The Effect of Cottage Development on Aquatic Macroinvertebrate Communities and Water Quality in Central Ontario Lakes

A thesis presented to

The Faculty of Graduate Studies

of

Lakehead University

By

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In partial fulfillment of requirements

for the degree of

Master of Science in Biology

January 12, 2023

Abstract

In Canada, over 3 million freshwater lakes provide a wide range of ecological, financial, aesthetic, and public health services for their citizens, communities, and tourists. This is especially true for regions like south-central Ontario, commonly referred to as 'cottage country', that depend on these ecosystems directly for activity, business, and tourism. However, with property ownership growing in the area, as well as tourism, the region is experiencing more human development and subsequent activities associated with it. Forms of these developmental activities include land clearing for cottage or roadway development at the shore or surrounding watershed, installation of wells and septic systems, riparian zone alteration for aesthetic or recreation purposes, and increases in foreign materials that are a by-product of this human activity (like increased fertilizer use on farmland and gardens, oil or gasoline contamination and contaminants originating from the cottages themselves). In this study, we aim to gather baseline data on local macroinvertebrate communities and the physicochemical properties of water in interior lakes in the Muskoka region of central Ontario, and to compare developed and isolated lakes to assess the impact of cottage development. Using an RDA analysis, I found that, among the water variables assessed, conductivity, pH and dissolved oxygen concentration had a significant effect on the composition of benthic macroinvertebrate communities. Of the environmental variables assessed, free-floating and rooted floating macrophytes appear to have the greatest effect on macroinvertebrate community composition. We learned that four of the six water variables assessed changed significantly from season to season, providing an insight on the seasonal trends in these interior lakes. When comparing isolated and developed lakes, we found that pH, water conductivity and available nitrogen concentration differed significantly between them. Of the five benthic macroinvertebrate indices calculated and assessed, only EOT biotic index differed significantly between isolated and developed lakes. Taxa richness, diversity, CIGH and HBI showed no significant differences between lake types. Water chemistry variables that were determined to have a significant effect on community composition are those commonly altered by developmental activities (pH, conductivity and available nitrogen). It is expected that, as these regions experience more development in subsequent decades, differences between lake types will become more pronounced, particularly in terms of biotic indicators.

Lay Summary

Cottages have become a major way of life for many Ontarians, however, their impacts on the local environment are often overlooked. As areas around lakes undergo developmental activities, more attention needs to be paid to how these lakes are responding both in terms of their water quality and also in terms of aquatic life. The goal of this research is to compare water quality and macroinvertebrate communities in lakes of central Ontario with, and without, cottage development. I found that conductivity (the ability for water to pass an electrical current, usually a function of the concentration of dissolved ions), lake pH and dissolved oxygen concentration have the largest impact on the community composition of local benthic (bottom-dwelling) communities. I have also detected the importance of aquatic macrophytes in the composition of benthic communities, with free-floating, as well as rooted aquatic plants playing major roles.

After determining the seasonal shifts in water quality and benthic communities, we compared these variables directly between lakes experiencing and not experiencing developmental activities. I found that pH, conductivity, and available nitrogen were significantly different between developed and isolated lakes. When benthic macroinvertebrate communities were compared, organisms belonging to EOT taxa (taxa that are considered to prefer good quality water) were significantly different between developed and isolated lakes. The results suggested that differences between isolated and developed lakes are beginning to become evident, and if development continues, the differences between the two lake types will become more and more pronounced.

Acknowledgments

First, I would like to thank my incredible supervisor Dr. Nandakumar Kanavillil for his consistent support and guidance during this long journey. Even though the majority of work completed for this thesis was done with COVID restrictions in place, Dr. Kanavillil's support was always felt, and his aid was instrumental in this project. I would also like to acknowledge my committee members, Dr. Stephen Hecnar and Dr. Sreekumari Kurissery for their constructive feedback and valued knowledge, and my external reviewer, Dr. Lesley Lovett-Doust.

I want to also thank some amazing staff members at Lakehead University, who were kind enough to set aside time out of their busy days to help me with my studies. Firstly, thank you Dr. Victoria Te Brugge for your immense help as both a resource for information and help in the laboratory, and for being a kind mentor and friendly face to chat to in times of stress and confusion. Thank you, Dr. Usha Menon, for helping with organizing materials needed for my studies and for being flexible with lab space and materials. Thank you, Dr. Gerardo Reyes for all your help with statistical analyses and Dr. Michael Rennie for insights on statistical analysis and experimental design.

Thank you to Dr. Chris Jones whose expertise in benthic macroinvertebrate sampling for the Ministry of the Environment also helped me a great deal in terms of both experimental design and the statistical analyses of my macroinvertebrate data.

The support of my incredible family was critical. Thank you to my amazing father, Kai, as well as the rest of the Wisch family. Thank you to my wonderful partner Mackenzie for her constant support in the field and during long hours, as well as to her family for their unwavering support. Lastly, I would like to thank my late mother, whose passion for the outdoors is the driving force behind my own passion today.

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Chapter 1. Introduction

1.1 Overview

Canada is uniquely positioned as a country, possessing over 3 million freshwater lakes covering 7.6% of its total surface area (Palmer *et al.,* 2010). In-land lakes provide enormous ecological, financial, aesthetic, and public health services for the surrounding communities, so their degradation is a major concern for Canadians. This is especially true for regions like south-central Ontario, which is considered Ontario's "cottage country" (District Municipality of Muskoka, 2005). This region relies on water-related activities and businesses centred around water bodies as a major source of revenue (Palmer *et al.,* 2010). Keeping these lentic ecosystems protected is therefore of great importance. This issue is also of concern for the property owners themselves. It has been shown that water quality matters to potential buyers in Ontario, with buyers willing to pay approximately 2% more for a property for each 1-foot increase in water clarity (Clapper and Caudill, 2014). The greatest threat to the freshwater ecosystems in this area is activities associated with development, and in the case of this region, in the form of cottage development (Lesauteur, 1968; Hicks and Frost, 2010). This is mainly due to the wide range of impacts these developmental activities have on the lake environment, both in the riparian zone surrounding the lake and the littoral zone of the lake itself (Hicks and Frost, 2010). Therefore, the present study was undertaken to gain a better understanding of macroinvertebrate communities in Central Ontario lakes and to assess how these communities, and the water quality of the lakes they live in, are affected by cottage development and related activities.

Presently, there is a lack of macroinvertebrate assessments in this region. There are only a few comparative studies that assess the conditions of developed and undeveloped lakes. Therefore, this study will address these gaps by studying macroinvertebrate communities and water quality in both developed and isolated lakes in the south-central region of Ontario and use these findings to better understand the effect of developmental activities that affect these crucial ecosystems.

1.2 Factors Affecting Aquatic Ecosystems

Aquatic ecosystems are affected by both biotic and abiotic factors.

1.2.1 Biotic Factors

Ecosystems are dynamic environments where many variables work together to create a functional environment. The organisms living in these ecosystems, whether they be fish, macroinvertebrates, plants, algae, etc., interact with one another and their environment in a variety of ways. One of the most common interactions that animals and plants engage in is competition. There are two general forms of competition between species in a natural environment, real, and apparent competition (Holomuzki *et al.,* 2010). Real competition can occur directly through interference competition while indirect competition occurs through resource exploitation (Holomuzki *et al.,* 2010). Competition for resources can occur within the same species, or between different species that share similar feeding or living strategies. In terms of macroinvertebrates, herbivorous snails and larval caddisflies have strong exploitative effects on their environment (Holomuzki *et al.,* 2010). An abundance of snails in an environment can lead to periphytic food depletion, resulting in a reduced growth of competitors thereby changing the overall diversity of consumers (Holomuzki *et al.,* 2010). The competitors affected by a shortage of periphyton include chironomids and caddisflies, which share similar feeding strategies and resources (Holomuzki *et al.,* 2010). A similar situation can arise with high density grazing by larval Helicopsyche caddisflies, which can greatly depress periphyton abundance (Holomuzki *et al.,* 2010). This can cause a decrease in grazer growth, pupal size, and the rate of population recruitment, which can have serious ramifications for the populations in benthic communities (Holomuzki *et al.,* 2010). The other form of competition, apparent competition, is so-called because it does not result in resource limitations and the interaction is negative for both species involved (Holomuzki *et al.,* 2010). Essentially, apparent competition occurs when prey species indirectly depress each other by attracting the presence of a shared predator (Holomuzki *et al.,* 2010). Apparent competition is a relatively new concept being examined in benthic communities that does not yet have many concrete examples. There have been some documented instances of apparent competition in marine shores, but little work has been done to examine this form of competition in freshwater ecosystems. Predation is another form of interaction that occurs between a predator and it prey. Freshwater predators can limit prey abundances, alter prey size distribution, behaviour and age structure, and cause changes to entire food webs (Holomuzki *et al.,* 2010). This process forms the basis for a typical trophic cascade (Holomuzki *et al.,* 2010**)**

1.2.2 Abiotic Factors

In lentic ecosystems, abiotic factors may include variables such as temperature, light intensity, various aspects of water chemistry, littoral conditions, presence of woody debris etc. While these variables are unrelated to living things, they can still be affected by organisms in the environment and vice versa. These abiotic factors can also be greatly impacted or altered by anthropogenic activities through directly altering the environment; for example, by removing woody debris from the littoral zone, or indirectly as a result of nearby agricultural operations or cottage development (Hadley *et al.,* 2013; Krynak and Yates, 2020).

Intensive agriculture occurring near a lake or water source can have severe impacts on the ecosystem aitits water chemistry. Intensive agriculture can lead to excess nutrients (commonly phosphorus and nitrogen) and other substances such as pesticides being released into the local water systems (Hadley *et al.,* 2013). Continued intensive agriculture can lead to the flourishing of certain feeding groups (Krynak and Yates, 2020). When compared to healthy water systems that show diverse feeding groups, impacted water systems show the dominance of certain feeding groups leading to changed community interactions (Lento *et al.,* 2012). This stress can also result in a decrease in diversity and in turn, changes in the body mass spectrum of benthic invertebrate communities (Krynak and Yates, 2020). Krynak and Yakes (2020) observed that populations of Ephemerellidae and Hydropsyche are the groups most affected by agricultural activities. Areas affected by intense agricultural activities were seen to lack large predatory benthic species (Krynak and Yates, 2020). Body size and the accompanying body-mass spectrum of a healthy benthic community are extremely important for allowing the community to function properly. Body size is a major mediating factor in determining how effectively animals obtain resources, with larger organisms more effectively obtaining food compared to smaller individuals (Krynak and Yates, 2020). Changes in the size spectrum of benthic communities can also cause serious consequences for ecosystem processes (e.g. nutrient cycling) and resource availability (Krynak and Yates, 2020). These factors emphasize the importance of a healthy and variable body-mass spectrum within a benthic community which, in turn, enites its resiliency in times of stress (Krynak and Yates, 2020). When this spectrum is altered, the community is more susceptible to hardship and more likely to decrease in diversity and abundance. The effect of intensive agriculture on benthic invertebrate communities does, however, seem to involve a temporal lag. Krynak and Yates (2020) found a lack of short-term effects of agriculture on benthic communities, suggesting a lag in the time it takes those impacts to reach and affect the whole benthic community.

As phosphorus and nitrogen are common by-products of agriculture effluents, and outflow from septic systems and outhouses, these ions commonly find their way into waterways through surface runoff. Large amounts of phosphorus and nitrogen in a water body can be extremely detrimental to water health and the organisms inhabiting in it. These nutrients, when in excess, can result in toxic cyanobacterial ("algal") blooms and increased plant growth (Nelligan *et al.,* 2019). Eutrophication occurs when a water body experiences excessive plant or algal growth. Once these algae die off, the microbial decomposition of their organic debris depletes dissolved oxygen, creating an anoxic environment (Hadley *et al.,* 2013).

Another factor that uniquely acts on the lakes in this study area is the underlying geology of the Precambrian shield. The gradual weathering of the shield bedrock results in a poor buffering capacity of shield waters (Neff and Jackson, 2013). Typically, these shield waters are characterized by low conductivity, which can also result in acidification and calcium depletion (Neff and Jackson, 2013). These systems not only differ chemically from off-shield sites, but also differ in biological characteristics such as fish and macroinvertebrate community composition (Neff and Jackson,.

1.2.3 Cottage Development and Its Effects

Cottage development is prevalent and widespread across the central Ontario region. This region of Ontario has an abundance of freshwater inland lakes which provide great opportunities for residential and recreational property development. As cottages are developed and subsequently lived in, a variety of impacts are created and imposed on the local environment. Described below are some of those impacts.

Coarse woody debris, also known as "coarse woody habitat" (CWH) is a key component of a lake's littoral zone and serves many roles. Coarse woody debris comprises essentially any fallen trees or logs that are now present in the littoral zone of a lake. Decomposition of wood debris in water is a slow process taking decades to centuries to complete (Marburg *et al.,* 2006). For this reason, the removal of littoral wood debris can quickly remove tens to hundreds of years of input and these regions can never recover from the loss of these structures, forever altering that littoral zone. Coarse woody habitat supports periphyton growth, increases the retention of organic sediment, and serves as a substrate for benthic invertebrate production, making this resource an important habitat (Marburg *et al.,* 2006). Not only does CWH support lower-level trophic species, but it also plays an important role in the life histories of many fish species by providing protection, nesting, and spawning sites, and providing greater prey availability (Sass *et al.,* 2006). As lakeshores are developed, the removal of the littoral woody debris is often done for aesthetic or recreational reasons (Sass *et al.,* 2006). Woody debris density commonly decreases with increasing building density around a lake, and once a lake reaches more than 9 buildings/km, lakes rarely have sufficient woody debris remaining (Marburg *et al.,* 2006). Traditionally, CWD can be quite variable among undeveloped lakes; however, that natural variability is significantly decreased with increasing building density (Marburg *et al.,* 2006). Overall, the best predictor of littoral coarse woody debris is the density of riparian coarse wood, given tit wood is its source (Marburg *et al.,* 2006). Unfortunately, cottage development also has an impact on riparian coarse wood, due to land-clearing for building or view/aesthetic purposes, or to create more recreational areas. Since so many lower trophic organisms rely on CWH for food and habitat resources, its removal not only has a massive impact on their abundance but also on their predators. The growth rates of common lentic predators such as *Micropterus salmoides* (largemouth bass) and *Lepomis macrochirus* (bluegill), were observed to be significantly higher in undeveloped lakes with abundant CWH compared to populations in developed lakes where that habitat has been disturbed (Sass *et al.,* 2006). Removal of CWH causes predatory fish, specifically bass in this case, to shift from a predominantly aquatic prey diet to a diet that is increasingly subsidized by terrestrial prey (Sass *et al.,* 2006). Bass diets shifted from 17-19% terrestrial prey pre-CWH removal to a staggering 51-55% post removal. Perch fishes rely on CWH for spawning substrate, foraging and refuge (Sass *et al.,* 2006). Following CWH removal, researchers observed an increase in mortality, a decrease in prey availability and a loss of spawning substrate for perch populations (Sass *et al.,* 2006). These combinations of effects greatly reduced the reproductive potential of perch to levels at or below its replacement rate, which over time would result in population collapse (Sass *et al.,* 2006).

Similar to the removal of coarse woody debris, the removal of aquatic macrophytes in and around the littoral zone is very common in cottage development. Macrophyte biomass and species richness both have a negative relationship with cottage development (Hicks and Frost, 2010). Aquatic macrophytes are important components of littoral zones that provide many ecosystem services. Important littoral macrophyte ecosystem services include stabilizing sediment, the storage and release of nutrients, providing energy and nutrients for consumers and providing habitat for a wide variety of macroinvertebrates, fish and amphiphans (Hicks and Frost, 2010). The removal of these macrophytes has severe negative impacts on local benthic macroinvertebrate populations (Hicks and Frost, 2010). Riparian plant life is also key for aquatic environments as it acts as a buffer protecting the lake from surrounding land use. As surface runoff discharges along the riparian area, riparian plant life reduces the flow of nutrients and sediment before they make their way to the deeper water body (Owens *et al.,* 2021). These riparian buffers are effective at reducing nonpoint source pollution, which is key to reducing the effects of anthropogenic development (Owens *et al.,* 2021). The removal of these key areas is also associated with cottage development, as buffers are cleared for roads, driveways, and properties.

Hydrocarbons are another pollutant commonly found in the waters and sediments of urban water bodies. Hydrocarbons are common derivatives of crude oil sources such as gasoline, kerosene, fuel oil, asphalt, etc. These sources are composed of saturated straight-chained hydrocarbons, alkanes, cycloalkanes, and aromatics (Pettigrove and Hoffman, 2005). The aromatic components are the most toxic of these substances due to their low molecular weight and solubility. Heavier hydrocarbons are more likely to be absorbed into the sediment because they do not mix well with water and can become an issue for macroinvertebrates that live on that substrate (Pettigrove and Hoffman, 2005). A study performed by Pettigrove and Hoffman (2005) assessed high molecular weight hydrocarbon concentration in sediments and its effect on benthic macroinvertebrate communities. They found that at a total petroleum hydrocarbon (TPH) concentration of 860 mg/kg, there was a significant increase in the abundance of opportunistic species. The opportunistic species observed were *Polypedilum vespertinus* and *Cricotopos albitarsis,* two Diptera species (Pettigrove and Hoffman, 2005). However, at concentrations of 860 to 1870 mg/kg TPH, a reduction in the abundance of all pollution-sensitive taxa was observed (Pettigrove and Hoffman, 2005). Once concentrations became higher than 1870 mg/kg, there were substantial losses in terms of the presence and abundance of most aquatic invertebrates in the area, regardless of life history or tolerance (Pettigrove and Hoffman, 2005). Hydrocarbons can also directly affect macroinvertebrates by coating them and smothering them, which does not alter cellular activities but instead inhibits activities such as movement, feeding and respiration (Pettigrove and Hoffman, 2005). Smothering via hydrocarbon contamination can also lead to decreases in the abundance and diversity of communities (Pettigrove and Hoffman, 2005). Although cottage areas are usually not considered to be 'urban', where many hydrocarbon pollutants are present, it is still likely that developed, and even some isolated lakes would be exposed to hydrocarbon pollutants. Most developed lakes in Ontario have boat launches with a high boat presence throughout the summer months. Coupled with this possible source of hydrocarbon contamination, many areas surrounding these lakes are used by ATVs in summer and snowmobiles in winter. Generators on cottage properties are another possible source of hydrocarbon pollution in the area, as some lakes have no electric power access despite high densities of cottages. Considerable work has been done to create macroinvertebrate sensitivity rankings to assess hydrocarbon pollution more efficiently. The most sensitive macroinvertebrates to hydrocarbons according to these rankings include Planariidae, Ceriodaphnia, Daphnia and Diplostrasca (Gerner *et al.,* 2017). The macroinvertebrates that are most tolerant of hydrocarbons appear to be Lymnaea, Ceratopogonidae, Zygoptera, Basommatophora and Tricladida (Gerner *et al.,* 2017).

A by-product of any human dwelling that needs to be heavily considered is that of septic waste and how it is disposed of at a property. In the region of this study there are two general methods to contain septic waste; one being a properly built septic tank and the other is a simpler 'outhouse'. Properly built septic systems are called Class IV septic systems, which involve a septic tank along with a series of leaching beds (Ontario, 2022). Waste from a property will slowly moves through the tank where solids will settle and liquids will travel to the leaching bed, which consists of perforated PVC pipes surrounded by stone and sand (Ontario, 2022). This effluent would have experienced bacterial breakdown and several filtering processes as it slowly leeches into the underlying soil (Ontario, 2022). After a duration of typically five years, the remaining solids and debris will need to be pumped from the tank and disposed of properly.

Tanks are typically inspected following draining, with a typical tank expected to last 25 years (MOE *et al.,* 2010). An issue with septic tanks is that due to the relatively isolated nature of a lot of the properties, liberties tend to be taken with the inspection and care of older tanks (MOE *et al.,* 2010). Along with this, property owners may feel that due to their isolated nature, they can get away with simple holding tanks or a more traditional 'outhouse'. Holding tanks do not provide any form of treatment or proper leeching, and therefore pose a massive risk if accidentally damaged (Ontario, 2022). 'Outhouses' also possess no treatment capabilities and rather than contained in a structure, the waste is in immediate contact with the underlying soil (MOE *et al.,* 2010).

For waterfront properties, a constant worry for homeowners is the threat of shoreline erosion. A common mitigation technique for property erosion is shoreline armouring. Shoreline armouring is the implementation of engineered shore structures that serve to reduce erosion by reducing the effects of waves, storms, and floods (Chhor *et al.,* 2019). Generally, there are two common practices for shoreline armouring, riprap revetments and retaining walls (Chhor *et al.,* 2019). Riprap revetments consist of sloped barriers of rock or rubble that lie parallel to the shoreline. This is a relatively cost-effective approach that requires little excavation of the shoreline to construct (Chore *et al.,* 2019). Retaining walls are fully consolidated vertical barriers constructed of concrete or stone that run perpendicular to the waterline (Chhor *et al.,* 2019). This method of armouring is more costly and requires a fair amount of excavation of the shoreline substrate (Chhor *et al.,* 2019). Chhor *et al.* (2019) examined the effects of shoreline armouring on the littoral fish and benthic invertebrates inhabiting the affected area. They found that submergent and emergent macrophytes and coarse woody debris were greatly affected by shoreline armouring and were all less abundant along the modified shorelines compared to natural environments (Chhor *et al.,* 2019). The abundance of fish and benthic communities were not generally affected by shoreline armouring; however, it did cause an increase in the abundance of Amphipoda, Isopoda, Ephemeroptera and Cladocera when compared to natural shorelines (Chhor *et al.,* 2019). While this may not seem like a problem, these results suggest that the human modification of shorelines has the potential to change the community structure of littoral ecosystems.

A complementary engineering activity associated with cottage development is the construction and maintenance of roadways. With urbanization comes an increase in the amount of impervious cover on the ground, in the form of paved roads, driveways and walkways. These impermeable surfaces do not function like normal riparian areas and can drastically alter local hydrology by increasing the frequency and size of surface flow events (Gal *et al.,* 2019). The runoff from these impermeable surfaces contains higher concentrations of sediment, nutrients, and chemical pollutants since the flow is no longer percolating through the surrounding soil on its way to the water body (Gal *et al.,* 2019). These influxes of various nutrients and pollutants can alter water chemistry, salinity and the community composition of the water system (including macroinvetebrates, amphibians and fish) (Sanzo and Hecnar, 2006; Mantyka-Pringle *et al.,* 2014). High nutrient loads resulting from high runoff, combined with warmer water temperatures resulting from climate change are considered the leading drivers of decline in macroinvertebrate communities worldwide (Mantyka-Pringle *et al.,* 2014).

1.2.4 Climate Change

Global warming has resulted in an increase in annual mean air temperature and has already affected the duration of ice-free periods for lakes in south-central Ontario (Mantyka-Pringle *et al.,* 2014). This will have a direct effect on seasonal activities such as "ice-fishing" in the region. Longer ice-free periods could result in longer growing periods for plants and animals and create stronger thermal stratification in the water column (Hadley *et al.,* 2013). These factors can have significant impacts on water-column mixing, light availability, and the distribution of nutrients throughout the system (Hadley *et al.,* 2013). Between 1981 and 2005, the mean air temperature in south-central Ontario increased by ~0.75ºC (Palmer *et al.,* 2010). As water temperature increases, the dissolved oxygen capacity of that water also decreases (Ito and Momii, 2014). This could affect many members of the benthic community that require higher levels of dissolved oxygen to survive (Mantyka-Pringle *et al.,* 2014).

1.2.5 Water Chemistry

An important component of any water ecosystem is its water chemistry. Water chemistry refers to the chemical characteristics of water that dictate the type of life that thrives in that environment. Typical aspects of water quality that are studied in lentic systems include pH, conductance, and dissolved oxygen, as well as nutrients like total phosphorus and available nitrogen (Palmer *et al.,* 2010). The pH is the measure of how acidic or basic a substance is; it is a direct measure of the concentration of hydrogen ions in a solution and is inversely plotted on a logarithmic scale. Aquatic organisms are sensitive to acidification because of the high level of hydrogen ions. Highly acidic environments can cause H^+ to interfere with the uptake and regulation of other ions used by an organism (Lento *et al.,* 2008). Invertebrates like gastropods and molluscs are highly susceptible to water acidification because the uptake of calcium ions can be severely affected, and calcium is a critical component of shell development and structure (Lento *et al.,* 2008).

The ability of water to pass an electrical current is known as water conductivity. The conductivity of lentic water can be altered by the presence of a variety of anions (like chloride, nitrate, sulphate, etc.) and cations (like sodium, calcium, iron, etc.) (EPA, 2012). The conductivity of a lake is mainly affected by the local geology, with weathering of the bedrock providing dissolved ions directly into the water. For our study area, the bedrock of the Precambrian shield has a low buffering capacity, and these waters are usually considered to have low conductivity because of this (Neff and Jackson, 2013). Developmental activities, especially the use of road salt in winter, can affect the conductivity of lake water. As cottage development occurs, service roads will be built, with the majority being gravel roads, to begin with. As development continues, more paved surfaces are constructed, and with this, more surface runoff results. In the cottage country of south-central Ontario, road salting is extensive during the winter months, with NaCl being the most common form of road salt applied (District Municipality of Muskoka and FedNor, 2004). As road salting occurs, sodium and chloride ions are released into the environment and find their way into the surrounding water systems via surface runoff. The greatest influx of these ions into the water occurs during the spring melt (the "freshet") and this can lead to spikes in conductivity. Other substances that can contribute to ion accumulation in this area are $MgCl_2$ which is used to reduce road salt scatter (it causes aggregation of fine dust particles, and a less slippery surface), and $CaCl_2$ which is used as a dust suppressant on gravel roads (District Municipality of Muskoka and FedNor, 2004). The effect of high conductivity on macroinvertebrates is not completely understood, but work is being carried out to improve knowledge in this area. One interesting finding is that waters with high conductance experience high fluctuations in the levels of dissolved solids which requires organisms to constantly burn energy for osmoregulation (Armstead *et al.,* 2016). This constant energy demand can be stressful for aquatic organisms and result in population decline.

Dissolved oxygen is another important water chemistry parameter affecting living organisms. (Ministry of the Environment, 2019). Dissolved oxygen levels can be affected by anthropogenic factors in a variety of ways. The removal of large amounts of littoral vegetation, a common practice in cottage development, can decrease levels of dissolved oxygen in lakes because it directly removes a source of dissolved oxygen, a byproduct of photosynthesis (Neff and Jackson, 2013). Aquatic oxygen concentration can be further affected by nutrient loading, enhanced thermal stability, and increased surface water temperatures (Nelligan *et al.,* 2018). Low dissolved oxygen concentrations represent a serious risk to aquatic organisms by making gas exchange more difficult, especially for macroinvertebrates with filamentous gills, e.g. some Trichoptera.

Phosphorus is a commonly assessed nutrient in lentic ecosystems because of its prevalent human use and the impact it can have on aquatic systems. Phosphorus is a critical nutrient in plant growth and is widely used in agriculture (and gardening) as a fertilizer. Shoreline clearing, fertilizer use, erosion and land runoff as well as malfunctioning septic systems are all factors that can contribute to increased phosphorus loads in lakes (Ministry of the Environment, 2019). The most common sources of phosphorus in inland lakes are domestic waste and plant fertilizers (Palmer *et al.,* 2010). High concentration of total phosphorus in lakes can result in increased plant growth, and cyanobacterial blooms if the lake is receiving high phosphorus, but low available nitrogen (Ministry of the Environment, 2019). More importantly though, higher phosphorus concentrations ultimately result in eutrophication, which can make the ecosystem relatively less healthy (Ministry of the Environment, 2019). Available nitrogen, in the form of nitrate and nitrite, is another common nutrient used in the agriculture industry and thus is also a common nutrient in aquatic ecosystems. Nitrogen, like phosphorus, is a critical component in plant growth and can affect aquatic ecosystems in a similar way.

1.3 Previous Research in the Study Area

1.3.3 Central-Ontario Water Quality

Historically, the study area has been experiencing both anthropogenic and climatic impacts over the last couple of decades. With the increasing popularity of the area since the mid-1970s, Central Ontario has experienced widespread forest clearance, increased shoreline development, acidic deposition, and arrival of invasive species (Hadley *et al.,* 2013). These factors can have enormous impacts on the aquatic ecosystems in the area, so monitoring these ecosystems is crucial in understanding how they are affected. While there is no historical data available specifically on each of the lakes observed in this study, over recent decades there have been a couple of studies that assessed water chemistry of lakes similar to those in the Muskoka-Haliburton region, located on Precambrian Shield bedrock in central Ontario (Hadley *et al.,* 2013; Palmer *et al.,* 2010). Lake chemistry in the region is changing in response to the changes in anthropogenic activities and climate change (Hadley *et al.,* 2013). Therefore, it is important to monitor the health of lakes individually so that appropriate mitigating strategies can be adopted (Palmer *et al.,* 2010). In general, the water quality of lakes in south-central Ontario changed significantly from 1981 to 2005 (Palmer *et al.,* 2010). These water quality changes have occurred at a regional scale and therefore affected lakes over a large gradient (Palmer *et al.,* 2010).

Mitigation strategies adopted over the past 25-30 years have resulted in some improvements in water quality (specifically acidity) in the lakes of south-central Ontario (Palmer *et al.,* 2010). This was achieved through a 50% reduction in sulphate (SO_4^{2-}) deposition (Palmer *et al.,* 2010). Despite this decrease in acidic deposition, the majority of lakes in the study area did not see significant improvements in alkalinity or pH. This is most likely due to a widespread (prior) loss of calcium and magnesium ions, caused by the leaching of catchment soils, which depleted exchangeable cation reserves (Palmer *et al.,* 2010).

Climate change has also had an impact on water quality in this area. As air temperature increases, lakes in the area experience increased organic matter production and decomposition, as well as increased dissolved organic carbon release from the soils (Palmer *et al.,* 2010). Between the study years 1981 and 2005, the mean air temperature in the study region increased by approximately 0.75C^o (Keller *et al.,* 2008; Palmer *et al.,* 2010). Long-term increases in average air temperature resulting from climate change were also correlated with increases in ammonia (NH_3) and ammonium (NH_4^{2-}) concentrations (Palmer *et al.,* 2010). It appears that terrestrial mineralization of ammonium may be enhanced during warm, dry periods, which are correlated with increasing air temperatures (Qiu and McComb, 1996).

1.3.2 Effect of Cottage Development on Ontario Water Quality

Lakeshore development around south-central Ontario has had a significant impact on water quality over the last 25 years (Palmer *et al.* 2010). As development occurs, more roads are constructed and associated road salting increases. Conductivity, as a result, was observed to be higher around developed lakes and has increased as years passed (Palmer *et al.,* 2010). Despite this, Na^+ and Cl^- concentrations were among the lowest in Canada and remain below detrimental levels (Palmer *et al.,* 2010) (Molot and Dillon, 2008). While it is reassuring to see that these ion levels are relatively low in the area, it could mean that the area is more susceptible to large influxes of ions, which could greatly alter water conductivity. The rapid construction of roads coupled with the areas of low buffering capacity could suggest that these lakes are at risk of increased conductivity (Neff and Jackson, 2013). Another aspect of water quality linked to development that was studied over the last 25 years in the area was total phosphorus (Palmer *et al.,* 2010). According to Palmer *et al* (2010) despite the increased development activities in the south-central Ontario, the amount of total phosphorus decreased over time in both developed and undeveloped lakes. Little is known as to what is driving this decrease in total phosphorus and this is currently the focal point of research (Palmer *et al.,* 2010). The present study also hopes to explore this phenomenon and gain a better understanding of fluctuations in water quality in the lakes of this region.

Cottage development also has a profound impact on the surrounding watershed, which adds further stress to lakes experiencing such development. Anthropogenic stress at the watershed level typically results in the reduction or complete removal of natural vegetation as well as increased road density and impermeable surfaces (Kovalenko *et al.,* 2014). Watershed-level human development (other than agriculture) has been shown to have a significant effect on the functional diversity of macroinvertebrate communities. Interestingly, increased settlement seems to have a greater effect on the functional diversity of macroinvertebrates than does increased agricultural activity (Kovalenko *et al.,* 2014). The negative effect of watershed development was strong enough to be detected against the background of geographic differences and the presence of additional stressors acting on individual lake samples in the Laurentian Great Lakes System (Kovalenko *et al.,* 2014). This tells us that the impact of watershed development can be strong and consistent.

1.3.3 Previous Macroinvertebrate Research in Central Ontario

Ontario researchers have a long history of using sampling of benthic macroinvertebrates to assess lake systems, and using those findings to gain a better understanding of the communities that are affected. The most common assessment done using benthic macroinvertebrates is stream assessments done with the Ontario Benthos Biomonitoring Network (OBBN). The OBBN is a key resource for these assessments in Ontario as they provide a variety of data collection resources and identification keys, as well as outlines of the appropriate sampling procedures to ensure the procedures are consistent among researchers (OBBN, 2007).

Over the past two decades, central Ontario has seen more whole lake assessments with studies on macroinvertebrates. Kovalenko *et al.* (2014) studied benthic macroinvertebrates in coastal wetlands along the Great Lakes in Canada and the United States. They assessed the impact of watershed-scale anthropogenic stress on macroinvertebrate functional diversity in the summers of 2002 and 2003. They found that the functional diversity of macroinvertebrate communities was negatively impacted by anthropogenic activities in watersheds with more developmental activities than on those with no, or fewer developmental activities (Kovalenko *et al.,* 2014).

A major factor that remains present in Central Ontario is the residue of massive acidic deposition from the mid-1900s. Eastern Canada, from Ontario to the Atlantic provinces, was once greatly affected by acid deposition due to its proximity to industries generating sulphate emissions (Lento *et al.,* 2011). These emissions coupled with the low buffering capacity of the pre-Cambrian shield have caused many lakes in the region to drop in pH (Lento *et al.,* 2011). Fortunately, as mentioned earlier, emission control programs and legislation have resulted in a drastic reduction in sulphate emissions. Lento et al. (2011) examined how lakes in central Ontario are recovering from acidic deposition. They observed that the percentage of Chironomidae in the microbenthic community has decreased over the study period in all but one lake (Lento *et al.,* 2011). Chironomidae are a tolerant Diptera family and therefore this finding tells us that the health of these lakes is improving. This finding was further supported by the increase in Ephemeropteran, Plecopteran and Trichopteran species (or EPT index values) over the study period (Lento *et al.,* 2011). The EPT index looks at macroinvertebrates that are known to be relatively sensitive to pollutants and harsh conditions. Therefore, an increase in EPT and a decrease in Chironomidae suggest that lakes are improving in terms of their water quality/health (Lento *et al.,* 2011).

1.4 Macroinvertebrates

In lentic systems, macroinvertebrates most commonly remain in the littoral zone due to the presence of macrophytes, coarse woody debris, and other sources of refuge and food. Benthic macroinvertebrates (visible with the naked eye, with a length >0.25mm) include organisms belonging to various phyla and orders, and thus are extremely diverse (Gal *et al.,* 2019). Due to their diversity, it is important to understand the life history and feeding habits of particular taxa to understand how they interact with one another and ultimately how they are affected by changes in their local environment.

1.4.1 Order Coleoptera

The Order Coleoptera is a highly diverse order of the Phylum Arthropoda which includes 25% of all known animal species, and 40% of all known insects (Thorp and Rogers, 2015). The Coleoptera consists of the beetles, which are extremely diverse, living on almost all continents in a wide variety of habitats. The focus with respect to this study is on aquatic Coleoptera, which constitute 5% of the Order Coleoptera. An aquatic beetle is defined as a Coleopteran that has at least one life-history stage that is entirely dependent on an aquatic habitat (Thorp and Rogers, 2015). Many aquatic Coleoptera species found in the study area possess aquatic larval stages, where the species lives underwater as a larva, and then become terrestrial as an adult. However, there are also examples of Coleoptera in our study area that remain aquatic throughout their entire life cycle. It is estimated by evolutionary biologists that aquatic beetles have invaded water in at least ten different instances, causing considerable diversity in their adaptations to, and strategies for, living in water (Thorp and Rogers, 2015). Therefore, it is important to understand each family's individual strategies.

The Family Dytiscidae are predaceous diving beetles, with 173 aquatic genera, making it the largest aquatic beetle family. Adults and larvae usually reside in similar habitats; medium or small pools of streams or lentic habitats. Despite this, adults and the larvae rarely inhabit the same area. Larvae are generally elongated, with agile walking legs, urogomphi (projections found on the terminal abdominal segment) and large, sickle-shaped, hollow mandibles (Thorp and Rogers, 2015). Larvae are usually yellow or white, with some having dark markings. During development, these larvae pass through 3 instars or developmental stages; the first possesses a set of 'egg-busters' on their front clypeus and the last instar possesses functional spiracles on their lateral margin segments. Larvae carry out gas exchange by periodically raising the tip of their abdomens out of the water (Thorp and Rogers, 2015). The larvae can inject digestive enzymes into their prey through their hollow mandibles and they feed on the prey once it is sufficiently liquefied (Thorp and Rogers, 2015). Adults are also highly predaceous but lack this digestive enzyme ability. Prey for these beetles include mosquito larvae and small Crustacea. Dytiscidae larvae can be generalized into four habitats and predatory behaviour categories. These include swimmers, floaters, crawlers, and burrowers (Thorp and Rogers, 2015). Larval pupation commonly occurs near the waterline, either under stones or wood that the late instars dig under using their mandibles.

The Family Gyrinidae consists of the whirligig beetles. These beetles are found in oxygen-rich habitats, most likely because of the high oxygen requirements of the larvae's tracheal gills (Thorp and Rogers, 2015). Adult whirligig beetles are found in lentic and lotic systems, but the larvae commonly avoid habitats with turbulent water and dense macrophyte growth. Interestingly, these beetles can live at greater depths than other Coleopterans. Larvae are elongated, slender, cylindrical, and generally unpigmented (Thorp and Rogers, 2015). Their main source of food is soft-bodied insects living on the bottom of the substrate. These include chironomid larvae, odonate nymphs and tubificid worms. Larvae digest their food extra-intestinally with the aid of their hollow mandibles. In the larva's third instar, it leaves the water to pupate on plant stems or on the shore itself.

The Family Haliplidae are known as the "crawling water beetles" and are strictly water beetles throughout their entire life cycle. Both the larvae and adults live in mostly stagnant or slow-moving water. Larvae cannot swim and are often overlooked because of their highly effective camouflage among benthic algae. Larvae are entirely herbivorous, feeding on all sorts of filamentous algae and Characeans. They use their highly specialized mouthparts to suck out the contents of unicellular algae. Gas exchange is carried out via their tracheal gills while on the benthic substrate. The Family Hydrophilidae are known as the "water scavenger beetles". Their larvae are voracious predators with extra-oral (external) digestion and they feed on a wide variety of aquatic animals. Females have the unique characteristic of producing silk cocoons or silk cases that will serve to contain their eggs.

The Family Psephenidea are the water pennies, whose larvae are flattened with expanded lateral margins in the thoracic and abdominal segments that give the appearance of a penny. These larvae are "scrappers" that feed on detritus and algae (Thorp and Rogers, 2015). They are found in fast-flowing water and are very sensitive to pollution or eutrophication.

1.4.2 Order Diptera

The order Diptera is one of the most speciose insect orders in the world with over 159,000 species distributed globally (Thorp and Rogers, 2015). A minimum of 30 families in the order are aquatic Diptera from North America, showing great diversity in habitat selection by residing in almost all possibly aquatic habitat types (lakes, ponds, marshes, cold and hot springs, seepages, groundwater zones, and streams of any velocity) (Thorp and Rogers, 2015). Most larvae are free-living and actively move throughout their environment, but some are inactive and may be hidden in sediments, within saturated wood or inside silken tubes attached to the substrate (Thorp and Rogers, 2015). Diptera larvae are distinguished by a lack of jointed thoracic legs and fleshy long bodies, however, given these characteristics, the larvae can differ greatly among families (Thorp and Rogers, 2015). Due to this great diversity, only families that are commonly found in our study area are described below.

The Family Chironomidae is one of the most widespread and well-known families of dipteran larvae due to their considerable range of habitat and feeding preferences. Chironomidae can live in lentic, lotic, wood, terrestrial and even intertidal marine environments, and show a similar diversity in feeding strategies, which includes all aquatic functional groups (Thorp and Rogers, 2015). Chironomidae are known for their high resiliency, which makes these larvae common in various aquatic environments and often results in them being the first colonizers in areas following droughts or floods (Serra *et al.,* 2017). Several Chironomid species also possess haemoglobin analog, which gives them a high tolerance of low oxygen concentrations (Panis *et al*., 1995; Serra *et al.,* 2017). Chironomidae are one of the most used bioindicators of poor water quality globally due to the factors listed above (Mandaville, 2002).

Mosquitos belong to the Family Culicidae. Culicidae larva live in lentic habitats. They have a planktonic life and show a variety of feeding strategies: filterers, predators, and collector-gatherers (Thorp and Rogers, 2015). The family Dixidae are known as the "meniscus midges" and are seen inhabiting in lentic and lotic margins (Thorp and Rogers, 2015). Meniscus midges are grazers and collector-gatherers, feeding on plant material in the water column (Thorp and Rogers, 2015). Empididae is the family of the dance flies; these inhabit a wide range of lentic, lotic, and even terrestrial habitats (Thorp and Rogers, 2015). They are predators of other insects, showing a preference for Simuliids, and have a worldwide distribution (Thorp and Rogers, 2015). Muscidae is the family of the house flies. These common flies have aquatic larval species that inhabit lentic and lotic habitats, where they feed by collecting and gathering plant material or through predation on Limnophora, another genus of flies (Thorp and Rogers, 2015). The family Psychodidae includes the moth, and sand flies. These flies are scrapers and collector-gatherers and inhabit marshes, tree holes and shallow pools (Thorp and Rogers, 2015). The family Tipulidae is the family of the crane flies. These inhabit a wide variety of lentic and lotic systems and exhibit a variety of feeding strategies, from predators to shredders and collector-gatherers (Thorp and Rogers, 2015).

The typical development of Dipteran larvae consists of an egg, at least three larval stages followed by the pupal and adult stages (Thorp and Rogers, 2015). Larval development is usually highly dependent on environmental conditions, as seen with a lot of other benthic invertebrate larvae. The development time for Dipteran larvae can range anywhere from a few days to a few years and can be interrupted by diapause, which is also seen in Trichoptera larvae (Thorp and Rogers, 2015). Most lentic Diptera are associated with benthic substrates in the littoral zone (Thorp and Rogers, 2015). Many families (including Tipulidae and Tabanidae) inhabit saturated sediments and respire through spiracles, so they must remain near the surface in order to access the air above (Thorp and Rogers, 2015). To avoid this narrow living zone, many families have adopted strategies to overcome this, which include smaller body sizes and relative inactivity (Thorp and Rogers, 2015). Some Chironomids took this a step further by having a hemoglobin analog present, which enables them to take in oxygen even in near-anoxic environments (Panis *et al.,* 1995; Thorp and Rogers, 2015).

1.4.3 Order Ephemeroptera

Ephemeroptera is an order in the class Insecta and is commonly known as mayflies. The mayflies have a dominant larval stage that is always aquatic. All nymphs have 1 pair of antennae, three ocelli and 2 compound eyes, these structures help with identification to the order level (Barber-James *et al.,* 2008). The family Heptageniidae are flattened dorsoventrally which enables them to live underneath the stones and pebbles to avoid the rough currents of streams (Thorp and Rogers, 2015). Other families, like Baetidae and Siphlonuridae, are pisciform ("fish-like") and actively swim throughout their habitat, whether that be a lentic or lotic system (Thorp and Rogers, 2015). Along with these two common life history strategies, other mayfly families are highly adapted to a specific environment, like some members of the family Ephemeridae, that have developed mandibular mouthparts to loosen the substrate. and flattened legs for digging (Barber-James *et al.,* 2008; Thorp and Rogers, 2015).

Ephemeroptera larvae are known to commonly inhabit both lentic and lotic systems with most species feeding on detritus and periphyton (Thorp and Rogers, 2015). Mayflies can be categorized into two broad feeding categories; collectors, and scrapers. Collector mayflies are filter collectors; they use setae on their mouthparts to collect food (Leptophlebiidae) or use their forelegs to act as filters (Isonychiidae and Oligoneuriidae). The scraper mayflies predominately feed on periphyton, which they scrape and consume from the substratum surface or the surfaces of macrophytes. Mayfly larvae that inhabit lentic systems reside strictly in the littoral zone (Thorp and Rogers, 2015). In general, mayflies are negatively affected by a low pH, especially during their crucial emergence or egg hatching periods (Rowe *et al.,* 1988). Given the adults' narrow mating period of a few days to weeks, depending on the species, any delay in emergence can have significant consequences for their reproductive success (Thorp and Rogers, 2015). An increase in winter temperatures and a subsequent decrease in summer temperatures can greatly affect their life cycles (Thorp and Rogers, 2015; Barber-James *et al.,* 2008).

1.4.4 Order Hemiptera

The order Hemiptera are the 'true bugs'; they represent the fifth largest order within the class Insecta (Thorp and Rogers, 2015). Hemiptera includes terrestrial and aquatic true bugs. Most of the members of the order are terrestrial. The order initially evolved terrestrially before some families invaded aquatic habitats, and because of this, respiration, mating behaviour and the general physiology of the aquatic species are built on terrestrial plans and therefore show interesting adaptations (Thorp and Rogers, 2015). In temperate zones, aquatic Hemiptera have a univoltine life cycle, breeding only once a year. Their eggs are typically laid in the spring, and larvae develop during the summer and fall, with individuals overwintering as adults (Thorp and Rogers, 2015). Due to this cycle through the seasons, species can be susceptible to dramatic temperature changes, whether that is caused by climate change or events such as a sudden influx of water from a dam release (Idigoras Chaumel *et al.,* 2019). Some of the smaller taxa in the order, like water striders, water boatmen and backswimmers are multivoltine, producing multiple generations in a year (Thorp and Rogers, 2015). The majority of aquatic Hemiptera are predators, feeding on benthic invertebrates. As for any predator, this diet and lifestyle do make

Hemiptera predisposed to factors resulting in extinction. These include factors like dependence on particular prey species, small population sizes, large body size and a relatively low reproductive efficiency (Thorp and Rogers, 2015).

1.4.5 Order Megaloptera

The order Megaloptera belongs to the class Insecta and is composed of the Alderflies, Fishflies and Dobsonflies (Thorp and Rogers, 2015). All members of this order have aquatic larvae that are generalist predators of other aquatic invertebrates (Thorp and Rogers, 2015). These species are also known for their extended aquatic larval periods and brief terrestrial pupae and adult stages (Liu, 2018). Along with exclusively aquatic larvae, the Megaloptera are also characterized by a prognathous adult head and the broad area of the hind wing (Thorp and Rogers, 2015). The family Corydalidae has 6 species in Canada and is found in the Boreal Shield region of Ontario (Liu, 2018). The family Sialidae is also found in the Boreal Shield area of Ontario and live in both lentic and lotic habitats (Liu, 2018).

1.4.6 Order Odonata

Odonata is an order of flying insects consisting of dragonflies and damselflies. They are obligate carnivorous predators that feed on a wide variety of prey (Thorp and Rogers, 2015). Odonate larvae have roughly the same anatomy as adults, with a head possessing eyes, antennae, and mouthparts; 3 pairs of legs on the thorax and wing sheets in later developed instars (Cannings, 2019). The diet and microhabitats of the larvae vary with different families, but the distinct foraging modes amongst larvae can be divided into active, sedentary, visual, and tactile strategies (Thorp and Rogers, 2015). An active larva searches for prey by climbing or swimming. A sedentary larva stays motionless on the substrate, in a “sit-and-wait” mode until a suitable prey approaches. Visual larvae are dependent on eyesight for identifying and capturing prey and the larva depends on stimuli from mechanoreceptors on its legs, antennae and mouthparts to detect the presence of prey (Thorp and Rogers, 2015). It is important to note that some larvae can combine different feeding modes together while others remain in distinct groups (Thorp and Rogers, 2015). Odonata larvae are relatively abundant benthic macroinvertebrates because they can inhabit almost all types of freshwater habitats.

There are four distinct larval types: claspers, sprawlers, hiders, and burrowers. These terms indicate where they usually live, and their typical feeding strategies (Thorp and Rogers, 2015). Claspers exhibit thigmotaxis, where they secure their body on substrates like twigs or stones (Thorp and Rogers, 2015). To help with this, they usually possess “adapted flattened femora” (flattened first joint of the limb), which helps them to stay in place even in strong currents (Thorp and Rogers, 2015). Claspers usually live among woody detritus, roots or between stones in running water. Families that fall in the clasper category are Aeshnidae, Calopterygidae and Libellulidae (Thorp and Rogers, 2015). Sprawlers possess long legs that help support the body on, or in, a matrix of detritus or vegetation. Hiders cover their body with detritus and sit in cavities between stones or other submerged objects (Thorp and Rogers, 2015). Larvae that prefer to live among leaf litter usually have a dorsoventrally flattened body with long legs to help them spread out as flat as possible. Families that can be considered hiders include Macromiidae, Corduliidae, Libellulidae and some Gomphidae (Thorp and Rogers, 2015). Burrowers are like hiders; however, they have a compact body and execute digging movements with their legs to hide their body underneath the sediment (Thorp and Rogers, 2015). Most Gomphidae, all Cordulegastridae, Chlorogomphidae and some Libellulidae belong to this type of life form (Thorp and Rogers, 2015). Generally, you can tell where, in the sediment, a larva is living based on the length of its hind legs. Shallow burrowers in finer substrates have longer legs, whereas species digging in coarse sediment and deeper burrowers have shorter legs relative to the length of the abdomen (Thorp and Rogers, 2015).

1.4.7 Order Plecoptera

Order Plecoptera are known as stoneflies. Characteristics of their larvae include 2 antennae, cerci (paired sensory “pincer” structures attached to the abdomen) and 2 tarsal claws, the presence of gills and a drab body colour (Thorp and Rogers, 2015). Most stonefly species have larvae that are associated with cold waters and are at their most diverse in temperate mountain areas (Thorp and Rogers, 2015). In North America over recent years, we have seen significant radiation of species into warmer-water streams. Most notably this can be seen in the family Perlidae, which have a greater gill surface area, allowing sufficient gas exchange despite the lower oxygen content of warmer waters (Thorp and Rogers, 2015). Most species of the order Plecoptera occupy fast-flowing, riffle areas of streams where coarse mineral substrates dominate the stream bed (Thorp and Rogers, 2015). Stoneflies are very environmentally sensitive and are considered among the most sensitive freshwater invertebrates (Mandaville, 2002). This makes them valuable indicators of water quality. Stoneflies as mentioned above, have a high oxygen requirement and thus are relatively intolerant of high temperatures.

1.4.8 Order Trichoptera

The order Trichoptera are caddisflies whose larva appear caterpillar-like, with an elongated and cylindrical body form. During development, these larvae usually undergo 5 larval instars (Thorp and Rogers, 2015). Feeding strategies vary considerably amongst different families, some with very specific strategies and others being omnivores. Generally, larvae feed on algae, decomposing and living plant tissue and other aquatic invertebrates (Thorp and Rogers, 2015). The families Rhyacophilidae and Hydrobiosidae, the free-living caddisflies, are primarily predators that actively hunt other aquatic invertebrates for food (Thorp and Rogers, 2015). In addition to these families, predation is widespread in the order Trichoptera, with some Polycentropoids and Hydrosychids even using their silken nets to trap invertebrate prey (Thorp and Rogers, 2015). Leptoceridae larvae are also predaceous and have adapted sharply toothed mandibles to aid in their lifestyle (Thorp and Rogers, 2015). Herbivory is also common in Trichoptera and includes various adapted strategies. Scraper species have specialized mandibles to scrape periphyton off surfaces (Thorp and Rogers, 2015). Other strategies include piercers, with specialized mandibles to pierce plant tissue or cells to suck fluid, and chewer-shredders, who have strong mandibles that bare teeth for chewing living macrophyte tissue (Thorp and Rogers, 2015). Scrapping and grazing are exclusive in the families Glossosomatidae, Helicopsychidae, Apataniidae, Goeridae and Cenoidae, and occur in select members of Hydroptilidae and Leptoceridae. (Thorp and Rogers, 2015). Trichoptera larvae respiration is cutaneous and occurs across the soft body surfaces (Thorp and Rogers, 2015). Some species also possess filamentous gills that are located on the abdomen (Thorp and Rogers, 2015). In waters with high temperatures, and consequently low dissolved oxygen, larvae tend to have more gill filaments than conspecifics in lower temperatures (Thorp and Rogers, 2015). The order Trichoptera can enter a state of diapause in response to recurring periods of difficult environmental conditions (Thorp and Rogers, 2015). The most common environmental condition inducing diapause in Trichoptera larvae is photoperiod, in terms of shorter daylight hours. Diapause causes a suspension of normal development, which delays metamorphosis and adults do not emerge until fall (Thorp and Rogers, 2015). When emergence occurs in this case, the adults' eggs are close to fully developed and thus are laid a few days later. Due to this delay in maturation and egg-laying, diapause can function to synchronize oviposition periods in fall

(Thorp and Rogers, 2015). Trichopteran larvae represent an important component of lake and river ecosystems. They serve an important role in nutrient processing and energy flow, as well as being an important food source for a variety of aquatic predators (Mandaville, 2002). Some Trichoptera larvae can produce silk, which is mainly used in the construction of cases, feeding or the production of pupal shelters/cocoons (Gal *et al.,* 2019). Case-making caddisflies use silk to bind together material to construct their cases, which act as an area of refuge from predators by camouflaging with the surrounding structures (Gal *et al.,* 2019). Most of the threats faced by caddisflies are related to the impacts of human activities on the aquatic ecosystems (Thorp and Rogers, 2015). These anthropogenic threats include point-source discharge of pollutants, agricultural and urban pollution, and changes in water flow regimes resulting from control points like dams or dikes (Thorp and Rogers, 2015).

1.4.9 Order Decapoda

The order Decapoda is a large crustacean order that includes shrimp, crayfish, lobster, and crab. In our study area, the family Cambaridae is by far the most abundant of the Decapods in the area and is the most diverse freshwater crayfish family found in North America (Thorp and Rogers, 2015). The family Cambaridae diverged from their marine ancestors around 239 million years ago. Crayfish occupy four main habitats in the natural environment, of which well-oxygenated rivers, streams and lakes are the most common, in both cool and warm water habitats (Thorp and Rogers, 2015). Other common habitats include poorly oxygenated cool and warm water habitats like vegetated eutrophic ponds, wetlands, water ditches and backwaters of streams (Thorp and Rogers, 2015). Crayfish are also capable of inhabiting burrows and caves, but this is less common. Stream and lake-dwelling cambarid crayfish are strongly associated with cervices among stones, weed beds, leaf litter, brush piles, log jams, and roots of riparian trees (Thorp and

Rogers, 2015). This is because these areas offer a suitable level of protection from predation and provide food sources (Thorp and Rogers, 2015). Due to this habitat preference, the lake-wide population density of crayfish is highest in the littoral zone of lakes (Thorp and Rogers, 2015). The freshwater crayfish diet is composed of allochthonous and autochthonous organic matter, dead and living animals, macrophytes and microorganisms (Thorp and Rogers, 2015). Feeding is done by collecting material from the substrate using their mandibles and maxillipeds (Thorp and Rogers, 2015). If given a choice, crayfish will commonly prefer to consume animal tissue over plant material, and juveniles consume a higher proportion of animal material than do adults (Thorp and Rogers, 2015). Adults usually feed on more plant material, like benthic algae, vascular plant material and detritus (Thorp and Rogers, 2015).

1.4.10 Isopods, Amphipods, and Hirudinea

The Isopods are an order of the Malacostracan crustaceans that are commonly known as sowbugs. Isopods are ecologically important to benthic communities due to their high trophic level. By consuming plant and animal material, Isopods transfer and store metabolic energy in trophic systems and accumulate toxins from the water and sediment (Plahuta *et al.,* 2017). Freshwater Isopods are sediment-dwellers and are in constant contact with the substrate. Due to this, Isopods are in constant contact with contaminants from water and sediment. Isopods are, however, considered robust organisms that can readily tolerate changes in pH and dissolved oxygen concentrations (Plahuta *et al.,* 2017). They are considered to have intermediate sensitivity to contaminants but show negligible biotransformation (Plahuta *et al.,* 2017). Biotransformation is the process of an organism slowly accumulating contaminants (e.x. metals, contaminants, etc.) through bioaccumulation, and then transferring potentially hazardous levels of these contaminants when consumed by another organism (Cain *et al.,* 2003).

The Amphipoda, commonly known as scuds, is another order of malacostracan crustaceans (Birmingham *et al.,* 2005). These invertebrates have no carapace and are mostly detritivores or scavengers (Thorp and Rogers, 2015). Amphipoda bear three pairs of swimmerets on the abdomen, have a linearly flattened body, and offspring undergo direct development; that is, they do not have a larval stage, and eggs are retained by the female until juveniles hatch and are released (Vainola *et al.,* 2008). Epibenthic taxa are the most diverse and commonly found type of Amphipoda in our study area (Vainola *et al.,* 2008). Amphipoda are abundant and ecologically important members of soft-bottom benthic communities (de-la-Ossa-Carretero *et al.,* 2012). Despite their niche specificity, in general, Amphipoda are tolerant of varying physicochemical characteristics in sediment and water (Thorp and Rogers, 2015). Due to their low dispersion and mobility, these animals live in direct contact with the sediment and thus are exposed to whatever contaminants are in the water and those absorbed into the sediment. Amphipoda are sensitive to pollutants and toxicants and are considered more sensitive to these substances than the other benthic invertebrates (Thorp and Rogers, 2015). Amphipoda are also known to have a general sensitivity to sewage contamination and therefore has been shown to decrease in abundance and diversity near areas of discharge (Vainola *et al.,* 2008). This decrease in abundance and diversity was related to a decrease in redox potential near the sewage outfall (Vainola *et al.,* 2008). Lower redox potential has significant effects on the environment; it creates a greater demand for oxygen in sediments and can release toxins, thereby stressing plant and animal life (see Pezeshki & DeLaune, 2012).

