut microbiomes (Nalepa *et al.*, 2001). Two studies have also investigated changes in gut microbiomes throughout developmental life stages, finding that gut

bacterial taxa such as *Pantoea* and *Stenotrphomonas* (two genera of Gammaproteobacteria) are maintained from the larval stage into the pupal and adult stages in *Ips pini* and *Agrilus planipennis* bark beetles (Delalibera *et al.*, 2007; Vasanthakumar *et al.*, 2008). Developmental stages have been known to impact gut microbiomes of some invertebrates more than others; certain adult-larval pairs have different diets but still cluster distinctly together on principal coordinates analysis (PCoA) plots, while other adult-larval pairs have identical diets but do not show similar bacterial communities (Colman *et al.*, 2012).

Vertical transmission of gut bacteria from parent to offspring is supported by studies investigating the effects of taxonomy on the gut microbiome. More specifically, both lab-reared and wild cockroaches share at least 25 bacterial species within their gut which are termed their "bacterial core", "core microbiota", or "core microbiome" (Pérez-Cobas *et al.*, 2015). These core microbiomes are observed consistently across all individuals regardless of diet, suggesting that these particular bacteria may have been passed down vertically from parent to offspring. Another possibility for the existence of a core microbiome is that some bacterial individuals may have co-evolved with host invertebrate taxa over time as has been observed between the cockroach *Blatetella germanica* and the bacterial strain *Blattabacterium Bge* (Pérez-Cobas *et al.*, 2015). In this case, the specific endosymbiotic bacterium plays a vital role in nutrient acquisition for the cockroaches and it is also able to recycle nitrogenous wastes produced by the cockroach (Pérez-Cobas *et al.*, 2015). The concept of co-evolution between gut bacteria and hosts also suggests that core microbiomes are essential to host organisms for survival. Thus, any hosts that do not possess these core bacterial individuals within in their gut

microbiomes may be selected against and are not analyzed in gut microbiome studies. Overall, it is likely that a combination of horizontal and vertical factors plays a role in the process by which the gut microbiomes of invertebrates are colonized.

Studies on gut microbiomes of terrestrial invertebrates have revealed significant differences among taxa, shaping the composition and abundance of the gut microbiome. One such study showed evidence of this when comparing the gut microbiomes of 21 invertebrate taxa (Yun *et al.*, 2014). Specifically, they revealed significant differences in the relative abundance of anaerobic bacteria between individuals from the orders Coleoptera, Hemiptera, Blattaria, and Mantodea (Yun *et al.*, 2014). Individuals from the orders Isoptera and Hymenoptera were both also found to contain distinct bacterial taxa not present in the gut microbiomes of other invertebrate orders (Colman *et al.*, 2012). Additionally, analyses of the gut microbiomes of six *Drosophila* species revealed that the bacterial genera *Myroides* and *Actinobacter* are limited exclusively to *Drosophilia ananassa* (Singhal *et al.*, 2017). Bacterial richness and evenness (measures of alpha diversity) within the gut microbiome also differed significantly among eight invertebrate orders and were more similar among closely related invertebrates (Jones *et al.*, 2013). Wild populations of several *Drosophila* species have also revealed different levels of bacterial diversity; *Drosophila melanogaster* possessed the most diverse gut bacterial communities, while *Drosophila bipectinate* possessed the least diverse communities (Singhal *et al.*, 2017). Differences in bacterial diversity have also been revealed across 12 mosquito species, with *Anopheles crucians* containing significantly higher and more evenly distributed bacterial diversity, and *Aedes albopictus* possessing significantly lower bacterial diversity (Muturi *et al.*, 2017). Beta diversity, or bacterial community

composition, has also been shown to differ significantly among terrestrial invertebrate taxa. For example, using PCoA plots, the gut microbiomes of the mosquito species *Aedes albopictus* and *Culex pipiens* clustered together and separately from 10 other mosquito species (Muturi *et al.*, 2017). Similarly, termites that are more closely related feature a more similar gut community structure than termites from different subfamilies (Mikaelyan *et al.*, 2015).

Little is known about whether habitat affects the gut microbiomes of invertebrates. Across 21 invertebrate orders from four habitat types (“sky”, “ground”, “underground”, and “aquatic”), there were differences in the relative abundance of anaerobic bacteria, but not of aerobic or facultative anaerobic bacteria or of the bacterial richness observed within the gut (Yun *et al.*, 2014). The authors stated that this variation is most likely associated with the oxygen availability within each habitat type, as the aquatic habitat which featured the greatest abundance of anaerobes has much less available oxygen than the sky habitat which featured invertebrates containing the lowest abundance of anaerobic gut bacteria (Yun *et al.*, 2014).

Studies investigating the effects of diet on the gut microbiomes of terrestrial invertebrates have generally revealed significant differences, though inconsistencies in the terminology used to classify dietary habits among research groups makes them difficult to compare. Specifically, the use of trophic guilds such as carnivorous, scavengers, detritivorous, nectarivorous, pollinivorous, dead-wood xylophagous, live-wood xylophagous, haematophagous, artificially fed, and naturally fed organisms are generally used in only a few studies. In contrast, diet types such as omnivores, herbivores, and predators are more commonly shared among studies, making it possible

to assess how this affects gut microbiomes (Colman *et al.*, 2012; Jones *et al.*, 2013; Yun *et al.*, 2014; Kim *et al.*, 2017; Ayayee *et al.*, 2018). One study found that individual termites from 18 different species in the same feeding group showed striking similarities in the dominant gut bacterial phyla (Mikaelyan *et al.*, 2015). Additionally, omnivorous invertebrates have significantly greater gut bacterial diversity than both carnivorous or herbivorous invertebrates (Yun *et al.*, 2014).

Diet can also explain some of the variation in beta diversity among the gut microbiomes of terrestrial invertebrates. Separate comparisons among eight terrestrial invertebrate orders and 18 termite species showed that beta diversity was significantly different among diet types, with PCoA plots revealing that invertebrates feeding on similar materials clustered strongly together, while those feeding on different materials did not (Jones *et al.*, 2013; Mikaelyan *et al.*, 2015). Finally, detritivores showed significant similarities, clustering together in a PCoA, while other diet types such as omnivores, herbivores, and predators did not show significant similarities in bacterial community composition (Colman *et al.*, 2012). Significant differences among diet types in terrestrial invertebrates have also been observed in a lab-based setting. The dominant gut bacterial genera differed between Coleoptera feeding on different diets; invertebrates feeding on natural wood-based diets had *Enterococcus*-rich microbiomes, while artificially-fed invertebrates were dominated by *Lactococcus* (Kim *et al.*, 2017).

1.1.3 Current gut microbiome research on aquatic invertebrates

Aquatic benthic macroinvertebrates, such as crustaceans or insects, are vital members of aquatic ecosystems. They are important for processes such as nutrient

cycling and the breakdown of plant materials, and they serve as sources of food for larger predatory organisms such as fishes or birds (Wallace and Webster, 1996). Aquatic invertebrates are also used as indicator species to monitor the quality of waterways, including the accumulation of heavy metals and insecticides in food webs (Wallace and Webster, 1996; Dahl *et al*., 2004). Additionally, the community structure can provide information about past and current water quality, as many invertebrate taxa have differing tolerances to contaminants (Luoma *et al.*, 2009).

To date, only one study has focused exclusively on examining the gut microbiomes of aquatic invertebrates in a freshwater environment (Ayayee *et al.*, 2018). This study examined 10 invertebrate families from two streams, to determine whether differences in functional feeding group (filter feeders, grazers/collectors, predators, and omnivores) corresponded to differences in the composition of the gut microbiome. As with many previous terrestrial studies, the authors found the bacterial phyla Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria to be abundant across the gut microbiomes of these samples (Ayayee *et al.*, 2018). The authors revealed that 79 of the 548 observed bacterial families differed significantly in abundance among samples from different functional feeding groups (Ayayee *et al.*, 2018). Gut bacterial richness and evenness also differed significantly among functional feeding groups, with grazers/collectors and filter feeders generally having the greatest richness, and predators and omnivores having the lowest (Ayayee *et al.*, 2018). Significant differences in beta diversity were revealed among the gut microbiomes of omnivores, predators, and filter feeders (Ayayee *et al.*, 2018). Overall, the author’s findings suggest that functional feeding group-specific internal gut conditions may impact the successful gut colonization

of select bacteria. These results also suggest that, among sampling sites, there may be stable and consistent functional feeding group-specific bacterial communities (Ayayee *et al.*, 2018).

Although hypotheses concerning the colonization of invertebrate gut microbiomes have primarily involved terrestrial invertebrates, hypotheses have been proposed specific to aquatic invertebrates. Specifically, current research suggests that functional feeding group and internal gut conditions may impact successful gut colonization by bacteria (Ayayee *et al.*, 2018). Filter feeders and grazers, for example, are adapted to consume fine particulate organic matter and biofilms, respectively (Cummins and Klug, 1979). While nearly all foods contain bacteria by nature, this food is known to contain diverse mixtures of microorganisms including bacteria, fungi, algae, and viruses, so it is thought that these dietary habits introduce specific bacteria into the gut that could then colonize the gut microbiome (Ayayee *et al.*, 2018). Additionally, differences in the internal gut pH due to diet or the shape of the gut tract, as a result of host invertebrate taxonomy, may also affect the bacteria that are able to exist within the gut microbiome (Ayayee *et al.*, 2018). It should be noted, however, that further research is needed to determine the processes of internal sorting involved during initial bacterial colonization and later establishment of gut microbiomes across developmental stages (Ayayee *et al.*, 2018).

1.1.4 Rationale for this study

Currently, there is very little research regarding the composition of the gut bacteria of aquatic benthic macroinvertebrates even though they are key members of aquatic food webs. Some of the previous research has been conducted using culture-

dependent methods (Anand *et al.*, 2010; Taglavia *et al.*, 2014) but these methods can be time-intensive and often produce biased results as a large proportion of bacteria cannot be cultured (Wade, 2002; Reeson *et al.*, 2003). In contrast, more novel culture-independent methods involving genetic sequencing of specific hypervariable regions of the *16S rRNA* gene have recently been developed and provide a comprehensive and accurate breakdown of the relative abundances of bacterial individuals present in the gut microbiome of invertebrates (Yun *et al.*, 2014).

Similarly, a method of taxonomic identification – DNA barcoding – is beneficial in aquatic invertebrate gut microbiome studies when identifying aquatic invertebrates and the bacterial individuals found within their gut microbiomes. DNA barcoding uses short pre-determined regions of a specific gene (the specific gene and region are dependent upon the type of organism being identified) to identity an organism down to the level of genus and species following genetic sequencing (Hebert *et al.*, 2003). Traditional taxonomic identification techniques, which have been used in some previous invertebrate gut bacterial works often rely on determining taxonomic differences using morphological identification keys, and require an advanced knowledge of the morphological characteristics of the specific organism being identified (Colman *et* al., 2012; Duguma *et al.*, 2015; Ayayee *et al.*, 2018). DNA barcoding mitigates several of the issues with traditional taxonomic identification, since it is much more reliable, less time-intensive, requires only a very small volume of genetic material, and is accessible to a wide variety of users since an advanced knowledge of the morphological characteristics distinguishing a particular taxon from others is not required (Hebert *et al.*, 2003). DNA metabarcoding – a closely related technique to DNA barcoding – allows for the taxonomic identification of

samples consisting of many species; it provides users with the same benefits of DNA barcoding, while allowing a large number of species to be identified in parallel (Pavan-Kumar *et al*., 2015). In the case of gut microbiome studies, this means that the previously mentioned culture-dependent methods, which are quite time-consuming, can instead be replaced by this more efficient culture-independent methodology.

