CYANOBACTERIA PRESENCE IN FOUR LAKES IN THE HALIFAX REGIONAL MUNICIPALITY (HRM), NOVA SCOTIA

by

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For Rob and Doug:

I wouldn't have been here if it wasn't for you two. Thanks for believing in me.

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ABSTRACT

The major objective of this project is to assess the presence of cyanobacteria (organisms that can pose serious challenges for drinking water treatment systems), in Halifax water supplies using DNA typing to identify cyanobacteria species. Four lakes in the Halifax Regional Municipality (HRM), NS, were chosen due to their differing water chemistry. A total of 35 taxa of cyanobacteria and one taxa of unassigned bacteria were detected, of which, 15 genera of cyanobacteria were identified. Of those genera, 11 have been associated with cyanotoxins, which are harmful to humans and animals. Supplementary data showed that two of the four lakes had higher pH, alkalinity, TN, TP and turbidity means than the others, and had detectable cyanotoxins and algal blooms after periods of rain followed by long periods of warm, dry, relatively calm weather. This thesis will provide a foundation for future experiments into lake and cyanotoxin management.

LIST OF ABBREVIATIONS

BMAA	β-methylamino-L-alanine
BSC	biological safety cabinet
DOC	dissolved organic carbon
MC-LR	microcystin-LR
MDL	minimum detection limit
MIB	2-methylisoborneol
NOM	natural organic matter
NTU	Nephelometric Turbidity Units
OTU	operational taxanomic unit
PCR	polymerase chain reaction
PES	polyethersulfone
rRNA	ribosomal ribonucleic acid
SUVA	specific ultraviolet absorbance
TOC	total organic carbon
TN	total nitrogen
TP	total phosphorous

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CHAPTER 1 INTRODUCTION

1.1. CYANOBACTERIA

Cyanobacteria, also known as blue-green algae, are a phylum of single celled bacteria that have existed for roughly 3.5 billion years and range in size between 0.5 μm to 40 μm in diameter (Maier *et al*, 2000; Waterbury, 2006; Percival and Williams, 2014). Characteristic of this phylum are the presence of phycobiliproteins, specialized accessory pigments that are arranged into light-harvesting complexes called phycobilisomes (Whitton and Potts, 2000; Rastogi *et al*, 2015; Hunter *et al*, 2017). These pigments, phycocyanin, phycoerythrin and allophycocyanin, aid chlorophyll-α in photosynthesis, and give cyanobacteria their blue-green color.

Other notable characteristics of certain cyanobacteria include the presence of gas vacuoles, heterocysts and/or akinetes (Whitton and Potts, 2000; Percival and Williams, 2014). Gas vacuoles are comprised of large numbers of ridged, hollow gas vesicles that are stacked together in hexagonal arrays. These gas vacuoles allow cyanobacteria to be buoyant and thus be move throughout the water column more efficiently than non-motile microorganisms, even in low light conditions. Heterocysts, which aid in nitrogen fixation, typically develop when nitrogen becomes a limited resource, giving cyanobacteria an ecological advantage over eukaryotic algae in nitrogen poor environments. In extremely adverse conditions, cyanobacteria capable of developing heterocysts can also develop akinetes, dormant, thick walled cells that can withstand desiccation and extreme temperatures.

Cyanobacteria can exist in a variety of forms in salt and fresh water environments, and in soil (Whitton and Potts, 2000; Waterbury, 2006; Moreira *et al*, 2014). Although single celled, cyanobacteria can form colonies to form filamentous chains, coccoidal spheres, free floating planktonic colonies, or mats. They can also form complexes with other organisms such as green algae, heterotrophic microbes, fungi, or organic material to

form phytoplankton, lichens, periphyton and the like. Examples of these complexes can be seen in Figure 1.1.

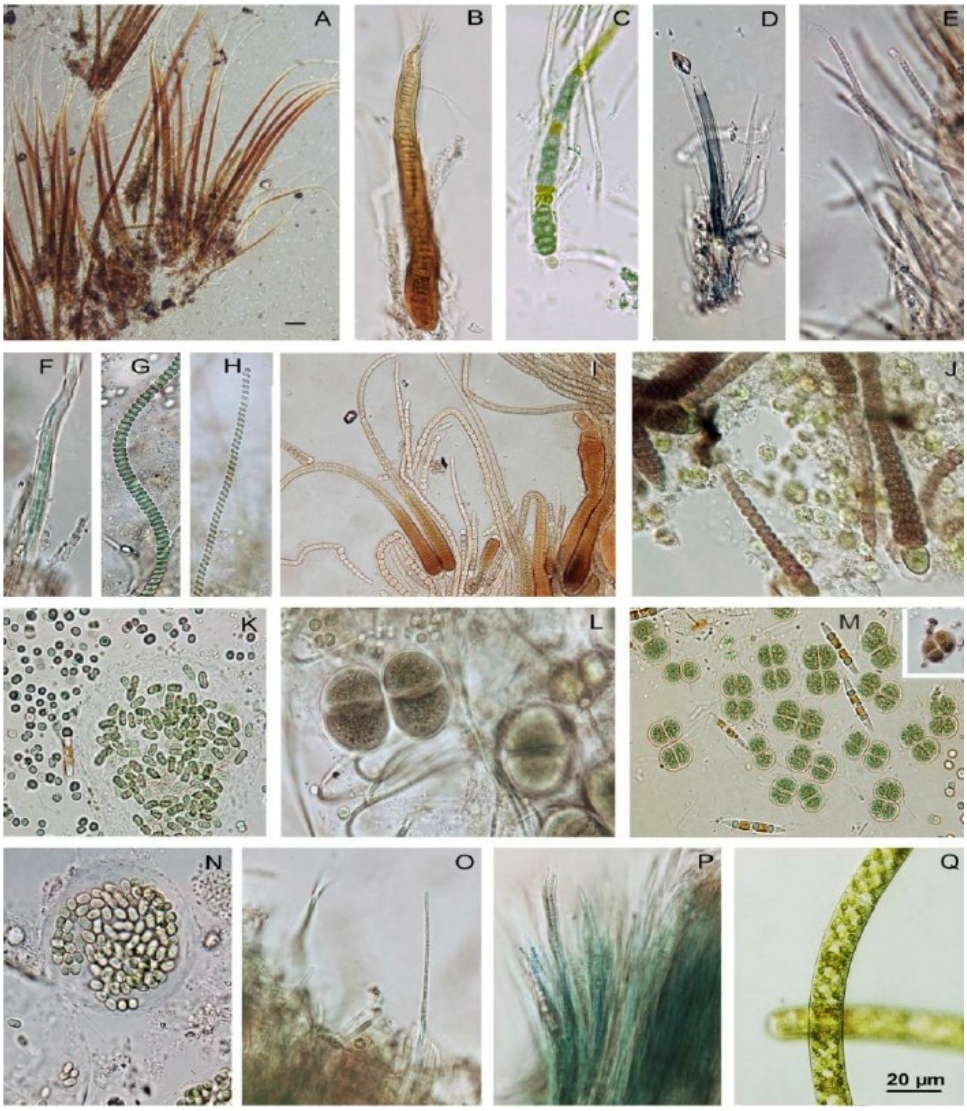


Figure 1.1. Cyanobacteria assemblages, both coccoid and filamentous, found in mats and laboratory cultures in Abed *et al* (2013).

Cyanobacteria serve as important atmospheric nitrogen fixers and are major contributors to the global oxygen supply (Maier *et al*, 2000; Whitton and Potts, 2000; Percival and Williams, 2014). Certain taxa, such as Spirulina (*Arthrospira*), are considered super food due to high concentrations of essential fat, protein, minerals and vitamins (Raposo *et al*, 2013; Gutiérrez-Salmeán *et al*, 2015). However, cyanobacteria can also cause serious health problems and pose challenges to water treatment processes.

1.2. CONCERNS ASSOCIATED WITH CYANOBACTERIA

1.2.1. Algal Blooms and Cyanotoxins

Algal blooms can block water treatment filters, cause a variety of taste and odour problems, and can negatively impact lake biota by forming surface scum (Whitton and Potts, 2000). Surface blooms deoxygenate water and blocks sunlight from reaching lower water strata, making lakes uninhabitable for flora and fauna alike Algal blooms can remain for hours, days, or even months under the right conditions.

Although algal blooms can be caused by other organisms, such as flagellates, diatoms and green algae, blooms caused by cyanobacteria come with a particular health risk: cyanotoxins (World Health Organization (WHO), 1999; Whitton and Potts, 2000; Carmichael and Boyer, 2016; Buratti *et al*, 2017). Cyanotoxins are toxins produced by cyanobacteria and can be categorized into five toxin classes:

i. Hepatotoxins – Microcystin and nodularin are cyclic peptides that can cause life threatening liver haemorrhages at acute doses and promote tumour growth. They are the most common cyanotoxin produced by cyanobacteria.

ii. Neurotoxins – The alkaloids anatoxin and saxitoxin, and the non-proteinogenic amino acid β-methylamino-L-alanine (BMAA), target nerve axons and synapses. They can cause a loss of muscle control and death by respiratory distress.

iii. Cytotoxins – Cylindrospermopsin, an alkaloid, invokes widespread tissue damage to the liver, kidneys and lymphatic tissue.

iv. Dermatotoxins – Aplysiatoxin and lyngbyatoxin are also alkaloids, and can cause skin irritation and dermal lesions.

v. Endotoxins – All cyanobacteria can cause gastrointestinal distress.

1.2.2. Geosmin and 2-methylisoborneol

Cyanobacteria have also been associated with the organic compounds geosmin and 2-methylisoborneol (MIB), saturated cyclic tertiary alcohols that can cause water to have a musty flavour and taste, and that are difficult to remove with conventional treatment processes (Izaguirre *et al*, 1982; Izaguirre and Taylor, 2004). It has been reported that both compounds are metabolites produced by cyanobacteria, actinomycetes and proteobacteria (Suurnäkki *et al*, 2015; Lee *et al*, 2017).

1.3. FACTORS THAT INFLUENCE BLOOMS

1.3.1. Eutrophication

Eutrophication in lakes occurs when excessive amounts of nutrients, in particular nitrogen (N) and phosphorus (P), are added to the water system from sources such as fertilizer run off, manure, and sewage (Paerl *et al*, 2015, 2016; Schindler *et al*, 2016). Lakes where phosphorus is high in ratio to other nutrients such as carbon and nitrogen, tend to be more favourable environments for cyanobacteria growth (Schindler *et al*, 2016; Verhamme *et al*, 2016; Lin *et al*, 2017).

For example, toxic algal blooms influenced by eutrophication due to high levels of phosphorus from fertilizer, manure and sewage have become a yearly occurrence in Lake Erie (Kelly, 2015; Barber, 2016). Most notably, in 2014, Toledo, Ohio, was forced to shut down their water supply systems due to a massive, severely toxic, algal bloom. For three days, there was a ban on drinking and tap water due to high levels of microcystin from the algal bloom. More than 400,000 residents in Toledo, and 30,000 residents in South-East Michigan were effected.

In Nova Scotia, it is believed that discharge from mink farms and mink food processing facilities have been the cause of eutrophication in watersheds in Yarmouth and Digby County (Taylor, 2009; Brylinsky, 2011). The discharge contains very high

concentrations of phosphorus, making it the most likely source of phosphorus available for cyanobacteria to consume. What complicates matters is that half of all Canadian mink farms are located in Nova Scotia, of which, 85% of provincial production originated out of Yarmouth and Digby County as of 2012, making these areas high risk for algae and cyanobacteria blooms (CBC News, 2012).

1.3.2. Climate Change and Lake Recovery from Acidification

Rising temperatures and longer seasons caused by climate change results in higher temperatures and prolonged thermal stratification within lakes, preferred growth conditions for cyanobacteria (Whitton and Potts, 2000; Pick, 2016). Drought conditions can also be beneficial to cyanobacteria if periods of intense rainfall occurred prior to the drought, which would affect nutrient flow into watersheds and prolong stratification in lakes (Paerl *et al*, 2016; Pick, 2016).

Another factor influencing the rise in algal and cyanobacteria blooms could also lie in the process of lake recovery from acidification. There are several environmental factors that affect lake recovery: watershed disturbance, surrounding forest type, wetland coverage, soil type, bedrock geology, lake topography, hydrology, water chemistry, and magnitude of acid reduction (Jeffries *et al*, 2003a; Ginn *et al*, 2015). Atmospheric acid deposition of sulfur oxides (SO_x) and nitrogen oxides (NO_x) via acid rain is particularly concerning, as the long range transport of these chemicals can cause an increase of nutrients into nutrient limited ecosystems (Walker *et al*, 2003).

However, due to emissions regulations in the United States (Clean Air Act), Canada (Eastern Canada Acid Rain Program), and Europe (United Nation's Economic Commission for Europe's Convention on Long Range Transboundary Air Pollution), atmospheric acid deposition of SO_x and NO_x in the form of acid rain has decreased across North America and Europe since the 1980's (Stoddard *et al*, 1999; Jeffries *et al*, 2003a). As of 2003, total emissions of sulfur dioxide (SO_2) in North America had decreased by roughly 40% since the 1980's (Jeffries *et al*, 2003a). Although NO_x affects acidification,

SO_x plays a more important role in acid deposition and has experienced a larger decrease than NO_x (Jeffries *et al*, 2003a,b).

In Nova Scotia, lakes are typically acidic due to naturally occurring organic acids and bedrock geology, whereby silica-based gneiss and granite are acid sensitive due to limited carbonate sources and low base cation concentrations (Jeffries *et al*, 2003b; Ginn *et al*, 2015). Lake pH has been further depressed by long-range transport of sulphate (SO_4^{2-}) from industrial centers in northeastern United States, the Laurentian Great lakes Area and local oil refineries and power generating stations, as well as by natural acidification from organic anions (Ginn *et al*, 2015).

According to studies performed during the 1990's, there were no trends showing lake recovery from acidification in lakes in Nova Scotia (Jeffries *et al*, 2003a). However, according to Anderson *et al* (2017), several drinking water supplies in Nova Scotia have been showing signs of lake recovery in recent years. Sulfur oxide deposition has been decreasing, resulting in increased dissolved organic carbon (DOC). There has also been a rise in natural organic matter (NOM), colour and geosmin occurrence. Lake recovery from acidification could potentially mean the return of fish populations to water bodies, and the increase of cyanobacteria activity, as the chemical and biological effects of acidification are decreased (Jeffries *et al* 2003a,b).

1.4. ALGAL BLOOMS IN NOVA SCOTIA

As mentioned previously, lakes in Nova Scotia are typically acidic due to naturally occurring organic acids and bedrock geology (Jeffries *et al*, 2003b; Ginn *et al*, 2015). However, due to influencing factors like eutrophication from mink farms, climate change, and potential lake recovery from acidification, blooms, even toxic blooms caused by cyanobacteria, have not been unusual to the province within the last decade (Whitton and Potts, 2000; Taylor, 2009; Brylinsky, 2011; Pick, 2016; Anderson *et al*, 2017).

Mattatall Lake, shared between Colchester County and Cumberland County has experienced algal blooms since 2005 (Mathieson, 2015; Campbell, 2016; Tetanish, 2016). It has also been reported that these blooms can persist into November or even January. However, in 2017, there was no bloom observed at Mattatall Lake (Sullivan, 2017). Locals observed that unlike in previous years, the lake was clear, vegetation had begun to grow on the bottom of the lake, and a species of frog had returned. Yarmouth County and Digby County have experienced algal blooms every year since 2007 (Province of Nova Scotia (PNS), 2007, 2016f; Wendland, 2011; Lavoie, 2014; Allen, 2016). Of note, blooms have been observed in Lake Fanning, Parr Lake, Ogden Lake and Lake Vaughan in Yarmouth County, and Porcupine Lake in Digby County.

Other areas of note include Inverness County, where Lake Ainslie has had algal blooms in 2009 and 2010, and both the Southwest Margaree River and Margaree River have exhibited blooms in 2009 (PNS, 2009a,b, 2010). Lochaber Lake, located in both Antigonish County and Guysborogh County, experienced an algal bloom in 2012 (Lochaber Community Development Association, 2012). In Kings County, Lumsden Pond in the Lumsden Pond Provincial Park in Wolfville was reported to have algal blooms in 2013 and 2016, while Lake Torment has experienced blooms since at least 2014 (PNS, 2013b, 2014, 2015, 2016b,d; Tetanish, 2016). In 2015, an algal bloom was reported in Sherbrooke Lake in Lunenburg County, and in 2016 an algal bloom was reported in Middle River in Pictou County (PNS, 2016e; Tetanish, 2016).

Algal blooms have even been reported in Halifax County, albeit only in recent years. Algal blooms were reported at Powder Mill Lake in Waverley in 2016, and in Oathill Lake in Dartmouth in 2017 (PNS, 2016c; Meloney, 2017). However, the bloom in Oathill Lake was determined to be a green algae bloom, and was not definitively associated with cyanobacteria.

1.5. RESEARCH OBJECTIVES

Information regarding cyanobacteria, cyanotoxins and algal blooms is limited in Atlantic Canada. This is problematic as climate change and human activity have exhibited impacts on water chemistry, and resultantly, the ecology of biota in aquatic environments. As lakes recover from acidification, more organisms, such as cyanobacteria, have an opportunity to thrive, which increases the risk of cyanotoxins being present in drinking water supplies and recreational waterways. Short and long term preventative measures and treatment activities designed to combat blooms and cyanotoxins cannot be established without first developing an inventory of cyanobacteria present in lakes.

The major objective of this study was to provide a baseline for future research and inquiry into cyanobacteria population ecology which will enable future best practices for lake management in Nova Scotia. To meet this objective, the study was divided into three subtasks: a) determine the presence and taxa of cyanobacteria in three Halifax water supplies and one recreational lake using DNA typing, and determine b) water chemistry and c) environment data to provide context to cyanobacteria population data.

CHAPTER 2 METHODOLOGY

2.1. SAMPLE LOCATIONS

2.1.1. Fletchers Lake

Fletchers Lake, also known as Lake Fletcher, shown in Figure 2.1., is located in Wellington and Fall River, NS, in the Collin's Park Watershed area, and supplies the Collin's Park Water Treatment Plant, built in 2010 (Government of Canada (GC), 2016a; Halifax Water, 2016; Halifax Water, 2017). It has an area of 100 ha and a maximum depth of 12 m (PNS, 2016g). Fletchers Lake serves as the water supply for residents of Wellington, and serves as the lake into which treated wastewater from the plant is discharged.

The Collin's Park Watershed is not considered a protected watershed area. According to the Halifax Regional Municipality (HRM) (2017d), "[the] goal of source water protection is to maintain, or improve, the quality of drinking water resources before it reaches the supply plant," cost effectively and sustainably, for present and future use. They do this by implementing the following: the Nova Scotia Environment Act (PNS, 2013a) and the Halifax Regional Water Commission Act (PNS, 2017c), which restricts fishing, hunting and motor vehicle use within the protected watershed area; the Protection of Property Act (PNS, 2002), which allows land around the lakes within the watershed to be purchased in an effort to reduce activities such as illegal dumping, off-highway use, and trespassing; Best Management Practices (Halifax Water, 2010), which attempt to strike a balance water treatment objectives with forest ecosystem conservation; and finally, source water monitoring, whereby source water sampling and analysis are routinely conducted, along with security checks by patrols and citizen watch groups (HRM, 2017d). Not being a protected watershed, The Collin's Park Watershed area is likely more susceptible to anthropogenic forces than other, protected watersheds.

A section of the watershed overlaps with the Waverly-Salmon River Long Lake Wilderness Area and the Waverly Game Sanctuary. The southern tip and eastern edges share the shoreline with residential houses and Highway 2 (Nova Scotia Trunk 2), while the rest of the lake is surrounded by forest.

With regards to forest composition, Fletchers Lake is primarily surrounded by tolerant mixedwoods, but spruce (of note, red and black spruce), hemlock and pine trees were also present (PNS, 2017d). With regards to geology and mineral presence, Fletchers Lake overlies the Halifax Formation as well as areas of Middle-Late Devonian muscovite biotite monzogranite (PNS, 2017a). Slate, siltstone and minor sandstone can be found in the area. Also present in these areas: granite, granodiorite, diorite, diabase, gabbro, building stone, aggregate, tin, copper, lead, zinc and base metals (PNS, 2017b).

Between June and October 2016, water samples were collected from Fletchers Lake by Michael Brophy (MASc student, Dalhousie University), on foot from Fletchers Lake's tributaries, while raw water was collected from the Collin's Park Water Treatment Plant.

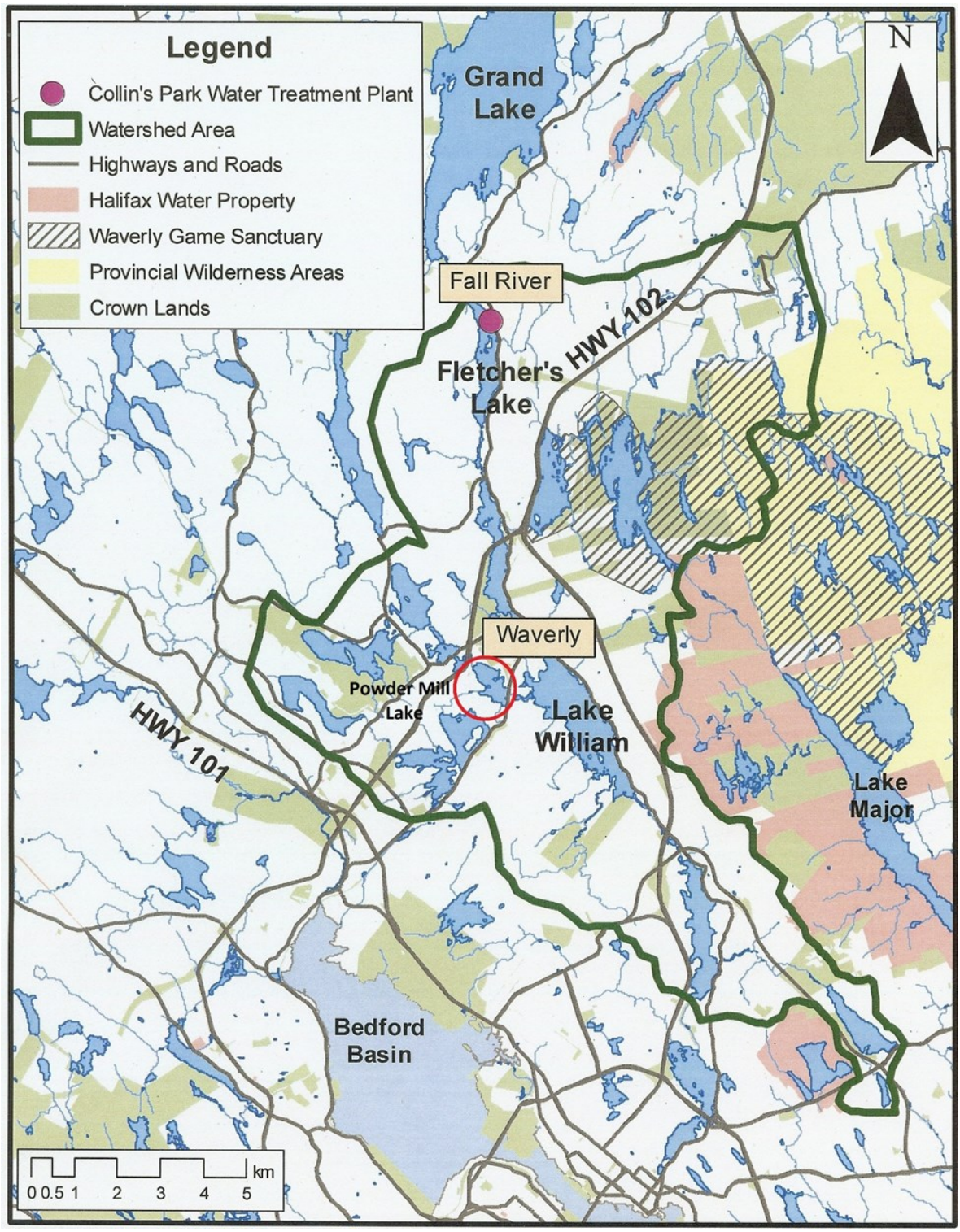


Figure 2.1. Map of the Collin's Park Public Water Supply area, featuring location of Fletchers Lake and Powder Mill Lake (circled in red) within the watershed (HRM, 2017a).

2.1.2. Powder Mill Lake

Powder Mill Lake, formerly known as Fish Lake, circled in Figure 2.1., is located in Waverley, NS, and is also within the Collin's Park Watershed area (Halifax Water, 2016). It has an area of 43 ha and a maximum depth of 13 m (GC, 2016c; PNS, 2016g). Powder Mill Lake does not act as a water supply source, but rather a recreational lake for swimming and fishing (PNS, 2016a). It also collects run off from the Nova Scotia Firefighters School and Highway 2 (Nova Scotia Trunk 2). Residential houses, forest and Powder Mill Park share Powder Mill Lake's remaining shoreline. A gravel quarry, owned by Rocky Lake Quarry Ltd., is also in the area.

Powder Mill Lake is primarily surrounded by a mixture of spruce and pine trees, as well as tolerant mixedwoods (PNS, 2017d). With regards to geology and mineral presence, Powder Mill Lake overlies part of the Goldenville Formation (PNS, 2017a). Quartzose sandstone with a chlorite-rich matrix and gold bearing quartz veins can be found in the Goldenville Formation. Also present in this area: greywacke, quartzite, slate, aggregate, tungsten, zinc and lead (PNS, 2017b).

The first three water samples taken from Powder Mill Lake were collected on foot from the inlet stream, next to Highway 2 and from the shoreline of Powder Mill Park, in July 2016. However, subsequent samples collected between July and November 2016, were collected from in-lake using an inflatable kayak. Samples from Powder Mill Lake were collected by the author.