The order Hirudinea of Phylum Annelida is known as the leeches. These infamous freshwater predators and ectoparasites inhabit lakes, swamps, rivers, streams, and even moist soils (Thorp and Rogers, 2015). Leeches are most abundant along the shallow vegetated shoreline of lakes or large rivers (Kazanci *et al.,* 2015). They possess two suckers; one on the anterior end, and the other on the posterior end of their soft, worm-like bodies. The anterior sucker can be prominent or simple, consisting of expanded lips while the posterior sucker is generally directed ventrally and is wider than the body at the point of attachment to the substrate (Thorp and Rogers, 2015). Given the leech's diverse habitat selection, they inhabit a wide range of physicochemical conditions and therefore show a high level of physiological plasticity (Thorp and Rogers, 2015). Leeches also show a level of conformity with respect to dissolved oxygen, with their oxygen uptake usually being proportional to whatever the oxygen concentration of the water is (Thorp and Rogers, 2015). Many leech species can survive in anoxic conditions for more than 60 days and in hyperoxia for a considerable amount of time as well (Thorp and Rogers, 2015). Due to their high level of conformity to various conditions, it is said that a large presence of leeches in a habitat, with few other species, can indicate extremely poor habitat quality (Thorp and Rogers, 2015). Research has even shown that leeches may even prefer polluted environments over areas of higher water quality (Kazanci *et al.,* 2015).

1.4.11 Order Gastropoda and Pelecypoda

The order Gastropoda is the single shelled member of the Phylum Mollusca. The family Lymnaeids are the most diverse pulmonated group in the northern US and Canada. These freshwater snails have large teeth on their radula which are useful for cropping strands of filamentous algae (Thorp and Rogers, 2015). The Lymnaeids are scrapers, which feed on algae (Thorp and Rogers, 2015). The Pulmonates are a family of Gastropods that commonly live in slow-moving, silty habitats (Thorp and Rogers, 2015). These Gastropods use aerial respiration and have shells with high drag coefficients; this allows them to fit well in this specific environment (Thorp and Rogers, 2015). Pulmonates are oviparous and reproduce once and die with their entire life cycle occurring over the course of a year (Thorp and Rogers, 2015). The family Physidae is another family found in the area and has a worldwide distribution (Thorp and Rogers, 2015). These Gastropods have small shells that are sinistral with raised spires (Thorp and Rogers, 2015). This unique, thin shell shape is most likely an adaptation to deter shell-invading predators like crayfish (Thorp and Rogers, 2015). Their tentacles and foot are slender and their radular teeth are smaller and more complicated than the Lymnaeids. With the help of these teeth, the Physidae are better at harvesting tightly attached periphyton species like diatoms and detritus (Thorp and Rogers, 2015). This family also has a faster rate of crawling, which helps with dispersal, and this coupled with their early maturity and high feeding productiveness allows Physidae to be widespread (Thorp and Rogers, 2015). Planorbids are another widespread and diverse family of snails found in this area. Planorbids possess a planospiral shell which ranges from 1 to 30mm (Thorp and Rogers, 2015). This group also possesses hemoglobin, used as a respiratory pigment, which gives them their characteristic reddish colour –in contrast to the blue colour of snails containing hemocyanin as the respiratory pigment (Thorp and Rogers, 2015). The Planorbids are detritivores and feeds on bacteria (Thorp and Rogers, 2015). Gastropod habitat selection appears to be heavily based on shell shape, life history and physiology, which explains why different families exhibit different adaptations to microhabitats (Thorp and Rogers, 2015). Research has shown that as lake area, watershed area and lake order increase, Gastropod diversity also increases (Thorp and Rogers, 2015). Lake Order (LO) measures landscape position in terms of increasing numbers of contributing streams (Martin and Soranno, 2006). This is most likely due to the presence of a wide range of habitats in which different species of Gastropods can live, while avoiding non-favourable conditions (Thorp and Rogers, 2015). Water hardness and pH are considered to be the major factors that determine the distribution of freshwater snails (Lento *et al.,* 2008).

The order Pelecypoda is a diverse order of the Mollusca which includes the mussels and clams. The Unionoidae are freshwater mussels with an interesting parasitic life cycle that is unique to bivalves (Thorp and Rogers, 2015). The larval stage of Unionoidae is known as the glochidium. Once released through the excurrent aperture, they attach to a host (typically the gills of a fish) (Thorp and Rogers, 2015). This relationship allows larvae to travel distances much greater than they could travel on their own. After a few days, the glochidia detach from the fishes' gills and drift to the substrate, where they settle as juvenile mussels (Thorp and Rogers, 2015). Freshwater mussels spend their entire life either partially or entirely buried in mud, sand, or gravel in just about any habitat that has permanent water (Thorp and Rogers, 2015). Freshwater mussels are commonly found in lotic waters, but some species are commonly found in lakes. As freshwater mussels are sessile filter-feeders, they are susceptible to negative effects of sewage effluent or other forms of anthropogenic pollutants (Thorp and Rogers, 2015).

Sphaeriidae are another family found in our study area and are known as fingernail clams. These clams have direct development, so the external appearance of juveniles is as miniature versions of their adult form (Thorp and Rogers, 2015). Sphaeriids have the capacity for fertilization and birth year-round, but these processes are usually influenced by environmental variables (Thorp and Rogers, 2015). Fingernail clams are found in the same habitats as freshwater mussels but are more capable of living in ephemeral habitats such as temporary ponds (Thorp and Rogers, 2015).

In general, freshwater bivalves are found in water with near-neutral pH, although they can live in ranges of 5.6 to 8.3 (Thorp and Rogers, 2015). Acidic conditions can erode their calcareous shell and cause considerable mortality (Lento *et al.,* 2008).

1.5 Benthic Macroinvertebrates and Bioassessment

A critical aspect of bioassessment of a waterbody by sampling benthic macroinvertebrates is understanding the tolerance of these various types of macroinvertebrates. When we speak of 'tolerance' with respect to bioassessment, we are talking about the animal's tolerance of persistent organic pollutants (POPs). Since we are identifying specimens to the family level in this study, we will be using tolerances calculated at the family level (see Table 1, Appendix I). These family tolerance values were developed by weighting species according to their relative abundance and individual tolerance capabilities, which are summarized as a single value for the family. These tolerance values range from 0 to 10 for families. These values increase as the quality of the water decreases (Hilsenhoff, 1988) (see Table 2, Appendix I). A protocol manual developed by Mandaville (2002) summarizes the tolerance values determined by various authors in the 1980s and 1990s, in order to make the data readily understandable to future researchers (see Table 1.1).

1.5.1 Biotic Indices

Once sampling of benthic macroinvertebrates has been carried out and specimens have been identified, data are usually arranged or transformed into a biotic index. These biotic index systems have been created to assign a numerical score for specific benthic organisms that are known as indicators (Mandaville, 2002). Indicator organisms have specific environmental and physical requirements, so the presence or absence, abundance and behaviour can all be examined to see if those requirements are being met (Mandaville, 2002). Biotic indices use a combination of these indicator species that share common requirements to evaluate the system from which they were collected. For example, the EPT index is a commonly used biotic index to assess waterbodies. The EPT index stands for Ephemeroptera, Plecoptera and Trichoptera, which are insect groups that are all considered to be sensitive to pollution (Mandaville, 2002). The EPT index is calculated by determining the percent of all specimens collected that belong to these three taxa (Jones *et al.,* 2017). Therefore, the higher the number generated by the EPT index, the higher the water quality. For lake sampling, Plecoptera are not very common, therefore, a more useful index of water quality is the EOT. This index substitutes Plecoptera for Odonata, which are much more prevalent in lake systems and share similar tolerance levels (Jones *et al.,* 2017).

CIGH is another biotic index used in bioassessment, whose principles are based on fundamental macroinvertebrate knowledge. CIGH stands for Corixidae, Isopods, Gastropods and Hirudinae (leeches), which are all macroinvertebrates that are considered tolerant to organic pollution. Therefore, if a large percentage of the sample is composed of these taxa, we could conclude that the sample originated from a poor-quality environment.

Taxa richness is another commonly used biotic index for macroinvertebrates. Taxa richness indicates the health of an aquatic community by its diversity. As diversity increases, it is assumed that habitat diversity, suitability and water quality all increase with it. Therefore, a high taxa richness value would indicate a sample area of high water quality (Mandaville, 2002). The total diversity of a sample is calculated using the total number of different macroinvertebrate families represented in a sample (Mandaville, 2002; Paller *et al.,* 2020). For our data, richness will be standardized, so richness scales with sample size. This is required because of varying sample sizes from our lakes. For the purpose of our study, taxa richness refers to the richness of different macroinvertebrate families present. Identification and richness calculations were performed at the family level to aid in the identification process, and because genus and family richness generally correlate well with species richness in a sampled community (Paller *et al*., 2020).

1.5.2 Benthic Macroinvertebrates as Tools for Bioassessment

Freshwater research in Canada has an extensive history in the use of macroinvertebrates to assess the health of aquatic ecosystems. This form of ecological assessment grew in popularity in the 1980s and has become more widespread in national research as more knowledge has been gained on the subject. Once researchers and government institutions saw the possibility and benefits of sampling environments using macroinvertebrate samples, the Canadian Aquatic Biomonitoring Network (CABIN) was created. CABIN is a national biomonitoring program developed by Environment Canada which is used to standardize the biomonitoring sampling protocols and data analysis to ensure consistent sampling methods and comparable data (CABIN, 2011). Benthic macroinvertebrates are used as tools for bioassessment for a variety of reasons. Firstly, macroinvertebrates are generally sedentary and long-lived, so they stay put in their environment for a relatively long period of time, allowing them to integrate with whatever conditions are present (Mandaville, 2002). Macroinvertebrates are also ubiquitous and rich in numbers, so their sampling can be carried out across many different habitats with, generally, great success (Mandaville, 2002. Benthic macroinvertebrates are also stored relatively easily. When submerged in ethanol, macroinvertebrates remain relatively consistent in their appearance and anatomical structures (OBBN, 2011). Due to these factors, macroinvertebrates remain the most common organisms sampled to assess the health of an ecosystem.

1.6 Specific Aims and Research Rationale

1.6.1. Research Objectives and Questions

This research will address the current gap in terms of recent research on macroinvertebrate communities and water quality in the lakes of central Ontario, as well as examining how cottage development has affected these ecosystems by using a variety of sampling and analyses to address the following objectives:

1. Provide baseline knowledge of macroinvertebrate communities and the water quality of interior lakes in central Ontario. In addition, examine the general effect these water quality variables have on macroinvertebrate communities in the region.
2. Directly compare developed and isolated lakes to assess the effect cottage development has had on freshwater lakes in Ontario.

Based on these objectives, numerous research questions arise:

1. What is the typical macroinvertebrate community composition of inland lakes in central Ontario?
2. Does macroinvertebrate community composition differ between lakes experiencing cottage developmental activities compared to isolated lakes with little or no cottage development?
3. Does the water chemistry of central Ontario inland lakes change with cottage development?

1.6.2 Research Rationale

A review of the literature shows that there are limited studies and little data on the macroinvertebrate communities of interior lakes in central Ontario; there are also very few recent studies that compare lakes adjacent to developed and un-developed ecosystems.

A report by Gal *et al.* (2019) provided an evidence-based overview of studies that have assessed macroinvertebrate communities to examine the effects of urbanization. This was done by reviewing 197 papers on the subject, to examine the outcomes of how human developmental activities influence the diversity of freshwater macroinvertebrate communities (Gal *et al.,* 2019). This review found that macroinvertebrates were indeed the group most often used for bioassessment (Gal *et al.,* 2019). The authors also reviewed where these assessments were being carried out, with 90% of studies being done on stream habitats and 10% on pond habitats (Gal *et al.,* 2019). Interestingly, this review found that lake communities had been completely ignored, with 0% of the studies reviewed being carried out on lake habitats (Gal *et al.,* 2019). The authors also examined how each study presented their results; over 95% of studies relied entirely on an examination of taxon richness (Gal *et al.,* 2019). This means that, despite the large number of bioassessments that were carried out, there was little variation in methodology or approach between the studies. While this does allow data from different studies to be readily compared, it prevents the analysis of different types of environments or distinctive aspects of the systems being studied. Given the ecological, economic and cultural importance of lakes, it is surprising that there has been little focus on macroinvertebrate communities as a tool to assess the impacts of urbanization on lakes in central Ontario. In the present study, I hope to address this gap, by focusing entirely on lentic ecosystems to assess the impacts of cottage development on lake systems. I will use a variety of biotic indices, rather than just the simple metric of Taxa Diversity to examine the health of lake systems. Thus, this study should provide a better understanding of how community composition of benthic invertebrates changes in lentic systems when they are exposed to the effects of human settlement and land development.

Chapter 2. General Methodology

2.1 Study Location

The town of Kearney, Ontario (45°3320 N 79°1327 W) was selected as the study area because this watershed includes several lakes both with, and without cottage development activities. In addition, the town of Kearney has a large "cottage population" compared to its population of permanent residents. According to the Government of Canada census data for 2021, a total of 1195 private dwellings occupied the town. Of these, only 460 were considered occupied by 'usual residents' (Statistics Canada, 2022). This region has also seen an increase in the number of cottages in recent years, increasing from 588 non-permanent residential homes in 2011 to 735 in 2021 (Statistics Canada, 2022).

Lake selection was made using a wide variety of resources. Google Earth was used to visually identify possible sampling locations and their accessibility (Google Earth, 2022). Lake fact sheets created and distributed by the Ministry of Natural Resources were used to determine which lakes were deemed "isolated" versus "populated" using the crown land cover percentage value (MNR, 2021). Thus, lakes with more than 70% crown land cover (where cottage development is not permitted) were classified as isolated lakes, while lakes with less than 70% percent crown land cover were classified as populated lakes. However, no isolated lake has a crown land cover percentage less than 80% and no populated lake has a crown land cover percentage higher than 30%, so it is assumed that these lakes are indeed experiencing very different intensities of anthropogenic impacts (MNR, 2011).

Another major determining factor in lake selection, especially for the isolated lakes, was accessibility. Several 'isolated' lakes in the area have little to no means of access, with many of the isolated lakes eventually chosen being only accessible via narrow ATV or snowmobile trails.

Local geology was also taken into consideration when selecting lakes for sampling. The central region of Ontario has various types of underlying geology that would affect bedrock type and weathering rates, and thus local water chemistry. In order to study this possible effect, lakes with the two most common types of geology in the area, Felsic Igneous and Migmatite rocks, were selected.

After carefully considering all lakes in the area with their variety of characteristics, 6 isolated and 6 populated lakes were selected for sampling (Figure 1). The isolated sites sampled were Buck Lake, Spruce Lake, Bluesky Lake, Tea Lake, Charlie Mann's Lake and Mining Cooperation Lake (see Figure 1, isolated are coloured in blue). The 6 populated lakes sampled were Beaver Lake, Perry Lake, Sand Lake, Loon Lake, Lower Raven Lake and Grass Lake (see Figure 1, populated are coloured in yellow).

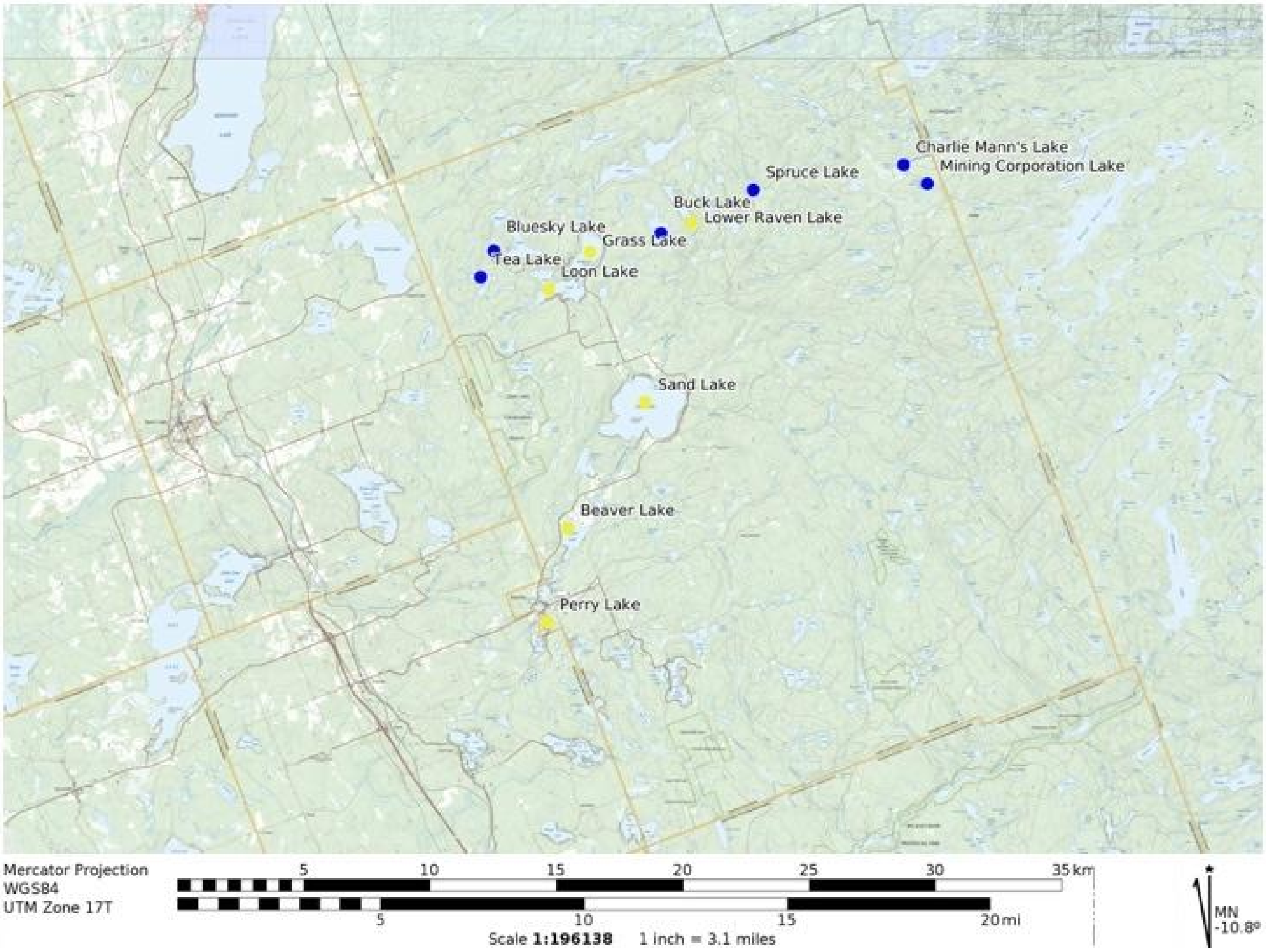

Figure 1. Map of sampling sites in the town of Kearney, District of Parry Sound, Ontario. Map generated using the program Caltopo (2021). Blue pins illustrate isolated lakes and yellow pins illustrate populated lakes. Town of Kearney boarder represented by yellow box encompassing study sites.

2.2 Sampling Site Descriptions

Most of the lakes in the study area are located within the Magnetawan River watershed. This is a large watershed north of Huntsville, Ontario, that begins at Magnetawan Lake in Algonquin Park and runs east to west, eventually draining into Georgian Bay. Ten of our 12 study lakes are located within the Magnetawan River watershed. Tea and Bluesky Lake are located along the border of the watershed, cut off by a single hill. Therefore, these lakes remain exposed to similar effects in terms of underlying geology, aerial deposition, etc. as the other lakes located in the watershed. All lakes are located within, or in very close proximity to, the town of Kearney. Located 36 km north of Huntsville, Kearney lies in the southeast portion of the Almaguin Highlands and encapsulates an area of 529 square kilometers (Town of Kearney, 2021). With a population of only 882 year-round residents, the town is predominantly made up of dense forests and lakes, with a heavy cottage population during the summer months and with high snowmobile traffic in winter (Town of Kearney, 2021). Ice Fishing is prevalent in the area during winter as well which is also associated with snowmobile activity. We were unable to access information regarding the lake order of each lake sampled and therefore did not estimate lake order, due to the inaccuracy of estimating from satellite imagery, especially in such a dense collection of lakes. For summary of study lake characteristics see Appendix Table 1.

2.2.1 Developed Lakes: Site Descriptions

Site 1: Beaver Lake

Beaver Lake is a 295-ha medium depth lake located in the town of Kearney at the coordinates 45.630 N 79.723 W. Beaver Lake has a maximum depth of 10 meters, a mean depth of 4.4 meters and a perimeter of 19 km (Ministry of Natural Resources, 2017). Beaver Lakes drainage basin is the Lower Magnetawan River and it lake order is This lake has a crown land cover percentage of only 2%, meaning it has a high degree of shoreline development that includes both commercial and residential structures (Ministry of Natural Resources, 2017). The surrounding watershed has a drainage area of 65.7 km^2 and is mostly composed of dense woodland but there is a small farm with some agricultural land on the northeast shore, which appears to be cropland. The water level of Beaver Lake is regulated by two MNR-operated dams, and the water from Beaver Lake flows directly into Perry Lake located to the south in the centre of the town of Kearney which is only 2.5 km away. A relatively busy, paved road runs along the west and north shorelines of the lake. A boat launch on the north-west shoreline provides public access. Major fish species include northern pike, smallmouth bass, walleye, black crappie, and lake whitefish (Ministry of Natural Resources, 2017).

Site 2: Perry Lake

Perry Lake is a 68-ha lake located in the heart of the town of Kearney at the coordinates 45.546 N 79.227 W. Perry Lake has a maximum depth of 13 m and sits at 335 m above sea level (Ministry of Natural Resources, 2017). This lake has a crown land cover percentage of 0% with a high level of urban, commercial, and residential development (Ministry of Natural Resources, 2017). The watershed area is 382 km^2 and since Perry Lake is surrounded by the town of Kearney itself, it is surrounded by a lot more impermeable surfaces compared to other lakes in the area. Therefore, it is reasonable to say that this lake will experience a much higher level of surrounding surface runoff influent. Multiple boat launches and public beaches are located on the shores of Perry Lake. Major fish species include brook trout, lake whitefish, smallmouth and largemouth bass, northern pike, and walleye (Ministry of Natural Resources, 2017). The drainage basin of Perry Lake is the Magnetawan River.

Site 3: Sand Lake

Sand Lake is a 580-ha, deep lake that is one of the largest in the area. It is located in the town of Kearney at the coordinates 45.6272 N 79.1719 W and sits at 339 m above sea level (Ministry of Natural Resources, 2017). Due to its large size and proximity to neighbouring towns and highways, Sand Lake is a very popular cottage and resort lake destination. Two resorts are located on the shoreline of Sand Lake; one has a large dock with a boat launch, as well as a large, manicured sand beach with few remaining macrophytes. The 12.2 km perimeter is highly developed both commercially and residentially, resulting in 0% crown land cover remaining around the lake (Ministry of Natural Resources, 2017). The surrounding watershed is measured at 242 km^2, and is mostly composed of dense woodland, with some cottages, farming, logging operations and hills encapsulating the region. The water level of Sand Lake is not regulated by any dams. Major fish species include lake trout, lake whitefish, brook trout, northern pike, rainbow trout, burbot, smallmouth and largemouth bass (Ministry of Natural Resources, 2017).

Site 4: Loon Lake

Loon Lake is a 156-ha lake with high cottage development, located in the town of Kearney at the coordinates 45.669 N 79.219 W. Loon Lake has a mean depth of 11m with a maximum depth of 29m (Ministry of Natural Resources, 2017). The lake sits at 412 m above sea level and has a 37 km^2 watershed surrounding it. This consists mainly of dense woodland, cottages, dirt roads, ATV trails and rolling hills. The 8 km perimeter of the lake is mostly inhabited by seasonal cottages, with residential shoreline development being the dominant development surrounding the lake. The lake is surrounded by only 10% of crown land (Ministry of Natural Resources, 2017). A dam located on the south-east corner of the lake regulates water level through the Magnetawan River Water Management Plan (Ministry of Natural Resources, 2017). Numerous boat launches are to be seen, both on the north and south ends of the lake. Major fish species include lake trout, brook trout, round whitefish, smallmouth bass, burbot, white sucker, and some presence of rainbow smelt (Ministry of Natural Resources, 2017).

Site 5: Grass Lake

Grass Lake is a popular cottage lake in the town of Kearney with a surface area of 138-ha at the coordinates 45.679 N 79.203 W. It has an average depth of 11m and a maximum depth of

37m, lying 372 m above sea level (Ministry of Natural Resources, 2017). Located just north-east of Loon Lake, the two lakes are connected via a small channel, that boaters and wildlife can readily cross. Grass Lake has a considerable amount of cottage development, with only 2% crown land cover and a high degree of residential shoreline development (Ministry of Natural Resources, 2017). The lake has a perimeter of 6.4 km and has a watershed area of 28 km^2 (Ministry of Natural Resources, 2017). The surrounding area is mostly composed of dense woodland, dirt and paved roads, large hills, and other lakes. Major fish species include lake trout, smallmouth bass, burbot, yellow perch, brook trout, lake whitefish and northern pike (Ministry of Natural Resources, 2017). The inlet of Grass Lake is controlled by a MNR owned dam, the drainage basin for this lake is the Magnetawan River (Ministry of Natural Resources, 2017).

Site 6: Lower Raven Lake

Lower Raven Lake is a 39-ha lake located in the town of Kearney at the coordinates 45.692 N 79.151 W (Ministry of Natural Resources, 2017). Lower Raven Lake has an average depth of 6 m and a maximum depth of 16 m (Ministry of Natural Resources, 2017). Although it lies directly east of Buck Lake, one of the "undeveloped lakes" in the area (see below) this lake is slightly different, with more residential development, and only 30% crown land cover (Ministry of Natural Resources, 2017). Its small 3 km perimeter is mostly developed, but these regions are still on the low end of shoreline and residential development. The public does have access, but most of the access points are via narrow and rough dirt roads, making it more isolated to the public than the lakes described above, that have better accessibility. The surrounding watershed is only 3.4 km^2 in area, and comprises dense woodland, dirt roads and small cottages. The major fish species present in Lower Raven Lake are yellow perch, brook trout, lake trout and splake (Ministry of Natural Resources, 2017).

2.2.2 Isolated Lakes: Site Descriptions

Site 7: Buck Lake

Buck Lake is a secluded 96-ha lake in the town of Kearney at the coordinates 45.6900 N 79.1672 W. It has an average depth of 14 m and a maximum of 46 m. It is only accessible by rugged narrow dirt roads, so the main form of development on this lake is recreation camps (hunting cabins/camps), which are still relatively low in impact. The perimeter of 8.4 km is mostly crown land, with an 80% crown land cover (Ministry of Natural Resources, 2017). The surrounding watershed is a small 2.4 km^2 area due to hills surrounding the local area, making the lake somewhat isolated not only in terms of human factors, but also by environmental factors (Ministry of Natural Resources, 2017). The major fish species is lake trout (Ministry of Natural Resources, 2017).

Site 8: Spruce Lake

Spruce Lake is an isolated lake with 2 small cottage developments in the town of Kearney at the coordinates 45.703 N 79.118 W. Spruce Lake has a surface area of 20-ha with a mean depth of 8m and a maximum depth of 16 m (Ministry of Natural Resources, 2017). This lake is only accessible via a crown land trail branching off a neighbouring dirt road; however, this trail, which runs close to the shoreline, is used year-round by ATVs and snowmobiles. This could be a possible source of pollution along with the 2 small cottages. The crown land cover of 97% is composed of dense woodland with small wetlands and large hills in the distance (Ministry of Natural Resources, 2017). The watershed for this lake is only 4 km^2 in area and is located 482 m above sea. The major fish species present in Spruce Lake is brook trout (Ministry of Natural Resources, 2017).

Site 9: Bluesky Lake

Bluesky Lake is a small lake located in the town of Kearney at 415 m above sea level and at the coordinates 45.748 N 79.178 W. Bluesky Lake is relatively small and shallow, with an area of 33 ha, a mean depth of 4 m and a maximum depth of 8 m. This lake does not allow motorized boats (according to signage at lake property) to access the water and is said by locals to have some of the best water quality in the area. With a perimeter of 4 km, there are a few cottages around the lake, but it still has a 95% crown land cover (Ministry of Natural Resources, 2017). The surrounding watershed has an area of 11.4 km^2 and is mostly composed of woodland, some wetland-like areas to the south-west, and hills of crown land (Ministry of Natural Resources, 2017). The major fish species present in Bluesky Lake is brook trout (Ministry of Natural Resources, 2017).

Site 10: Tea Lake

Tea Lake is a secluded lake located in the town of Kearney at an elevation of 412 m above sea level, at the coordinates 45.672 N 79.255W. Tea Lake has an average depth of 5 m, a maximum depth of 19 m and a lake area of 34-ha (Ministry of Natural Resources, 2017). Tea Lake is accessible via narrow ATV trails, and has a low level of shoreline development with only a few recreational camps (hunting camps) and a crown land cover of 95% (Ministry of Natural Resources, 2017). ATV and snowmobile trails near the lake are used year-long and could serve as a pollution source. The major fish species are brook trout and lake trout (Ministry of Natural Resources, 2017).

Site 11: Charlie Mann's Lake

Charlie Mann's Lake is a secluded lake in the District of Parry Sound and within a few kilometers of the western border of Algonquin Park at coordinates 45.712 N 79.042 W. This lake has an area of 8 ha, an average depth of 3 m and a maximum depth of 6 m (Ministry of Natural

Resources, 2017). A dirt logging road with a short bridge spanning a channel is the only form of development near the lake, as it has 100% crown land coverage (Ministry of Natural Resources, 2017). The 1.5 km long perimeter is undisturbed and possesses dense macrophyte vegetation, dense tree stands and wetlands. The major fish species found in Charlie Mann's Lake are yellow perch and brook trout (Ministry of Natural Resources, 2017).

Site 12: Mining Corporation Lake

Mining Corporation Lake, despite its name, is an isolated and relatively untouched 7-ha lake located near the western edge of Algonquin Park, in the District of Parry Sound, at coordinates 45.705 N 79.303 W. The lake has a mean depth of 3 m and a maximum depth of 10 m with a very small watershed of only 0.5 km^2 (Ministry of Natural Resources, 2017). The only form of development near this lake is an ATV/snowmobile trail that runs along the northern edge of the lake, this trail also serves as the only point of access. Logging roads, with some still being active, are in the region of the lake, but given its small watershed area, these should not affect the lake itself. The perimeter of 1.4 km consists mostly of densely vegetated littoral zones, tall surrounding trees, and dense surrounding woodland. The major fish species present in Mining Corporation Lake is yellow perch (Ministry of Natural Resources, 2017).

2.3 Macroinvertebrate Sampling Methods

Macroinvertebrate samples were collected at two sites per lake, with three replicate samples taken at each site. This yielded a total of 3 macroinvertebrate samples per site and 6 samples per lake. This procedure was carried out over the course of three seasons to assess macroinvertebrate communities at different life stages, namely: Fall 2020 (November 7 – 10), Spring 2021 (May 10, 14-15, 18-19) and Summer 2021 (August 17-18, 21, 23-24).

Macroinvertebrate sampling was carried out in accordance with the Ontario Benthos Biomonitoring Network protocols (OBBN, 2007) to ensure sample collection was done as consistently and accurately as possible (OBBN, 2007). At each sampling site, a transect running from shore to 1-meter depth was created at three points per sampling site, generating our replicate samples for the site. Each of those transects was sampled using a 500µm D-shaped dip net for 10 minutes utilizing the travelling kick and sweep method (OBBN, 2007). Using this method, the samples were collected along the transect by kicking up the substrate and bumping the net along the substrate to gather any suspended organisms (OBBN, 2007). Subsequent replicate samples were taken slightly upstream of the previous sample locations to minimize the impact of the first sampling transects.

Once collected, samples were initially sorted in the field, where large debris were removed, then samples were then transferred to large, sealed plastic bags. Back at the laboratory, samples were cleaned, and all visible macroinvertebrates were transferred to 50 mL centrifuge tube and filled with 70% ethanol. Ethanol was used to fix the macroinvertebrates in their current state, making identification easier later. Once all samples were fixed and preserved, sample counting, and identification were carried out at a later time. One by one, samples were emptied into tubs and identical specimens were grouped together. Once all specimens were sorted, the identification of those organisms was carried out with the aid of a variety of taxonomic keys and resources from both online and literary sources (Peckarsky *et al.,* 1990; APHA, 1995; Schmid, 1998; Jessup *et al.,* 2002; Voshell, 2002; Birmingham *et al.,* 2005; Merritt *et al.,* 2008; Morse *et al.,* 2020; Oksanen et al., 2020). Total number of macroinvertebrate individuals sampled at each site can be found in the appendix, where information is organized as a table (see Appendix Table 3).

2.4 Water Quality Variables and Sampling

Immediately prior to macroinvertebrate sampling, real-time water chemistry measurements were made at each sampling site for each lake sampled. Water temperature (ºC), pH, conductivity (µs/cm) and dissolved oxygen (mg/L) were all measured using a variety of multiprobes.

Dissolved oxygen and water temperature at each site were measured using a multiprobe (HQ40d multiprobe made by HACH). This multiprobe features a probe that can be fully submerged, so measurements were made at a 1-meter depth, as close to the substrate as possible to characterize the macroinvertebrate habitat. Conductivity was measured by using a conductivity meter (SympHony SB90M5 multiprobe made by VWR). This probe cannot be fully submerged, so measurements were made just below the water surface. Lastly, pH was measured using a pH meter (SympHony SP70P made by VWR). This probe also has water immersion limitations, so measurements were carried out near the water surface. At the beginning of each day, all probes were calibrated using appropriate standard solutions. The water chemistry data collected were immediately recorded on OBBN lake-field sheets in the field and input to Microsoft Excel Spreadsheet software back at the laboratory.

At each site, for every lake, water samples were collected in 150 mL screw-top bottles. Before collecting, bottles were rinsed with lake water three times before the final sample was taken. Water samples were collected near the substrate of the lake, between 0.5m and 1m depth. This was done to ensure that collected water samples closely represent the habitat conditions experienced by benthic organisms. Water samples were labelled according to their site and replicate number, and immediately frozen.

2.4.1 Nutrient Analysis Protocols

In the lab, water samples collected in 150 mL bottles were thawed in batches and nutrient analyses were carried out to assess the total amount of total phosphorus (TP) and available nitrate/nitrite (NO_3 + NO_2) content present in the water. Nutrient analysis was carried out following the protocols of the American Public Health Association (APHA, 1995).

All glassware used for the nutrient analysis process was acid washed using 70% HCl and then thoroughly rinsed in deionized water to reduce cross-contamination. Water samples were allowed to reach room temperature before processing, where they were then placed into appropriate glassware to carry out nutrient analysis once the glass was fully air-dried. Replicate samples were completed for both TP and (NO_3 + NO_2) in order to reduce error and improve accuracy.

The total phosphorus nutrient analysis involved two major steps. First, the conversion of phosphorus to dissolved orthophosphate, which is known as the digestion. This was followed by colorimetric determination of dissolved orthophosphates. The digestion stage of this process involved 30% sulphuric acid (H_2SO_4) and ammonium persulphate. Together these oxidize all organic matter, releasing phosphorus as orthophosphate. Following the reduction of the digested sampled using a hot plate, 1 Phos ver 3 pillow was added to each sample and thoroughly mixed to reduce the complex. The final sample was loaded into a cuvette and assessed using a Beckman Coulter UV/vis spectrophotometer, where absorbance was measured at 880nm. Using a standard curve and the associated regression analysis equation generated from phosphate standards, the TP concentrations at each of the sites sampled were calculated for each season.

Available nitrate analysis was carried out using the cadmium reduction method (APHA, 1995). 15mL subsamples were taken from defrosted water samples from each site sampled. A NitraVer 6 pillow was emptied into a test tube, shaken for 3 minutes, and allowed to rest for 2 minutes. Then, 10mL of the supernatant was transferred to a clean acid-washed test tube, making sure no cadmium pellets from the Nitra Ver 6 pillow were transferred with the solution. A NitriVer 3 pillow was then added and shaken gently for 30 seconds, after which the samples were left for 15 minutes to allow for a colour change. After this waiting period, the sample was transferred to an acid-washed 10mL cuvette and placed in the Beckman Coulter UV/vis spectrophotometer where absorbance was measured at 507nm. As for the TP analysis, a standard curve and associated regression analysis equation were generated and used to determine the nitrate concentration for each of the samples assessed.

2.5 Environmental Variables

During lake sampling, several variables related to habitat quality and substrate characteristics were also recorded. This was also done on the same standard OBBN lake-field sheets which were used to record all data related to lakes while in the field. These variables include time of sample collection, coordinates, collection method, etc. as well as environmental characteristics of the study site such as surrounding land use, dominant and second dominant substrate type, and habitat characteristics (e.g. noting the presence of woody debris, detritus, macrophytes, algae, etc.). These variables were used to assess the quality of benthic habitat at each sampling site, which would have its own impact on the macroinvertebrate distribution and composition. Once returned from the field, data recorded on lake field sheets were input to a Microsoft Excel file for subsequent statistical analysis.

2.6 Statistical Analysis

Following data collection and organization in Microsoft Excel, all remaining data analysis and generation of graphs and figures were carried out using the statistical analysis software R (R Core Team, 2021; Microsoft Corporation, 2022). Altogether, a total of 222 separate sample readings were recorded over the three seasons for 18 environmental variables (including water chemistry and habitat quality). In addition, 48 families of macroinvertebrates were evaluated from each sample site. The first step of the analysis was to calculate the various biotic indices that can be used to better understand the macroinvertebrate communities inhabiting the lakes (see section 2.6.1, below, for details). Following that, a variety of statistical tests were used to assess differences between lake types as well as to explore any associations between various water and environmental variables. These are explained below.

2.6.1 Analysis of Macroinvertebrate Indices

As mentioned earlier, indices are used to aid in the assessment of the health and composition of macroinvertebrate communities as an indicator of the health of the ecosystem in which they live. These indices evaluate selected groups of macroinvertebrates or aspects of the groups to assess certain aspects of that community, whether in terms of the presence (or absence) of sensitive taxa, tolerant taxa or the overall richness of the group. For statistical analysis, several R packages were utilized for analysis and plotting: R Stats, vegan and ggplot2 (R Core Team, Oksanen *et al.,* 2020, Wickham, 2016).

(a) Taxa Richness

Richness is simply a count of all different orders or families present in a sample. Taxa richness indicates the health of an aquatic community by its diversity. As water quality increases, it is expected that species diversity, habitat diversity, and taxa richness all increase (Mandaville, 2002). Therefore, a high taxa richness would indicate a sample area of high quality. Taxa richness is calculated as the total number of different taxa represented in a sample. For this study, richness was standardized, which acknowledges that richness scales with sample size. This is necessary due to the varying sample sizes from our lake samples. The calculation of richness was done using R, after counting the number of families present in a sample (Paller *et al.*, 2020).

(b) <u>CIGH</u>

CIGH = [(Corixidae + Isopods + Gastropods + Hirudinea)/count] x 100

The acronym "CIGH" typically stands for Corixidae, Isopoda, Gastropoda and Hirudinidae, and is a biotic index that utilizes the counts of these pollution-tolerant taxa to assess the quality of an environment (Mandaville, 2002). Since these groups are known to be relatively tolerant to pollutants, a high CIGH value may indicate the sample comes from a polluted, and therefore poor-quality environment (Mandaville, 2002). The four taxa are added together, divided by the total sample count and then multiplied by 100 to give the percent of the sample that is composed of the pollution-tolerant taxa. To calculate CIGH, we used the software program R where tallies of the four taxa are input and added together, before being divided by the total sample count and converted to a percentage (R Core Team, 2021).

(c) <u>EOT</u>

EOT = [(Ephemeroptera + Odonata + Trichoptera)/Count] x 100

EOT stands for Ephemeroptera, Odonata and Trichoptera. This biotic index is a modified version of the common EPT index, but replaces Plecoptera with Odonata, as Odonata have similar sensitivities to pollutants and disturbances, but are more common in lentic systems (like lakes) than Plecoptera (Mandaville, 2002; Jones *et al.*, 2017). The orders used in the EOT index are all considered to be relatively sensitive to pollutants, so a high EOT value may indicate the sample comes from a healthy, high-quality environment (Jones *et al.*, 2017). Tallies of the three taxa are added together, divided by the total sample count and then multiplied by 100 to give the percent of the sample that is composed of the pollution-sensitive taxa. To calculate EOT, we used R to select and add the three taxa together before dividing the sum by total sample count and then converting the value into a percentage.

(d) Shannon Diversity Index

H = $-\sum_{i=1}^{S} p_i \, log_b p_i$

The Shannon Diversity Index, also referred to as the Shannon Index or the Shannon and Wiener Index, is the standard index for calculating the diversity of a sample community (Oksanen *et al.,* 2020). In this equation, p_i is the proportion of species i and S is the number of species (Oksanen *et al.,* 2020). To calculate the Shannon Diversity Index for each sample, the function “diversity” from the package ‘vegan’, was used (R Core Team, 2021; Oksanen *et al.*, 2020).

(e) Hilsenhoff Biotic Index

HBI = $\frac{\sum n_1 \, x \, a_i}{N}$

The Hilsenhoff Biotic Index is used to give an estimated overall tolerance of a sampled community from a given area. In this equation, ‘n’ is the number of specimens in a given family, ‘a’ is the tolerance value of that family, and N is the total number of specimens in the sample (Hilsenhoff, 1988). Tolerance values for individual families were obtained from Mandaville (2002) who compiled tolerance data from several sources (see Appendix, Table 4). The results of this index will be a value from 0 to 10, which corresponds to an inferred water quality and degree of organic pollution based on which families are present, and their tolerances (see Appendix, Table 5) (Mandaville, 2002). The online resource ‘macroinvertebrates.org’ was used as an aquatic invertebrate identification resource to identify families that were missing from Mandaville (2002) (Morse *et al.*, 2020).

2.6.2 Wilcoxon Rank-Sum Test

Once index values were computed for all samples in all seasons sampled, the next step was to compare the values for “isolated lakes” with those for “populated lakes” in order to determine whether significant differences are present. Since there were problems with the data in terms of non-normality, where transformations did not solve the issue for almost all index values, whether grouped by type or by season and type, it was not appropriate to apply traditional statistical comparison tests. To address the non-normality of the data, Wilcoxon rank-sum tests were used. The Wilcoxon Rank-Sum test is a non-parametric test used in place of a two-sample t-test where we cannot assume that the data are normally distributed (Oksanen *et al.*, 2020).

To carry out this test, the function ‘wilcox.test’ from the package ‘R Stats’ was used (R Core Team, 2021). Since the data collected were not paired, the Wilcoxon rank-sum test was used to compare the isolated and populated lake variables together; this approach is equivalent to the Mann-Whitney U test (R Core Team, 2021). The null hypothesis of the Wilcoxon rank-sum test is that the distributions of ‘x’ and ‘y’ differ by a location shift of μ, or the mean of the group, with the alternative hypothesis being that ‘x’ and ‘y’ differ by some other location shift (R Core Team, 2021). If the test output differs with a p-value of less than 0.05, we can consider that there is a significant difference between groups ‘x’ and ‘y’.

2.6.3 Kruskal-Wallis Test

The Kruskal-Wallis test, equivalent to the Mann-Whitney U test, is used for the comparison of more than two groups. The Kruskal-Wallis test is a non-parametric alternative to a traditional one-way ANOVA, which tests whether samples originate from the same sampling distribution. In this test, data from all groups are ranked together and the sum of those ranks is determined for each group (Dinno, 2015).

2.6.4 Dunn's Test

The Dunn's test is a *post hoc* test used following a significant Kruskal-Wallis test, to determine which groups are causing the significant difference. The Dunn's test looks for stochastic dominance among multiple pairwise comparisons among 'k' groups [m = k(k-1)/2] (Dinno, 2015).

The Dunn's test also adjusts the p-value to account for the increased probability of falsely rejecting the null hypothesis. In the case of our data, a Bonferroni adjustment was applied. A Bonferroni adjustment multiplies the p-values by the number of comparisons being made, which counteracts the multiple comparisons problem which can lead to an incorrect rejection of the null hypothesis (Dinno, 2015).

2.6.5 Redundancy Analysis (RDA)

A redundancy analysis (RDA) was used to assess the effect of various environmental variables on the taxa composition of natural communities. Multivariate methods, like the RDA, provide a method to structure community samples and associated environmental variables by separating systemic variation from the background "noise" (Braak and Verdonschot, 1995). This is especially important when dealing with natural community data because, generally, species occur only in select samples and the association between species and environmental variables is generally nonlinear (Ter Braak and Verdonschot, 1995).

The redundancy analysis (RDA) is a linear constrained ordination method based on Euclidean distances (Zeleny, 2021). The RDA analysis calculation is a set of multiple linear regression analyses, where species abundance is regressed against environmental variables, one at a time (Zeleny, 2021). To run an RDA analysis, the data must fit a linear distribution. A detrended correspondence analysis (DCA) was used to assess linearity. If the axis 1 gradient length of ordinated species data exceeds 4.0, then the distribution is expected to be unimodal and therefore a CCA (Canonican Correlation Analysis) is an ideal test (Zeleny, 2022). If the gradient length is between 3.0 and 4.0, an RDA and CCA will give similar results (Zeleny, 2022). The DCA on the taxa data in the present study yielded a value of 3.1, which would suggest that an RDA or CCA would produce similar results. For this reason, it was decided that we would simply carry out a redundancy analysis (RDA).

The data for the RDA included the individual taxa counts collected from sampling sites and the water chemistry variables collected at each site. For this analysis, family abundance data were transformed using the Hellinger transformation. This procedure is a "convex standardization" that simultaneously helps minimize the effects of data sets that include very different total abundances of organisms in different samples. To do this, the program calculates a sample total standardization (all values in a row are divided by the row sum), and then takes the square root of the values (see Legendre and Gallagher, 2001). Environmental data were standardized because the variables were measured in different units of measurement, and the CCA required standardized environmental variables (Ter Braak and Verdonschot, 1995). The function 'decostand' from the package 'vegan' was used for all data transformations during the analysis (Oksanen *et al.*, 2020).

Two forms of scaling are common with RDA analysis ordination plots, for the purpose of our study and analysis, 'Scaling 1' is used in all RDA plots generated. 'Scaling 1' is object focused, where the distance between objects are Euclidean distances and angles between vectors of response variables and explanatory variable reflect linear correlation (Zeleny, 2021).

Following the initial RDA analysis, each environmental variable assessed was reassessed using a reduction forward selection analysis. Forward selection reduces the number of explanatory variables contributing to the model by individually assessing each and removing variables with weak explanatory capabilities or variables that were highly correlated with other variables (Dray *et al.*, 2022). Forward selection was carried out using the 'forward.sel' function from the R package 'adespatial' (Dray et *al.,* 2022).

Results of the RDA will shows us the variation in community composition that can be explained by the environmental variables tested; however, permutation tests are needed to understand which of these are significant and should be considered for further interpretation. To aid in the interpretation of the RDA results, an ANOVA-like permutation test is run (Oksanen *et al.,* 2020). The function 'anova.caa' from the package 'vegan' was used for this permutation test (Oksanen *et al.,* 2020). This permutation test is based on the Monte Carlo permutation test but allows users more control over what the permutation test is examining (Zeleny, 2022). This function was used test the variation explained by individual explanatory variables after removing any variation explained by all other variables in the model (Zeleny, 2020). This was used to test the effect of each environmental variable independently and showed us which environmental factors were affecting the community composition independently. This permutation test tests the significance of variance explained by environmental factors, as well as performing variance partitioning which determines variance explained by different groups of environmental variables (Zeleny, 2020).

Chapter 3. The Effect of Water Chemistry on Macroinvertebrate Abundance and Community Composition

3.1 Introduction

When comparing ecosystems, it is important to understand the factors contributing to the environments individually, and the impact these factors have on life in those environments. Therefore, as the first step in this study, I set out to understand the role water chemistry has on the macroinvertebrates that inhabit the systems. As mentioned earlier; pH, dissolved oxygen concentration, water temperature, conductivity, total phosphorus and available nitrogen are the components of water chemistry that were assessed at each of the study sites.

pH is a measure of H^+ ion concentration, and when pH is low, elevated levels of H^+ ions are present in the system –it is acidic. High H^+ levels can directly affect organisms inhabiting the environment as these protons interfere with the regular uptake of other ions. This is a particular problem for organisms that rely on calcium and sodium for growth and reproduction (Lento *et al.,* 2008). Decreasing pH has been well documented to reduce the diversity and abundance of aquatic insects (Rowe *et al.,* 1988). Elevated H^+ ions, and decreasing pH, has also been shown, experimentally, to decrease the hatching success of various macroinvertebrate eggs (Rowe *et al.,* 1988). For example, mayfly eggs experienced increased levels of incomplete hatches at a pH of 4 and 4.5, fish experienced decreased hatching success at pH 4.0-5.5, amphibians experienced decreased hatching success at pH 3.5-5.0, molluscans at pH 5.0-5.5 and crayfish at pH 4.5-5.5 (Peterson *et al.,* 1980; Berrill *et al.,* 1985; Servos *et al.,* 1985; Freda, 1986; Rowe *et al.,* 1998). pH itself can also result in alterations of available nutrients and affect how specific chemicals act in the water, causing a plethora of possible negative effects (Lento *et al.,* 2008). It is well documented that the majority of lakes in central Ontario are still recovering from high levels of acidic deposition from the early- to mid-1900s. Following efforts to reduce and limit sulphate emissions, these lakes have begun to rebound and improve in terms of their acidity levels (Hall and Smol, 1996; Lento *et al.,* 2008). However, if anthropogenic stress continues to increase in the area, we could see these improvements become slow, or reverse entirely (Dillon *et al.,* 2007). Re-acidification can also occur during drought and rewetting cycles, where stored sulphate is released from dried soils into adjacent water bodies (Lento *et al.,* 2008). This phenomenon will likely become more frequent as climate change affects precipitation cycles in the area (Lento *et al.,* 2008).

Dissolved oxygen is a critical component of any aquatic ecosystem as many organisms living in the system require oxygen to survive. Many aquatic macroinvertebrates that reside in freshwater lakes require dissolved oxygen for respiration, and many (including some trichopteran species, mayflies, etc.) possess intricate gill structures to aid in this uptake (Thorp and Rogers, 2015). These intricate gill structures make the uptake of oxygen more efficient, so these organisms tend to be able to tolerate low oxygen concentrations better than organisms that lack these structures. As dissolved oxygen concentration decreases in an environment, less oxygen is available for respiration, which can result in community collapse, where only certain species of macroinvertebrates are able to thrive in the environment. Dissolved oxygen concentration in water is regulated by a variety of factors, including atmospheric and hydrostatic pressure, turbulence, temperature, ice cover, etc. (Canadian Council of Ministers of the Environment, 1999). Dissolved oxygen in aquatic environments can also be affected by the presence of aquatic macrophytes (Fujibayashi *et al.,* 2020). As these plants carry out photosynthesis, carbon dioxide is absorbed, and oxygen is released (Casey, 2011). The presence of macrophytes can therefore improve dissolved oxygen concentrations as well as other ecosystem services like sediment stabilization, providing habitat and nutrient uptake (Fujibayashi *et al.,* 2020). However, large amounts of aquatic macrophytes can also be detrimental to dissolved oxygen. As aquatic macrophytes die, their biomass is deposited on the sediment or the floor of the littoral zone, resulting in a buildup of organic material. This organic debris undergoes bacterial degradation, which consume dissolved oxygen. This dead biomass can also stimulate the release of nutrients from the sediment which subsequently promotes eutrophication as well as sedimentary organic carbon enrichment, which results in oxygen consumption (Fujibayashi *et al.,* 2020). Due to the adverse effects associated with dead and decaying aquatic macrophytes, a balance must be maintained where macrophytes are present, but they are continuously harvested (e.g., by herbivores) before dying and decaying (Fujibayashi *et al.,* 2020). In large and deep freshwater systems, like the lakes being sampled in this study, oxygenation variability depends on wind circulation and currents that help move oxygenated water from the surface to deeper areas (Canadian Council of Ministers of the Environment, 1999). As temperatures increase in the summer months, dissolved oxygen concentrations decrease in the upper strata of the water column, the epilimnion (Canadian Council of Ministers of the Environment, 1999). However, as temperatures begin to decline in fall, dissolved oxygen concentrations increase (Canadian Council of Ministers of the Environment, 1999).

To establish water quality guidelines for in large freshwater systems like lakes, authorities focus on the lowest acceptable dissolved oxygen concentration (in mg/L) possible to support life. These values are a minimum of 5.5 mg/L (6 mg/L for early life stages) in warm waters and a minimum of 6.5 mg/L (9.5 mg/L for early life stages) in cold waters (Canadian Council of Ministers of the Environment). These guidelines ensure early life stages, as well as adults of a variety of macroinvertebrates and other forms of life, are still able to thrive in the environment (Canadian Council of Ministers of the Environment, 1999). If values drop below these levels, we can expect early-stage organisms to be impacted first, resulting in less offspring survival, which in turn will have a drastic effect on population size. If this trend continues, adults will also be greatly impacted, resulting in a whole population collapse due to low oxygen levels.

Conductivity is the measure of the ability of an electric current to travel through the water. Essentially, this depends on the number of dissolved cations and anions present in the water. The negative and positive charges associated with these ions (anions and cations, respectively) allow the electrical current to travel. Conductivity is an important aspect of water quality because water with high conductance experiences higher fluctuations in terms of levels of dissolved solids (Armstead *et al.,* 2016). This is an issue because a high dissolved solids concentration requires organisms to constantly regulate these ions through osmoregulation. This in turn exerts an energy demand on the organisms and can become stressful over time (Armstead *et al.,* 2016). Conductivity is an important aspect of water quality in the region of central Ontario because of the presence of the Precambrian shield. The bedrock in the area causes a large number of lakes in the area to have a low buffering capacity, making them susceptible to influxes of anions and cations, commonly in the form of road salt, applied in winter (Neff and Jackson, 2013).

Phosphorus and nitrogen are the two most frequently assessed nutrients in lake systems. These nutrients are of interest because both are critical nutrients in plant growth and as such are widely used as key ingredients in fertilizers (Ministry of the Environment, 2019). Excessive application of fertilizers can result in these nutrients running off the treatment area and entering nearby water sources. This effect can be intensified by shoreline clearing, erosion and non-permeable surfaces, all of which are common problems that arise as a result of human developmental activities (Marburg *et al.,* 2006).

A review of current literature identified a lack of studies conducted on the impacts of cottage development on Ontario's interior lakes, and, more broadly, a lack of basic water chemistry and macroinvertebrate data for these lakes. There have been, however, a few studies that have assessed lake conditions and how those lakes have changed with respect to time and developmental activities. While historical data in the region of central/south-central Ontario is not very common, a few studies have been carried out in the region that assess changes in macroinvertebrate and diatom populations with respect to water chemistry over time (Lento *et al.*, 2008; Palmer *et al.*, 2011; Hadley *et al.*, 2013; Nelligan *et al.*, 2019). In general, these reports show water quality in the region of central Ontario has changed significantly from 1981 to 2007 (Palmer *et al.,* 2011; Hadley *et al.,* 2013).

A major aspect of the history of water quality in central Ontario relates to the large amounts of acidic sulphur deposition seen in the area throughout the industrial revolution, until air quality regulations were introduced in the 1990s (Dillon *et al.,* 2007). Lakes in the regions affected by these depositions have begun to recover and have subsequently seen some improvements in lake water pH levels. For example, a study carried out by Palmer *et al.* (2011) sampled lakes in central Ontario in 2005 and compared those findings with records from 1981 to assess how these lakes have changed over time. Comparing 1981 and 2005 data, the authors noted an increase in lake pH, which was attributed in part to a 50% reduction in sulphate deposition in that same time frame (Palmer *et al.,* 2011). These trends are further supported by Lento *et al* (2008), who observed pH gradually increasing from 1993 to 1998 in 17 lakes in central Ontario. The relationship between reduced sulphate deposition and increasing lake pH is not as linear as it appears, however. A study by Hadley *et al.* (2013) compared water chemistry data collected from freshwater lakes in the Muskoka-Haliburton area from the years 1992 and

2007. In contrast to the findings of Palmer *et al.* (2011), Hadley *et al.* (2013) found that water pH actually *declined* in 72% of the lakes sampled, by an average of 0.12 pH units. Hadley *et al.* (2013) attributed these differences in pH trends to possible re-acidification events that occurred in the spring of 2007. The region of central Ontario may experience these re-acidification events as excess sulfur may have been stored in wetlands and the littoral zones of lakes. There it may have oxidized during summer droughts, and was later mobilized by rainfall in the autumn months (Hadley *et al.*, 2013). We therefore expect that, in general, pH has tended to increase in central Ontario from the 1990s, but the possibility of re-acidification in specific regions remains possible and depends on other environmental variables such as the presence of nearby wetlands and precipitation and air temperature.