Finally, most existing invertebrate gut microbiome research has been conducted in habitat types different from those found in Canada. These have included terrestrial studies, which have been conducted in regions such as Hawaii in the United States (Jones *et al.*, 2013), China (Chen *et al.*, 2016), India (Singhal *et al.*, 2017), Italy (Taglavia *et al.*, 2014), and Korea (Yun *et al.*, 2014); as well as an aquatic study conducted in Ohio in the United States (Ayayee *et al.*, 2018). The lack of information known about the gut microbiomes of aquatic invertebrate taxa found in the Saint John River – a large Canadian freshwater river that is tidally influenced – presents opportunities to address current taxonomic, geographic, and habitat-related gaps associated with freshwater aquatic ecosystems. The only previous aquatic invertebrate gut microbiome study was performed in two small Ohio tributaries – Tinder's Creek (known to be heavily polluted with more than 70% of the watershed's land use being involved in commercial and industrial processes) and the West Branch of the Mahoning River (surrounded by deciduous forest and very little anthropogenic disturbance) (Ayayee *et al.*, 2018). Given that the habitat of the Saint John River differs so greatly from the previously studied habitat types, it will be interesting to see whether there are differences in the gut microbiomes of aquatic invertebrates between these habitats as a result.

1.1.5 Background information on the Saint John River

The Saint John River, located mainly in the province of New Brunswick (NB), Canada, is 673 kilometers in length with a drainage area of 54,986 square kilometers (Kidd *et al.*, 2011). The Mactaquac Aquatic Ecosystem Study (MAES), a whole-river ecosystem study spanning multiple years, is a large-scale research project through the Canadian Rivers Institute at the University of New Brunswick in partnership with NB Power. This work is supporting an environmental impact assessment related to the removal or refurbishment of the prematurely ageing Mactaquac Dam – a large 372 MW run-of-the-river hydroelectric facility in the Saint John River (Chateauvert *et al.*, 2015). As part of the MAES work, a variety of aquatic components are evaluated both upstream and downstream of the dam to assess fish passage, environmental flows, and benthic food webs, while performing baseline community assessments and developing indices to support environmental assessments. The sampling for this thesis was in collaboration with the MAES research on macroinvertebrate communities at several sites on the river downstream of the Mactaquac Dam.

1.1.6 Objectives and predictions

The objectives of this thesis are to:

1) Characterize the gut microbiomes of 16 aquatic benthic macroinvertebrate orders in the Saint John River.
2) Assess the degree to which the composition of the gut microbiome is affected by a) taxonomy, b) water flow velocity, c) microhabitat, d) functional feeding group, and e) sampling year.

3) Compare and contrast these findings to previous information for terrestrial and aquatic invertebrate species.

Based on the literature, my predictions are that:

a) Taxonomically-related aquatic invertebrates will possess gut microbiomes that are more similar in relative abundance and diversity than those that are taxonomically unrelated;

b) Aquatic invertebrates sampled from sites with similar water flow velocities will possess gut microbiomes that are more similar than those from sites with greater differences in water flow velocities;

c) Aquatic invertebrates sampled within the same microhabitat type will possess gut microbiomes that are more similar than those from different microhabitat types;

d) Aquatic invertebrates belonging to the same functional feeding group will possess gut microbiomes that are more similar than those from different functional feeding groups;

e) Aquatic invertebrates collected during the same sampling year will possess gut microbiomes that are more similar than those collected during different sampling years.

This project is the first to test explicit hypotheses about whether taxonomic, habitat-based, dietary and temporal factors result in significant changes to the gut microbiomes of aquatic benthic invertebrates in the Saint John River. This research reveals new knowledge about aquatic benthic macroinvertebrates and their associations with bacteria, which will aid in establishing baseline information for this system and facilitate future

studies seeking to understand how, and if, the gut microbiomes of aquatic invertebrates may be used as an indicator of human disturbance.

1.2 Methods

1.2.1 Field collections

A subset of six sites along a 20 kilometer reach of the Saint John River immediately downstream of the Mactaquac Dam were selected from the core benthic macroinvertebrate sites monitored for the MAES project (Figure 1; Appendix I). Based on previous measurements, sites 1-3 had high water flow velocities (ten measurements recorded for each site during MAES sampling between September 22-28, 2016 with a mean across all three sites of 0.394 ± 0.341 m/s), while sites 4-6, which were located further downstream, had lower velocities (ten measurements recorded for each site during MAES sampling between September 20-21, 2016 with a mean across all three sites of 0.013 ± 0 m/s). Aquatic invertebrate sampling took place from October 18-20, 2016. A kick net (mesh size of 400 µm) was used to collect invertebrates belonging to several taxa from each of the six sampling sites following modified Canadian Aquatic Biomonitoring Network (CABIN) field sampling protocols (Environment Canada, 2012). It should be noted that microhabitat was not specifically quantified or taken into consideration during this sampling year; however, sites 1-3 (high-flow) generally contained more cobble and gravel in the substrate, while sites 4-6 (low-flow) generally consisted of a mixture of macrophytes and silt/sand. Upon collection, each invertebrate was rinsed using 95% ethanol (non-denatured ethyl alcohol) and was placed into separate 1.5 mL

microcentrifuge tubes filled with 95% ethanol. Each microcentrifuge tube was stored in a cooler of dry ice for 3-10 hours until storage in a -20℃ lab freezer was possible.

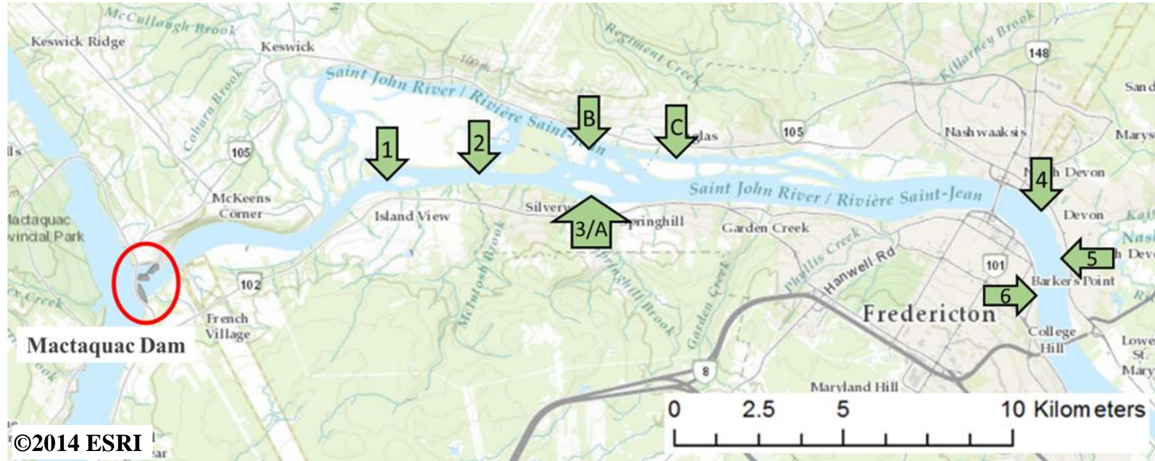

Figure 1 – Map showing the location of the 6 sampling sites (labelled 1-6) from 2016 and the 3 sampling sites (labelled A-C) from 2017. All sites are located downstream of the Mactaquac Dam in the Saint John River.

Three sites within the Saint John River were sampled in 2017, each containing distinct microhabitats (Figure 1). Within each of these sites, aquatic invertebrates were collected from three distinct microhabitat types: 1) primarily cobble/gravel, 2) macrophyte, and 3) silt/sand. Water chemistry variables, as well as aquatic invertebrates, were sampled from each of the microhabitats at the three sites on August 30-31, 2017. Snapshot measurements of water chemistry were taken using a calibrated YSI Multi-Meter; the specific variables measured included water temperature, dissolved oxygen, specific conductance, pH, and turbidity (Appendix II). Aquatic invertebrate sampling followed an identical protocol to that used in the sampling during the 2016 sampling year, as described previously.

1.2.2 Laboratory processing

Prior to DNA extraction, all pipettes and equipment were cleaned with RNase Away® Decontamination Reagent. Individual invertebrate samples were first removed from their 1.5 mL microcentrifuge tubes using sterile forceps. Each invertebrate was then surface rinsed with 95% ethanol, followed by a rinse with distilled water to remove excess ethanol that could prevent optimal DNA extraction results. These rinse steps were done to remove environmental bacterial cells present on the exterior surface of invertebrates, as only bacterial cells from within the gut microbiome are of interest in this study (Hammer *et al.*, 2015). It should be noted, however, that independent tests were not performed to verify the successful removal of environmental bacteria from the samples. Previous reports indicate, however, that following genetic sequencing, the high bacterial biomass within the gut microbiome often masks the detection of any outstanding environmental bacteria following surface rinsing (Hammer *et al.*, 2015). Once rinsed, whole invertebrate samples were individually homogenized using a Retsch™ MM 400 Mixer Mill and both the bacterial and host aquatic invertebrate DNA were extracted from each sample using the Omega BioTek E.Z.N.A.® Soil DNA Kit, following the protocol outlined by the manufacturer.

The extracted host aquatic benthic macroinvertebrate DNA was amplified using the mitochondrial cytochrome c oxidase subunit I (*COI*) genetic barcode. A polymerase chain reaction (PCR) was done using a primer pair designed specifically for use in identifying invertebrate organisms (Folmer *et al.*, 1994):

LCO1490 5’– GGTCAACAAATCATAAAGATATTGG –3’
HCO2198 5’– TAAACTTCAGGGTGACCAAAAAATCA –3’

These primers were designed to produce a product size of 710 base pairs (bp) following PCR. This particular reaction involved an initial hot-start step of 95℃ for 2 minutes, followed by 35 cycles of 95℃ for 1 minute, 40℃ for 1 minute, and 72℃ for 1.5 minutes, and was finalized with an extension step at 72℃ for 7 minutes (Folmer *et al*., 1994). Sanger sequencing down to genus- and species-level identity of the aquatic invertebrates was completed by the Genome Québec Innovation Centre at McGill University in Montreal, Québec.

To determine the composition of the bacterial DNA present in the gut microbiome of each aquatic benthic macroinvertebrate, the *16S rRNA* genetic barcode was used in conjunction with Illumina© MiSeq PE 300 next-generation sequencing. Specifically, the V3-V4 hypervariable region of the *16S rRNA* gene was targeted, as suggested by Illumina©; the V3-V4 region of the *16S rRNA* gene is roughly 464 bp in length. To amplify this target region, modified primers containing tags necessary for Illumina© MiSeq protocols were used in a PCR (Herlemann *et al*., 2011; Klindworth *et al*., 2013):

Bakt_341F 5'– ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG –3'
Bakt_805R 5'– TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC –3'

This reaction involved an initial hot-start step of 95℃ for 2 minutes, followed by 30 cycles of 95℃ for 30 seconds, 62.8℃ for 30 seconds, and 72℃ for 1 minute, and was finalized with an extension step at 72℃ for 8 minutes. The products of this reaction were sent to Genome Québec for Illumina© MiSeq PE 300 sequencing.