2.1.3. Pockwock Lake

Pockwock Lake, shown in Figure 2.2., is located in Upper Hammonds Plains, NS, in the Pockwock Lake and Tomahawk Lake Watershed area, and supplies the J. Douglas Kline Water Supply Plant, built in 1977 (Halifax Water, 2016; Halifax Water, 2017). It has an area of 902 ha and a maximum depth of 43 m (PNS, 2016g; Scott, 2004). Pockwock Lake serves as the water supply for the residents of Halifax, Bedford,

Sackville, Fall River, Waverly and Timberlea. The watershed is mostly owned by the Crown, with a small percentage being considered Halifax Water property. A third of this watershed is also shared by the Pockwock Wilderness Area (PNS, 2016h). The Pockwock Lake and Tomahawk Lake Watershed area is designated as a protected watershed area (HRM, 2017d)

Pockwock Lake is primarily surrounded by spruce and pine trees, but hemlock and tolerant mixedwoods are also present (PNS, 2017d). With regards to geology and mineral presence, Pockwock Lake overlies part of the Goldenville Formation and Halifax Formation, as well as areas of Mid-Late Devonian granodiorite, and Mid-Late Devonian biotite monzogranite (PNS, 2017a). Slate, siltstone and minor sandstone can be found in the Halifax Formation area, while quartzose sandstone with a chlorite-rich matrix and gold bearing quartz veins can be found in the Goldenville Formation. Also present in these areas: greywacke, quartzite, granite, diorite, diabase, gabboro, aggregate, tungsten, zinc, lead, building stone, tin and copper (PNS, 2017b).

Water samples were collected from Pockwock Lake between June and November 2016, by Dave Redden (MASc student, Dalhousie University), in-lake with a motorized boat and on foot from its tributaries.

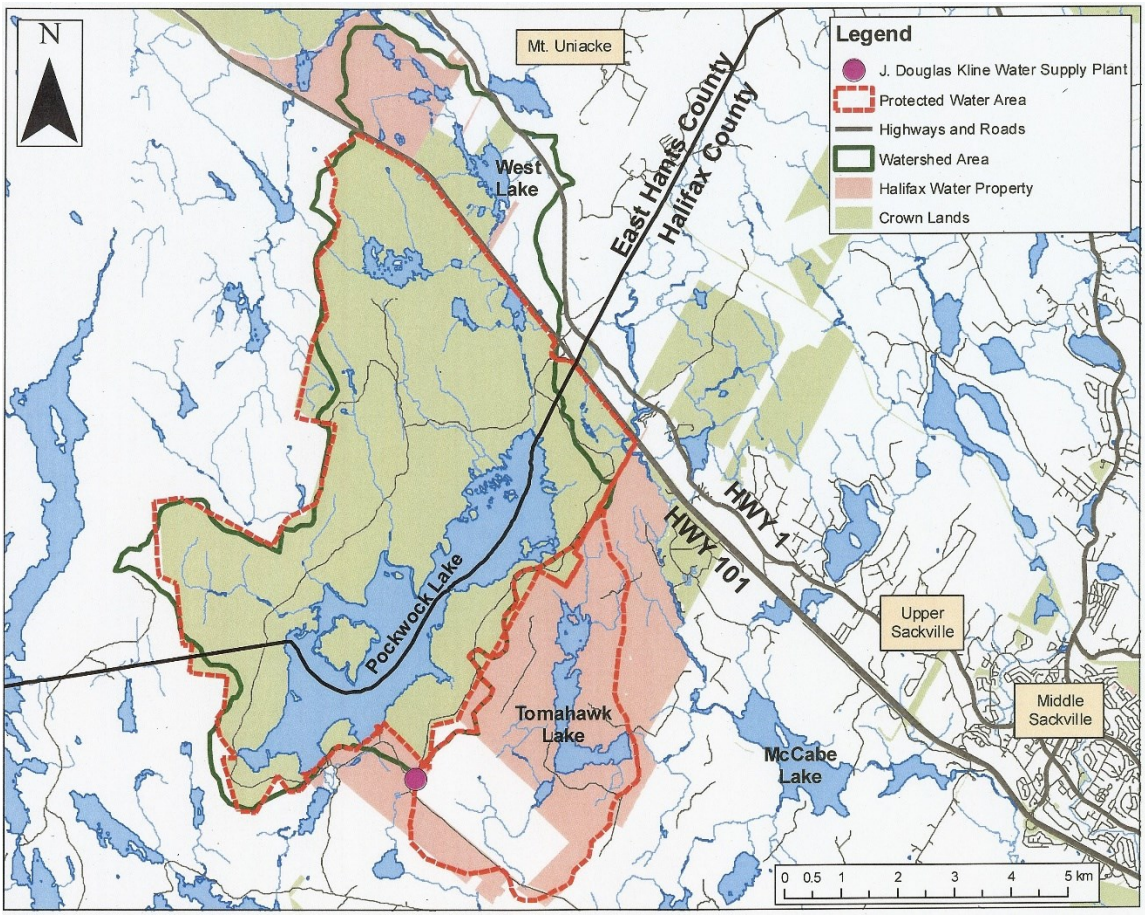


Figure 2.2. Map of the Pockwock Lake and Tomahawk Lake Watershed areas, featuring Pockwock Lake within the watershed (HRM, 2017c).

2.1.4. Lake Major

Lake Major, shown in Figure 2.3., is located in Dartmouth, NS, in the Lake Major Watershed area, and supplies the Lake Major Water Supply Plant, built in 1999 (Halifax Water, 2016; Halifax Water, 2017). It has an area of 377 ha and a maximum depth of 65 m (PNS, 2016g). Lake Major serves as the water supply for residents in Dartmouth, Eastern Passage, Cole Harbour, Westphal, Cherry Brook, Montague Mines and North Preston. Like the Pockwock Lake and Tomahawk Lake Watershed area, the Lake Major Watershed area is also designated as a protected watershed area (HRM, 2017d). About a third of the watershed area overlaps with the Waverley-Salmon River Long Lake Wilderness Area and another third overlaps with the Waverley Game Sanctuary (Halifax Water, 2016; Halifax Water, 2017). The remaining area is mostly owned by Halifax Water, followed by the Crown.

Lake Major is primarily surrounded by a mixture of spruce (including red and black spruce), hemlock and pine trees, but tolerant mixedwoods are also present (PNS, 2017d). With regards to geology and mineral presence, Lake Major also overlies on the Goldenville and Halifax Formations like Pockwock Lake, as well as areas of Mid-Late Devonian muscovite biotite monzogranite (PNS, 2017a,b).

Water samples were collected between June and October 2016, from Lake Major by Lindsay Anderson (PhD student, Dalhousie University), on foot from Lake Major's tributaries and from the pump house at the Lake Major Water Supply Plant.

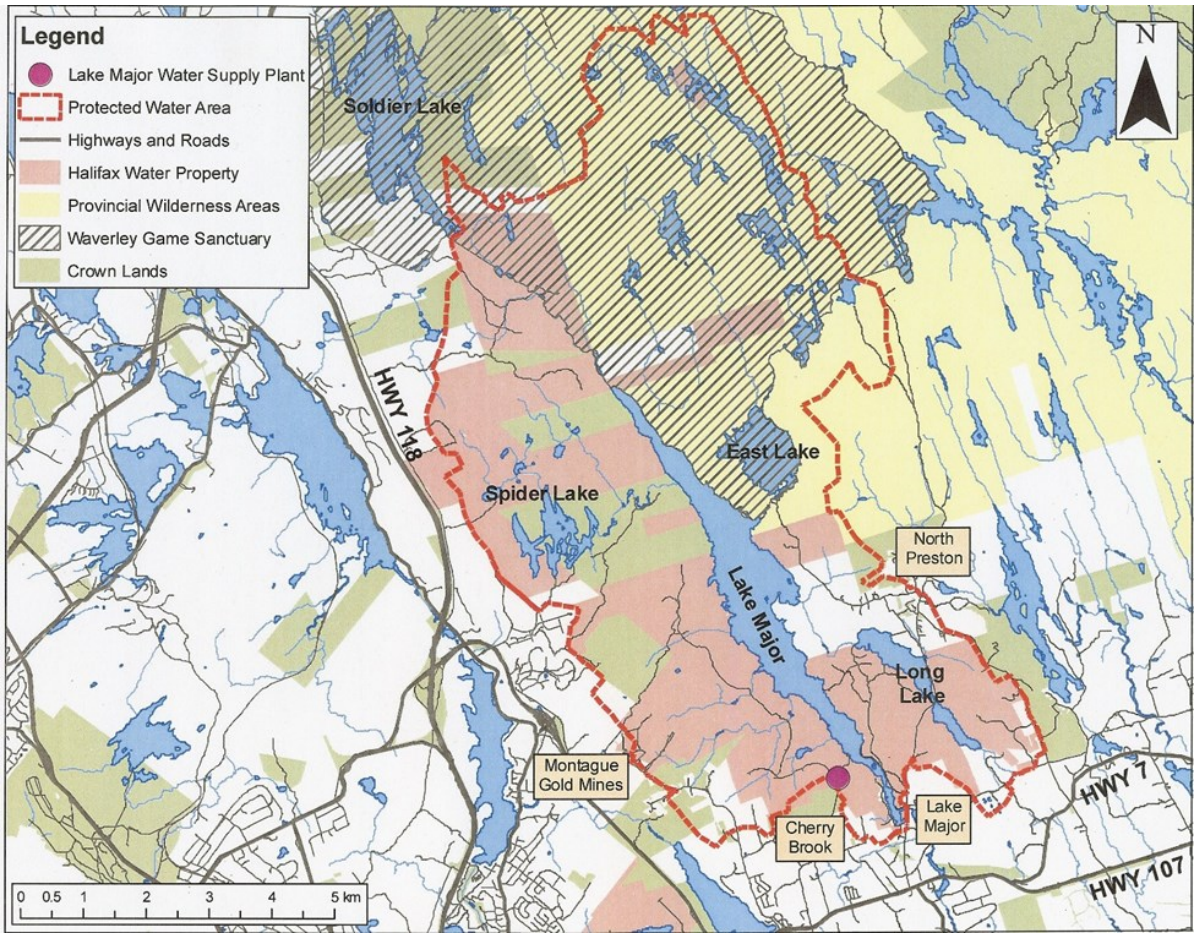


Figure 2.3. Map of the Lake Major Watershed area, featuring location of Lake Major within the watershed (HRM, 2017b).

2.2. CYANOBACTERIA POPULATION ANALISYS

2.2.1. Sample Collection

Due to diel vertical migration and the presence of gas vacuoles in bloom forming cyanobacteria, cyanobacteria of interest were most likely to be in surface water in the late morning (Whitton and Potts, 2000; Ringelberg, 2010; Sainmont *et al.*, 2012). Therefore, samples for cyanobacteria identification were planned to have been collected during the late morning/early afternoon. Unfortunately, due to scheduling conflicts for transportation to and from the lakes, as well as restricted access to water crafts, samples were not always collected during the late morning/early afternoon. They were, however, always collected during daylight hours.

According to the World Health Organization (WHO) (1999), photosynthetic organisms should be collected in amber glass containers for species identification, and to be kept cool to restrict photosynthetic and metabolic activity. Using glass containers also helps keep the organism from adhering to the inner container walls. WHO (1999), also suggests that for microscopic identification and quantification of cyanobacteria that is representative of the population present in each lake, 100 mL of water is required from each designated sample site. Therefore, hypothetically, collecting more than 100 mL of water per site would increase the likelihood of gathering enough genetic material for cyanobacteria identification that would be representative of the population present. However, as previously mentioned, there was limited space available in each water craft used. Therefore, for the sake of consistency, it was determined that 200-250 mL per sampling site was needed for cyanobacteria population analysis.

Pre-washed 500 mL amber glass bottles that had been washed in the dishwasher were used to collect most of the water samples needed for cyanobacteria population analysis. This provided sufficient sample volumes. However, once again, due to limited space available in each water craft used, and because 500 mL amber glass bottles were not always available, 500 mL or 1 L polyethylene bottles were also used. In some

instances, the excess water intended for water quality analysis which had been collected in 1 L polyethylene bottles, was used for cyanobacteria population analysis.

2.2.2. Isolation and Filtration

Cyanobacteria isolation was primarily performed within a Thermo Fisher Scientific 1300 Series A2 biological safety cabinet (BSC) (Thermo Fisher Scientific, Marietta, OH) to avoid sample contamination, and performed as soon as possible. All glassware, filter paper, tinfoil and tweezers were sterilized in an autoclave prior to use. Filtration procedures that needed to take place outside of the BSC were kept to an absolute minimum, also to avoid contamination.

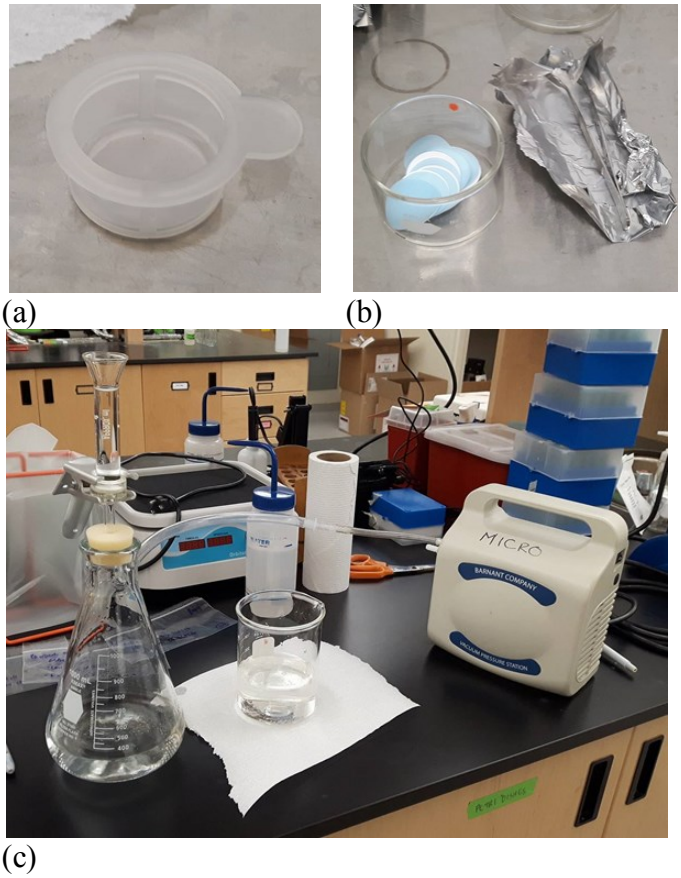


Figure 2.4. (a) A 70µm nylon mesh filter; (b) autoclaved tweezers and 0.22 µm PES filter discs; (c) set up used for vacuum filtration of sample water through a PES filter.

Cyanobacteria range in size between 0.5 µm to 40 µm (Maier *et al.*, 2000). Therefore, water samples collected for cyanobacteria identification were initially decanted through a 70 µm Falcon nylon mesh cell strainer to remove larger debris. The filtrate was then filtered through a 0.22 µm Millipore Express Plus PES filter disc via vacuum filtration through a glass microanalysis filter holder assembly (Advantec MFS, Inc., Pleasanton, CA). Polyethersulfone (PES) filters were chosen because of their low protein biding properties, which would allow for easier DNA extraction (MO BIO Laboratories, 2016a,b). Theoretically, this process would isolate cyanobacteria of all sizes onto the filter disc. Filters and microanalysis filter holder assembly can be seen in Figure 2.4.

2.2.3. Storage

Once the filter had become clogged (or the water sample ran out), the filter disc was removed from the glass filter holder assembly, and wrapped in sterile tinfoil, like the filter seen in Figure 2.5. It was then placed into a marked plastic baggie and kept in the freezer at -20°C until ready for DNA extraction.

Figure 2.5. Example of a PES filter post vacuum filtration being prepared for storage.

2.2.4. DNA Extraction

Prior to extraction, tweezers for handling filters, a scoopula spatula for weighing out glass beads, weigh dishes to cut the filters on, a jar of 1.5 mL centrifuge tubes, and boxes of pipette tips were autoclaved. Frozen filters were removed from the freezer and

thawed in the BSC. Once thawed, DNA extraction was performed using an Omega Bio-tek E.Z.N.A. Water DNA Kit (Omega Bio-tek Inc., Norcross, GA), following the procedure laid out by Omega Bio-tek (2014) in their product manual with minor adjustments.

For example, the protocol called for centrifugation at 4,000 x *g* in Step 9 of the procedure. However, the centrifuge available for use, a Thermo IEC Centra CL2 centrifuge (Thermo Fisher Scientific, Marietta, OH), could only reach a top speed of 3,500 x *g*. Thus, centrifugation at 3,500 x *g* at Step 9 was followed instead of centrifugation at 4,000 x *g*. Another adjustment made concerned centrifugation tubes. In Step 10, the procedure required that the cleared supernatant from Step 9 be transferred to a new, 50 mL centrifuge tube. However, due to the large number of samples that needed to be processed, 15 mL centrifuge tubes were used at Step 10. The Thermo IEC Centra CL2 centrifuge had holders for four 50 mL centrifuge tubes or eight 15 mL centrifuge tubes. Using 15 mL centrifuge tubes at this point decreased the amount of time required to perform extractions and increased the number of samples that could be processed in a day. These changes whould have little to no effect on results.

Following a final centrifugation 14,000 x *g* using a Hettich Mikro 200 centrifuge (Hettich Lab Technology, Beverly, MA), the 1.5 mL microcentrifuge tube containing the extracted DNA was capped and stored in the freezer at -20°C until ready for submission.

2.2.5. Cyanobacteria DNA Sequencing

Extracted cyanobacteria DNA was sent to the Centre for Comparative Genomic and Evolutionary Bioinformatics, Integrated Microbiome Resource laboratory (CGEB-IMR) at Dalhousie University, for high throughput sequencing.

Primers used for taxa identification were the traditional 16S rRNA universal bacterial primers 515F-926R (targeting the V4-V5 regions), and the primer pair CYA359F-CYA781R (targeting the V3-V4 regions) to specifically amplify cyanobacteria

genetic material (Nübel *et al.*, 1997; Walters *et al*., 2015). Sequencing procedures and library composition followed methods outlined by Comeau *et al*. (2017). In summary, two separate DNA dilutions per sample were amplified via polymerase chain reaction (PCR) using a high fidelity polymerase, verified by gel, purified, normalized and pooled for sequencing using a 300+300 bp v3 kit on an Illumina MiSeq.

After sequencing, low-quality and chimeric reads were removed before samples underwent open-reference operational taxonomic unit (OTU) picking at an identity level of 97% (Comeau *et al*., 2017). OTU's were associated with cyanobacteria taxa using QIIME's default GreenGenes 13.8, which dates to 2013. Following sample normalization (i.e. equal number of reads per sample), OTU tables, taxonomic summaries, alpha-diversity rarefaction plots, and beta-diversity plots were generated. However, for this project, only OTU tables and taxonomic summaries were focused on.

Once results were received, cyanobacteria isolates were organized by their affiliated orders, except for OTU's associated with the class Chloroplast. Organisms within this class are named according to the eukaryotes they reside in at the order level and down. This would create confusion when conducting population analysis, so it was decided to simply organize the chloroplasts by their class, and not their orders. Cyanobacteria isolates were converted from either percentages or OTU counts, into decimals. From these decimals, proportion of reads in percentages could be established by determining the ratio between an isolate detected and the total number of isolates detected for a given month. An example of this procedure can be seen in Figure 2.6.

$$\left(\frac{\text{0.55 OTU's associated with the order Nostocales in July}}{\text{5.00 OTU's detected in July}}\right) \times 100 = \text{OTU's associated with the order Nostocales represented 11\% of all reads in July.}$$

Figure 2.6. Example calculation for the determination of ratios between detected cyanobacteria isolates.

Once proportions were determined, column graphs showing monthly proportion of reads (%) for each order/class of cyanobacteria detected per lake were made. Average proportion of reads were also determined, but were not graphed.

2.3. WATER CHEMISTRY

2.3.1. Sample Collection

It was determined that ten water quality parameters were to be monitored: pH, total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (TN), total phosphorous (TP), turbidity, UV_{254}, specific ultraviolet absorbance (SUVA), colour, and alkalinity.

According to the American Public Health Association (APHA), American Water Works Association (AWWA), and the Water Environment Federation (WEF) (2012), of these parameters, 40 mL was required to analyse TOC and TN, 40 mL was required to analyse DOC, 10 mL was required to analyse TP, between 50-100 mL was required to analyse turbidity, and 40 mL was required to measure alkalinity. To meet these requirements, as well as have enough left over to conduct analysis on UV_{254}, colour, and to have extra water if needed, 1 L of water was collected at each sampling site using a 1 L polyethylene bottle that had been pre-washed in the dishwasher and rinsed with ethanol prior to use in the field.

The measurement of pH was performed *in situ,* while the measurement of TOC, DOC, TN, TP, turbidity, UV_{254}, colour, and alkalinity were performed in the lab within 24 to 48 hours after collection. Laboratory analysis followed procedures laid out by the APHA, AWWA and WEF (APHA *et al*, 2012). SUVA was calculated when convenient after DOC and UV_{254} results were determined.

Water chemistry data for Fletchers Lake was provided by Michael Brophy (MASc student, Dalhousie University), and water chemistry data for Powder Mill Lake came

from the author. Water chemistry data for Pockwock Lake was provided by Dave Redden (MASc student, Dalhousie University), while water chemistry data for Lake Major was provided by Lindsay Anderson (PhD student, Dalhousie University) and Halifax Water.

2.3.2. Alkalinity and pH

Roughly 40mL of raw water sample was used to measure alkalinity with a Mettler Toledo T50 automated titrator (Mettler Toledo, Columbus, OH) that had a minimum detection limit (MDL) of 2.44 $CaCo_3$ mg/L, and had been calibrated to stock solutions with pH of 4, 7 and 10, prior to use. The measurement of pH was performed *in situ* by submerging a YSI 650 MDS multiparameter sonde, model number 12C100865 (YSI Inc., Yellow Springs, OH), over the side of the water craft being used, and recording the data presented on screen when the readings stabilized. The sonde was calibrated for pH once a month.

2.3.3. Total Organic Carbon and Dissolved Organic Carbon

Raw water samples intended for TOC measurement were poured head-space free into 40 mL pre-cleaned glass vials and preserved with two to three drops of concentrated phosphoric acid. Samples intended for DOC measurement were first filtered through a 0.45µm GVS UltraSep PES filter disc, which had been pre-rinsed with 500mL of Milli-Q water. Filtered samples were then poured head-space free into 40mL pre-cleaned glass vials as well, and preserved with two to three drops of concentrated phosphoric acid. TOC and DOC were measured using a Shimadzu TOC-V CPH analyzer with an ASI-V autosampler (Shimadzu Corporation, Kyoto, Japan), that had an MDL of 0.25 ppm.

2.3.4. Total Nitrogen and Total Phosphorus

Raw water samples intended for TN measurement were poured head-space free into 40mL pre-cleaned glass vials and preserved with two to three drops of concentrated phosphoric acid. TN was measured using a Shimadzu TNM-1 total nitrogen measuring

unit also hooked up to the ASI-V autosampler, which had an MDL of 0.12 ppm. About 10mL of raw water sample intended for TP measurement was poured into pre-cleaned Fisher Scientific general purpose polypropylene test tubes and preserved with two to three drops of trace metal grade nitric acid. TP was measured using a Thermo Scientific XSeries 2 inductively coupled plasma mass spectrometer (ICP-MS) with an ASX-520 autosampler, which had an MDL of 10 ppb.

2.3.5. UV_{254}, Specific Ultraviolet Absorbance, Turbidity and Colour

Raw water samples were used to measure turbidity with a HACH 2100AN turbidometer (Hach Company, Loveland, CO), which had been initially zeroed with Milli-Q water and had an MDL of 0.12 NTU. In contrast, UV_{254} and colour water samples were first filtered through a 0.45µm GVS UltraSep PES filter disc, which had been pre-rinsed with 500mL of Milli-Q water. UV_{254} and colour were measured with a HACH DR5000 spectrophotometer 190 nm. SUVA was calculated when convenient after DOC and UV_{254} were determined.

2.4. ENVIRONMENT DATA

Hourly environment data for temperature and wind speed were collected from the Bedford Range weather station, located at 44°44'45.09"N, 63°39'42.08"W, in Bedford, NS, near the Sackville River and Bicentennial Drive overpass. Data was acquired through the Environment Canada website (GC, 2016b). Daily accumulated precipitation data was collected from the Weather Network website for Bedford, NS (The Weather Network (TWN), 2017).

CHAPTER 3 RESULTS

3.1. CYANOBACTERIA POPULATION ANALYSIS

3.1.1. Fletchers Lake

A total number of 19 samples were collected from Fletchers Lake on September 14 and 29, and October 19, of 2016, for cyanobacteria population analysis. However, of the 19 samples, only 14 successfully amplified during PCR. See Appendix A for details regarding sample sites, collection dates, and sample amplification success.

Figure 3.1. depicts the overall proportion of all cyanobacteria OTU reads detected in the samples, by month. Reads are organized by their affiliated orders, except for OTU's affiliated with chloroplasts, which are organized by their associated class of chloroplast instead of their orders. See Appendix A for tabulated proportion of reads per month and details concerning order composition per month.