Overall, Palmer *et al.* (2011) found water conductance had decreased in 30 of 36 lakes assessed in the region from 1981 to 2005 despite an increase in Na^+ and Cl^- ions in over 80% of the lakes studied (Palmer *et al.*, 2011). Hadley *et al.* (2013) also observed similar trends in lake conductivity from 1992 to 2008, with conductivity significantly decreasing during that time. Interestingly, in the lakes assessed, they also observed a decrease in base cations, usually meaning Na, K, Ca and Mg (Hadley *et al.,* 2013).

Total phosphorus (TP) has been assessed in numerous studies over time in the region of central Ontario. From 1981 to 2005, Palmer *et al.* (2011) found that the total phosphorus decreased in regional lakes by nearly 20%. In contrast, Hadley *et al.* (2013) found no significant change in TP from 1992 to 2008. Hadley *et al.* (2013) suggest that this difference is due to the fact that the Dorset lakes, studied by Palmer *et al.* (2011) seem to have experienced a shift in nutrient loads, with oligotrophication, the tendency to become clearer and less nutrient-rich, in the region ceasing in the early 1990s.

Lento *et al.* (2008) studied similar water chemistry characteristics in central Ontario lakes and their effect on benthic macroinvertebrate communities from 1993 to 1998. They found significant differences in benthic communities over the course of the study, with more than 66% of benthic community variation being explained each year. This finding suggests that these changes in benthic communities may be directly related to changes in water chemistry (Lento *et al.,* 2008).

The research objectives of the present study are to provide a baseline knowledge of macroinvertebrate communities and the water quality of interior lakes in central Ontario, as well as to examine the general effect these water quality variables have on macroinvertebrate communities in the region. Based on those objectives, the research question being asked is, "Is the macroinvertebrate composition of Central Ontario inland lakes limited by environmental variables?" Our hypothesis is that the composition of macroinvertebrate communities is directly affected by changes in environmental parameters, and, therefore, lakes that are subjected to developmental activities will have less diverse macroinvertebrate communities than lakes without human development activities.

3.2 Methods

3.2.1 Study Locations

The lakes sampled were as follows: Beaver Lake, Town of Kearney (45.630 N 79.723 W); Perry Lake, Town of Kearney (45.546 N 79.227 W); Sand Lake, Town of Kearney (45.6272 N 79.1719 W); Loon Lake, Town of Kearney (45.669 N 79.219 W); Grass Lake, Town of Kearney (45.679 N 79.203 W); Buck Lake, Town of Kearney (45.6900 N 79.1672 W); Lower Raven Lake, Town of Kearney (45.692 N 79.151 W); Spruce Lake, Town of Kearney (45.703 N 79.118 W); BlueSky Lake, Town of Kearney (45.748 N 79.178 W); Tea Lake, Town of Kearney

(45.672 N 79.255 W); Charlie Mann's Lake, District of Parry Sound (45.712 N 79.042 W); Mining Corporation Lake, District of Parry Sound (45.705 N 79.303 W). For more detailed descriptions of the lakes sampled please see Chapter 2 (General Methods), above.

3.2.2 Sampling of Water Chemistry

At each sampling site, a HQ40d multiprobe manufactured by HACH was used to measure dissolved oxygen (mg/L) and water temperature (ºC); a SympHony SB90M5 multiprobe by VWR was used to measure water conductivity (µs/cm), and pH was measured by a SympHony SBSP70P by VWR. The probes were calibrated daily before field measurements were taken.

In addition to i*n situ* sampling of water chemistry, water samples were collected from each sampling site for subsequent nutrient analysis in the laboratory. These water samples were immediately frozen following collection and later thawed to room temperature to assess their total phosphorus and available nitrogen content (see chapter 2 for detailed methodology).

3.2.3 Sampling of Macroinvertebrates

Macroinvertebrate samples were collected at two separate sites per lake, with three replicate samples taken per site. This process was carried out in all 12 lakes. All lakes were sampled in each of three seasons; Fall 2020 (November 7 – 10), Spring 2021 (May 10, 14-15, 18-19) and Summer 2021 (17-18, 21, 23-24). Macroinvertebrate sampling was carried out in accordance with the Ontario Benthos Biomonitoring Networks (OBBN) protocol manual to ensure consistency in how samples were collected and preserved (OBBN, 2007). Following collection, macroinvertebrate samples were cleaned of external debris, and 70% ethanol was added for preservation. Identification of taxa was carried out in the laboratory using various identification resources (Peckarsky *et al.,* 1990; APHA, 1995; Schmid, 1998; Jessup *et al.,* 2002;

Voshell, 2002; Birmingham *et al.,* 2005; Merritt *et al.,* 2008; Morse *et al.,* 2020; Oksanen et al., 2020).

3.2.4 Data analysis

3.2.4.1 Kruskal-Wallis Test and Dunn's Test

The Kruskal-Wallis test was used for the non-parametric analysis of water chemistry variables to assess whether seasonal averages were significantly different from one another. This test was used to compare more than two groups, and assesses whether samples originate from the same sampling distribution (Dinno, 2015). A Dunn's test was used as a post hoc test if the results of the Kruskal-Wallis test came back significantly different. The Dunn's test performs multiple pairwise comparisons using z-test statistics and is useful in assessing which specific groups are significantly different from the others in a Kruskal-Wallis test (Dinno, 2015).

3.2.4.2 RDA Analysis

A redundancy analysis (RDA) was used to assess the effect of various environmental variables on the composition of benthic communities. For this chapter, RDA analyses were used to assess the effect of six water chemistry variables: water temperature (ºC), dissolved oxygen concentration (mg/L), pH, conductivity (µS/cm), total phosphorus (µg/L) and available nitrogen (µg/L). A lake type distinction variable (isolated and developed lake type) was also added to the RDA model (0 = isolated, 1 = developed). RDA analysis was performed using the statistical software 'R' and the package 'vegan' (R Core Team, 2021; Oksanen et al., 2020). The function 'anova.cca' was used to run a permutation test following the RDA to assess which water variables were having a significant effect (Oksanen et al., 2020).

3.3 Results

3.3.1 Physicochemical Properties of Lake Water

During the study period, the average water temperature was found to be 8.93ºC ± 0.226 SE in fall, 10.73ºC ± 0.25 SE in spring and 24.99ºC ± 0.44 SE in summer (see Table 6). A Kruskal-Wallis test showed a significant difference in water temperature among seasons (Chi-Square = 175.54, df = 2, p-value <0.001). A Dunn's post hoc test revealed that all three seasons were significantly different from one another (Fall-Spring: z statistic = -5.20, p-value <0.001, Fall-Summer: z statistic = -13.13, p-value <0.001 and Spring-Summer: z statistic = -8.012, p-value < 0.001).

Average dissolved oxygen concentration peaked in spring with a mean of 10.80 mg/L ± 0.16 SE, but this season also showed the most variation (see Table 7). The second highest value of dissolved oxygen was in fall, with an average value of 10.06 mg/L; summer showed the lowest concentration with a mean of 8.13 mg/L (see Table 7). Dissolved oxygen seemed to vary among sites more than among seasons. A Kruskal-Wallis test was used to compare the seasonal variation of dissolved oxygen in lakes, and it was found to differ significantly among seasons (Chi-Square = 130.37, df = 2, p < 0.001). A Dunn's test was used to evaluate the results of the Kruskal-Wallis test to examine which seasons if any, were significantly different from the rest. The results indicated that the dissolved oxygen concentration in summer samples was significantly different from the fall and spring samples. In pairwise comparisons (summer-spring: z = 10.64, p-value <0.001 and summer-fall: z = 8.83, p-value <0.001).

Average pH readings ranged from 6.06 – 6.81 among different lakes, and from 6.14 – 6.90 among seasons. Average pH was found to be at its lowest in fall (6.14), highest in spring (6.90) and in between the two above values in summer (6.54) (see Table 8). A Kruskal-Wallis test was used to assess the differences in pH among the seasons sampled. This test found a significant difference in pH among the three seasons (Chi-Square = 99.22 df = 2, p-value <0.001). The Dunn’s test showed that the three seasons were all significantly different in pairwise comparisons, suggesting that pH changed significantly from one season to other (fall-spring: z statistic= -9.93 p-value <0.001, fall-summer: z statistic= -4.37, p-value <0.001 and spring-summer: z statistic = 5.61, p-value <0.001).

Average conductivity among the seasons ranged very little from 22.13 µS/cm in the spring to its highest value in fall, of 24.16 µS/cm (see Table 9). However, it differed more widely among different lakes, ranging from 14.01µS/cm – 38.36µS/cm (see Table 9). As with the previous variables, a Kruskal-Wallis test was used to assess the difference in conductivity among different seasons; the results showed no significant differences (chi-square = 1.52, df = 2, p-value > 0.1).

Average total phosphorus (µg/L) showed a range of values from 40.85 µg/L to 92.32 µg/L in spring and fall, respectively (see Table 10). There was, however, a large amount of variation among lakes, with a standard error of 33.92 in fall, 8.45 in spring and 10.23 in summer, respectively (see Table 10). As a result, despite the large range of values between seasons, no significant difference was found among the three seasons (Kruskal-Wallis test chi-square = 2.80, df = 2, p-value > 0.1). This is likely due to the wide range of values noted in different lake sites within the same season.

Average total nitrogen (µg/L) showed the highest value in spring with an average of 51.41 µg/L ±4.99 SE) (see Table 11). The fall and summer averages showed similar values with a mean of 34.17 µg/L ± 6.77 SE and 36.78 µg/L ± 7.26 SE, respectively (see Table 11). As with the phosphorus results, there was considerable variation among lakes, even in the same season. A

Kruskal-Wallis test found a significant difference in the average available nitrogen among seasons (chi-square = 27.43, df = 2, p-value < 0.001). The Dunn's post hoc test revealed that the difference in available nitrogen among seasons are due to the spring concentrations being significantly higher than the fall and summer averages (fall-spring: z statistic = -3.99, p-value <0.001 and spring-summer: z statistic = 4.92, p-value < 0.001).

Table 6. Water temperature (ºC) data from 12 lakes in central Ontario during the Fall of 2020 and the Spring & Summer of 2021. Water temperature was measured in the littoral zone of the lake at a depth of around 0.5m. Average water temperatures for the three seasons (with standard errors, S.E.) and average water temperature for each lake were calculated (with S.E.). ISO = isolated, POP = populated by human settlement.

			Water Temperature (ºC)			
Lake Name	**Site Number**	**Lake Type**	**Fall**	**Spring**	**Summer**	**Lake Mean (S.E.)**
Spruce	Site 1	ISO	10	9.8	26	15.417 (3.460)
Spruce	Site 2	ISO	9.6	10.4	26.7	
Charlie Mann's	Site 1	ISO	8.3	9.8	27.2	14.883 (3.684)
Charlie Mann's	Site 2	ISO	7.8	10.5	25.7	
Mining Corp.	Site 1	ISO	10.8	10.7	25.5	15.3 (3.184)
Mining Corp.	Site 2	ISO	10.1	9.5	25.2	
Tea	Site 1	ISO	8.5	10.6	25.6	14.867 (3.366)
Tea	Site 2	ISO	8.7	10.5	25.3	
Bluesky	Site 1	ISO	8.4	11.2	26.4	15.15 (3.526)
Bluesky	Site 2	ISO	7.8	11.2	25.9	
Buck	Site 1	ISO	8.6	9.8	25.8	14.7 (3.475)
Buck	Site 2	ISO	8.3	10.2	25.5	
Beaver	Site 1	POP	7.9	11.2	24.1	14.383 (3.126)
Beaver	Site 2	POP	8	11	24.1	
Sand	Site 1	POP	7.6	11.4	23.7	13.938 (1.961)
Sand	Site 2	POP	11.6	10.2	18.6	
Sand	Site 3	POP	NA	10.2	18.2	
Loon	Site 1	POP	8.5	10.3	26	14.95 (3.497)
Loon	Site 2	POP	7.9	11.2	25.8	
Grass	Site 1	POP	9.3	9.7	24.3	14.55 (2.910)
Grass	Site 2	POP	11.2	9.7	23.1	
Lower Raven	Site 1	POP	8.6	9.7	26.4	14.933 (3.604)
Lower Raven	Site 2	POP	8.5	10.2	26.2	
Perry	Site 1	POP	9.1	14.6	24.2	15.617 (2.640)
Perry	Site 2	POP	9.1	14.2	22.5	
Mean per Season (S.E.)			8.925 (0.226)	10.712 (0.249)	24.72 (0.444)	

Table 7. Dissolved oxygen concentration (mg/L) in 12 lakes in central Ontario in Fall 2020 and Spring & Summer 2021. Dissolved oxygen was measured using a portable multiprobe and measurement was done in the littoral zone at a 1-meter depth just above the substrate. Measurments were taken in each of the two sites sampled per lake per season. The average dissolved oxygen for the three seasons sampled was calculated (with standard deviations, S.E.) as well as the average dissolved oxygen for each lake (with S.E.). ISO = isolated, POP = populated by human settlement.

			Dissolved Oxygen Concentration (mg/L)			
Lake Name	**Site Number**	**Lake Type**	**Fall**	**Spring**	**Summer**	**Lake Mean (S.E.)**
Spruce	Site 1	ISO	8.55	9.28	7.96	8.803 (0.407)
Spruce	Site 2	ISO	10.26	9.25	7.52	
Charlie Mann's	Site 1	ISO	9.43	10.4	8.08	9.578 (0.640)
Charlie Mann's	Site 2	ISO	8.66	12.32	8.58	
Mining Corp.	Site 1	ISO	9.91	11.3	7.79	9.645 (0.572)
Mining Corp.	Site 2	ISO	10.07	10.68	8.12	
Tea	Site 1	ISO	11.15	9.18	7.76	9.722 (0.630)
Tea	Site 2	ISO	11.6	10.29	8.35	
Bluesky	Site 1	ISO	10.63	10.45	9.42	10.173 (0.285)
Bluesky	Site 2	ISO	10.95	10.38	9.21	
Buck	Site 1	ISO	10.14	10.41	7.41	9.453 (0.669)
Buck	Site 2	ISO	10.27	11.13	7.36	
Beaver	Site 1	POP	10.63	10.58	8.81	10.187 (0.479)
Beaver	Site 2	POP	11	11.45	8.65	
Sand	Site 1	POP	9.9	19.2	8.14	11.038 (1.400))
Sand	Site 2	POP	9.46	13	7.9	
Sand	Site 3	POP	NA	13.2	7.5	
Loon	Site 1	POP	9.57	10.51	8.68	9.482 (0.384)
Loon	Site 2	POP	8.87	10.7	8.56	
Grass	Site 1	POP	9.51	7.71	8.04	8.74 (0.401)
Grass	Site 2	POP	10.31	8.35	8.52	
Lower Raven	Site 1	POP	9.78	9.91	7.34	9.085 (0.527)
Lower Raven	Site 2	POP	10.07	9.9	7.51	
Perry	Site 1	POP	10.28	10.48	8.23	9.487 (0.493)
Perry	Site 2	POP	10.35	9.88	7.7	
Mean per Season (S.E.)			10.05625 (0.155)	10.7976 (0.430)	8.1256 (0.115)	

Table 8. pH data from twelve lakes in central Ontario, sampled in Fall 2020 and Spring & Summer 2021. pH was measured using a portable multiprobe which was calibrated daily. Measurements were done in the littoral zone of the lake at two sites in each lake, in each of the three seasons. The average pH for the three seasons sampled was calculated as well as the average pH for each lake (both with accompanying standard errors, S.E.). ISO = isolated, POP = populated by human settlement.

			pH			
Lake Name	**Site Number**	**Lake Type**	**Fall**	**Spring**	**Summer**	**Lake Mean (S.E.)**
Spruce	Site 1	ISO	5.88	6.97	6.3	6.462 (0.205)
Spruce	Site 2	ISO	6.07	7.15	6.4	
Charlie Mann’s	Site 1	ISO	5.61	6.53	5.85	6.055 (0.214)
Charlie Mann’s	Site 2	ISO	5.44	6.78	6.12	
Mining Corp.	Site 1	ISO	6.21	7.3	5.9	6.37 (0.253)
Mining Corp.	Site 2	ISO	5.75	6.96	6.1	
Tea	Site 1	ISO	6.43	6.74	6.7	6.577 (0.0996)
Tea	Site 2	ISO	6.24	6.45	6.9	
Bluesky	Site 1	ISO	6.36	6.92	7.1	6.795 (0.169)
Bluesky	Site 2	ISO	6.25	6.84	7.3	
Buck	Site 1	ISO	5.67	6.86	6.54	6.352 (0.277)
Buck	Site 2	ISO	5.34	6.94	6.76	
Beaver	Site 1	POP	6.52	7.3	6.92	6.775 (0.144)
Beaver	Site 2	POP	6.58	6.98	6.35	
Sand	Site 1	POP	6.7	7.19	6.29	6.588 (0.103)
Sand	Site 2	POP	6.44	6.68	6.43	
Sand	Site 3	POP	NA	6.65	6.32	
Loon	Site 1	POP	6.44	6.81	7.12	6.805 (0.148)
Loon	Site 2	POP	6.32	6.91	7.23	
Grass	Site 1	POP	6.7	6.52	6.23	6.537 (0.107)
Grass	Site 2	POP	6.56	6.93	6.28	
Lower Raven	Site 1	POP	5.36	6.83	6.51	6.292 (0.255)
Lower Raven	Site 2	POP	5.65	6.72	6.68	
Perry	Site 1	POP	6.44	7.35	6.33	6.73 (0.169)
Perry	Site 2	POP	6.37	6.96	6.93	
Mean per Season (S.E.)			6.13875 (0.089)	6.8908 (0.048)	6.5436 (0.081)	

Table 9. Seasonal water conductivity (µS/cm) data from 12 central Ontario lakes in Fall, Spring and Summer 2020-2021. Water conductivity was measured in the lake's littoral zone at two sites per lake each season. The average conductivity for the three seasons sampled and the average conductivity for each lake over the course of the study period were calculated (with standard errors, S.E.). ISO = isolated, POP = populated by human settlement.

			Conductivity (µS/cm)			
Lake Name	**Site Number**	**Lake Type**	**Fall**	**Spring**	**Summer**	**Lake Mean (S.E.)**
Spruce	Site 1	ISO	16.22	15.95	15.94	15.905 (0.373)
Spruce	Site 2	ISO	17.02	16.07	14.23	
Charlie Mann's	Site 1	ISO	20.8	23.12	23.62	21.788 (0.548)
Charlie Mann's	Site 2	ISO	21.51	20.12	21.56	
Mining Corp.	Site 1	ISO	16.31	12.13	14.39	14.57 (0.658)
Mining Corp.	Site 2	ISO	16.43	13.95	14.21	
Tea	Site 1	ISO	15.89	17.53	16.48	16.498 (1.083)
Tea	Site 2	ISO	13.61	21.08	14.4	
Bluesky	Site 1	ISO	16.68	18.65	12.45	15.328 (1.085)
Bluesky	Site 2	ISO	17.32	14.67	12.2	
Buck	Site 1	ISO	14.19	14.14	13.75	14.007 (0.115)
Buck	Site 2	ISO	14.25	13.56	14.15	
Beaver	Site 1	POP	36.6	34.8	41.2	37.533 (0.891)
Beaver	Site 2	POP	37.4	36.6	38.6	
Sand	Site 1	POP	35.4	42.4	43.2	38.363 (2.332)
Sand	Site 2	POP	50.2	30.2	35.4	
Sand	Site 3	POP	NA	32	38.1	
Loon	Site 1	POP	25.54	20.48	19.98	21.622 (0.833)
Loon	Site 2	POP	21.79	21.51	20.43	
Grass	Site 1	POP	28.58	17.86	19.6	20.89 (1.651)
Grass	Site 2	POP	21.36	17.53	20.41	
Lower Raven	Site 1	POP	15.08	15.76	15.17	15.738 (0.223)
Lower Raven	Site 2	POP	15.86	16.02	16.54	
Perry	Site 1	POP	42.7	37	41.3	40.033 (2.574)
Perry	Site 2	POP	49.2	30.2	39.8	
Mean per Season (S.E.)			24.164 (2.341)	22.133 (1.7436)	23.084 (2.201)	

Table 10. Total phosphorus concentration (µg/L) in water samples collected from 12 lakes in central Ontario during the Fall of 2020 and Spring & Summer of 2021. Total phosphorus was determined from water samples collected at each of the two sites sampled in each lake, each season. All available phosphorus in samples was converted and assessed using nutrient analysis techniques. The average total phosphorus (µg/L) for each season was determined as well as the average total phosphorus from each lake (each with accompanying standard errors). ISO = isolated, POP = populated by human settlement.

			Total Phosphorus (µg/L)			
Lake Name	**Site Number**	**Lake Type**	**Fall**	**Spring**	**Summer**	**Lake Mean (S.E.)**
Spruce	Site 1	ISO	20.549	44.66075	2.0015	13.977 (11.17)
Spruce	Site 2	ISO	696.9145	16.22125	58.26225	
Charlie Mann’s	Site 1	ISO	97.212	53.31625	5.711	36.52 (14.11)
Charlie Mann’s	Site 2	ISO	34.76875	18.69425	9.4205	
Mining Corp.	Site 1	ISO	44.0425	63.8265	70.009	58.37 (14.37)
Mining Corp.	Site 2	ISO	115.14125	48.9885	8.184	
Tea	Site 1	ISO	559.663	43.42425	72.482	121.74 (88.1)
Tea	Site 2	ISO	26.7315	23.64025	4.4745	
Bluesky	Site 1	ISO	52.07975	57.02575	97.83025	53.21 (11.01)
Bluesky	Site 2	ISO	18.69425	60.117	33.53225	
Buck	Site 1	ISO	33.53225	81.84	91.64775	41.47 (15.09)
Buck	Site 2	ISO	1.38325	34.1505	79.901	
Beaver	Site 1	POP	65.063	219.6255	68.15425	88.45 (28.26)
Beaver	Site 2	POP	102.158	53.31625	22.40375	
Sand	Site 1	POP	11.8935	23.022	39.71475	56.25 (26.73)
Sand	Site 2	POP	14.3665	3.238	162.12825	
Sand	Site 3	POP	NA	4.4745	191.186	
Loon	Site 1	POP	116.996	10.657	160.2735	65.27 (24.62)
Loon	Site 2	POP	50.225	14.3665	39.0965	
Grass	Site 1	POP	42.18775	87.32	29.82275	45.90 (8.85)
Grass	Site 2	POP	50.225	31.05925	34.76875	
Lower Raven	Site 1	POP	8.80225	42.806	8.80225	18.90 (6.33)
Lower Raven	Site 2	POP	1.38325	29.2045	22.40375	
Perry	Site 1	POP	21.7855	25.495	32.29575	23.13 (4.03)
Perry	Site 2	POP	29.82275	4.4745	24.87675	
Mean per Season (S.E.)			92.32 (33.92)	40.85 (8.45)	54.78 (10.23)	

Table 11. Total available nitrogen concentration (µg/L) from water samples collected from twelve lakes in central Ontario in Fall 2020 and Spring and Summer 2021. Water samples were collected at two sites per lake per season. Nutrient analysis techniques were used to convert all forms of nitrogen in water samples to available nitrogen and concentration was determined using spectrophotometry where samples were compared to a standard curve. The average available nitrogen for each season and the average for each lake was calculated with standard error provided. ISO = isolated, POP = populated by human settlement.

			Total Available Nitrogen (µg/L)			
Lake Name	**Site Number**	**Lake Type**	**Fall**	**Spring**	**Summer**	**Lake Mean (S.E.)**
Spruce	Site 1	ISO	30.62725	21.73685	166.20585	67.39 (26.97)
Spruce	Site 2	ISO	23.95945	24.5151	137.31205	
Charlie Mann's	Site 1	ISO	31.73855	40.0733	16.736	31.00 (3.21)
Charlie Mann's	Site 2	ISO	30.0716	31.73855	35.6281	
Mining Corp.	Site 1	ISO	21.73685	66.18885	20.62555	31.37 (7.52)
Mining Corp.	Site 2	ISO	18.40295	37.8507	23.4038	
Tea	Site 1	ISO	19.51425	21.1812	22.2925	23.40 (2.92)
Tea	Site 2	ISO	20.0699	19.51425	37.8507	
Bluesky	Site 1	ISO	23.95945	72.301	33.4055	47.67 (7.75)
Bluesky	Site 2	ISO	44.5185	67.8558	43.96285	
Buck	Site 1	ISO	18.40295	22.84815	20.62555	22.48 (1.40)
Buck	Site 2	ISO	20.0699	27.29335	25.6264	
Beaver	Site 1	POP	27.29335	95.08265	18.9586	50.54 (14.66)
Beaver	Site 2	POP	45.07415	95.6383	21.1812	
Sand	Site 1	POP	85.6366	134.5338	22.2925	65.77 (16.59)
Sand	Site 2	POP	127.31035	34.5168	25.07075	
Sand	Site 3	POP	NA	74.5236	22.2925	
Loon	Site 1	POP	24.5151	47.8524	63.96625	34.42 (7.20)
Loon	Site 2	POP	26.7377	25.07075	18.40295	
Grass	Site 1	POP	17.8473	59.52105	30.0716	47.76 (18.54)
Grass	Site 2	POP	23.95945	135.08945	20.0699	
Lower Raven	Site 1	POP	35.6281	32.84985	22.2925	31.00 (1.95)
Lower Raven	Site 2	POP	30.62725	34.5168	30.0716	
Perry	Site 1	POP	42.2959	40.62895	20.62555	29.42 (4.07)
Perry	Site 2	POP	30.0716	22.2925	20.62555	
Mean per Season (S.E.)			34.17 (4.99)	51.41(6.77)	36.78 (7.26)	

3.3.2 Effect of Water Quality on the Composition of Macroinvertebrate Communities

To gain a better understanding of the relationship between water chemistry and benthic community composition in the area, a Redundancy Analysis (RDA) was carried out (see Figure 2). Data used for the RDA analysis included six water chemistry variables, recorded at each site sampled, as well as tallies of the abundance of members of macroinvertebrate families sampled at every site for each replicate. The macroinvertebrate data were summarized in a table which also indicates the presence or absence of particular benthic macroinvertebrate families (see Table 12). Replicate samples from the same lakes were grouped together for each season. By examining the macroinvertebrate data, we can see that the families with higher numbers of benthic macroinvertebrates were Corduliidae of the Order Odonata (found in 34 of 36 samples), Sphaeriidae of the Order Sphaeriida (found in 34 of 36 samples) and Chironomidae of the Order Diptera (found in 33 of 36 samples) (see Table 12). The most consistently represented (most times during the study) order of macroinvertebrates over the course of the study was the Order Odonata. Eight families of Order Odonata were present 141 times out of a possible 288 (see Table 12).

The main output of an RDA analysis is the associated ordination diagram that was generated (see Figure 2). In this ordination diagram, the six different environmental variables are represented by blue arrows. The red arrows with associated family names represent the individual family count averages derived from the community composition data. The RDA axes seen in the ordination diagram are linear combinations of the environmental variables assessed by the model. The constrained axis RDA1, the main explainable axis in the RDA, separated conductivity and water temperature from the other four water chemistry variables. The other axis, RDA2, separated water temperature and total phosphorus from the other four water chemistry variables (pH, dissolved oxygen, available nitrogen, and total phosphorus). Conductivity and water temperature were positively correlated with the first synthetic gradient, RDA1, while total phosphorus, available nitrogen, pH, and dissolved oxygen were negatively correlated with that axis (Figure 2). Water temperature appears to be highly correlated with the second constrained axis RDA2, with total phosphorus being slightly positively correlated as well (see Figure 2).

From the results, we can see six constrained axes generated by the model, and from their eigenvalues, the percentage of explained variance is calculated (see Table 13). The constrained axis RDA1 explains 4.49% of variance, RDA2 explains 2.91%, RDA3 explains 1.94%, RDA4 explains 0.68%, RDA5 explains 0.42% and RDA6 explains 0.28% of variance in benthic taxa composition. A permutation test was run by 'axis' to see if any axes explained a significant difference in explained variance (see Table 13). It was determined that RDA1 ($p < 0.001$), RDA2 ($p < 0.001$), and RDA3 ($p < 0.001$) were able to explain significant variation in benthic community composition.

Another permutation test was run on the same RDA model, but instead assessed the effect of the individual water chemistry variables on benthic community composition (see Table 10). By doing this, we can determine the percentage of variance in community composition explained by separate water chemistry variables; we can also test the significance of any effect. This permutation test found water temperature explained 2.18% of variance, dissolved oxygen explained 0.90%, pH explained 2.41%, conductivity explained 4.06%, total phosphorus explained 0.37% and available nitrogen explained 0.79% of variance in benthic community composition (see Table 13). Water temperature ($p < 0.001$), dissolved oxygen ($p<0.01$), pH ($p <$

0.001), conductivity ($p < 0.001$) and available nitrogen ($p < 0.05$) explained a significant amount of variance in benthic community composition (see Table 13).

Three similar RDA analyses were carried out following the initial test, but instead of assessing the whole study data, I assessed the effects of water chemistry seasonally by dividing the data into the three seasons sampled.

First, I assessed the data collected from the fall of 2020. The ordination diagram generated from the fall RDA analysis showed the first constrained axis, RDA1, separating the variables total phosphorus and water temperature from the other four water chemistry variables (see Figure 3). The second axis, RDA2, separated none of the water chemistry variables with all variables falling in the lower half of the axis (see Figure 3). Six constrained axes were generated by the Fall RDA model and were assessed using the permutation test (see Table 14). The constrained axis RDA1 was found to explain the most variance with 9.32% of variance in benthic community composition being explained. The other axes: RDA2 explained 5.22% of variance, RDA3 explained 3.89% of variance, RDA4 explained 2.92% of variance, RDA5 explained 1.84% of variance and RDA6 explained 0.96% of variance in benthic community composition. The constrained axes RDA1 (p-value < 0.001), RDA2 (p-value <0.01), RDA3 (p-value <0.01) and RDA4 ($p<0.05$) were found to have statistically significant p-values. Other permutation tests were used to assess the effect of individual environmental variables on the community composition of macroinvertebrates in the Fall of 2020 (see Table 14). The six water chemistry variables were found to explain the following percent variance in benthic community composition: water temperature explained 3.12%, dissolved oxygen explained 2.59%, pH explained 7.02%, conductivity explained 4.49%, total phosphorus explained 2.10% and available nitrogen explained 4.84% (see Table 14). All six water chemistry variables had p-values less than 0.05 in the analysis of fall data.

Another RDA analysis was carried out, to assess the effect of water chemistry on benthic macroinvertebrate community composition in the Spring of 2021. The RDA1 axis separated water temperature, which was negatively correlated with the axis, from the other five water chemistry variables, which were positively correlated (see Figure 4). The second axis RDA2 did not separate any variables, with all variables being positively correlated with it (see Figure 4). The combination of the six water chemistry variables accounted for 13.3% of the variance in benthic macroinvertebrate communities in the spring of 2021. Permutation tests were again used to assess the effect of axes and the individual variables on the macroinvertebrate communities sampled in the Spring of 2021. The axes RDA1 explained 8.42% of variance in the benthic communities, RDA2 explained 5.91% of variance, RDA3 explained 2.85% of variance, RDA4 explained 1.97% of variance, RDA5 explained 0.73% of variance and RDA6 explained 0.47% of variance in benthic community composition (see Table 15). The axes RDA1 (p-value = 0.001) and RDA2 (p-value = 0.001) were found to have a significant effect on benthic communities. When assessing individual water chemistry variables, water temperature explained 5.05% of variance in benthic community composition, dissolved oxygen explained 2.25%, pH 5.02%, conductivity 4.20%, total phosphorus 2.51% and available nitrogen explained 1.31% (see Table 15).

The ordination diagram generated from the summer 2021 data RDA analysis showed the first axis, RDA1, separating water temperature, total phosphorus, available nitrogen and pH from conductivity and oxygen (see Figure 5). The second axis, RDA2, separated water temperature from the other 5 water chemistry variables (see Figure 5). The combination of the six water chemistry variables accounts for 20.07% of variance observed in benthic macroinvertebrate communities. The permutation tests done on the Summer RDA identified the explained variance and significance of axes and water chemistry variables. The axis RDA1 explained 11.53% of variance, RDA2 explained 7.67%, RDA3 4.22%, RDA4 2.11%, RDA5 1.00% and RDA6 explained 0.30% of variance in benthic macroinvertebrate community composition (see Table 16). The axes RDA1 (p-value < 0.001), RDA2 (p-value < 0.001) and RDA3 (p-value <0.01) were found to have a significant effect on macroinvertebrate community variance (see Table 16). Water temperature explained 2.18% of variance, dissolved oxygen explained 0.90% of variance, pH explained 2.41% of variance, conductivity explained 4.06% of variance, total phosphorus explained 0.37% of variance and available nitrogen explained 0.79% of variance in benthic community composition (see Table 13). Water temperature (p-value < 0.001), dissolved oxygen (p-value <0.01), pH (p-value < 0.001), conductivity (p-value < 0.001) and available nitrogen (p-value <0.05) were found to have a significant effect on benthic community composition variance in the summer of 2021 (see Table 16).

Table 12. Presence and absence of benthic macroinvertebrates sampled over the course of three seasons. In each lake, benthic macroinvertebrate communities were sampled at 2 separate sites, with three replicates taken at each site, during three seasons (Fall, Spring and Summer). Replicate samples were combined for the purpose of this table. Check marks indicate that the family was present and sampled from that site at that season, X indicates that the family was not present. For complete macroinvertebrate data with number of specimens of each family found, see Appendix I.

			Ephemeroptera					
Site	**Development Type**	**Season**	**Baetidae**	**Ephermerellidae**	**Ephermeridae**	**Heptageniidae**	**Ploymitarcyidae**	**Siphlonuridae**
Spruce	ISO	Fall	✕	✕	✓	✓	✕	✕
Charlie Mann’s	ISO	Fall	✕	✕	✕	✕	✕	✕
Buck	ISO	Fall	✕	✕	✕	✕	✕	✕
Mining Corp.	ISO	Fall	✕	✕	✕	✕	✕	✕
Tea	ISO	Fall	✕	✓	✕	✕	✕	✕
Bluesky	ISO	Fall	✕	✕	✕	✓	✕	✕
Beaver	POP	Fall	✕	✕	✓	✓	✕	✕

Sand	POP	Fall	✗	✗	✗	✗	✗	✗
Loon	POP	Fall	✗	✗	✗	✗	✗	✗
Grass	POP	Fall	✗	✗	✓	✓	✗	✗
Lower Raven	POP	Fall	✓	✗	✗	✓	✗	✗
Perry	POP	Fall	✗	✗	✗	✗	✗	✗
Spruce	ISO	Spring	✗	✗	✓	✓	✗	✗
Charlie Mann’s	ISO	Spring	✗	✗	✓	✓	✗	✗
Buck	ISO	Spring	✗	✗	✗	✓	✗	✗
Mining Corp.	ISO	Spring	✗	✗	✗	✓	✗	✗
Tea	ISO	Spring	✗	✓	✗	✗	✗	✓
Bluesky	ISO	Spring	✗	✓	✗	✗	✗	✗
Beaver	POP	Spring	✗	✓	✓	✓	✗	✗
Sand	POP	Spring	✗	✗	✗	✓	✗	✗
Loon	POP	Spring	✗	✓	✓	✗	✗	✗
Grass	POP	Spring	✗	✗	✓	✓	✗	✗
Lower Raven	POP	Spring	✗	✗	✗	✓	✗	✗
Perry	POP	Spring	✗	✓	✗	✓	✗	✗
Spruce	ISO	Summer	✗	✗	✗	✗	✗	✗
Charlie Mann’s	ISO	Summer	✗	✗	✗	✗	✗	✗
Buck	ISO	Summer	✗	✗	✗	✗	✗	✗
Mining Corp.	ISO	Summer	✗	✗	✗	✗	✗	✗
Tea	ISO	Summer	✗	✗	✗	✗	✗	✗
Bluesky	ISO	Summer	✗	✗	✗	✗	✗	✗
Beaver	POP	Summer	✗	✗	✓	✗	✓	✗
Sand	POP	Summer	✗	✗	✗	✗	✗	✗
Loon	POP	Summer	✗	✗	✗	✗	✗	✗
Grass	POP	Summer	✗	✗	✓	✗	✗	✗
Lower Raven	POP	Summer	✗	✗	✗	✗	✗	✗
Perry	POP	Summer	✗	✗	✓	✗	✗	✗

			Odonata							
Site	**Development Type**	**Season**	**Aeshnidae**	**Calopterygidae**	**Coenagrionidae**	**Cordulegastridae**	**Corduliidae**	**Gomphidae**	**Lestidae**	**Libellulidae**
Spruce	ISO	Fall	✓	✗	✓	✗	✓	✓	✗	✗
Charlie Mann’s	ISO	Fall	✓	✓	✓	✗	✓	✓	✗	✓
Buck	ISO	Fall	✓	✓	✓	✗	✓	✓	✗	✗
Mining Corp.	ISO	Fall	✓	✗	✓	✗	✓	✓	✗	✓

Tea	ISO	Fall	✓	✗	✗	✗	✓	✓	✗	✓
Bluesky	ISO	Fall	✓	✗	✓	✗	✓	✓	✗	✓
Beaver	POP	Fall	✓	✗	✓	✗	✓	✓	✗	✗
Sand	POP	Fall	✓	✗	✗	✗	✓	✗	✗	✓
Loon	POP	Fall	✓	✗	✓	✗	✓	✗	✗	✗
Grass	POP	Fall	✓	✗	✓	✗	✓	✗	✗	✗
Lower Raven	POP	Fall	✓	✗	✓	✗	✓	✓	✗	✗
Perry	POP	Fall	✓	✗	✓	✗	✓	✓	✗	✗
Spruce	ISO	Spring	✓	✗	✓	✗	✓	✓	✗	✓
Charlie Mann’s	ISO	Spring	✓	✗	✓	✗	✓	✓	✗	✓
Buck	ISO	Spring	✓	✗	✗	✗	✓	✓	✗	✓
Mining Corp.	ISO	Spring	✓	✗	✓	✗	✓	✓	✗	✓
Tea	ISO	Spring	✓	✗	✓	✗	✓	✓	✗	✗
Bluesky	ISO	Spring	✓	✗	✓	✗	✓	✓	✓	✓
Beaver	POP	Spring	✓	✗	✓	✓	✓	✓	✗	✗
Sand	POP	Spring	✗	✗	✓	✗	✓	✓	✗	✗
Loon	POP	Spring	✗	✗	✓	✗	✓	✓	✗	✓
Grass	POP	Spring	✗	✗	✓	✗	✓	✓	✗	✓
Lower Raven	POP	Spring	✗	✗	✓	✗	✓	✓	✗	✗
Perry	POP	Spring	✓	✗	✓	✗	✓	✓	✓	✗
Spruce	ISO	Summer	✓	✗	✗	✗	✓	✓	✗	✗
Charlie Mann’s	ISO	Summer	✗	✗	✓	✗	✓	✓	✗	✗
Buck	ISO	Summer	✓	✗	✓	✗	✗	✓	✗	✗
Mining Corp.	ISO	Summer	✓	✗	✓	✗	✓	✓	✗	✗
Tea	ISO	Summer	✓	✗	✓	✗	✓	✓	✗	✗
Bluesky	ISO	Summer	✗	✗	✗	✗	✓	✗	✗	✗
Beaver	POP	Summer	✓	✗	✗	✓	✓	✓	✗	✓
Sand	POP	Summer	✗	✗	✓	✗	✗	✗	✗	✗
Loon	POP	Summer	✓	✗	✗	✗	✓	✓	✗	✗
Grass	POP	Summer	✓	✗	✓	✗	✓	✓	✗	✗
Lower Raven	POP	Summer	✓	✗	✗	✗	✓	✓	✗	✗
Perry	POP	Summer	✓	✗	✓	✗	✓	✓	✗	✗

			Trichoptera					Hemiptera		
Site	**Development Type**	**Season**	**Dipseudi-psidae**	**Hydropsy-chidae**	**Hydropt-ilidae**	**Limneph-ilidae**	**Rhyacoph-ilidae**	**Corixidae**	**Hirudinae**	**Gastropoda**
Spruce	ISO	Fall	✗	✗	✓	✗	✗	✗	✗	✓

Charlie Mann’s	ISO	Fall	✗	✓	✗	✗	✗	✗	✓	✓
Buck	ISO	Fall	✗	✗	✗	✗	✗	✗	✓	✓
Mining Corp.	ISO	Fall	✗	✗	✗	✗	✓	✗	✓	✓
Tea	ISO	Fall	✗	✓	✗	✓	✓	✗	✓	✓
Bluesky	ISO	Fall	✗	✓	✗	✗	✓	✗	✗	✓
Beaver	POP	Fall	✗	✓	✗	✓	✓	✗	✓	✓
Sand	POP	Fall	✗	✓	✗	✗	✓	✗	✗	✓
Loon	POP	Fall	✗	✗	✗	✗	✗	✗	✗	✗
Grass	POP	Fall	✗	✓	✗	✗	✓	✗	✗	✓
Lower Raven	POP	Fall	✗	✗	✗	✓	✓	✗	✓	✗
Perry	POP	Fall	✗	✓	✗	✓	✗	✗	✗	✓
Spruce	ISO	Spring	✗	✗	✗	✗	✗	✗	✗	✗
Charlie Mann’s	ISO	Spring	✗	✗	✗	✓	✓	✗	✗	✓
Buck	ISO	Spring	✗	✗	✗	✓	✓	✗	✓	✓
Mining Corp.	ISO	Spring	✗	✗	✗	✓	✓	✗	✓	✓
Tea	ISO	Spring	✗	✗	✗	✗	✓	✗	✓	✗
Bluesky	ISO	Spring	✓	✗	✗	✓	✓	✗	✗	✓
Beaver	POP	Spring	✗	✗	✗	✓	✓	✗	✓	✓
Sand	POP	Spring	✗	✗	✗	✗	✗	✗	✗	✗
Loon	POP	Spring	✗	✗	✗	✗	✓	✗	✗	✓
Grass	POP	Spring	✗	✗	✗	✗	✓	✗	✓	✓
Lower Raven	POP	Spring	✗	✗	✗	✓	✓	✗	✗	✗
Perry	POP	Spring	✗	✗	✗	✓	✓	✗	✓	✓
Spruce	ISO	Summer	✗	✗	✗	✗	✗	✗	✗	✓
Charlie Mann’s	ISO	Summer	✗	✗	✗	✗	✗	✗	✓	✓
Buck	ISO	Summer	✗	✗	✗	✗	✗	✗	✓	✓
Mining Corp.	ISO	Summer	✗	✗	✗	✗	✗	✗	✗	✓
Tea	ISO	Summer	✗	✗	✗	✗	✗	✓	✓	✗
Bluesky	ISO	Summer	✗	✗	✗	✓	✗	✗	✗	✓
Beaver	POP	Summer	✗	✗	✗	✗	✗	✗	✗	✓
Sand	POP	Summer	✗	✗	✗	✗	✗	✗	✓	✓
Loon	POP	Summer	✗	✗	✗	✗	✗	✗	✓	✓
Grass	POP	Summer	✗	✗	✗	✗	✗	✗	✗	✗
Lower Raven	POP	Summer	✗	✗	✗	✗	✗	✗	✗	✓
Perry	POP	Summer	✗	✗	✗	✗	✗	✗	✗	✓

				Coleoptera					
Site	Development Type	Season	Asellidae	Elmidae	Gyrinidae	Haliplidae	Hydrophilidae	Psephenidae	Ptilodactylidae
Spruce	ISO	Fall	✗	✗	✗	✗	✗	✗	✗
Charlie Mann’s	ISO	Fall	✗	✓	✓	✓	✓	✓	✓
Buck	ISO	Fall	✗	✗	✗	✗	✗	✗	✗
Mining Corp.	ISO	Fall	✗	✗	✗	✗	✗	✗	✗
Tea	ISO	Fall	✗	✗	✗	✗	✗	✗	✓
Bluesky	ISO	Fall	✗	✗	✗	✗	✗	✗	✗
Beaver	POP	Fall	✓	✗	✗	✓	✓	✗	✗
Sand	POP	Fall	✗	✗	✗	✗	✗	✗	✗
Loon	POP	Fall	✓	✗	✗	✗	✗	✗	✗
Grass	POP	Fall	✗	✗	✗	✗	✗	✗	✗
Lower Raven	POP	Fall	✗	✗	✗	✗	✗	✗	✗
Perry	POP	Fall	✓	✗	✗	✗	✗	✗	✗
Spruce	ISO	Spring	✗	✗	✗	✗	✗	✗	✗
Charlie Mann’s	ISO	Spring	✗	✗	✗	✗	✗	✗	✗
Buck	ISO	Spring	✗	✗	✗	✗	✗	✗	✗
Mining Corp.	ISO	Spring	✗	✗	✗	✗	✗	✗	✗
Tea	ISO	Spring	✗	✓	✗	✗	✗	✗	✗
Bluesky	ISO	Spring	✗	✗	✗	✓	✗	✗	✗
Beaver	POP	Spring	✗	✗	✗	✗	✗	✗	✗
Sand	POP	Spring	✗	✗	✗	✗	✗	✗	✗
Loon	POP	Spring	✗	✗	✗	✗	✗	✗	✗
Grass	POP	Spring	✗	✗	✗	✗	✗	✗	✗
Lower Raven	POP	Spring	✗	✗	✗	✗	✗	✗	✗
Perry	POP	Spring	✗	✗	✗	✗	✗	✗	✗
Spruce	ISO	Summer	✗	✗	✗	✗	✗	✗	✗
Charlie Mann’s	ISO	Summer	✓	✗	✗	✗	✗	✗	✗
Buck	ISO	Summer	✗	✗	✗	✓	✗	✗	✗
Mining Corp.	ISO	Summer	✓	✗	✗	✗	✗	✗	✗
Tea	ISO	Summer	✗	✗	✗	✗	✗	✗	✗
Bluesky	ISO	Summer	✗	✗	✗	✓	✗	✗	✗
Beaver	POP	Summer	✓	✗	✓	✓	✗	✗	✗
Sand	POP	Summer	✓	✗	✗	✗	✗	✗	✗
Loon	POP	Summer	✓	✗	✗	✗	✗	✗	✗
Grass	POP	Summer	✓	✗	✗	✓	✗	✗	✗

Lower Raven	POP	Summer	×	×	×	×	×	×	×
Perry	POP	Summer	✓	×	×	×	×	×	×

			Diptera					**Hemiptera**		
Site	**Development Type**	**Season**	**Atheri -cidae**	**Chironom-idae**	**Stratio-myidae**	**Tipulidae**	**Tabanidae**	**Naucoridae**	**Notonectidae**	**Veliidae**
Spruce	ISO	Fall	×	✓	×	×	×	×	×	×
Charlie Mann’s	ISO	Fall	×	✓	×	✓	×	×	✓	×
Buck	ISO	Fall	×	✓	×	×	×	×	×	×
Mining Corp.	ISO	Fall	✓	✓	×	×	×	×	×	×
Tea	ISO	Fall	✓	✓	×	×	×	×	×	×
Bluesky	ISO	Fall	✓	✓	×	×	×	×	×	×
Beaver	POP	Fall	×	×	×	×	✓	×	×	×
Sand	POP	Fall	✓	×	×	×	×	×	×	×
Loon	POP	Fall	×	✓	×	×	×	×	×	×
Grass	POP	Fall	×	✓	×	×	×	×	×	×
Lower Raven	POP	Fall	✓	✓	×	×	×	×	×	×
Perry	POP	Fall	✓	✓	×	×	×	×	×	×
Spruce	ISO	Spring	×	✓	✓	×	×	×	×	×
Charlie Mann’s	ISO	Spring	×	✓	✓	×	×	×	✓	×
Buck	ISO	Spring	×	✓	×	×	×	×	×	×
Mining Corp.	ISO	Spring	×	✓	✓	×	×	×	✓	×
Tea	ISO	Spring	×	✓	✓	×	×	×	×	×
Bluesky	ISO	Spring	×	✓	×	×	×	×	×	×
Beaver	POP	Spring	×	✓	×	×	×	×	×	×
Sand	POP	Spring	×	✓	✓	×	×	×	×	×
Loon	POP	Spring	×	✓	×	×	×	×	×	✓
Grass	POP	Spring	×	✓	×	×	×	×	×	×
Lower Raven	POP	Spring	×	✓	✓	×	×	×	×	×
Perry	POP	Spring	×	✓	✓	×	×	×	×	×
Spruce	ISO	Summer	×	✓	✓	×	×	×	×	×
Charlie Mann’s	ISO	Summer	×	×	×	×	×	×	×	×
Buck	ISO	Summer	×	✓	×	×	×	×	✓	×
Mining Corp.	ISO	Summer	×	✓	×	×	×	×	×	×
Tea	ISO	Summer	×	✓	×	×	×	×	×	×
Bluesky	ISO	Summer	×	✓	×	×	×	×	×	×
Beaver	POP	Summer	×	✓	×	×	×	×	×	×
Sand	POP	Summer	×	✓	×	×	×	×	×	×

Loon	POP	Summer	×	✓	×	×	×	×	×	×
Grass	POP	Summer	×	✓	×	×	×	✓	✓	×
Lower Raven	POP	Summer	✓	✓	×	×	×	×	×	×
Perry	POP	Summer	×	✓	×	×	×	×	×	×

			Megaloptera		Lepioptera			
Site	Development Type	Season	Corydalidae	Sialidae	Cambaridae	Amphipoda	Oligochaeta	Lymnaeidae
Spruce	ISO	Fall	×	×	×	✓	×	✓
Charlie Mann’s	ISO	Fall	×	×	×	✓	×	✓
Buck	ISO	Fall	✓	×	×	✓	×	×
Mining Corp.	ISO	Fall	×	×	×	✓	×	✓
Tea	ISO	Fall	×	×	×	✓	✓	✓
Bluesky	ISO	Fall	✓	×	✓	✓	✓	✓
Beaver	POP	Fall	×	✓	×	✓	×	✓
Sand	POP	Fall	×	×	×	×	×	✓
Loon	POP	Fall	×	×	✓	✓	✓	×
Grass	POP	Fall	×	✓	×	✓	×	×
Lower Raven	POP	Fall	✓	×	×	✓	×	×
Perry	POP	Fall	×	✓	✓	✓	✓	✓
Spruce	ISO	Spring	×	×	×	✓	✓	×
Charlie Mann’s	ISO	Spring	×	×	×	✓	×	✓
Buck	ISO	Spring	✓	✓	×	✓	×	✓
Mining Corp.	ISO	Spring	×	✓	×	✓	✓	×
Tea	ISO	Spring	×	×	×	✓	✓	×
Bluesky	ISO	Spring	×	×	×	✓	✓	×
Beaver	POP	Spring	×	×	✓	✓	✓	✓
Sand	POP	Spring	×	✓	×	×	✓	✓
Loon	POP	Spring	×	×	×	✓	✓	✓
Grass	POP	Spring	×	×	×	×	✓	✓
Lower Raven	POP	Spring	×	×	×	✓	×	×
Perry	POP	Spring	×	✓	✓	✓	×	✓
Spruce	ISO	Summer	×	×	×	✓	×	✓
Charlie Mann’s	ISO	Summer	×	×	×	✓	×	✓
Buck	ISO	Summer	✓	×	×	✓	×	×
Mining Corp.	ISO	Summer	×	×	×	✓	×	✓
Tea	ISO	Summer	×	×	✓	×	✓	×
Bluesky	ISO	Summer	×	×	×	✓	✓	✓

Beaver	POP	Summer	✓	✓	✓	✓	✓	✓
Sand	POP	Summer	×	✓	×	✓	✓	✓
Loon	POP	Summer	×	×	×	✓	✓	✓
Grass	POP	Summer	×	×	✓	✓	×	×
Lower Raven	POP	Summer	✓	×	×	×	×	✓
Perry	POP	Summer	✓	✓	×	✓	×	✓

Site	**Development Type**	**Season**					
Spruce	ISO	Fall	Planorbidae	Physidae	Sphaeriidae	Unionidae	Platyhelminthes
Charlie Mann’s	ISO	Fall	×	×	✓	✓	×
Buck	ISO	Fall	×	×	✓	✓	×
Mining Corp.	ISO	Fall	✓	×	×	✓	×
Tea	ISO	Fall	✓	×	✓	✓	×
Bluesky	ISO	Fall	×	×	✓	×	×
Beaver	POP	Fall	✓	×	✓	×	×
Sand	POP	Fall	✓	×	✓	✓	✓
Loon	POP	Fall	✓	×	✓	✓	✓
Grass	POP	Fall	×	×	✓	✓	✓
Lower Raven	POP	Fall	✓	×	×	×	×
Perry	POP	Fall	×	×	✓	×	×
Spruce	ISO	Spring	✓	×	✓	✓	×
Charlie Mann’s	ISO	Spring	×	×	✓	✓	×
Buck	ISO	Spring	✓	✓	✓	×	×
Mining Corp.	ISO	Spring	×	×	✓	×	×
Tea	ISO	Spring	✓	×	✓	✓	×
Bluesky	ISO	Spring	×	×	✓	×	×
Beaver	POP	Spring	✓	×	✓	×	×
Sand	POP	Spring	✓	×	✓	✓	×
Loon	POP	Spring	✓	×	✓	✓	×
Grass	POP	Spring	×	×	✓	×	×
Lower Raven	POP	Spring	×	×	✓	✓	×
Perry	POP	Spring	×	×	✓	✓	×
Spruce	ISO	Summer	×	×	✓	✓	×
Charlie Mann’s	ISO	Summer	✓	×	✓	×	×
Buck	ISO	Summer	✓	×	✓	×	×

Mining Corp.	ISO	Summer	✓	×	✓	×	×
Tea	ISO	Summer	✓	×	✓	✓	×
Bluesky	ISO	Summer	×	×	✓	✓	×
Beaver	POP	Summer	✓	×	✓	×	×
Sand	POP	Summer	✓	×	✓	✓	×
Loon	POP	Summer	✓	×	✓	×	×
Grass	POP	Summer	×	×	✓	✓	×
Lower Raven	POP	Summer	×	×	✓	×	×
Perry	POP	Summer	✓	×	✓	×	×

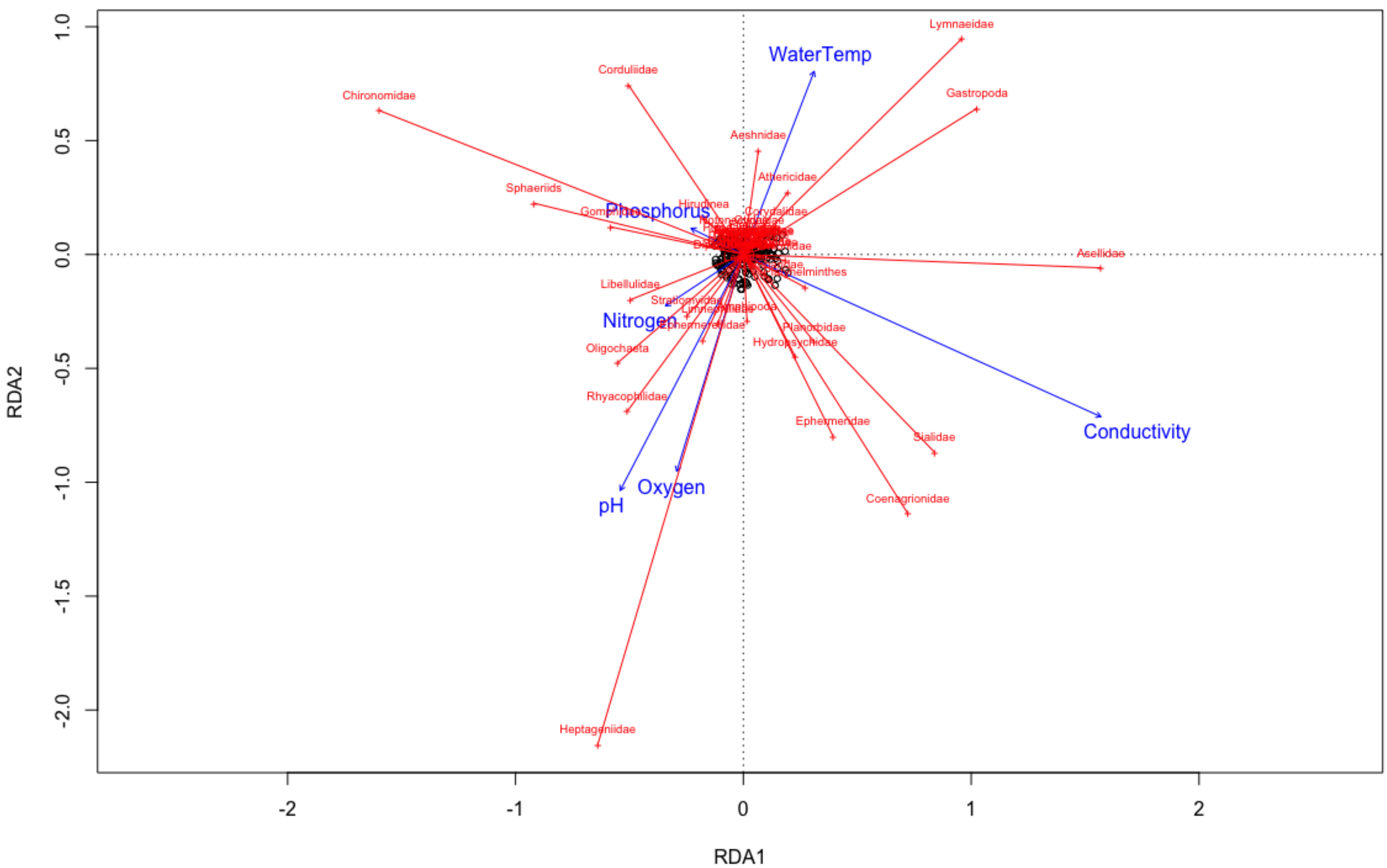

Figure 2. Whole study RDA ordination diagram on the effect of water chemistry on macroinvertebrate composition in benthic communities of central Ontario. Taxa data were Hellinger-transformed and environmental variables were standardized. Data include sampling

over three seasons, and include families of benthic invertebrates and water chemistry. Blue text and arrows represent the environmental variables that were assessed; red text represents different families of macroinvertebrates sampled. Family scores are scaled by eigenvalues while site scores (black dots clustered in the centre) are unscaled. The combination of all 7 water chemistry variables accounts for 9.82% of variation in benthic community composition.