1.2.3 Analysis of aquatic invertebrate sequence reads

Sanger sequence trimming and analyses were carried out using the bioinformatics software Molecular Evolutionary Genetics Analysis (MEGA7; Kumar *et al*., 2015). Low

quality base calls, such as those featuring peaks with uneven spacing and height or that were ambiguous in nature, were manually trimmed from both ends of each sequence, leaving a high-quality sequence for identification. The Basic Local Alignment Search Tool (BLAST) algorithm was used to assign genus- and species-level identity to the aquatic invertebrates. Upon taxonomic identification of each sample, the invertebrates were assigned to functional feeding groups according to the classification used by Merritt *et al.* (2008).

1.2.4 Analysis of gut bacterial sequence reads

Illumina© sequence reads were processed for quality. Low-quality sequences shorter than 200 bp or possessing a Phred quality score lower than 30 over a 50 bp sliding window were removed using Trimmomatic (v0.38; Bolger *et al.*, 2014). Unless otherwise stated, all subsequent sequence processing and analyses were completed using the bioinformatics software QIIME (Quantitative Insights Into Microbial Ecology - v1.9.1) and statistical analyses were carried out using R (v3.5.1) and R Studio (v1.1.456) (Caporaso, Kuczynski *et al*., 2010; RStudio Team, 2016; R Core Team, 2018). The resulting high-quality sequences were aligned to the Greengenes core reference alignment using the default PyNAST aligner method in QIIME (DeSantis *et al*., 2006; Caporaso, Bittinger *et al*., 2010). Operational taxonomic units (OTUs – a proxy for bacterial species) were generated de novo using the default uclust method in QIIME at a 97% sequence similarity (Edgar, 2010). All OTUs present fewer than 25 times in at least one aquatic benthic macroinvertebrate sample were discarded in order to remove singleton individuals and chimeric sequences. Chimeric sequences were filtered out from the

remaining high-quality sequences using the default ChimeraSlayer method in QIIME (Haas *et al*., 2011). Taxonomic assignment of OTUs was done using the Greengenes core reference alignment in conjunction with the default uclust method in QIIME (DeSantis *et al*., 2006; Edgar, 2010; McDonald *et al.*, 2012; Werner *et al*., 2012). Sequences corresponding to gene fragments from Archaea, chloroplasts, mitochondria, or eukaryotic organisms – possibly resulting from undigested food particles within aquatic invertebrates or from the tissues of the host aquatic invertebrates themselves – were removed following taxonomic identification.

1.2.5 Data analyses

Following sequence processing, the remaining high-quality sequence reads from all invertebrate samples from both the 2016 and 2017 sampling years underwent rarefaction to a sequencing depth of 10,000 sequence reads. Rarefaction curves confirmed adequate sampling depth across samples, indicating that much of the bacterial diversity was able to be captured from each host invertebrate sample at a depth of 10,000 sequence reads. Additionally, the Good’s coverage index was used to confirm that the level of sequence coverage resulting from a sampling depth of 10,000 sequence reads was adequate, covering approximately 95% of sequences per sample (Appendix III).

When performing the remaining statistical analyses on the gut microbiomes of these aquatic invertebrates, samples were organized into several test groups specific to a particular hypothesis. These test groups were formed to reduce the number of confounding factors following statistical analyses. The factors being assessed in relation to bacterial diversity included: host invertebrate genus-, family-, and order-level

taxonomy, habitat (water flow velocity and microhabitat type), functional feeding group, and sampling year. Table 1 lists and describes the test groups that were used in this project to independently evaluate the impacts of various factors on the gut microbiomes of aquatic invertebrates.

The relative abundances of bacterial sequences observed within the gut microbiomes of all aquatic invertebrates were compared using the group_significance command in QIIME (Caporaso, Kuczynski *et al*., 2010). The Kruskal–Wallis one-way analysis of variance test was run to determine whether the relative abundances of each bacterial OTU differed significantly among the various categories of invertebrates being compared for each factor. The Benjamini-Hochberg procedure was used to correct for multiple comparisons using a false discovery rate of 5%.

Alpha diversity was assessed across all test groups to estimate how the diversity in the gut microbiome of individual aquatic invertebrates was impacted by the various factors. Specifically, the observed OTU metric was used to estimate the bacterial richness of OTUs within individual samples, while Shannon's diversity index was used to assess both the abundance and evenness of the bacterial OTUs within individual samples. Mean alpha diversity values were calculated for samples corresponding to a) host invertebrate taxonomy (order-, family-, and genus-level), b) water flow velocity (low-flow vs high-flow), c) microhabitat type (cobble/gravel vs macrophyte vs silt/sand), d) functional feeding group (collectors vs piercers vs predators vs scrapers vs shredders), and e) sampling year (2016 vs 2017). The Kruskal–Wallis one-way analysis of variance test was run within the R environment to analyze differences among the groups of samples mentioned above across each of the alpha diversity metrics that were run (R Core Team,

2018). The Dunn's test of multiple comparisons using rank sums was run post-hoc using the *dunn.test* package within the R environment to determine which specific pairs of sample groups differed significantly from one another (Dinno, 2017; R Core Team, 2018). The Benjamini-Hochberg procedure was used to correct for multiple comparisons across all alpha diversity analyses, using a false discovery rate of 5%.

Beta diversity was assessed among test groups to determine how the dissimilarity in the gut bacterial community composition differed among individual aquatic invertebrates with various factors (several levels of host taxonomy, water flow velocity, microhabitat type, functional feeding group, and sampling year – as described in the previous paragraph). Three metrics were used to assess beta diversity among invertebrate samples: Bray–Curtis dissimilarity, unweighted UniFrac, and weighted UniFrac. Bray–Curtis dissimilarity calculates beta diversity using abundance data derived from gut bacterial OTUs, while both UniFrac metrics incorporate phylogenetic relationships among gut bacterial sequences. The weighted UniFrac metric specifically accounts for both the phylogenetic relationships among gut bacteria as well as the abundances of those bacterial OTUs, while the unweighted UniFrac metric uses phylogenetic information and presence/absence data from the bacterial OTUs to calculate beta diversity. Significant differences in beta diversity, as well as the effect size (R^2), for all test groups were determined using the Adonis statistical test – a more robust version of the permutational multivariate analysis of variance (PERMANOVA) test, as it allows for input of both numeric and categorical variables – in QIIME with 1000 permutations (Caporaso, Kuczynski *et al.*, 2010). The Benjamini-Hochberg procedure was then used to correct for multiple comparisons across all beta diversity analyses, using a false discovery rate of

5%. Principal Coordinates Analysis (PCoA) plots were generated using the *ape* package within the R environment to visualize the clustering patterns present within the distance matrices generated from the gut bacterial communities among aquatic benthic macroinvertebrates belonging to the various groups of factors (Paradis *et al.*, 2004; R Core Team, 2018).

Table 1 – List of the individuals and sample sizes included in each of the test groups used to separately evaluate the impacts of host invertebrate taxonomy (genus-, family, and order-level), habitat (water flow velocity and microhabitat type), functional feeding group, and sampling year on the gut microbiomes of aquatic invertebrates.

Factor	Test group	Sample size (n)	Individuals	Similarities among individuals
Taxonomy (genus)	1	12	4 genera – 3 *Amnicola* vs 3 *Polypedilum* vs 3 *Sialis* vs 3 *Trichocorixa*	Collected from site 5 in 2016, same water flow velocity
	2	15	5 genera – 3 *Agnetina* vs 3 *Ceratopsyche* vs 3 *Chimarra* vs 3 *Ephemerella* vs 3 *Macronema*	Collected from site 2 in 2016, same water flow velocity
	3	55	9 genera – 10 *Gammarus* vs 3 *Goera* vs 4 *Gyrinus* vs 4 *Lepidostoma* vs 4 *Lumbriculus* vs 8 *Lymnaea* vs 3 *Mystacides* vs 7 *Nectopsyche* vs 12 *Physella*	Collected from site A in 2017, same microhabitat type
	4	43	8 genera – 3 *Erpobdella* vs 11 *Gammarus* vs 5 *Lepidostoma* vs 6 *Lymnaea* vs 4 *Nebrioporus* vs 3 *Nectopsyche* vs 8 *Physella* vs 3 *Stenelmis*	Collected from site C in 2017, same microhabitat type
	5	43	7 genera – 3 *Ceratopsyche* vs 5 *Erpobdella* vs 9 *Gammarus* vs 7 *Lepidostoma* vs 4 *Lymnaea* vs 5 *Nectopsyche* vs 10 *Physella*	Collected from site B in 2017, same microhabitat type
Taxonomy (family)	1	12	4 families – 3 Amnicolidae vs 3 Chironomidae vs 3 Corixidae vs 3 Sialidae	Collected from site 5 in 2016, same water flow velocity
	2	16	4 families – 3 Ephemerellidae vs 6 Hydropsychidae vs 4 Perlidae vs 3 Philopotamidae	Collected from site 2 in 2016, same water flow velocity
	3	59	9 families – 3 Chironomidae vs 10 Gammaridae vs 3 Goeridae vs 4 Gyrinidae vs 4 Lepidostomatidae vs 11 Leptoceridae vs 4 Lumbriculidae vs 8 Lymnaeidae vs 12 Physidae	Collected from site A in 2017, same microhabitat type
	4	48	9 families – 5 Chironomidae vs 4 Dytiscidae vs 3 Elmidae vs 3 Erpobdellidae vs 11 Gammaridae vs 5 Lepidostomatidae vs 3 Leptoceridae vs 6 Lymnaeidae vs 8 Physidae	Collected from site C in 2017, same microhabitat type
	5	48	8 families – 5 Chironomidae vs 5 Erpobdellidae vs 9 Gammaridae vs 3 Hydropsychidae vs 7 Lepidostomatidae vs 5 Leptoceridae vs 4 Lymnaeidae vs 10 Physidae	Collected from site B in 2017, same microhabitat type

Factor	Test group	Sample size (n)	Individuals	Similarities among individuals
Taxonomy (order)	1	12	4 orders – 3 Caenogastropoda vs 3 Diptera vs 3 Hemiptera vs 3 Megaloptera	Collected from site 5 in 2016, same water flow velocity
	2	17	3 orders – 4 Ephemeroptera vs 4 Plecoptera vs 9 Trichoptera	Collected from site 2 in 2016, same water flow velocity
	3	66	7 orders – 10 Amphipoda vs 4 Coleoptera vs 3 Diptera vs 3 Ephemeroptera vs 20 Heterobranchia vs 4 Lumbriculida vs 22 Trichoptera	Collected from site A in 2017, same microhabitat type
	4	58	7 orders – 11 Amphipoda vs 3 Arhynchobdellida vs 8 Coleoptera vs 5 Diptera vs 3 Ephemeroptera vs 14 Heterobranchia vs 14 Trichoptera	Collected from site C in 2017, same microhabitat type
	5	49	5 orders – 9 Amphipoda vs 5 Arhynchobdellida vs 5 Diptera vs 15 Heterobranchia vs 15 Trichoptera	Collected from site B in 2017, same microhabitat type
Water flow velocity	1	8	5 low-flow vs 3 high-flow	Genus *Sialis*, same functional feeding group, collected in 2016
	2	15	11 low-flow vs 4 high-flow	Family Chironomidae, same functional feeding group, collected in 2016
Microhabitat type	1	30	9 cobble/gravel vs 10 macrophyte vs 11 silt/sand	Genus *Gammarus*, same functional feeding group, collected in 2016
	2	30	7 cobble/gravel vs 12 macrophyte vs 11 silt/sand	Genus *Physella*, same functional feeding group, collected in 2016
	3	17	10 macrophyte vs 7 silt/sand	Genus *Lymnaea*, same functional feeding group, collected in 2016
	4	13	3 cobble/gravel vs 10 silt/sand	Genus *Nectopsyche*, same functional feeding group, collected in 2016