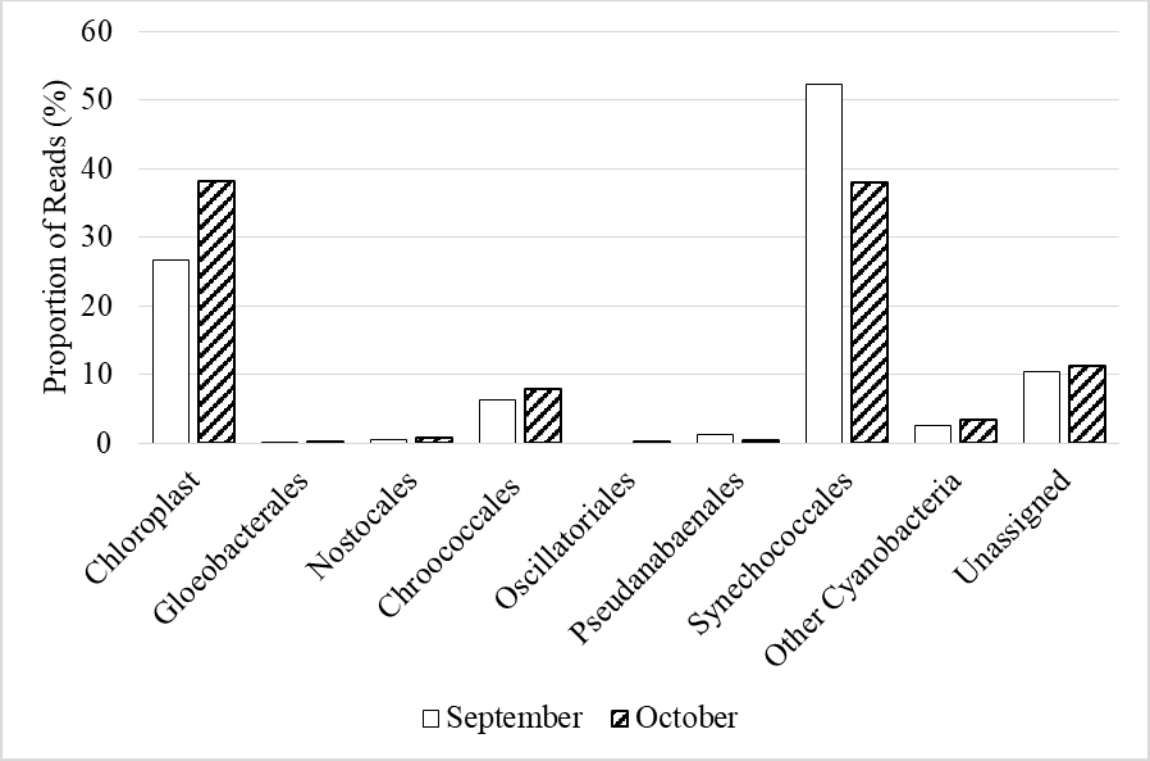


Figure 3.1. Overall proportion of OTU reads detected in samples from Fletchers Lake, for September and October. See Appendix A for tabulated proportion of reads per month and order composition per month.

Overall, OTU's that were associated with the order Synechococcales made up 52% of reads in September (mean, 50%), then decreased to 38% in October (mean, 35%). OTU's associated with the class Chloroplast represented 27% of the reads in September (mean, 28%) and increased to 38% of the reads in October (mean, 38%). OTU's associated with unassigned OTU's represented 10% of reads in September (mean, 12%), and 11% in October (mean, 14%). OTU's associated with the order Chroococcales represented 6.3% of reads in September (mean, 6.3%), and 7.9% of reads in October (mean, 7.4%). OTU's associated with the orders Gloeobacterales, Nostocales, Oscillatoriales, Pseudanabaenales and other cyanobacteria not designated to a particular order, represented less than 5% each (overall and on average), of all OTU's detected in September and October.

3.1.1.1. Class Chloroplast

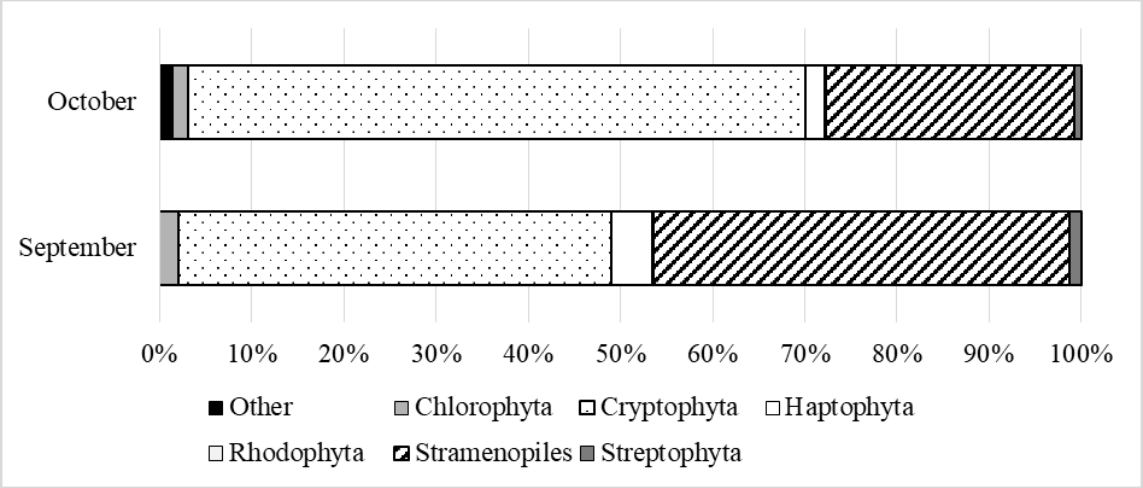


Figure 3.2. Taxa composition for the class Chloroplast for Fletchers Lake samples.

As seen in Figure 3.2., OTU's associated with Cryptophyta made up 47% of the Chloroplast reads in September (mean, 40%), then increased to 67% in October (mean, 49%). OTU's associated with Stramenopiles represented 45% of Chloroplast reads in September (mean, 49%), then decreased to 27% representation in October (mean, 41%). OTU's simply associated with the class Chloroplast without further identification, and OTUs associated with Chlorophyta, Haptophyta, Rhodophyta, and Streptophyta represented less than 5% each (even on average), of all detected OTU's affiliated with the class Chloroplast in September and October. The exception being OTU's associated with Haptophyta, which had a mean of 6.8% in September.

3.1.1.2. Order Gloeobacterales

OTU's associated with the genus *Gloeobacter* made up 100% of the Gloeobacterales reads in September (mean, 17%) and remained at 100% of reads for October (mean, 38%).

3.1.1.3. Order Nostocales

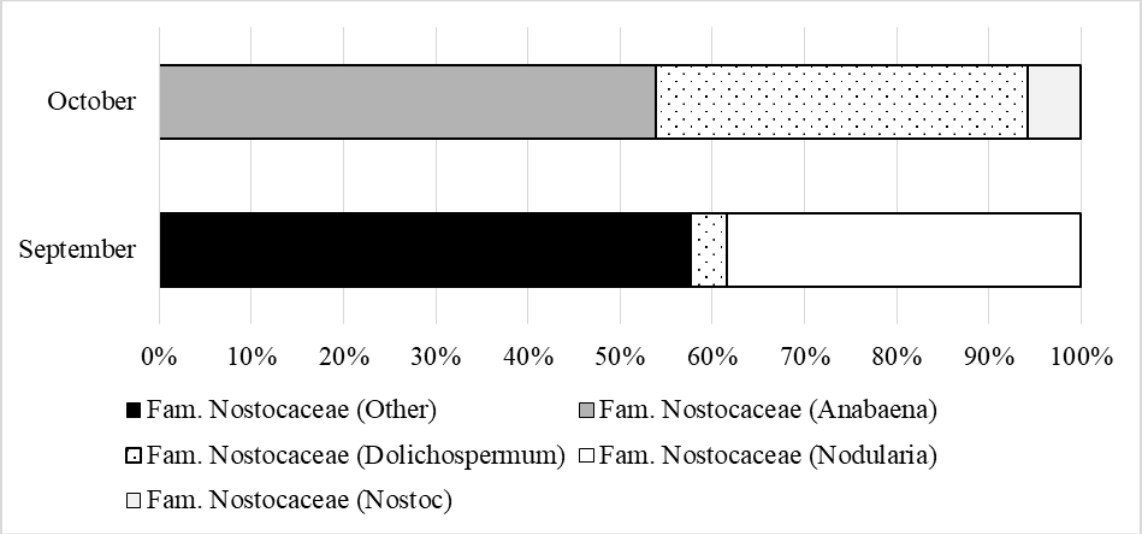


Figure 3.3. Taxa composition for the order Nostocales for Fletchers Lake samples.

As seen in Figure 3.3., OTU's simply associated with the family Nostocaceae without further identification made up 58% of Nostocales reads in September (mean, 10%), but were not detected in October. OTU's associated with the genus *Nodularia* made up 38% of Nostocales reads in September (mean, 6.7%), yet were undetected in October. OTU's associated with the genus *Dolichospermum* made up 3.9% of the Nostocales reads in September (mean, 17%), then increased to 40% in October (mean, 8.9%). OTU's associated with the genus *Anabaena* were not detected in September, but were found to represent 54% of Nostocales reads in October (mean, 29%). OTU's associated with the genus *Nostoc* were not detected in September, but did, however, represent 5.8% of Nostocales reads in October (mean, 13%).

3.1.1.4. Order Chroococcales

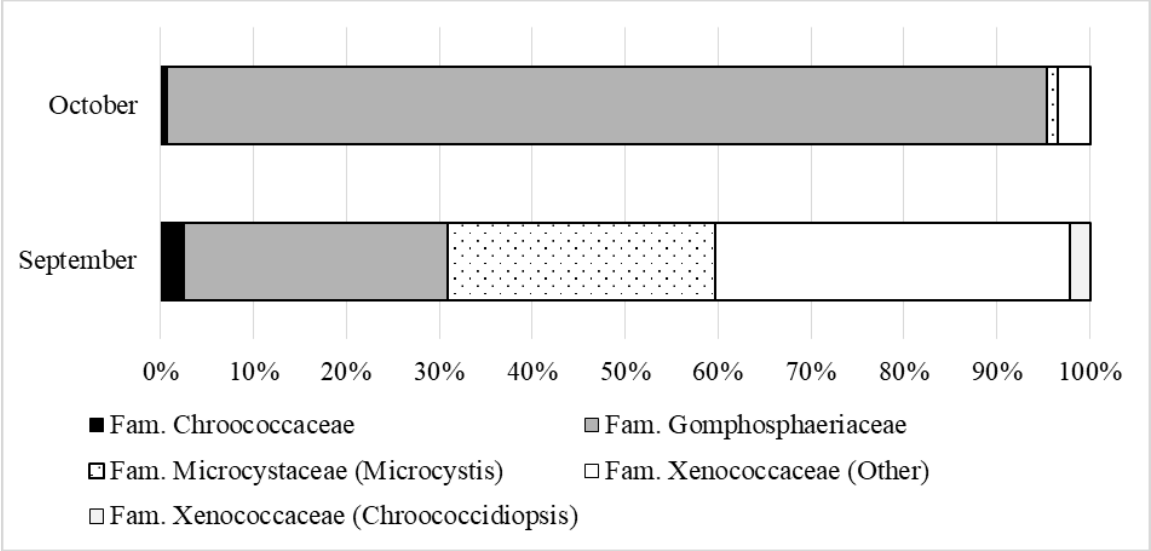


Figure 3.4. Taxa composition for the order Chroococcales for Fletchers Lake samples.

As seen in Figure 3.4., OTU's simply associated with the family Xenococcaceae without further identification made up 38% of the Chroococcales reads in September (average, 32%), but decreased to 3.4% in October (average, 15%). OTU's associated with the genus *Microcystis* made up 29% of the Chroococcales reads in September (average, 21%), then decreased to 1.2% of reads in October (average, 5.0%). OTU's associated with the family Gomphosphaeriaceae made up 28% of Chroococcales reads in September (average, 37%), then increased to represent 95% of reads in October (average, 52%). OTU's associated with the family Chroococcaceae and the genus *Chroococcidiopsis* represented less than 5% each (even on average), of all detected OTU's affiliated with the order Chroococcales in September and October. The exception being OTU's associated with the family Chroococcaceae, which had a mean of 9.6% in September.

3.1.1.5. Order Oscillatoriales

OTU's associated with the genus *Phormidium* were not detected in September. However, in October, they represented 100% of Oscillatoriales reads (mean, 25%).

3.1.1.6. Order Pseudanabaenales

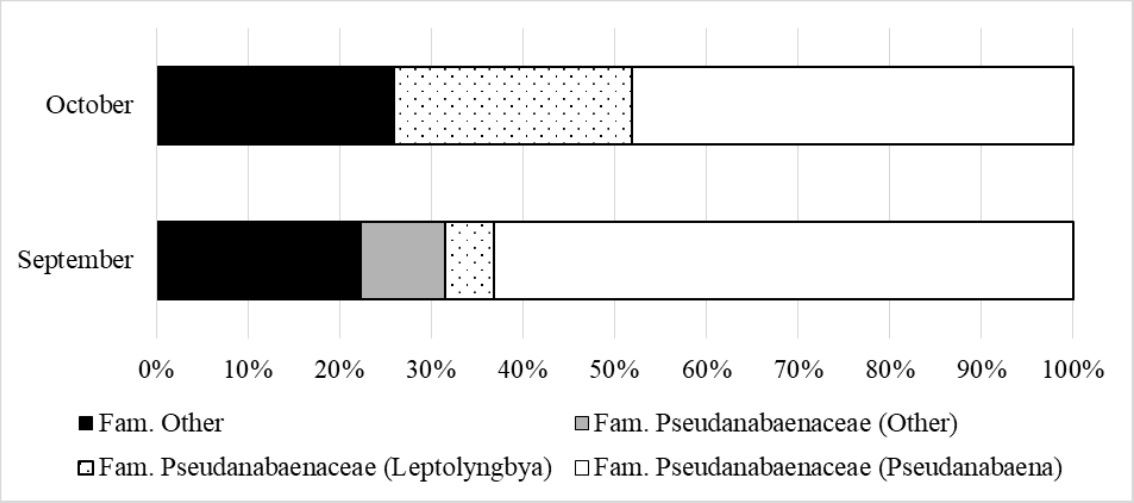


Figure 3.5. Taxa composition for the order Pseudanabaenales for Fletchers Lake samples.

As seen in Figure 3.5., OTU's associated with the genus *Pseudanabaena* made up 63% of the Pseudanabaenales reads in September (mean, 33%), but decreased to 48% in October (mean, 31%). OTU's simply associated with the order Pseudanabaenales without further identification made up 22% of Pseudanabaenales reads in September (mean, 13%), the increased to 26% representation in October (mean, 8.0%). OTU's simply associated with the family Pseudanabaenaceae without further identification made up 9.2% of the Pseudanabaenales reads in September (mean, 2.2%), and were not detected in October. OTU's associated with the genus *Leptolyngbya* represented 5.3% of the Pseudanabaenales reads in September (mean, 1.2%), then increased to 26% representation in October (mean, 23%).

3.1.1.7. Order Synechococcales

OTU's associated with the genus *Synechococcus* made up 99.97% of the Synechococcales reads in September (mean, 83%), then 99.96% of reads in October (mean, 94%). OTU's associated with the family Chamaesiphonaceae made up 0.033% of

the Synechococcales reads in September (mean, 17%), and 0.035% in October (mean, 6.3%).

3.1.2. Powder Mill Lake

A total number of 55 samples were collected from Powder Mill Lake on July 12 and 28, August 16, 24 and 30, September 10, 16 and 23, October 2, 18 and 30, and November 7, 13 and 21, of 2016, for cyanobacteria population analysis. See Appendix B for details regarding sample sites, collection dates, and sample amplification success.

Figure 3.6. depicts the overall proportion of all cyanobacteria OTU reads detected in the samples, by month. Reads are organized by their affiliated orders, except for OTU's affiliated with chloroplasts, which are organized by their associated class of chloroplast instead of their orders. See Appendix B for tabulated proportion of reads per month and details concerning order composition per month.

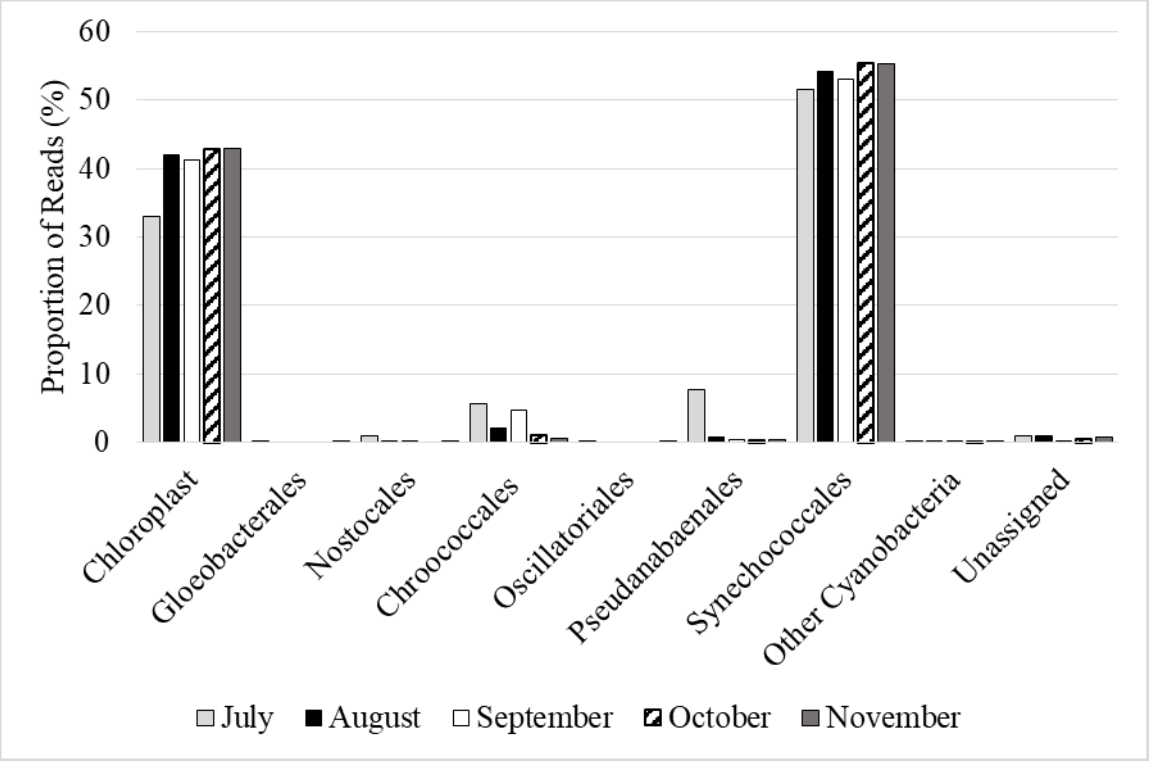


Figure 3.6. Overall proportion of OTU reads detected in samples from Powder Mill Lake, for July, August, September, October and November. See Appendix B for tabulated proportion of reads per month and order composition per month.

Overall, OTU's that were associated with the order Synechococcales made up 52% of the reads in July (mean, 52%), increased to 54% in August (mean, 54%), decreased slightly to 53% in September (mean, 53%), increased to 55% in October (mean, 55%) and 55% in November (mean, 55%). OTU's associated with the class Chloroplast represented 33% of the reads in July (mean, 33%), increased to 42% in August (mean, 42%), decreased slightly to 41% in September (mean, 41%), increased to 43% in October (mean, 43%), and 43% in November (mean, 43%). OTU's that were associated with the order Gloeobacterales, Nostocales, Chroococcales, Oscillatoriales, Pseudanabaenales, unassigned OTU's and other cyanobacteria not designated to a particular order represented less than 5% each (overall and on average), of all OTU's detected in July, August, September, October, and November. Exceptions being: OTU's affiliated with the order Chroococcales, which represented 5.6% of reads in July (mean,

5.6%), and those associated with the order Pseudanabaenales, which represented 7.6% of reads in July (mean, 7.6%).

3.1.2.1. Class Chloroplast

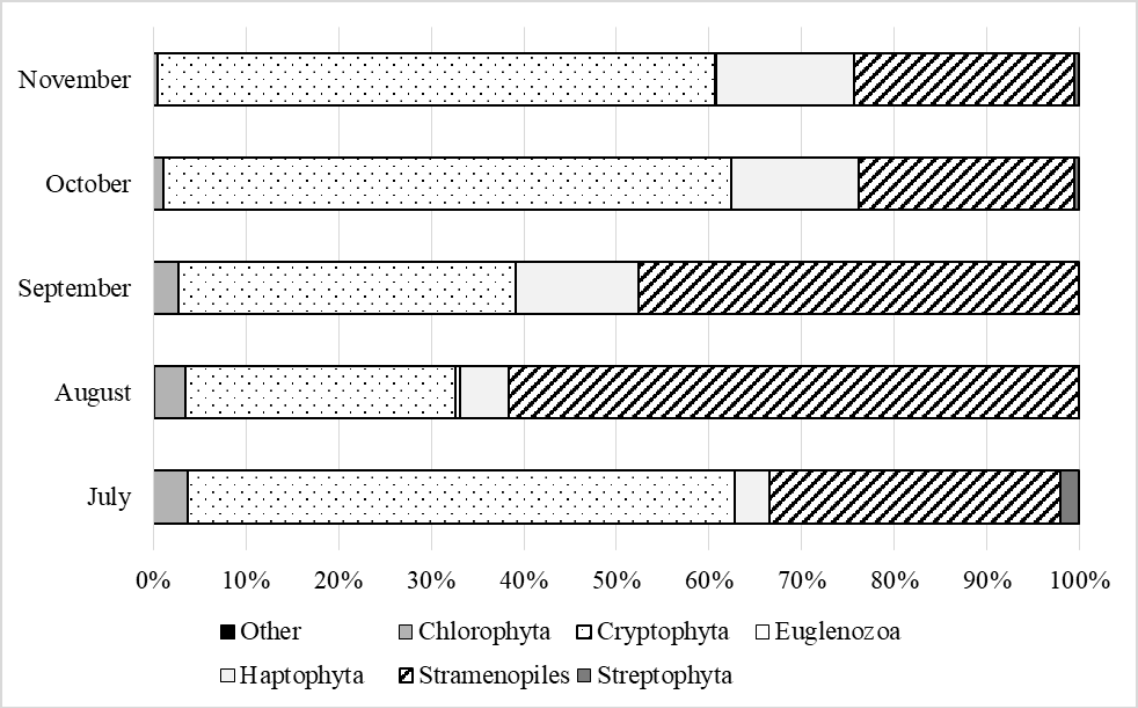


Figure 3.7. Taxa composition for the class Chloroplast for Powder Mill Lake samples.

As seen in Figure 3.7., OTU's associated with Cryptophyta made up 59% of the Chloroplast reads in July (mean, 52%), decreased to 29% in August (mean, 29%), increased to 36% in September (mean, 37%), increased to 61% of reads in October (mean, 62%), then decreased slightly to 60% in November (mean, 60%). OTU's associated with Stramenopiles represented 31% of Chloroplast reads in July (mean, 38%), increased to 62% of reads in August (mean, 61%), decreased to 48% in September (mean, 47%), decreased again to 23% in October (mean, 23%), then increased slightly to 24% of reads in November (mean, 24%). OTU's associated with Haptophyta made up 3.7% of the Chloroplast reads in July (mean, 4.4%), increased to 5.3% in August (mean, 5.4%), increased again to 13% in September (mean, 13%), increased slightly to 14% in October (mean, 14%), and continued to increase to 15% in November (mean, 15%). OTU's simply associated with the class Chloroplast without further identification and

OTU's affiliated with Chlorophyta, Euglenozoa and Streptophyta represented less than 5% each (even on average), of all detected OTU's affiliated with the class Chloroplast in July, August, September, October and November.

3.1.2.2. Order Gloeobacterales

OTU's associated with the genus *Gloeobacter* represented 100% of the Gloeobacterales reads in July (mean, 14%) and in November (mean, 8.3%). They were not detected in August, September or October.

3.1.2.3. Order Nostocales

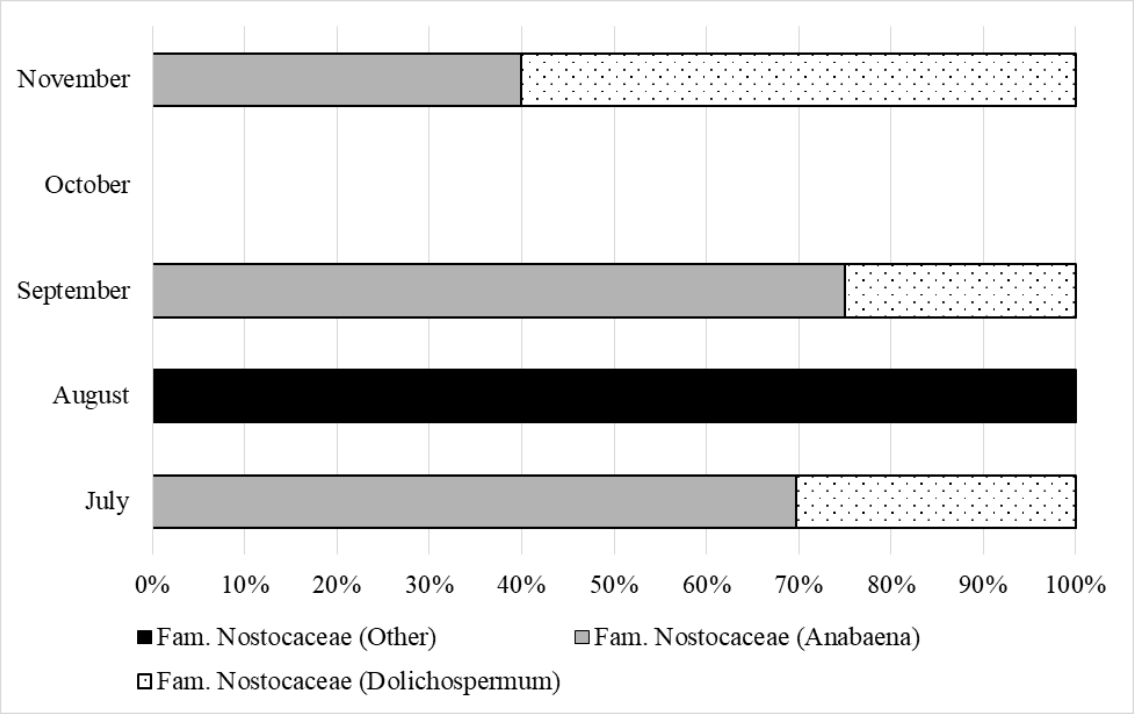


Figure 3.8. Taxa composition for the order Nostocales for Powder Mill Lake samples.