Table 13. 'By Axis' and 'By Terms' permutation tests for whole study water chemistry RDA analysis. Results are assessing constrained axes, and environmental variables independently using the anova.cca function from the R package "vegan". 999 permutations were used for both tests. Macroinvertebrate data were Hellinger-transformed, and water chemistry data were standardized. PR(>F) column represents the p-value associated with the calculated F statistic. Axes RDA1, RDA2 and RDA3 had p-values less than alpha 0.05. Water chemistry variables WaterTemp, Oxygen, pH, Conductivity and Nitrogen had p-values less than alpha 0.05.

'By Axis'	Df	Variance	% Explained Variance	F	Pr(>F)
RDA1	1	0.02732	4.49	10.6514	0.001***
RDA2	1	0.01769	2.91	6.8972	0.001***
RDA3	1	0.0118	1.94	4.5989	0.001***
RDA4	1	0.00413	0.68	1.6114	0.412
RDA5	1	0.00258	0.42	1.0066	0.763
RDA6	1	0.00168	0.28	0.6534	0.843
Residual	212	0.54379			
'By Terms'	**Df**	**Variance**	**% Explained Variance**	**F**	**Pr(>F)**
WaterTemp	1	0.01325	2.18	5.164	0.001***
Oxygen	1	0.0055	0.90	2.1438	0.009**
pH	1	0.01468	2.41	5.7232	0.001***
Conductivity	1	0.02471	4.06	9.6327	0.001***
Phosphorus	1	0.00226	0.37	0.8815	0.601
Nitrogen	1	0.00481	0.79	1.8737	0.025*
Residual	212	0.54379			

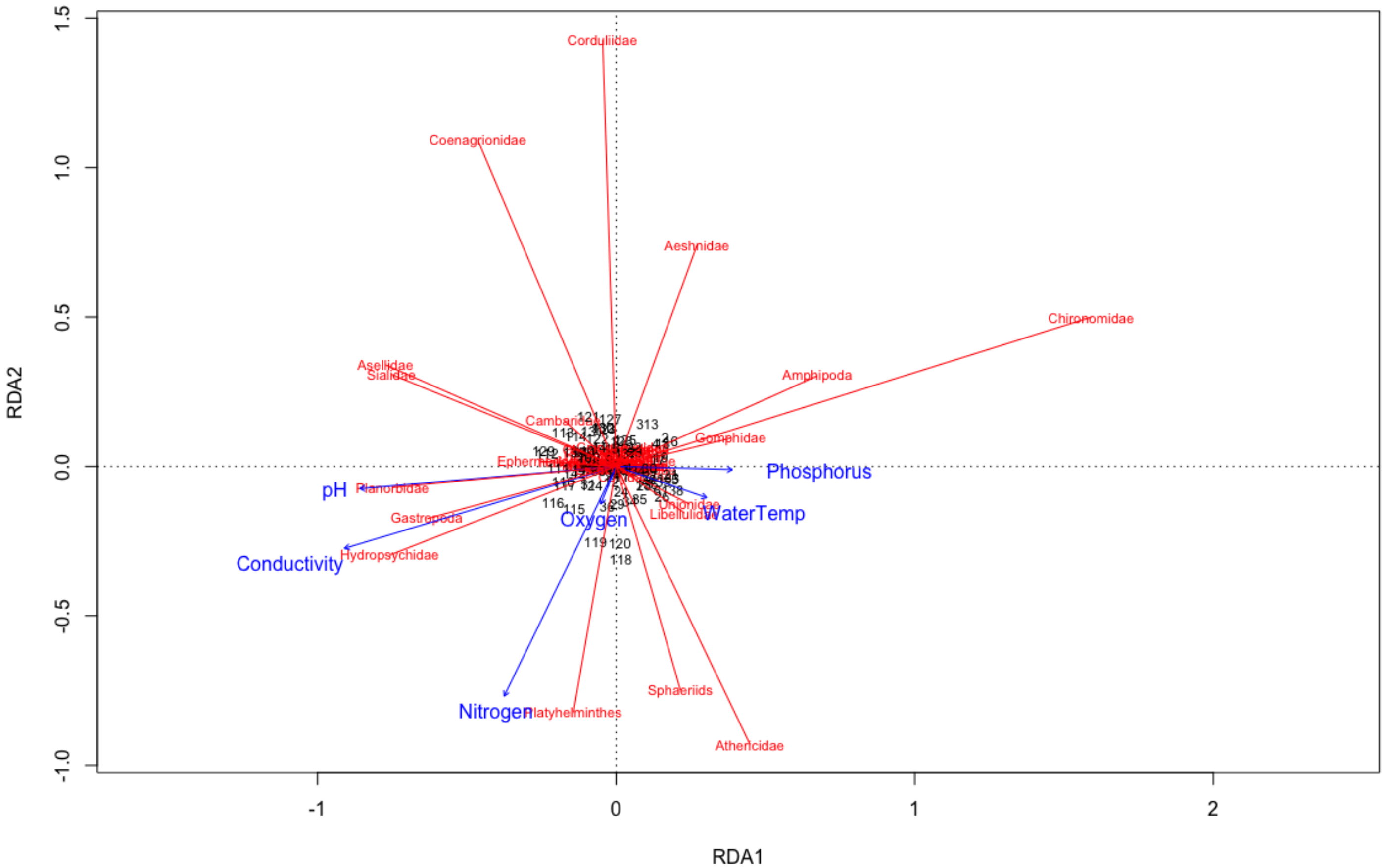

Figure 3. RDA ordination diagram showing the effect of water chemistry on benthic macroinvertebrate community composition in Fall. Blue arrows and text represent water chemistry variables and red text represents different families of macroinvertebrates. Family scores are scaled by eigenvalues while site scores (black dots clusters in the center) are unscaled. The combination of 7 water chemistry variables explains 17.15% of variance in benthic communities in the Fall 2020 season.

Table 14. 'By Axis' and 'By Terms' permutation test for Fall 2020 water chemistry RDA analysis. Results are assessing constrained axes and water chemistry variables independently using the 'anova.cca' function from the R package "vegan". 999 permutations were used for both tests. Macroinvertebrate data were Hellinger transformed, and water chemistry data were standardized. PR(>F) column represents the p-value associated with the calculated F statistic. Constrained axes RDA1, RDA2, RDA3 and RDA4 had p-values less than alpha 0.05. All water chemistry variables assessed had p-values less than 0.05.

"By Axis"	Df	Variance	% Explained Variance	F	Pr(>F)
RDA1	1	0.05816	9.32	7.982	0.001***
RDA2	1	0.0326	5.22	4.474	0.002**
RDA3	1	0.02427	3.89	3.3306	0.007**

RDA4	1	0.01826	2.93	2.5061	0.035*
RDA5	1	0.01148	1.84	1.5757	0.249
RDA6	1	0.006	0.96	0.8242	0.646
Residual	65	0.47358			
"By Terms"	**Df**	**Variance**	**% Explained Variance**	**F**	**Pr(>F)**
WaterTemp	1	0.01948	3.12	2.6735	0.002**
Oxygen	1	0.01614	2.59	2.2154	0.010**
pH	1	0.04383	7.02	6.0159	0.001***
Conductivity	1	0.028	4.49	3.8428	0.001***
Phosphorus	1	0.01312	2.10	1.8009	0.039*
Nitrogen	1	0.03019	4.84	4.1439	0.001***
Residual	65	0.47358			

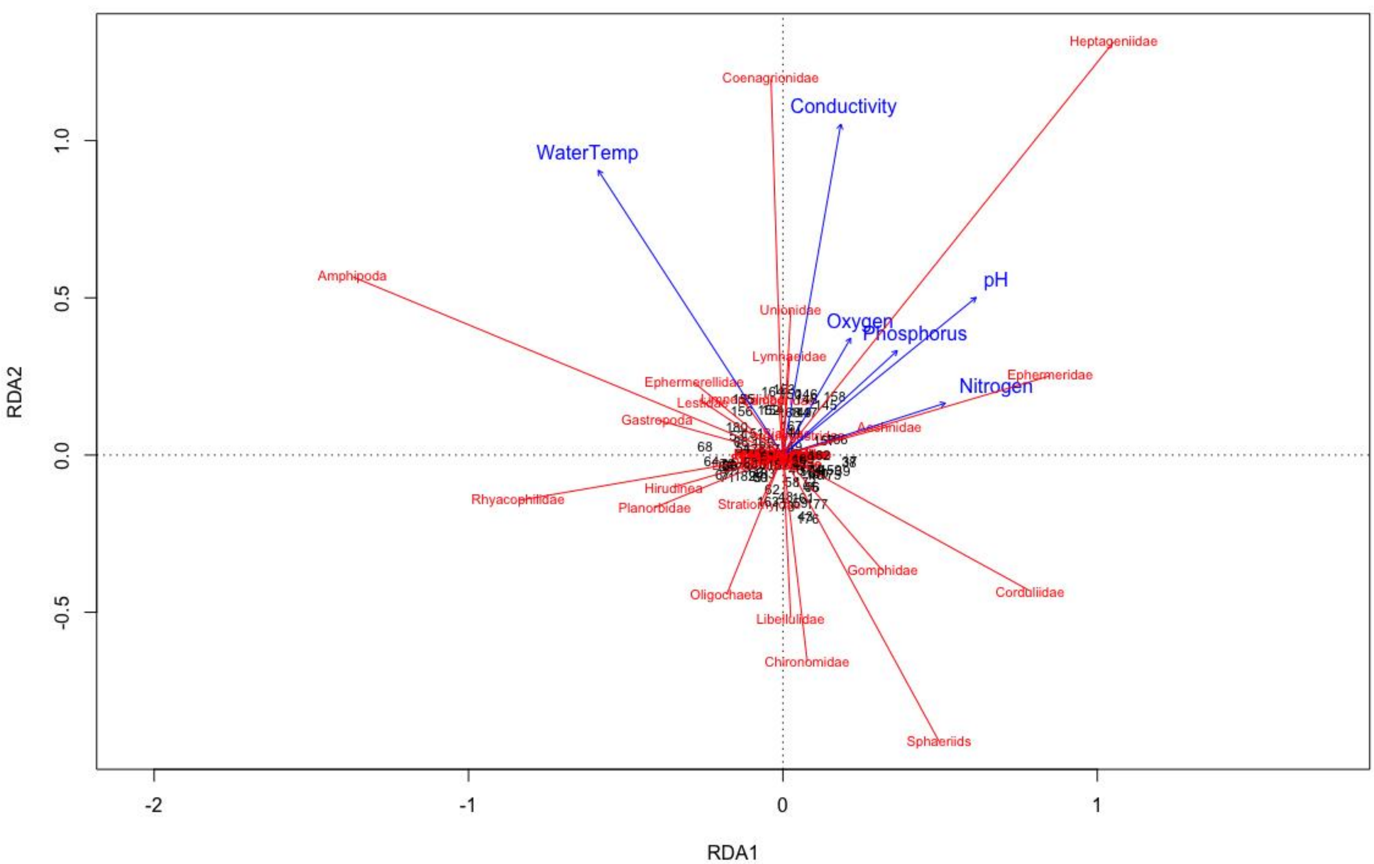

Figure 4. RDA analysis of the effect of water chemistry on macroinvertebrate community composition in spring. Macroinvertebrate data were Hellinger transformed and water chemistry data were standardized. Blue arrows and text represent environmental variables and red text and lines represent families of macroinvertebrates sampled. Family scores are scaled by eigenvalues while site scores (back dots clustered in the center) are unscaled. The combination of all six water chemistry variables explains 13.3% of variance in benthic macroinvertebrate communities.

Table 15. 'By Axis' and 'By Terms' permutation tests for Spring 2021 water chemistry RDA analysis. Results assess constrained axes and environmental variables independently using the anova.cca function from the R package vegan. 999 permutations were used for both tests. Macroinvertebrate data were Hellinger transformed and water chemistry data were standardized. PR(>F) column represents the p-value associated with the calculated F statistic. Constrained axes RDA1 and RDA2 had p-values less than alpha 0.05. Water chemistry variables water temperature, dissolved oxygen, pH, conductivity and total phosphorus had p-values less than 0.05.

"By Axis"	**Df**	**Variance**	**% Explained Variance**	**F**	**Pr(>F)**
RDA1	1	0.0432	8.42	7.1839	0.001 ***
RDA2	1	0.03036	5.91	5.049	0.001 ***
RDA3	1	0.01461	2.85	2.4295	0.098
RDA4	1	0.01011	1.97	1.6812	0.371
RDA5	1	0.00374	0.73	0.6227	0.99
RDA6	1	0.00239	0.47	0.3968	0.981
Residual	68	0.40893			
"By Terms"	**Df**	**Variance**	**% Explained Variance**	**F**	**Pr(>F)**
WaterTemp	1	0.02592	5.05	4.3106	0.001***
Oxygen	1	0.01157	2.25	1.9237	0.035*
pH	1	0.02577	5.02	4.2845	0.001***
Conductivity	1	0.02158	4.20	3.5877	0.001***
Phosphorus	1	0.01287	2.51	2.1405	0.013*
Nitrogen	1	0.00671	1.31	1.116	0.317
Residual	68	0.40893			

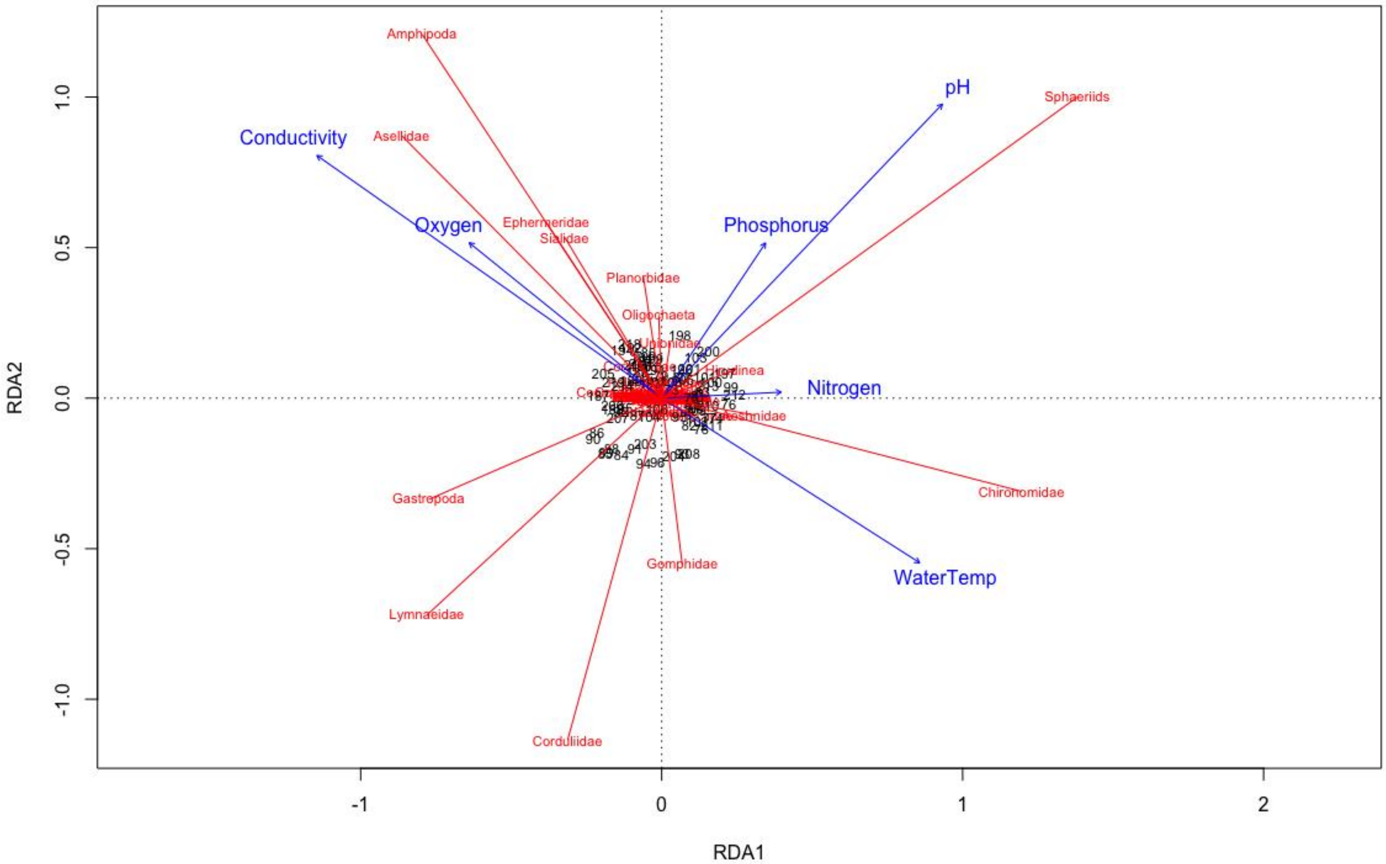

Figure 5. RDA ordination diagram showing the effect of water chemistry on macroinvertebrate community compositiiton in summer. Macroinvertebrate data were Hellinger transformed and water chemistry data were standardized. Blue arrows and text represent water chemistry variables while red text represents macroinvetebrate families sampled. Family scores are scaled by eigenvalues. The combination of all six water chemistry variables account for 20.07% of variance in summer benthic community composition.

Table 16. 'By Axis' and 'By Term' permutation tests for summer 2021 water chemistry RDA analysis. Results assess constrained axes and environmental variables independently using the anova.cca function from the R package vegan. 999 permutations were used for both tests. Macroinvertebrate data were Hellinger transformed and water chemistry data were standardized. PR(>F) column represents the p-value associated with the calculated F statistic. Constrained axes RDA1, RDA2 and RDA3 had p-values less than 0.05. Water chemistry variables: water temperature, dissolved oxygen, pH, conductivity and available nitrogen had p-values less than 0.05.

"By Axis"	Df	Variance	% Explained Variance	F	Pr(>F)
RDA1	1	0.06818	11.53	10.2398	0.001***

RDA2	1	0.04535	7.67	6.8105	0.001***
RDA3	1	0.02495	4.22	3.7475	0.005**
RDA4	1	0.0125	2.11	1.8768	0.287
RDA5	1	0.00591	1.00	0.8883	0.858
RDA6	1	0.00179	0.30	0.2692	0.992
Residual	65	0.43279			
"By Terms"	**Df**	**Variance**	**% Explained Variance**	**F**	**Pr(>F)**
WaterTemp	1	0.01325	2.18	5.164	0.001***
Oxygen	1	0.0055	0.90	2.1438	0.006**
pH	1	0.01468	2.41	5.7232	0.001***
Conductivity	1	0.02471	4.06	9.6327	0.001***
Phosphorus	1	0.00226	0.37	0.8815	0.552
Nitrogen	1	0.00481	0.79	1.8737	0.027*
Residual	212	0.54379			

3.4 Discussion

3.4.1 Physicochemical Properties of Lake Water

In the present study, water chemistry variables were collected from 12 lakes in central Ontario and seasonal averages were compared to gain an understanding of how these variables change. Four of the six water chemistry variables changed significantly from one season to another.

Water temperature (ºC) trended with air temperature as expected, with average water temperature steadily increasing from fall (8.93ºC), to spring (10.73ºC) and hitting it peak in summer (24.99ºC). Since none of the lakes experience large influxes from major anthropogenic sources such as industry or water treatment plants, there was little variation overall, among lakes. The summer had the largest error in water temperature between lakes, with a standard error of 0.444C^{o}, which is likely due to the fact that some lakes have more shoreline tree and plant cover, providing shade in the littoral regions where temperature was measured.

Average dissolved oxygen concentration (DO) in the littoral zone was similar in fall (10.7976mg/L) and spring (10.056mg/L). With the aid of a Kruskal-Wallis tests and a Dunn's post hoc test, we found that summer DO differed significantly from the other months with an average concentration of 8.126mg/L. The Canadian environmental quality guidelines outline the acceptable ranges of dissolved oxygen concentrations for freshwater systems (Canadian Council of Ministers of the Environment, 1999). For cold water periods, the lowest tolerable concentrations of DO for aquatic organisms were 9.5 mg/L for sensitive developmental stages and 6.5 mg/L for other life stages, respectively (Canadian Council of Ministers of the Environment, 1999). The three lakes sampled in the fall with the coldest water temperatures were Spruce Lake (8.55 mg/L), Charlie Mann's Lake (8.66 mg/L) and Loon Lake (8.87 mg/L). With the average water temperature being 8.5ºC for lakes sampled in the fall, we can argue that these DO measurements will remain at biologically stable levels, as cold water supports increased DO concentrations. For warm water systems, a DO concentration of 6 mg/L is the lowest concentration accepted to support sensitive taxa (Canadian Council of Ministers of the Environment, 1999). No lakes sampled in the spring or summer showed DO concentrations less than that critical value, therefore, dissolved oxygen concentration does not appear to be a limiting factor for local aquatic life.

The two major sources of dissolved oxygen in aquatic systems are the atmosphere and photosynthesis by aquatic vegetation (Canadian Council of Ministers of the Environment, 1999). Due to the importance of aquatic vegetation as a contributor to the dissolved oxygen concentration, one would expect a higher concentration of DO in the summer months, when plants are at their most productive. However, temperature is a major regulating factor in DO, especially in large systems like lakes. In the spring months, as temperatures begin to rise, the water column of a lake in the area would be close to 100% saturated (Canadian Council of Ministers of the Environment, 1999). When temperatures continue to rise in the summer months, the upper layer of the water column (also known as the epilimnion) warms at a faster rate and (process known as stratification) will result in a localized reduction of DO (Canadian Council of Ministers of the Environment, 1999). In fall, when temperatures drop, the metalimnion and hypolimnion mix with the epilimnion (vertical mixing). This will result in an increase in oxygen concentration in the epilimnion (Canadian Council of Ministers of the Environment, 1999). These standard trends in oxygen concentration associated with seasonal changes and air temperature match our measured trends nicely, with the lowest oxygen concentration occurring in the summer and the spring and fall having higher averages.

By examining the trends of pH levels in the lakes sampled in and around Kearney, Ontario, it appears the region experiences a seasonal shift in aquatic pH, with the lowest and most acidic conditions occurring in fall. Following winter and ice melt, we see pH spike and a subsequent leveling off in the summer before we can expect it to drop again in the fall. This spike in pH in spring, and the subsequent decrease over time is an interesting finding that does not follow historic trends in the region. Historically, the Precambrian Shield region of central Ontario experienced a drop in pH in spring; this was mostly attributed to the rapid melt of accumulated snow (Jeffries *et al.,* 1978). Jeffries *et al* (1978) sampled H^+, SO_4 and NO_3- levels in accumulated snow near lakes and rivers in Central Ontario in 1978 and assessed how the melting of these snowpacks altered pH in the region. They found that during mid-March of 1978 1-1.5m of snow cover, with an average pH of 4.0-4.3 (due to concentrations of acidic sulphate and protons, or H^+ ions), melted into nearby water systems, lowering the water pH. Coupled with the fact that the Precambrian bedrock underlying most watersheds has a low buffering capacity, and shows limited H^+ assimilation, the compounds within the snowmelt enter local water systems relatively unaltered (Jeffries *et al.,* 1978). This resulted in a drop in pH of 0.5, 1.2 and 0.3 pH units in the lakes studied in 1978. The authors suggested that this could have a significant effect on the species composition of local fish communities (especially species whose acid-sensitive life stages coincide with snowmelt periods, like northern pike, yellow perch, walleye, and rainbow trout), as well as macroinvertebrate communities (Jeffries *et al.,* 1978; Palmer *et al.,* 2011). Since the lakes assessed in our study did not experience the same pH decreases during spring snowmelt, it is possible that the effects of acidic compound accumulation in snow cover have decreased significantly since they were last assessed in the area in 1978. This is an interesting finding and good news for freshwater systems in the area. Future research in the area on sampling ions associated with acidic deposition could be valuable.

Ontario has experienced significantly less sulphate deposition since regulatory measures were introduced in the 1990s (Palmer *et al.,* 2011). For this reason, Ontario lakes have experienced a steady increase in aquatic pH since then. For example, Palmer *et al* (2011) found that 35 of 36 lakes sampled experienced increases in pH from 1981 to 2005. Lakes in our study appear to be trending in a similar direction, with no season having an average pH less than 6. Seasonal variation in pH can be a simple by-product of the influence from primary productivity in the lake, which increases pH (Casey, 2011). This could explain the higher pH levels recorded in spring and summer, when more photosynthesis would be occurring compared to the fall. Variation in pH between seasons may also be affected by more local factors, which would require a more detailed assessment of coastal soils and other immediate parameters to better explain the observed trends.

Conductivity did not vary much from season to season, with the lowest average value of 22.13 µS/cm in spring 2021, and the highest average of 24.16 µS/cm in fall 2020. In central Ontario's Canadian Shield lakes, conductivity generally ranges from 22 to 33 µS/cm with an average of 23.1 µS/cm; this fits with the values recorded in the present study (Palmer *et al.,* 2011). A long-term study on Ontario's boreal lakes from 1976 to 2002 found that lakes in the area are generally 'dilute' with conductivity ranging from 22 to 35 µS/cm (Dillon *et al.,* 2007). We can therefore assign the term "dilute" to the lakes assessed in this study. A biologically high-water conductivity for a lake system would be approximately between 100 and 200µS/cm (Armstead *et al.,* 2016), which was not seen in any of the lakes studied. Generally, lakes experience seasonal fluctuations in water conductivity, which tend to increase in warmer temperatures (Armstead *et al.,* 2016). This trend was seen, but it was a relatively small increase, from spring to summer, of ~2 µS/cm, increasing from ~23 µS/cm to ~24µS/cm. Due to the low buffering capacity of the underlying bedrock, conductivity can be a concern (Palmer *et al.,* 2011). If large amounts of ionic deposition were to suddenly occur in the area, for example, as a result of mining activities, lakes could be severely affected and the low buffering capacity could mean these systems would have a difficult time rebounding (Palmer *et al.,* 2011).

Lakes located on Ontario's Canadian Shield are typically classified as nutrient-poor environments (Reid and Girard, 1994). In 1994, lake sampling in central Ontario found that 11 of 12 lakes sampled were oligotrophic, with total phosphorus and available nitrogen concentrations being less than 10 µg/L (Reid and Girard, 1994). Furthermore, when sampling central Ontario lakes from 1976 to 2002, Dillon *et al.,* (2007) found that total phosphorus concentrations ranged from 4 to 14 µg/L, and an average of 6.5 µg/L was sampled in central Ontario in 2004/5 by Palmer et al (2011). According to the previous studies, from 1976 up to 2005, lakes in central Ontario have had consistent levels of total phosphorus, averaging in a range between 4 µg/L to just 14µg/L. In the present study the lakes in central Ontario during fall 2020 averaged a total phosphorus concentration of 92.32 µg/L (± 33.92 SE), with average concentrations in the following spring, 40.85 µg/L (± 8.45 SE), and summer, 54.78 µg/L (± 10.23 SE), respectively. These averages are much higher than that reported in the previous studies and are therefore of serious concern. Interestingly, the standard deviations (S.D.s) of all three seasons are very high, with 2 seasons having higher S.D. values than their averages! This suggests there may be considerable variation between lakes. This may be due to either increased ionic deposition in certain areas, or some error in the nutrient analysis process. After removing an "outlier" value in a water sample taken in fall from Spruce Lake site 2, which showed a very high total phosphorus concentration of ~696 µg/L, the fall value dropped to an average of 53.81 µg/L with a S.E. of 11.17. After that adjustment, the three seasons showed similar average total phosphorus concentration values, with the lowest values being evident in spring. These averages are still considerably higher than previously sampled lakes in the region of central Ontario. We found that phosphorus did not vary significantly from season to season, and variation between lakes was high. Inconsistent total phosphorus levels made the range of concentrations especially high and made comparisons difficult. The total phosphorus concentration of lakes in this region is highly variable, even among adjacent sub catchments and the drivers of TP change over time remain unclear (Eimers *et al.,* 2009). This high variation observed in TP could be attributed to sewage waste in the watershed which originates from cottages/hunting lodges (MOE *et al.,* 2010). This influence would likely follow a seasonal pattern, which would be correlated with activity at those properties. For cottages, high summer activity could drive the increased TP concentrations observed from spring to fall (~10µg/L increase on average). As cottage activity winds down, hunting cabins tend to increase in activity during the fall, which could explain the further increases observed in that season (MOE *et al*., 2010). While proper septic systems or practices would prevent contamination, regulations and testing of septic systems tends to be sparse in the area, therefore, the possibility of contamination exists (Roy *et al.,* 2017). There is also a natural leeching process involved with most Class IV (the standard) septic systems, even though that effluent is filtered and bacterially digested, the risk for contaminants, including nutrients, is possible (MOE *et al.,* 2010; Roy *et al.,* 2017). Lakes and associated phosphorus dynamics in south Central Ontario are a topic of much current research (e.g. Eimers *et al., 2009;* Raney and Eimers*,* 2013*;* Crossman *et al.,* 2016). The fluctuations seen within lakes from season to season is an interesting observation, however. Typically, lakes do not vary by that much from season to season let alone yearly. Palmer *et al.* (2011) compared lakes over the course of 30 years, and found the maximum rate of change in TP per year for any lake to be 0.29µg/L. The seasonal changes in the present study were much greater than that, which deserves further investigation.

Measured nitrogen concentrations showed values similar to those reported in earlier studies (Hall and Smol, 1966; Nelson and McCraken, 2002; Palmer *et al.,* 2011). Hall and Smol (1966) found high nitrogen concentrations in central Ontario lakes in 1992, with an average value of 101.76 µg/L, and a range from 2.0 to 247.00 µg/L. Nelson and McCracken (2002) sampled 59 lakes of three different sizes in the Canadian Shield, small (area of 9 – 35 ha), medium (area of 42 – 94.7 ha) and large (area of 106 – 2818.8 ha). This sampling, done from the fall of 1992 to the end of 1995, found that medium sized lakes in the region had the highest available nitrogen concentration, with an average of 56 µg/L. Small lakes had the second highest average with 41 µg/L and large lakes had the lowest with 38µg/L (Nelson and McCracken, 2002). In 36 regional lakes, in 2004-2005, Palmer *et al* (2011) measured an average available nitrogen concentration of 43.3 µg/l, with a minimum of 9.1 µg/L and a maximum of 120.7 µg/L. This range is similar to that noted in the present study and suggests these systems have nitrogen concentrations that are typical for the region.

While the changes in total phosphorus concentration between seasons were not found to be significant, we note that both total phosphorus and available nitrogen were at their highest concentrations in the region in spring. This is not a typical trend in the region, as nutrient concentrations generally peak in late summer (Casey, 2011). However, spring peaks in TP are not unknown, and may be related to overturn, or mixing of layers, of the lake (Casey, 2011). Elevated levels of TP in spring or fall could be due to internal loading of phosphorus from lake sediments and the subsequent mixing of water layers (Casey, 2011). Lakes in our study are mostly located on low buffering underlying bedrock, so this explanation is unlikely to hold for our study lakes.

It is important to mention that the nutrient analysis of water samples is a delicate process which requires considerable accuracy and precision. It is possible that some of the measurements of total phosphorus and available nitrogen in this study, particularly values that were “outliers”, were incorrect due to errors in the laboratory procedures. In each case of an outlier, both replicate samples showed similar results. This suggests contamination of the sample in it entirety is also possible.

3.4.2. Relationship Between Physiochemical Water Variables and the Composition of Benthic Macroinvertebrate Communities.

The seasonal ordination diagrams generated from indivudal RDA analyses show differing relationships between water chemistry variables and their respective constrained axes (Figures 2, 3 & 4). However, the main explanatory variables, namely water temperature, conductivity and pH, appear to be the most correlated, whether positively or negatively, with the constrained axes in the various seasons. Conversely, total phosphorus, dissolved oxygen and available nitrogen had smaller correlations between the axes for most of the seasons. The largest outlier of this would be the effect of total nitrogen in the fall RDA analysis, which is observed in the ordination diagram where nitrogen is strongly negatively correlated with the axis RDA2.

When water chemistry variables were individually assessed, we found that five of the six water chemistry variables had a significant effect on variation in the benthic macroinvertebrate community, with total phosphorus being non-significant as a variable. Water temperature explained 2.18% of the variance in benthic community composition, dissolved oxygen explained

0.90%, pH explained 2.41%, conductivity explained 4.06%, total phosphorus explained 0.37% and available nitrogen explained 0.79% (see Table 13).

When assessed seasonally, we observed a similar effect of each water chemistry variable with each explaining similar levels of variance with differing significance. However, some differences between seasons were observed. In the fall samples, all water chemistry variables assessed were found to have a significant effect on benthic community composition. In the spring, five of the six water chemistry variables assessed had a significant effect on benthic community composition, with no significant effect for available nitrogen. Then, in the summer, total phosphorus was the only water chemistry variable found to not have a significant effect on benthic community composition.

pH explained the largest portion of variance in the fall RDA analysis (7.02%), the second most in the spring (5.02%) and the second most in the summer (2.41%). These results, coupled with pH explaining the second most amount of variance in the whole study RDA analysis (2.41%), allows us to conclude that, among the water chemistry variables tested, pH is one of the most important contributors to benthic community variance in our sampling region. pH explained a significant level of variance over the course of the whole study as well as in each season independently. Acidity is considered to be one of the most limiting variables in water chemistry when it comes to aquatic life (Palmer *et al.,* 2011). This is especially true for systems that experience fluctuations from pH < 6 to pH > 6, which are considered biologically significant thresholds (Palmer *et al.,* 2011). In the present study eight of the twenty-five lake sites (two sites each per lake) sampled fluctuated across this range, with at least one of the seasons sampled having a pH below 6 followed by a season above 6. Six of these eight lakes were considered to be isolated, with only two developed lakes moving across this threshold. Crossing these barriers can be considered significant for benthic macroinvertebrate families and thus is likely to have an effect on the composition of a given community. In a study that confirms the importance of pH in central Ontario's lakes, Lento *et al* (2008) identified a strong temporal relationship between lake benthic macroinvertebrates and water chemistry variables related to pH, like alkalinity, sodium and calcium ion concentrations, and pH itself. This is supported by our findings, where pH had a significant effect on benthic community composition by generating the second greatest amount of variance as indicated in the model (2.41%). As suggested by Lento *et al.* (2008), we can assume that the effect pH is having on benthic community composition is not only due to the direct influence pH has on benthic life but also the influence pH has on a variety of dissolved ions. Anthropogenic acidification remains a primary determinant of water quality in the area of central Ontario and has a profound impact on aquatic life (Palmer *et al.,* 2011).

Water temperature consistently explained a significant amount of variance in fall (3.12%), spring (5.05%) and summer (2.18%) as well as during the course of the entire study, by explaining 2.18% of variation in the whole study of benthic community composition. The effect water temperature has on benthic communities is well documented, as it changes seasonally in association with air temperature. Water temperature is a major factor in influencing macroinvertebrate emergence, egg deposition and hatching, and therefore has a major effect on which taxa are present in the system (Thorp and Rogers, 2015), as well as the timing of these activities. The fall months are common emergence periods for a variety of benthic macroinvertebrate species (Thorp and Rogers, 2015). For this reason, we typically see variation in community composition as well as differences in population sizes and maturity in different seasons (Thorp and Rogers, 2015). We expect water temperature to have a clearer, significant effect when the data from the three different seasons are combined (data from the whole study), as each season could affect different life stages of the benthic community. In the present study, however, the effect of water temperature on benthic community composition remained consistent even when the individual seasons were tested separately. Looking at the variance in community composition explained by water temperature from season to season, we see that temperature has its largest effect in the spring, and least effect in summer. A variety of factors contribute to the rate at which winter ice melts, as well as the rate at which the temperature of the water body increases (Dillon *et al.,* 2007; Hadley *et al.,* 2013; Nelligan *et al.,* 2019). These contributing factors include differing tree canopy and macrophyte cover around the littoral zone, influx of water from external sources, human activity, and surface runoff (Dillon *et al.,* 2007; Hadley *et al.,* 2013; Nelligan *et al.,* 2019). The resulting variation in water temperature could cause variability in community composition of macroinvertebrates in the lake's littoral zone. When organisms undergo life stage changes during spring (e.g. emergence, mating, egg laying or hatching), it is natural that this season would show the greatest effect of differences in water temperature. Water temperature was the only variable in the whole study RDA analysis that was correlated with both RDA axes, which suggests that it may have played a major role in driving changes or differences in community structure (Lento *et al.,* 2008).

Conductivity was one of the most consistent water chemistry variables across the three seasons sampled in terms of its contribution to explained variance in benthic community composition. In all three seasons, the fall, spring and summer of the study, conductivity explained 4.49%, 4.20% and 4.06% of community composition variance respectively with the effect being statistically significant in all seasons. This is also reflected in the whole study RDA analysis, where conductivity explained the greatest amount of variance in community composition, at 4.06%. This could be due to significant differences in conductivity among lakes, with some averaging as low at 15 µs/cm and others being as high as 40µs/cm, but this parameter maintained a predictable range, with no extreme outliers. Despite this range, no lakes assessed could be deemed highly conductive, with values in the range of 100 and 200 µs/cm (Eimers *et al.,* 2009). However, conductivity was still seen to have an effect on species composition, which is interesting. When conductivity is high in a lake, aquatic organisms may struggle to maintain consistent osmoregulation, which can pose serious risks to a benthic community (Armstead *et al.,* 2016). Another reason for the significant effect of conductivity on benthic community composition could be the low buffering capacity of the lakes themselves and their surrounding watersheds. Any influx of dissolved solids will be more impactful as they enter water systems relatively unaltered (Palmer *et al.,* 2011). The unique geological composition of the region, coupled with the impact of dissolved solids on conductivity could be the reason behind the ecological significance of this water chemistry variable.

Dissolved oxygen was found to have a significant effect on benthic community composition throughout the study (explaining 0.90% of variance), as well as season-by-season in fall (2.59%), spring (2.25%), and summer (0.90%) samples. The lower measured impact of dissolved oxygen concentration on the variance of microbenthic community, accompanied by relatively highly significant p-values compared to other water chemistry variables, shows that the effect of dissolved oxygens on benthic community variance is significant but less intense than conductivity, pH and water temperature. In a manner similar to water temperature, dissolved oxygen concentration tends to shift through the year in relation to other environmental variables. As pointed out earlier in the chapter, water temperature is a major mediating factor affecting dissolved oxygen concentration, so it is not surprising that this environmental variable is having a similar effect on benthic community composition. Dissolved oxygen can have an interesting effect on benthic community composition as some species are better able to tolerate lower concentrations by having morphological advantages over others. For example, many Trichopteran taxa possess external, feather-like gills, which increase the surface area available for gas exchange (Morse *et al.,* 2020). For this reason, they can survive in areas of wider variability of dissolved oxygen concentrations, while taxa without these appendages, like some Plecopterans and Odonatans would not (Morse *et al.,* 2020). The three lakes with the lowest consistent DO concentrations were examined to see if this was the case in our study. These were: Spruce Lake (8.8 mg/L), Grass Lake (8.74 mg/L) and Lower Raven Lake (9.09 mg/L). In Spruce Lake, only one season (fall 2020) yielded a Trichopteran specimen in a sample, a member of the family Hydroptilidae. In Grass Lake, two Trichopteran specimens were found in fall samples (Hydropsychidae and Rhyacophilidae) and one in the spring (Rhyacophilidae). In Lower Raven Lake, specimens of the Trichopteran families Limnephilidae and Rhyacophilidae were found in Fall and Spring samples. In the summer samples, no Trichopteran families were found in any of these three lakes. Of the three Trichopteran families sampled at the three lakes mentioned, Limnephilidae are the only family that possesses the distinctive filamentous external gills mentioned above. While this family was present in lakes with low DO concentrations, Trichopterans possessing these structures were no more likely to be present than other families in the order. It is possible that since DO levels are not reaching biologically critical levels, benthic macroinvertebrates that possess external gills do not experience a significant advantage over fellow Trichopterans lacking these structures. We do see a low Trichopteran presence in the summer months, when water temperatures are at their highest, and dissolved oxygen concentration is at its lowest. However, this can also be attributed to the natural life cycle of Trichopteran species, who emerge and become terrestrial in the summer months (Thorp and

Rogers, 2014). A variety of other factors can influence dissolved oxygen concentration levels in lake systems besides water temperature; these include water turbidity and the presence or absence of aquatic macrophytes.

Since these systems are relatively lentic, experiencing slow water flow, aquatic macrophytes contributing to dissolved oxygen through photosynthesis is a likely source. Lakes experiencing different development activities or differing shoreline types could be associated with contrasting macrophyte presence in their littoral zones. This in turn could result in differing DO concentrations which would in turn have contrasting impacts on their local macroinvertebrate communities (Nelligan *et al.,* 2019; Palmer *et al.,* 2011).

Available nitrogen was found to have a significant effect on benthic community variance during the whole study (0.79%), as well as during two of the seasons, in samples from fall (4.84%) and summer (0.79%). In spring, available nitrogen was found to explain 1.31% of community variance overall, however, this effect was not statistically significant ($p = 0.317$). Clearly, available nitrogen had its greatest impact on benthic community variance in the fall of 2020. Interestingly, the fall samples had the lowest average available nitrogen concentration as well as the lowest standard deviation between lakes sampled. The reason behind this powerful effect in fall is unclear. Lakes in the region differ in terms of littoral and watershed characteristics; this could result in some experiencing higher inputs of nutrient loads through overland runoff. Generally, there is a negative relationship between high nutrient concentrations and invertebrate taxon richness (Ouyang *et al.*, 2018). However, the effect can vary widely between differing ecoregions or even differing systems, as it appears invertebrates respond differently to similar nutrient concentrations (Ouyang *et al.*, 2018). It is possible that available nitrogen concentrations are not directly impacting benthic community composition but rather influence other factors that are affecting the benthic community. For example, nitrogen is a critical nutrient for plant growth and therefore could be influencing macrophyte abundance or algae growth (Griffith, 2011). These conditions could be favorable for some benthic macroinvertebrates, but unfavorable for others.

Total phosphorus was the only water chemistry variable assessed that was found to not have a significant effect on community composition variance over the whole study period. However, when assessed seasonally, total phosphorus explained a significant amount of variance in the fall and spring seasons by explaining 2.10% and 2.51% of variance respectively. While total phosphorus had a significant effect in those seasons, its effect was the least of the variables tested in the fall and second lowest factor in spring. Available nitrogen and total phosphorus are critical nutrients for plant growth. For this reason, the significant effect of total phosphorus in the spring could be because lakes with higher TP concentrations experience more rapid macrophyte growth in their littoral regions in spring (Grant *et al.,* 1999; Griffith, 2011). The macrophytes could serve as habitat for benthic macroinvertebrates and support diverse community composition. This effect could continue into the fall, with lakes with lower TP experiencing fall macrophyte die-off sooner than lakes with elevated TP concentrations. While this has not been specifically observed in aquatic macrophytes, TP is critical to the early growth period of the spring, as well as being a critical plant growth nutrient during flowering and seed production in the later part of the growing season (Grant *et al.,* 1999; Griffith, 2011). The amount of phosphorus used by a plant is much higher than that of the other “macronutrients”, nitrogen and potassium, in the late growing season, underscoring its importance at this time (Griffith, 2011).

3.5 Conclusions Based on the Results in Chapter 3

Of the twelve physicochemical water property variables assessed, four were found to change significantly from one season to another.

3.5.1 Water Temperature

Water temperature (ºC) changed significantly from season to season with a steady increase from fall, through spring, to summer. This trend is as expected and parallels trends in air temperature. Water temperature also had a significant effect on the composition of benthic communities in the area and explained 2.18% of variance in community composition. Seasonally, water temperature explained 3.12% of community variance in the fall, 5.05% in the spring and 2.18% in the summer leading to significant effect in the community composition. This is an expected result as macroinvertebrates have different emergence and life-stage period depending on the seasons.

3.5.2 Dissolved Oxygen

Dissolved oxygen differed significantly with the seasons. DO was found to have a significant effect on community composition, explaining 0.90% of variance in benthic community composition in the whole study period. Seasonally, the effect of DO on community composition remained significant (2.59% in the fall, 2.25% in the spring and 0.90% in the summer, respectively). Although photosynthesis generates more oxygen in lake water in summer, the warm water temperatures resulted in the lowest DO. Dissolved oxygen concentration did not influence benthic community composition as it appears no regions of sampling had a biologically detrimental level.

3.5.3 Conductivity

Conductivity did not differ significantly from season to season or from lake to lake, staying relatively stable throughout the study period. While conductivity did not reach detrimental levels at any point in any lake, it was found to have a significant impact on benthic community composition and explained the most variance in benthic community composition in the model (4.06%). This value remained relatively consistent when assessed seasonally, with conductivity explaining 4.49% of community variance in fall, 4.20% in spring and 4.06% in summer, with its effect being significant across all seasons.

3.5.4 pH

pH was found to change significantly from season to season; it was highest in spring and lowest in fall. pH was found to have a significant effect on benthic community composition and explained the second greatest portion of variance in benthic community composition (2.41%). On a season-by-season basis, pH explained 7.02% of community variance in fall, 5.02% in spring and 2.41% in summer. The effect of pH on community variance was found to be significant over the whole study as well as in each season independently. This, coupled with the lack of closely associated taxa in the ordination plot suggests it is one of the most significant water chemistry variables assessed and has a great influence on benthic communities in the area.

3.5.5. Total Phosphorus

Total phosphorus showed considerable variation between lakes and between sites in the same lake but it did not vary significantly from season to season. Total phosphorus was the only variable assessed that did not have a significant effect on benthic community composition, explaining only 0.37% of the variance. On a season-by-season basis, TP was found to have a significant effect in fall (explaining 2.10% of variance) and in spring (explaining 2.51% of variance), but in summer, TP did not have a significant effect, only explaining 0.37% of community variance.

3.5.6. Available Nitrogen

Available nitrogen levels were found to be significantly different in spring compared to fall and summer. Spring peaks in nutrient content could be due to spring overturn in the lakes. Available nitrogen had a significant effect on benthic community variance over the course of the whole study, explaining 0.79%. On a season-by-season basis, available nitrogen has a significant effect in fall (explaining 4.84% of variance) and summer (explaining 0.79% of variance). Available nitrogen did not have a significant effect on community variance in the spring sample collected in 2021 (explaining 1.31%).

In general, it appears pH, conductivity and water temperature are the three most significant water parameters in terms of their consistent effect on benthic macroinvertebrate community composition in the region in and around Kearney in south-central Ontario Canada.

Chapter 4. Comparing Developed and Isolated Lakes in Terms of Differences in Water Chemistry and Macroinvertebrate Communities.

4.1 Introduction

Anthropogenic activities have altered natural environments and landscapes throughout human history. As human development continues, more stress is applied to these ecosystems in the form of directly altering the ecosystems themselves, or through indirect effects such as altering the surrounding landscape.

Fallen trees or wood debris that find their way into the littoral zone of a lake are known as coarse woody debris or coarse woody habitat (CWH). This debris plays a crucial role in supporting biodiversity in the lake as it provides a habitat for fish spawning, benthic macroinvertebrates and periphyton, as well as increasing organic sediment retention and plant life (Marburg *et al.,* 2006; Sass *et al.,* 2006). As lakeshores experience increasing human development activities, wood debris in the littoral zone is commonly removed to make these areas more accessible or aesthetically pleasing (Sass *et al.,* 2006). This removal affects organisms throughout the food chain, as aquatic predators may shift their diets in response to CWH removal, to become more dependent on terrestrial prey items. This in turn can alter the composition of the macroinvertebrate community (Sass *et al.,* 2006).

Aquatic macrophytes are another critical component of the lake littoral zone, where most biodiversity is to be found (Hicks and Frost, 2010). Aquatic macrophytes stabilize sediment, store nutrients, provide energy for consumers and provide habitat for a variety of macroinvertebrates and fish (Hicks and Frost, 2010). Human development negatively impacts the aquatic macrophytes as they are often removed (Hicks and Frost, 2010).

Riparian plant life is also crucial, as it reduces the inflow of nutrients and sediment from nearby surfaces into local lakes (Owens *et al.,* 2021). Their removal is also associated with increased human development as riparian plant life is commonly removed to allow the building of roads, driveways, and property aesthetics (Owens *et al.,* 2021).

Shoreline armouring is a form of littoral zone modification done by property owners that can have severe impacts on local biota. Especially in recent years, with extreme weather events and higher water levels in some locations, property owners have sought to mitigate shoreline erosion to maintain pre-existing infrastructure and to enhance property values. Shoreline armouring, commonly in the form of retaining walls and riprap revetments, acts to reduce the effects of waves, preventing local erosion. Riprap revetments consist of sloped barriers of rock or rubble while retaining walls are large vertical concrete structures. Both of these cause significant reduction of pre-existing littoral coarse woody debris as well as submerged and emergent macrophytes (Chore *et al.,* 2019). Shoreline armouring has also been found to alter the community composition of benthic macroinvertebrate communities by increasing certain taxa while others become less abundant (Chore *et al.,* 2019).

An increase in impermeable surfaces is highly likely as we build roads, sidewalks and driveways to get to and from properties and services (Gal *et al.,* 2019). The negative aspect of these impermeable surfaces is that they do not absorb water run off transporting soil and other substances (Palmer *et al.,* 2011). Since more of these materials end up in the lake, this can result in higher concentrations of sediment, suspended solids, nutrients and chemical pollutants, which in turn can alter water chemistry and quality (Gal *et al.,* 2019).

Contaminants originating from human activities are another stress that human development can impose on an environment. Nutrients like phosphorus and nitrogen are commonly used as fertilizers, which, when used in high amounts, can run off local surfaces and enter nearby lakes and rivers (Palmer *et al.,* 2011). In regions of human development and activity, a common source of phosphorus contamination is human waste. This is especially true in regions like central Ontario where outhouses are common alternatives to town sewage or septic tanks (Palmer *et al.,* 2010). Higher concentrations of phosphorus and nitrogen in lakes can result in increased plant growth and when plants die and decompose, this can ultimately result in hypoxia in the environment.

Hydrocarbons are common derivatives of oil sources like gasoline, kerosene, fuel oil and asphalt, and are common contaminants associated with human development (Pettigrove and Hoffman, 2005). Human activities such as ATV and snowmobile riding, gas-fuelled generator use, paving of roads, and boat engines are all possible sources of hydrocarbons, which commonly take place at or near the water's edge (Pettigrove and Hoffman, 2005). Hydrocarbon contamination can also arise from road activity (e.x. vehicle exhaust and tire wear) (Pettigrove and Hoffman, 2005). High molecular weight hydrocarbons are likely to be absorbed into the substrate while lightweight aromatic components of hydrocarbons are more toxic due to their solubility in water (Pettigrove and Hoffman, 2005). Researchers began to notice the effects of hydrocarbon contamination in water at 860 mg/kg total petroleum hydrocarbon (TPH), where there was a significant increase in the abundance of opportunistic species (Pettigrove and Hoffman, 2005). These opportunistic taxa included various species of the Dipteran family Chironomidae, which is known for it tolerance to unfavourable conditions (Pettigrove and

Hoffman, 2005). This trend continued as concentration increased, and at 1870 mg/kg TPH, they detected a substantial loss of most aquatic invertebrates (Pettigrove and Hoffman, 2005).

A survey of literature on central Ontario's lake ecosystems, their inhabitants, and the effect of cottage development showed a dearth of sufficient information. While there are a few studies on factors associated with human development, and their impact on water chemistry, fish and macroinvertebrate populations, a thorough study is lacking. We have seen that water quality in the region has changed significantly over the last 25 years owing to human developmental activities. We also know that the community composition of benthic organisms is highly correlated with water chemistry (Palmer *et al.,* 2010; Lento *et al.,* 2008). Therefore, an increase in human developmental activities will very likely have a significant impact on the benthic macroinvertebrate community.

The research objective addressed in this part of study is to assess the effect of cottage development on freshwater lentic water systems by directly comparing developed and isolated lakes in Central Ontario. I pose the following two questions:

1. Does the composition of macroinvertebrate communities differ between lakes that experience cottage developmental activities versus isolated lakes, and
2. Does the water chemistry of Central Ontario's inland lakes differ with respect to the presence or absence cottage development?

I hypothesize that due to the known effects of human development activities, lakes surrounded by human development will have lower water quality and a diminished macroinvertebrate community compared to isolated lakes in the region.

4.2 Methods

4.2.1 Study Locations

The sampling sites are as follows: Beaver Lake, Town of Kearney (45.630 N 79.723 W); Perry Lake, Town of Kearney (45.546 N 79.227 W); Sand Lake, Town of Kearney (45.6272 N 79.1719 W); Loon Lake, Town of Kearney (45.669 N 79.219 W); Grass Lake, Town of Kearney (45.679 N 79.203 W); Buck Lake, Town of Kearney (45.6900 N 79.1672 W); Lower Raven Lake, Town of Kearney (45.692 N 79.151 W); Spruce Lake, Town of Kearney (45.703 N 79.118 W); Bluesky Lake, Town of Kearney (45.748 N 79.178 W); Tea Lake, Town of Kearney (45.672 N 79.255 W); Charlie Mann's Lake, District of Parry Sound (45.712 N 79.042 W); Mining Corporation Lake, District of Parry Sound (45.705 N 79.303 W). Detailed information on each of these lakes is provided above, in Chapter 2 (Materials and Methods).

4.2.2 Sampling of Macroinvertebrates

Macroinvertebrate samples were collected for each lake at two different sites, with three replicate samples being taken from each site. A total of 12 lakes were sampled this way during three different seasons (Fall 2020, and Spring and Summer 2021). To ensure consistency, macroinvertebrate sampling was carried out in accordance with the OBBN protocols (OBBN, 2007). Following collection, samples were initially cleaned and preserved in 70% ethanol. Back in the lab, samples were sorted, and identified with the help of a variety of identification keys and resources. Macroinvertebrate data were organized to generate different biotic indices (specifically EOT, CIGH, Richness, Diversity and HBI) to evaluate different aspects of the macroinvertebrate communities sampled.

4.2.3 Sampling of Water Chemistry

Before sampling each site for macroinvertebrates, water chemistry analysis was carried out at each site using a variety of multiprobes to assess lake water temperature (ºC), pH, conductivity (µs/cm) and dissolved oxygen concentration (mg/L). Along with these in-field assessments, water samples were collected at each site in 150 ml bottles and frozen for later nutrient analysis in the laboratory. Nutrient analyses were made of total phosphorus concentration (µg/L) and available nitrogen concentration (µg/L). The methods for the analyses are described above, in Chapter 2.

4.2.4 Assessing Habitat Quality

At each lake sampling site, habitat characteristics were recorded on a scale from 0 to 2 in accordance with the OBBN protocols (see Table 17 for an explanation) (OBBN, 2007). These habitat variables will be used in an RDA analysis to assess their effect with respect to benthic macroinvertebrate community composition.

Table 17. Explanation of habitat quality recordings in accordance with OBBN protocol manual (OBBN, 2007). Nine habitat quality characteristics were recorded at each site sampled, on a scale from 0 to 2, with associated explanations.

		Scale Description		
Habitat Variable	**Abbreviation**	**0**	**1**	**2**
Coarse Woody Debris	Wood Debris	Not Present	Sparse	Highly Present
Detritus	Detritus	Not Present	Sparse	Highly Present
Macrophytes - Emergent	E. Macrophytes	Not Present	Sparse	Highly Present
Macrophytes - Rooted Floating	RF. Macrophytes	Not Present	Sparse	Highly Present
Macrophytes - Submergent	S. Macrophytes	Not Present	Sparse	Highly Present
Macrophytes - Free Floating	FF. Macrophytes	Not Present	Sparse	Highly Present
Algae - Floating	F. Algae	Not Present	Sparse	Highly Present
Algae - Filamentous	Fil. Algae	Not Present	Sparse	Highly Present
Algae - Attached	A. Algae	Not Present	Sparse	Highly Present

4.2.5 Statistical Analysis

Since most of the water chemistry data were not normally distributed, it was decided that Wilcoxon tests would be the appropriate non-parametric alternative to a two-sample t-test. The Wilcoxon rank sum test, also known as the Mann-Whitney-Wilcoxon test, is a statistical test used to compare two groups that does not require the assumption of normally distributed data (R Core Team, 2021). For this chapter, Wilcoxon rank sum tests were used to compare different environmental variables and macroinvertebrate community characteristics between developed and isolated lakes. Statistical analysis was carried out using the software 'R' (R Core Team, 2021). For all significance tests, the alpha value of 0.05 was used to assign a statistically significant difference between sample groups.