Factor	Test group	Sample size (n)	Individuals	Similarities among individuals
Functional feeding group	1	22	5 collectors vs 5 predators vs 4 scrapers vs 8 shredders	Order Trichoptera, collected from site A in 2017, same microhabitat type
	2	15	3 collectors vs 12 shredders	Order Trichoptera, collected from site B in 2017, same microhabitat type
Sampling year	1	15	3 2016 vs 12 2017	Genus *Physella*, same functional feeding group, collected from site 3/A in 2016 and 2017
	2	8	5 2016 vs 3 2017	Order Ephemeroptera, same functional feeding group, collected from site 3/A in 2016 and 2017

1.3 Results

1.3.1 Sequencing results

The gut microbiomes of 264 aquatic benthic macroinvertebrates were sequenced following the sampling efforts of October 18-20, 2016 and August 30-31, 2017. These invertebrates represent 16 orders, 31 families, and 44 genera (Figure 2). A total of 23,445,019 *16S rRNA* V3-V4 high quality bacterial sequences were obtained from the gut microbiomes of the 264 samples, with individual samples containing an average of 88,807 (±45,406) sequence reads and ranging from 11,706 – 244,742 sequence reads per sample. The 23,445,019 high quality sequence reads cluster into a total of 19,986 unique bacterial operational taxonomic units (OTUs), as defined at a 97% sequence similarity level.

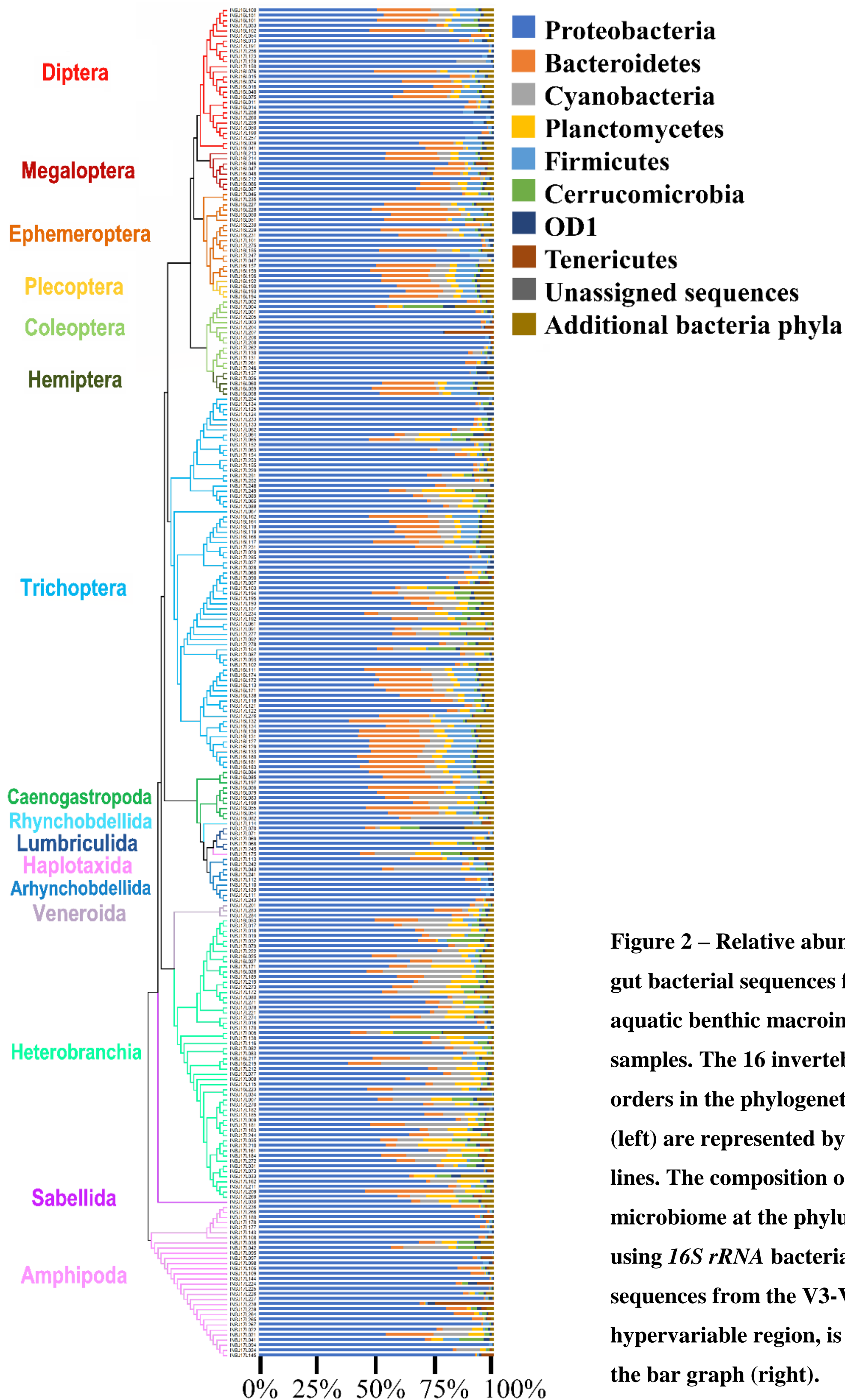

Figure 2 – Relative abundance of gut bacterial sequences from 264 aquatic benthic macroinvertebrate samples. The 16 invertebrate orders in the phylogenetic tree (left) are represented by coloured lines. The composition of the gut microbiome at the phylum level, using *16S rRNA* bacterial sequences from the V3-V4 hypervariable region, is shown in the bar graph (right).

1.3.2 Gut bacterial relative abundance

A total of 48 bacterial phyla, 118 classes, 203 orders and 240 families were found within the gut microbiomes of the 264 aquatic benthic macroinvertebrates. On average, these samples were largely dominated by a small number of bacterial phyla; 92.92% of the bacterial sequences was accounted for by only five phyla (relative abundance values averaged across bacterial sequences sampled from all relevant samples from Figure 2). Proteobacteria accounted for 73.15% of the detected sequences, while Bacteroidetes, Cyanobacteria, Planctomycetes, and Firmicutes accounted for 8.14%, 5.18%, 3.38%, and 3.07%, respectively. Additional phyla in low relative abundances accounted for a combined 7.08% of the gut microbiome, while unassigned bacteria represent a negligible portion (less than 0.01%) of the detected bacterial sequences. Three classes of bacteria from the phylum Proteobacteria were dominant within the gut microbiome of the invertebrates, representing 68.14% of the total gut bacterial abundance. These classes include Gammaproteobacteria (56.11%), Betaproteobacteria (6.31%), and Alphaproteobacteria (5.11%). Unassigned bacterial sequences account for 5.71% of the detected sequences at the class level, while additional classes present in low abundances represent 26.75% of the sequences within the gut microbiome. At the bacterial order level, Enterobacteriales alone represents 55.92% of the detected sequences within the gut microbiomes of these samples, with orders such as Bacteroidales, Burkenholderiales, Stramenopiles and Clostridiales accounting for 4.77%, 4.06%, 2.13%, and 1.93%, respectively. Unassigned bacterial sequences have a relative abundance of 8.02%, with additional orders at smaller abundances accounting for the remaining 23.17% of the sequences. The bacterial family Enterobacteriaceae represents 55.13% of the observed

sequences within the gut microbiomes of these invertebrates, with families such as Comamonadaceae, Pirellulaceae, Porphyromonadaceae and Rikenellaceae representing 2.99%, 1.24%, 1.13%, and 1.13% of the bacterial sequences, respectively; unassigned sequences represent 17.92% and additional bacterial families account for 20.46%.

The relative abundances of several bacterial OTUs differed significantly among invertebrate taxa in three of the five test groups evaluating host invertebrate genus, family, and order (Table 2). Similarly, one of the two test groups showed differences in the relative abundance of gut bacterial OTUs over time (Table 2). For functional feeding group comparisons, differences were only present in less than 1% of the bacterial OTUs shared among the gut microbiomes of these invertebrates (Table 2). The test groups evaluating impacts of water flow velocity and microhabitat type showed no differences in the relative abundances of bacterial OTUs between different water flow levels or among microhabitat types (Table 2).

Table 2 – Percentage of bacterial OTUs that had significantly different relative abundances among the categories of each factor. Corrections for multiple comparisons were made using the Benjamini-Hochberg procedure and a false discovery rate of 5%.

Factor	Test group	% of significantly different bacterial OTUs (number of significantly different OTUs/total number of OTUs)
Taxonomy (genus)	1	0%
	2	0%
	3	0.02% (1/5258)
	4	15.20% (499/3283)
	5	38.21% (1629/4263)
Taxonomy (family)	1	0%
	2	0%
	3	0.04% (2/5259)
	4	18.37% (610/3321)
	5	39.17% (1720/4391)

Factor	Test group	% of significantly different bacterial OTUs (number of significantly different OTUs/total number of OTUs)
Taxonomy (order)	1	0%
	2	0%
	3	0.39% (23/5890)
	4	7.08% (275/3886)
	5	6.62% (295/4459)
Water flow velocity	1	0%
	2	0%
Microhabitat type	1	0%
	2	0%
	3	0%
	4	0%
Functional feeding group	1	0%
	2	0.95% (24/2537)
Sampling year	1	11.77% (321/2728)
	2	0%

1.3.3 Gut bacterial alpha diversity

Several factors affected the alpha diversity of the gut microbiomes of individual aquatic benthic macroinvertebrates. The genus of a host affected both the number of observed OTUs and the Shannon diversity index (2 of 5 groups; Figure 3; Table 3). Family and order-level taxonomy also showed similar results in which two of the five groups had significant differences in alpha diversity (Table 3). One test group on the impacts of water flow velocity showed no significant differences in alpha diversity, while the other showed a significant difference in only the Shannon diversity index (Table 3). Specifically, low-flow sites had a significantly lower Shannon diversity index value than high-flow sites (Figure 4). All four of the test groups on microhabitat type showed no significant differences in alpha diversity among invertebrates collected from any of the

three microhabitats (Table 3). Neither of the test groups evaluating the impact of functional feeding group on the gut microbiome revealed significant differences in alpha diversity among invertebrates belonging to different functional feeding groups (Table 3). Finally, sampling year did not affect alpha diversity of these samples (Table 3).

Table 3 – *P* values from Kruskal-Wallis statistical tests measuring alpha diversity in the gut microbiomes of aquatic invertebrates. Corrections for multiple comparisons were made using the Benjamini-Hochberg procedure and a false discovery rate of 5%. *P* values depicting significant differences are bolded.