As seen in Figure 3.8., OTU's associated with the genus *Anabaena* represented 70% of Nostocales reads in July (mean, 48%), were not detected in August, represented 75% of reads in September (mean, 78%), were not detected in October, but did represented 40% of reads in November (mean, 13%). OTU's associated with the genus

Dolichospermum represented 30% of Nostocales reads in July (mean, 24%), were not detected in August, represented 25% of reads in September (mean, 22%), were not detected in October, and represented 60% of reads in November (mean, 21%). OTU's simply associated with the family Nostocaceae without further identification made up 100% of Nostocales reads in August (mean, 17%), but were not detected for the remainder of the season.

3.1.2.4. Order Chroococcales

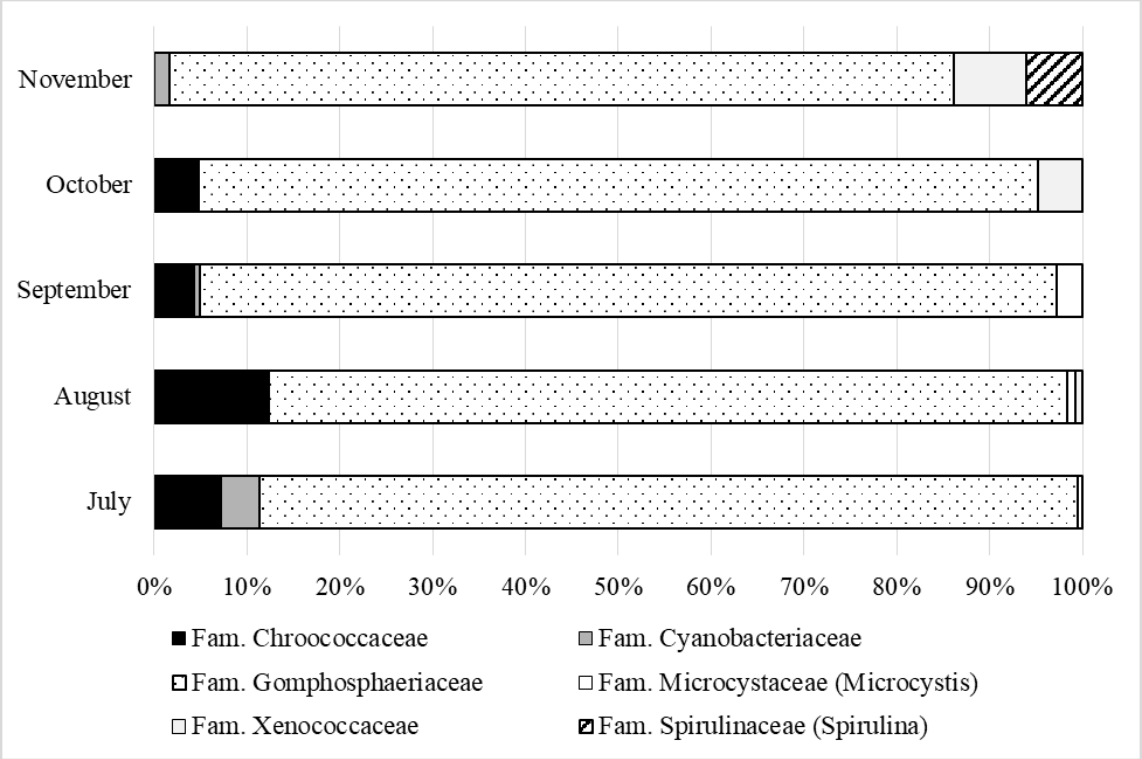


Figure 3.9. Taxa composition for the order Chroococcales for Powder Mill Lake samples.

As seen in Figure 3.9., OTU's associated with the family Gomphosphaeriaceae made up 88% of the Chroococcales reads in July (mean, 87%), decreased to 86% in August (mean, 85%), increased to 92% in September (mean, 91%), decreased to 90% in October (mean, 89%), then decreased to 85% in November (mean, 73%). OTU's associated with the family Chroococcaceae represented 7.2% of Chroococcales reads in July (mean, 7.1%), decreased to 12% in August (mean, 13%), decreased again to 4.3% in

September (mean, 4.6%), increased slightly to 4.8% in October (mean, 5.0%), then were not detected in November.

OTU's that were associated with the family Cyanobacteriaceae and Xenococcaceae, and the genus *Microcystis* and *Spirulina*, represented less than 5% each (overall and on average), of all OTU's associated with the order Chroococcales detected in July, August, September, October, and November. Exceptions being: OTU's affiliated with the family Cyanobacteriaceae, which represented a mean of 5.2% of Chroococcales reads in July; OTU's affiliated with the family Xenococcaceae had a mean of 5.6% in October and represented 7.8% of reads in November (mean, 8.6%); and OTU's associated with the genus *Spirulina* represented 6.1% of reads in November (mean, 8.9%).

3.1.2.5. Order Oscillatoriales

OTU's affiliated with the genus *Phormidium* represented 100% of the Oscillatoriales reads in July (mean, 14%), and in November (mean, 8.3%). They were not detected in August, September or October.

3.1.2.6. Order Pseudanabaenales

OTU's associated with the genus *Pseudanabaena* made up 98.7% of Pseudanabaenales reads in July (mean, 94.5%), increased to 100% in August (mean, 100%), decreased to 90% in September (mean, 94.4%), increased to 100% in October (mean, 75%), and decreased to 97.6% in November (mean, 75%). OTU's associated with the order Pseudanabaenales without further identification, the family Pseudanabaenaceae without further identification, and the genus *Leptolyngbya*, represented less than 5% each (overall and on average), of all OTU's associated with the order Pseudanabaenales detected in July, August, September, October, and November. Exceptions being: OTU's affiliated with the family Pseudanabaenaceae without further identification represented an average of 8.3% of Pseudanabaenales reads in November, and OTU's associated with

the genus *Leptolyngbya* represented a mean of 5.1% of reads in July, and represented 10% of reads in September (mean, 5.6%).

3.1.2.7. Order Synechococcales

OTU's associated with the genus *Synechococcus* represented 99.94% of Synechococcales reads in July (mean, 99.95%), increased slightly to 99.97% in August (mean, 99.97%), increased again to represent 100% of reads in September (mean, 100%) and October (mean, 100%), then decreased to 99.95% in November (mean, 99.95%). OTU's affiliated with the family Synechococcaceae without further identification and with the genus *Prochlorococcus* represented less than 1% each (overall and on average), of all detected OTU's associated with the order Synechococcales in July, August, September, October, and November.

3.1.3. Pockwock Lake

A total of 114 samples were collected from Pockwock Lake on July 19 and 21, August 2, 4, 16, 18, 29 and 30, September 13 and 14, and October 5 and 12, of 2016, for cyanobacteria population analysis. However, of the 114 samples, only 35 successfully amplified during PCR. See Appendix C for details regarding sample sites, collection dates, and sample amplification success.

Figure 3.10. depicts the overall proportion of all cyanobacteria OTU reads detected in the samples, by month. Reads are organized by their affiliated orders, except for OTU's affiliated with chloroplasts, which are organized by their associated class of chloroplast instead of their orders. See Appendix C for tabulated proportion of reads per month and details concerning order composition per month.

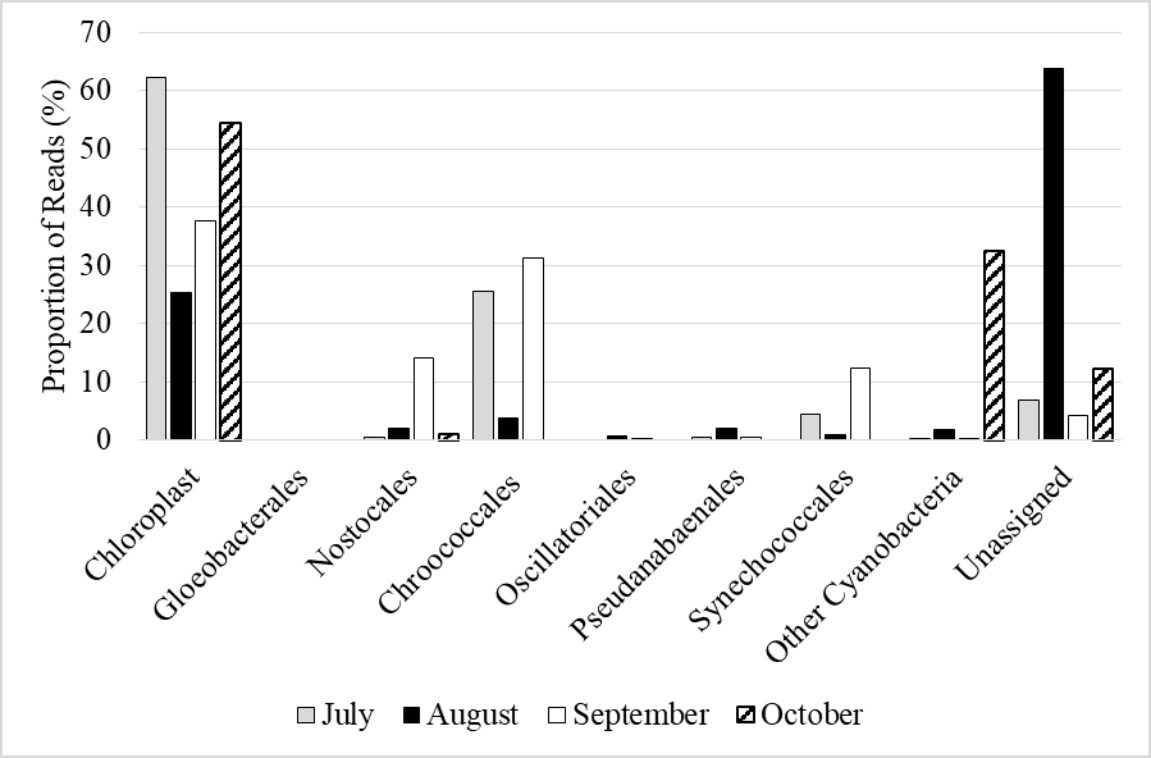


Figure 3.10. Overall proportion of OTU reads detected in samples collected from in-lake and from tributaries at Pockwock Lake, for July, August, September and October. See Appendix C for tabulated proportion of reads per month and order composition per month.

Overall, OTU's associated with the class Chloroplast represented 62% of the reads in July (mean, 62%), decreased to 25% in August (mean, 25%), increased to 38% in September (mean, 38%), and increased again to 54% in October (mean, 54%). OTU's associated with the order Chroococcales represented 25% of reads in July (mean, 25%), decreased to 3.7% in August (mean, 3.7%), increased to 31% in September (mean, 31%), then were not detected in October. OTU's associated with unassigned OTU's represented 6.9% of reads in July (mean, 6.9%), increased to 64% in August (mean, 64%), decreased to 4.3% in September (mean, 4.3%), then increased to 12% in October (mean, 12%). OTU's associated with the order Gloeobacterales, Nostocales, Oscillatoriales, Pseudanabaenales, Synechococcales, and OTU's associated with other cyanobacteria without further identification represented less than 5% each (overall and on average), of all OTU's detected in July, August, September, and October. Exceptions include: OTU's affiliated with the order Nostocales, which represented 14% of reads in September (mean,

14%), OTU's affiliated with the order Synechococcales which represented 12% of reads in September (mean, 12%), and OTU's affiliated with other cyanobacteria not designated to a particular order which represented 32% of reads in October (mean, 32%).

3.1.3.1. Class Chloroplast

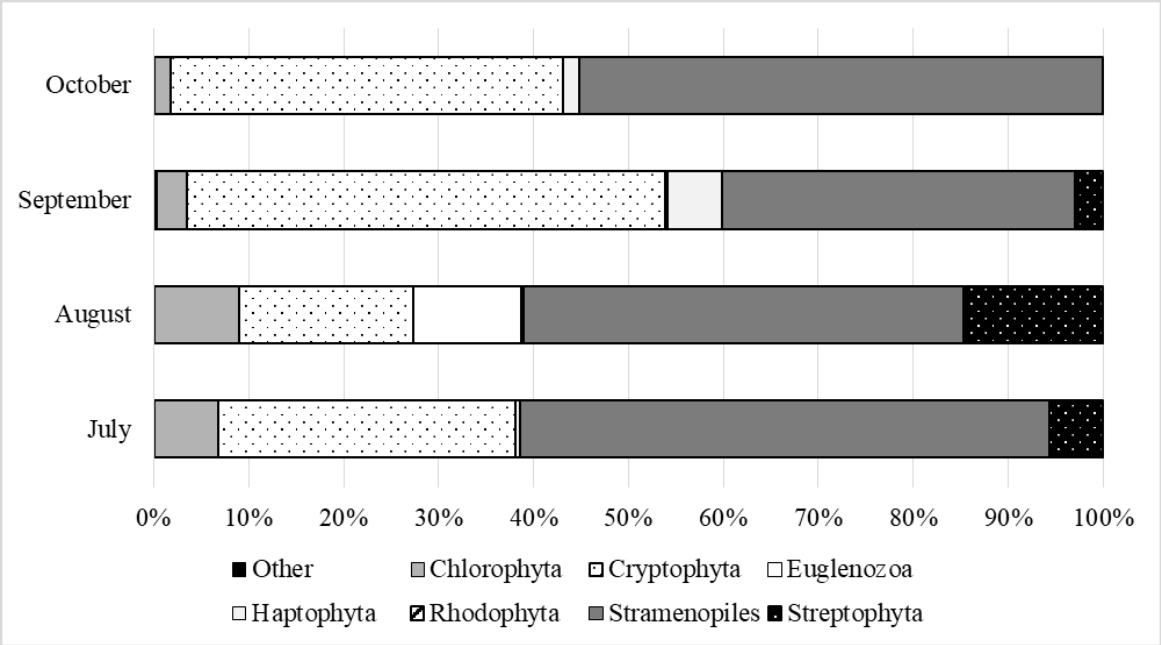


Figure 3.11. Taxa composition for the class Chloroplast for Pockwock Lake samples.

Seen in Figure 3.11., OTU's associated with Stramenopiles represented 56% of Chloroplast reads in July (mean, 52%), decreased to 46% of reads in August (mean, 32%), decreased to 37% in September (mean, 41%), then increased to 55% in October (mean, 50%). OTU's associated with Cryptophyta made up 31% of the Chloroplast reads in July (mean, 37%), decreased to 18% in August (mean, 15%), increased to 50% in September (mean, 44%), but decreased to 41% of reads in October (mean, 48%). OTU's associated with Chlorophyta made up 6.8% of the Chloroplast reads in July (mean, 7.0%), increased to represent 9.0% of Chloroplast reads in August (mean, 5.5%), decreased to 3.2% in September (mean, 4.4%), and decreased again to 1.7% in October (mean, 1.3%). OTU's affiliated with Streptophyta represented 5.7% of Chloroplast reads in July (mean, 3.7%), then increased to 15% in August (mean, 8.5%), decreased to 3.0%

in September (mean, 1.7%), and were not detected in October. OTU's associated with the class Chloroplast without further identification, Euglenozoa, Haptophyta, and Rhodophyta represented less than 5% each of detected OTU's affiliated with the class Chloroplast, in July, August, September, and October. The exceptions being: OTU's associated with Euglenozoa represented 11% of Chloroplast reads in August (mean, 5.4%), and OTU's associated Haptophyta represented 5.7% of Chloroplast reads in September (mean, 7.9%).

3.1.3.2. Order Gloeobacterales

As mentioned previously, there were no detectable OTU's associated with the order Gloeobacterales.

3.1.3.3. Order Nostocales

OTU's affiliated with the genus *Dolichospermum* represented 100% of the Nostocales reads in July (mean, 33%) and August (mean, 14%), then decreased slightly to 99.7% in September (mean, 83%), and were not detected in October. OTU's associated with the genus *Nostoc* were not detected in July or August, but did represent 0.27% of Nostocales reads in September (mean, 0.21%), and increased to 100% representation in October (mean, 50%).

3.1.3.4. Order Chroococcales

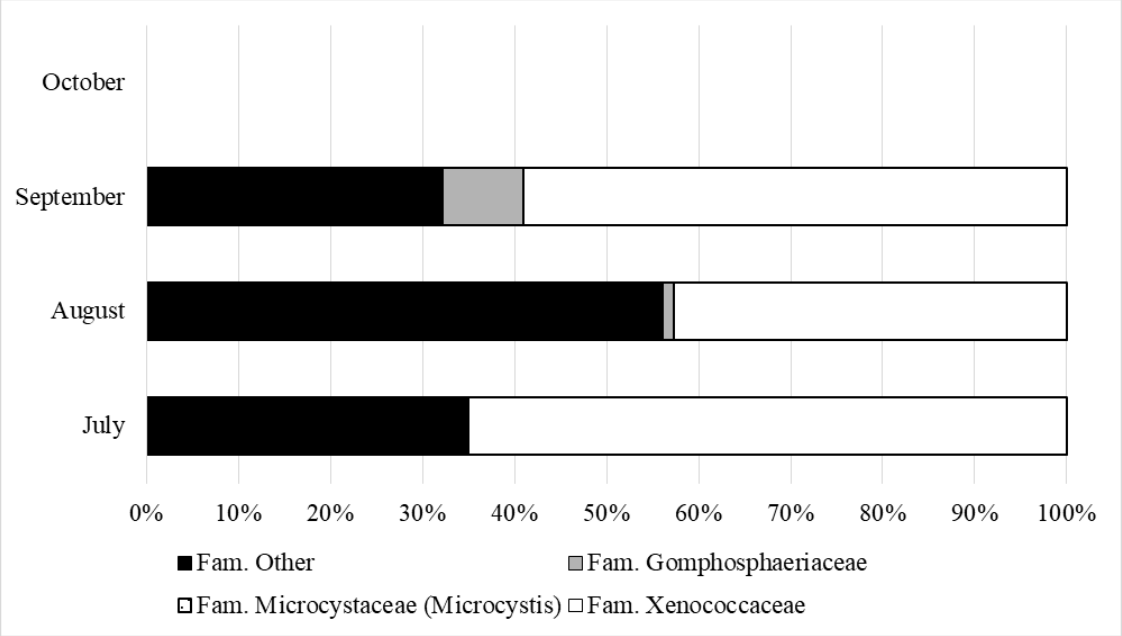


Figure 3.12. Taxa composition for the order Chroococcales for Pockwock Lake samples.

Seen in Figure 3.12., OTU's associated with the family Xenococcaceae represented 65% of Chroococcales reads in July (mean, 33%), decreased to 43% in August (mean, 6.9%), increased to 59% in September (mean, 64%), and were not detected in October. OTU's associated with the order Chroococcales without further identification represented 35% of Chroococcales reads in July (mean, 33%), increased to 56% in August (mean, 17%), decreased to 32% in September (mean, 29%), and were not detected in October. OTU's associated with the family Gomphosphaeriaceae and the genus *Microcystis* represented less than 5% each of detected OTU's affiliated with the order Chroococcales, in July, August, September, and October. The exception being OTU's associated with the family Gomphosphaeriaceae, which represented 8.7% of Chroococcales reads in September (mean, 7.5%).

3.1.3.5. Order Oscillatoriales

OTU's affiliated with the genus *Phormidium* were not detected in July or October, but did represent 99.7% of the Oscillatoriales reads for August (mean, 19%) and 100% in September (mean, 17%).OTU's associated with the family Phormidiaceae without further identification were only detected in August, and represented 0.28% of Oscillatoriales reads for that month (mean, 0.014%).

3.1.3.6. Order Pseudanabaenales

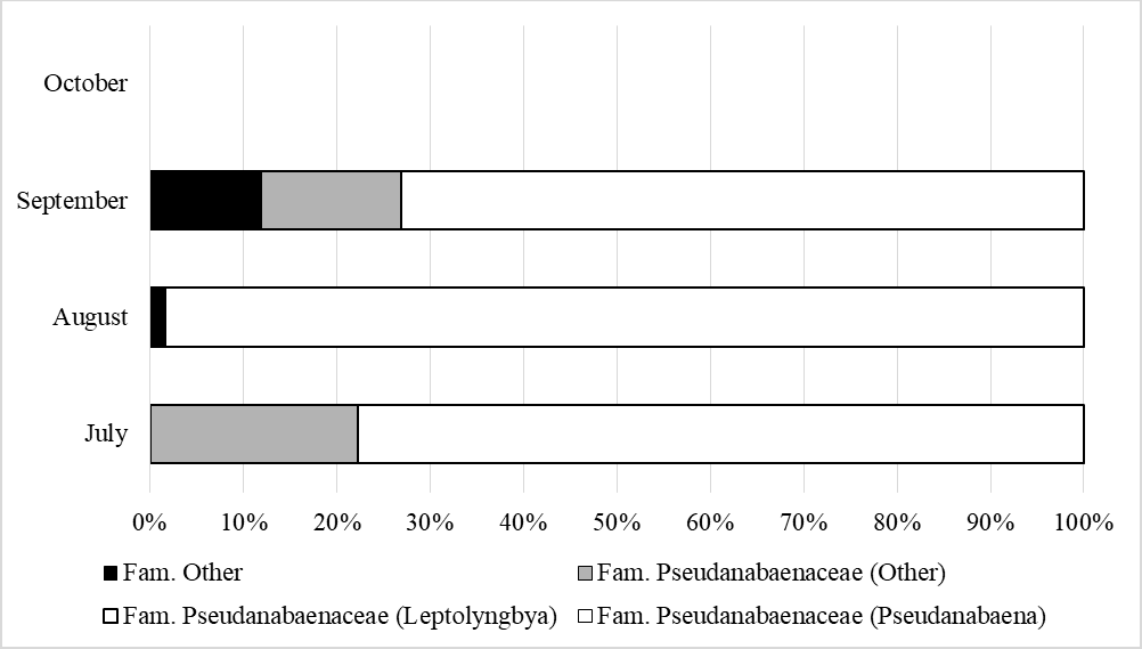


Figure 3.13. Taxa composition for the order Pseudanabaenales for Pockwock Lake samples.

As seen in Figure 3.13., OTU's associated with the genus *Pseudanabaena* represented 78% of Pseudanabaenales reads in July (mean, 13%), increased to 98% of reads in August (mean, 28%), decreased to 73% in September (mean, 47%), and were not detected in October. OTU's affiliated with the family Pseudanabaenaceae without further identification represented 22% of Pseudanabaenales reads in July (mean, 3.7%), were not detected in August, represented 15% of reads in September (mean, 17%), and were not detected in October.

OTU's associated with just the order Pseudanabaenales without further identification and the genus *Leptolyngbya*, represented less than 5% each (overall and on average), of all OTU's associated with the order Pseudanabaenales detected in July, August, September and October. Exception being those OTU's affiliated with the order Pseudanabaenales without further identification, which represented 12% of reads in September (mean, 19%).

3.1.3.7. Order Synechococcales

OTU's associated with the genus *Synechococcus* represented 100% of Synechococcales reads in July (mean, 50%), decreased slightly to 99.8% in August (mean, 24%), increased back to 100% representation in September (mean, 100%), and were not detected in October. OTU's affiliated with the genus *Paulinella* were only detected in August, and represented 0.23% of Synechococcales reads for that month (mean, 0.078%).

3.1.4. Lake Major

A total of seven samples were collected from Lake Major, and were collected on June 23, July 28 and October 19, of 2016, for cyanobacteria population analysis. However, of the seven samples, only two successfully amplified during PCR. The two successful samples were both collected on July 28. See Appendix D for details regarding sample sites, collection dates, and sample amplification success.

Figure 3.14 depicts the overall proportion of all cyanobacteria OTU reads detected in the samples. Reads are organized by their affiliated orders, except for OTU's affiliated with chloroplasts, which are organized by their associated class of chloroplast instead of their orders. See Appendix D for tabulated proportion of reads per month and details concerning order composition per month.

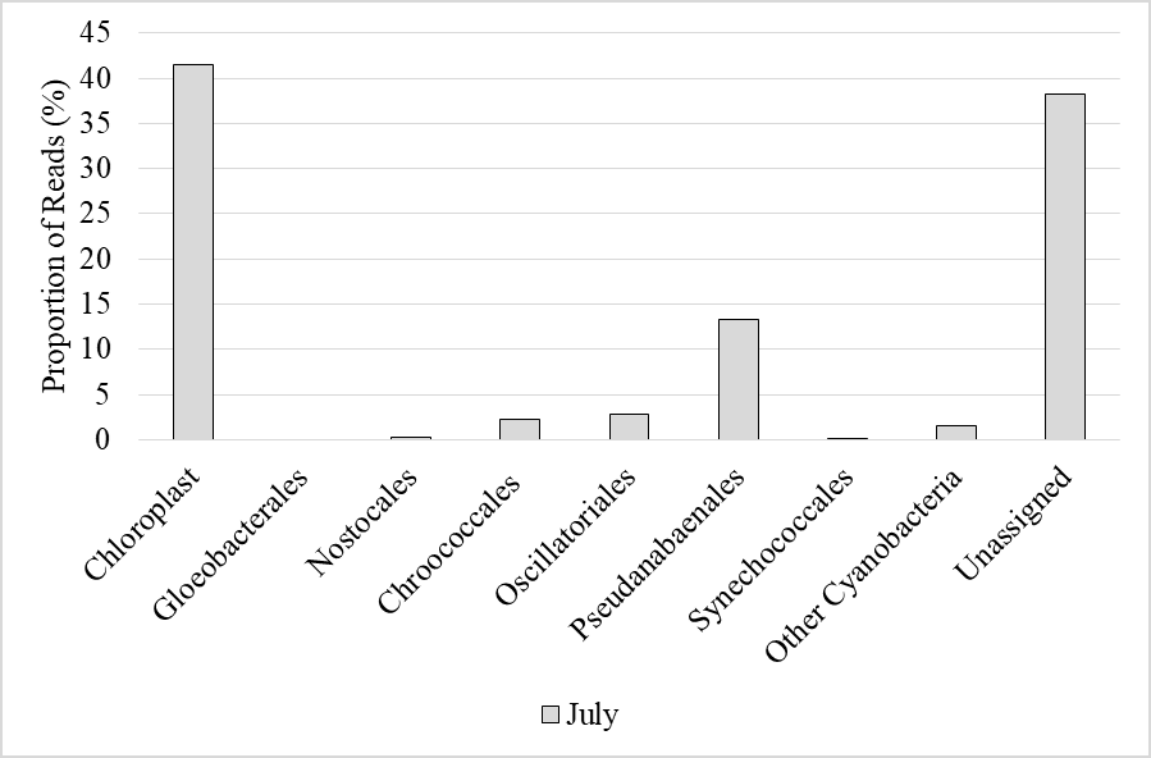


Figure 3.14. Overall proportion of OTU reads detected in samples collected from tributaries and the pump house at Lake Major for July. See Appendix D for tabulated proportion of reads per month and order composition per month.