4.2.5.1 RDA analysis

Redundancy analyses (RDAs) were used to assess the effects of water chemistry variables, habitat quality characteristics and lake type on benthic macroinvertebrate community composition. For this analysis, six water chemistry variables were assessed; water temperature (ºC), dissolved oxygen concentration (mg/L), pH, conductivity (µS/cm), total phosphorus (µg/L) and available nitrogen (µg/L). In addition, the analysis addressed nine habitat quality characteristics (scaled from 0 to 2, see Table 12), as well as the distinction as to whether the lake sampled was isolated or developed (0 - isolated, developed - 1). The function 'forward.sel' from the package 'adespatial' was used for forward selection (Dray et al 2022).

4.3 Results

4.3.1 Comparison of Water Chemistry in Isolated and Developed Lakes

To assess the potential differences between isolated and developed (populated) lakes in central Ontario, we compared numerous water chemistry variables and benthic macroinvertebrate community characteristics.

First, we assessed the difference between isolated and developed lakes with respect to average dissolved oxygen concentration over the whole study period (see Figure 6), as well as differences between lake types on a seasonal basis (Figure 7). We found no significant differences between lake types for the whole study ($W = 6048$, $p = 0.64$). In addition there were no significant differences between the two kinds of lake within individual seasons (Fall: $W = 414$, $p = 0.21$; Spring: $W= 342$, $p = 0.89$; Summer: $W = 309$, $p = 0.71$). For the whole study, isolated lakes averaged a dissolved oxygen concentration of 9.56 mg/L, while developed lakes had a similar average of 9.71 mg/L (Figure 6). Once the data were divided seasonally, isolated and developed lakes still had similar averages. In fall, isolated lakes averaged 10.14 mg/L of dissolved oxygen and developed lakes averaged 9.98 mg/L (Figure 7). In spring, isolated lakes averaged 10.42 mg/L while developed lakes had an average value of 10.97 mg/L (Figure 7). Lastly, in summer, isolated lakes had an average dissolved oxygen concentration of 8.13 mg/L, while developed lakes showed an average of 8.17 mg/L (Figure 7).

For the whole study period, the pH of isolated lakes showed an average value of 6.44, while developed lakes showed an average of 6.63 (Figure 8). The Wilcoxon rank sum test used to compare these lake types showed a statistically significant difference between the two ($W = 4635$, $p = 0.009$**). On a season-by-season basis, isolated lakes in fall averaged a pH of 5.94 while developed lakes averaged a pH of 6.34, this difference was statistically significant (W =

133.5, $p = 0.002$*, Figure 9). The average pH in spring was 6.87 in isolated lakes and 6.93 in developed lakes; this difference was not statistically significant ($W = 261$, $p = 0.73$, Figure 9). Lastly, the average summer pH in isolated lakes was 6.50 compared to 6.61 in developed lakes; this difference was not statistically significant ($W = 303$, $p = 0.64$, Figure 9).

Over the whole study, average conductivity differed significantly between isolated and developed lakes ($W = 1503$, $p < 2.2e\text{-}16$***), with isolated lakes having an average conductivity of 16.35 µS/cm and developed lakes averaging 29.21 µS/cm (Figure 10). On a season-by-season basis, isolated and developed lakes were statistically different from one another in all three seasons (Fall: $W = 21$, $p = 9.708e\text{-}07$; Spring: $W = 42$, $p = 4.94e\text{-}06$; Summer: $W = 54$, $p = 1.194e\text{-}05$, Figure 8). Average conductivity values in fall were 16.69 µS/cm in isolated lakes and 31.64 µS/cm in developed lakes (Figure 11). In spring, the average conductivity for isolated lakes was 16.65 µS/cm in contrast to 26.68 µS/cm for developed lakes (Figure 11). In summer, the average conductivity in isolated lakes was 15.62 µS/cm compared to 29.30 µS/cm in developed lakes (Figure 11).

Total phosphorus concentration (TP) was also compared in isolated and developed lakes across the whole study period, as well as on a season-by-season basis. Overall, the average total phosphorus in isolated lakes was 75.18 µg/L, and in developed lakes 47.34 µg/L; the difference between these values was not statistically significant ($W = 6444$, $p = 0.183$) (Figure 12). A similar effect is seen when we compare TP concentrations on a season-by-season basis; in no season did the two lake types differ significantly (Fall: $W = 778.5$, $p = 0.14$; Spring: $W = 805.5$, $p = 0.08$; Summer: $W = 594$, $p = 0.5463$, Figure 13). In fall, average total phosphorus concentrations were 141.73µg/L in isolated lakes and 42.91 µg/L in developed lakes (Figure 13). In spring samples, the average total phosphorus concentration was 39.35 µg/L in isolated lakes and 45.382 µg/L in the developed lakes (Figure 13). In summer, isolated lakes had an average of 44.45 µg/L and developed lakes averaged 53.73 µg/L (Figure 13).

The last water chemistry variable assessed to compare isolated and developed lakes was available nitrogen concentration. Over the whole study, available nitrogen concentration in isolated lakes averaged 37.22 µg/L compared to developed lakes which averaged 44.13 µg/L (Figure 14). This difference between lake types was statistically significant according to the Wilcoxon rank sum test ($W = 4900.5$, $p = 0.04$, Figure 14). On a season-by-season basis, it is evident that available nitrogen concentration was significantly different between isolated and developed lakes (Fall: $W = 333$, $p = <0.001$*; Spring: $W = 360$, $p = 0.001$*; Summer: $W = 900$, $p = 0.004$*) (Figure 15). The average available nitrogen concentrations in fall samples were 25.26 µg/L in isolated, and 43.08 µg/L in developed lakes (Figure 15). In spring, isolated lakes had an average available nitrogen concentration of 37.76 µg/L while developed lakes had an average of 63.13 µg/L (Figure 15). Lastly, in summer, isolated lakes had an average of 48.64 µg/L of available nitrogen and populated lakes had an average concentration of 26.14µg/L (Figure 15).

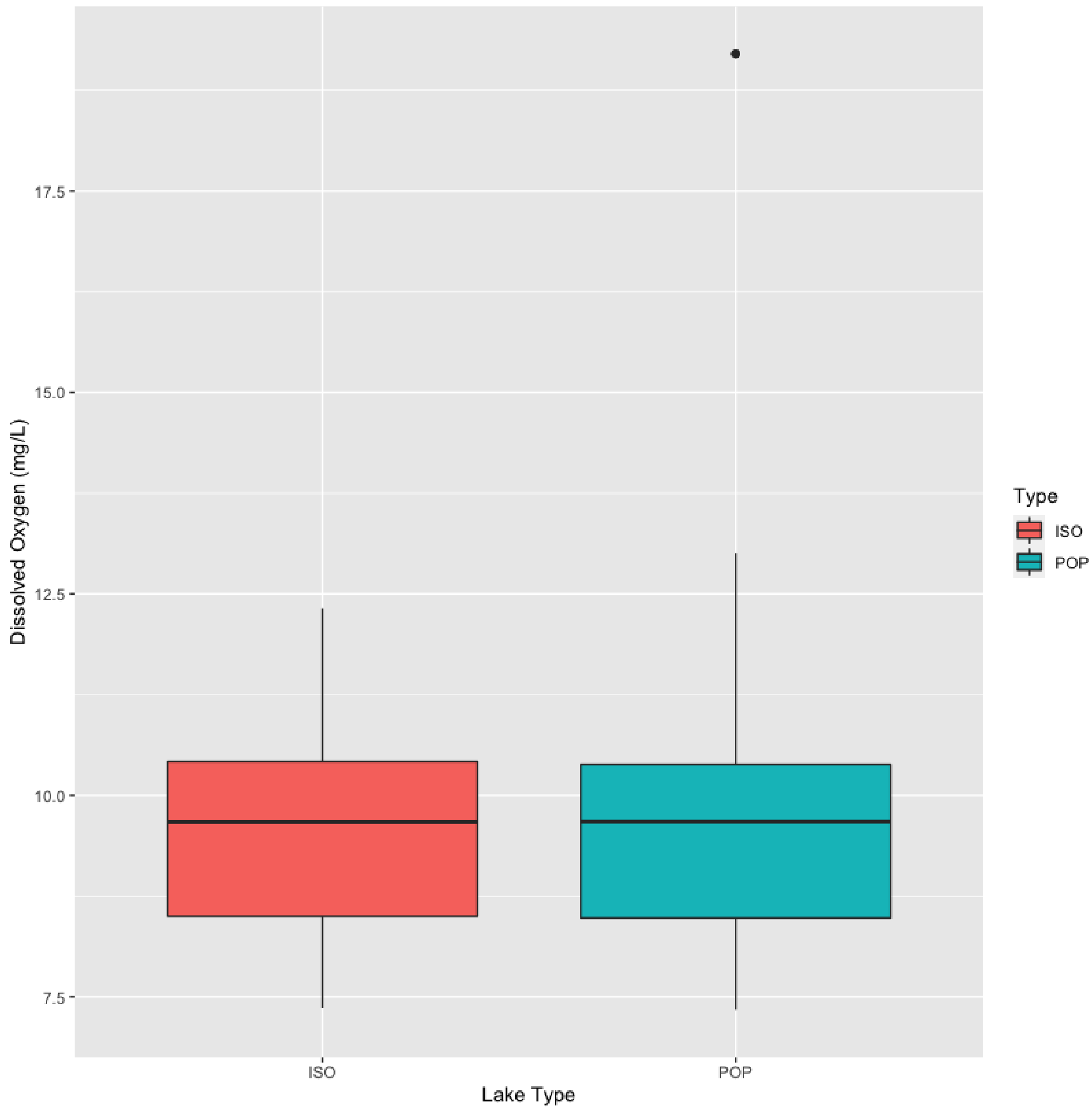

Figure 6. Dissolved oxygen concentrations (mg/L) in isolated (red) versus populated lakes (blue). Data include 3 seasons of sampling (Fall, Spring, Summer, 2020-21), where 6 isolated and 6 populated lakes were sampled at two locations per lake, for each season. The average dissolved oxygen concentration in isolated lakes was 9.56 mg/L while developed lakes had an average of 9.71 mg/L. A Wilcoxon rank sum test was used to compare the average dissolved oxygen concentration between isolated and populated lakes ($W = 6048$, $p = 0.64$). Any dots located outside the boxplot represent outlier samples of a single value from an individual site.

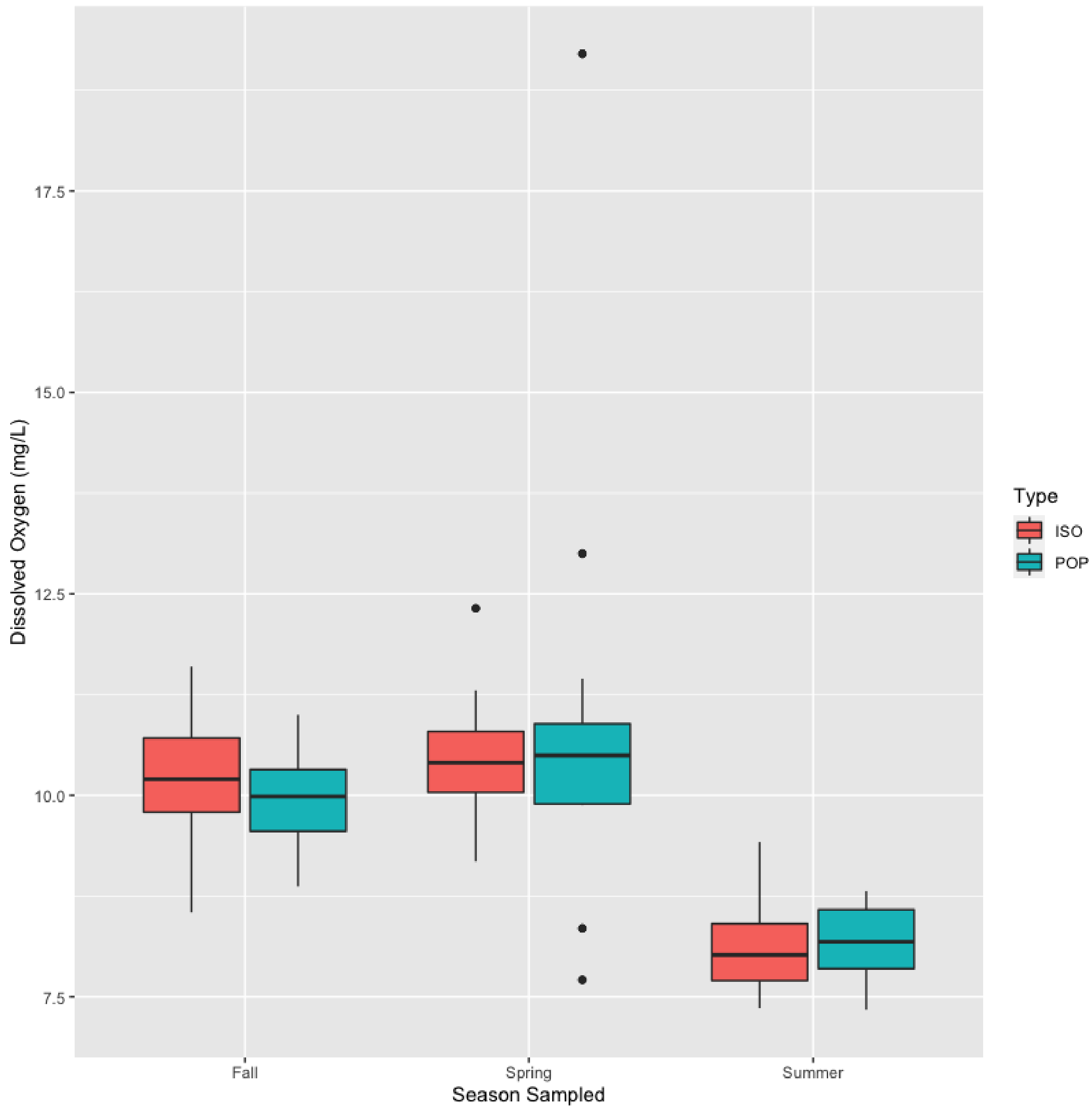

Figure 7. Dissolved oxygen concentration (mg/L) in 6 isolated (red) and 6 populated (blue) lakes, grouped by season sampled (Fall, Spring & Summer, left to right). For each season dissolved oxygen was measured at two sites per lake. A Wilcoxon rank sum test was used to compare dissolved oxygen concentration between isolated and populated lakes for each season separately. Fall ($W = 414$, $p = 0.21$), Spring ($W = 342$, $p = 0.89$), Summer ($W = 309$, $p = 0.71$). Floating dots are outliers that did not fit within the range encapsulated by the boxplot. These outliers are samples from individual sites sampled.

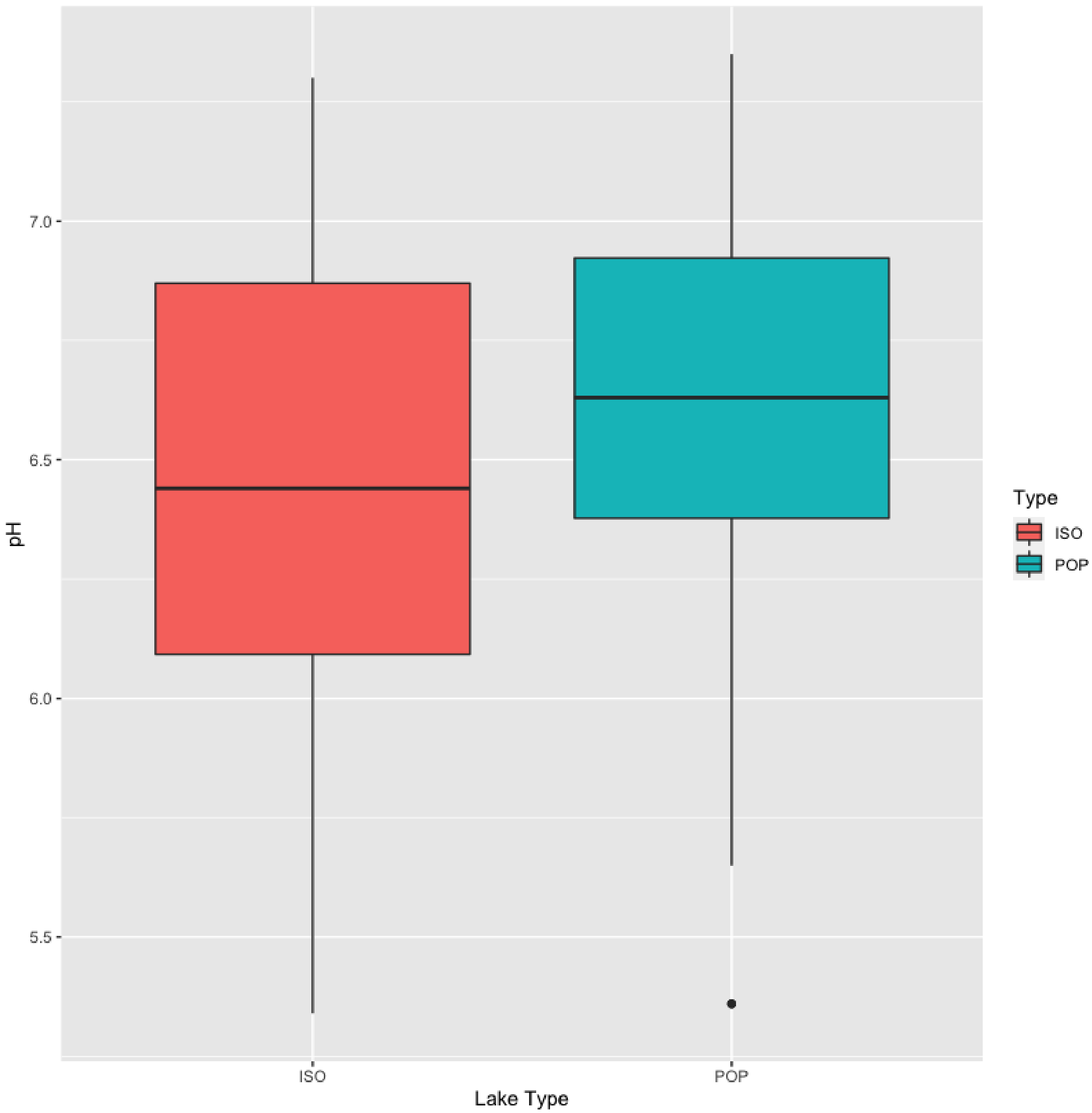

Figure 8. Average lake pH in isolated (red) and populated (blue) lakes from Fall 2020 to Summer 2021. Each of 6 isolated and 6 populated lakes were sampled at two sites per season. Isolated lakes had an average pH of 6.44, while populated lakes show an average of 6.63. A Wilcoxon rank sum test was used to compare the average pH of isolated and populated lakes ($W = 4635$, $p = 0.009$*). Black dots represent outlier samples that did not fit within the range of the boxplot. These outliers represent an individual data point collected from one site sampled.

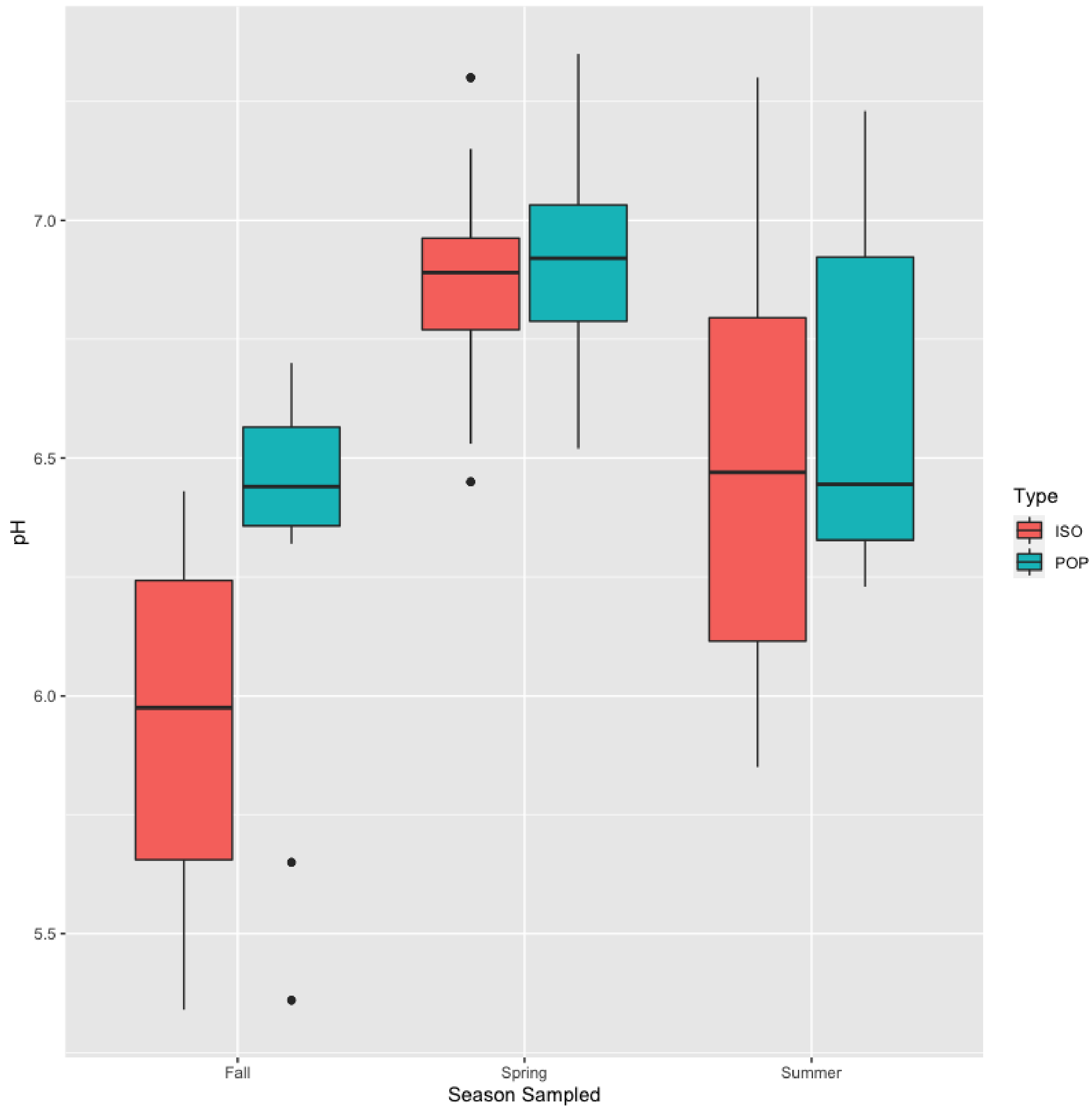

Figure 9. Average lake pH in 6 isolated (red) and 6 populated (blue) lakes, grouped by season sampled (fall, spring and summer, from left to right). Each lake was sampled at two different sites per season from Fall 2020 to Summer 2021. A Wilcoxon rank sum test was used to compare the average pH in isolated versus populated lakes for each season separately. Fall ($W = 133.5$, $p = 0.002$*), Spring ($W = 261$, $p = 0.73$) and Summer ($W = 303$, $p = 0.64$). Black dots represent outlier data points that did not fit within the range represented by the boxplot. These outliers represent the data for an individual site at a given lake.

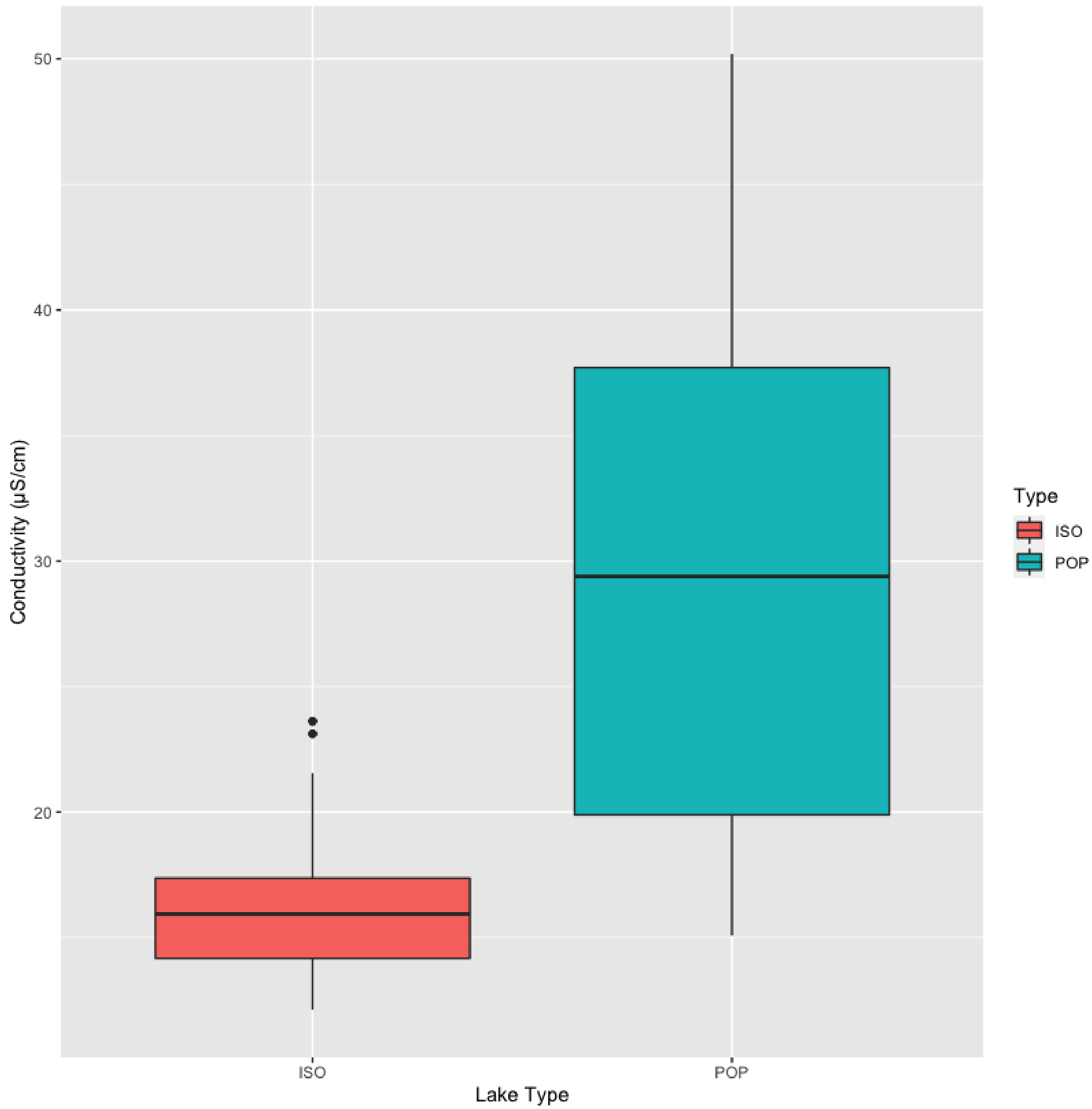

Figure 10. Average lake conductivity (µs/cm) in 6 isolated (red) and 6 populated (blue) lakes. Samples were taken at two sites per lake for three seasons (Fall 2020, and Spring and Summer 2021). A Wilcoxon rank sum test was used to compare average conductivity in the two lake types ($W = 1503$ $p = < 2.2e\text{-}16$*). Black dots represent outliers that did not fit in the range encapsulated by the boxplot. These outliers represent data from a individual site.

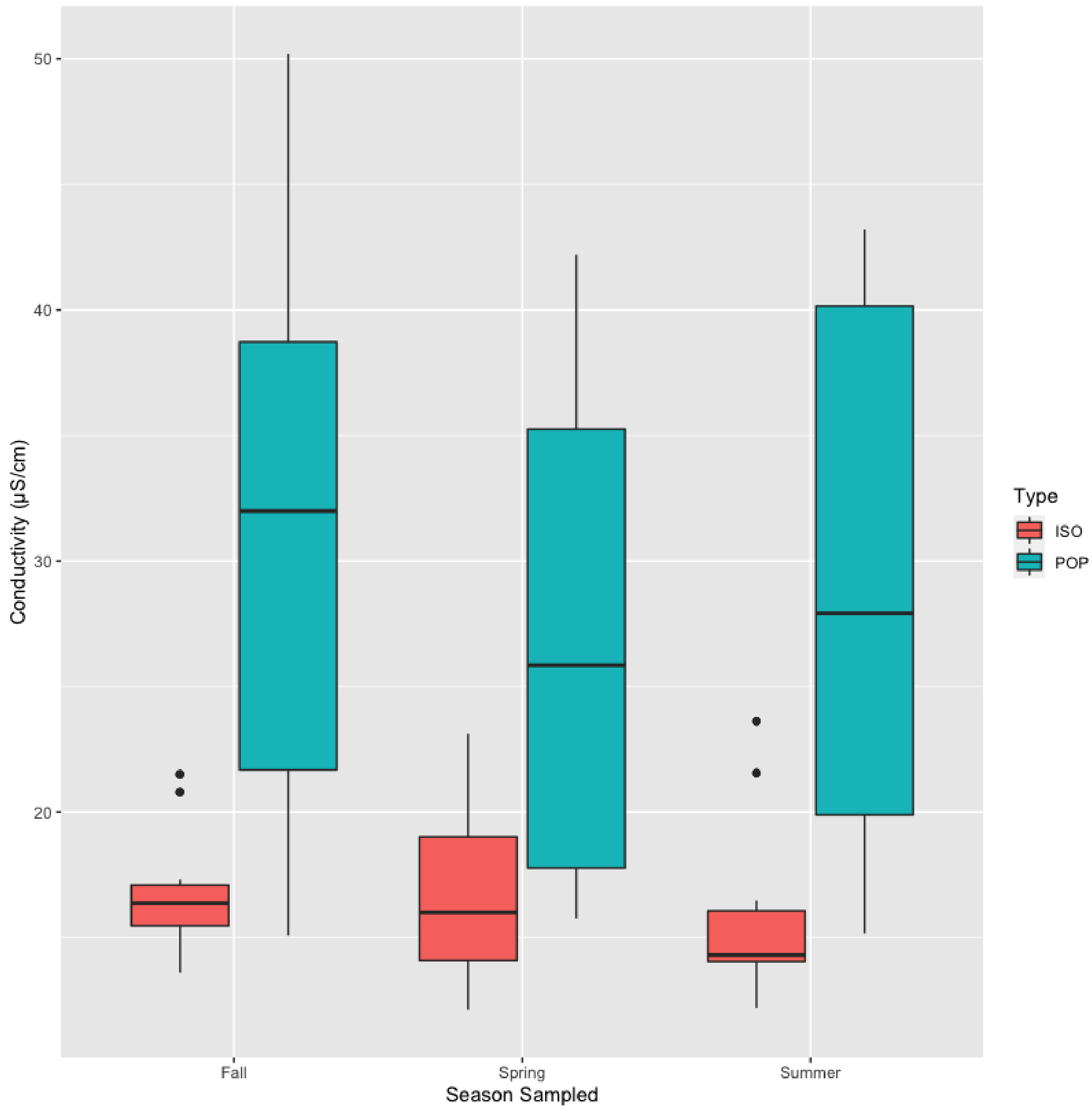

Figure 11. Average lake conductivity (µs/cm) in 6 isolated and 6 populated lakes, grouped by season (fall 2020, and spring and summer 2021, from left to right). Conductivity was sampled at two different sites per lake. A Wilcoxon rank sum test was used to compare average conductivity between the isolated and populated groups for each season independently, Fall (W = 21, p = <9.708e-07*), Spring (W = 42, p = <4.94e-06*) and Summer (W = 54, p = <1.194e-05*). Black dots represent outlier samples that did not fit within the range encapsulated by the boxplots. These outlier represent the readings collected from a single site.

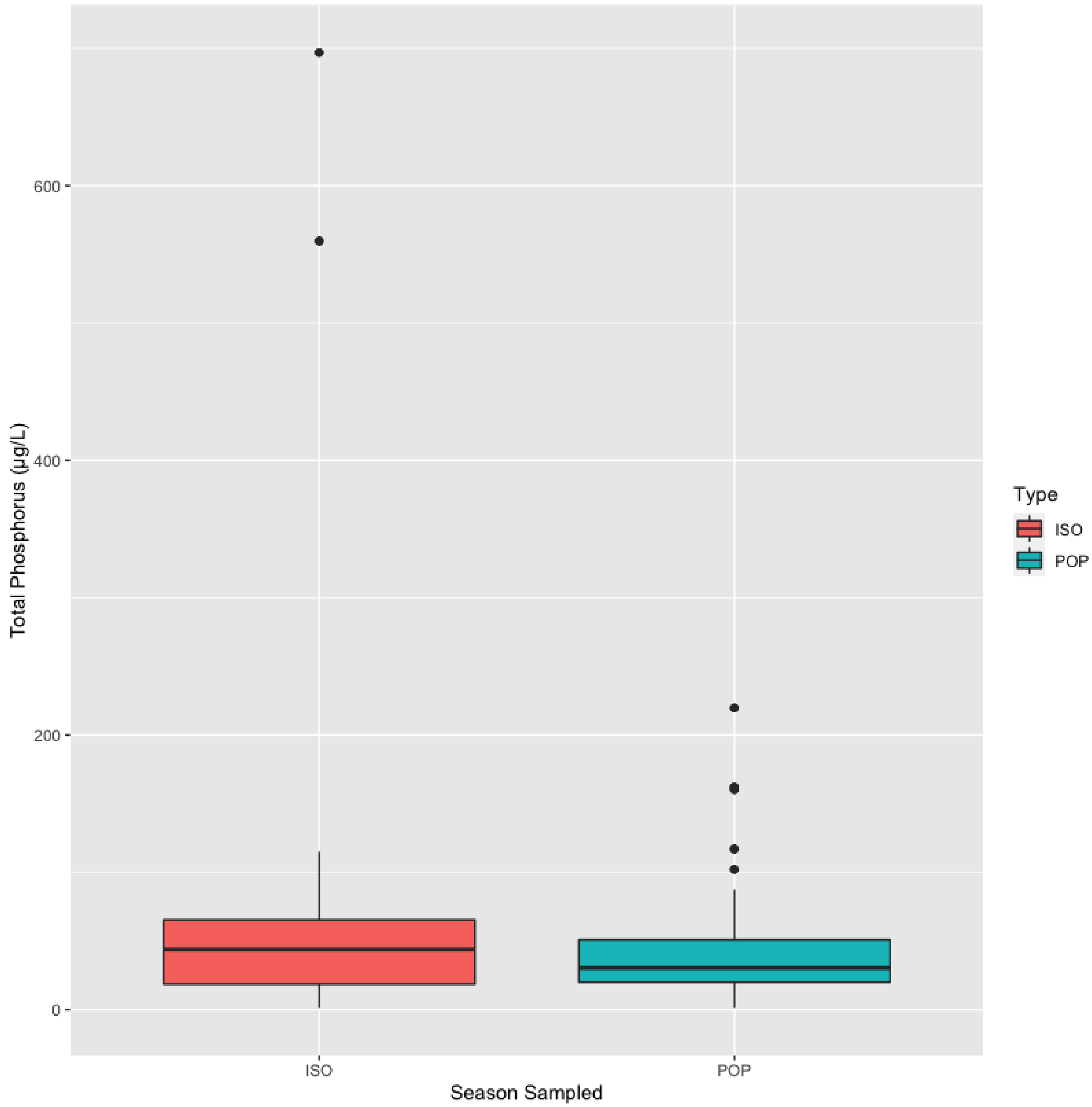

Figure 12. Average total phosphorus concentration (µg/L) in 6 isolated versus 6 populated lakes in three seasons (Fall 2020, Spring and Summer 2021). Water sampling was conducted at each site with two sites being sampled per lake for each season. Phosphorus concentration was determined through the nutrient analysis from water samples collected during sampling. To compare average phosphorus levels between the two lake types, a Wilcoxon rank sum test was used: ($W = 6444$, p-value = 0.18). Black dots represent outlier samples that did not fit the range of the boxplots. These outliers represent a indivudal reading collected from an indivual site.

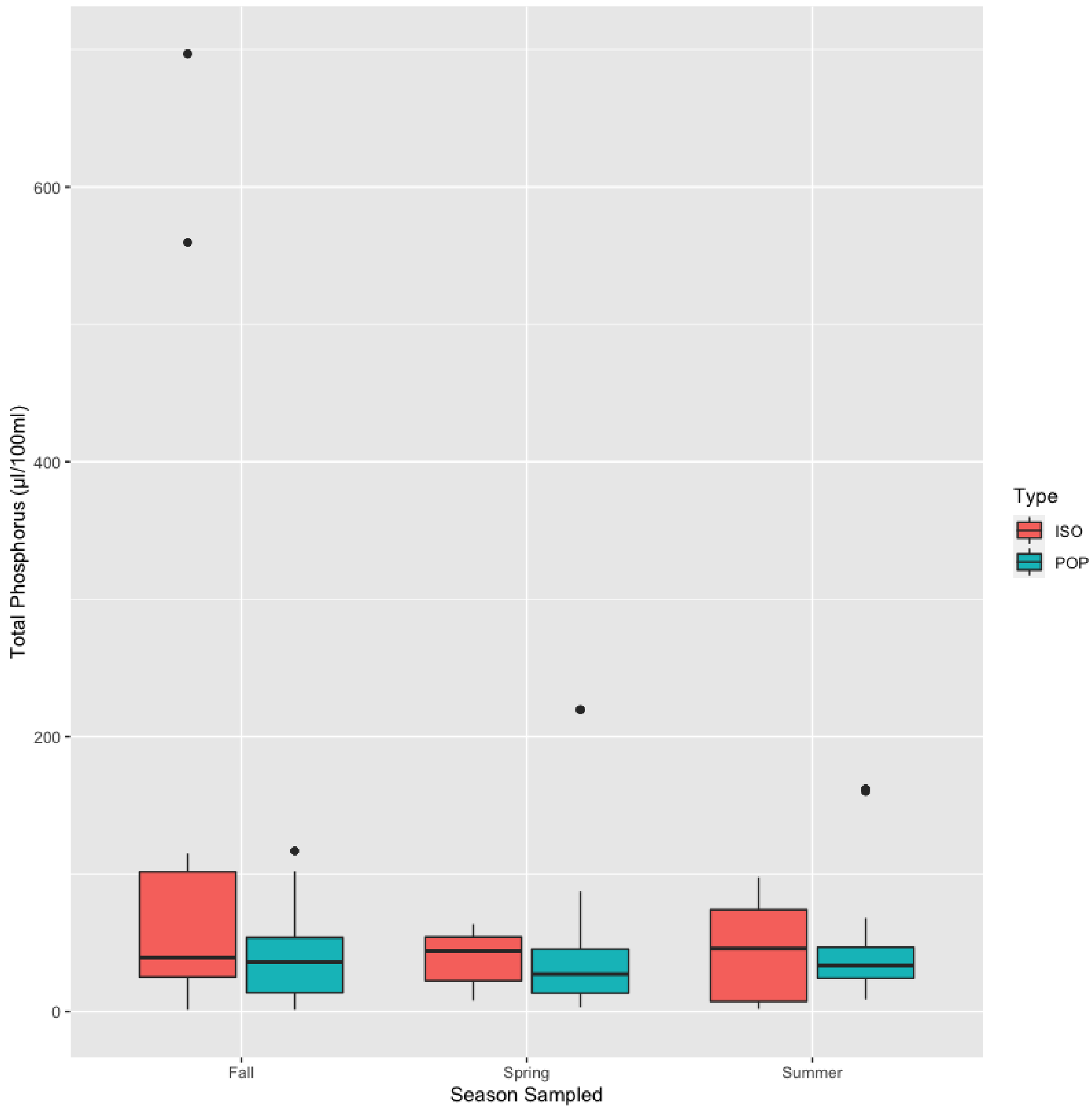

Figure 13. Average total phosphorus concentration (µg/L) in 6 isolated (red) and 6 populated (blue) lakes, sampled in three different seasons (Fall 2020, Spring and Summer 2021 respectively). Wilcoxon rank sum tests were used to compare the phosphorus concentrations of isolated and populated lakes, for each season, separately: Fall ($W = 778.5$, $p = 0.14$), Spring ($W = 805.5$, $p = 0.077$), Summer ($W = 594$, p-value $= 0.55$). Black dots represent outliers that do not fit within the boxplot range. These outliers represent a indivudal reading collected from an indivual site.

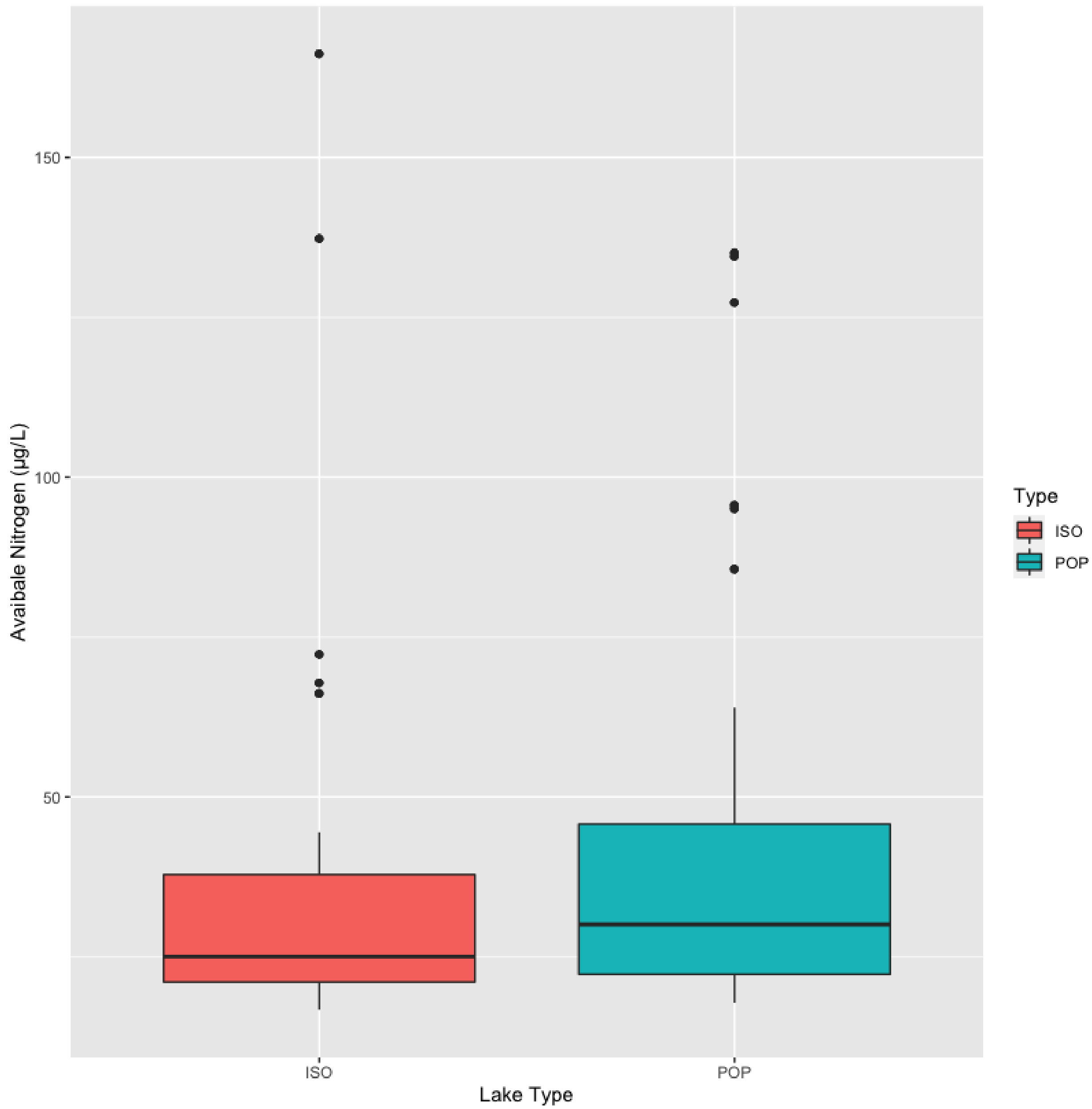

Figure 14. Average available nitrogen concentration (μg/L) in 6 isolated versus 6 populated lakes in three seasons (Fall 2020, Spring and Summer 2021). Water sampling was conducted at each site with two sites being sampled per lake for each season. To compare average nitrogen concentrations between the two lake types, a Wilcoxon rank rum test was used: (W = 4900.5, p-value = 0.043). Black dots represent outliers that do not fit the range encapsulated by boxplot. These outliers represent a indivudal reading collected from an indivual site.

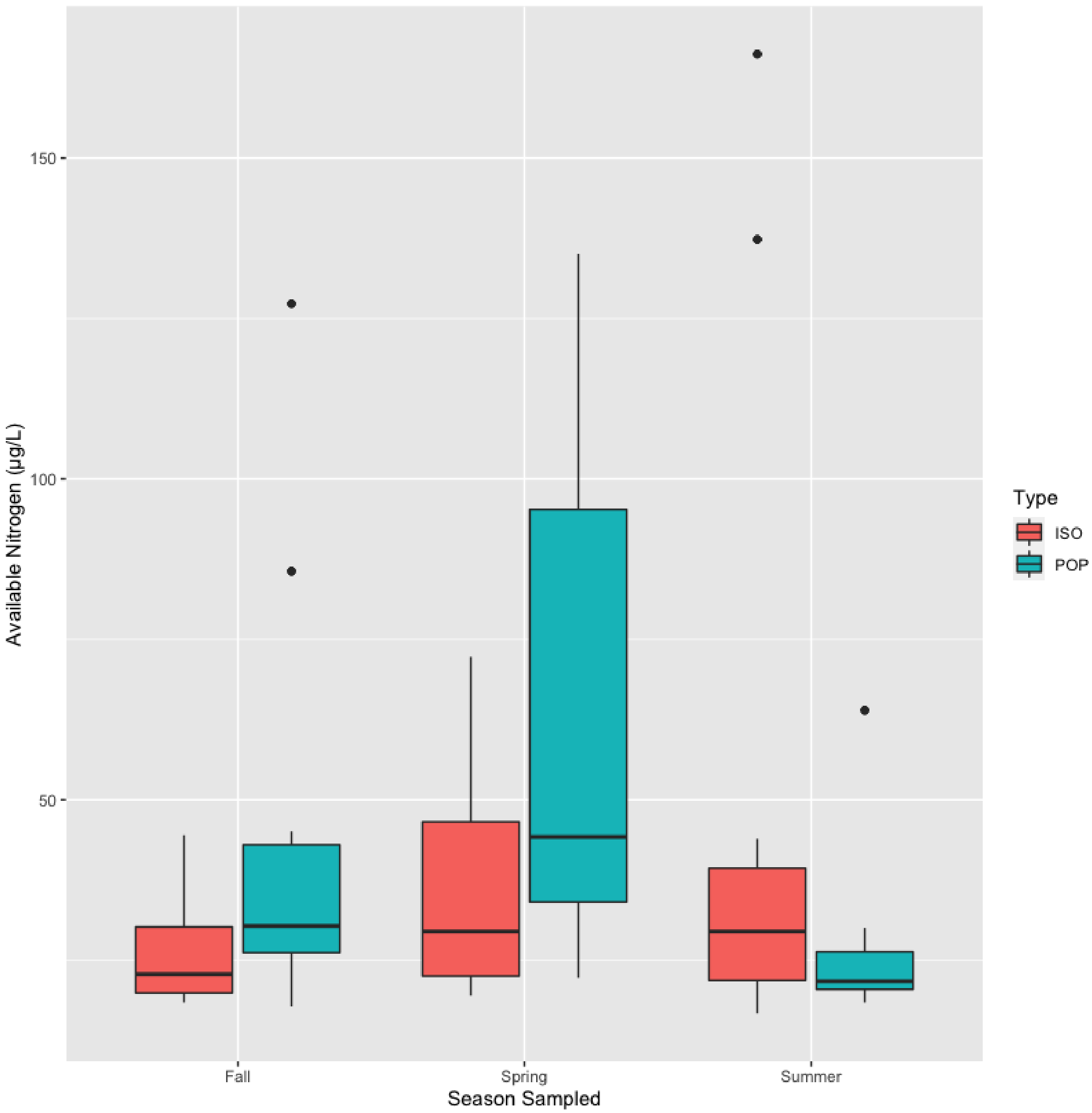

Figure 15. Average available nitrogen concentration (µg/L) from 6 isolated (red) and 6 populated (blue) lakes, sampled in three different seasons (Fall 2020, and Spring and Summer 2021 respectively). Wilcoxon rank sum tests were used to compare the nitrate/nitrite concentrations of isolated and populated lakes to one another, for each season separately: Fall ($W = 333$, $p = <0.001$*), Spring ($W = 360$, $p = 0.001$*), Summer ($W = 900$, p-value = 0.004*). Black dots represent outliers that do not fit within the range of the boxplots generated. These outliers represent a indivudal reading collected from an indivual site.

4.3.2. Comparison of Macroinvertebrate Communities in Isolated and Developed Lakes

To gain a better understanding of how human impacts are affecting lake benthic communities, we compare the benthic macroinvertebrate communities sampled in isolated and developed lakes. The biotic indices used to characterize benthic communities were taxa richness, Shannon Diversity Index, EOT (the proportion of organisms known to be highly sensitive to pollution and disturbance in lentic systems, that were Ephemeroptera, Odonata and Trichoptera), CIGH (the proportion of known pollution-tolerant taxa, Corixidae, Isopoda, Gastropoda and Hirudinidae) and HBI (the Hilsenhoff Biotic Index).

In the study overall, isolated lakes had an average taxa richness of 2.97 while developed lakes had an average of 3.00. This difference was not statistically significant, according to the Wilcoxon rank sum test ($W = 5475.5$, $p = 0.43$, Figure 16). On a season-by-season basis, isolated lakes had an average taxa richness of 2.92 in fall while developed lakes had an average of 2.89. This difference was not statistically significant ($W = 338$, $p = 0.94$, Figure 17). In spring, isolated lakes had an average taxa richness of 3.12 and developed lakes had an average of 3.15, again a difference that was not statistically significant ($W = 339$, $p = 0.93$, Figure 17). In summer, isolated lakes had an average taxa richness of 2.86, while the developed lakes averaged 2.97, which again was not a statistically significant difference ($W = 271$, $p = 0.33$, Figure 17).

The next macroinvertebrate community characteristic examined was diversity, calculated using the Shannon Diversity Index. For the whole study, isolated lakes had an average diversity of 1.63 and populated lakes had an average of 1.61 (Figure 18). These values were not significantly different ($W = 5802$, p-value $= 0.95$, Figure 18). On a seasonal basis, in fall, isolated lakes had an average diversity of 1.65 compared to 1.59 in the developed lakes (Figure 19). This difference was not statistically significant ($W = 353$, $p = 0.76$, Figure 19). In spring, isolated lakes had an average diversity of 1.83 compared to an average of 1.74 in developed lakes (Figure 19). This measure did not differ significantly different between isolated and developed lakes in the spring (W = 410, p = 0.23, Figure 19). In summer, isolated lakes had an average diversity of 1.40 while developed lakes had an average diversity of 1.51 (Figure 19). As in the previous seasons, isolated and developed lakes did not differ significantly from each other in summer (W = 243, p-value = 0.24, Figure 19).

The average EOT values for isolated and developed lakes differed significantly (W = 4555, p = 0.005, Figure 20). During the course of the whole study, isolated lakes had an average EOT percentage of 21.07 while developed lakes had an average percentage of 49.08 (Figure 20). When data were divided according to season, EOT values in isolated and developed lakes were significantly different in fall (W = 174, p = 0.01**) and spring (W = 114, p = <0.001***, Figure 21). However, EOT did not differ significantly between isolated and developed lakes in summer (W = 320.5, p-value = 0.70). In fall, the average EOT percentage in isolated lakes was 29.72 compared to 49.08 in developed lakes (Figure 21). In spring, isolated lakes had an average EOT percentage of 37.02 while developed lakes had an EOT percentage of 52.66 (Figure 21). In summer, the average EOT percentages in isolated and developed lakes were determined to be 26.47 and 24.63 respectively, a non-significant difference (Figure 21).

The percentage of benthic samples composed of CIGH was calculated for the entire study period, as well as for each of the three seasons of the study. Overall, the average CIGH percent in isolated lakes was 10.90 compared to 9.30 in developed lakes (Figure 22). This was not a statistically significant difference (W = 6416, p = 0.19, Figure 22). In fall, isolated lakes had an average CIGH percentage of 9.57 while developed lakes had a percentage of 11.24 (Figure 23). This difference was not statistically significant (W = 276, p = 0.72). In spring, isolated lakes had an average CIGH percentage of 6.52 compared to an average of 2.36 in developed lakes. This difference was statistically significant (W = 253, p = 0.02*, Figure 23). Finally, in summer, isolated lakes had an average CIGH percentage of 16.61, while developed lakes had an average of 14.3; this difference was not statistically significant (W = 317, p = 0.52, Figure 23).

The Hilsenhoff Biotic Index (HBI) is a valuable tool for assessing the health of a benthic community based on the differential tolerance of pollution according to macroinvertebrate family (see Tables 1 and 2 in Appendix I). Basically, HBI values will range from 0 to 10, which corresponds to an inferred water quality and degree of organic pollution present in the area, all based on known pollution tolerance of different macroinvertebrate families (Mandaville, 2002). Overall, isolated lakes were found to have an average HBI value of 5.48 while developed lakes had an average HBI value of 5.41. This difference was not statistically significant; based on a Wilcoxon rank sum test (W = 5475.5, p = 0.43, Figure 24). The two types of lake were also compared in fall, spring and summer (Figure 25). None of the seasons showed statistically significant differences between the two lake types. In fall, isolated lakes had an average HBI value of 5.43 while developed lakes had an average of 5.40 (W = 338, p = 0.94, Figure 25). In spring, benthic communities sampled in isolated lakes had an average HBI value of 5.12 while developed lakes had an average of 5.19 (W = 339, p = 0.93, see Figure 25). Lastly, in summer, benthic communities sampled from isolated lakes had an average HBI value of 5.89 while developed lakes had an average of 5.66 (W = 271, p = 0.33, Figure 25).

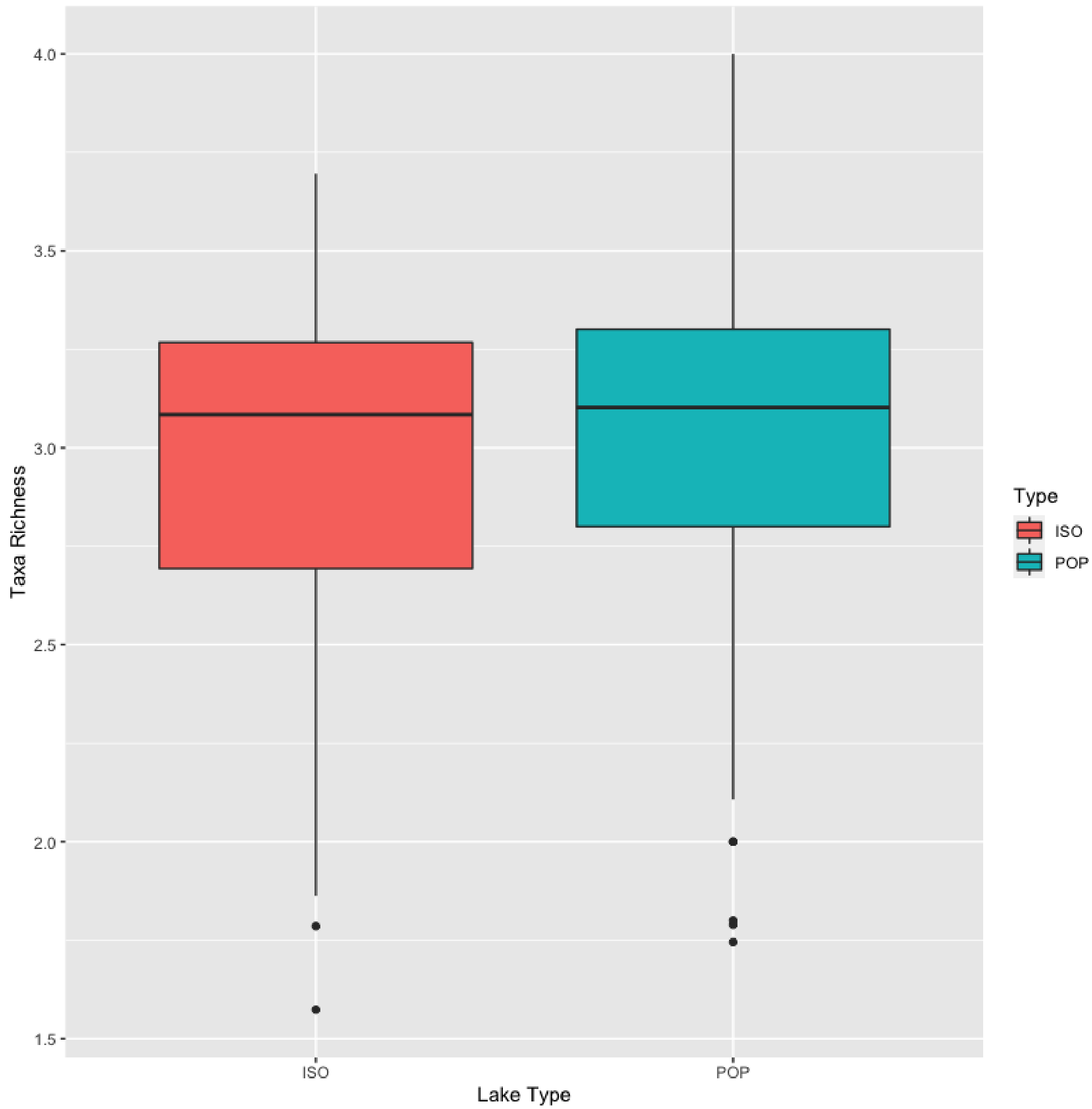

Figure 16. Average taxa richness of benthic macroinvertebrate communities sampled in 6 isolated and 6 populated lakes for three seasons: Fall 2020, Spring and Summer 2021. Three macroinvertebrate samples were taken per site, with two sites being sampled per lake for each season. Isolated lakes had an average HBI value of 5.48 and developed lakes had an average of 5.41. A Wilcoxon rank sum test was used to compare the average taxa richness of isolated and populated lakes sampled ($W = 5475.5$, $p = 0.44$). Richness was determined from the number of different families found within a given sample. Dots represent data points that fall outside of the range encapsulated by the boxplot. These outliers represent a single replicate sample collected from an indivual site.