Factor	Test group	Kruskal-Wallis *P* values	
		Observed OTUs	Shannon diversity index
Taxonomy (genus)	1	.050	.030
	2	.024	.075
	3	.667	.574
	4	**<.001**	**<.001**
	5	**<.001**	**<.001**
Taxonomy (family)	1	.041	.030
	2	.059	.328
	3	.114	.408
	4	**<.001**	**<.001**
	5	**<.001**	**<.001**
Taxonomy (order)	1	.049	.030
	2	.167	.703
	3	.131	.267
	4	**<.001**	**<.001**
	5	**<.001**	**<.001**
Water flow velocity	1	.654	.297
	2	.019	**.009**
Microhabitat type	1	.463	.026
	2	.552	.835
	3	.079	.696
	4	.063	.043

Factor	Test group	Kruskal-Wallis *P* values	
		Observed OTUs	Shannon diversity index
Functional feeding group	1	.594	.098
	2	.665	.885
Sampling year	1	.083	.194
	2	.025	.025

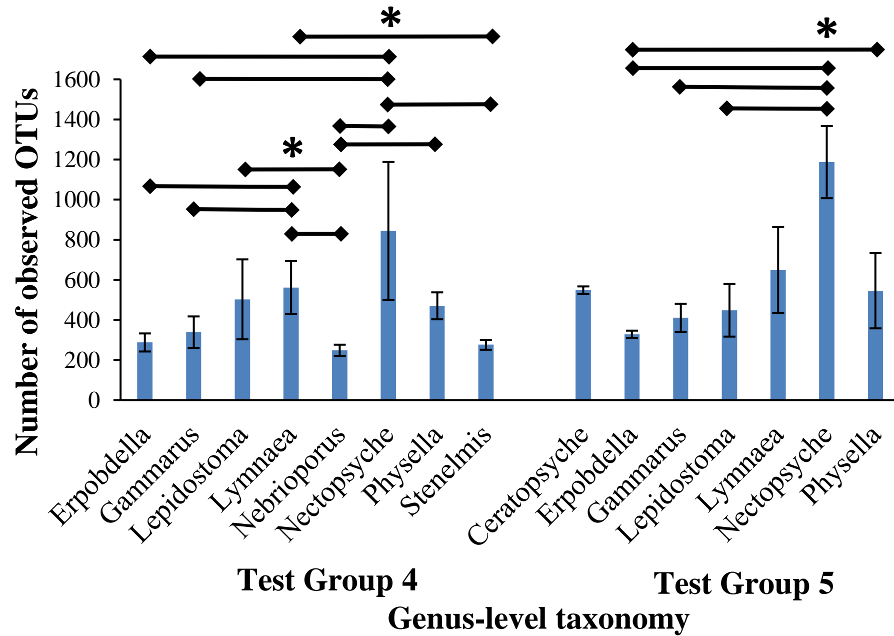

Figure 3 – Alpha diversity present within the gut microbiomes of various aquatic invertebrate genera from two test groups. The amount of bacterial diversity was determined by comparing the average number of OTUs within these individuals. Asterisks indicate statistically significant differences among indicated pairs of values, following corrections for multiple comparisons using the Benjamini-Hochberg procedure and a false discovery rate of 5%.

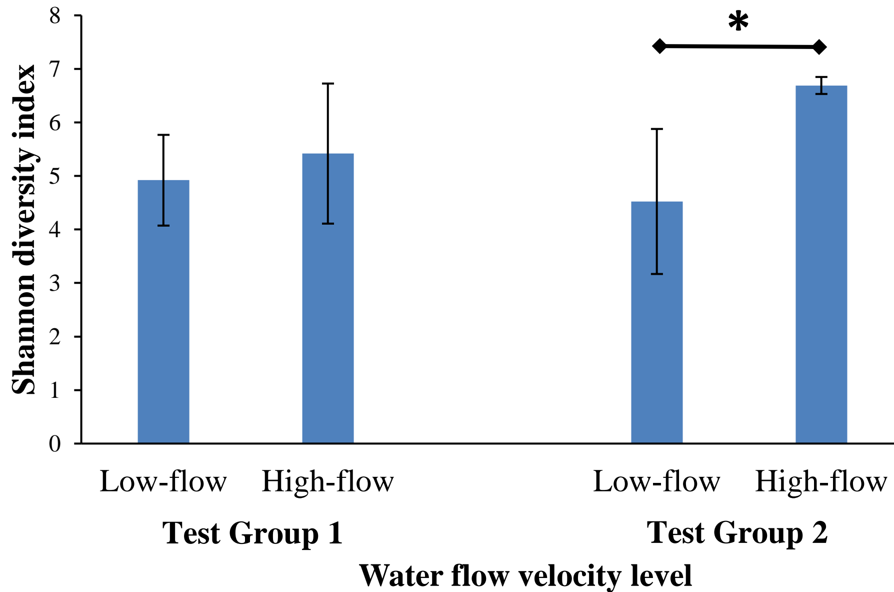

Figure 4 – Alpha diversity present within the gut microbiomes of aquatic invertebrates sampled from sites with either low flows or high flows. The amount of bacterial diversity was determined by comparing the average Shannon diversity index value within these individuals. Asterisks indicate statistically significant differences among indicated pairs of values, following corrections for multiple comparisons using the Benjamini-Hochberg procedure and a false discovery rate of 5%.

1.3.4 Gut bacterial beta diversity

Several significant differences in the beta diversity of the gut microbiomes of aquatic benthic macroinvertebrates were observed including differences among invertebrates belonging to different genera (4 of 5 groups; Table 4). The effect sizes (R^2) also showed that between 33.79% and 75.06% of the overall variation in distances can be explained by genus-level taxonomy (Table 4). In addition, PCoA plots display clear clustering among invertebrates of distinct genera (Figure 5A). Similarly, there were significant dissimilarities in gut bacterial community structure among individuals from different families (4 of 5 groups), with a fifth significant test group for the Unweighted UniFrac metric only (Table 4). The effect sizes show that between 16.54% and 75.16% of

the overall variation in distances could be explained by family-level taxonomy. PCoA plots constructed using the unweighted UniFrac distance matrices of these samples also show patterns of clustering in which invertebrate families cluster together, but distinctly from non-related families (Figure 5B). As with genus and family-level taxonomy, significant differences were revealed across all beta diversity metrics among aquatic invertebrates belonging to different orders (4 of 5 groups; Table 4). These taxonomic differences accounted for between 15.24% and 76.53% of the overall variation in distances, while PCoA plots of the unweighted UniFrac distance matrices display clustering in which invertebrate orders group together, but separately from non-related orders (Figure 5C).

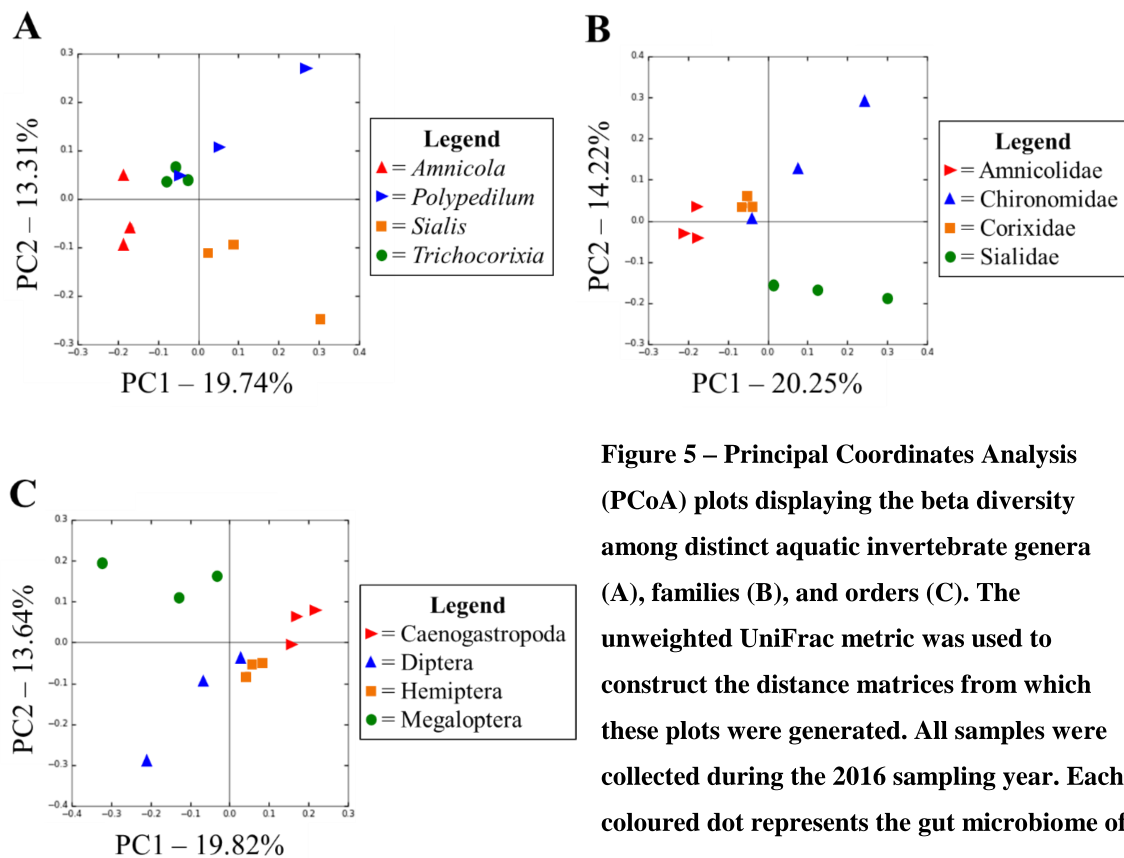

Figure 5 – Principal Coordinates Analysis (PCoA) plots displaying the beta diversity among distinct aquatic invertebrate genera (A), families (B), and orders (C). The unweighted UniFrac metric was used to construct the distance matrices from which these plots were generated. All samples were collected during the 2016 sampling year. Each coloured dot represents the gut microbiome of an individual aquatic invertebrate sample.

Beta diversity also differed within taxa among habitats. Specifically, there were significant differences in beta diversity across all three metrics among individuals sampled from low flows and high flows and between 11.74% and 36.80% of the overall variation in distances can be attributed to water flow (one group; Table 4). The PCoA plot generated from unweighted UniFrac distance matrix, however, shows overlap among samples from the two flow-types, suggesting that water flow velocity has a weak effect on beta diversity (Figure 6A). Additionally, only one group showed a significant dissimilarity in gut bacterial community structure among samples collected from different microhabitat types, with between 10.12% and 15.22% of the overall variation in distances attributed to microhabitat type (Table 4). The PCoA plots generated from these test groups displayed a great deal of overlap among samples collected from different microhabitats (Figure 6B). Few significant differences were revealed in the two test groups evaluating the impacts of functional feeding group on the gut microbiome; only an Adonis test of a distance matrix generated using the Weighted UniFrac metric for one of the test groups revealed a significant difference (Table 4). The effect size of the test group showing significant differences in beta diversity showed that 28.09% of the overall variation in distances can be attributed to functional feeding group (Table 4). Further, PCoA plots of the data from these test groups show no clear clustering of the samples according to functional feeding group (Figure 7A). All measures of beta diversity were shown to be significantly different between sampling years in both test groups with between 15.70% and 36.97% of the overall variation in distances attributed to sampling year (Table 4). PCoA plots of these two test groups reveal clear patterns of clustering among aquatic invertebrate samples collected during each sampling year (Figure 7B).