Overall, OTU's associated with the class Chloroplast represented 42% of the reads (mean, 34%), 38% of the reads were associated with unassigned OTU's (mean, 49%), and 13% were associated with the order Pseudanabaenales (mean, 11%). OTU's that were associated with the orders Gloeobacterales, Nostocales, Chroococcales, Oscillatoriales, and Synechococcales, and OTU's associated with other cyanobacteria not designated to a particular order represented less than 5% each (overall and on average), of all OTU's detected in July.

3.1.4.1. Class Chloroplast

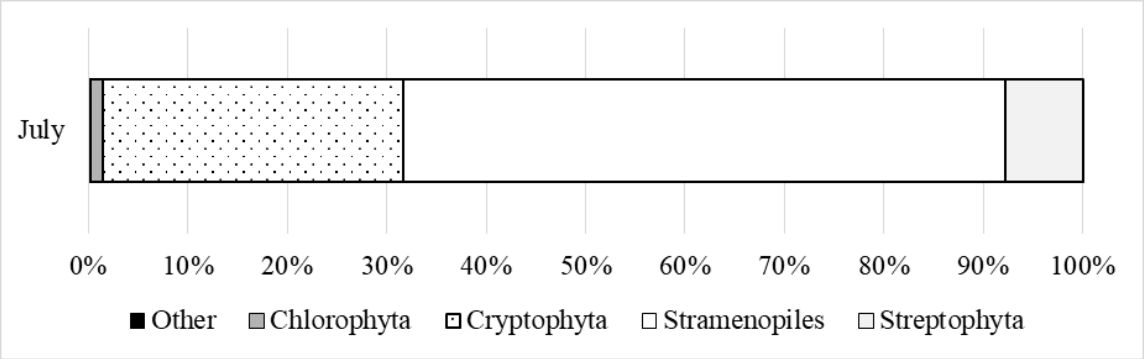


Figure 3.15. Taxa composition for the class Chloroplast for Lake Major samples.

Of the OTU's associated with the class Chloroplast, 61% were associated with Stramenopiles (mean, 66%), 30% were associated with Cryptophyta (mean, 29%), and 7.7% were associated with Streptophyta (mean, 3.9%). OTU's simply associated with the class Chloroplast without further identification and OTU's associated with Chlorophyta represented less than 5% each, of detected Chloroplast reads in July.

3.1.4.2. Order Gloeobacterales

As mentioned previously, there were no detectable OTU's associated with the order Gloeobacterales.

3.1.4.3. Order Nostocales

Of the OTU's associated with the order Nostocales, 50% were associated with the genus *Anabaena* (mean, 67%), and 50% were associated with the genus *Dolichospermum* (mean, 33%).

3.1.4.4. Order Chroococcales

Of the OTU's associated with the order Chroococcales, 100% were associated with the family Xenococcaceae (mean, 50%).

3.1.4.5. Order Oscillatoriales

Of the OTU's associated with the order Oscillatoriales, 100% were associated with the genus *Phormidium* (mean, 50%).

3.1.4.6. Order Pseudanabaenales

Of the OTU's associated with the order Pseudanabaenales, 99.5% were associated with the genus *Pseudanabaena* (mean, 50%) while 0.47% were associated with the genus *Arthronema* (mean, 50%).

3.1.4.7. Order Synechococcales

Of the OTU's associated with the order Synechococcales, 100% were associated with the genus *Synechococcus* (mean, 50%).

3.2. WATER CHEMISTRY

3.2.1. Fletchers Lake

3.2.1.1. Water Chemistry of Treatment Plant Water

Monthly means and standard deviations were determined for water samples collected from the Collin's Park Water Treatment Plant, and summarised in Appendix A. Data was provided by Michael Brophy (MASc student, Dalhousie University). Alkalinity and pH both increased from June to July, decreased from July to August, increased from August to September, and decreased from September to October, and showed an overall upward trend. The highest alkalinity mean was found to be 18.02 ± 0.94 $CaCO_3$ mg/L in September, and the lowest mean was found to be 15.59 $CaCO_3$ mg/L in August, while the highest pH mean was found to be 6.72 ± 0.40 in September, and the lowest value was found to be 5.29 in August.

TOC increased from June to July, decreased from July to September, and increased from September to October, and showed an overall downward trend. The highest TOC mean was determined to be 3.65 ± 0.11 mg/L in July, while the lowest mean was determined to be 3.08 ± 0.15 mg/L in September. TN decreased from June to September, and remained somewhat the same from September to October, having an overall downward trend. The highest TN mean was determined to be 0.24 ± 0.03 mg/L in June, and the lowest mean was found to be 0.13 ± 0.01 mg/L in September, and 0.13 mg/L in October.

SUVA decreased from June to September, and increased from September to October, and had an overall downward trend. The highest SUVA mean was determined to be 3.48 ± 0.38 m^{-1} absorbance per mg/L of DOC in June, and was lowest in September, with a mean of 2.12 ± 0.01 m^{-1} absorbance per mg/L of DOC. Turbidity decreased from June to August, and increased from August to October, with and overall upward trend. The highest turbidity mean was determined to be 1.95 NTU in October, and the lowest mean was determined to be 0.89 NTU in August. Colour was only collected August to October. Within that time frame, colour decreased from August to September, and increased from September to October, showing an overall upward trend. The highest colour value was found to be 9.50 NTU in October, while the lowest mean was found to be 5.67 ± 0.58 NTU in September.

Data for UV_{254} and TP were unavailable. However, according to Clement *et al* (2007), Fletchers Lake had TP values ranging between 9 µg/L and 12 µg/L in 2000.

3.2.1.2. Water Chemistry of Stream and Tributary Samples

Due to the large variation in values for tributary samples, it was decided to show the spread of data encountered in samples, along with the means and medians, rather than simply means and standard deviations. As seen in Appendix A, the highest alkalinity value was found to be 28.41 $CaCO_3$ mg/L in September, while the lowest value was

found to be 0.03 $CaCO_3$ mg/L in July. The highest pH value was found to be 7.34 in September, and the lowest value was found to be 3.57 in June.

The highest TOC value was determined to be 21.20 mg/L in June, while the lowest value was determined to be 1.98 mg/L in August. The highest DOC value was determined to be 20.55 mg/L in June, while the lowest value was determined to be 2.01 mg/L in August. The highest TN value was determined to be 0.61 mg/L in August, and the lowest value was found to be 0.06 mg/L in July and September.

The highest SUVA value was determined to be 6.71 m^{-1} absorbance per mg/L of DOC in July, and was lowest in September, with a value of 1.92 m^{-1} absorbance per mg/L of DOC. The highest turbidity value was determined to be 21.93 NTU in June, and the lowest value was determined to be 0.35 NTU in October. As mentioned previously, colour was only collected August to October. Within that time frame, the highest colour value was found to be 146.50 NTU in August, while the lowest value was found to be 6.00 NTU in September. Data for UV_{254} and TP were unavailable.

3.2.2. Powder Mill Lake

Monthly means and standard deviations were determined for water samples collected from in-lake collection points at Powder Mill Lake, and summarised in Appendix B. It should be noted that only pH was collected from July to November, while the other water quality parameters were collected only in October and November.

Alkalinity decreased from 24.38 ± 2.87 $CaCO_3$ mg/L in October to 21.47 ± 0.72 $CaCO_3$ mg/L in November. From July to August, pH increased, then decreased from August to October, and increased from October to November, having an overall upward trend. The highest pH mean was found to be 7.48 ± 0.15 in November, and the lowest mean was found to be 7.06 ± 0.27 in July.

TOC increased from 3.10 ± 0.36 mg/L in October to 3.53 ± 1.41 mg/L in November. TN increased slightly from 0.13 ± 0.01 mg/L in October, to 0.14 ± 0.01 mg/L in November. TP was determined to be below the detection limit of 10 µg/L in October and November.

UV_{254} increased slightly from 0.07 ± 0.01 cm^{-1} in October to 0.08 ± 0.001 cm^{-1} in November while SUVA increased from 2.11 ± 0.66 m^{-1} abs per mg/L of DOC in October to 2.47 ± 0.34 m^{-1} abs per mg/L of DOC in November. Turbidity increased from 0.67 ± 0.11 NTU in October to 0.93 ± 0.27 NTU in November, while colour increased from 6.25 ± 4.41 NTU in October to 9.64 ± 1.22 NTU in November.

3.2.3. Pockwock Lake

3.2.3.1. Water Chemistry of In-Lake Samples

Monthly means and standard deviations were determined for water samples collected from in-lake collection points at Pockwock Lake, and summarised in Appendix C. Data was provided by Dave Redden (MASc student, Dalhousie University). Alkalinity increased from June to July and decreased from July to August. There was no data for September, however, alkalinity was found to be higher in October than in August. There may be an overall upward trend. The highest alkalinity mean was found to be 1.85 ± 0.60 $CaCO_3$ mg/L in October, and the lowest mean was found to be 1.04 ± 0.50 $CaCO_3$ mg/L in June. Data for pH decreased from June to August, and there was no data for September. However, pH in October was greater than it was in August. There may be an overall upward trend. The highest pH mean was found to be 5.25 ± 0.04 in October, and the lowest mean was found to be 5.12 ± 0.22 in August.

TOC increased from June to July, and decreased from July to August. There was no data for September, but TOC for October was lower than that in August. There may be an overall downward trend. The highest TOC mean was determined to be 3.60 ± 0.36 mg/L in July, while the lowest mean was determined to be 2.88 ± 0.36 mg/L in October.

TN decreased from June to July, and there was no data for August. However, TN for October was higher than the TN in July. The only surface sample in September was reported to have a much higher concentration than other surface samples for Pockwock Lake. However, the value was closer in likeness to concentrations from at-depth samples, and may have been mislabelled as a surface sample. The highest TN mean was determined to be 0.09 ± 0.03 mg/L in June and 0.09 ± 0.01 mg/L in October, and the lowest mean was found to be 0.06 ± 0.01 mg/L in September.

UV_{254} decreased from June to July, increased from July to August, and decreased from August to October, showing an overall downward trend. The highest UV_{254} mean was determined to be 0.11 ± 0.002 cm^{-1} in June, and the lowest mean was found in October at 0.068 ± 0.0008 cm^{-1}. SUVA decreased from June to July, increased from July to September, and decreased from September to October. Data shows an overall downward trend. The highest SUVA mean was determined to be 3.43 ± 0.36 m^{-1} absorbance per mg/L of DOC in June, and was lowest in October, with a mean of 2.61 ± 0.11 m^{-1} absorbance per mg/L of DOC. Turbidity increased from June to August, there was no data for September, and turbidity in October was lower than it was in August. Data shows an overall downward trend. The highest turbidity mean was determined to be 0.71 ± 0.45 NTU in August, and the lowest mean was determined to be 0.45 ± 0.08 NTU in October. Colour decreased from June to August, greatly increased in September, and greatly decreased in October. Data showed an overall downward trend. The highest colour mean was found to be 37.89 ± 21.69 NTU in September, while the lowest mean was found to be 10.63 ± 0.72 NTU in October.

Data for TP was unavailable. However, according to Ginn *et al* (2015), Pockwock Lake had a TP concentration around 4 µg/L in 2005, 2006.

3.2.3.2. Water Chemistry of Stream and Tributary Samples

Due to the large variation in values for tributary samples, it was decided to show the spread of data encountered in samples, along with the means and medians, rather than

simply means and standard deviations. As seen in Appendix C, the highest alkalinity value was found to be 53.08 $CaCO_3$ mg/L in August, while the lowest value was found to be 0.03 $CaCO_3$ mg/L in July. The highest and lowest pH value was observed in June, at 5.89 and 3.90, respectively.

The highest and lowest TOC concentrations were observed in July, at 32.84 mg/L and 2.40 mg/L, respectively. The highest DOC concentration was determined to be 44.74 mg/L in October, while the lowest value was determined to be 2.30 mg/L in September. The highest TN concentration was determined to be 3.65 mg/L in August, and the lowest value was found to be 0.07 mg/L in July.

The highest UV_{254} value was found to be 1.18 cm^{-1} in July, and the lowest was found to be 0.07 cm^{-1} in July and August. The highest SUVA value was determined to be 6.92 m^{-1} absorbance per mg/L of DOC in July, and was lowest in August, with a value of 2.38 m^{-1} absorbance per mg/L of DOC. The highest turbidity value was determined to be 36.92 NTU in July, and the lowest value was determined to be 0.46 NTU in June and July. The highest and lowest colour value was observed in August, at 688 NTU and 3.00 NTU, respectively. Data for TP was unavailable.

3.2.4. Lake Major

3.2.4.1. Water Chemistry of Pump House Samples

Monthly averages and standard deviations were determined for water samples collected from the pump house at Lake Major, and summarised in Appendix D. Data was provided by Lindsay Anderson (PhD student, Dalhousie University) and Halifax Water. Data for pH increased from June to August, then decreased from August to November, showing an overall downward trend. The highest pH mean was found to be 5.59 ± 0.06 in August, and the lowest mean was found to be 5.25 in November.

TOC concentration decreased from June to September, and increased from September to October. TOC data showed an overall downward trend. The highest mean TOC concentration was determined to be 4.90 ± 1.18 mg/L in October, while the lowest mean was determined to be 3.98 ± 0.27 mg/L in September.

UV_{254} decreased from June to August, remained almost the same into September, and increased from September to November. UV_{254} data showed an overall downward trend. The highest UV_{254} mean was determined to be 0.24 ± 0.01 cm^{-1} in June, and the lowest mean was found to be 0.18 ± 0.002 cm^{-1} in August and 0.18 ± 0.01 cm^{-1} in September. SUVA decreased from June to August, increased from August to September, and decreased from September to October. SUVA data showed an overall downward trend. The highest SUVA mean was determined to be 5.11 ± 0.16 m^{-1} absorbance per mg/L of DOC in June, and was lowest in August, with a mean of 3.93 ± 0.15 m^{-1} absorbance per mg/L of DOC. Turbidity increased from June to July, decreased from July to September, increased from September to October, and finally decreased from October to November. Turbidity data showed an overall downward trend. The highest turbidity mean was determined to be 0.43 ± 0.08 NTU in July, and the lowest mean was determined to be 0.29 ± 0.08 NTU in September. Colour decreased from June to September, then increased from September to November. The highest colour mean was found to be 44.38 ± 1.94 NTU in June, while the lowest mean was found to be 26.33 ± 0.58 NTU in September.

Data for alkalinity, TN and TP were unavailable. However, according to Clement *et al* (2007), Lake Major had TP values ranging between 4 µg/L and 11 µg/L in 2000.

3.2.4.2. Water Chemistry of Stream and Tributary Samples

Once again, due to the large variation in values for tributary samples, it was decided to show the spread of data encountered in samples, along with the means and medians, rather than simply means and standard deviations. However, for this data set,

only July was reported, as tributary samples for cyanobacteria analysis were only collected in June and July, and tributary water quality data prior to July was not available.

As seen in Table 3.9., the highest pH value was found to be 6.20, and the lowest to be 4.50. The highest TOC concentration was found to be 16.07 mg/L, and the lowest concentration was found to be 3.09 mg/L, respectively. The highest DOC concentration was determined to be 13.62 mg/L, while the lowest concentration was determined to be 2.76 mg/L. The highest TP concentration was found to be 27.00 µg/L and the lowest was found to be 5.00 µg/L. The highest turbidity value was determined to be 22.60 NTU, and the lowest value was determined to be 0.68 NTU. The highest colour value was observed to be 50.00 NTU and the lowest was observed to be 12 NTU. Data for alkalinity, TN, UV_{254} and SUVA were unavailable.

3.3. ENVIRONMENT DATA

3.3.1. June

3.3.1.1. Temperature

After determining the daily maximum, minimum, median, upper quartile and lower quartile values for temperature data, a box-and-whisker graph, Figure 3.16(a), was made to show the hourly spread of temperatures per day for the month of June. It was also determined that the maximum temperature in June was 27.0°C, the minimum temperature was 3.2°C, and the mean temperature with standard deviation was 14.4°C ± 4.8°C.

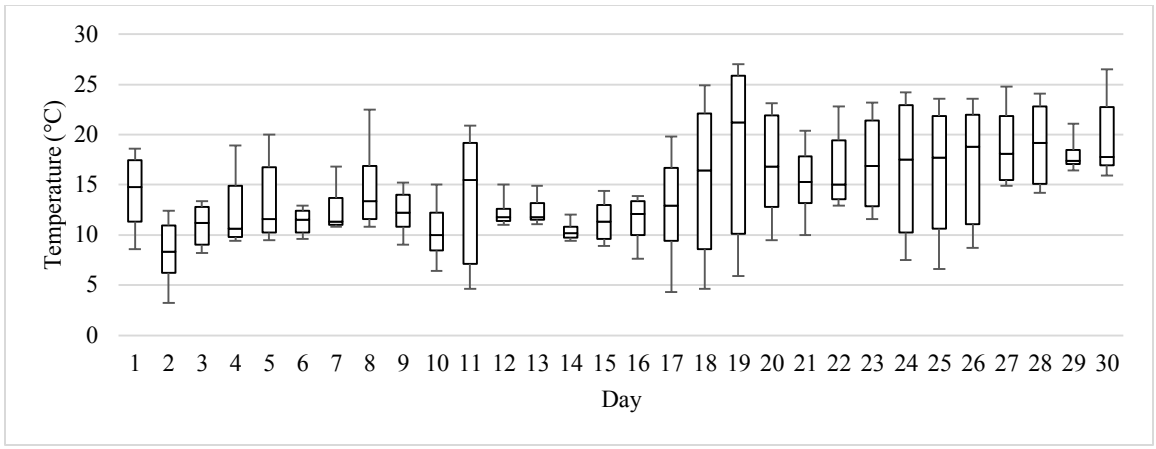
3.3.1.2. Precipitation

The maximum volume of precipitation achieved in one day during June was 18.3 mm, while the minimum volume of precipitation was found to be 0.0 mm. The mean volume of precipitation was 1.9 mm, and the total accumulated volume of precipitation in

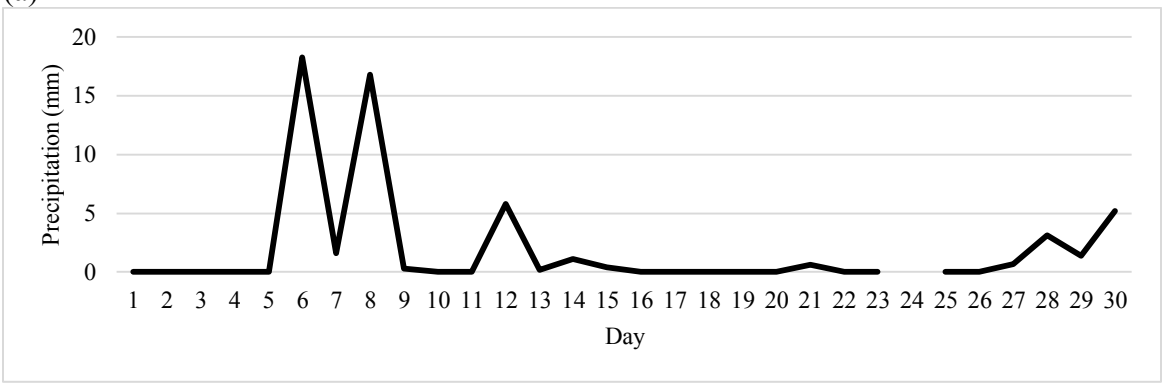
June was determined to be 55.5 mm. A line graph depicting daily precipitation data collected in June can be found in Figure 3.16(b).

3.3.1.3. Wind Speed

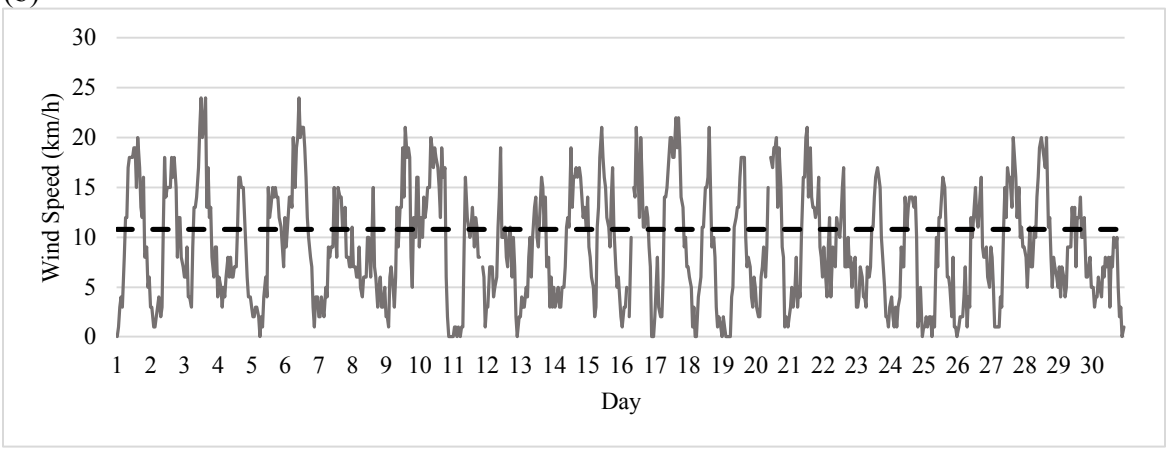
The maximum wind speed recorded at the Bedford Range weather station in June was 24.0 km/h, while the minimum wind speed was 0.0 km/h. The mean with standard deviation was 9.1 km/h ± 5.7 km/h. A time series graph depicting wind speed data for June can be seen in Figure 3.16(c). The dashed line in Figure 3.16(c), and shown in all subsequent time series graphs for wind speed in this report, depicts the wind speed threshold over which algal/cyanobacteria blooms are disrupted and dispersed, which is around 10.8 km/h (Whitton and Potts, 2000).



(a)



(b)



(c)

Figure 3.16. (a) Temperature data for June, collected from the Bedford Range weather station (GC, 2016b); (b) Precipitation data for Bedford, NS, for June, collected from The Weather Network's historical weather database (TWN, 2017); (c) Wind speed data for June, collected from the Bedford Range weather station (GC, 2016b).

3.3.2. July

3.3.2.1. Temperature

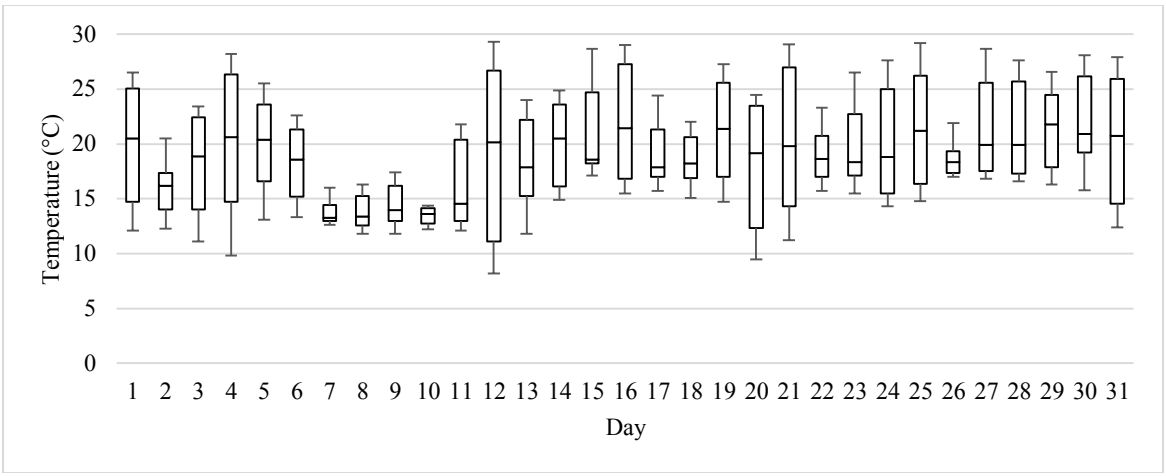
After determining the daily maximum, minimum, median, upper quartile and lower quartile values for temperature data, a box-and-whisker graph, Figure 3.17(a), was made to show the hourly spread of temperatures per day for the month of July. It was also determined that the maximum temperature in July was 29.3°C, the minimum temperature was 8.2°C, and the mean temperature with standard deviation was 19.0°C ± 4.7°C.