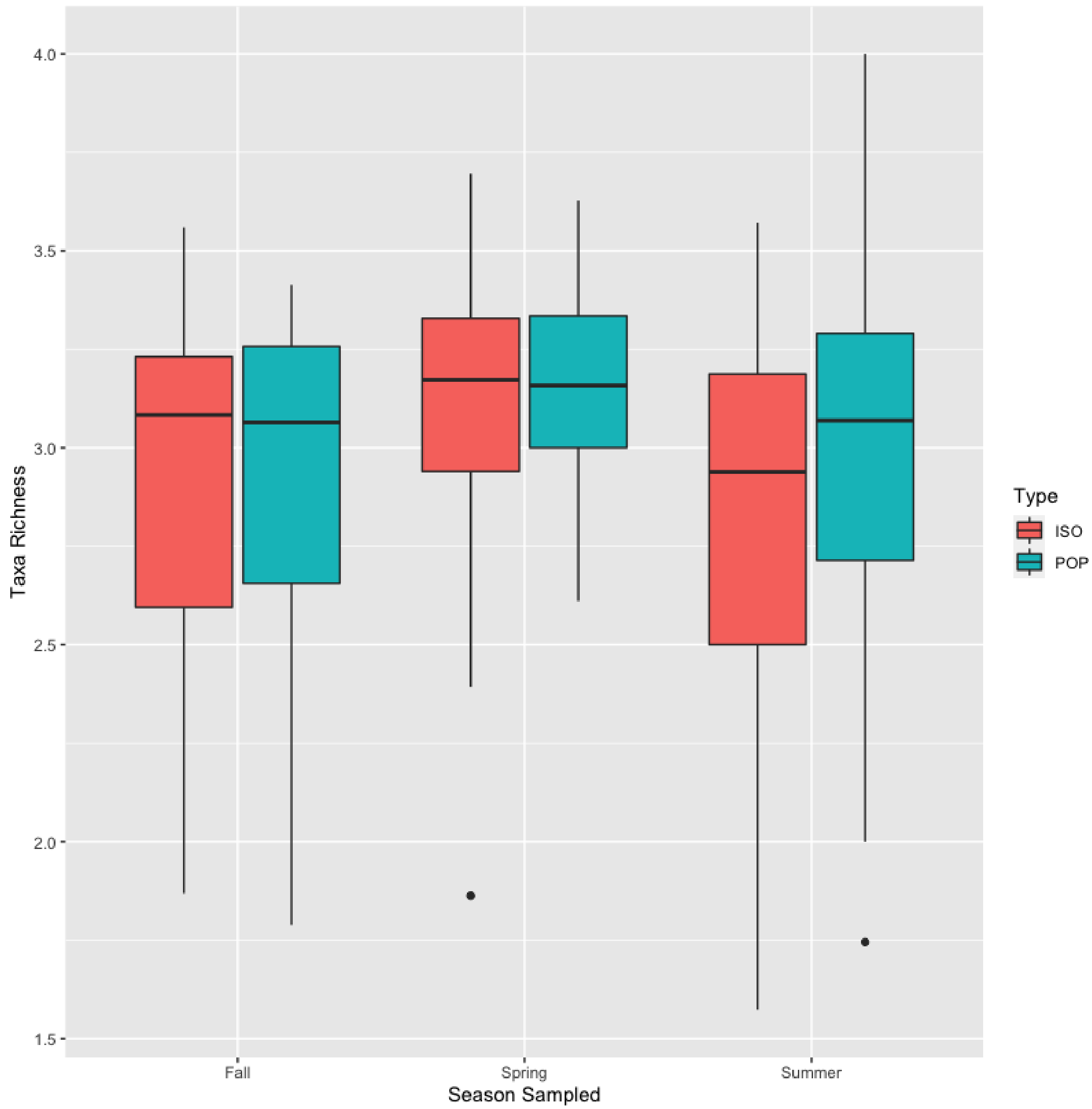

Figure 17. Average taxa richness of macroinvertebrate communities sampled in 6 isolated (red) and 6 populated (blue) lakes, grouped by season (fall, spring and summer respectively). Two sites per lake were sampled in each season. Wilcoxon rank sum tests were used to compare average taxa richness in isolated versus populated lakes for each season independently: Fall ($W = 338$, $p = 0.94$), Spring ($W = 339$, $p = 0.93$) and Summer ($W = 271$, $p = 0.33$). Richness was determined from the number of different families present in a given sample. Dots represent outlier samples. These outliers represent a single replicate sample collected from an indivual site.

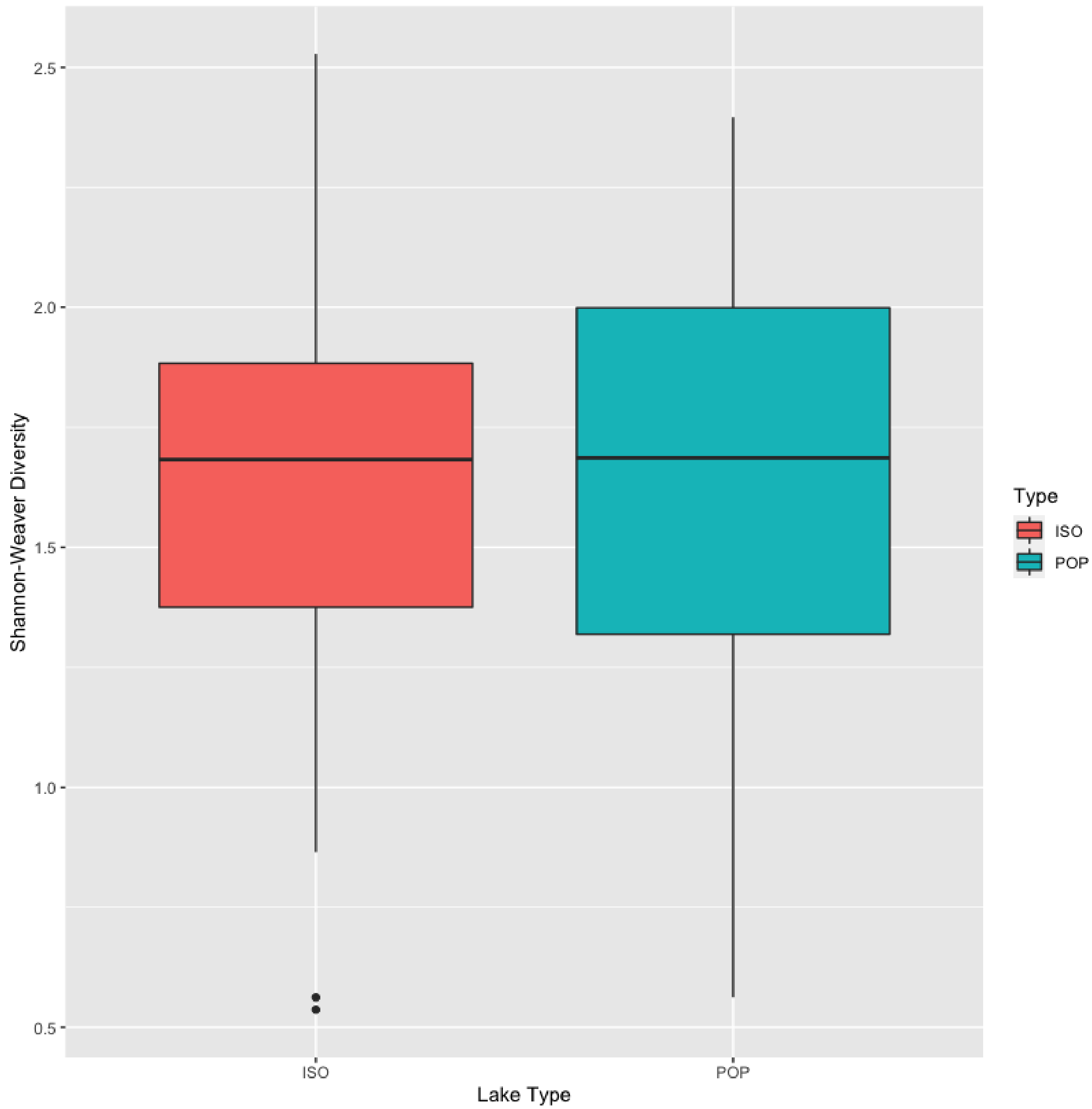

Figure 18. Average Shannon Diversity Index value for benthic macroinvertebrate communities sampled in 6 isolated (red) and 6 populated (blue) lakes in three seasons (fall 2020, spring and summer 2021). Benthic macroinvertebrate sampling was based on three replicates per site, and each lake was sampled at two separate sites. A Wilcoxon rank sum test was used to compare the isolated and populated index values ($W = 5802$, $p = 0.95$). Dots represent outlier samples, these outliers represent a single replicate sample collected from an indivual site.

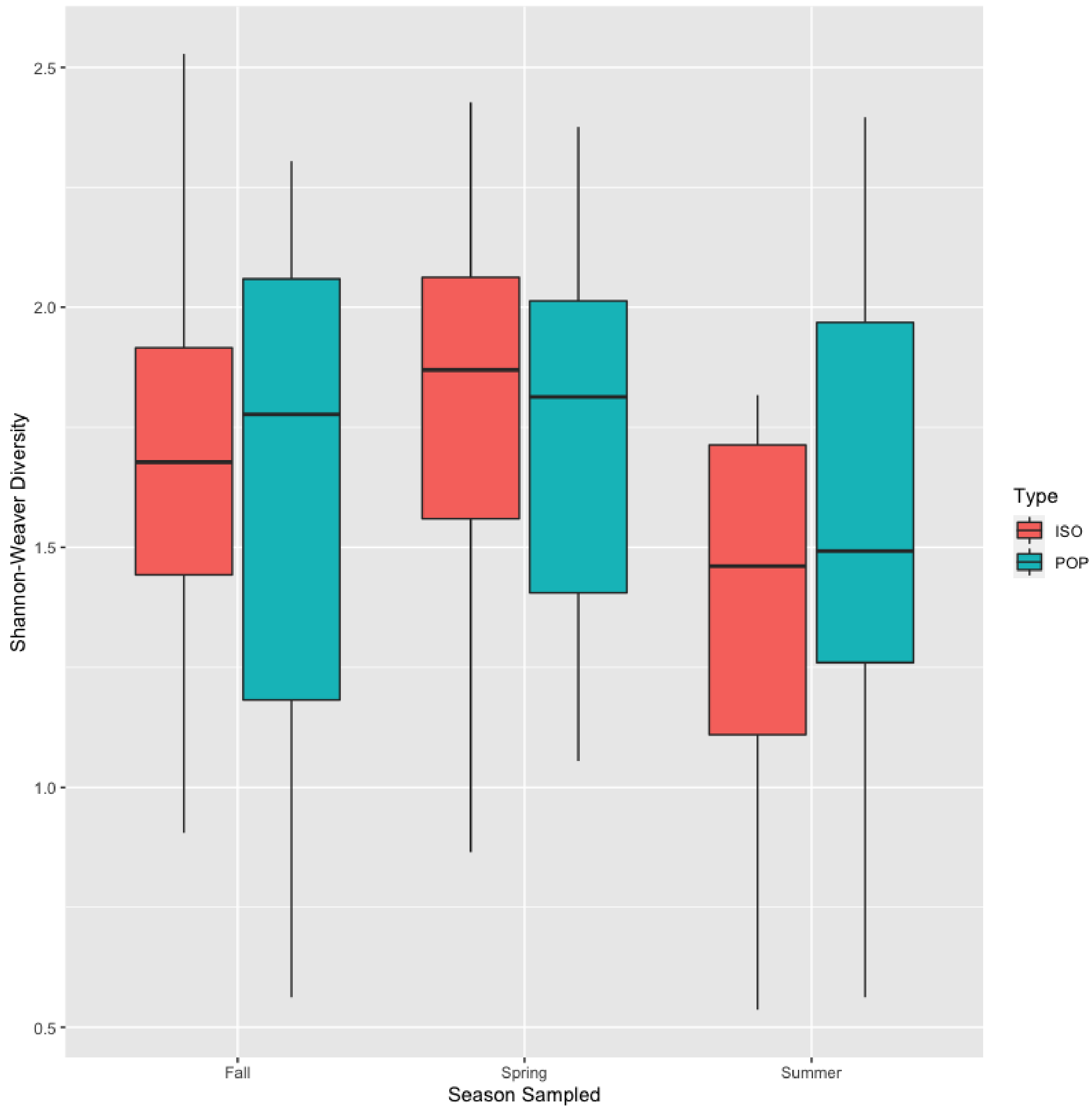

Figure 19. Average Shannon Diversity Index values for benthic macroinvertebrate communities in 6 isolated (red) and 6 populated (blue) sampled in each of three seasons (Fall 2020, Spring and Summer 2021). Macroinvertebrates were sampled three times per site, with each lake being sampled at two separate sites. Wilcoxon rank sum tests were used to compare the average index values for isolated and populated lakes for each season separately; Fall ($W = 353$, $p = 0.76$), Spring ($W = 410$, $p = 0.23$) and Summer ($W = 243$, $p = 0.24$). None of these differences are statistically significant.

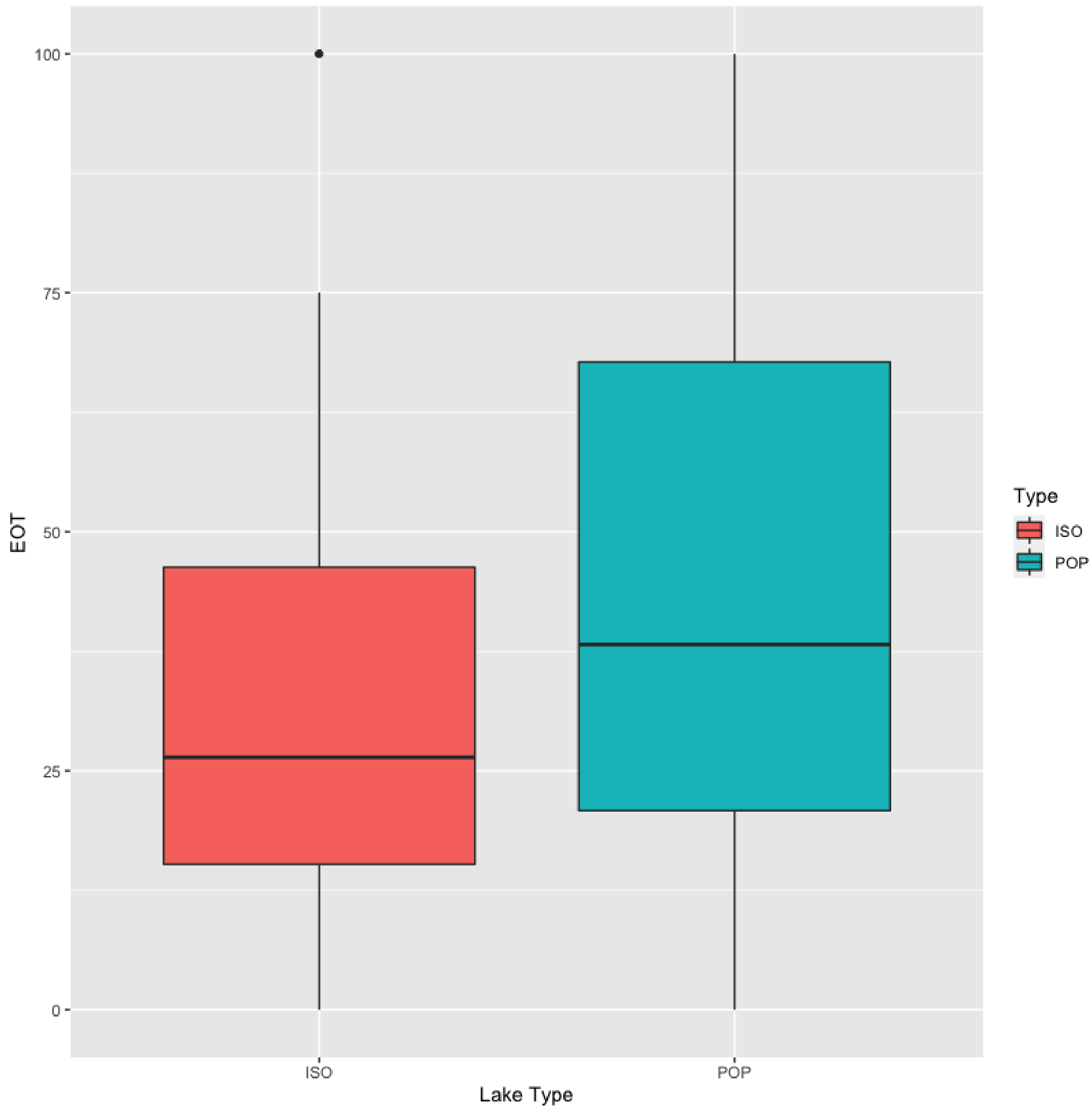

Figure 20. Average EOT index value for benthic macroinvertebrate communities sampled in 6 isolated (red) and 6 populated (blue) lakes in three seasons (fall 2020, spring and summer 2021). Benthic macroinvertebrates were sampled three times at each site, with two sites being selected for each lake per season. To compare average EOT values between isolated and populated lakes, a Wilcoxon rank sum test was used ($W = 4555$, $p = 0.005$**). Outlier samples are represented by black dots, these outliers represent a single replicate sample collected from an indivual site.. This difference is statistically significant.

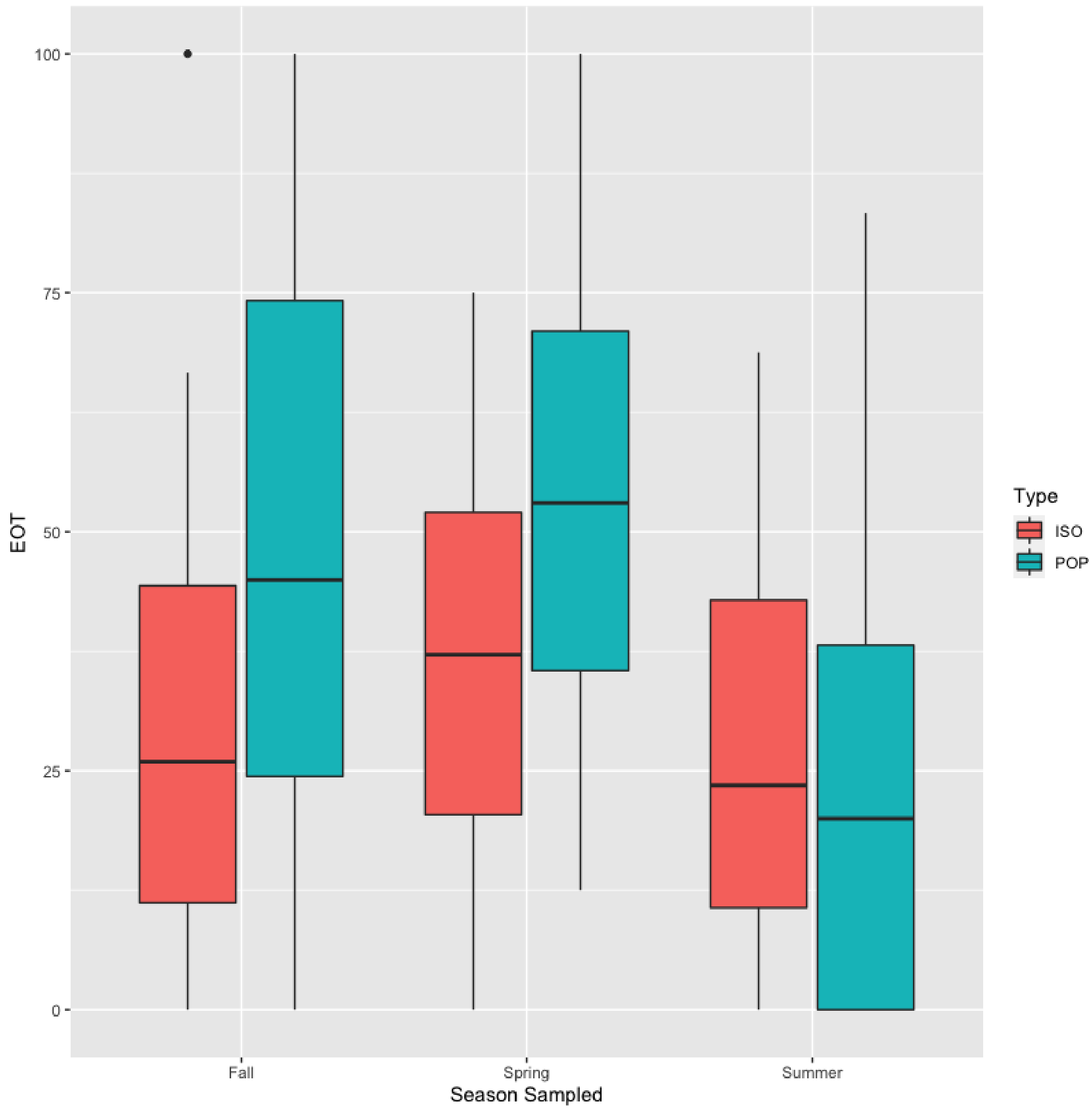

Figure 21. Average EOT index value for each season (fall 2020, and Spring and Summer 2021) sampled in 6 isolated (red) and 6 populated (blue) lakes. Benthic macroinvertebrate communities were sampled three times per site and each lake was sampled at two separate sites. Wilcoxon rank sum tests were used to compare the EOT values of isolated to populated lakes in each season: fall ($W = 174$, $p = 0.011$*), spring ($W = 114$, $p = <0.001$*) and summer ($W = 320.5$, $p = 0.70$). Outliers are represented by dots that do not fall within the boxplot range. These outliers represent a single replicate sample collected from an indivual site.

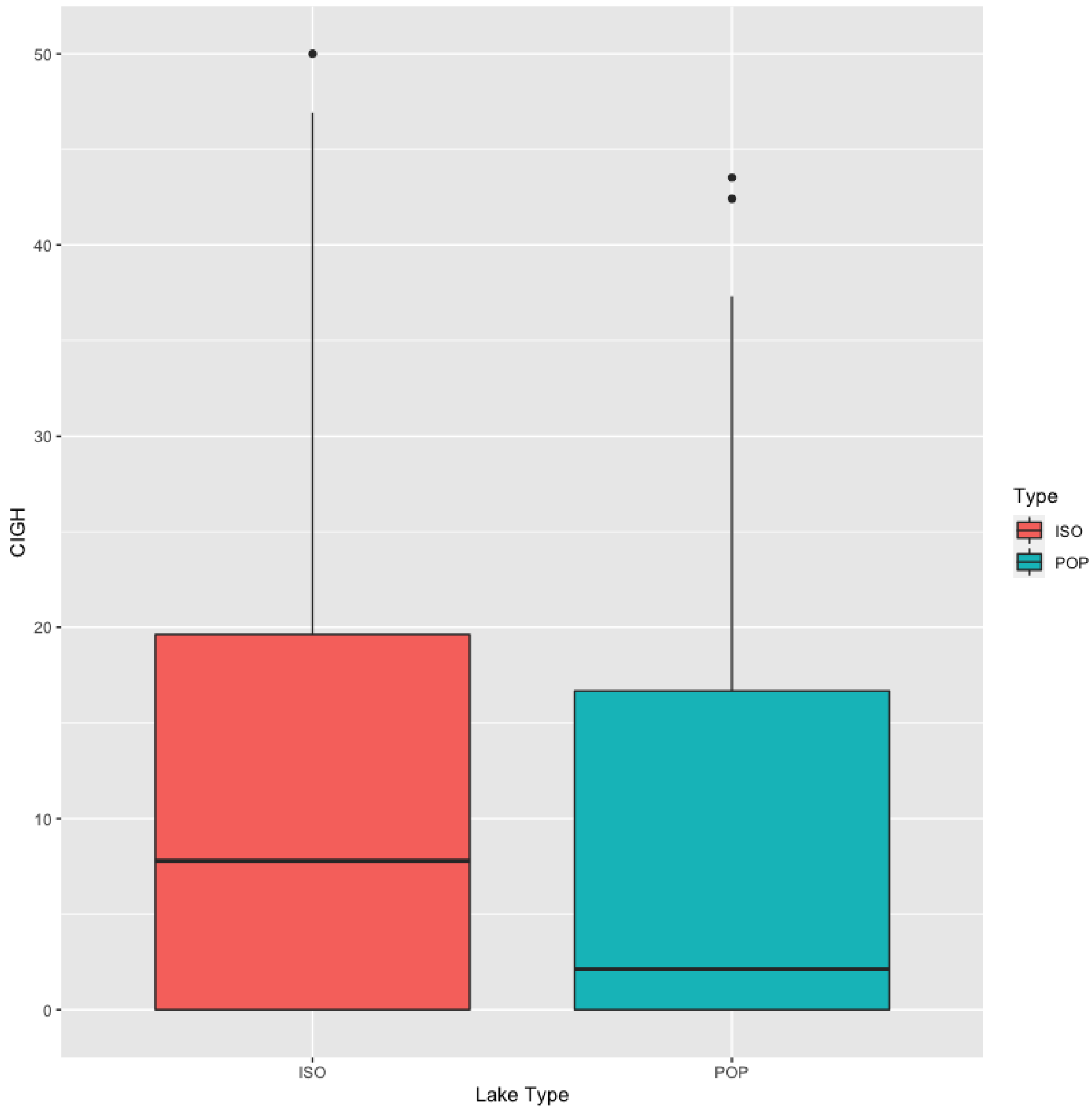

Figure 22. Average CIGH index values for benthic macroinvertebrate communities sampled in isolated (red) and populated (blue) lakes in three seasons (Fall 2020, Spring and Summer 2021). Benthic macroinvertebrates were sampled three times per site, with each lake being sampled at two different sites. To compare the average CIGH values between isolated and populated lakes, a Wilcoxon rank sum test was used ($W = 6416$, $p = 0.19$). Dots represent samples that do not fall within range outlined by boxplot, these outliers represent a single replicate sample collected from an indivual site.. The two lake types do not differ significantly overall.

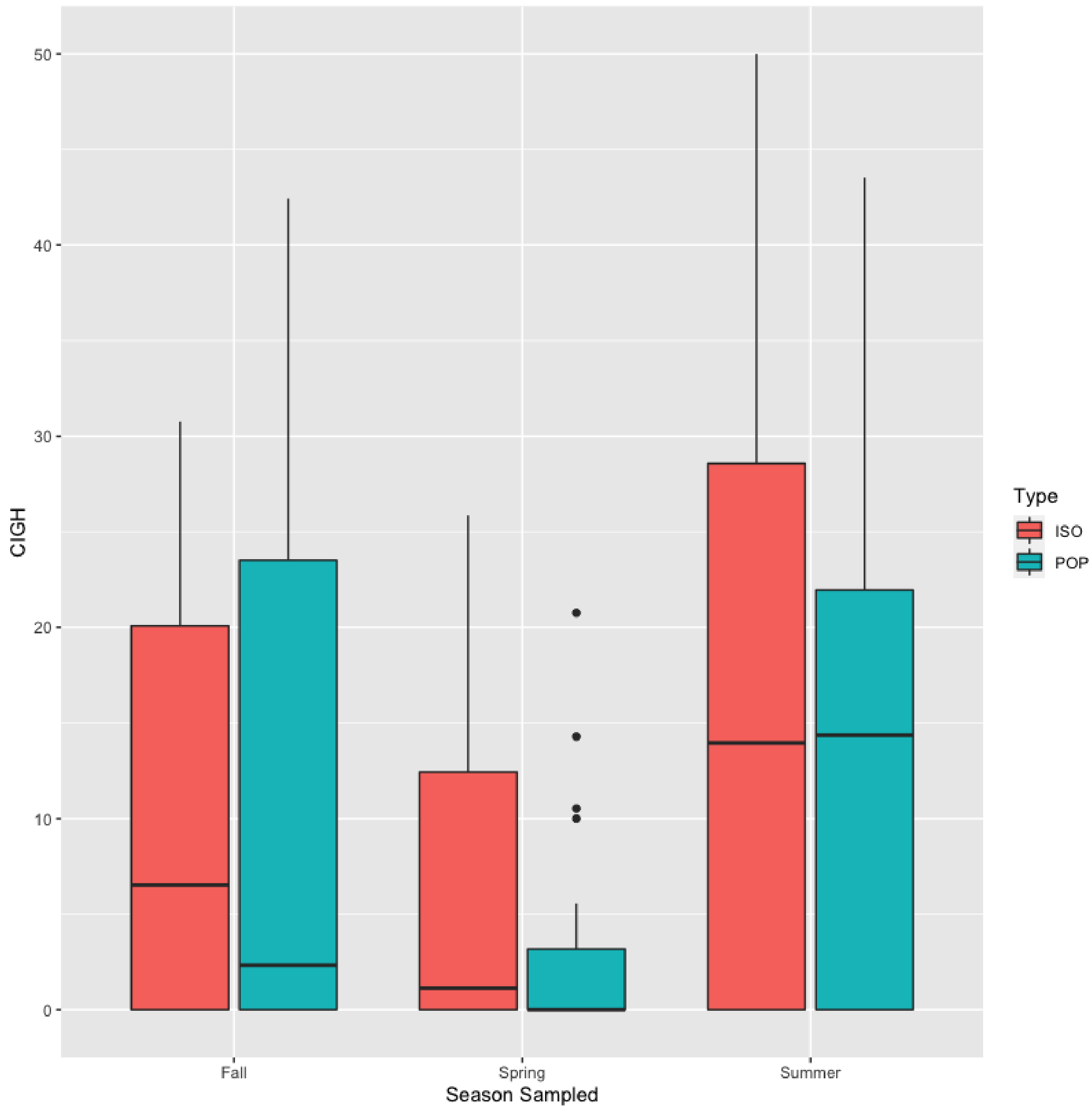

Figure 23. Average CIGH index values for each season (fall 2020, and spring and summer 2021) sampled in 6 isolated (red) and 6 populated (blue) lakes. Benthic macroinvertebrate communities were sampled three times per site, with two sites being sampled for each lake each season. Wilcoxon rank sum tests were used to compare the values of isolated and populated lakes for each season separately: Fall (W = 276, p = 0.72), Spring (W = 253, p-value = 0.01545*) and Summer (W = 317, p-value = 0.5201). Dots represent samples that did not fit within the range of the boxplot. These outliers represent a single replicate sample collected from an indivual site.

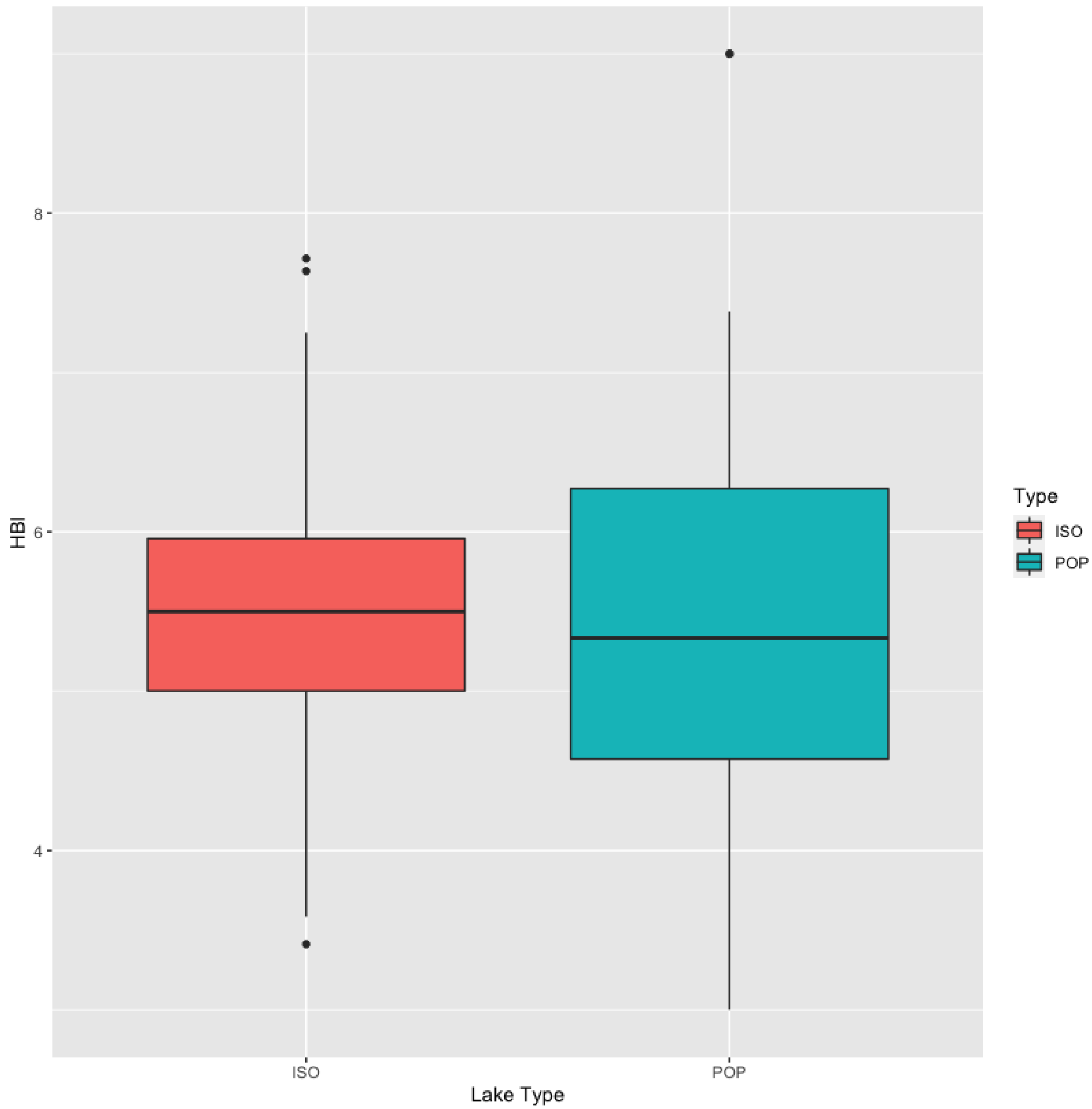

Figure 24. Average Hilsenhoff biotic index (HBI) values for isolated and developed lakes, sampled in Kearney, Ontario. Average HBI value for isolated lakes was 5.48 while the average HBI for developed lakes was 5.41. A Wilcoxon rank sum test was used to compare these means ($W = 6216$, $p = 0.64$). Dots represent outliers, these outliers represent a single replicate sample collected from an indivual site.

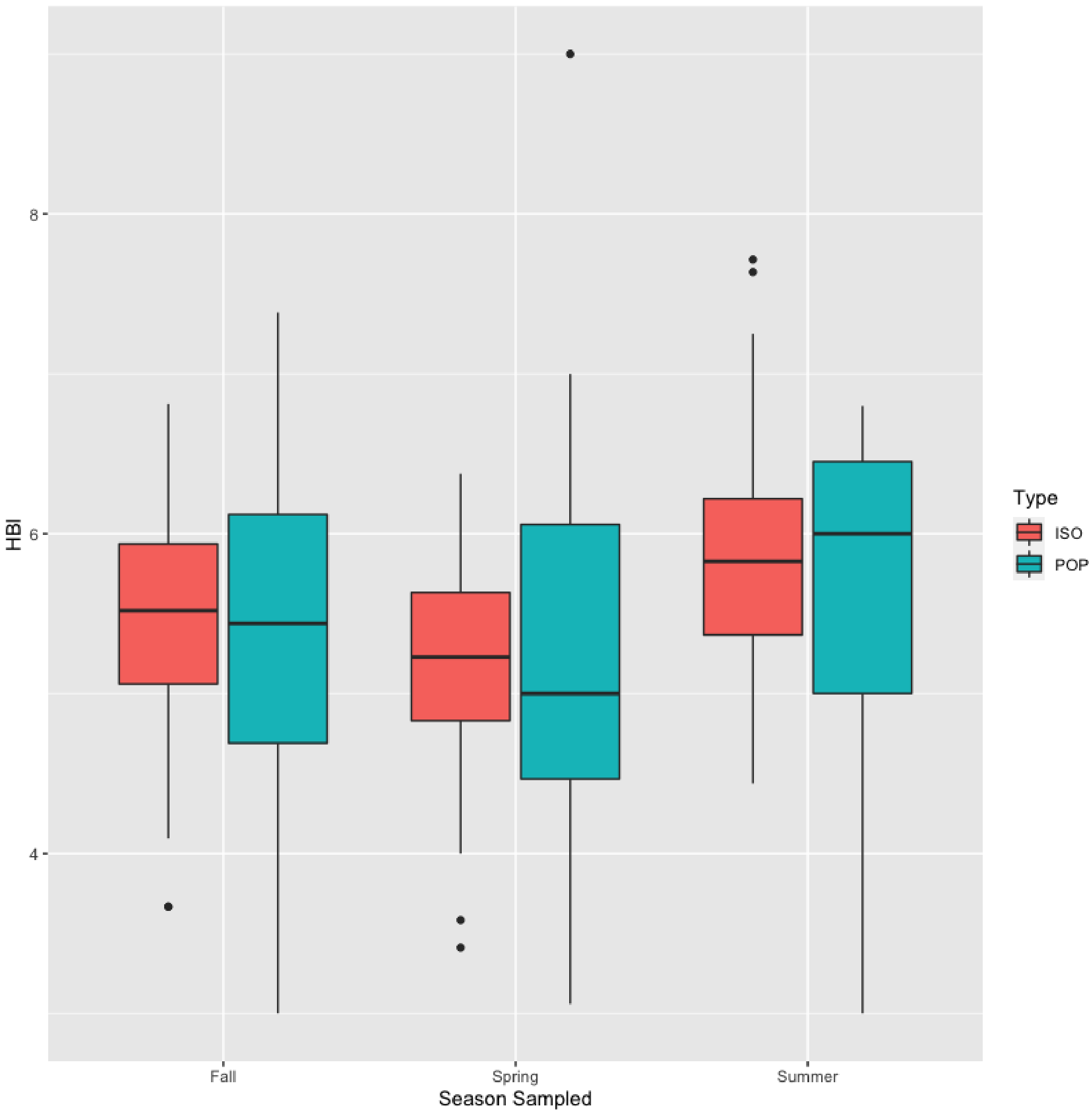

Figure 25. Average seasonal Hilsenhoff biotic index (HBI) value for isolated and developed lakes. Isolated lakes (red) had average HBI values of 5.43, 5.12 and 5.89 in the Fall, Spring and Summer respectively. Developed lakes (blue) had average HBI values of 5.40, 5.19 and 5.66 in the Fall, Spring and Summer, respectively. Wilcoxon rank sum tests were used to compare isolated and developed average seasonal HBI values. For fall, ($W = 670.5$, p-value = 0.8043); for spring, ($W = 719.5$, $p = 0.86$) and for summer, ($W = 689.5$, $p = 0.64$). Dots represent outlier samples, these outliers represent a single replicate sample collected from an indivual site.

4.3.3 Integrating the Contributions of Water Chemistry, Habitat Quality and Lake Type to Benthic Community Composition.

A whole study RDA analysis was carried out to assess the impacts a variety of water and limnological parameters on benthic community composition. Lake development type (isolated versus developed) was also included in this model to see if macroinvertebrates are affected by community composition as well as relationships with other environmental variables. The constrained axis RDA1 appears to separate the variables: dissolved oxygen, pH, nitrogen, phosphorus, wood debris, detritus and Fil. Algae from: development type, conductivity, water temperature, F.F. Macrophytes, R.F. Macrophytes, S. Macrophytes, E.Macrophytes, A. Algae and F. Algae (see Figure 26). The constrained axis RDA2 separates: lake development type, conductivity, oxygen, pH, nitrogen, E. Macrophytes, F.F. Macrophytes and F. Algae from: wood debris, detritus, water temperature, phosphorus, R.F. Macrophytes, S. Macrophytes, A. Algae and Fil. Algae (see Figure 26). The whole study RDA analysis was found to explain 20.53% of benthic community variance observed in the data (see Figure 26).

As in previous RDA analyses, results were assessed using post-hoc tests by 'axes' as well as by 'terms' (see Table 18). When the RDA analysis is assessed by axes, we see that sixteen constrained axes are generated by the model (see Table 18). The constrained axis RDA1 explained the most variance of the group, with 5.37% explained variance and a p-value < 0.001. The axis RDA2 explained the second most variance at 3.75% with an associated p-value of < 0.001. This trend of decreasing explained variance continued with the axis RDA3 which explained 2.78% of benthic community variance with an associated p-value of < 0.001. RDA4 was the last axis to have a p-value less than the critical value, with a p-value of 0.030, significant at $p<0.05$* and an explained variance of 1.86%. The axes RDA5 and RDA6 explained more than

1% of variance, with 1.63% and 1.11% explained variance respectively, but had larger, non-significant, p-values (0.082 and 0.585). The remaining 10 constrained axes explained less than 1% of benthic community variance observed by the RDA model (see Table 18).

When assessed according to individual terms, the post-hoc test of the RDA analysis lists the variance contributed by each variable independently and tests their significance (see Table 18). Of the environmental variables tested, conductivity was found to explain the most variance, at 4.06% of variance in benthic community composition and an associated p-value of < 0.001 (see Table 18). pH had the second largest effect on variance, explaining 2.41% (p-value < 0.001). Water temperature explained 2.18% of benthic community variance, with an associated p-value < 0.001. Lake development type explained 2.01% of variance in benthic community composition with an associated p-value < 0.001. The amount of free-floating macrophytes present (FF.Macrophytes) explained 1.22% of community variance with an associated p-value < 0.001. The presence of attached algae (A.Algae) explained 1.2% of benthic community variance with an associated p-value < 0.001. Wood debris presence explained 0.95% of community variance, with a p-value of 0.002, significant at $p<0.01$. Dissolved oxygen concentration explained 0.90% of benthic community variance, with a p-value of 0.005, significant at $p<0.01$. Detritus explained 0.84% of benthic community variance with a p-value of 0.011, significant at $p<0.05$. Nitrogen explained 0.79% of community variance with a p-value of 0.015, significant at $p<0.05$. The presence of emergent macrophytes (E.Macrophytes) explained 0.79% of variance with a p-value of 0.015, significant at $p<0.05$. The presence of filamentous algae (Fil.Algae) explained 0.78% of variance with an associated p-value pf 0.017, significant at $p<0.05$. The presence of submerged macrophytes (S.Macrophytes) explained 0.75% of benthic community variance with a p-value of 0.024, significant at $p<0.05$. Presence of filamentous algae (F.Algae) explained

0.74% of variance with a p-value of 0.024, significant at $p<0.05$. The presence of rooted-floating macrophytes (R.F. Macrophytes) and phosphorus concentrations were not statistically significant factors, explaining only 0.55% of community variance with a p-value pf 0.117 and 0.37% of variance with a p-value of 0.488, respectively.

Given the large number of environmental variables included in this RDA analysis, a forward selection procedure was carried out to remove variables with low explanatory capabilities (see Table 19). The cut-off point used for this forward selection was the adjusted R^2 value of the whole study, which was found to be 0.1423. Nine of the sixteen variables were selected using the forward selection of the RDA model, those variables being conductivity ($p < 0.001$), pH ($p <0.001$), water temperature ($p < 0.001$), development type ($p < 0.001$), attached algae ($p <0.001$), free-floating macrophytes ($p <0.001$), wood debris ($p <0.05$), detritus ($p <0.05$), and filamentous algae ($p <0.05$) (see Table 19). An ordination diagram was generated following forward selection to better visualize their effect on benthic community composition (see Figure 27). In the forward transformed ordination diagram, we can see that the association between benthic macroinvertebrate families and the environmental variables assessed are much clearer than the pre-selected RDA analysis. The environmental variable conductivity appears closely parallel to the horizontal RDA1 axis, suggesting this variable has the largest effect on community composition variance (see Figure 27).

An additional RDA ordination diagram was generated from the forward selected variables to illustrate the variables having the greatest impact on variance in benthic community composition (see Figure 28). This figure also displays individual site means on the RDA diagram to illustrate the differences between isolated samples (red) and samples collected from developed lakes (blue) (see Figure 28). This figure shows that isolated and developed lakes have similar placements with respect to environmental variables. Initial trends appear to show a closer relationship between isolated lake samples and wood debris, detritus and attached algae. In contrast, developed lake samples appear to show a strong relationship with increasing lake conductivity (see Figure 28).

Separate RDA analyses with subsequent forward selections were carried out on samples from each of the individual seasons to compare the effect of environmental variables on benthic community composition during different stages of development and background environmental conditions.

The first subsample assessed was samples from fall of 2020. The constrained axis RDA1 separated detritus, phosphorus, water temperature, wood debris, A.Algae and Fil.Algae (which were positively correlated with the axis) from oxygen, lake type, pH, conductivity, nitrogen, E.Macrophytes, S.Macrophytes and FF.Macrophytes (which were negatively correlated with the axis, see Figure 29). FF.Macrophytes, water temperature, pH, lake type and Fil.Algae (which were negatively correlated with the axis) were separated from the other 10 environmental variables by the constrained axis RDA2. The fall RDA analysis explained 36.84% of variance in benthic community composition. When assessed by axis, the constrained axis RDA1 accounted for 10.55% of explained variance with an accompanying p-value of less than 0.001 (see Table 20). RDA2 explained 9.88% of variance in benthic community composition, with a p-value of $<$ 0.001. RDA3 and RDA4 both explained more than 5% of variance, and both had p-values less than 0.001. The constrained axis RDA5 explained 4.34% of community variance with a p-value of 0.005 ($p<0.01$). RDA6 explained 3.79% of variance with an associated p-value of 0.016 ($p<0.05$). The constrained axes RDA7, RDA8 and RDA9 explained more than 1% of community variance independently and had associated p-values of 0.109, 0.74 and 0.848, respectively, which were not statistically significant. The remaining constrained axes generated by the RDA accounted for less than one percent of explained variance, and each had an associated p-value of 1.00. As in the overall RDA analysis (pooling all three seasons), a forward selection was carried out to reduce the number of environmental variables used in the model, removing those with low explanatory power. The forward selection process of the Fall RDA initially selected 13 variables based on their adjusted R^2 cumulative values, however, the selection of more than 10 variables in a forward selection is not recommended, so the top 10 variables were selected instead (Table 21) (Blanchet *et al,* 2008; Zeleny, D. 2021). The environmental variables selected by this process were: development type ($p < 0.001$), conductivity ($p < 0.001$), detritus ($p < 0.001$), nitrogen ($p < 0.001$), water temperature ($p < 0.001$), emergent macrophyte presence $p < 0.001$, oxygen ($p < 0.001$), attached algae presence ($p < 0.001$), pH ($p < 0.001$) and submergent macrophyte presence ($p < 0.01$), (see Table 21). By assessing the ordination diagram generated from the fall forward selected data, we can see water temperature, dissolved oxygen concentration and pH closely associated with the constrained axis RDA1, suggesting high explanatory capabilities, which are conformed by our post-hoc analysis (see Figure 30).

The spring RDA analysis explained 24.59% of variance in benthic community composition (see Figure 31). The constrained axes RDA1 and RDA2 are displayed on the spring RDA ordination diagram generated from spring data only (see Figure 31). RDA1 appears to separate the following environmental variables: water temperature, detritus, wood debris, FF.Macrophytes, S.Macrophytes, E.Macrophytes and RF. Macrophytes from: conductivity, development type, pH, phosphorus, oxygen and nitrogen. The second axis, RDA2, separates the variables detritus, E.Macrophytes and RF.Macrophytes (which are negatively associated with the axis) from the other environmental variables. For the spring RDA post-hoc analysis, the first constrained axis RDA1 explained 11.57% of variance in benthic community composition, with a p-value of < 0.001. RDA2 explained 7.72% of variance and had an associated p-value < 0.001 (Table 22). RDA3 explained 4.82% of benthic community variance with an associated p-value <0.01. The constrained axes RDA4, RDA5, RDA6, RDA7 and RDA8 explained more than 1% of benthic community variance, these axes had p-values of 0.062, 0.404, 0.69, 0.956 and 1.000 respectively, all of which were not statistically significant (see Table 22). The remaining axes did not explain more than 1% of variance, and all of these axes had associated p-values of 1.00 (see Table 22). When environmental variables were individually assessed in the post-hoc RDA test, it is clear that water temperature and pH made the highest contributions to explained variance, explaining 5.05% and 5.02%, respectively (see Table 22). Both of these variables had p-values <0.001. The next highest explained variance attributed to an individual environmental variable was conductivity, which explained 4.2% of benthic community variance with an associated p-value of < 0.001. Wood debris presence explained 3.92% of benthic community variance, with a p-value of < 0.001. Lake development type explained 3.31% of benthic community composition variance, $p<0.001$. Of all the macrophyte variables, the presence of rooted floating macrophytes (RF.Macrophytes) explained the most benthic community variance at 3.23%, with a p-value < 0.001. Emergent macrophytes explained 2.76% of variance, with a p-value <0.01. Phosphorus explained 2.51% of benthic community variance with a p-value <0.01. Dissolved oxygen concentration explained 2.25% of variance in the model, with a p-value <0.05. Free-floating macrophytes explained 1.91% of community variance with a p-value <0.05. The remaining variables, nitrogen, detritus and submergent macrophytes explained between 1.3% and 1.18% of variance, and had non-significant p-values of 0.207, 0.281 and 0.311 respectively. A forward selection was again carried out on the seasonal RDA analysis to narrow down variables that had strong explanatory effects. A forward selection for the spring RDA analysis selected nine variables from the previous 16. These were: wood debris ($p < 0.001$), water temperature ($p < 0.001$), pH, ($p < 0.001$) development type ($p < 0.001$), E.Macrophytes ($p <0.01$), RF.Macrophytes ($p <0.01$), conductivity ($p <0.01$), FF.Macrophytes ($p <0.05$) and S.Macrophytes ($p <0.05$) (see Table 23). The ordination diagram displaying the results of the spring forward selected RDA confirm the results found by the post-hoc test (Figure 32). It appears development type and conductivity are negatively associated with the constrained axis RDA2, suggesting a negative impact on community composition (Figure 32). Along with this, we see wood debris closely associated with the other habitat parameters forward selected by the model, as well as being positively associated with the constrained axis RDA2 (Figure 32).

The last seasonal data subset used for an independent RDA analysis was that collected in the summer of 2021 (see Figure 33). This RDA analysis was able to explain 27.78% of the variance observed in benthic community composition at that time. Looking at the ordination diagram, we can see that the constrained axis RDA1 separates the variables: oxygen, pH, nitrogen, phosphorus, wood debris, detritus and Fil.Algae from: development type, F.F.Macrophytes, conductivity, water temperature, R.F.Macrophytes, F. Algae, E.Macrophytes, S.Macrophytes and A.Algae. The constrained axis RDA2 separates nitrogen, pH, oxygen, development type, conductivity, F.Algae and E.Macrophytes from the other variables assessed. The first constrained axis, RDA1, explained 13.58% of the variance and had an associated p-value which was less than 0.001. RDA2 explained 8.91% of variance in community composition, with a p-value less than 0.001. RDA3 was explained 5.75% of benthic community composition with a p-value <0.05. The constrained axes RDA4, RDA5, RDA6, RDA7 and RDA8 had an explained variance percentage above or at 1%, and each had p-values greater than the alpha value of 0.05 (see Table 24). When assessed by terms independently, of all the environmental variables, it appears pH explains the most variance in the summer RDA model, at 9.12% of variance with an accompanying p-value < 0.001 (Table 24). Water temperature explained the second most variance in summer samples, by explaining 6.10% of variance with a p-value $<$ 0.001. Dissolved oxygen explained 4.78% of variance in benthic community composition with an associated p-value < 0.001. Conductivity explained 4.16% of variance in benthic community composition and had a p-value < 0.001. RF.Macrophytes and detritus each explained 2.73% and 2.71% of benthic community variance, with accompanying p-values of <0.05 and <0.01 respectively. FF.Macrophytes, development type, F.Algae, wood debris, S.Macrophytes, nitrogen and phosphorus concentration were found to each explain between 2.0 and 1.0% of variance in benthic community composition. These variables each had p-values that were greater than our alpha value of 0.05, so were not statistically significant (see Table 24). The forward selection carried out on the summer subset RDA analysis identified 5 environmental variables based on adjusted R^2 cumulative values. The variables selected by this process were: conductivity ($p < 0.001$), pH ($p < 0.001$), oxygen ($p < 0.001$), FF. Macrophytes (free floating macrophyte presence) ($p <0.01$) and water temperature ($p <0.01$) (see Table 25). The ordination diagram generated from the forward selected summer RDA analysis confirms the findings from the post-hoc tests (Figure 34). pH appears closely associated with the constrained axis RDA1, while conductivity appears closely associated with the positive end of the constrained axis RDA2 (Figure 34).

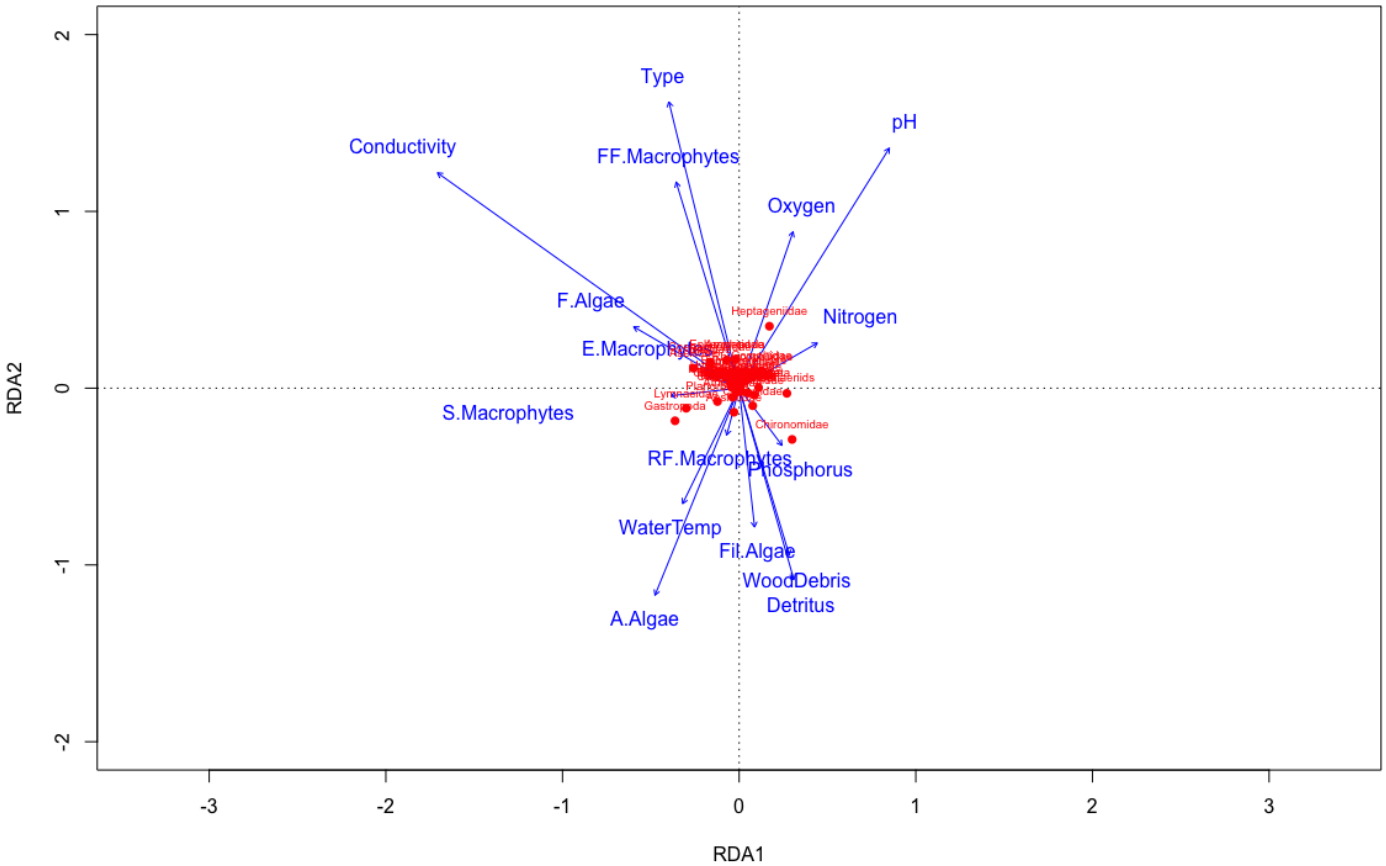

Figure 26. Whole study RDA analysis of the effect of water chemistry, habitat quality and lake type variables on benthic macroinvertebrate community composition. Sample data consist of three replicates for each of two sites in each of three seasons of sampling 12 lakes. Environmental data were standardized, and species data were Hellinger-transformed. Overall, the 16 environmental variables accounted for 20.53% of variance observed in benthic community composition during the course of the study.