Table 4 – *P* values from Adonis statistical tests measuring beta diversity using the unweighted and weighted UniFrac metrics and the Bray-Curtis dissimilarity metric. Corrections for multiple comparisons were made using the Benjamini-Hochberg procedure and a false discovery rate of 5%. *P* values depicting significant differences in beta diversity are bolded. R^2 (effect size) values display how much of the overall variation in distances can be explained by the factor being tested.

Factor	Test group	Unweighted UniFrac		Weighted UniFrac		Bray-Curtis dissimilarity	
		P values	R^2	*P* values	R^2	*P* values	R^2
Taxonomy (genus)	1	**.002**	**0.359**	**<.001**	**0.751**	**.002**	**0.681**
	2	**<.001**	**0.351**	**.006**	**0.414**	**<.001**	**0.384**
	3	.272	0.156	.279	0.160	.328	0.154
	4	**<.001**	**0.411**	**<.001**	**0.490**	**<.001**	**0.425**
	5	**<.001**	**0.338**	**<.001**	**0.498**	**<.001**	**0.464**
Taxonomy (family)	1	**<.001**	**0.374**	**.002**	**0.752**	**<.001**	**0.683**
	2	**<.001**	**0.255**	**.022**	**0.309**	**.023**	**0.262**
	3	**.026**	**0.165**	.083	0.171	.304	0.146
	4	**<.001**	**0.444**	**<.001**	**0.546**	**<.001**	**0.445**
	5	**<.001**	**0.348**	**<.001**	**0.513**	**<.001**	**0.471**
Taxonomy (order)	1	**<.001**	**0.375**	**<.001**	**0.765**	**<.001**	**0.686**
	2	**.009**	**0.152**	**.015**	**0.207**	**.027**	**0.170**
	3	.038	0.114	.261	0.104	.381	0.094
	4	**<.001**	**0.334**	**<.001**	**0.371**	**<.001**	**0.274**
	5	**<.001**	**0.239**	**<.001**	**0.288**	**.002**	**0.225**
Water flow velocity	1	.215	0.153	.313	0.137	.196	0.192
	2	**.025**	**0.117**	**.002**	**0.368**	**.007**	**0.317**
Microhabitat type	1	**.027**	**0.101**	**.025**	**0.128**	**.002**	**0.152**
	2	.213	0.076	.616	0.061	.419	0.069
	3	.235	0.070	.224	0.079	.325	0.063
	4	.088	0.125	.064	0.168	.077	0.154
Functional feeding group	1	.228	0.160	**.015**	**0.281**	.051	0.203
	2	.040	0.129	.243	0.093	.247	0.093
Sampling year	1	**.006**	**0.162**	**.003**	**0.370**	**<.001**	**0.288**
	2	**<.001**	**0.157**	**.006**	**0.328**	**.017**	**0.250**

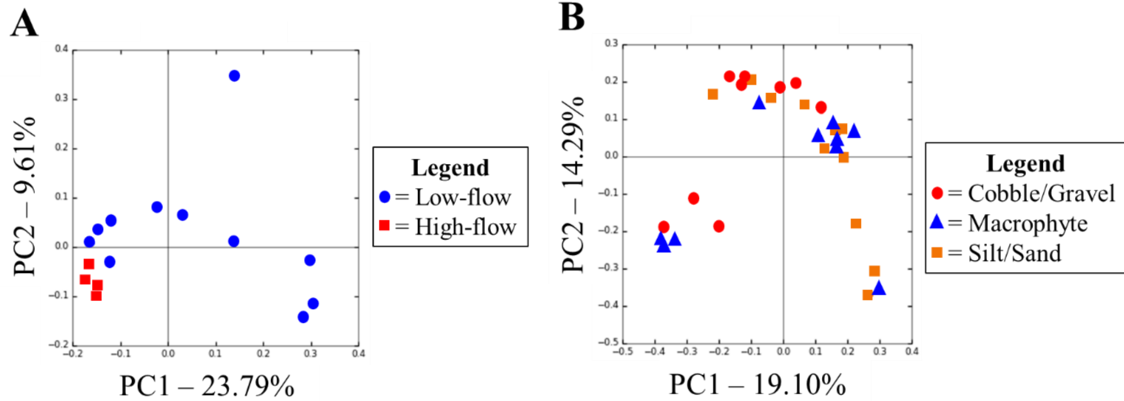

Figure 6 – Principal Coordinates Analysis (PCoA) plots displaying the beta diversity among invertebrates collected from distinct water flow velocity levels (A) and microhabitat types (B). The unweighted UniFrac metric was used to construct the distance matrices from which these plots were generated. Samples shown in A were collected during the 2016 sampling year, while those from B were collected in 2017. Each coloured dot represents the gut microbiome of an individual aquatic invertebrate sample.

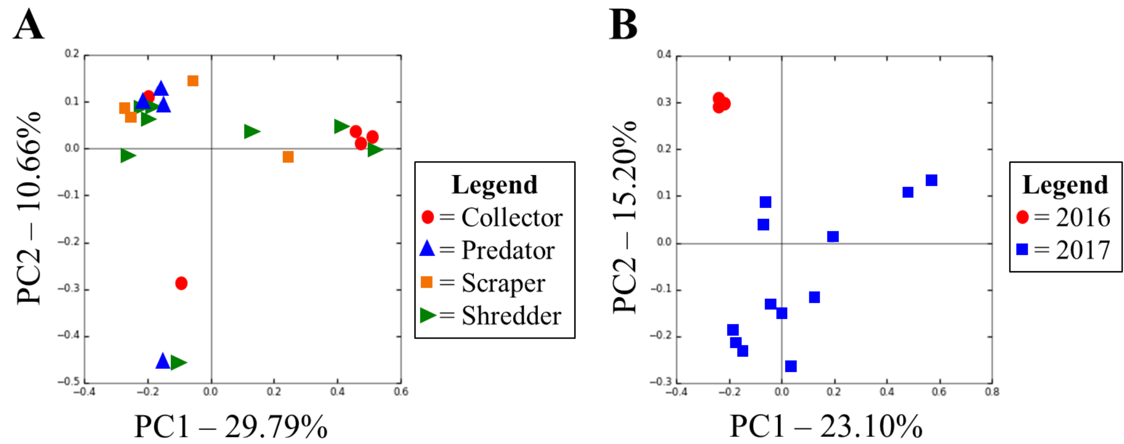

Figure 7 – Principal Coordinates Analysis (PCoA) plots displaying the beta diversity among invertebrates belonging to distinct functional feeding groups (A) or sampled during distinct sampling years (B). The unweighted UniFrac metric was used to construct the distance matrices from which these plots were generated. Samples shown in A were collected during the 2017 sampling year, while those from B were collected across both the 2016 and 2017 sampling years. Each coloured dot represents the gut microbiome of an individual aquatic invertebrate sample.

1.4 Discussion

1.4.1 Factors affecting the gut microbiomes of aquatic invertebrates

Gut microbiomes were characterized for 264 aquatic benthic macroinvertebrate individuals from the Saint John River. These individuals were also assessed to determine if host taxonomy, habitat, diet, and time affected gut microbiome composition. I found that the gut microbiomes of these aquatic invertebrates differed significantly according to host invertebrate taxonomy and sampling year. In contrast, measures of habitat, such as water flow velocity and microhabitat type, had weak, but significant, impacts on gut bacterial composition, while functional feeding group had no significant effect on the gut microbiome.

My finding that functional feeding group has essentially no significant impact on the gut microbiome of these samples is contrary to previous studies. Ayayee *et al.* (2018) found that measures of alpha and beta diversity differed significantly among functional feeding groups of aquatic invertebrates. More specifically, estimates of bacterial richness and evenness were greatest in grazer/collectors, and lowest in predators and omnivores, functional feeding groups clustered separately from one another in an NMDS plot, and omnivorous invertebrates – but less so for predacious invertebrates – had similar community compositions among streams (Ayayee *et al.*, 2018). In terrestrial invertebrates, Yun *et al.* (2014) found that omnivorous invertebrates had significantly higher alpha diversity than either carnivorous or herbivorous individuals. However, there are potential problems with the methodology used in these studies. Specifically, analyses by Ayayee *et al.* (2018) did not appear to have controlled for host invertebrate taxonomy,

increasing the possibility that taxonomy may confound the overall conclusions regarding the effects of functional feeding group. In my study, only invertebrates from the order Trichoptera were included in each test group used to determine the effects of functional feeding group, thus reducing the effects of taxonomy as a confounding factor.

As reported widely for terrestrial invertebrates, I found significant differences in the relative abundance, alpha diversity, and beta diversity among host invertebrate taxa at the levels of genus, family, and order. Core microbiomes, which are collections of specific bacterial species commonly shared among all individuals of a host invertebrate taxon (Pérez-Cobas *et al.*, 2015), are thought to be responsible for these observed differences. The precise methods by which core microbiomes develop in invertebrates is not currently known, however, it has been suggested that co-evolution between bacterial individuals and their invertebrate host may be the primary mechanism, as has been observed in cockroaches (Pérez-Cobas *et al.*, 2015). Colman *et al.* (2012) have also shown evidence for the existence of core microbiomes and co-evolution between gut bacteria and terrestrial invertebrates, as individual Isoptera and Hymenoptera feature distinct bacterial taxa that are not found in other orders. Previous studies involving fishes also support the existence of core microbiomes; more specifically, several bacterial taxa were commonly found, and measures of both alpha and beta diversity were similar in zebrafish from the lab and those collected from natural habitats (Roeselers *et al.*, 2011). Although taxonomic effects have been widely reported in previous studies involving terrestrial invertebrates, the test groups from my project also suggest that aquatic invertebrates have a core microbiome.

This is the first study to test for temporal variability on the gut microbiomes of aquatic invertebrates and I found that the year of sampling had a significant effect on bacterial relative abundance and beta diversity. While there are no previous studies against which to compare these data, bacterial communities in streams are known to vary over time (Olapade and Leff, 2005; Portillo *et al.*, 2012). Specifically, biofilms – which are collections of bacterial organisms that often adhere to surfaces such as rocks, small woody debris, and leaves – have significant differences in the abundances of Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria between seasons (Olapade and Leff, 2005). The authors suggest that temporal differences in aqueous dissolved organic materials and inorganic nutrients were most likely responsible for the observed differences among biofilms (Olapade and Leff, 2005). Temporal differences in alpha and beta diversity have also been found in free-floating communities of bacterioplankton in freshwater streams that appeared to be driven by changes in streamwater biogeochemistry (Portillo *et al.*, 2012). Given these previously observed temporal changes across bacterioplankton and biofilms in aquatic habitats due to biogeochemistry, it is possible that the significant temporal differences in the gut microbiomes of the aquatic invertebrates from my study may also be as a result of shifts in biogeochemical conditions. Further research should be done to determine the factors driving the differences in the gut microbiomes over time. These studies should also report sample collection dates – these are often missing – to ensure that temporal differences can be assessed and considered when interpreting these results.

Measures of habitat – including both water flow velocity and microhabitat type – were associated with few significant compositional differences to the gut microbiomes of

the aquatic invertebrates from my project. While this has not been studied previously using aquatic invertebrates, some evidence finds that changes in habitat are associated with compositional differences to the gut microbiomes of terrestrial invertebrates. Specifically, a previous study found that only the relative abundance of anaerobic gut bacteria differs among invertebrates sampled from different habitat types ("sky", "ground", "underground", "aquatic"); anaerobic bacteria were most abundant in invertebrates from aquatic habitats, while invertebrates from the sky and ground featured the lowest abundance of anaerobes (Yun *et al.*, 2014). It is important to note, however, that the differences among these habitat types were much larger than the differences among the microhabitats in my project.