3.3.2.2. Precipitation

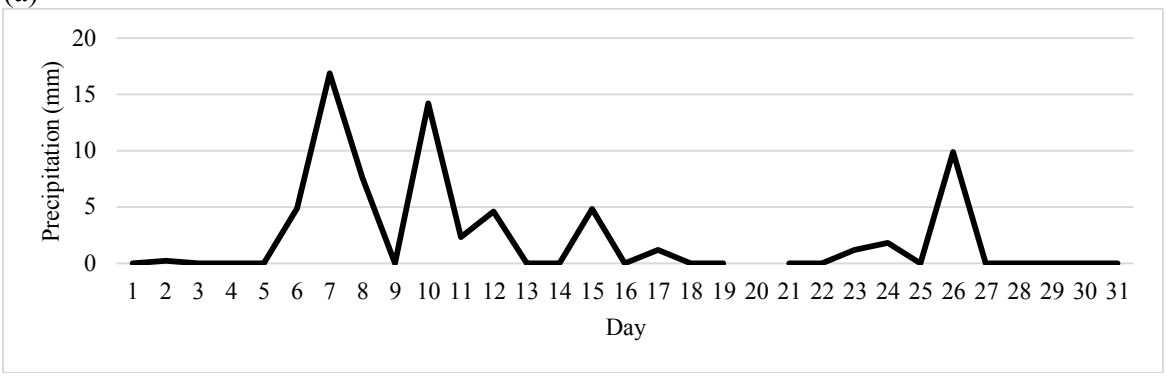
The maximum volume of precipitation achieved in one day during July was 16.9 mm, while the minimum volume of precipitation was found to be 0.0 mm. The mean volume of precipitation was 2.3 mm, and the total accumulated volume of precipitation was determined to be 69.6 mm. A line graph depicting daily precipitation data collected in July can be found in Figure 3.17(b).

3.3.2.3. Wind Speed

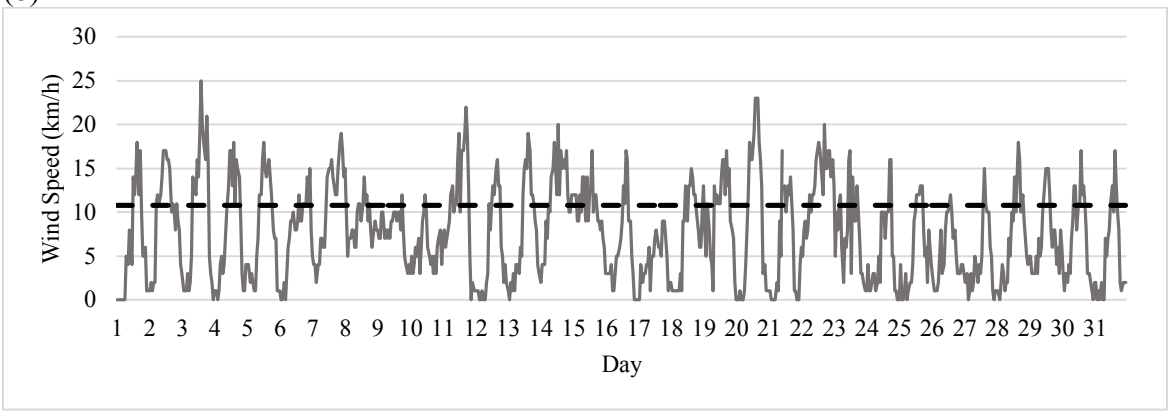
The maximum wind speed detected at the Bedford Range weather station in July was 25.0 km/h, while the minimum wind speed was 0.0 km/h. The mean with standard deviation was 7.7 km/h ± 5.4 km/h. A time series graph depicting wind speed data for July can be seen in Figure 3.17(c).



(a)



(b)



(c)

Figure 3.17. (a) Temperature data for July, collected from the Bedford Range weather station (GC, 2016b); (b) Precipitation data for Bedford, NS, for July, collected from The Weather Network's historical weather database (TWN, 2017); (c) Wind speed data for July, collected from the Bedford Range weather station (GC, 2016b).

3.3.3. August

3.3.3.1. Temperature

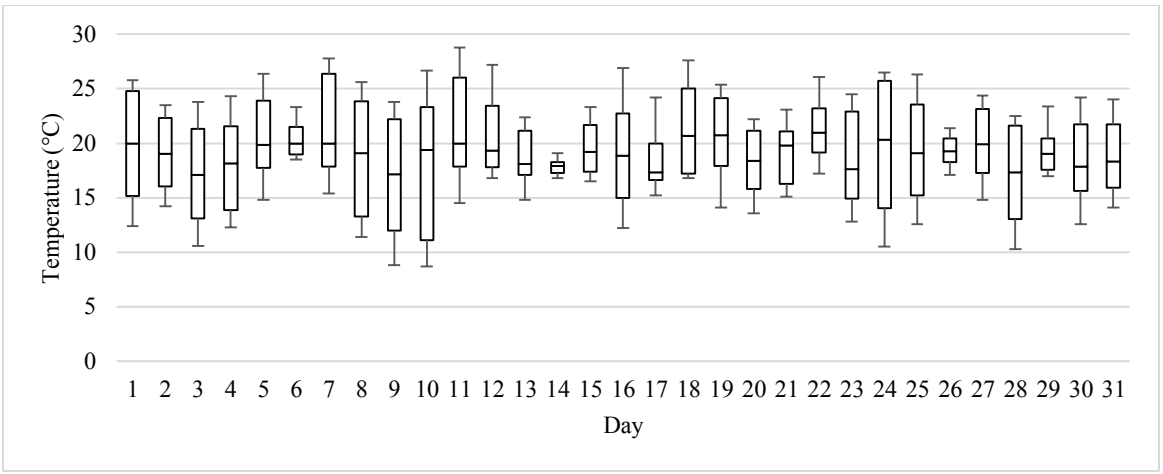
After determining the daily maximum, minimum, median, upper quartile and lower quartile values for temperature data for August, a box-and-whisker graph, Figure 3.18(a), was made to show the hourly spread of temperatures per day for the month. It was also determined that the maximum temperature in August was 28.8°C, the minimum temperature was 8.7°C, and the mean temperature with standard deviation was 19.3°C ± 3.9°C.

3.3.3.2. Precipitation

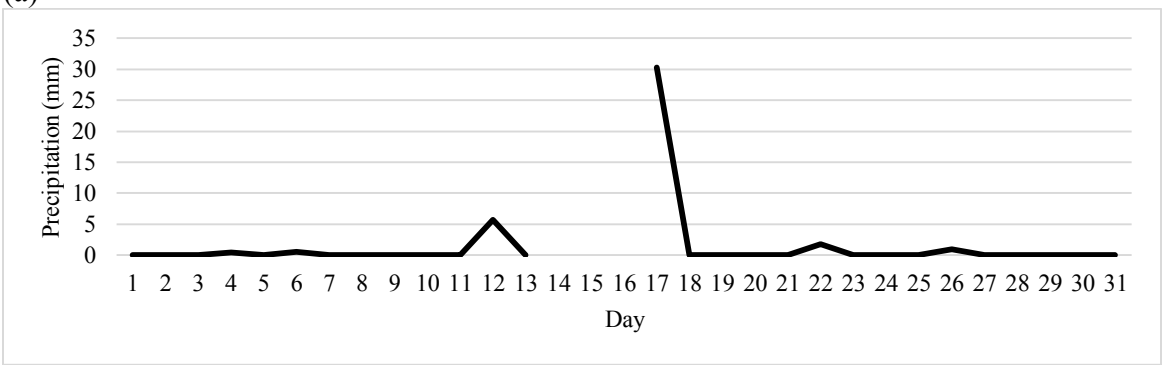
The maximum volume of precipitation achieved in one day during August was 30.3 mm, while the minimum volume of precipitation was found to be 0.0 mm. The mean volume of precipitation was 1.4 mm, and the total accumulated volume of precipitation was determined to be 39.7 mm. A line graph depicting daily precipitation data collected in August can be found in Figure 3.18(b).

3.3.3.3. Wind Speed

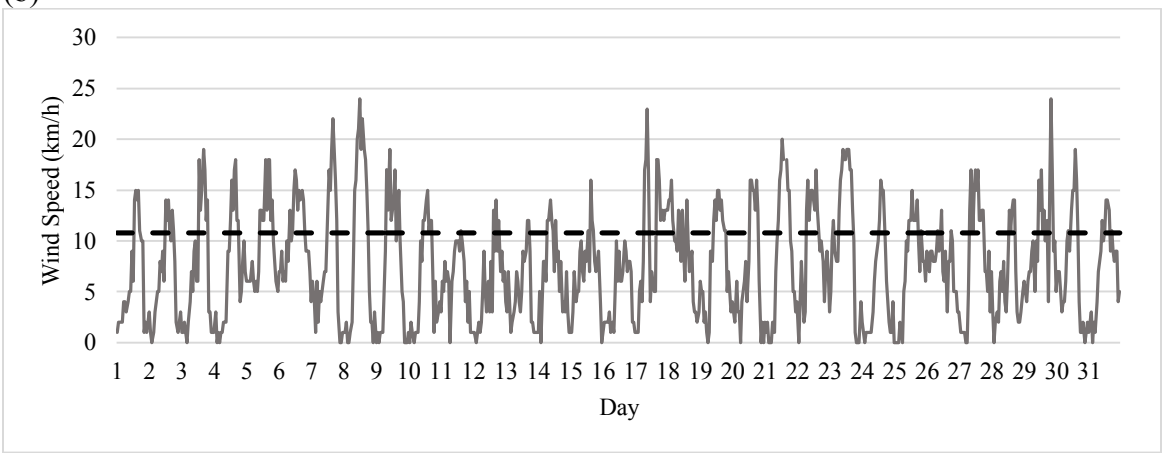
The maximum wind speed detected at the Bedford Range weather station in August was 24.0 km/h, while the minimum wind speed was 0.0 km/h. The mean with standard deviation was 7.6 km/h ± 5.3 km/h. A time series graph depicting wind speed data for August can be seen in Figure 3.18(c).



(a)



(b)



(c)

Figure 3.18. (a) Temperature data for August, collected from the Bedford Range weather station (GC, 2016b); (b) Precipitation data for Bedford, NS, for August, collected from The Weather Network's historical weather database (TWN, 2017); (c) Wind speed data for August, collected from the Bedford Range weather station (GC, 2016b).

3.3.4. September

3.3.4.1. Temperature

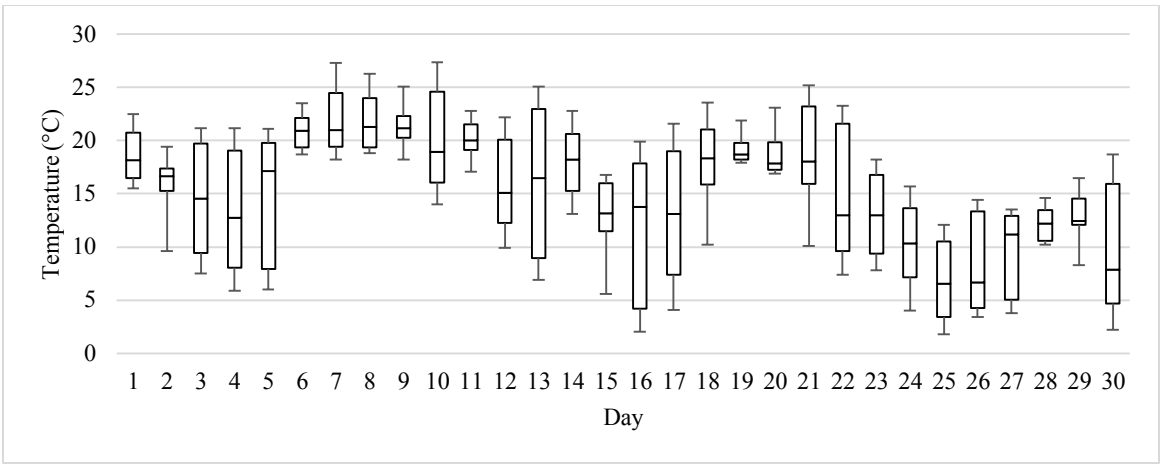
After determining the daily maximum, minimum, median, upper quartile and lower quartile values for temperature data, a box-and-whisker graph, Figure 3.19(a), was made to show the hourly spread of temperatures per day for the month of September. It was also determined that the maximum temperature in September was 27.4°C, the minimum temperature was 1.8°C, and the mean temperature with standard deviation was 15.5°C ± 5.8°C.

3.3.4.2. Precipitation

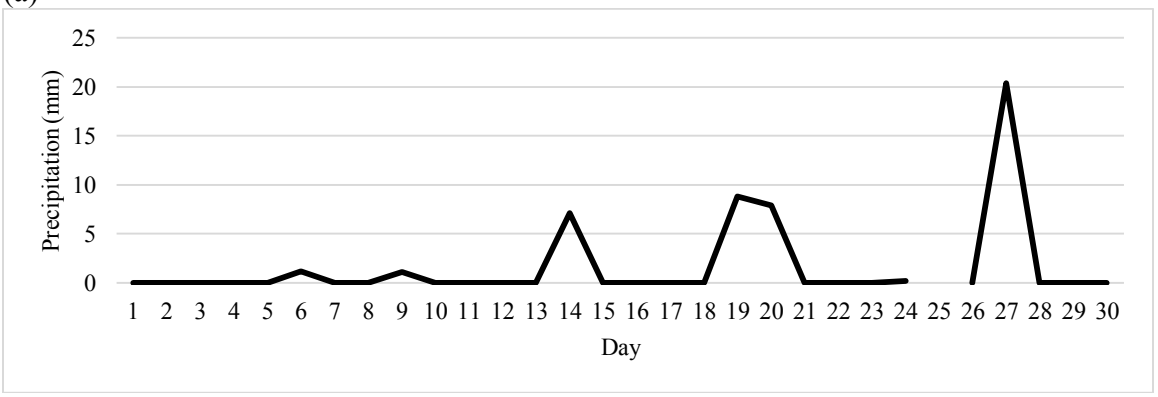
The maximum volume of precipitation achieved in one day during September was 20.4 mm, while the minimum volume of precipitation was found to be 0.0 mm. The mean volume of precipitation was 1.6 mm, and the total accumulated volume of precipitation was determined to be 46.7 mm. A line graph depicting daily precipitation data collected in September can be found in Figure 3.19(b).

3.3.4.3. Wind Speed

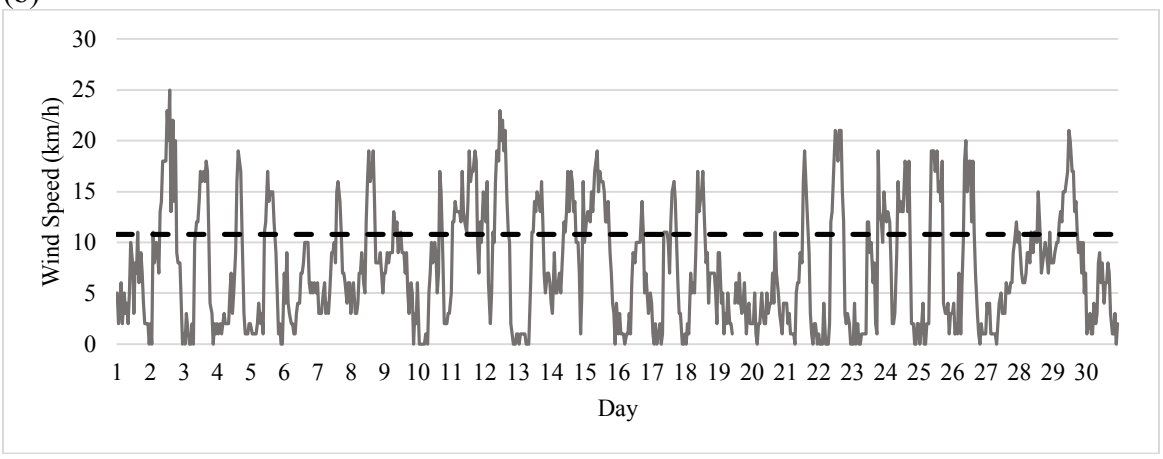
The maximum wind speed detected at the Bedford Range weather station in September was 25.0 km/h, while the minimum wind speed was 0.0 km/h. The mean with standard deviation was 7.5 km/h ± 5.8 km/h. A time series graph depicting wind speed data for September can be seen in Figure 3.19(c).



(a)



(b)



(c)

Figure 3.19. (a) Temperature data for September, collected from the Bedford Range weather station (GC, 2016b); (b) Precipitation data for Bedford, NS, for September, collected from The Weather Network's historical weather database (TWN, 2017); (c) Wind speed data for September, collected from the Bedford Range weather station (Government of Canada, 2016b).

3.3.5. October

3.3.5.1. Temperature

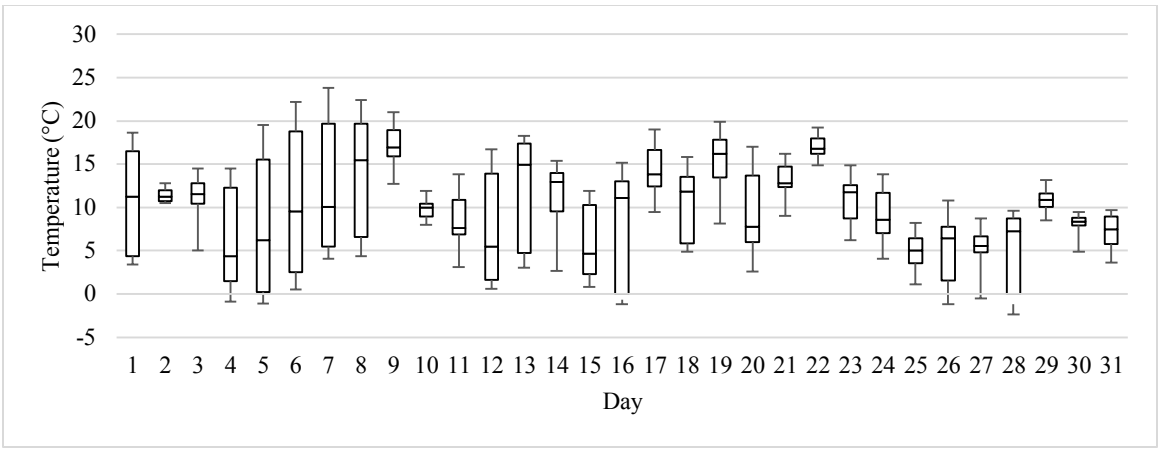
After determining the daily maximum, minimum, median, upper quartile and lower quartile values for temperature data for October, a box-and-whisker graph, Figure 3.20(a), was made to show the hourly spread of temperatures per day for the month. It was also determined that the maximum temperature in October was 23.8°C, the minimum temperature was -2.4°C, and the mean temperature with standard deviation was 10.0°C ± 5.4°C.

3.3.5.2. Precipitation

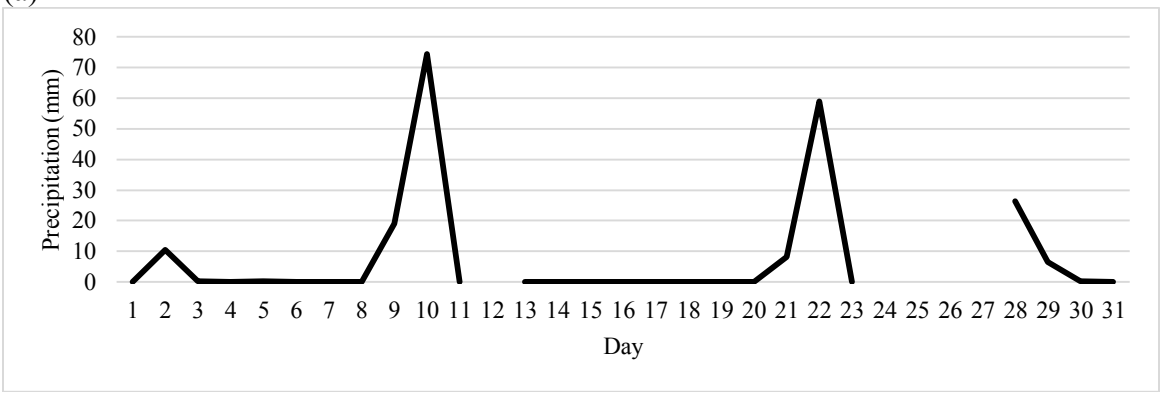
The maximum volume of precipitation achieved in one day during October was 74.5 mm, while the minimum volume of precipitation was found to be 0.0 mm. The mean volume of precipitation was 7.6 mm, and the total accumulated volume of precipitation was determined to be 204.7 mm. A line graph depicting daily precipitation data collected in October can be found in Figure 3.20(b).

3.3.5.3. Wind Speed

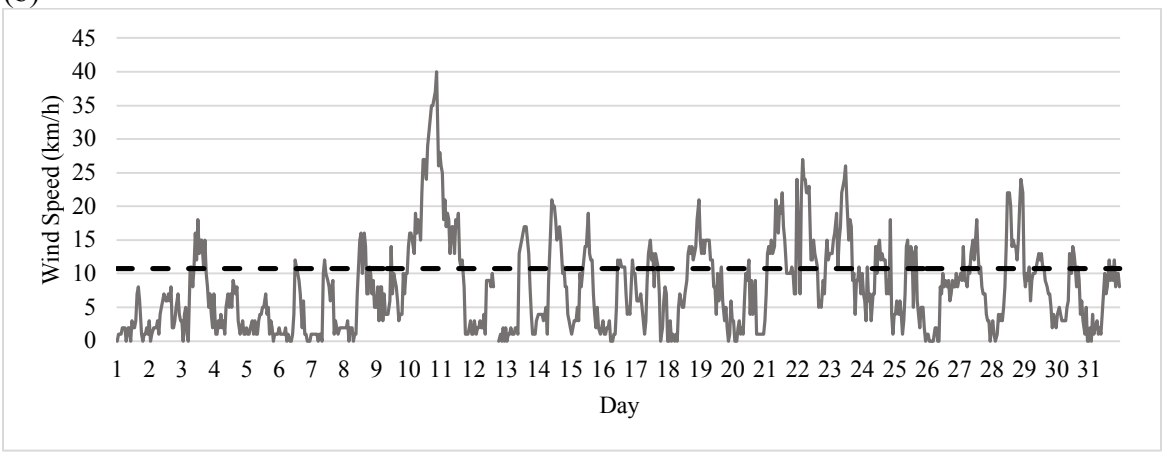
The maximum wind speed detected at the Bedford Range weather station in October was 40.0 km/h, while the minimum wind speed was 0.0 km/h. The mean with standard deviation was 7.9 km/h ± 6.7 km/h. A time series graph depicting wind speed data for October can be seen in Figure 3.20(c).



(a)



(b)



(c)

Figure 3.20. (a) Temperature data for October, collected from the Bedford Range weather station (GC, 2016b); (b) Precipitation data for Bedford, NS, for October, collected from The Weather Network's historical weather database (TWN, 2017); (c) Wind speed data for October, collected from the Bedford Range weather station (GC, 2016b).

3.3.6. November

3.3.6.1. Temperature

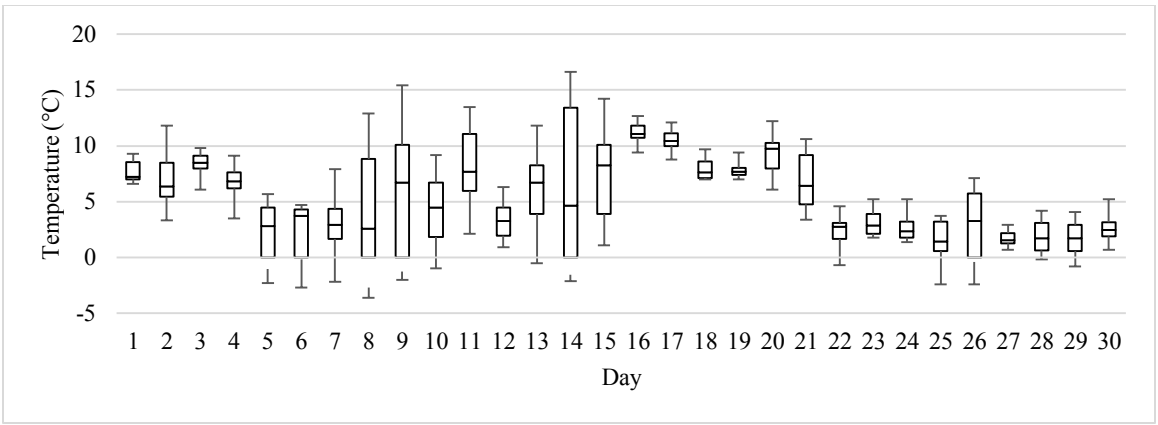
After determining the daily maximum, minimum, median, upper quartile and lower quartile values for temperature data, a box-and-whisker graph, Figure 3.21(a), was made to show the hourly spread of temperatures per day for the month of November. It was also determined that the maximum temperature in November was 15.1°C, the minimum temperature was -3.6°C, and the mean temperature with standard deviation was 5.0°C ± 4.0°C.

3.3.6.2. Precipitation

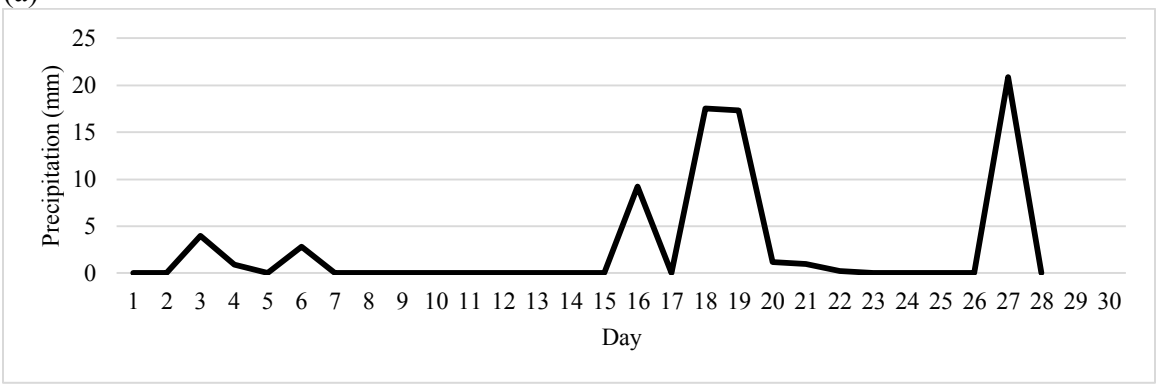
The maximum volume of precipitation achieved in one day during November was 20.9 mm, while the minimum volume of precipitation was found to be 0.0 mm. The mean volume of precipitation was 2.7 mm, and the total accumulated volume of precipitation was determined to be 75.0 mm. A line graph depicting daily precipitation data collected in November can be found in Figure 3.21(b).