Table 18. Permutation test results listed by 'axes' and 'terms' for whole study RDA analysis assessing various water chemistry and habitat variables effect on benthic community composition. Permutation tests were carried out using the R function 'anova.cca'. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

Axes	**Df**	**Variance**	**% Explained Variance**	**F**	**p-value**
RDA1	1	0.03270	5.36936996	13.6491	0.001***
RDA2	1	0.02285	3.75199094	9.5353	0.001***
RDA3	1	0.01694	2.78156352	7.0706	0.001***
RDA4	1	0.01135	1.8636804	4.7384	0.030*
RDA5	1	0.00994	1.63215711	4.1476	0.082
RDA6	1	0.00677	1.1116402	2.8245	0.585
RDA7	1	0.00601	0.98684751	2.5068	0.719
RDA8	1	0.00518	0.85056075	2.1618	0.836
RDA9	1	0.00338	0.5549991	1.4097	0.997
RDA10	1	0.00320	0.52544293	1.3336	0.996
RDA11	1	0.00203	0.33332786	0.8464	1.000
RDA12	1	0.00163	0.26764749	0.6799	1.000
RDA13	1	0.00145	0.23809133	0.6042	1.000
RDA14	1	0.00080	0.13136073	0.3322	1.000
RDA15	1	0.00054	0.08866849	0.2274	1.000
RDA16	1	0.00026	0.04269224	0.1085	1.000
Residual	202	0.48398			
Terms					
WaterTemp	1	0.01325	2.17569787	5.5285	0.001***
Oxygen	1	0.00550	0.90311987	2.2951	0.005**
pH	1	0.01468	2.41050903	6.1271	0.001***
Conductivity	1	0.02471	4.05747126	10.3125	0.001***
Phosphorus	1	0.00226	0.37110016	0.9438	0.488
Nitrogen	1	0.00481	0.78981938	2.0060	0.015*
Type	1	0.01224	2.00985222	5.1099	0.001***
WoodDebris	1	0.00576	0.94581281	2.4034	0.002**
Detritus	1	0.00509	0.83579639	2.1238	0.011*
E.Macrophytes	1	0.00479	0.7865353	1.9989	0.015*
RF.Macrophytes	1	0.00337	0.55336617	1.4058	0.117
S.Macrophytes	1	0.00456	0.74876847	1.9043	0.024*
FF.Macrophytes	1	0.00746	1.22495895	3.1151	0.001***
F.Algae	1	0.00450	0.73891626	1.8776	0.024*
Fil.Algae	1	0.00472	0.77504105	1.9706	0.017*
A.Algae	1	0.00732	1.20197044	3.0536	0.001***
Residual	202	0.48398			

Table 19. Results of forward selection of variables from whole study RDA analysis. Adjusted R^2 threshold used as cut-off during forward selection (0.1423264). Of the 16 variables assessed in the original RDA analysis, 9 were selected using forward selection. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

	Variables	**R^2**	**R^2 Cumulative**	**AdjR^2 Cumulative**	**F**	**p-value**
1	**Conductivity**	0.038608938	0.03860894	0.03417856	8.714601	<0.001***
2	**pH**	0.027018901	0.06562784	0.05697624	6.245994	<0.001***
3	**WaterTemp**	0.023590585	0.08921842	0.07650984	5.568817	<0.001***
4	**Type**	0.020446837	0.10966526	0.09302349	4.914582	<0.001***
5	**A.Algae**	0.018545981	0.12821124	0.10774672	4.531251	<0.001***
6	**FF.Macrophytes**	0.015492858	0.1437041	0.11946931	3.83569	<0.001***
7	**WoodDebris**	0.007670538	0.15137464	0.12322119	1.907182	0.03*
8	**Detritus**	0.007694948	0.15906959	0.12703414	1.921609	0.021*
9	**F.Algae**	0.007102492	0.16617208	0.13026561	1.780248	0.036*

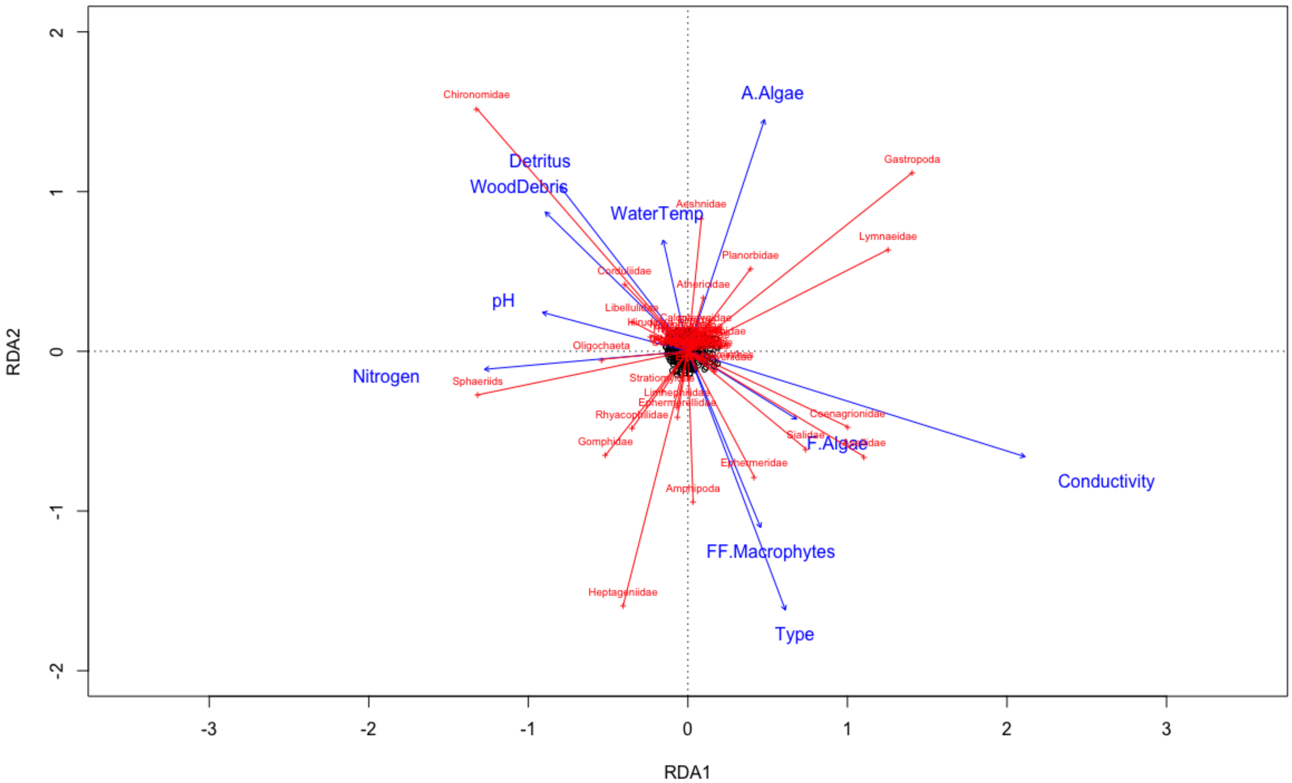

Figure 27. Whole study forward selected RDA analysis of the effect of water chemistry, habitat quality and lake type variables on benthic macroinvertebrate community composition. Sample data consist of three replicates for each of two sites in each of three seasons of sampling 12 lakes. Environmental data were standardized, and species data were Hellinger-transformed. Environmental variables forward selected were conductivity, pH, water temperature, type, attached algae, free flating macrophytes, wood debris, detritus and filamentous algae.

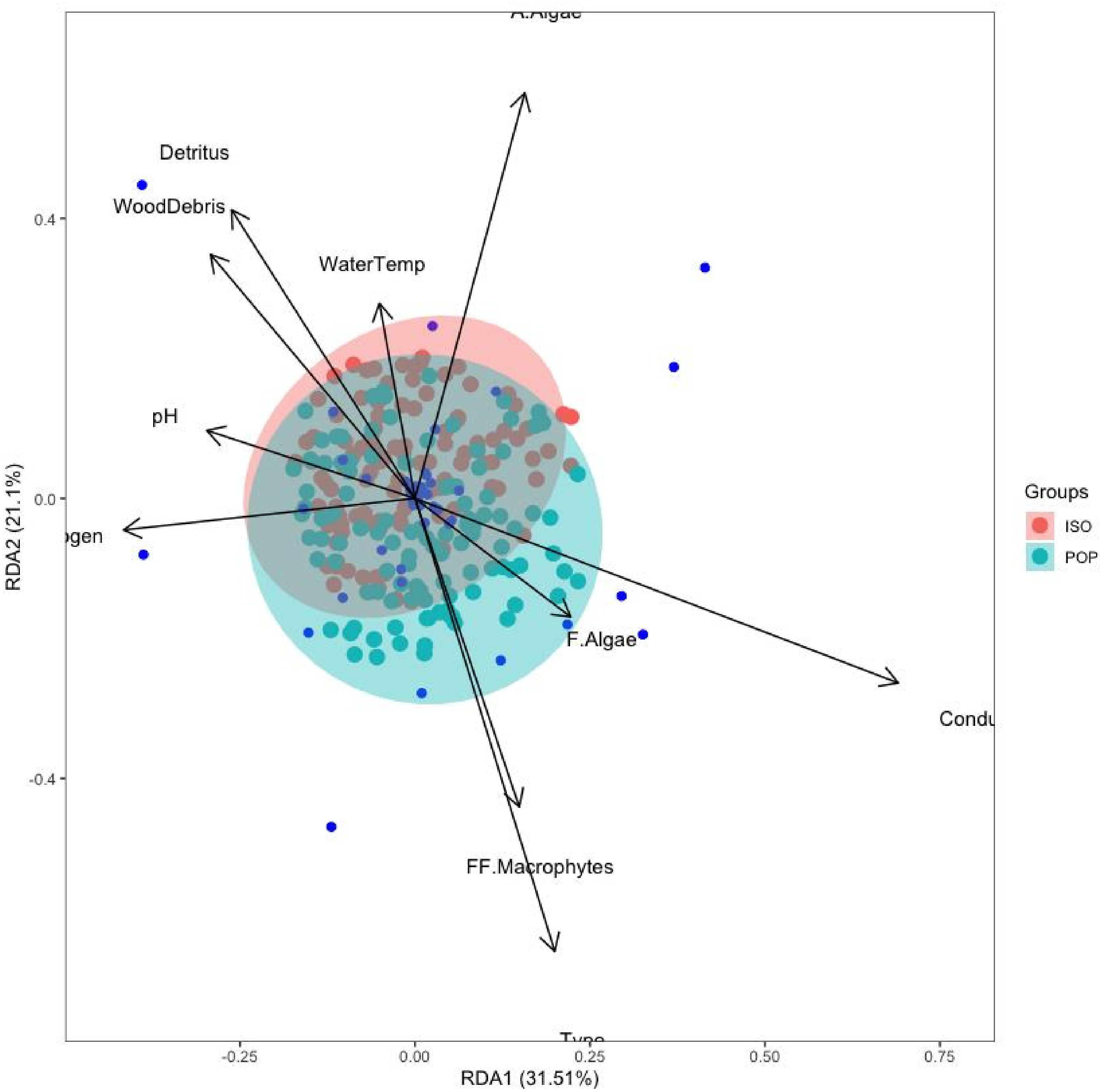

Figure 28. Plot of forward selected whole study RDA analysis assessing 9 variables related to water chemistry, habitat quality and lake development type. Red points and the associated pink bubble represent isolated sites, blue points and the associated pale blue bubble represent populated (or developed) sites. Small dark blue dots represent the placement of macroinvertebrate families sampled and their relationships with variables tested.

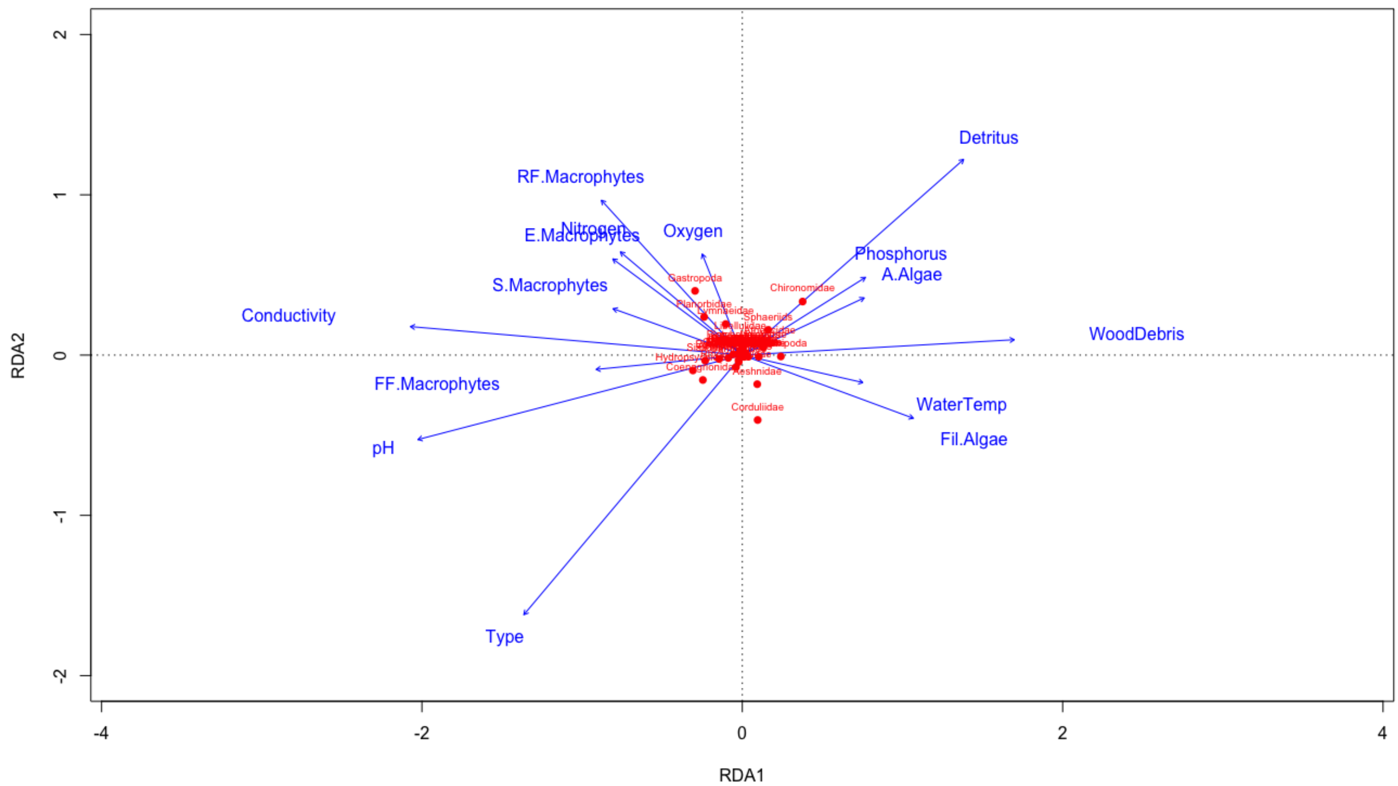

Figure 29. Fall season RDA analysis of water chemistry, habitat quality and lake type variables and their effect on benthic macroinvertebrate community composition. Samples were collected from 12 lakes, with 2 sites selected per lake which were each sampled 3 times. Environmental data were standardized, and species data were Hellinger-transformed. The fall RDA model explained 36.84% of the variance in benthic community composition in fall.

Table 20. Permutation test results done on 'axes' and 'terms' for the fall season RDA analysis assessing the effects of various water chemistry and habitat variables on the benthic community composition. Permutation tests were carried out using the R function 'anova.cca'. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

AXES	Df	Variance	% Explained Variance	F	p-value
RDA1	1	0.065894	10.5541176	11.8638	<0.001***
RDA2	1	0.061658	9.87564548	11.1011	<0.001***
RDA3	1	0.042430	6.79593301	7.6392	<0.001***
RDA4	1	0.035871	5.74539036	6.4584	<0.001***
RDA5	1	0.027158	4.3498456	4.8897	0.005**
RDA6	1	0.023689	3.79422242	4.2651	0.016*
RDA7	1	0.017983	2.88030317	3.2378	0.109
RDA8	1	0.011550	1.8499417	2.0795	0.74
RDA9	1	0.009985	1.5992786	1.7977	0.848
RDA10	1	0.005565	0.89133555	1.0019	1.000
RDA11	1	0.004571	0.73212844	0.823	1.000
RDA12	1	0.002758	0.44174365	0.4966	1.000
RDA13	1	0.002092	0.33507169	0.3766	1.000
RDA14	1	0.001518	0.24313519	0.2733	1.000
RDA15	1	0.000587	0.09401868	0.1057	1.000
Residual	56	0.311035			
TERMS					
Water.Temp.	1	0.019479	3.11991966	3.5070	<0.001***
Oxygen	1	0.016141	2.58527764	2.9061	<0.001***
pH	1	0.043831	7.02033978	7.8916	<0.001***
Conductivity	1	0.027998	4.48439399	5.0409	<0.001***
Phosphorus	1	0.013121	2.10156917	2.3624	0.005**
Nitrogen	1	0.030192	4.8358034	5.4358	0.001***
Type	1	0.035038	5.61197931	6.3084	0.001***
Wood.Debris	1	0.008534	1.36687686	1.5264	0.091
Detritus	1	0.029303	4.69341372	5.2758	<0.001***
E.Macrophytes	1	0.024331	3.89705659	4.3807	<0.001***
R.F.Macrophytes	1	0.008428	1.34989901	1.5174	0.098
S.Macrophytes	1	0.014030	2.24716222	2.5260	0.003**
F.F.Macrophytes	1	0.013056	2.09115823	2.3507	0.011*
Fil.Algae	1	0.007465	1.19565687	1.3441	0.178
A.Algae	1	0.022361	3.5815249	4.0260	<0.001***
Residual	56	0.311035			

Table 21. Results of forward selection carried out on Fall 2020 RDA analysis. 15 environmental variables were used in the original RDA analysis for fall 2020 data, but this table lists the 10 variables that had the greatest contributions. The adjusted R^2 threshold used as the cut-off for forward selection was 0.3683795. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

		R^2	R^2 Cumulative	Adj R^2 Cumulative	F	p-value
1	Type	0.07218816	0.07218816	0.05893371	5.446332	<0.001***
2	Conductivity	0.06487472	0.13706288	0.11205021	5.187349	<0.001***
3	Detritus	0.05089682	0.1879597	0.15213439	4.262083	<0.001***
4	Nitrogen	0.05109452	0.23905422	0.19362462	4.498787	<0.001***
5	WaterTemp	0.03426406	0.27331828	0.21826664	3.111992	<0.001***
6	E.Macrophytes	0.03316942	0.3064877	0.24247118	3.10883	<0.001***
7	Oxygen	0.0353171	0.3418048	0.2698147	3.434079	<0.001***
8	A.Algae	0.0289364	0.37074119	0.29083531	2.897048	<0.001***
9	pH	0.02752436	0.39826556	0.31091701	2.835986	<0.001***
10	S.Macrophytes	0.02382179	0.42208735	0.32734757	2.514445	0.003**

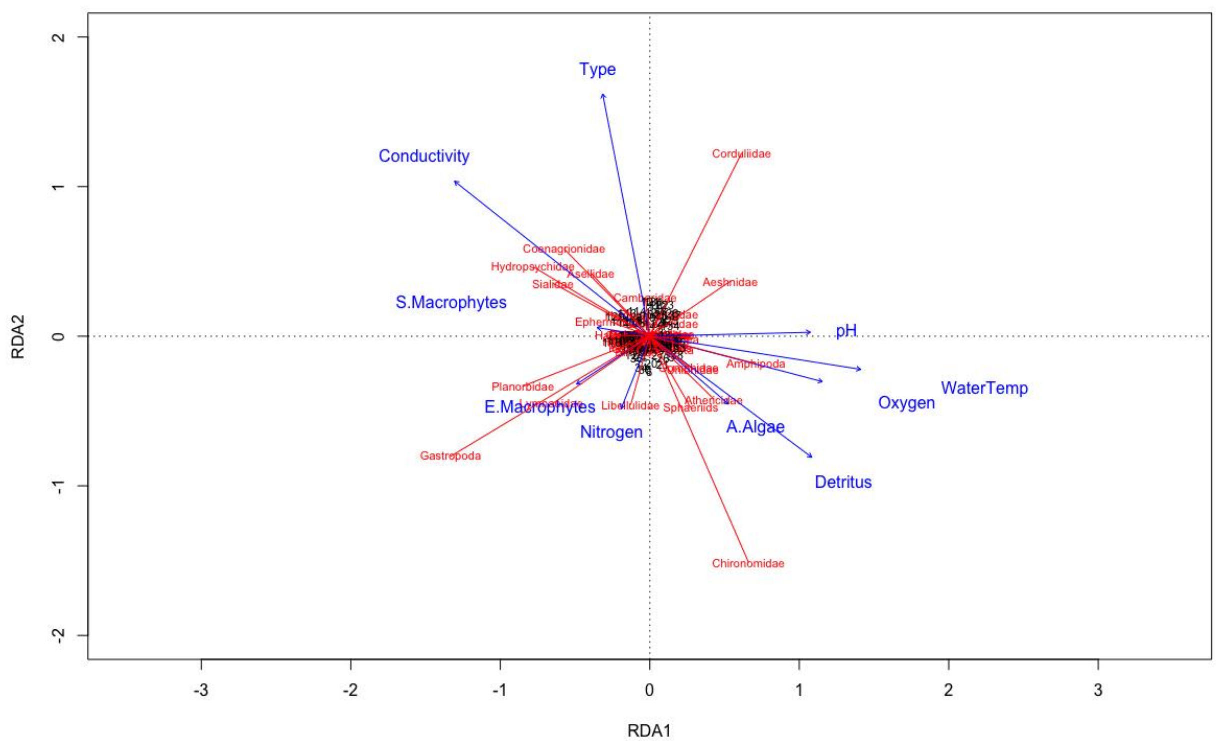

Figure 30. Fall season forward selected RDA analysis of water chemistry, habitat quality and lake type variables and their effect on benthic macroinvertebrate community composition.

Samples were collected from 12 lakes, with 2 sites selected per lake which were each sampled 3 times. Environmental data were standardized, and species data were Hellinger-transformed. Environmental variables forward selected in the fall season were development type, conductivity, detritus, nitrogen, water temperature, emergent macrophytes, oxygen concentration, attached algae, pH and submergent macrophytes.

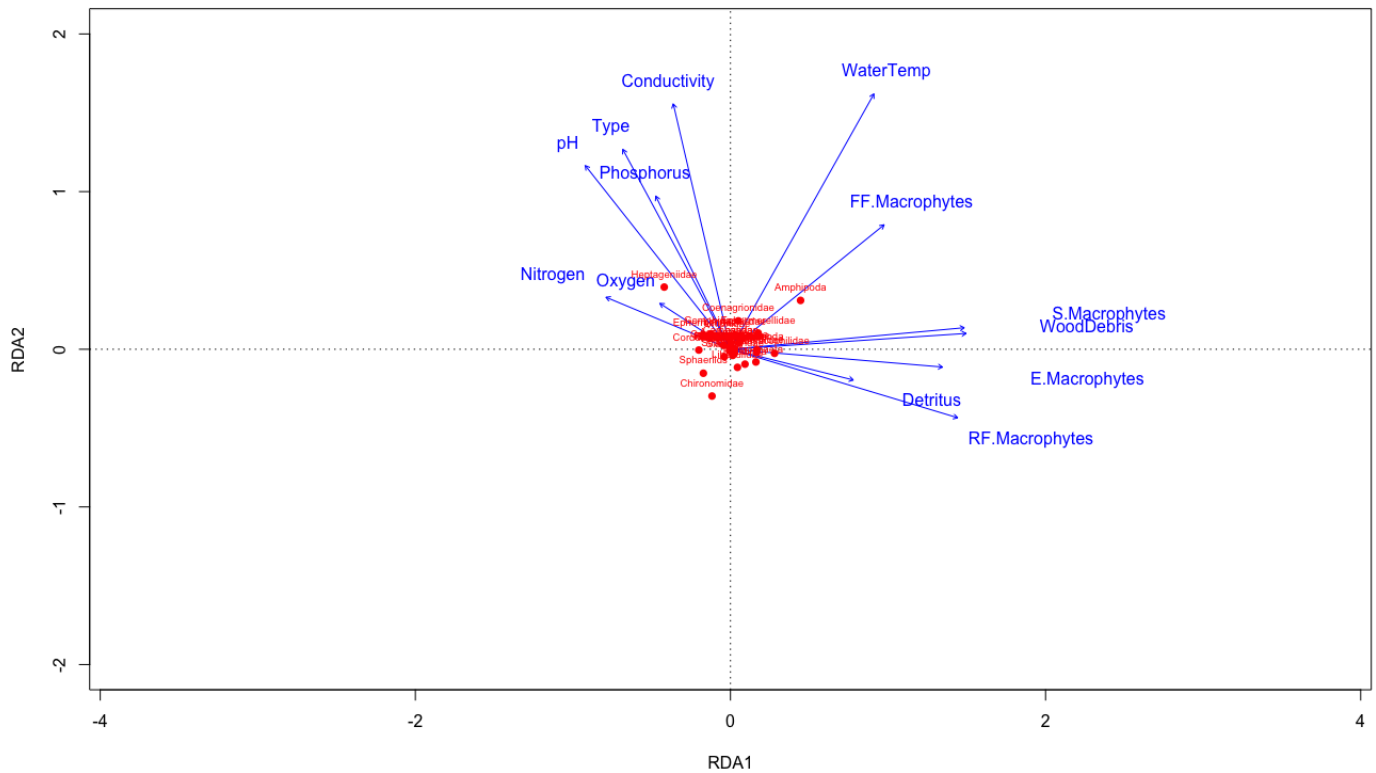

Figure 31. RDA analysis of spring data for water chemistry, habitat quality and lake type as variables affecting the composition of benthic macroinvertebrate communities. Sample data were from the 12 lakes sampled during the spring of 2021. For each lake, sites were sampled 3 times. Environmental data were standardized, and species data were Hellinger-transformed. The variables in the spring RDA analysis explained 24.59% of the variance in benthic community composition.

Table 22. Premutation test results done on ‘axes’ and ‘terms’ for spring RDA analysis assessing the effects of various water chemistry and habitat variables on the benthic community composition. Permutation tests were carried out using the R function ‘anova.cca’. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

AXES	Df	Variance	% Variance Explained	F	p-value
RDA1	1	0.0594	11.5708	11.3544	<0.001***
RDA2	1	0.0396	7.7178	7.5730	<0.001***

RDA3	1	0.0247	4.8153	4.7253	0.006**
RDA4	1	0.0198	3.8511	3.7785	0.062
RDA5	1	0.0144	2.8128	2.7600	0.404
RDA6	1	0.0117	2.2791	2.2363	0.69
RDA7	1	0.0083	1.6168	1.5868	0.956
RDA8	1	0.0051	1.0012	0.9817	1.000
RDA9	1	0.0044	0.8493	0.8343	1.000
RDA10	1	0.0033	0.6467	0.6337	1.000
RDA11	1	0.0019	0.3740	0.3664	1.000
RDA12	1	0.0010	0.1909	0.1877	1.000
RDA13	1	0.0006	0.1130	0.1106	1.000
Residual	61	0.31911			
TERMS					
WaterTemp	1	0.02592	5.04929	4.95520	<0.001***
Oxygen	1	0.01157	2.25387	2.21130	0.014*
pH	1	0.02577	5.02006	4.92530	<0.001***
Conductivity	1	0.02158	4.20384	4.12420	<0.001***
Phosphorus	1	0.01287	2.50711	2.46060	0.005**
Nitrogen	1	0.00671	1.30713	1.28290	0.207
Type	1	0.01695	3.30191	3.24070	<0.001***
WoodDebris	1	0.02013	3.92138	3.84860	<0.001***
Detritus	1	0.00610	1.18830	1.16630	0.281
E.Macrophytes	1	0.01416	2.75841	2.7063	0.006**
RF.Macrophytes	1	0.01658	3.22983	3.16940	<0.001***
S.Macrophytes	1	0.00608	1.18440	1.16250	0.311
FF.Macrophytes	1	0.00981	1.91101	1.87500	0.042*
Residual	61	0.31911			

Table 23. Forward selection results from spring subset RDA analysis. 13 environmental variables were used in the original spring RDA analysis; nine were retained for this table. Selection of variables was based on an adjusted R^2 threshold of 0.2458868. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

	Variables	R^2	R^2 Cumulative	Adj R^2 Cumulative	F	p-value
1	WoodDebris	0.0633276	0.0633276	0.05049647	4.935466	<0.001***
2	WaterTemp	0.05363841	0.116966	0.09243729	4.373519	<0.001***
3	pH	0.05021641	0.1671824	0.13199294	4.281087	<0.001***
4	Type	0.0350459	0.2022283	0.15664136	3.075082	<0.001***

5	E.Macrophytes	0.02506212	0.2272904	0.17129699	2.237951	0.007**
6	RF.Macrophytes	0.02599366	0.2532841	0.1873974	2.367124	0.003**
7	Conductivity	0.02680617	0.2800903	0.20487581	2.494775	0.003**
8	FF.Macrophytes	0.02267754	0.3027678	0.21825481	2.146656	0.011*
9	S.Macrophytes	0.02026495	0.3230328	0.22929883	1.945769	0.019*

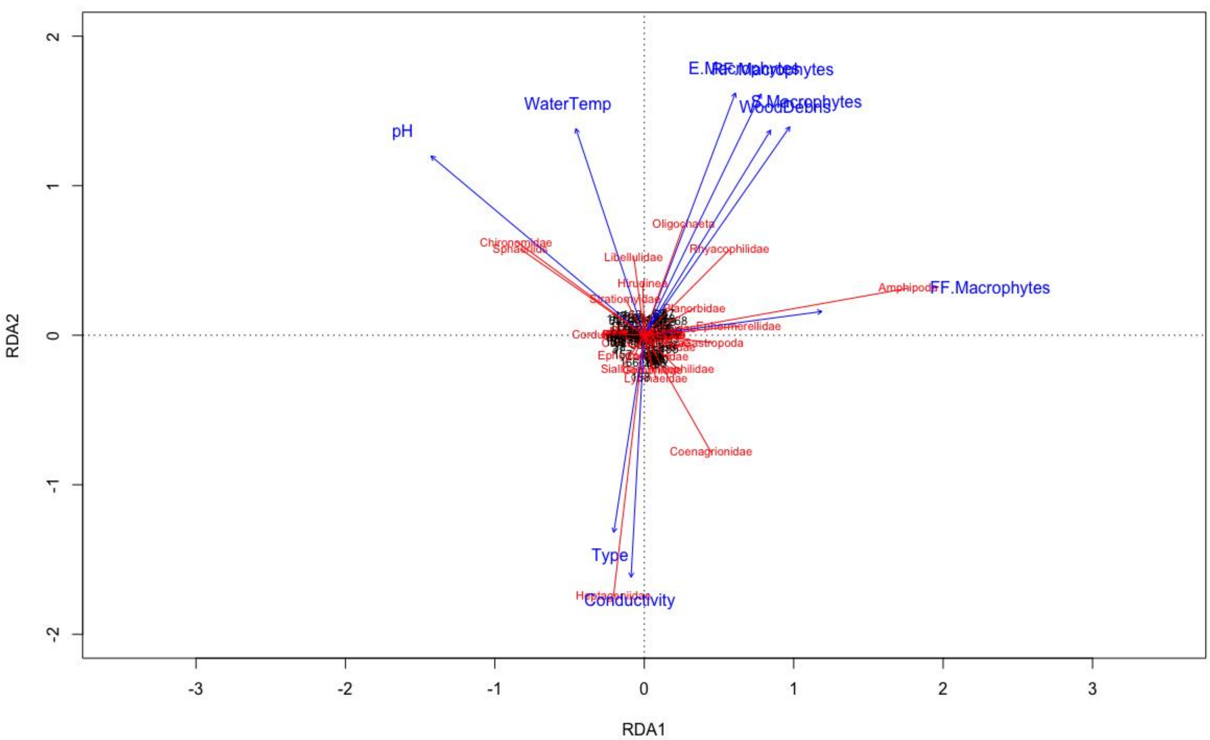

Figure 32. Forward selected RDA analysis of spring data for water chemistry, habitat quality and lake type as variables affecting the composition of benthic macroinvertebrate communities. Sample data were from the 12 lakes sampled during the spring of 2021. For each lake, sites were sampled 3 times. Environmental data were standardized, and species data were Hellinger-transformed. Environmental variables forward selected for the model were wood debris, water temperature, pH, development type, emergent macrophytes, rooted floating macrophytes, conductivity, free floating macrophytes and submergent macrophytes.

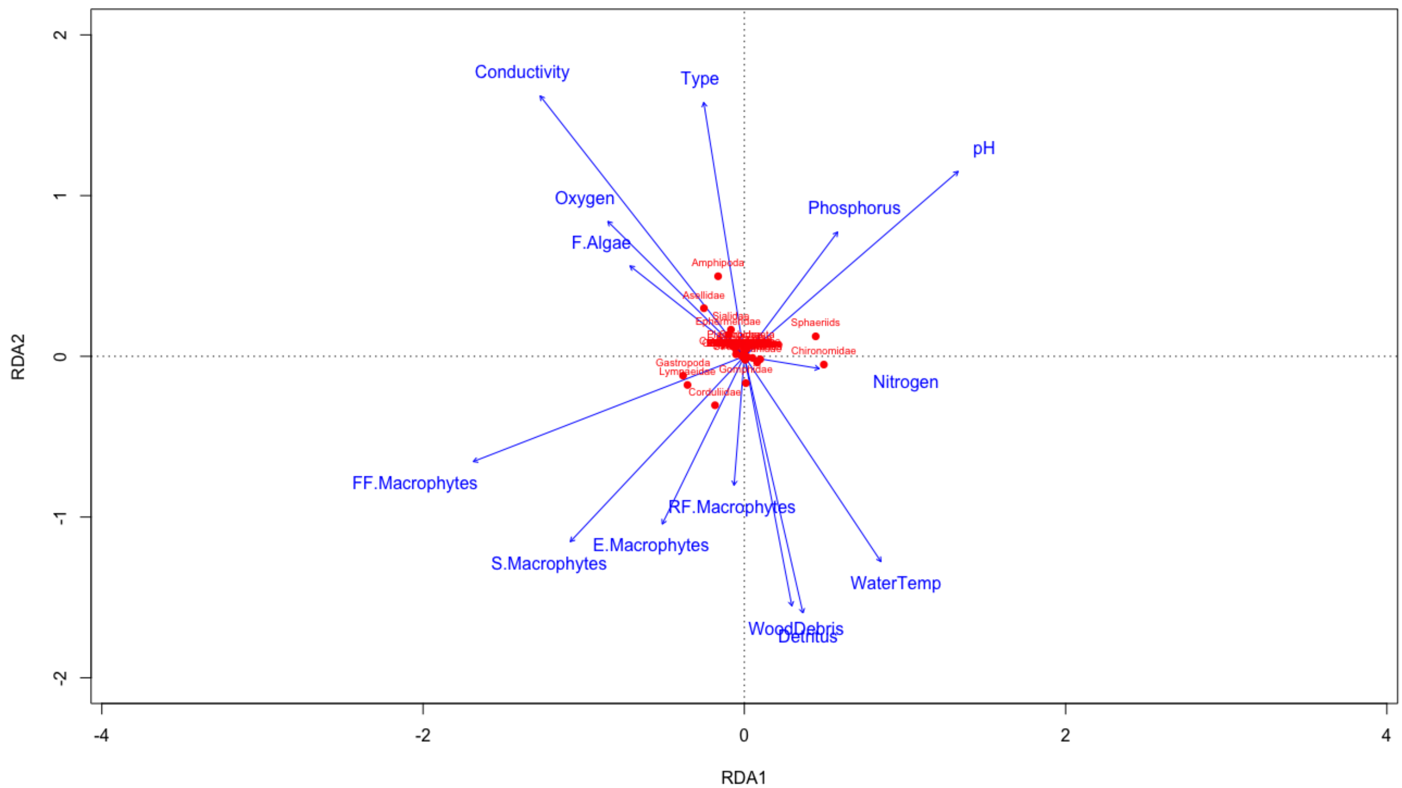

Figure 33. Summer RDA analysis of water chemistry, habitat quality and lake type variables as they affect benthic macroinvertebrate community composition. Sample data were collected from 12 lakes, from 2 sites per lake, with 3 replicate samples taken per site, in summer of 2021. Environmental data were standardized, and species data were Hellinger-transformed. Variables included in the summer RDA analysis account for 27.78% of the variance in benthic community composition at that time.

Table 24. Permutation test results done on ‘axes’ and ‘terms’ for summer RDA analysis assessing effects of various water chemistry and habitat variables on the benthic community composition at that time. Permutation tests were carried out using the R function ‘anova.cca’. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

AXES	Df	Variance	% Variance Explained	F	p-value
RDA1	1	0.0803	13.5763437	13.3452	<0.001***
RDA2	1	0.05269	8.90831319	8.7574	<0.001***
RDA3	1	0.03403	5.75346171	5.6559	0.017*
RDA4	1	0.02809	4.74918424	4.6692	0.062
RDA5	1	0.01938	3.27658208	3.2213	0.396
RDA6	1	0.00998	1.68732142	1.6579	0.995
RDA7	1	0.00826	1.39652053	1.3729	0.999

RDA8	1	0.00589	0.99582396	0.9787	1.000
RDA9	1	0.00375	0.63401356	0.6239	1.000
RDA10	1	0.00228	0.38548024	0.3794	1.000
RDA11	1	0.00168	0.28403807	0.2793	1.000
RDA12	1	0.00121	0.20457504	0.2017	1.000
RDA13	1	0.00072	0.1217306	0.1192	1.000
RDA14	1	0.00025	0.04226757	0.0408	1.000
Residual	57	0.34296			
TERMS					
WaterTemp	1	0.03592	6.07300455	5.9702	<0.001***
Oxygen	1	0.02827	4.77961689	4.6991	<0.001***
pH	1	0.05387	9.10781612	8.9535	<0.001***
Conductivity	1	0.02461	4.16081965	4.0896	<0.001***
Phosphorus	1	0.00771	1.30353188	1.2812	0.236
Nitrogen	1	0.00830	1.40328334	1.3794	0.189
Type	1	0.00969	1.63829104	1.6108	0.091
WoodDebris	1	0.00845	1.42864389	1.4042	0.17
Detritus	1	0.01605	2.71357803	2.6681	0.007**
E.Macrophytes	1	0.01205	2.0372969	2.0027	0.040*
RF.Macrophytes	1	0.01614	2.72879436	2.6826	0.008**
S.Macrophytes	1	0.00832	1.40666475	1.3822	0.182
FF.Macrophytes	1	0.01060	1.79214499	1.7614	0.065
F.Algae	1	0.00853	1.44216951	1.4178	0.185
Residual	57	0.34296			

Table 25. Forward selection results from the summer subset RDA analysis. 14 environmental variables were used in the original summer RDA analysis; five were retained for this analysis. The adjusted R^2 threshold used as a cut-off during forward selection was 0.277741. Statistical significance is indicated as follows: *** = p<0.001, ** = p<0.01, and * = p<0.05.

	Variables	R^2	R^2 Cumulative	Adj R^2 Cumulative	F	p-value
1	Conductivity	0.08349119	0.08349119	0.0703982	6.376789	<0.001***
2	pH	0.07337706	0.15686824	0.1324296	6.005013	<0.001***
3	Oxygen	0.05667102	0.21353926	0.1788425	4.899964	<0.001***
4	FF.Macrophytes	0.03656916	0.25010843	0.2053388	3.267318	0.002**
5	WaterTemp	0.02678244	0.27689087	0.2221099	2.444501	0.01*

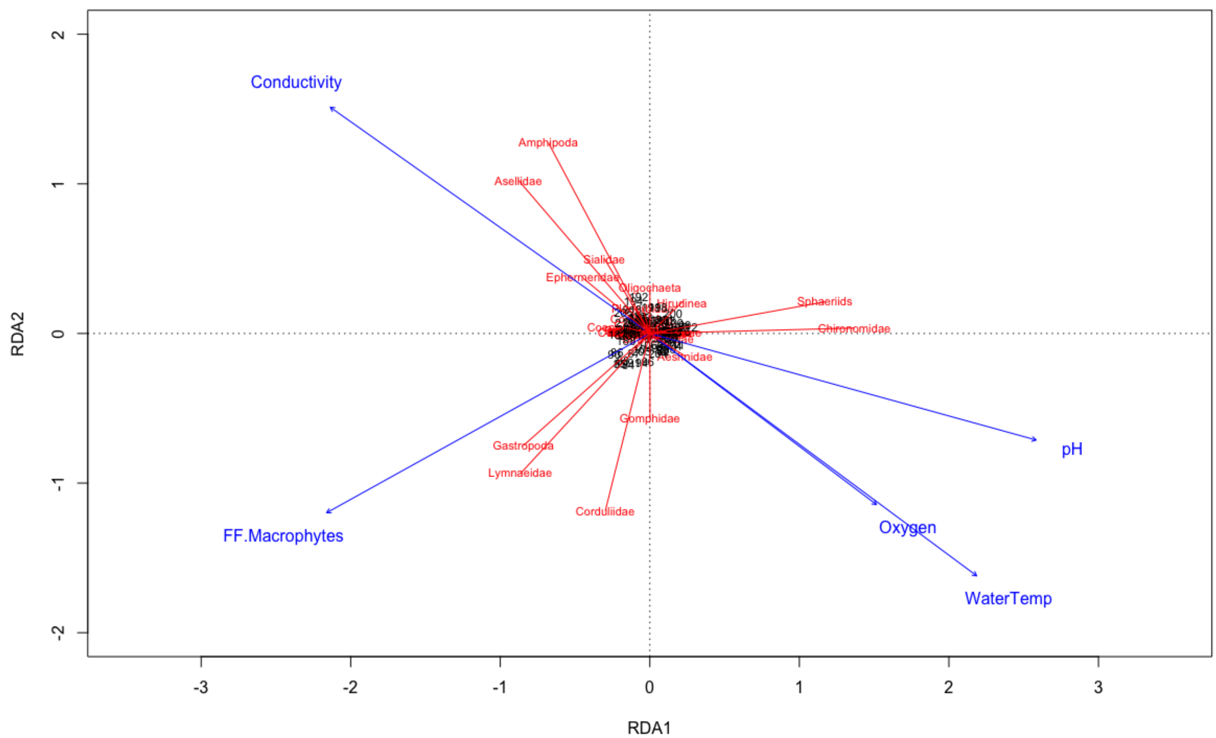

Figure 34. Forward selected summer RDA analysis of water chemistry, habitat quality and lake type variables as they affect benthic macroinvertebrate community composition. Sample data were collected from 12 lakes, from 2 sites per lake, with 3 replicate samples taken per site, in summer of 2021. Environmental data were standardized, and species data were Hellinger-transformed. The environmental variables forward selected for the model were conductivity, pH, oxygen concentration, free-floating macrophytes and water temperature.

4.4 Discussion

4.4.1 The Impact of Human Development on Water Chemistry in Lakes of Central Ontario

Over the course of this study, three of the five water chemistry variables were found to differ significantly between isolated and developed lakes in central Ontario. These were: pH, conductivity, and available nitrogen concentration.

In isolated sites, the average pH was found to be 6.44 over the course of the study. When divided according to season, pH in isolated lakes was lowest in fall (5.94), highest in spring (6.87), and in between these values in summer (6.50). In developed lakes, the average pH was found to be 6.63 over the study period. When examined on a seasonal basis, developed lakes showed similar trends to isolated lakes, with their lowest pH in fall (6.34), highest in spring (6.93) and in between these values in the summer (6.61). Overall, average pH was significantly different between isolated and developed lakes ($W = 4635$, $p < 0.01$), however, on a season-by-season basis, only the fall season showed significant differences between lake types ($W = 133.5$, $p < 0.01$). In the other two seasons sampled, average pH was consistently lower in isolated lakes than in developed lakes, however, the averages only differed between the two lake types by ~ 0.1 - 0.2 pH units. pH changed in a similar pattern according to season in both isolated and developed lakes. As mentioned above, in Chapter 3, these fluctuation patterns do not match with earlier published studies, (e.g. Jeffries *et al.,* 1978), that found pH to be at its highest in fall, rather than spring, as found in our study area. Despite this contrast, since both lake types trended in the same way, we can infer that higher pH levels in spring are a genuine feature of the Kearny region, and not a sampling anomaly.

Overall, pH was found to be lower in isolated lakes than in developed lakes for all three seasons sampled. A possible explanation for lower pH values in isolated lakes is that they are taking longer to recover from historic (airborne) acidic deposition compared to developed lakes. This could be because the shorelines and adjacent areas of isolated lakes are less disturbed than developed lakes. For this reason, stored sulphate from earlier periods of deposition may be retained in the littoral zone or nearby watershed (Lento *et al.,* 2008; Palmer *et al.,* 2011). When examined more closely, the fall season, when isolated and developed lakes differed significantly is largely responsible for the overall differences between the two types of lake. In fall, isolated lakes had a significantly lower pH than was seen in any lake type in the other seasons. In the fall samples, two isolated lakes (Charlie Mann's Lake and Buck Lake) had average pH levels well below 6 while only one developed lake (Lower Raven Lake) had an average pH less than 6. Charlie Mann's and Buck Lake appear to have skewed the average pH in isolated fall samples to generate this significant difference between the two types of lakes. If it were not for these two exceptionally acid results in fall, pH values would be quite similar in isolated and developed lakes over the course of the study. An explanation for the acidity of these two lakes is hard to pinpoint, but it could be the result of an unknown discharge source in the small hunting cabins located on Buck Lake, or the small dirt road that connects Kearney, Ontario to the neighbouring Algonquin Park, which is where Charlie Mann's Lake is located.

Differences between isolated and developed lakes with respect to lake conductivity (μS/cm) are very striking. Developed lakes in our sampling area consistently had average conductivity levels well above those in isolated lakes in the region. Over the whole study period, isolated lakes had an average conductivity of 16.35 μS/cm while developed lakes had an average conductivity of 29.21 μS/cm, a statistically significant difference at $p<0.001$ ($W = 1503$, p- < 2.2e-16*). Differences in average conductivity between isolated and developed lakes were also apparent in each season. In fall, average conductivity in isolated (16.69 μS/cm) and developed lakes (31.64 μS/cm) were significantly different from one another at $p<0.001$ ($W = 21$, $p = 9.708e-07$). In spring average conductivity in isolated lakes (16.65 μS/cm) and developed lakes (26.68 μS/cm) were still significantly different from each other at $p<0.001$ ($W = 42$, $p = 4.94e-06$). Finally, in summer, differences between the average conductivity in isolated lakes (15.62 μS/cm) and developed lakes (29.30) were again statistically significant at $p<0.001$ ($W = 54$, $p = 1.194e-05$).

Developed lakes not only had consistently higher conductivity than isolated lakes but also showed a greater range in average conductivity from season to season. This may be due to developed lakes in the region being more exposed and susceptible to influxes of base cations, due to the variety, and seasonality of the effects of human development (Lento *et al.,* 2008; Palmer *et al.,* 2011). As development proceeds, associated road building, land clearing and road maintenance can significantly affect local water conductivity (Palmer *et al.,* 2011). Increased impermeable surfaces are highly associated with human development and as these surfaces increase in an area, surface runoff generally increases (Lento *et al.,* 2008). A similar effect occurs with shoreline and watershed clearing. Property owners often clear shorelines and watershed macrophytes for aesthetic and recreational reasons (Palmer *et al.,* 2011). With less plant life to act as a buffer between nearby roads and the lake itself, base cations and other pollutants can readily enter these water systems relatively unaltered (Lento *et al.,* 2008). This, coupled with the region's already low buffering capacity due to the underlying geology, could result in these substances entering nearby lakes. This is especially true in spring, when accumulated base cations in snow (from the application of road salt throughout the winter) melt and enter nearby lakes (Lento *et al.,* 2008; Neff and Jackson, 2013).

Lakeshore development and the associated road salting were identified as the likely drivers of increased lake Na^+ and Cl^- in central Ontario in 2005, with conductivity and Na^+/Cl^- concentrations being highly correlated (Palmer *et al.,* 2011). We see a similar trend in the present study. While conductivity levels did not reach biologically detrimental levels over the course of this study or in studies done in the region in the past, these trends are of concern (Lento *et al.,* 2008; Palmer *et al.,* 2011; Neff and Jackson, 2013). As development continues in the area, we can expect road salting, the area of impermeable surfaces and extent of watershed/shoreline clearing all to increase. This in turn could cause lake conductivity in the area to continue to increase (Lento *et al.,* 2008; Palmer *et al.,* 2011; Neff and Jackson, 2013). These trends, coupled with the region's low buffering capacity, could drastically change lake water chemistry in the region in coming decades (Lento *et al.,* 2008; Neff and Jackson, 2013). Based on the results of our three-season sampling in an area of central Ontario, we can confidently say that human development has already had a significant effect on lake conductivity.

Differences in dissolved oxygen (DO) concentration (mg/L) in isolated and developed lakes in the present study were not statistically significant, with average concentrations of 9.56 mg/L and 9.71 mg/L for isolated and developed lakes, respectively ($W = 6048$, $p = 0.6389$). This pattern was evident in all three seasons; dissolved oxygen concentrations in isolated (10.14 mg/L) and populated lakes (9.98 mg/L) in fall only differed by ~ 0.15 mg/L ($W = 414$, $p = 0.206$). In the spring, average DO in isolated (10.42 mg/L) and developed lakes (10.97 mg/L) only differed by ~ 0.55 mg/L ($W = 342$, $p = 0.894$). Lastly, in the summer, where average DO was lowest for both isolated (8.13 mg/L) and developed (8.17 mg/L) lakes, barely differed ($W = 309$, $p = 0.71$).

In both lake types, dissolved oxygen concentration trended with its main mediating factor, water temperature. We did observe higher DO concentrations in spring compared to fall, which is unusual, but this may be due to the increased primary productivity seen in spring, as well as the fact that spring sampling was carried out before the lake warmed sufficiently to stratify (Canadian Council of Ministers of the Environment, 1999). This would result in higher DO concentrations near the surface of the water when sampling occurred, which would result in DO levels more in line with fall concentrations. However, we do not have data to determine when lake turnover occurred in the area and therefore cannot conclude this is the reasoning behind TP loads. No lakes or lake types exhibited biologically significant levels of DO, (i.e., they were not too low to support aquatic life) so we can conclude that this is not a driving factor for community composition in the area.

Over the whole study period, isolated lakes averaged a total phosphorus concentration (TP) of 75.18 µg/L and developed lakes averaged 47.34 µg/L. While these averages appear very different, there was considerable variability between lakes sampled within the same type and some notable outliers, so no statistically significant difference was found ($W = 6444$, $p = 0.14$). On a season-by-season basis, again, although the mean values seemed very different, there were no statistically significant differences. In fall, the total phosphorus concentration was more than three times higher in isolated lakes with an average of 141.73 µg/L compared to developed lakes with an average of 42.91 µg/L ($W = 778.5$, $p = 0.14$). The substantially high average of TP concentration found in isolated lakes in the fall appears to be outlier that may not reflect true concentrations in that region during the time of sampling. By examining individual TP readings from Fall 2020 samples, we observe two samples with extremely high TP levels relative to others, Spruce Lake site 2 (696.92µg/L) and Tea Lake site 1 (559.66µg/L), which are both considered to be isolated lakes. If these two samples are removed, the average TP concentration drops from 92.32 to 43.59, which is more in line with the spring and summer averages observed. Total phosphorus concentrations in isolated and developed lakes were more similar in the spring and summer months. In spring, isolated lakes had an average TP of 39.35µg/L while developed lakes had an average of 45.38 µg/L ($W = 805.5$, $p = 0.07674$). In summer, the average TP in isolated lakes was 44.45 µg/L and 53.73 µg/L in developed lakes ($W = 594$, $p = 0.55$).

In the last few decades, many oligotrophic Canadian Shield lakes in south-central Ontario have experienced declines in total phosphorus levels (Eimers *et al.,* 2009). Despite the region's increased human activity and development, some of these lakes have experienced up to 30%

reductions in TP concentration (Eimers *et al.,* 2009). These decreases in TP concentration have been seen both in lakes with shoreline development and those without, which suggests that the factors influencing TP concentration in the region operate on a regional scale rather than being lake-specific (Eimers *et al.,* 2009). Despite this trend of decreasing TP concentration in south-central Ontario, annual average TP was found to vary almost fivefold across eleven study catchments sampled by Eimers *et al* (2009), all of which were in close proximity to one another. Dissolved organic carbon (DOC), land-use, atmospheric deposition, Fe and Ca concentrations were all found to not have an effect on TP concentrations in the area and did not differ between the lakes with differences in TP (Eimers *et al.,* 2009). Therefore, the reasons for this observed regional variation in TP concentration of freshwater lakes in south-central Ontario remain unclear and are the focal point of ongoing research in the area (Eimers *et al.,* 2009). In contrast to the decreasing TP concentrations in south-central Ontario lakes, we observed relatively high TP concentrations in the lakes sampled for our study. Comparing our findings with previous reports on our area, it seems that TP concentration in the region have changed significantly. However, TP concentrations in the south-central Canadian Shield lakes are highly variable, even for lakes located in the same watershed or local region (Eimers *et al.,* 2009). Therefore, it is possible that the region we sampled in the present study has a naturally high concentrations compared to the Dorset lakes, that were the focus of previous studies in the region (Eimers *et al.,* 2008; Lento *et al.,* 2008; Palmer *et al.,* 2011). It may simply be that TP values are highly variable, or other, unidentified factors in the local area could be influencing high TP concentrations; however, those factors are, clearly, not well understood (Eimers *et al.,* 2009). In terms of seasonal trends in TP concentrations in this region, typically researchers have reported declines in TP concentrations in the spring (Eimers *et al.,* 2009; Palmer *et al.,* 2011). This is attributed to snowmelt, which is a dominant hydrologic event in this region and accounts for at least 50% of annual runoff (Eimers *et al.,* 2009). These runoff events decrease TP concentrations due to the water body having a higher influx of water and other materials, coupled with the fact that phosphorus is less mobile in soil than other nutrients/compounds, like nitrogen for example (Eimers *et al.,* 2009). This trend is observed in our isolated lakes, which had their lowest seasonal average TP concentration in the spring (39.35 µg/L). This decrease was not observed in the developed lakes, which had a higher TP concentration in the spring than they did in fall. It is possible that external factors imposed by human development are causing these trends in developed lakes, like inputs from septic systems, which may affect long-term changes in lake concentrations (Eimers *et al.,* 2009). Cottage activity is of course most prevalent during the summer months while cabin properties in the area are usually associated with hunting activity in the fall and early winter. During the summer we would expect the most activity in the area due to the high number of cottages. Cottages are more likely to have septic beds, while cabin properties are more likely to use an outhouse or 'composting toilet' (Roy *et al.,* 2017). The septic tanks associated with cottages are unlikely to release nutrients or contaminants during use in the summer or during freeze thaw events in the spring. Septic beds more common with cabin properties do not provide the same level of protection to the local environment. The material deposited at these areas will likely release into the environment through soil leaching or during spring thawing events (Hendry and Leggatt, 1982). It is important to note that due to the location and proximity to rural areas of most of the cottages in the area, septic tanks mat be built out of code or not inspected as frequently as local laws and regulations would require (Roy *et al.,* 2017). This could result in faulty septic tanks which could result in unexpected contaminants entering the local water systems (Hendry and Leggatt, 1982). However, this contradicts earlier studies which concluded that these factors have less of an impact than previously thought in the region (Eimers *et al.,* 2009; Palmer *et al.,* 2011). Regarding seasonal trends, typically TP concentrations are at their highest in summer, when runoff is at its lowest (Eimers *et al.,* 2009). The same trend was seen in the present study, with summer having the highest TP concentrations and TP concentrations increasing from spring to summer in isolated lakes.