Any habitat differences in gut microbiomes may be related to among-location differences in non-host associated bacteria. For example, bacterial phyla were significantly different across four streams that differed in hydrology and physiochemical conditions (Portillo *et al.*, 2012). Bacterioplankton communities are known to be affected by pH (Methé and Zehr, 1999), dissolved organic carbon concentrations (Judd *et al.*, 2006), and temperature (Adams *et al.*, 2010). While both water flow velocity and microhabitat type had little influence on the gut microbiomes of the aquatic invertebrates from my study, it is possible that testing additional measures of habitat (such as pH, dissolved organic carbon, and temperature which significant alter the composition of bacterioplankton) in future studies may prove to have greater impacts on gut microbiomes.

1.4.2 Common gut bacterial taxa

Similarities were observed among bacterial taxa in this project, when compared to the gut microbiomes of previously studied aquatic and terrestrial invertebrate taxa. The bacterial phyla Proteobacteria, Bacteroidetes, Cyanobacteria, Planctomycetes, Firmicutes, Actinobacteria, and Fusobacteria were abundant in the invertebrates from the current study and each has been previously found in terrestrial and aquatic invertebrates, with Proteobacteria being the most abundant phylum (Colman *et al.*, 2012; Jones *et al.*, 2013; Yun *et al.*, 2014; Mikaelyan *et al.*, 2015; Pérez-Cobas *et al.*, 2015; Kim *et al.*, 2017; Muturi *et al.*, 2017; Ayayee *et al.*, 2018). Although considerable variability was observed among studies at lower bacterial taxonomic levels, the bacterial classes Gammaproteobacteria, Betaproteobacteria, and Alphaproteobacteria and the bacterial family Enterobacteriacaea were all highly abundant in my samples and are commonly seen in previous studies of terrestrial and aquatic invertebrates (Colman *et al.*, 2012; Jones *et al.*, 2013; Yun *et al.*, 2014; Ayayee *et al.*, 2018). It should be noted that observed differences in the bacterial abundance and composition of gut microbiomes at lower taxonomic levels of bacteria could have been related to differences in the methodologies used among studies when collecting, storing, or performing lab work on invertebrates, as has been suggested by Hammer *et al.* (2015). Future studies could investigate this phenomenon by determining if the gut microbiomes of taxonomically identical invertebrates differ based on differences in the following methodologies. For example, experimental treatments could include surface rinsing some invertebrates but not others upon collection, using various storage media for preservation of samples prior to DNA

extraction, using different DNA extraction kits, or amplifying and analyzing different hypervariable regions in the *16S rRNA* gene.

1.4.3 Functional roles of gut bacterial taxa

Currently, very little is known about the functional roles that specific gut bacteria play in invertebrates, but the functions of gut microbiomes as a whole have been reported. Specifically, nitrogen fixation (Ayayee *et al.*, 2014), nitrogen recycling (Ayayee *et al.*, 2014), provisioning of essential amino acids lacking in diets (Ayayee *et al.*, 2015; Ayayee *et al.*, 2016), and degradation of harmful allochemicals produced by plants (Hammer and Bowers, 2015) are done by the gut microbiomes of terrestrial invertebrates. Although no similar functions have been demonstrated for aquatic invertebrates, Ayayee *et al.* (2018) believe that the gut bacterial functions in terrestrial invertebrates may be relevant in aquatic invertebrates given their terrestrial origins (Douglas, 2009). The current knowledge gaps also exist in fishes and amphibians, which often share similar aquatic habitats with aquatic invertebrates and which have been a larger focal point of previous gut bacterial study than invertebrate gut microbiomes. While fish microbiomes have been studied much more extensively than invertebrates, Talwar *et al.* (2018) argue that there needs to be a shift in focus to functional microbiomics since very little is known about the functional roles of individual gut bacteria. Studies of aquatic amphibian gut microbiomes have investigated gut bacterial changes during metamorphosis (Chai *et al.*, 2018), the effects of pollutants (Kohl *et al.*, 2015), and the effects of environmental temperatures (Kohl and Yahn, 2016) on gut bacteria, whereas research investigating the functional roles of individual gut bacteria is lacking. Further, Wong and Rawls (2012)

show that current information concerning the functions of bacteria within gut microbiomes is derived primarily from studies on mammals, and an understanding of their functional roles in other host taxa are limited. The human medical literature presents some information about the functions of specific bacterial taxa found within gut microbiomes. For example, Enterobacteriaceae accounted for nearly 56% of the total bacterial sequences across all aquatic invertebrate samples in my study, and this family indirectly modulates the level of fungal colonization that results in intestinal inflammation in patients with inflammatory bowel disease (Sovran *et al.*, 2018). It is unclear whether similar functions would also be performed by these bacteria within the gut microbiomes of aquatic invertebrates. Such gaps emphasize that more work must be done in this novel field of gut bacterial ecology to gain a better understanding of the roles served by these gut bacteria.

1.4.4 Potential limitations

There are several potential limitations associated with my study that may have an influence on my overall findings and conclusions. Firstly, the three low-flow sites are in much closer proximity to the City of Fredericton compared with the high-flow sites and are exposed to outputs from the Nashwaak River. Fredericton is more densely populated than the more rural land further upstream where my three high-flow sites were located, so there is a possibility that comparisons across these sites were affected by differences in population densities and outputs from the Nashwaak tributary near those sites. Another limitation of my project involves the number of samples that I was able to collect as it was not always possible to find a sufficient number of invertebrates from each taxon.

Some invertebrate samples were also lost during lab work due to unsuccessful DNA extraction and PCR amplification, or following genetic sequencing if sequence quality was too poor. This also caused limited sample sizes in the test groups. When referring to the relative abundances or the presence/absence of bacteria in the gut microbiome, it should be noted that it is most accurately referring to analyses performed on a subset of the gut microbiome from each invertebrate. The reason for this subsampling of DNA was mainly so that if PCR amplification or the genetic analyses of a sample did not work properly, then adequate DNA would remain for additional troubleshooting of issues. Another possible limitation is that when performing PCR amplification, PCR bias may have occurred in which highly abundant bacterial sequences would be inflated in relation to those bacterial sequences that are very rare, which may be essentially "drowned out" as a result. Finally, when aquatic invertebrates ingest food material it is likely that bacteria are ingested as well; it is not currently known, however, for what duration these bacteria reside in the gut microbiome or whether these bacteria serve a functional role in the gut while they are present. As a result, it is possible that some bacteria described as belonging to the gut microbiome may in actuality be transient species that are associated with ingested food materials. It is therefore important that caution be taken in interpreting the results, particularly when attempting to gain further insights into the functional significance of bacteria sampled from within aquatic invertebrate gut microbiomes.

1.4.5 Conclusions and future research

This study presents basic research concerning the gut bacterial composition across a diverse range of aquatic invertebrate taxa from the Saint John River in New Brunswick,

Canada. Most notably, I found almost no significant differences in either the relative abundance or the diversity of the gut microbiome among invertebrates from different functional feeding groups. Although this is contrary to previous findings involving aquatic invertebrates, concerns with certain methodologies raise some doubt regarding those previously-reached conclusions. Additionally, my results support the growing body of literature showing significant differences in the gut microbiomes of invertebrates among host taxa at the genus, family, and order levels, and the growing belief that core microbiomes and co-evolution among distinct host invertebrate taxa drive those differences, though further study is needed to determine the origins of core microbiomes and to identify the specific bacterial organisms that are essential to them. I also observed temporal differences in the gut microbiomes of these aquatic invertebrates, though the source of these differences is not clear. Finally, measures of habitat – including water flow velocity and microhabitat type – reveal weak but significant differences to the gut microbiomes of these aquatic invertebrates, though these factors appear to be of lesser importance in comparison to those – such as water quality – known to affect natural bacterial communities.

My project establishes a baseline of natural variability and diversity of the gut microbiomes of aquatic benthic macroinvertebrates. Since this is the first study to test some of these factors, similar testing in different rivers would be useful to establish the scope of these patterns. Now that baseline information is known, additional hypotheses can be generated with the goal of addressing aspects of conservation and management, as well as water quality. Specifically, one could assess the effects of water temperature, which will likely become more relevant given concerns of increasing climate change, or

determine how wastewater effluents, improperly treated sewage, or nutrient run-off from croplands affect invertebrate gut microbiomes. Future studies should also consider collecting substrate or water samples from all sites in which invertebrates are collected. This approach would allow environmental bacterial profiles to be established, which could provide insights on the colonization and function of the gut microbiome of invertebrates. This would aid in determining similarities among the gut microbiomes of aquatic invertebrates, both compositionally and functionally, and environmental bacteria – a rich area for future research.

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Appendix I

Sampling site locations within the Saint John River in which aquatic benthic macroinvertebrates and water chemistry variables were collected in 2016 and 2017.

Site ID	Year Sampled	Longitude (°)	Latitude (°)
1	2016	45.97	-66.805
2	2016	45.971	-66.778
3/A	2016 and 2017	45.968	-66.753
4	2016	45.967	-66.632
5	2016	45.956	-66.625
6	2016	45.94	-66.632
B	2017	45.975	-66.751
C	2017	45.976	-66.728

Appendix II

Raw water chemistry data collected during the 2017 sampling season

Site ID	Microhabitat	Date and time	Water temperature (°C)	Dissolved oxygen (%)	Dissolved oxygen (mg/L)	Specific conductance (mS/cm)	pH	Turbidity (NTU)
3/A	Macrophytes	August 30, 2017 @ 1300h	21.6	111.5	9.83	123.0	7.26	3.0
	Cobble/Gravel	August 30, 2017 @ 1305h	21.7	111.7	9.85	122.6	7.91	2.2
	Silt/Sand	August 30, 2017 @ 1310h	21.5	406.8	9.43	118.8	7.99	1.7
B	Macrophytes	August 30, 2017 @ 1710h	21.4	112.3	9.93	116.7	7.99	1.8
	Cobble/Gravel	August 30, 2017 @ 1715h	21.3	105.4	9.36	117.4	8.14	1.6
	Silt/Sand	August 30, 2017 @ 1720h	21.4	127.9	11.40	116.6	8.90	7.2
C	Cobble/Gravel	August 31, 2017 @ 1220h	21.7	111.4	9.83	118.8	8.32	1.6
	Macrophytes	August 31, 2017 @ 1225h	21.9	113.5	9.94	119.2	8.33	2.3
	Silt/Sand	August 31, 2017 @ 1230h	20.9	102.2	9.12	118.3	8.19	1.6

Appendix III

Raw data showing the Good's coverage index values for 264 aquatic invertebrate samples following rarefaction of gut bacterial sequences to a sampling depth of 10,000.