3.3.6.3. Wind Speed

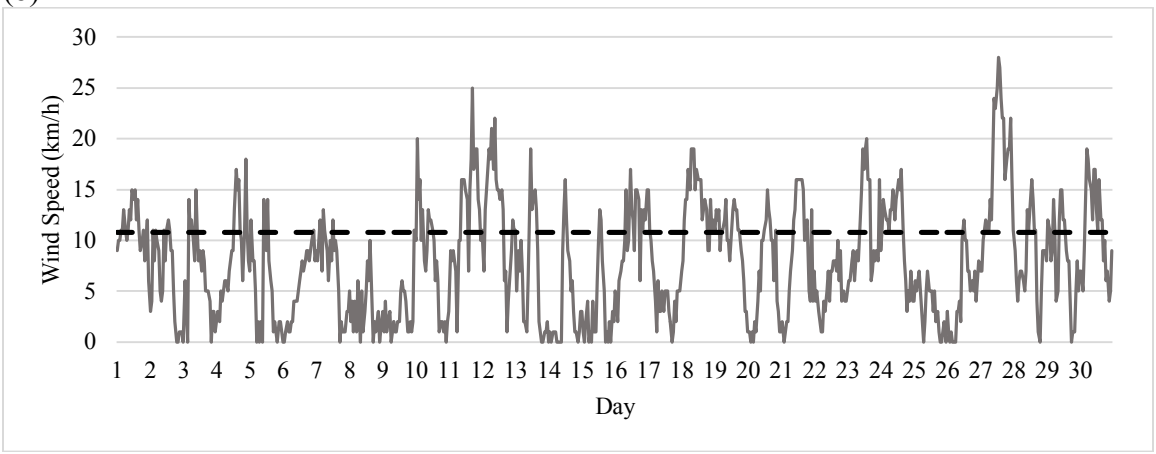
The maximum wind speed detected at the Bedford Range weather station in November was 28.0 km/h, while the minimum wind speed was 0.0 km/h. The mean with standard deviation was 7.9 km/h ± 5.5 km/h. A time series graph depicting wind speed data for November can be seen in Figure 3.21(c).



(a)



(b)



(c)

Figure 3.21. (a) Temperature data for November, collected from the Bedford Range weather station (GC, 2016b); (b) Precipitation data for Bedford, NS, for November, collected from The Weather Network's historical weather database (TWN, 2017); (c) Wind speed data for November, collected from the Bedford Range weather station (GC, 2016b).

CHAPTER 4 DISCUSSION

4.1. CYANOBACTERIA POPULATION ANALYSIS

4.1.1. Cyanobacteria Presence

The most dominant group of cyanobacteria isolates observed typically within and between samples would represent around 50% of the OTU reads, while the second most dominant group of isolates would represent around 25% to 30% of OTU reads. The third most dominant isolates would represent around 10% to 20% of OTU reads, while all remaining groups would represent less than 5% of reads, each. Of note, of the dominant groups of isolates observed, OTU's associated with the class chloroplast were usually the most dominant or second most dominant group amongst all samples collected in 2016.

Samples from tributaries and the Collin's Park Water Treatment Plant at Fletchers Lake usually showed four dominant groups of cyanobacteria isolates that each represented over 5% of OTU reads detected in September and October. Meanwhile, samples from in-lake sites and one tributary at Powder Mill Lake usually showed two dominant groups above 5%, with the exception of July, which had four dominant groups above 5%. Of note, the two most dominant cyanobacteria isolate groups observed in samples from Fletchers Lake and Powder Mill Lake, every month, were associated with the order Synechococcales and the class Chloroplast. This may or may not be because Powder Mill Lake flows into Lake William, which flows into Thomas Lake, which flows into Fletchers Lake (Soil and Water Conservation Society of Metro Halifax, 2017). Samples from tributaries and in-lake sites at Pockwock Lake usually showed two to three dominant groups of cyanobacteria isolates with proportions of reads over 5%, except for September, which showed four dominant groups above 5%. Meanwhile, samples from Lake Major tributaries had three dominant groups above 5%.

Table 4.1. Comparison of cyanobacteria isolates detected between June and November, 2016.

Affiliated Cyanobacteria Taxa	Fletchers Lake	Powder Mill Lake	Pockwock Lake	Lake Major
Class Chloroplast				
Other	x	x	x	x
Chlorophyta	x	x	x	x
Cryptophyta	x	x	x	x
Euglenozoa		x	x	
Haptophyta	x	x	x	
Rhodophyta	x		x	
Stramenopiles	x	x	x	x
Streptophyta	x	x	x	x
Ord. Gloeobacterales				
Fam. Gloeobacteraceae (*Gloeobacter*)	x	x		
Ord. Nostocales				
Fam. Nostocaceae (Other)	x	x		
Fam. Nostocaceae (*Anabaena*)	x	x		x
Fam. Nostocaceae (*Dolichospermum*)	x	x	x	x
Fam. Nostocaceae (*Nodularia*)	x			
Fam. Nostocaceae (*Nostoc*)	x		x	
Ord. Chroococcales				
Fam. Other			x	
Fam. Chroococcaceae	x	x		
Fam. Cyanobacteriaceae		x		
Fam. Gomphosphaeriaceae	x	x	x	
Fam. Microcystaceae (*Microcystis*)	x	x	x	
Fam. Spirulinaceae (*Spirulina*)		x		
Fam. Xenococcaceae (Other)	x	x	x	x
Fam. Xenococcaceae (*Chroococcidiopsis*)	x			
Ord. Oscillatoriales				
Fam. Phormidiaceae (Other)			x	
Fam. Phormidiaceae (*Phormidium*)	x	x	x	x

Ord. Pseudanabaenales				
Fam. Other	x	x	x	
Fam. Pseudanabaenaceae (Other)	x	x	x	
Fam. Pseudanabaenaceae (*Leptolyngbya*)	x	x	x	
Fam. Pseudanabaenaceae (*Arthronema*)				x
Fam. Pseudanabaenaceae (*Pseudanabaena*)	x	x	x	x
Ord. Synechococcales				
Fam. Chamaesiphonaceae	x			
Fam. Synechococcaceae (Other)		x		
Fam. Synechococcaceae (*Prochlorococcus*)		x		
Fam. Synechococcaceae (*Paulinella*)			x	
Fam. Synechococcaceae (*Synechococcus*)	x	x	x	x
Other Cyanobacteria	x	x	x	x
Unassigned	x	x	x	x

As seen in Table 4.1., a total of 35 taxa of cyanobacteria and one taxa of unassigned bacteria (which may or may not include cyanobacteria isolates) were associated with detected OTU's. Of which, 15 genera of cyanobacteria were identified. Of those genera, 11 have been associated with the cyanotoxins microcystin, nodularin, anatoxin, saxitoxin, BMAA and cylindrospermopsin (WHO, 1999; Jakubowska and Szeląg-Wasielewska, 2015; Carmichael and Boyer, 2016; Buratti *et al*, 2017).

Table 4.2. Summary table of cyanobacteria genera associated with cyanotoxins detected between June and November 2016.

Sample Location	Affiliated Toxin Producing Cyanobacteria Genera	Associated Cyanotoxin
Fletchers Lake	*Anabaena*	Microcystin, anatoxin, saxitoxin, cylindrospermopsin
	Dolichospermum	Microcystin, anatoxin, cylindrospermopsin
	Nodularia	Nodularin
	Nostoc	Microcystin
	Microcystis	Microcystin, anatoxin
	Phormidium	Possibly microcystin, anatoxin, saxitoxin
	Leptolyngbya	Possibly microcystin
	Pseudanabaena	Possibly microcystin
	Synechococcus	Microcystin

Powder Mill Lake	*Anabaena*	Microcystin, anatoxin, saxitoxin, cylindrospermopsin
	Dolichospermum	Microcystin, anatoxin, cylindrospermopsin
	Microcystis	Microcystin, anatoxin
	Spirulina	Possibly microcystin
	Phormidium	Possibly microcystin, anatoxin, saxitoxin
	Leptolyngbya	Possibly microcystin
	Pseudanabaena	Possibly microcystin
	Prochlorococcus	BMAA
	Synechococcus	Microcystin
Pockwock Lake	*Dolichospermum*	Microcystin, anatoxin, cylindrospermopsin
	Nostoc	Microcystin
	Microcystis	Microcystin, anatoxin
	Phormidium	Possibly microcystin, anatoxin, saxitoxin
	Leptolyngbya	Possibly microcystin
	Pseudanabaena	Possibly microcystin
	Synechococcus	Microcystin
Lake Major	*Anabaena*	Microcystin, anatoxin, saxitoxin, cylindrospermopsin
	Dolichospermum	Microcystin, anatoxin, cylindrospermopsin
	Phormidium	Possibly microcystin, anatoxin, saxitoxin
	Pseudanabaena	Possibly microcystin
	Synechococcus	Microcystin

According to WHO (1999), Carmichael and Boyer (2016) and Buratti *et al* (2017), microcystin producing genera include *Anabaena*, *Dolichospermum*, *Nostoc*, *Microcystis*, *Synechococcus* and potentially *Leptolyngbya*, *Pseudanabaena*, *Spirulina*, and *Phormidium*. Nodularin is produced by *Nodularia*. Anatoxins are produced by *Anabaena*, *Dolichospermum*, *Mircrocystis*, and *Phormidium*, while saxitoxins are produced by *Anabaena* and *Phormidium*. Cylindrospermopsin has been associated with *Anabaena* and *Dolichospermum*. According to Jakubowska and Szeląg-Wasielewska (2015), BMAA is produced by *Prochlorococcus* genera. As seen in Table 4.2., cyanobacteria isolates associated with toxic cyanobacteria were detected in samples collected from Fletchers Lake, Powder Mill Lake, Pockwock Lake, and Lake Major,

However, samples collected from Fletchers Lake showed that OTU's associated with toxin producing cyanobacteria each represented less than 2% of all OTU reads, each month (except for *Synechococcus*, which represented 52% of reads in September (mean, 50%), and 38% of reads in October (mean, 35%)). Samples collected from Powder Mill Lake showed that OTU's affiliated with toxin producing cyanobacteria each represented less than 1% of all OTU reads, each month (except for *Pseudanabaena*, which represented 7.5% of reads in July (mean, 7.5%), and *Synechococcus*, which represented between 52% and 55% of reads through July to November (mean, between 52% and 55%)). Pockwock Lake samples showed that OTU's affiliated with toxin producing cyanobacteria each represented less than 2% of all OTU reads, each month (except for *Synechococcus*, which represented 4.4% of reads in July (mean, 4.4%), and 12% of reads in September (mean, 12%), and *Dolichospermum*, which represented 14% of reads in September (mean, 14%)). OTU's associated with toxin producing cyanobacteria in Lake Major samples each represented less than 3% of all OTU reads (except for *Pseudanabaena*, which represented 13% of reads in July (mean, 11%)).

There were also toxic cyanobacteria detected in this study that have been detected in lakes in Quebec and New England in previous years (Fortin *et al*, 2010; Zamyadi *et al*, 2012; Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs, 2014; University of New Hampshire, 2015; Pick, 2016). *Anabaena*, *Phormidium*, *Microcystis* and *Nostoc* genera have been detected in New England, while *Microcystis*, *Anabaena*, *Dolichospemum* and *Pseudanabaena* genera have been detected in Quebec.

4.1.2. PCR Amplification Failure

4.1.2.1. Low Cyanobacteria Concentration

Samples from Fletchers Lake had the highest PCR amplification success at 74%, whereas only 58% of samples from Powder Mill Lake amplified successfully. Samples from Pockwock Lake and Lake Major had low amplification success in comparison, whereby only 31% of Pockwock Lake samples amplified and only 29% of Lake Major

samples amplified. There are several potential reasons as to why so many samples did not amplify.

During sample processing, the CYA359F-CYA781R primers would often form dimers with themselves instead of forming complexes with targeted cyanobacterial DNA. This would suggest that there was a very low concentration of cyanobacteria DNA in the failed samples, providing a very limited amount of cyanobacteria genetic material for the CYA359F-CYA781R primers to bind too. This is likely indicative of naturally low concentrations of cyanobacteria at the sample sites.

For example, tributary samples from Pockwock Lake and Lake Major successfully amplified more often than in-lake surface samples or samples from the pump house, but often depicted OTU's associated with chloroplasts (i.e. cyanobacterium associated with eukaryotes) and unassigned bacteria in higher proportions than other OTU's. Figure 4.1. shows the initial filtering step used to remove material larger than 70 µm from a tributary sample from Lake Major.

Figure 4.1. A tributary sample undergoes an initial filtration to remove debris larger than 70 µm prior to vacuum filtration for cyanobacteria isolation.

4.1.2.2. Naturally Occurring Inhibitors

During processing, it was also observed that the 16S rRNA universal bacterial primers 515F-926R were being compromised as well. This would suggest the presence of naturally occurring PCR inhibitors in the water, such as compounds from plants, dead biomass, soil, sewage sludge, and/or waste water (Schrader *et al*, 2012; Rački *et al*, 2014; Jones *et al*, 2015). Examples of these compounds include humic, fulminic, and tannic acids, heavy metals, polyphenols, some polysaccharides and pectin.

4.1.3. Potential Seasonality Observed in Failed Sample Occurrence

It was observed that failed samples from Powder Mill Lake tended to occur late August to mid-October. Similarly, failed samples from Pockwock Lake happened most often between late August and early October, and were usually surface samples. This could suggest potential seasonality to cyanobacteria presence in HRM whereby peak season for surface bloom forming cyanobacteria may occur in the late spring early summer, instead of late summer/early autumn as experienced in other areas of Canada and internationally (Gladyshev *et al*, 2010; Gonçalves *et al*, 2011; Pan *et al*, 2013). This may also suggest that if an algal bloom is observed in these later months in HRM, they may be caused by other organisms, such as diatoms. Or perhaps, if a toxic cyanobacteria "bloom" were to occur in late summer/early autumn, it may occur in the water column, as not all toxic cyanobacteria create surface blooms (Pick, 2016).

Failed samples from Fletchers Lake typically came from specific sites in September and October. This would suggest that those sites likely exhibit conditions that may not be suitable for cyanobacteria. Failed samples from Lake Major included losses to both the tributary set and the pump house water set.

4.2. WATER CHEMISTRY

4.2.1. Alkalinity and pH

With regards to treatment plant samples from Fletchers Lake, in-lake samples from Powder Mill Lake and Pockwock Lake, and pump house samples from Lake Major, overall, Powder Mill Lake samples exhibited the highest mean alkalinity concentration, followed by Fletchers Lake, then Lake Major. Pockwock Lake samples had the lowest mean concentration of alkalinity. This would indicate that Powder Mill Lake has a better acid-neutralizing capacity than the other lakes in this study, while Pockwock Lake has the least acid-neutralizing capacity (APHA *et al*, 2012).

As mentioned previously, most lakes in Nova Scotia under normal conditions would not exhibit algal or cyanobacteria blooms, as Nova Scotian lakes are typically acidic due to naturally occurring organic acids and bedrock geology (Jeffries *et al*, 2003b; Ginn *et al*, 2015). Pockwock Lake and Lake Major are good examples. However, Fletchers Lake and Powder Mill Lake are more neutral, which is concerning, as freshwater cyanobacteria tend to prefer neutral to alkaline water sources with a pH between 6 and 10 (Chandra and Rajashekhar, 2016). This difference may be the result of activities from the Collin's Park Water Treatment Plant, the Nova Scotia Fire Fighters School, or the Rocky Lake gravel quarry. It could also be the result of run off from road ways and residences near the lakes.

4.2.2. Total Organic Carbon and Dissolved Organic Carbon

Results for TOC and DOC were very similar, therefore, only the TOC concentrations were reported. Once again, with regards to non-stream/non-tributary samples, overall, Lake Major samples exhibited the highest mean TOC concentration, followed by Fletchers Lake, then Powder Mill Lake. Pockwock Lake samples had the lowest mean TOC concentration. As TOC is used as a measurement of natural organic matter, it can be said that Lake Major has a higher concentration of organic matter than

the other lakes in this study, and that Pockwock Lake has the lowest concentration (Leenheer and Croué, 2003).

4.2.3. Total Nitrogen and Total Phosphorus

Results for TN and TP can be used to determine trophic status (Canadian Council of Ministers of the Environment (CCME), 2004; Nürnberg, 1996; Rast and Thornton, 2005). According to the indexes laid out by the CCME (2004) and Nürnberg (1996), summarized in Table 4.3., TP concentrations depicted in Clement *et al* (2007) for samples from Fletchers Lake were determined to be oligotrophic/mesotrophic in 2000. Meanwhile, TN concentrations for tributary and stream samples from Fletchers Lake in 2016 ranged in trophic status from oligotrophic to mesotrophic/eutrophic. Mean TP and TN concentrations for in-lake samples from Powder Mill Lake indicate samples were oligotrophic. TP concentrations depicted in Ginn *et al* (2015) for samples from Pockwock Lake were determined to be ultra-oligotrophic/oligotrophic in 2005/2006, while mean TN concentrations from in-lake samples from 2016 were oligotrophic. TN concentrations for tributary and stream samples from Pockwock Lake in 2016 ranged in trophic status from oligotrophic to hyper-eurotrophic. TP concentrations depicted in Clement *et al* (2007) for samples from Lake Major were determined to be ultra-oligotrophic/oligotrophic in 2000. In 2016, TP concentrations for tributary/stream samples ranged in trophic status from oligotrophic to meso-oligotrophic.

Table 4.3. Trophic status indexes for TP and TN concentrations according to the CCME (2004) and Nürnberg (1996).

Trophic Status	CCME (2004)	Nürnberg (1996)	
	TP (µg/L)	TP (µg/L)	TN (µg/L)
Ultra-oligotrophic	< 4	-	-
Oligotrophic	4-10	< 10	< 350
Mesotrophic	10-20	10-30	350-650
Meso-eutrophic	20-30	-	-
Eutrophic	35-100	30-100	651-1200
Hyper-eutrophic	> 100	> 100	> 1200

Picoplankton have an advantage in oligotrophic lakes when light conditions are favorable and grazing impacts are low (Callieri and Stockner, 2000, 2002). They also suggest that single celled picocyanobacteria prefer oligotophic to mesotrophic conditions, while colony forming picocyanobacteria prefer mesotrophic to eutrophic conditions. As seen in this study, *Synechococcus*, a single celled, toxin producing picocyanobacteria, was associated with large proportions of OTU reads in samples from Fletchers Lake and Powder Mill Lake. However, this was not the case for samples from Pockwock Lake and Lake Major. In contrast, OTU's associated with *Prochlorococcus*, another single celled, toxin producing picocyanobacteria, were only detected in Powder Mill Lake samples, and only in a very small proportion compared to other cyanobacteria.

4.2.4. UV_{254}, Specific Ultraviolet Absorbance, Turbidity and Colour

UV_{254}, unlike TOC and DOC, only roughly indicates overall NOM (Leenheer and Croué, 2003). Like mean TOC concentration, pump house samples from Lake Major had the highest mean UV_{254} reading. In-lake samples from Pockwock Lake had higher mean UV_{254} readings than Powder Mill Lake. SUVA can be used to describe the presence and aromaticity of DOC compounds (Leenheer and Croué, 2003). For example, high SUVA values are indicative of high concentrations of hydrophobic, aromatic NOM, like humic substances (Hansen et al, 2016). As mentioned previously, humic substances are naturally occurring PCR inhibitors (Schrader *et al*, 2012; Rački *et al*, 2014; Jones *et al*, 2015). Therefore, one would expect that the failed samples mentioned earlier would be more likely to occur in lakes with high SUVA values, and during times when SUVA is high. However, this was not always the case. Although samples from the pump house at Lake Major did fail, and had higher mean SUVA values than the other lakes in this study, samples from the Collin's Park Treatment Plant at Fletchers Lake had the second highest mean SUVA values, and did not fail throughout the season. In-lake samples from Pockwock Lake had higher mean SUVA values than in-lake samples from Powder Mill Lake, and had a lower amplification success rate than samples from Powder Mill Lake. Also interesting to note, is that failed samples from Powder Mill Lake and Pockwock

Lake more often occurred during August through to October, when mean SUVA was lower than in other months.

Turbidity measures the intensity of suspended and colloidal matter (APHA, 2012). Samples collected from the Collin's Park Treatment Plant had the highest mean turbidity readings, followed by in-lake samples from Powder Mill Lake, and in-lake samples from Pockwock Lake. Samples from the pump house at Lake Major had the lowest mean turbidity readings. Therefore, it can be said that samples from Fletchers Lake had a higher intensity of scattered light than other lakes in this study. Meanwhile, Lake Major had a lower intensity of scattered light than the other lakes. When turbidity is removed, the true colour of samples can be ascertained (APHA, 2012). Lake Major had the highest mean colour value, followed by Pockwock Lake, and Fletchers Lake. Powder Mill Lake had the lowest mean colour of the lakes in this study. Theoretically, photoautotrophs, like cyanobacteria, may be hindered by altered wavelengths through the water column. Alternatively, because of the phycobiliproteins that cyanobacteria possess, they may have an advantage over other photosynthetic organisms in aquatic environments with intense light scattering. With this in mind, it could be possible that suspended and colloidal matter could contain or be made of cyanobacteria.

4.2.5. Data Range for Tributary and Stream Samples

With regards to tributary and stream samples, Pockwock Lake tributaries usually had wider ranges of water quality values than Fletchers Lake and Lake Major, while Lake Major tributary samples typically had the smallest range of values. Exceptions being with pH, where Fletchers Lake tributaries exhibited the widest range of values, and with SUVA, where Pockwock Lake tributaries and Fletchers Lake tributaries had similar SUVA ranges.

4.3. ENVIRONMENT DATA

Environmental conditions that are preferential to cyanobacteria include intense rainfall events followed by calm weather with warm water temperature, decreased mixing and turbulence, minimal grazing by zooplankton, and low mean irradiance (Whitton and Potts, 2000). This pairing of rainfall events with drought/drought like conditions affects nutrient loads into lakes, and prolongs stratification (Paerl *et al*, 2016).

This was seen in 2016 as severe drought conditions were declared in Nova Scotia that year (Ward, 2016). This particularly unusual event marked the driest summer since the late 1800's. As this drought occurred, an algal bloom at Powder Mill Lake was reported on July 8 (PNS, 2016c). Prior to this event, precipitation events between June 6 and 8 were followed by almost four weeks of dry weather, and increased temperatures. However, wind was inconsistent, cycling almost equally above and below the 10.8 km/h threshold for bloom dispersal (Whitton and Potts, 2000).

In early September, it was noted that there was increased vegetation/algae growth along the shoreline of Fletchers Lake. Despite there not being an official bloom, microcystin, specifically microcystin-LR (MC-LR), was detected in July, 2016 (Brohpy, 2017). According to Brophy (2017), MC-LR concentrations increased above the maximum allowable concentration (MAC) of 1.5 µg/L set by Health Canada for MC-LR in drinking water, from July to September, then decreased below detection limit after September/October (Health Canada, 2017). However, MC-LR concentrations remained below 20 µg/L, the MAC set by Health Canada for total microcystin concentration allowed in recreational water supplies, throughout 2016 (Health Canada, 2012). Prior to this event, August had one major rain event, with the remainder of the month being quite dry and warm. Similarly, the beginning of September was quite dry and warm, as well. Wind seemed slightly more likely to be around or under the 10 km/h threshold for bloom dispersal leading up to early September.

CHAPTER 5 CONCLUSIONS

In general, information regarding cyanobacteria, cyanotoxins and algal blooms is limited in Atlantic Canada. This is problematic in the face of climate change and human activities. The objective of this study was to provide a baseline for future research into cyanobacteria population ecology which will enable future best practices for lake management in Nova Scotia.

Results of this study have shown the presence of at least 35 taxa of cyanobacteria and one taxa of unassigned bacteria, of which, 15 genera of cyanobacteria were identified. Of those genera, 11 have been associated with cyanotoxins. However, OTU's affiliated with toxin producing cyanobacteria typically represented 1-3% of all OTU's detected each month (with some exceptions).

Results have also shown that samples which failed PCR amplification may have been the result of naturally low cyanobacteria DNA concentrations and/or presence of naturally occurring inhibitors, and may have exhibited seasonality, as PCR amplification failure typically happened to samples collected between August and September, from in-lake sample sites. Also, samples collected from Fletchers Lake and Powder Mill Lake had a higher success rate for PCR amplification than samples collected from Pockwock Lake and Lake Major.

Supplementary water quality and environmental data showed that Fletchers Lake and Powder Mill Lake, both unprotected water bodies, had higher pH, alkalinity, TN, TP and turbidity averages than Pockwock Lake and Lake Major. It was also determined that there were periods of rain followed by long periods of warm, dry, relatively calm weather prior to microcystin detection at Fletchers Lake, and prior to the algal bloom detection at Powder Mill Lake.

Ultimately, cyanobacteria remain unpredictable and more experiments will need to be made to better understand these organisms. This thesis will hopefully provide the

baseline necessary for which those experiments can be built for the benefit of Nova Scotia as it develops best practices for lake and cyanotoxin management.

For future research, there are many avenues down which experiments can be made concerning cyanobacteria. Most notably, now that there is an idea of which cyanobacteria are present in lakes in HRM, laboratory cultures can be made to identify cyanobacteria to the species level. From there, the following experiments could be run:

1) A partnership with another laboratory would allow researchers to delve into bioinformatics and gene expression for toxin production, which would be more useful as not all toxic cyanobacteria create blooms (Pick, 2016).