The average available nitrogen over the course of the entire study was found to be 37.22 μg/L in isolated lakes and 44.12 μg/L in developed lakes. This difference was statistically significant at $p<0.05$ with a Wilcoxon rank sum test ($W = 4900.5$, $p = 0.04254$). On a season-by-season basis, the two lake types were significantly different in all three seasons. In fall, isolated lakes had an average available nitrogen concentration of 25.26 μg/L while developed lakes had an average of 43.08 μg/L ($W = 333$, $p <0.001$). In spring, isolated lakes averaged 37.76 μg/L of available nitrogen while developed lakes averaged 63.13 ($W = 360$, $p <0.001$). In summer, isolated lakes had average available nitrogen of 48.64 μg/L compared to 26.14 μg/L in developed lakes, $p<0.01$ ($W = 900$, $p = 0.004$). While these concentrations match with those in earlier reports (Eimers *et al.,* 2009; Palmer *et al.,* 2011), the seasonal trends and differences between isolated and developed lakes are interesting and merit closer examination (Casey, 2011; Palmer *et al.,* 2011). Overall, isolated lakes had significantly lower concentrations of available nitrogen in fall and spring. Interestingly, this trend reversed in the summer, when developed lakes showed lower concentrations (see Figure14). This may be because, in summer, runoff is at its lowest in the year (Eimers *et al.,* 2009). Therefore, possible sources of nitrogen for developed lakes such as runoff from agricultural sources and malfunctioning septic systems will be contributing very little at that time despite the fact that they are important sources of available nitrogen (Eimers *et al.,* 2009). The isolated lakes maintain relatively constant concentrations of available nitrogen in the following seasons (fall and spring) because these systems rely on natural inputs and do not experience the same seasonal influx from anthropogenic sources.

In the isolated lakes sampled, available nitrogen steadily increased over the course of the study from fall to the following summer, while developed lakes increased from fall to spring, but then decreased to their lowest average in the summer. As with TP concentrations, we expected available nitrogen to peak in the summer months as well, but they did not, mostly attributed to low outflow during that time (Eimers *et al.,* 2009). As mentioned in the previous chapter, a possible explanation for elevated nutrient concentrations in spring, seen in both lake types, is that the lake overturn caused nutrient-rich water from the bottom of the lake to appear at the surface (Canadian Council of Ministers of the Environment, 1999). This coupled with snow melt runoff and the "defrosting" of septic beds containing waste from the previous summer may have caused elevated nutrient concentrations at the surface waters during sampling (Palmer *et al.,* 2011).

4.4.2 The Impact of Human Development on the Benthic Communities of central Ontario

One of the five variables related to benthic communities in central Ontario was found to be significantly different between isolated and developed lakes in central Ontario. The average EOT was found to be significantly different between isolated and developed lakes for the whole study as well as for each season individually. Overall, isolated lakes had an average EOT percentage of 37.22, while developed lakes averaged 44.12, $p<0.05$ ($W = 4900.5$, $p = 0.043$). This was a very interesting finding as we had expected isolated lakes to have a higher EOT percentage, as the taxa involved are viewed as being relatively sensitive to organic pollutants (i.e. pesticides, hydrocarbons, waste). This pattern was also evident when data were assessed seasonally in fall and spring. In fall, isolated lakes had an average EOT percentage of 25.26 while developed lakes had an average percent of 43.08 ($W = 333$, $p <0.001$).

Looking at the data more closely, we see that developed lakes had a high number of members of the Odonata family, so this important contributor to the EOT calculation is highly abundant in the area. Generally, members of the order Odonata are relatively tolerant of high water conductivities and can generally tolerate pH environments as low as 4 (Thorp and Rogers, 2015; Morse *et al.,* 2020). Since isolated and developed lakes differ in terms of conductivity and pH, and Odonata are relatively tolerant of changes in these variables, the EOT index did not indicate that developed lakes were more contaminated than isolated lakes; on the contrary, the EOT score was consistently higher for developed lakes. A possible explanation for the increased presence of Odonata specimen in developed lakes is an increased light availability in the littoral zone. As we know, littoral zone clearing is a common practice for these developed lakes and therefore these littoral regions may have an increase in light availability when compared to isolated lakes in the area. Increased light availability in the littoral zone has been shown to promote early-stage amphibian presence, which are a typical prey of many Odonate larvae (Eakin, 2019). The EOT values found in both lake types suggest that there is no evidence to suggest that there are concerning levels of synthetic organic compounds (organic pollutants) (Mandaville, 2002; Jones et al., 2017). We can assume this given that EOT taxa are useful at identifying these compounds due to their vulnerability and sensitivities (Mandaville, 2002; Jones et al., 2017).

The CIGH biotic index was used to assess the percent of sampled communities that are composed of taxa that are known to be pollution-tolerant (OBBN, 2011). Overall, the average CIGH percentage for benthic communities in isolated lakes was found to be 10.90 and 9.30 in developed lakes, these values do not differ significantly ($W = 6416$, $p = 0.1853$). Looking at individual seasons, there were no significant differences in the fall (isolated lakes had a CIGH percentage of 9.57 and 11.24 in developed lakes, $W = 276$, $p = 0.7196$). In spring, isolated lakes had a CIGH percentage of 6.52 while developed lakes had an average of 2.36; this was the only season in which there was a significant difference between lake types ($W = 253$, $p = 0.01545$). In summer samples, the average CIGH percentage in both types of lakes increased, with an average of 16.61 in isolated, and 14.30 in developed lakes, but this difference was not statistically significant ($W = 317$, $p = 0.5201$).

Looking at the average CIGH in developed and isolated lakes, we can assume that organic pollutants are at a low enough level in both lake types such that they do not impact local benthic communities. While we did not sample for these pollutants, nevertheless this biotic index can be used as a possible indicator, for individual lakes, of poor water quality or high levels of organic pollutants. Since there are no significant differences in CIGH percentage between the isolated and developed lakes in this study, it is reasonable to conclude that the developed lakes do not contain detrimental contaminants that would harm macroinvertebrate life. There is a risk that this may change if human development activities in the region continue, however. It is interesting that isolated lakes exhibited a higher CIGH percentage in spring compared to the developed lakes at that time. This was likely due to a higher number of Isopoda and Gastropoda sampled in some isolated lakes. While these macroinvertebrates are used as indicators because they are considered to be tolerant of pollutants, they are also commonly found in healthy systems (Thorp and Rogers, 2015). The lowest CIGH percentages were seen in spring for both lake types; this is likely due to low numbers of Asellidae, the family of Isopods sampled, and Gastropoda being sampled in the spring. This is likely due to the simple fact that both orders are less abundant in the spring, as most of the population at that time consists of small juveniles, which we did not capture in our sampling procedure (Thorp and Rogers, 2015).

When we compare the EOT and CIGH index values with each other, it is clear that for both isolated and developed lakes, the average EOT percentage of benthic communities is larger than CIGH percentage. This tells us that the benthic communities in the area of study, regardless of development level, have more taxa which are pollution-sensitive than that are pollution-tolerant, suggesting these systems are supporting healthy benthic macroinvertebrate communities.

Average taxa richness over the course of the study did not differ significantly, with an average of 2.97 in isolated lakes and 3.00 in developed lakes ($W = 5475.5$, $p = 0.44$). When data were divided seasonally, the finding of no significant difference between the two lake types continued. In fall, isolated lakes had an average taxa richness of 2.92 and developed lakes had an average of 2.89. In the spring, taxa richness slightly increased in both lake types, with isolated lakes having an average of 3.12 and developed having an average of 3.15. Lastly in the summer, taxa richness decreased again to near fall levels, with isolated lakes averaging 2.86 and developed averaging 2.97. Average taxa richness values were relatively close between isolated and developed lakes over the course of the study period, with few changes observed from season to season. This, coupled with the repeatable nature of our sampling methods, indicate benthic communities in isolated and developed lakes have a similar number of families present in a given sample location. Taxa richness is relatively low, compared to some published reports on the area, since taxa richness is scaled with sample size. For this calculation, the smallest sample size collected during the study period was used, which in the present study was only 4, found in Sand Lake. Therefore, while it is useful to examine taxa richness across the present study, it would not be valid to compare taxa richness values in this study with previous published reports (Eimers *et al., 2009;* Palmer *et al.,* 2011).

The Shannon diversity index is a more complex measure of the diversity of taxa present in a community, as it considers both taxa richness and the number and distribution of those taxa found (Oksanen et al., 2020). Therefore, the Shannon Index is more useful for explaining the typical spread and diversity of taxa found in different sites (Oksanen et al., 2020). Overall, isolated lakes had an average diversity of 1.63 and developed lakes had an average diversity of 1.61. These values did not differ significantly with respect to lake type ($W = 5802$, $p = 0.95$). This pattern was also evident on a season-by-season basis. In fall, the average diversity of isolated lakes (1.65) and developed lakes (1.59) did not differ significantly ($W = 353$, $p = 0.76$). In spring, isolated lakes had an average diversity of 1.83 while developed lakes had an average of 1.73, values that do not differ significantly ($W = 410$, $p = 0.2325$). Finally, in summer, isolated lakes had an average diversity of 1.40 while developed lakes had an average of 1.51, again a difference that was not statistically significant ($W = 243$, $p = 0.2416$).

Diversity differed very little between lake types both for the whole study, and on a season-by-season basis. Generally, diversity trended in the same directions in both lake types, with the highest diversity being recorded in spring and the lowest seen in summer. These results suggest that benthic communities in the region, whatever the development level of the surrounding shoreline, experience similar seasonal shifts in terms of both taxa sampled, and in terms of the number of specimens sampled. This reinforces the finding that benthic communities are relatively similar in composition and abundance in the two different lake types.

The Hilsenhoff Biotic Index (HBI) was relatively consistent from season to season within lake types as well as between isolated and developed lakes, both over the course of the study as well as when data were examined on a season-by-season basis. Overall, isolated lakes had an average HBI value of 5.48 while developed lakes had an average of 5.41, a difference that was not statistically significant (W = 5475.5, $p = 0.44$) (see Figure 24,25). When these HBI values are compared with Hilsenhoff's standardized family biotic index values (see Tables 1 and 2 in Appendix I), both lake types would be considered to have 'good' water quality with 'some organic pollution likely' (Hilsenhoff, 1988). This classification was consistent between both lake types in the fall and spring, but in summer the index increased above the 5.51 boundary, in both lake types, indicating water quality was merely "fair" at that time. Based on the fact that the Hilsenhoff biotic index takes into account family level tolerance levels and their relative abundance, this index is our best approximation on general community tolerance in lakes we have assessed so far. Due to this, we can say that isolated and developed lakes have relatively similar benthic community composition in terms of pollution tolerance, which would also suggest there are similar levels of organic pollutants present in both lake types (Hilsenhoff, 1988). Given that, we can assume that the cottage development activities occurring in the region in and around Kearney, Ontario are not increasing the levels of organic pollutants to levels that influence benthic community composition. However, as mentioned earlier, using Hilsenhoff's family biotic values (see Tables 1 and 2 in Appendix I), the two lake types would both fall into the category of being merely "good", with "some organic pollution likely" (Hilsenhoff, 1988). This is a surprising finding, as that would suggest that, regardless of development activities experienced by a given lake, there is a source of organic pollution in the whole region that is having some tangible effect on benthic macroinvertebrate communities, whatever the developmental level of the surrounding lake shoreline. Possible sources of these organic pollutants include the nickel and copper ore processing occurring in Sudbury, Ontario, approximately 165 km from the study site. There is evidence that suggests that these contaminants have reached waterbodies in the Muskoka region, which is just slightly south of our location (Scheider *et al.,* 1980). Over the last 40 years, these activities and the measured concentrations of these organic contaminants have decreased, the possibility of these remaining in the systems or surrounding landscape is possible (Scheider *et al*., 1980). For the purpose of this study, we were unable to measure the concentrations of organic contaminants in our study lakes. Due to this, we are unable to conclude on whether organic pollutants are having an effect on benthic macroinvertebrate communities in the area. A second possible explanation of our observed HBI values are that the combination of relatively insoluble substrates in the region, low aquatic nutrient levels and short growing seasons has resulted in relatively little primary production (Verberk *et al.,* 2008; Eimers *et al.,* 2009; Palmer *et al.,* 2011). Wisconsin, where the HBI index was developed, is quite similar in terms of climate zones to Southern Ontario so regional climatic factors are not likely to be explanatory. The HBI was intitially applied to lotic systems, and here we apply the HBI to lentic systems; but again, many studies have done this in the past (Eimers *et al.,* 2009; Lento *et al.,* 2011)

While this index is extremely useful as a measure of the tolerance of a sampled benthic communities of organic pollutants, the HBI does not take into account family tolerances to other water chemistry variables such as pH, dissolved oxygen concentration, conductivity, etc. (Hilsenhoff, 1988). Therefore, even if the results of the HBI showed differences in benthic community composition between isolated and developed lakes, any such differences could be due to a variety of water chemistry or habitat quality differences, and not necessarily attributable to differing levels of organic pollutions (Hilsenhoff, 1988).

4.4.3 RDA Analyses of Water and Environmental Variables

In the overall RDA analysis, the 16 environmental variables used in the model were capable of explaining 20.53% of variance in benthic community composition. Seasonal subset

RDA analyses were also performed for Fall 2020, Spring 2021, and Summer 2021, which, respectively, explained 36.84%, 24.59% and 27.78% of the variance in benthic community composition. The ordination diagram identifies some variables that appear to be closely associated with each other, and others that orient in opposite directions.

Clearly, wood debris and detritus are closely associated with each other; this follows from the observation that both suggest a littoral zone that had experienced some level of disturbance, as it would tend to impact both variables in a similar manner. Interestingly, lake development type appears to be negatively associated with those variables. This indicates that developed lakes have a negative association with increasing wood debris and detritus. This supports the assumption that lake development is the driving force behind decreases in these important littoral zone characteristics. The negative relationship between lake development and wood debris/detritus appears to persist through each of the seasons, a finding that supports the view that these environmental variables will tend to remain relatively consistent and unchanged over time, unless disturbed by an outside factor (Marburg *et al.,* 2006; Sass *et al.,* 2006). This is especially true for wood debris, which results from growing trees along the shoreline falling into the littoral zone, so its input takes long periods of time (Marburg *et al.,* 2006; Sass *et al.,* 2006). A decrease of wood debris and detritus in the littoral zone is likely to cause subsequent change in the benthic macroinvertebrate community of the lake. Wood debris and detritus are key habitat characteristics of a healthy lentic littoral zone, and provide refuge, egg deposition and hatching habitats and food resources for both macroinvertebrates and higher trophic predators (Marburg *et al.,* 2006; Sass *et al.,* 2006). Interestingly, wood debris and detritus presence appear to be closely correlated with an increase in Chironomidae individuals (see Figure 27). This trend is observed both in the whole study RDA analysis as well as seasonally. This could be a possible explanation behind CIGH percentages not differing significantly between isolated and developed lakes. Chironomidae individuals appear to favor the littoral coverage supplied by wood debris and detritus present in isolated systems. With water chemistry not reaching biologically detrimental levels in developed or isolated lakes, differences in littoral conditions appear to be enough to drive Chironomidae numbers in isolated systems.

Wood debris had a significant effect on benthic community composition in the overall RDA analysis, accounting for 0.95% of the explained variance. The same was true of the spring season (3.92% explained variance, p-value < 0.001). In both cases wood debris was selected in their subsequent forward selection. Detritus also had a significant effect on community composition during the study as a whole, $p< 0.05$ (0.95% of explained variance and $p = 0.011$), as well as in the fall season (4.69% of explained variance) and summer (2.71% of explained variance, $p = 0.007$). Detritus was therefore selected by the subsequent forward selection in the whole study RDA analysis, as well as in the fall season, specifically. It appears that development is indeed having an effect on wood debris and detritus levels in the region of study, with these materials being removed from developed lakeshores; however, the impact this has on local benthic community composition can be argued. While both variables were found to have a significant effect on benthic community composition during the course of the whole study, it appears that its effect is not consistent from season to season. This could be due to varying water levels during the different seasons of sampling, which, in any lake, could shift the littoral zone to regions with more or less wood debris and detritus. Detritus is also most abundant in the late fall/spring, so this could also influence the amount of detritus, as well as it effect.

Developed lake type appears to be negatively associated with most of the variables based on macrophytes and algae. This is to be expected, based on the known impact lake development has on littoral zone plant life (Hicks and Frost, 2011). Interestingly, free floating macrophytes appears to be closely associated with developed lakes (see Figure 22). Free floating macrophytes were generally defined as macrophytes growing offshore that were not attached to the substrate and therefore could be less susceptible to shoreline modifications from property owners. Macrophytes that are more associated with the littoral zone and its substrate tend to decline with increasing shoreline development (Hicks and Frost, 2011). Similar to the effect of wood debris and detritus, littoral zone macrophytes, especially those that are rooted to the substrate, are critical food and habitat resources for benthic macroinvertebrate communities (Hicks and Frost, 2011). Four different macrophyte types were assessed in the RDA analysis (emergent, rooted floating, submergent and free-floating) while three types of algae were assessed (floating, filamentous and attached). Emergent, submergent and free floating macrophytes had a significant effect on benthic community composition in the whole study RDA analysis. Free floating macrophytes were the most significant of these macrophyte types, explaining 1.23% of variance in community composition with a p-value < 0.001, so this variable was also selected by the subsequent forward selection. On a season-by-season basis, submergent macrophytes were also found to be statistically significant in fall (where they were also selected by the fall RDA forward selection). When assessing seasonal samples individually, it appears that emergent macrophytes have the most consistent effect on benthic macroinvertebrate community composition, with a statistically significant effect in each individual season as well as during the study overall. This is to be expected as these macrophytes are attached to the substrate of the littoral zone and extend through the water column to the surface. This allows these macrophytes to provide numerous ecosystem services to macroinvertebrates as well as other species in the ecosystem, allowing the region to flourish (Hicks and Frost, 2011). Algae were less abundant at sampling locations in the present study, compared to the different macrophyte types. For this reason, not all seasonal subsets had algae present and therefore the variable could not be included in the RDA analysis. No algae were present at the time of the spring surveys. Of all the algal types, attached algae, commonly found on local rocks or plant life, were the most common in the study area. The fall season also appears to be the time when these algae were most common in the area. Since some seasons did not have particular types of algae present, it is possible that the relatively small sample size was responsible for skewing their observed importance in the overall RDA analysis.

Conductivity appears to be positively correlated with lake development type, as the two variables appear to be closely related in the overall RDA analysis (see Figure 22), as well as, separately in the spring and summer RDA analyses (see Figures 25 & 26). This is an expected relationship as increasing development around a lake is expected to increase overland runoff of substances like road salts. This, coupled with more activity associated with development results in these roads being maintained more in the winter months which in turn results in the unimpeded runoff of these substances into local water systems (Palmer *et al.,* 2011). Increased conductivity can influence benthic macroinvertebrate communities by increasing dissolved solid concentrations, forcing organisms to burn more energy to constantly regulate their internal concentrations through osmoregulation (Armstead et al., 2016). Increased conductivity in the region of study is cause for concern due to the low buffering capacity of the underlying bedrock of these lakes (Neff and Jackson, 2013).

Although the measured conductivity levels were lower than levels known to be harmful (biologically determinant levels, see Chapter 3), we do see that conductivity has a significant effect on benthic community composition over the study overall (4.06% explained variance, p-value < 0.001), as well as during each individual season (fall, 4.48% of explained variance, p-value < 0.001; spring, 4.20% of explained variance, p-value < 0.001, and summer, 4.16% of explained variance, p-value < 0.001). Explained variance remained relatively consistent in the whole study analysis as well as individually in each season. Conductivity was also selected by the RDA forward selection both during the whole study and in each individual season. These results suggest that conductivity had a significant and persistent impact on benthic community composition, regardless of season and the developmental stage of the macroinvertebrates.

Dissolved oxygen concentration appears to be negatively associated with increasing water temperature, which is an expected relationship based on the relationship between water temperature and the ability of that water to hold oxygen in solution (Palmer *et al.,* 2011). Dissolved oxygen had a significant ($p<0.01$) effect on benthic community composition during the course of the study overall (0.90%, p-value = 0.005**) but this parameter was not forward selected for the forward selection process due to other variables having higher explanatory capabilities. In the individual seasons, values in fall (2.59%, p-value < 0.001) and summer (4.78%) experienced a significant effect of dissolved oxygen concentration. For each of these two seasons, dissolved oxygen was one of the variables selected from the forward selection process. Dissolved oxygen appears to impact community variance most in summer, when it explained 4.78% of variance (p-value < 0.001). When water temperatures are at their highest, littoral conditions like macrophyte abundance, canopy coverage and lake inflows can modify summer lake temperatures. Interestingly, in the summer RDA ordination diagram, it appears developed lakes are associated with higher dissolved oxygen concentrations; this may be because developed lakes tend to have lower water temperatures, a point that is supported in the summer season ordination diagram (Figure 26).

In both the overall study analysis and season-by-season data, it appears pH tends to increase with lake development. This is an interesting finding, that isolated lakes have a lower pH and are more acidic than developed lakes. This finding supports the earlier observation, above, where the average pH of the two lake types was compared. Although this trend was observed in the RDA ordination diagram, differences in pH between isolated and developed lakes over the study overall was a mere 0.191 pH units. While this difference between lake types was previously determined to be statistically significant, this was largely attributable to differences in the fall samples (see Chapter 3).

The additional ordination diagram generated from the whole study RDA analysis was used to better visualize similarities and differences between the isolated and developed lake types (see Figure 23). In this figure, we see that isolated and developed lakes have quite similar characteristics and relationships to the environmental variables tested in the RDA analysis. We can see that increasing detritus and wood debris seems to cluster closer to isolated lake types, while increasing conductivity is more closely associated with developed lakes. Dissolved oxygen appears to divide the two lake development types in half, suggesting that dissolved oxygen concentration varied in a similar way in both groups of lakes. This figure offers a useful visual representation of the data and helps confirm previously stated findings based on statistical comparisons.

In general, the RDA analyses suggests that conductivity, pH and water temperature are the water variables having the greatest impact on variance in benthic community composition. Lake development type also appears to have a strong and consistent impact on variance in the benthic macroinvertebrate community in the study overall. Of the environmental variables assessed, free-floating macrophytes explained the most variance in the benthic macroinvertebrate community in the overall RDA analysis. However, when season-by-season analyses are made, it appears emergent macrophytes have the most consistent impact on benthic macroinvertebrate community composition compared to the other macrophyte and algal types assessed.

4.5 Conclusions Based on Chapter 4

The purpose of this chapter was to compare isolated lakes with developed lakes to see if human development in the region is influencing lake water chemistry, and/or the benthic communities of these systems.

Of the five water chemistry variables assessed, three were found to differ significantly between isolated and developed lakes. Conductivity was the parameter that differed most between lake types, both in the study overall, and in each individual season (fall, spring and summer). Isolated lakes had an average conductivity of 16.35 μS/cm, while developed lakes had an average of 29.21 μS/cm. From season to season, average conductivity in isolated lakes only varied by ~1μS/cm (15.615 - 16.69). In contrast, developed lakes varied in average conductivity by ~5μS/cm with averages ranging from 26.68 to 31.64 μS/cm. Conductivity was also seen to be associated with developed lakes in our whole study RDA analysis as well as season-by-season (see Figure 22). Conductivity explained a consistent amount of variance in benthic community composition both season-by-season and overall, as it was one of the most significant water chemistry variables assessed. We interpret these results as follows: human development activities cause developed lakes to have more roads and impermeable surfaces, and they tend to be closer to the lakeshore. These roads require winter maintenance in the form of road salt application, which in spring, is transported as water in snowbanks melts, and runs off into the nearby lake systems. This increase in impermeable surfaces coupled with the low buffering capacity of Canadian Shield Lakes, means that developed lakes experience increased base cation loads which in turn generate greater water conductivity.

pH was also found to be significantly different between isolated and developed lakes. This significant difference was noted over the course of the whole study, but, when assessed seasonally, only the fall samples showed a significant difference in pH between isolated and developed lakes. RDA analyses suggest that pH is having a significant and consistent effect on benthic community composition in the region across all seasons studied. pH was also consistently selected as an important explanatory variable by the forward selection process. While its effect is significant, differences in pH between lake types is less clear. When individual lakes are compared, it appears that two of the isolated lakes, in fall, have an average pH <6, and they were the major drivers of the observed significant difference. The other 10 lakes had similar average pH levels and all lakes had similar averages for the remainder of the study. The factors causing Buck Lake and Charlie Mann's Lake to have such a low pH in fall are unclear, as it appears these lakes are not uniquely susceptible to factors not found near other lakes studied. However, Buck Lake does have a small hunting cabin located on it southern shore, while Charlie Mann's Lake is in close proximity to a frequented gravel logging road. It is possible that these characteristics are having an unknown effect on these two water bodies, altering lake pH.

Available nitrogen concentration was the other water chemistry variable that differed significantly between isolated and developed lakes. Isolated lakes had lower nitrogen concentrations in the fall and spring compared to developed lakes but had a higher concentration in the summer than developed lakes. We had expected isolated lakes to have lower available nitrogen concentrations than developed lakes since they were less likely to experience anthropogenic inputs of nitrogen, like fertilizer runoff , human waste from improper septic systems, and wastewater entering the lake by overland runoff. While isolated lakes had an observed spike in available nitrogen in the summer, developed lakes had a clear drop in available nitrogen during that time. This could be because precipitation and runoff are lowest in the summer, so anthropogenic sources would not be contributing to the system at that time. In contrast isolated systems peaked at this time due to typical seasonal patterns in nutrient concentration, likely caused by internal loading of nutrients from materials decomposing in the littoral zone and sediments. The other water chemistry variables assessed, namely dissolved oxygen, and total phosphorus, were not found to differ between lake types.

We compared various aspects of benthic macroinvertebrate communities, in the form of biotic indices, between the two lake types. The biotic index, EOT, was the only variable to differ significantly between isolated and developed lakes. Interestingly, EOT values were, on average, higher in developed lakes than in isolated lakes, which was not expected given the premise that EOT taxa are sensitive to pollution. On closer examination, we found that the order Odonata was abundant in the area in general, particularly in developed lakes. While members of the order Odonata are regarded as pollution-sensitive, they are relatively tolerant of the pH and conductivity ranges recorded in developed lakes in the region. Therefore, we can conclude that developed lakes in the study area are not limited by any organic pollutants, and water chemistry remains in an acceptable range for most benthic macroinvertebrates found in the area.

The other biotic indices used, CIGH, taxa richness, diversity and HBI, were all found not to differ significantly between lake types.

Despite the fact that these indices did not differ significantly between lake types, the calculated HBI values for isolated and developed lakes offered some insight into the inferred degree of organic pollution present in the water bodies. According to this index, both isolated and developed lakes can be considered to likely have “some organic pollution” overall, and a “fairly substantial amount of pollution present” in one season, namely summer (Hilsenhoff, 1988), with the caution that these lakes are located in a geological area that offers little in the way of buffering capacity should there be aerial fallout of contaminants, e.g. from active ore smelting in the Sudbury area (Scheider *et al.,*1981).

The characteristics and abundance of various macrophytes and algae were assessed in terms of their effect on benthic community composition. Over the whole study, the presence of free-floating macrophytes had the largest impact on variance in benthic community composition, with emergent macrophytes and submergent macrophytes also having significant effects. Looking at seasonal RDA analyses, it appears that emergent macrophytes are the most consistent in terms of their effect on community composition from season to season. While all algae types had a significant effect on benthic community composition, these organisms were not present in every season. Therefore, any algae present were represented by smaller samples, a feature that can skew their observed importance in the overall RDA analysis.

Overall, we can conclude that the major difference observed between isolated and developed lakes in the region of study is lake conductivity. However, conductivity levels, as well as pH and other chemistry variables do not appear to have reached a biologically detrimental levels where it would impact the benthic macroinvertebrate communities of the region. We can confidently say, however, that human development does indeed have an effect on the local environment of the region. If such development continues or becomes more intense, these water chemistry and environmental variables will continue to diverge between isolated and developed lakes and may reach biologically detrimental levels. If that occurs, the difference in benthic macroinvertebrate communities will become evident, with major implications for local biodiversity and ecosystem services as well as recreational fisheries.

References

American Public Health Association (APHA). 1995. Standard methods for the examination of water and wastewater. *American Public Health Association, American Water Works Association, and Water Pollution Control Federation*. 19th edition, Washington, D.C.

Armstead, M.Y., Bitzer-Creathers, L. and Wilson, M. 2016. The effects of elevated specific conductivity on the chronic toxicity of mining influenced streams using Ceriodahina dubia. *PLoS One*, 11(11), 1-16. doi: 10.1371/journal.pone.0165683

Barber-James, H.B., Gattolliat, J.L., Sartori, M. and Hubbard, M.D. 2008. Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. *Hydrobiologia*; 595: 339-350. DOI 10.1007/s10750-007-9028-y

Berrill, M., Hollett, L.J., Margosian, A. and Hudson, J. 1985. Variation in tolerance to low environmental pH by the crayfish Orconectes rusticus, O. propinquus and Cambarus robustus. *Canadian Journal of Zoology*. 62: 2360-2363.

Birmingham, M., Heimdal, D., Hubbard, T., Krier, K., Leopold, R., Luzier, J., Neely, J., Soenen, B. and Wilton, T. 2005. Benthic macroinvertebrate key. *IOWATER volunteer water quality monitoring*.

Blanchet, F.G., Legendre, P. and Borcard, D. 2008. Forward selection of explanatory variables. *Ecology*; 89(9): 2623-2632.

Caltopo. 2021. CAL TOPO Online Mapping. https://caltopo.com

Canadian Benthos Biomonitoring Network (CABIN). 2011. Laboratory Methods. *Environment Canada*.

Canadian Council of Ministers of the Environment. 1999. Canadian water quality guidelines for the protection of aquatic life: Dissolved oxygen (freshwater). In: Canadian environmental quality guidelines, 1999, *Canadian Council of Ministers of the Environment, Winnipeg*.

Cannings, R.A. 2019. Odonata of Canada. *Zookeys*. 819. 227-241. doi: 10.3897/zookeys.819.25780

Casey, R. 2011. Water quality conditions and long-term trends in Alberta lakes. *Alberta Environment and Water, Edmonton*.

Chao, A. and Shen, T.J. 2003. Nonparametric estimation of Shannon's index of diversity when there are unseen species in sample. *Environmental and Ecological Statistics*: 10, 429-443. https://doi.org/10.1023/A:1026096204727

Chhor, A.D., Glassman, D.M., Smol, J.P., Vermaire, J.C. and Cooke, S.J. 2019. Ecological consequences of shoreline armoring on littoral fish and benthic macroinvertebrate communities in an Eastern Ontario Lake. *Aquatic Sciences.* 82:73. https://doi.org/10.1007/s00027-020-00740-0

Clapper, J. and Caudill, S.B. 2014. Water quality and cottage prices in Ontario. *Applied Economics*. 46(10): 1122-1126. http://dx.doi.org/10.1080/00036846.2013.851778

Dinno, A. 2015. Nonparametric pairwise multiple comparisons in independent groups using Dunn's test. *The Stata Journal*. 15: 292-300.

District Municipality of Muskoka. 2005. Muskoka economic profile. *Planning and Economic Development Department, Bracebridge, Ont*.

District Municipality of Muskoka and FedNor. 2004. *Muskoka water web*. [online]. Available at muskokawaterweb.ca.

Eimers, M.C., Watmough, S.A., Paterson, A.M., Dillon, P.J. and Yao, H. 2009. Long-term declines in phosphorus export from forested catchments in south-central Ontario. *Canadian Journal of Fisheries and Aquatic Science*. 66: 1682-1692. doi: 10.1139/F09-101

Environment Canada. (2007). *Environment Canada water* [online]. Available from ec.gc.ca/eau-water/.

Freda, J. 1986. The influence of acidic pond water on amphibians: a review. *Water Air and Soil Pollution*. 30: 439-450. https://doi.org/10.1007/BF00305213

Fujibayashi, M., Takakai, F., Masuda, S., Okano, K. and Miyata, N. 2020. Restoration of emergent macrophytes on the benthic environment of the littoral zone of a eutrophic lake. Ecological Engineering. 155. https://doi.org/10.1016/j.ecoleng.2020.105960

Gal, B., Szivak, I., Heino, J. and Schmera, D. 2019. The effect of urbanization on freshwater macroinvertebrates – knowledge gaps and future research directions. *Ecological Indicators*. 104: 357-364. https://doi.org/10.1016/j.ecolind.2019.05.012

Gerner, N.V., Cailleaud, K., Basseres, A., Liess, M. and Beketov, M.A. 2017. Sensitivity ranking for freshwater invertebrates towards hydrocarbon contaminants. *Ecotoxicology.* 26:1216-1226. doi: 10.1007/s10646-017-1847-7

Grant, C.A., Flaten, D.N., Tomasiewicz, D.J. and Sheppard, S.C. 1999. The importance of early season phosphorus nutrition. *Canadian Journal of Plant Science*. 81: 211-224. https://doi.org/10.4141/P00-093

Griffith, B. 2011. Efficient fertilizer use manual. http://www.back-to-basics.net/efu/pdfs/Phosphorus.pdf.

Hadley, K.R., Paterson, A.M., Hall, R.I. and Smol, J.P. (2013). Effects of multiple stressors on lakes in south-central Ontario: 15 years of change in lake water chemistry and sedimentary diatom assemblages. *Aquatic Science*. 75:349-360. doi:10.1007/s00027-012-0280-5

Hendry, G.S. and Leggatt, E.A. 1982. Some effects of shoreline cottage development on lake bacteriological water quality. *Water Research*: 16(7); 1217-1222. https://doi.org/10.1016/0043-1354(82)90140-3

Hicks, A.L. & Frost, P.C. (2011). Shifts in aquatic macrophyte abundance and community composition in cottage developed lakes of the Canadian Shield. *Aquatic Botany*: 94; 9-16. doi:10.1016/j.aquabot.2010.09.005

Hilsenhoff, W.L. (1988). Rapid field assessment of organic pollution with a family-level biotic index. J.N. Am. Benthol. Soc. 7(1):65-68.

Holomuzki, J.R., Feminella, J.W. and Power, M.E. 2010. Biotic interactions in freshwater benthic habitats. *Society for Freshwater Science*. 29(1): 220-244. https://doi.org/10.1899/08-044.1

Idigoras Chaumel, A.L., Armanini, D.G., Schwindt, J.A. and Yates, A.G. 2019. Interannual variation of benthic macroinvertebrate communities at long-term monitoring sites impacted by human activities: implications for bioassessment. *Diversity*. 11 (167). doi: 10.3390/d11090167

Ito, Y. and Momii, K. 2014. Impacts of regional warming on long-term hypolimnetic anoxia and dissolved oxygen concentration in a deep lake. *Hydrological Processes*. 29(9): 2232-2242. https://doi.org/10.1002/hyp.10362

Jeffries, D.S., Cox, C.M. and P.J. Dillon. 1978. Depression of pH in lakes and streams in central Ontario during snowmelt. J. Fish. Res. Board Can. 36: 640-646.

Jessup, B.K., Markowitz, A. and Stribling, J.B. (2002). Family-level key to the stream invertebrates of Maryland and surrounding areas. *Tetra Tech Inc*, Owings Mills, Maryland.

Jones, C., Somers, K.M., Craig, B. & Reynoldson, T.B. (2007). Ontario Benthos Biomonitoring Network: protocol manual. *Ontario Ministry of the Environment.*

Jones, C., Plewes, R., Murison, L., MacDougall, M.J., Sinclair, S., Davies, C., Bailey, J.L., Richardson, M. and Gunn, J. 2017. Random forests as cumulative effects models: A case study of lakes and rivers in Muskoka, Canada. *Journal of Environmental Management*. 201: 407-424. http://dx.doi.org/10.1016/j.jenvman.2017.06.011

Kazanci, N., Ekingen, P., Dugel, M. and Turkmen, G. 2015. Hirudinea (Annelida) species and their ecological preferences in some running waters and lakes. *International Journal of Environmental Science and Technology*. 12: 1087-1096. https://doi.org/10.1007/s13762-014-0574-3

Keller, W., Paterson, A., Somers, K., Dillon, P., Geneberry, J. And Ford, A. 2008. Relationship between dissolved organic carbon concentrations, weather, and acidification in small Boreal Shield lakes. *Canadian journal of Fish and Aquatic Sciences.* 65(5): 786-795. https://doi.org/10.1139/f07-19

Kovalenko, K.E., Brady, V.J., Ciborowski, J.J.H., Ilyushkin, S. and Johnson, L.B. 2014. Functional changes in littoral macroinvertebrate communities in response to watershed-level anthropogenic stress. *PLOS ONE*. 9(7). doi:10.1371/journal.pone.0101499

Krynak, E.M. and Yates, A.G. (2020). Intensive agriculture alters the biomass size spectrum and body-mass of benthic insects: evidence from a reciprocal transfer experiment. *Hydrobiologia*; 847: 1221-1235. https://doi.org/10.1007/s10750-020-04178-1(0123456789().,-volV()0123458697().,-volV)

Legendre, P. and Legendre, L. 2012. Numerical Ecology. 3rd Ed. Elsevier.

Lento, J., Dillon, P.J., Somers, K.M. and Reid, R.A. 2008. Changes in littoral benthic macroinvertebrate communities in relation to water chemistry in 17 Precambrian Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. 65(5). doi: 10.1139/F08-033

Lesauteur, T. 1968. Water pollution in summer cottage areas. *Canadian Public Health Association*. 59(7). 276-277.

Liu, X. (2018). Megaloptera of Canada. *Zookeys*; 819: 393-396.

Mandaville, S.M. (2002). Benthic macroinvertebrates in Freshwaters- taxa tolerance values, metrics and protocols. *Soil and Water Conservation Society of Metro Halifax.*

Mantyka-Pringle, C.S., Martin, T.G., Moffatt, D.B., Linke, S. and Rhodes, J.R. 2014. Understanding and predicting the combined effects of climate change and land-use change on freshwater macroinvertebrates and fish. *Journal of Applied Ecology*. 51: 572-581. doi: 10.1111/1365-2664.12236

Marburg, A.E., Turner, M.C. and Kratz, T.K. (2006). Natural and anthropogenic variation in coarse wood among and within lakes. *Journal of Ecology*; 94: 558-568. doi: 10.1111/j.1365-2745.2006.01117.x

Merritt. R.W., Cummins, K.W. and Berg, M.B. 2008. An introduction to the aquatic insects of North America. *4th Edition. Kendall/Hunt Publishing.*

Microsoft Corporation. (2022). Microsoft Excel. Retrieved from: https://office.microsoft.com/excel

Ministry of the Environment, Conservation and Parks. (2019). Lakeshore capacity assessment handbook: protecting water quality in inland lakes. Queens Printer of Ontario.

Ministry of the Environment, Conservation and Parks. (2018). Ecological impacts of cottages in Algonquin Provincial Park. *Queens Printer of Ontario*. ISBN: 978-1-4606-2961-1.

Morse, J., Broomall, M., Wenzel, J., Kautz, A., Louw, M. (Eds) (2020) The Atlas of Common Freshwater Macroinvertebrates of Eastern North America. Available from https://www.macroinvertebrates.org/ (Oct 6, 2022).

Neff., M.R. and Jackson, D.A. 2013. Regional-scale patterns in community concordance: testing the roles of historical biogeography versus contemporary abiotic controls in determining stream community composition. *Canadian Journal of Fish and Aquatic Sciences*. 70: 1141-1150. doi: 10.1139/cjfas-2012-0497

Nelligan, C., Jeziorski, A., Ruhland, K.M., Paterson, A.M. and Smol, J.P. (2019). Long-term trends in hypolimnetic volumes and dissolved oxygen concentrations in Boreal Shield lakes of south-central Ontario, Canada. *Canadian Journal of Fish and Aquatic Science;* 76: 2315-2325. doi: 10.1139/cjfas-2018-0278

Paller, M.H., Blas, S.A. and Kelley, R.W. (2020). Macroinvertebrate taxonomic richness is minimally disturbed streams on the southeastern USA Coastal Plain. *Diversity*; 12: 459-474.

Palmer, M.E., Yan, N.D., Paterson, A.M. and Girard, R.E. (2010). Water quality changes in south-central Ontario lakes and the role of local factors in regulating lake response to regional stressors. *Canadian journal of fisheries and aquatic science*; 68: 1038-1050. doi: 10.1139/f2011-041

Panis, L.I., Goddeeris, B. and Verheyen, R. (1995). The hemoglobin concentration of Chironomus cf.Plumosus l. (Diptera: Chironomidae) larvae from two lentic habitats. *Netherland Journal of Aquatic Ecology*; 29: 1-4. https://doi.org/10.1007/BF02061785

Peckarsky, B.L., Fraissinet, P.R., Penton, M.A. and Conklin, D.J. 1990. Freshwater macroinvertebrates of Northeastern North America. *Cornell University Press*: Ithaca, New York.

Peterson, R.H., Daye, P.G. and Metcalfe, J.L. 1980. Inhibition of Atlantic salmon (Salmo solar) hatching at low pH. Can. J. Fish. Aquat. Sci. 37: 770-774.

Pettigrove, V. and Hoffman, A. 2005. Effects of long-chain hydrocarbon-polluted sediment on freshwater macroinvertebrates. *Environmental Toxicology and Chemistry*. 24(10). 2500-2508.

Qiu, S. and McComb, A.J. 1996. Drying-induced stimulation of ammonium release and nitrification in refolded lake sediment. Mar. Fresh. Res. 47(3): 531-536. https://doi.org/10.1071/MF9960531

Oksanen, J.F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B. and Wagner, H. 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Owens, M.C., Williams, C.J. and Haynes, J.M. 2020. Effects of forested buffers on benthic macroinvertebrate indicators of water quality in the Western Finger Lakes, New York. *Inland Waters*. 11(1). 78-88. https://doi.org/10.1080/20442041.2020.1804786

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raney, S.M. and Eimers, C. (2013). Unexpected declines in stream phosphorus concentrations across southern Ontario. *Canadian Journal of Fisheries and Aquatic Science*; 71(3). DOI:10.1139/cjfas-2013-0300

Rowe, L., Hudson, J. and Berrill, M. 1988. Hatching success of mayfly eggs at low pH. *Canadian Journal of Fish and Aquatic Science*. 45: 1649-1652.

Rychia, A., Benndorf, J. and Bucznski, P. 2011. Impact of pH and conductivity on species richness and community stricture of dragonflies (Odonata) in small mining lakes. *Fundamental Applied Limnology.* 179: 41-50. doi:10.1127/1863-9135/2011/0179-0041

Roy, J.W., Spoelstra, J., Robertson, W.D., Klemt, W. and Schiff, S.L. 2017. Contribution of phosphorus to Georgian Bay from groundwater of a coastal beach town with decommissioned septic systems. *Journal of Great Lakes Research*. 43 (6). 1016-1029.

Sass, G.G., Kitchell, J.F., Carpenter, S.R., Hrabik, T.R., Marburg, A.E. and Turner, M.G. (2006). Fish community and food web responses to a whole-lake removal of coarse woody habitat. *Feature Fish Habitat:* 21,7; 321-330. DOI: 10.1577/1548-8446(2006)31[321:FCAFWR]2.0.CO;2

Scheider, W.A., Jeffries, D.S. and Dillon, P.J. (1981). Bulk deposition in the Sudbury and Muskoka-Haliburton areas of Ontario during the shutdown of Inco ltd in Sudbury. *Atmospheric Environment*; 15(6): 945-956.

Schmid, F. 1998. The insects and arachnids of Canada. *NRC Research Press.*

Serra, S.R.Q., Graca, M.A.S., Doledec, S. and Feio, M.J. (2017). Chironomidae traits and life history strategies as indicators of anthropogenic disturbance. *Environmental Monitoring and Assessment*; 189: 326-342. doi:10.1007/s10661-017-6027-y

Servos, M.R., Rooke, J.B. Mackie, G.L. 1985. Reproduction of selected Mollusca in some low alkalinity lakes in south-central Ontario. Canadian Journal of Zoology. 63: 511-515.

Shackleton, M., Holland, A., Stitz, L. and Mclnerney, P. 2019. Macroinvertebrate responses to conductivity in different bioregions of Victoria, Australia. *Environmental Toxicology and Chemistry.* 38(6): 1334-1342. https://doi.org/10.1002/etc.4400

Smokorowski, K.E., Pratt, T.C., Cole, W.G., Mallory, E.C. 2006. Effects on periphyton and macroinvertebrates from removal of submerged wood in three Ontario lakes. *Canadian Journal of Fish and Aquatic Science*. 63: 2038-2049. https://doi.org/10.1139/f06-104

Ter Braak, C.J.F. 1986. Canonical Correspondence Analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology.* 67, 1167-1179.

Ter Braak, C.J.F. and Verdonschot, P.F.M. 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences*. 57(3): 256-289.

Thorp, J.H. and Rogers, D.C. 2015. Ecology and general biology: Thorp and Covich's Freshwater Invertebrates: Fourth Edition. *Elsevier Inc.* ISBN: 978-0-12-385026-3

Town of Kearney. 2021. *Kearney's History and Heritage.* Retrieved from: https://townofkearney.ca/heritage-buildings/

United States Environmental Protection Agency (EPA). 2012. Conductivity. EPA Web Archive. Retrieved from: https://archive.epa.gov/water/archive/web/html/vms59.html

Vainola, R., Witt, J.D.S., Grabowski, M., Bradbury, J.H. and Sket, B. (2008). Global diversity of damphipods (Amphipoda; Crustacea) in freshwater. *Hydrobiologia*; 595: 241-255. https://doi.org/10.1007/s10750-007-9020-6

Verberk, W., Siepel, H. and Esselink, H. 2008. Life-history strategies in freshwater macroinverterbates.

Voshell, J.R. 2002. A guide to common freshwater invertebrates of North America. McDonald and Woodward Publishing Company: Blacksburg, Virginia.

Zeleny, D. 2021. RDA, tb-RDA, CCA and db-RDA (constrained ordination). Analysis of community ecology data in R. Retrieved from: https://www.davidzeleny.net/anadat-r/doku.php/en:rda_cca

Zeleny, D. 2022. Monte Carlo permutation test in constrained ordination. Analysis of community ecology data in R. Retrieved from: https://www.davidzeleny.net/anadat-r/doku.php/en:monte_carlo

Zeleny, D. 2022. Ordination analysis. Analysis of community ecology data in R. Retrieved from: https://www.davidzeleny.net/anadat-r/doku.php/en:ordination

Appendix I

Table 1. Developed lakes sampled for study with summary of associated physical properties.

Lake Name	**Beaver Lake**	**Perry Lake**	**Sand Lake**	**Loon Lake**	**Grass Lake**	**Lower Raven Lake**
Category	POP	POP	POP	POP	POP	POP
Crown Land Cover %	2%	0%	0%	10%	2%	30%
Location	45.630 N 79.723 W	45.546 N 79.227 W	45.672 N 79.1719 W	45.668 N 79.219 W	45.679 N 79.203 W	45.692 N 79.151 W
Drainage Basin	Lower Magnetawan River	Magnetawan River	Magnetawan River	Magnetawan River	Magnetawan River	Magnetawan River
Elevation (m asl)	277.8	335	339	412	372	396
Surface Area (ha)	295	68	580	156	138	39
Perimeter (km)	19		12.2	8	6.4	3
Max Depth (m)	10.7	13	59	29	37	16
Average Water Temperature (ºC)	14.383 (±3.126 SE)	15.617 (±2.640 SE)	13.938 (±1.961 SE)	14.95 (±3.497 SE)	14.55 (±2.910 SE)	14.933 (±3.604 SE)
Average Dissolved Oxygen (µg/L)	10.187 (±0.479 SE)	9.487 (±0.493 SE)	11.038 (±1.400 SE)	9.482 (±0.384 SE)	8.74 (±0.401 SE)	9.085 (±0.527 SE)
Average pH	6.775 (±0.144 SE)	6.73 (±0.169 SE)	6.588 (±0.103 SE)	6.805 (±148 SE)	6.537 (±0.107 SE)	6.292 (±0.255 SE)
Average Conductivity (µS/cm)	37.533 (±0.891 SE)	40.033 (±2.574 SE)	38.363 (±2.332 SE)	21.622 (±0.833 SE)	20.89 (±1.651 SE)	15.738 (± 0.223 SE)

Total Phosphorus Concentration (µg/L)	88.45 (±28.26 SE)	23.13 (±4.02 SE)	56.25 (±26.73 SE)	65.27 (±24.62 SE)	45.90 (±8.85 SE)	18.90 (±6.33 SE)
Available Nitrogen Concentration (µg/L)	50.54 (±14.66 SE)	29.42 (±4.07 SE)	65.77 (±16.59 SE)	34.42 (±7.20 SE)	47.76 (±18.54 SE)	31.00 (±1.95 SE)

Table 2. Isolated lakes sampled for study with summary of associated physical properties.

Lake Name	**Buck Lake**	**Spruce Lake**	**Bluesky Lake**	**Tea Lake**	**Charlie Mann's Lake**	**Mining Corporation Lake**
Category	ISO	ISO	ISO	ISO	ISO	ISO
Crown Land Cover %	80%	97%	95%	95%	100%	100%
Location	45.6900 N 79.1672 W	45.703 N 79.118 W	45.748 N 79.178 W	45.672 N 79.255 W	45.712 N 79.042 W	45.705 N 79.030 W
Drainage Basin	Magnetawan River	Magnetawan River	North Magnetawan River	North Magnetawan River	Magnetawan River	Magnetawan River
Elevation (m asl)	423	482	415	412	404	442
Surface Area (ha)	96	20	33	34	8	7
Perimeter (km)	8.4	2	4	4	1.5	1.4
Max Depth (m)	46	16	8	19	6	10
Average Water Temperature (ºC)	14.7 (±3.475 SE)	15.417 (±3.460 SE)	15.15 (±3.526 SE)	14.867 (±3.366 SE)	14.883 (±3.684 SE)	15.3 (±3.184 SE)
Average Dissolved Oxygen (µg/L)	9.453 (±0.669 SE)	8.803 (±0.407 SE)	10.173 (±0.285 SE)	9.722 (±0.630 SE)	9.578 (±0.640 SE)	9.645 (±0.572 SE)

Average pH	6.352 (±0.277 SE)	6.462 (±0.205 SE)	6.795 (±0.169 SE)	6.577 (±0.0996 SE)	6.055 (±0.214 SE)	6.37 (±0.253 SE)
Average Conductivity (µS/cm)	14.007 (±0.115 SE)	15.905 (±0.373 SE)	15.328 (±1.085 SE)	16.498 (±1.083 SE)	21.788 (±0.548 SE)	14.57 (±0.658 SE)
Total Phosphorus Concentration (µg/L)	41.47 (±15.09 SE)	13.977 (±11.17 SE)	53.21 (±11.01 SE)	121.74 (±88.1 SE)	36.52 (±14.11 SE)	58.37 (±14.37 SE)
Available Nitrogen Concentration (µg/L)	22.48 (±1.40 SE)	67.39 (±26.97 SE)	47.67 (±7.75 SE)	23.40 (±2.92 SE)	31.00 (±3.21 SE)	31.37 (±7.52 SE)

Table 3. Average count of benthic macroinvertebrate samples collected for all sample periods. Average count is mean of three replicates samples taken. Sorted by development type and season sampled.

Lake	Site	Season	Development Type	Average Count
Spruce	S1	Fall	ISO	10.00
Spruce	S2	Fall	ISO	26.00
Charlie's	S1	Fall	ISO	57.33
Charlie's	S2	Fall	ISO	53.00
Mining Corporation	S1	Fall	ISO	45.67
Mining Corporation	S2	Fall	ISO	54.00
Tea	S1	Fall	ISO	21.00
Tea	S2	Fall	ISO	20.67
BlueSky	S1	Fall	ISO	69.67
Buck	S2	Fall	ISO	55.33
Spruce	S1	Spring	ISO	24.33
Spruce	S2	Spring	ISO	23.33
Charlie's	S1	Spring	ISO	22.00
Charlie's	S2	Spring	ISO	32.33
Mining Corporation	S1	Spring	ISO	28.00
Mining Corporation	S2	Spring	ISO	29.00

Tea	S1	Spring	ISO	27.67
Tea	S2	Spring	ISO	14.67
BlueSky	S1	Spring	ISO	66.67
BlueSky	S2	Spring	ISO	63.33
Spruce	S1	Summer	ISO	16.00
Spruce	S2	Summer	ISO	10.00
Charlie's	S1	Summer	ISO	29.33
Charlie's	S2	Summer	ISO	14.00
Mining Corporation	S1	Summer	ISO	13.00
Mining Corporation	S2	Summer	ISO	14.67
Tea	S1	Summer	ISO	7.00
Tea	S2	Summer	ISO	9.00
BlueSky	S1	Summer	ISO	31.67
BlueSky	S2	Summer	ISO	34.33
Beaver	S1	Fall	POP	42.00
Beaver	S2	Fall	POP	43.33
Sand	S1	Fall	POP	16.67
Sand	S2	Fall	POP	7.33
Loon	S1	Fall	POP	9.67
Loon	S2	Fall	POP	19.00
Grass	S1	Fall	POP	25.00
Grass	S2	Fall	POP	16.33
Lower Raven	S1	Fall	POP	31.67
Lower Raven	S2	Fall	POP	31.67
Perry	S1	Fall	POP	54.33
Perry	S2	Fall	POP	73.33
Beaver	S1	Spring	POP	67.00
Beaver	S2	Spring	POP	36.00
Sand	S1	Spring	POP	6.67
Sand	S2	Spring	POP	7.00
Sand	S3	Spring	POP	9.67
Loon	S1	Spring	POP	19.67
Loon	S2	Spring	POP	18.00
Grass	S1	Spring	POP	14.67
Grass	S2	Spring	POP	15.00
Lower Raven	S1	Spring	POP	28.00
Lower Raven	S2	Spring	POP	29.67

Perry	S1	Spring	POP	48.00
Perry	S2	Spring	POP	73.33
Beaver	S1	Summer	POP	35.67
Beaver	S2	Summer	POP	21.33
Sand	S1	Summer	POP	6.33
Sand	S2	Summer	POP	4.67
Sand	S3	Summer	POP	0.00
Loon	S1	Summer	POP	13.67
Loon	S2	Summer	POP	9.00
Grass	S1	Summer	POP	10.33
Grass	S2	Summer	POP	11.67
Lower Raven	S1	Summer	POP	13.00
Lower Raven	S2	Summer	POP	13.00
Perry	S1	Summer	POP	32.00
Perry	S2	Summer	POP	56.67

Table 4. Tolerance values of macroinvertebrate families exposed to organic pollution. Table copied directly from Mandaville (2002) who compiled tolerance data from Bode *et al*. (1996); Hauer and Lamberti (1996); Hilsengoff (1988); Plafkin *et al*. (1989). Values range from 0 to 10, with 0 being not tolerant to pollutants and 10 being very tolerant.

Plecoptera		**Trichoptera**		**Amphipoda**	
Capniidae	1	Brachycentridae	1	Gammaridae	4
Chloroperlidae	1	Calamoceratidae	3	Hyalellidae	8
Leuctridae	0	Glossosomatidae	0	Talitridae	8
Nemouridae	2	Helicopsychidae	3		
Perlidae	1	Hydropsychidae	4	**Isopoda**	
Perlodidae	2	Hydroptilidae	4	Asellidae	8
Pteronarcyidae	0	Lepidostomatidae	1		
Taeniopterygidae	2	Leptoceridae	4	**Decapoda**	6
		Limnephilidae	4		
Ephemeroptera		Molannidae	6	**Acariformes**	4
Baetidae	4	Odontoceridae	0		
Baetiscidae	3	Philpotamidae	3	**Mollusca**	
Caenidae	7	Phryganeidae	4	Lymnaeidae	6
Ephemerellidae	1	Polycentropodidae	6	Physidae	8
Ephemeridae	4	Psychomyiidae	2	Sphaeridae	8
Heptageniidae	4	Rhyacophilidae	0		
Leptophlebiidae	2	Sericostomatidae	3		
Metretopodidae	2	Uenoidae	3		
Oligoneuriidae	2				
Polymitarcyidae	2				
Potomanthidae	4				
Siphlonuridae	7				
Tricorythidae	4				

		Diptera			
		Athericidae	2		
		Blephariceridae	0		
		Ceratopogonidae	6		
		Blood-red Chironomidae	8		
		(Chironomini)			
Odonata		Other Chironomidae	6		
		(including pink)			
Aeshnidae	3	Dolochopodidae	4		
Calopterygidae	5	Empididae	6		
Coenagrionidae	9	Ephydridae	6		
Cordulegastridae	3	Muscidae	6		
Corduliidae	5	Psychodidae	10	**Oligochaeta**	8
Gomphidae	1	Simuliidae	6		
Lestidae	9	Syrphidae	10	**Hirudinea**	
Libellulidae	9	Tabanidae	6	Bdellidae	10
Macromiidae	3	Tipulidae	3	*Helobdella*	10
Megaloptera		**Coleoptera**		**Polychaeta**	
Corydalidae	0	Dryopidae	5	Sabellidae	6
Sialidae	4	Elmidae	4		
		Psephenidae	4		
Lepidoptera				**Turbellaria**	4
Pyralidae	5	**Collembola**		Platyhelminthidae	4
		Isotomurus sp.	5		
Neuroptera				**Coelenterata**	
Sisyridae				Hydridae	
Climacia sp.	5			*Hydra* sp.	5

Table 5. Explanation of family-level biotic index used to evaluate water quality. Table taken directly from Hilsenhoff (1988).

Biotic Index	Water Quality	Degree of Organic Pollution
0.00 - 3.50	Excellent	No apparent organic pollution
3.51 - 4.50	Very Good	Possible slight organic pollution
4.51 - 5.50	Good	Some organic pollution
5.51 - 6.50	Fair	Fairly significant organic pollution
6.51 - 7.50	Fairly Poor	Significant organic pollution
7.51 - 8.50	Poor	Very significant organic pollution
8.51 - 10.00	Very Poor	Severe organic pollution