Sample name	Good's coverage index	Error	Sample name	Good's coverage index	Error
INVSJ16L011	0.962	0.001	INVSJ16L100	0.945	0.002
INVSJ16L013	0.962	0.002	INVSJ16L101	0.950	0.002
INVSJ16L014	0.961	0.002	INVSJ16L102	0.948	0.001
INVSJ16L015	0.958	0.002	INVSJ16L111	0.945	0.002
INVSJ16L016	0.955	0.001	INVSJ16L113	0.947	0.002
INVSJ16L025	0.928	0.002	INVSJ16L117	0.946	0.002
INVSJ16L027	0.936	0.002	INVSJ16L118	0.946	0.001
INVSJ16L028	0.929	0.002	INVSJ16L119	0.948	0.002
INVSJ16L039	0.955	0.002	INVSJ16L127	0.940	0.002
INVSJ16L040	0.954	0.001	INVSJ16L129	0.942	0.002
INVSJ16L041	0.962	0.001	INVSJ16L130	0.943	0.002
INVSJ16L046	0.970	0.001	INVSJ16L131	0.940	0.002
INVSJ16L047	0.961	0.002	INVSJ16L132	0.942	0.001
INVSJ16L048	0.955	0.002	INVSJ16L133	0.944	0.002
INVSJ16L050	0.956	0.002	INVSJ16L134	0.934	0.002
INVSJ16L051	0.948	0.001	INVSJ16L138	0.946	0.002
INVSJ16L053	0.934	0.003	INVSJ16L151	0.941	0.002
INVSJ16L054	0.941	0.002	INVSJ16L155	0.941	0.002
INVSJ16L055	0.944	0.003	INVSJ16L157	0.948	0.002
INVSJ16L056	0.947	0.002	INVSJ16L159	0.945	0.002
INVSJ16L058	0.950	0.002	INVSJ16L162	0.949	0.002
INVSJ16L059	0.949	0.001	INVSJ16L164	0.944	0.002
INVSJ16L060	0.954	0.001	INVSJ16L166	0.945	0.001
INVSJ16L074	0.949	0.002	INVSJ16L171	0.946	0.002
INVSJ16L075	0.948	0.002	INVSJ16L172	0.939	0.002
INVSJ16L076	0.944	0.001	INVSJ16L174	0.942	0.003
INVSJ16L079	0.944	0.002	INVSJ16L180	0.938	0.001
INVSJ16L082	0.930	0.002	INVSJ16L181	0.934	0.002
INVSJ16L083	0.936	0.002	INVSJ16L183	0.934	0.002
INVSJ16L084	0.939	0.002	INVSJ16L190	0.942	0.002
INVSJ16L085	0.943	0.002	INVSJ16L192	0.941	0.002
INVSJ16L086	0.949	0.002	INVSJ16L193	0.942	0.002
INVSJ16L087	0.944	0.002	INVSJ16L194	0.941	0.002

(Appendix III continued)

Sample name	Good's coverage index	Error
INVSJ16L196	0.947	0.002
INVSJ16L212	0.965	0.001
INVSJ16L213	0.947	0.002
INVSJ16L214	0.946	0.002
INVSJ16L217	0.928	0.002
INVSJ16L219	0.937	0.001
INVSJ16L223	0.936	0.002
INVSJ16L227	0.941	0.002
INVSJ16L228	0.945	0.003
INVSJ16L229	0.943	0.002
INVSJ16L230	0.954	0.001
INVSJ16L231	0.938	0.002
INVSJ17L001	0.961	0.002
INVSJ17L002	0.961	0.002
INVSJ17L003	0.962	0.002
INVSJ17L004	0.936	0.002
INVSJ17L006	0.947	0.001
INVSJ17L007	0.937	0.002
INVSJ17L008	0.960	0.002
INVSJ17L009	0.955	0.003
INVSJ17L016	0.970	0.001
INVSJ17L017	0.962	0.001
INVSJ17L018	0.963	0.001
INVSJ17L019	0.963	0.002
INVSJ17L021	0.960	0.003
INVSJ17L022	0.958	0.001
INVSJ17L024	0.944	0.003
INVSJ17L026	0.965	0.001
INVSJ17L027	0.971	0.002
INVSJ17L028	0.966	0.001
INVSJ17L029	0.967	0.001
INVSJ17L030	0.965	0.001
INVSJ17L031	0.963	0.001
INVSJ17L032	0.967	0.001
INVSJ17L033	0.959	0.002
INVSJ17L034	0.965	0.001
INVSJ17L035	0.942	0.002

Sample name	Good's coverage index	Error
INVSJ17L038	0.954	0.002
INVSJ17L041	0.967	0.001
INVSJ17L042	0.970	0.001
INVSJ17L043	0.968	0.001
INVSJ17L046	0.964	0.001
INVSJ17L047	0.963	0.001
INVSJ17L050	0.968	0.002
INVSJ17L053	0.971	0.001
INVSJ17L054	0.970	0.001
INVSJ17L057	0.969	0.002
INVSJ17L060	0.952	0.002
INVSJ17L061	0.957	0.001
INVSJ17L062	0.963	0.002
INVSJ17L063	0.963	0.002
INVSJ17L064	0.967	0.001
INVSJ17L065	0.970	0.001
INVSJ17L066	0.968	0.001
INVSJ17L067	0.968	0.001
INVSJ17L068	0.963	0.001
INVSJ17L069	0.965	0.001
INVSJ17L070	0.936	0.002
INVSJ17L071	0.967	0.002
INVSJ17L073	0.966	0.001
INVSJ17L077	0.967	0.001
INVSJ17L078	0.965	0.002
INVSJ17L079	0.959	0.001
INVSJ17L080	0.955	0.002
INVSJ17L082	0.948	0.001
INVSJ17L083	0.944	0.002
INVSJ17L087	0.952	0.001
INVSJ17L088	0.953	0.001
INVSJ17L089	0.950	0.001
INVSJ17L090	0.958	0.001
INVSJ17L091	0.951	0.002
INVSJ17L092	0.964	0.002
INVSJ17L093	0.970	0.001
INVSJ17L094	0.962	0.001

(Appendix III continued)

Sample name	Good's coverage index	Error	Sample name	Good's coverage index	Error
INVSJ17L095	0.966	0.002	INVSJ17L155	0.955	0.002
INVSJ17L097	0.962	0.001	INVSJ17L157	0.950	0.001
INVSJ17L098	0.966	0.001	INVSJ17L161	0.968	0.001
INVSJ17L101	0.965	0.002	INVSJ17L162	0.969	0.002
INVSJ17L102	0.953	0.002	INVSJ17L163	0.970	0.001
INVSJ17L103	0.948	0.002	INVSJ17L170	0.970	0.001
INVSJ17L104	0.926	0.002	INVSJ17L171	0.968	0.001
INVSJ17L106	0.963	0.001	INVSJ17L172	0.958	0.002
INVSJ17L108	0.964	0.002	INVSJ17L175	0.956	0.002
INVSJ17L109	0.960	0.002	INVSJ17L177	0.967	0.001
INVSJ17L110	0.968	0.001	INVSJ17L178	0.968	0.001
INVSJ17L111	0.968	0.001	INVSJ17L180	0.967	0.001
INVSJ17L112	0.969	0.001	INVSJ17L181	0.946	0.001
INVSJ17L113	0.977	0.002	INVSJ17L182	0.969	0.001
INVSJ17L114	0.971	0.001	INVSJ17L184	0.958	0.001
INVSJ17L115	0.962	0.001	INVSJ17L185	0.959	0.001
INVSJ17L116	0.964	0.002	INVSJ17L189	0.951	0.002
INVSJ17L118	0.962	0.001	INVSJ17L190	0.967	0.001
INVSJ17L121	0.963	0.002	INVSJ17L191	0.969	0.001
INVSJ17L122	0.962	0.002	INVSJ17L192	0.917	0.002
INVSJ17L123	0.968	0.001	INVSJ17L193	0.931	0.002
INVSJ17L124	0.970	0.001	INVSJ17L194	0.933	0.002
INVSJ17L125	0.971	0.001	INVSJ17L195	0.935	0.003
INVSJ17L129	0.969	0.002	INVSJ17L197	0.965	0.002
INVSJ17L130	0.965	0.001	INVSJ17L198	0.965	0.002
INVSJ17L131	0.965	0.001	INVSJ17L201	0.965	0.001
INVSJ17L133	0.958	0.003	INVSJ17L204	0.967	0.001
INVSJ17L134	0.968	0.002	INVSJ17L205	0.968	0.001
INVSJ17L137	0.972	0.001	INVSJ17L206	0.970	0.002
INVSJ17L138	0.967	0.001	INVSJ17L207	0.972	0.001
INVSJ17L139	0.971	0.001	INVSJ17L208	0.977	0.001
INVSJ17L143	0.965	0.002	INVSJ17L209	0.971	0.001
INVSJ17L144	0.965	0.001	INVSJ17L210	0.966	0.002
INVSJ17L145	0.968	0.001	INVSJ17L211	0.967	0.001
INVSJ17L150	0.969	0.002	INVSJ17L212	0.966	0.001
INVSJ17L152	0.969	0.001	INVSJ17L219	0.967	0.001
INVSJ17L154	0.960	0.002	INVSJ17L221	0.970	0.002

(Appendix III continued)

Sample name	Good's coverage index	Error	Sample name	Good's coverage index	Error
INVSJ17L222	0.962	0.001	INVSJ17L254	0.972	0.001
INVSJ17L224	0.973	0.001	INVSJ17L256	0.970	0.002
INVSJ17L225	0.970	0.002	INVSJ17L257	0.969	0.002
INVSJ17L226	0.968	0.001	INVSJ17L258	0.972	0.001
INVSJ17L227	0.969	0.002	INVSJ17L259	0.972	0.001
INVSJ17L229	0.959	0.002	INVSJ17L260	0.971	0.001
INVSJ17L231	0.966	0.002	INVSJ17L261	0.969	0.001
INVSJ17L233	0.972	0.001	INVSJ17L262	0.967	0.001
INVSJ17L234	0.970	0.001	INVSJ17L264	0.960	0.002
INVSJ17L235	0.972	0.002	INVSJ17L265	0.963	0.001
INVSJ17L236	0.972	0.001	INVSJ17L266	0.967	0.001
INVSJ17L238	0.973	0.001	INVSJ17L267	0.970	0.001
INVSJ17L239	0.969	0.002	INVSJ17L269	0.965	0.002
INVSJ17L241	0.969	0.002	INVSJ17L270	0.966	0.001
INVSJ17L242	0.970	0.002	INVSJ17L271	0.962	0.002
INVSJ17L243	0.973	0.001	INVSJ17L272	0.962	0.001
INVSJ17L244	0.969	0.002	INVSJ17L273	0.962	0.002
INVSJ17L245	0.972	0.002	INVSJ17L274	0.957	0.002
INVSJ17L246	0.972	0.001	INVSJ17L275	0.968	0.001
INVSJ17L247	0.972	0.0015	INVSJ17L276	0.968	0.001
INVSJ17L248	0.974	0.001	INVSJ17L277	0.917	0.002
INVSJ17L249	0.964	0.001	INVSJ17L278	0.948	0.002
INVSJ17L251	0.947	0.003	INVSJ17L283	0.967	0.001
INVSJ17L252	0.958	0.002	INVSJ17L284	0.964	0.001
INVSJ17L253	0.963	0.001	INVSJ17L285	0.965	0.001

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Investigating the gut microbiomes of aquatic macroinvertebrates in the Saint John River. Kroetsch, S.A., Kidd, K.A., Pavey, S.A. 17-May-18. CRI @ UNB Presentation Series, Fredericton, NB.

Investigating the effects of functional feeding group, taxonomy, and habitat on the gut microbiomes of aquatic macroinvertebrates in the Saint John River, New Brunswick. Kroetsch, S.A., Kidd, K.A., Pavey, S.A. 16-Mar-17. M.Sc. Proposal Presentation, Saint John, NB.