2) The determination of cyanobacteria population ecology within the water column and throughout lakes and tributaries might be used to identify areas of potential risk (i.e. areas or ecological niches where toxic cyanobacteria taxa may be more likely appear). It would also be beneficial to compare cyanobacteria population and water chemistry data between pre- and post-treatment plant samples, and distribution system samples

3) Correlations between water chemistry and cyanobacteria presence could be made. For example, further research with TOC and DOC could be used to determine sources of dissolved organic matter (DOM) that would affect aquatic food webs, especially with regards to cyanobacteria and the grazers that consume them (Hansen *et al*, 2016). Paired with colour, turbidity, UV_{254}, and SUVA data, research would provide insight into the impact of DOM on light scattering throughout the water column on organisms that photosynthesize.

4) Correlations between metals and cyanobacteria presence could also be made. For example, Sorichetti *et al* (2014) suggests that there may be a correlation between iron (Fe) availability, cyanobacteria growth, and competition for Fe that may be more influential than TN and TP concentration.

5) Further studies into using geosmin and MIB to monitor cyanobacteria occurrence, and utilize flow cytometry or FEEM to monitor phycocyanin and phycoerythrin to monitor cyanobacteria as well.

6) Conduct further studies into other organisms that can cause harmful algal blooms.

REFERENCES

Abed, R., Barry, M., Kindi, S., and Golubic, S. (2013). Structure and cyanobacterial species composition of microbial mats in an Arabian Desert stream. *African Journal of Microbiology Research*, 7(15): 1434-1442.

Allen, C. (2016, July 05). Algae bloom continues in Yarmouth County and beyond. *The Digby Courier*. Retrieved from: http://www.digbycourier.ca/news/regional/algae-bloom-continues-in-yarmouth-county-and-beyond-37688/

Anderson, L., Krkošek, W., Stoddart, A., Trueman, B., and Gagnon, G. (2017). Lake recovery through reduced sulfate deposition: A new paradigm for drinking water treatment. *Environmental Science and Technology*, 51 (3), 1414−1422.

American Public Health Association, American Water Works Association, and Water Environment Federation (2012). Standard Methods for the Examination of Water and Wastewater. Washington, DC: APHA.

Barber, S. (2016, Jun 13). Lake Erie's toxic algae bloom forecast for summer 2016. *EcoWatch*. Retrieved from: http://www.ecowatch.com/lake-eries-toxic-algae-bloom-forecast-for-summer-2016-1891172391.html

Brophy, M. (2017). Understanding water quality and the presence of mycrocystin-LR in a small drinking water supply. Halifax, NS; Dalhousie University.

Brylinsky, M. (2011). Water quality survey of ten lakes located in the Carleton River watershed area of Digby and Yarmouth Counties, Nova Scotia. Nova Scotia Department of the Environment. Retrieved from: https://www.novascotia.ca/nse/surface.water/docs/Yarmouth.Area.Lakes.Water.Quality.Assessment.pdf

Buratti, F., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., and Funari, E. (2017). Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Archives of Toxicology*, 91(3): 1049-1130.

Callieri, C. and Stockner, J. (2000). Picocyanobacteria success in oligotrophic lakes: fact of fiction? *Journal of Limnology*, 59(1): 72-76.

Callieri, C. and Stockner, J. (2002). Freshwater autotrophic picoplankton: a review. *Journal of Limnology*, 61(1): 1-14.

Campbell, F. (2016, Sep 10). No answers for toxic blue-green algae bloom on Mattatall Lake. *Local Xpress.* Retrieved from: https://www.localxpress.ca/local-news/no-answers-for-toxic-blue-green-algae-bloom-on-lake-mattatall-410559

Canadian Council of Ministers of the Environment (2004). Canadian water quality guidelines for the protection of aquatic life: Phosphorus: Canadian Guidance Framework for the Management of Freshwater Systems. In: Canadian environmental quality guidelines. Winnipeg, MB: Canadian Council of Ministers of the Environment.

Carmichael, W. and Boyer, G. (2016). Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. *Harmful Algae*, 54: 194-212.

CBC News (2012, Apr 02). Mink farms likely polluted lakes, study finds. *CBC News*. Retrieved from: http://www.cbc.ca/news/canada/nova-scotia/mink-farms-likely-polluted-lakes-study-finds-1.1238053

Chandra, S. and Rajashekhar, M. (2016). Effect of pH on freshwater cyanobacteria isolated from different habitats of southern Karnataka. International Journal of Life Sciences and Technology, 9(7): 56-64.

Clement, P., Keizer, P., Gordon, D., Clair, T., and Hall, G. (2007). Synoptic water quality survey of selected Halifax Regional Municipality Lakes on 28–29 March 2000. Canadian Technical Report of Fisheries and Aquatic Science. 2726. Dartmouth, NS; Bedford Institute of Oceanography.

Comeau, A., Douglas, G., and Langille, A. (2017). Microbiome helper: A custom and streamlined workflow for microbiome research. *American Society for Microbiology*, 2(1): 1-11.

Fortin, N., Aranda-Rodriguez, R., Jing, H., Pick, F., Bird, D., and Greer, W. (2010). Detection of microcystin-producing cyanobacteria in Missisquoi Bay, Quebec, Canada, using quantitative PCR. *Applied and Environmental Microbiology*, 76(15): 5105-5112.

Ginn, B., Rajaratnam, T., Cumming, B., and Smol, J. (2015). Establishing realistic management objectives for urban lakes using paleolimnological techniques: an example from Halifax Region (Nova Scotia, Canada). *Lake and Reservoir Management*, 31(2): 92-108.

Gladyshev, M., Sushchik, N., Makhutova, O., Dubovskaya, O., Kravchuk, E., Kalachova, G., and Khromechek, E. (2010). Correlations between fatty acid composition of seston and zooplankton and effects of environmental parameters in a eutrophic Siberian reservoir. *Limnologica*, 40: 343-357.

Gonçalves, R., Villafañe, V., Medina, C., Barbieri, E., and Helbling, W. (2011). Plankton dynamics and photosynthesis in a eutrophic lake in Patagonia (Argentina): influence of grazer abundance and UVR. *Latin American Journal of Aquatic Research*, 39(1): 117-130.

Government of Canada (2016a). Fletchers Lake (formerly Lake Fletchers). Retrieved from: http://www4.rncan.gc.ca/search-place-names/unique/fadd4af0af5f11d892e2080020a0f4c9

Government of Canada (2016b). Hourly data reports for Bedford Range, Nova Scotia. Retrieved from: http://climate.weather.gc.ca/climate_data/hourly_data_e.html?hlyRange=2004-09-24%7C2016-12-04&dlyRange=%7C&mlyRange=%7C&StationID=43123&Prov=NS&urlExtension=_e.html&searchType=stnProx&optLimit=specDate&Month=12&Day=4&StartYear=1840&EndYear=2016&Year=2016&selRowPerPage=25&Line=0&txtRadius=25&optProxType=navLink&txtLatDecDeg=44.881111111111&txtLongDecDeg=63.508611111111&timeframe=1

Government of Canada (2016c). Powder Mill Lake (formerly Fish Lake). Retrieved from: http://www4.rncan.gc.ca/search-place-names/unique/96ea4042afe711d892e2080020a0f4c9

Gutiérrez-Salmeán, G., Fabila-Castillo, L., and Chamorro-Cevallos, G. (2015). Nutritional and toxicological aspects of Spirulina (*Arthrospira*). *Nutrición Hospitalaria*, 32(1): 34-40.

Halifax Regional Municipality (2017a). Collin's Park Public Water Supply Area. Retrieved from: https://www.halifax.ca/sites/default/files/documents/home-property/water/CollinsParkMap.pdf

Halifax Regional Municipality (2017b). Lake Major Watershed Area. Retrieved from: https://www.halifax.ca/sites/default/files/documents/home-property/water/LakeMajorMap.pdf

Halifax Regional Municipality (2017c). Pockwock Lake and Tomahawk Lake Watershed Area. Retrieved from: https://www.halifax.ca/sites/default/files/documents/home-property/water/PockwockTomahawkMap.pdf

Halifax Regional Municipality (2017d). Source Water Protection. Retrieved from: https://www.halifax.ca/home-property/halifax-water/water-services/source-water-protection

Halifax Water (2017a). Water Supply and Treatment. Retrieved from: http://www.halifax.ca/HalifaxWater/WaterQuality/treatment.php

Halifax Water (2016). Halifax Water Watersheds. Retrieved from: http://www.halifax.ca/halifaxwater/sourcewater/watersheds.php

Halifax Water (2010). Halifax Water Best Management Practices for Halifax Water and Crown Managed Lands. Halifax, NS: Halifax Water.

Hansen, A., Kraus, T., Pellerin, B., Fleck, J., Downing, B., and Bergamaschi, B. (2016). Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation. *Limnology and Oceanography*. 61: 1015-1032.

Health Canada (2012). Guidelines for Canadian Recreational Water Quality, Third Edition. Ottawa, ON; Health Canada

Health Canada (2017). Guidelines for Canadian Drinking Water Quality: Summary Table. Ottawa, ON; Health Canada,

Hunter, P., Matthews, M., Kutser, T., and Tyler, A. (2017). Remote sensing of cyanobacterial blooms in inland, coastal and ocean waters. In: Meriluoto, J., Spoof, L. and Codd, G. (eds). Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. Chichester, UK: John Wiley & Sons, Ltd.

Izaguirre, G. and Taylor, W. (2004). A guide to geosmin- and MIB-producing cyanobacteria in the United States. *Water Science and Technology*, 49(9): 19-24.

Izaguirre, G., Hwang, C., Krasner, S., and McGuire, M. (1982). Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. *Applied and Environmental Microbiology*, 43(3): 708-714.

Jakubowska, N. and Szeląg-Wasielewska, E. (2015). Toxic picoplanktonic cyanobacteria – review. *Marine Drugs*, 13: 1497-1518.

Jeffries, D., Clair, T., Couture, S., Dillon, P., Dupont, J., Keller, W., McNicol, D., Turner, M., Vet, R., and Weeber, R. (2003a). Assessing the recovery of lakes in southeastern Canada from the effects of acidic deposition. *Ambio*, 32(3): 176-182.

Jeffries, D., Brydges, T., Dillon, P., and Keller, W. (2003b). Monitoring the results of Canada/U.S.A. acid rain control programs: some lake responses. *Environmental Monitoring and Assessment*, 88: 3-19.

Jones, L., Uyua, N., and Manrique, J. (2015). The peril of PCR inhibitors in environmental samples: the case of *Didymosphenia geminata*. *Biodiversity and Conservation*, 24: 1541-1548.

Kelly, M. (2015, Jul 31). One-year anniversary of Toledo's water system shutdown due to Lake Erie's algae bloom; "we are all Toledo." *NRDC.* Retrieved from: https://www.nrdc.org/media/2015/150731

Lavoie, J. (2014, Aug 15). Mink farm pollution key culprit in rendering Nova Scotia lakes unswimmable: Report. *Desmog Canada*. Retrieved from: https://www.desmog.ca/2014/08/15/mink-farm-pollution-key-culprit-rendering-nova-scotia-lakes-unswimmable-report

Lee, J., Rai, P., Jeon, Y., Kim, K., and Kwon, E. (2017). The role of algae and cyanobacteria in the production and release of odorants in water. *Environmental Pollution*, 227: 252-262.

Leenheer, J. and Croué, J. (2003). Characterizing dissolved aquatic organic matter. *Environmental Science and Technology*, 18-26.

Lin, W., Zhao, D., and Luo, J. (2017). Distribution of alkaline phosphatase genes in cyanobacteria and the role of alkaline phosphatase on the acquisition of phosphorus from dissolved organic phosphorous for cyanobacterial growth. *Journal of Applied Phycology*, DOI: 10.1007/s10811-017-1267-3.

Lochaber Community Development Association (2012). Blue-green algae alert for Lochaber Lake. Retrieved from: https://www.lochaber.ca/blue-green-algae-alert-for-lochaber-lake/

Maier, R.M., Pepper, I.L., and Gerba, C.P. (2000). Environmental Microbiology. San Diego, CA; Academic Press.

Mathieson, D. (2015, Jun 18). Neurotoxin producing algae found in Mattatall Lake. *Amherst News*. Retrieved from: http://www.cumberlandnewsnow.com/news/local/neurotoxin-producing-algae-found-in-mattatall-lake-31266/

Meloney, N. (2017, Oct 09). Ecosystem revival set back by unexpected algae bloom in Dartmouth lake. *CBC News*. Retrieved from: http://www.cbc.ca/news/canada/nova-scotia/plankton-oathill-lake-dartmouth-bob-rutherford-algae-1.4346536

Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs (2014). Bilan de la gestion des épisodes de fleurs d'eau d'algues bleu-vert, de 2007 à 2012. Quebéc City, QC; Gouvernement du Québec.

MO BIO Laboratories (2016a). DNeasy Power Water Kit. Retrieved from: https://mobio.com/products/dna-isolation/water/powerwaterr-dna-isolation-kit.html

MO BIO Laboratories (2016b). DNeasy Power Water Sterivex Kit. Retrieved from: https://mobio.com/products/dna-isolation/water/powerwaterr-sterivextm-dna-isolation-kit.html

Moreira, C., Ramos, V., Azevedo, J., and Vasconcelos, V. (2014). Methods to detect cyanobacteria and their toxins in the environment. *Applied Microbiology and Technology*, 98(19): 8073-8082.

Nübel, U., Garcia-Pichel, F., and Muyzer, G. (1997). PCR Primers to amplify 16S rRNA genes from cyanobacteria. *Applied and Environmental Microbiology*, 63(8): 3327-3332.

Nürnberg, G. (1996). Trophic state of clear and colored, soft- and hardwater lakes with special consideration of nutrients, anoxia, phytoplankton and fish. *Lake Reservoir Management*. 12(4): 432–447.

Omega Bio-tek, Inc. (2014). E.Z.N.A. Water DNA Kit Product Manual. Norcross, GA: Omega.

Paerl, H., Gardner, W., Havens, K., Joyner, A., McCarthy, M., Newell, S., Qin, B., and Scott, J. (2016). Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. *Harmful Algae*, 54: 213-222.

Paerl, H., Xu, H., Hall, N., Rossignol, K., Joyner, A., Zhu, G., and Qin, B. (2015) Nutrient limitation dynamics examined on a multi-annual scale in Lake Taihu, China: Implications for controlling eutrophication and harmful algal blooms. *Journal of Freshwater Ecology*, 30(1): 5-24.

Pan, J., Bournod, C., Cuadrado, D., Vitale, A., and Piccolo, M. (2013). Interaction between estuarine microphytobenthos and physical forcings: the role of atmospheric and sedimentary factors. *International Journal of Geosciences*, 4: 352-361.

Percival, S. and Williams, D. (2014). Cyanobacteria. In: Percival, S., Yates, M., Williams, D., Chalmers, R. and Gray, N. Microbiology of Waterborne Diseases, Microbiological Aspects and Risks. London, UK: Elsevier Ltd.

Pick, F. (2016). Blooming algae: a Canadian perspective on the rise of toxic cyanobacteria. *Canadian Journal of Fisheries and Aquatic Sciences*, 73(7): 1149-1158.

Province of Nova Scotia (2017a). Geoscience and Mines Branch, Geoscience Atlas Interactive Map. Retrieved from: https://fletcher.novascotia.ca/DNRViewer/?viewer=Geoscience

Province of Nova Scotia (2017b). Geoscience and Mines Branch, Mineral Resource Land Use Atlas Interactive Map. Retrieved from: https://fletcher.novascotia.ca/DNRViewer/?viewer=MRLU

Province of Nova Scotia (2017c). *Halifax Regional Water Commission Act, Chapter 55 of the Acts of 2007, amended 2012, c. 60; 2016, c. 23, An Act Respecting the Halifax Regional Water Commission*. Retrieved from the Nova Scotia Legislature website: https://nslegislature.ca/sites/default/files/legc/statutes/halifax%20regional%20water%20commission.pdf

Province of Nova Scotia (2017d). Significant Species and Habitats Database, Provincial Landscape Viewer. Retrieved from: https://nsgi.novascotia.ca/plv/

Province of Nova Scotia (2016a). Angler's Handbook and 2016 Summary of Regulations. Retrieved from: https://novascotia.ca/fish/documents/Anglers_Handbook_2016.pdf

Province of Nova Scotia (2016b). Blue green algae bloom in Lake Torment. Retrieved from: https://novascotia.ca/news/release/?id=20160624010

Province of Nova Scotia (2016c). Blue green algae bloom in Powder Mill Lake. Retrieved from: https://novascotia.ca/news/release/?id=20160708002

Province of Nova Scotia (2016d). Blue-green algae in Kings County. Retrieved from: https://novascotia.ca/news/release/?id=20160923005

Province of Nova Scotia (2016e). Blue green algae in Pictou County. Retrieved from: https://novascotia.ca/news/release/?id=20160817010

Province of Nova Scotia (2016f). Blue green algae in Yarmouth County. Retrieved from: https://novascotia.ca/news/release/?id=20160818007

Province of Nova Scotia (2016g). Nova Scotia Lake Survey Program Interactive Map. Retrieved from: http://nse.maps.arcgis.com/apps/webappviewer/index.html?id=7ded7a30bef44f848e8a4fc8672c89bd

Province of Nova Scotia (2016h). Pockwock Wilderness Area. Retrieved from: https://novascotia.ca/nse/protectedareas/wa_pockwock.asp

Province of Nova Scotia (2015). Blue green algae bloom in Lake Torment. Retrieved from: https://novascotia.ca/news/release/?id=20150814003

Province of Nova Scotia (2014). Blue green algae bloom in Lake Torment. Retrieved from: https://novascotia.ca/news/release/?id=20140627010

Province of Nova Scotia (2013a). *Environment Act, Chapter 1 of the Acts of 1994-1995 amended 1998, c. 18, s. 557; 1999 (2nd Sess.), c. 12, s. 76; 2001, c. 6, s. 103; 2004, c. 3, ss. 20, 21; 2006, c. 2, s. 3; 2006, c. 30; 2011, c. 61, An Act to Reform the Environmental Laws of the Province and to Encourage and Promote the Protection, Enhancement and Prudent Use of the Environment*. Retrieved from the Nova Scotia Legislature website: https://nslegislature.ca/sites/default/files/legc/statutes/environment.pdf

Province of Nova Scotia (2013b). Lumsden Pond Beach Re-Opened. Retrieved from: https://novascotia.ca/news/release/?id=20130816010

Province of Nova Scotia (2010). Suspected algae bloom spotted in Lake Ainslie. Retrieved from: https://novascotia.ca/news/release/?id=20100901004

Province of Nova Scotia (2009a). Algae bloom reported in Margaree Rivers. Retrieved from: https://novascotia.ca/news/release/?id=20090923009

Province of Nova Scotia (2009b). Lake Ainslie algae test results. Retrieved from: https://novascotia.ca/news/release/?id=20090925012

Province of Nova Scotia (2007). Algae bloom in three Nova Scotia Lakes. Retrieved from: https://novascotia.ca/news/release/?id=20070718004

Province of Nova Scotia (2002). *Protection of Property Act, Chapter 363 of the revised statutes, 1989, amended 2001, c. 12, s. 47*. Retrieved from the Nova Scotia Legislature website: https://nslegislature.ca/sites/default/files/legc/statutes/protect.htm

Rački, N., Dreo, T., Gutierrez-Aguirre, I., Blejec, A., and Ravnikar, M. (2014). Reverse transcriptase droplet digital PCR shows high resilience to PCR inhibitors from plant, soil and water samples. *Plant Methods*, 10(1): 1-10.

Raposo, M., Costa de Morais, R., and Bernardo de Morais, A. (2013). Health applications of bioactive compounds from marine microalgae. *Life Sciences*, 93: 479-486.

Rast, W. and Thornton, J. (2005). The phosphorus loading concept and the OECD eutrophication programme: Origin, application, and capabilities. In: O'Sullivan, P. and Reynolds, C. (eds). The lakes handbook: Lake restoration and rehabilitation. Malden, MA: Blackwell Publishing.

Rastogi, R., Sonani, R., and Madamwar, D. (2015). Effects of PAR and UV radiation on the structure and functional integrity of phycocyanin, phycoerythrin, and allophycocyanin isolated from the marine cyanobacterium *Lyngbya* sp. A09DM. *Photochemistry and Photobiology*, 91(4): 837-844.

Ringelberg, J. (2010). Diel vertical migration of zooplankton in lakes and oceans causal explanations and adaptive significances. London: Springer.

Sainmont, J., Thygesen, U., and Visser, A. (2012). Diel vertical migration arising in a habitat selection game. *Theoretical Ecology*, 6(2): 241-251.

Scott, R. (2004). Pockwock Lake water quality assessment: Effects of forest harvesting activity on surface water quality, final report. Halifax, NS: CWRS.

Schindler, D., Carpenter, S., Chapra, S., Hecky, R., and Orihel, D. (2016). Reducing phosphorous to curb lake eutrophication is a success. *Environmental Science and Technology*, 50(17): 8923-8929.

Schrader, C., Schielke, A., Ellerbroek, L., and Johne, R. (2012). PCR inhibitors – Occurrence, properties and removal. *Journal of Applied Microbiology*, 113: 1014-1026.

Soil and Water Conservation Society of Metro Halifax (2017). Narrative on lakes/rivers within the Halifax municipality. Retrieved from: http://lakes.chebucto.org/WATERSHEDS/narrative.html#shubenacadieriver

Sorichetti, R., Creed, I., and Trick, C. (2014). Evidence for iron-regulated cyanobacterial predominance in oligotrophic lakes. *Freshwater Biology*. 59: 679-691.

Stoddard, J., Jeffries, D., Lükewille, A., Clair, T., Dillon, P., Driscoll, C., Forsius, M., Johannessen, M., Kahl, J., Kellogg, J., Kemp. A, Mannio, J., Monteith, D., Murdoch, P., Patrick, S., Rebsdorf, A., Skjelkvåle, B., Stainton, M., Traaen, T., van Dam, H., Webster, K., Wieting, J., and Wilander, A. (1999). Regional trends in aquatic recovery from acidification in North America and Europe. *Nature*, 401: 575-578.

Sullivan, H. (2017, Nov 09). Mattatall Lake appears clear of green toxic algae blooms. *Truro Daily News*. Retrieved from: http://www.trurodaily.com/news/mattatall-lake-appears-clear-of-green-toxic-algae-blooms-160654/

Suurnäkki, S., Gomez-Saez, G., Rantala-Ylinen, A., Jokela, J., Fewer, D., and Sivonen, K. (2015). Identification of geosmin and 2-methylisoborneol in cyanobacteria and molecular detection methods for the producers of these compounds. *Water Research*, 68: 56-66.

Taylor, D. (2009). A water quality survey of nine lakes in the Carleton River watershed area, Yarmouth County, Nova Scotia. Water and Wastewater Branch, Nova Scotia Environment. Retrieved from: https://novascotia.ca/nse/surface.water/docs/Yarmouth.Area.Lakes.Report.with.Appendices.pdf

Tetanish, R. (2016, Feb 09). Growth of blue-green algae across Nova Scotia a concern: Dal AC prof. *Annapolis County Spectator*. Retrieved from: http://www.annapoliscountyspectator.ca/news/local/growth-of-blue-green-algae-across-nova-scotia-a-concern-dal-ac-prof-165/

The Weather Network (2017). Historical weather: Bedford, NS. Retrieved from: https://www.theweathernetwork.com/ca/weather/historical-weather/nova-scotia/bedford

University of New Hampshire (2015). Toxic cyanobacteria of New England, "The Dirty Dozen." Center for Freshwater Biology. Retrieved from: http://cfb.unh.edu/CyanoKey/indexCyanoQuickGuide.html

Verhamme, E., Redder, T., Schlea, D., Grush, J., Bratton, J., and DePinto, J. (2016). Development of the Western Lake Erie Ecosystem Model (WLEEM): Application to connect phosphorus loads to cyanobacteria biomass. *Journal of Great Lakes Research*, 42(6): 1193-1205.

Walker, T., Crittenden, P., and Young, S. (2003). Regional variation in the chemical composition of winter snow pack and terricolous lichens in relation to sources of acid emissions in the Usa river basin, northeast European Russia. *Environmental Pollution*, 125(3): 401-412

Walters, W., Hyde, E., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J., Jansson, J., Caporaso, J., Fuhrman, J., Apprill, A., and Knight, R. (2015). Improved bacterial 16S rRNA Gene (V4 and V4-V5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *American Society for Microbiology*, 1(1): 1-10.

Ward, R. (2016, Sep 18). Nova Scotia drought unusual, catches people unprepared. *CBC News*. Retrieved from: http://www.cbc.ca/news/canada/nova-scotia/drought-agriculture-canada-weather-dry-water-wells-farms-1.3767563

Waterbury, J. (2006). The Cyanobacteria—Isolation, Purification and Identification. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K., Stackebrandt, E. (eds). The Prokaryotes. New York, NY: Springer.

Wendland, S. (2011, Jan 25). Murky waters. *Halifax Media Co-op*. Retrieved from: http://halifax.mediacoop.ca/fr/story/murky-waters/5815

Whitton, B. and Potts, M. (Eds.) (2000). The Ecology of Cyanobacteria: Their diversity in time and space. Netherlands: Kluwer Academic Publishers.

World Health Organization (WHO) (1999). Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. London, UK: E & FN Spon.

Zamyadi, A., McQuaid, N., Prévost, M., and Dorner, S. (2012). Monitoring of potentially toxic cyanobacteria using an online multi-probe in drinking water sources. *Journal of Environmental Monitoring*, 14: 579-588.

APPENDIX A

Fletchers Lake

Collection dates, Sample Sites, Monthly Proportion of Reads and Monthly Order Composition for Cyanobacteria Population Analysis, Along with Monthly Water Chemistry Data for Treatment Plant Water and Tributary Samples

Fletchers Lake - Collection Dates and Sample Sites		
14-Sep-16	**29-Sep-16**	**19-Oct-16**
FI	DWR	DWR
	FBB*	FBB*
	FD	FD
	FI	FI
	FND	FND
	FO	FO
	FTB*	FTB*
		INWW
		MILO
		OUTWW*
		SOLO
* = Sample failed to amplify during PCR		

Fletchers Lake - Monthly Proportion of Reads	SEPTEMBER			OCTOBER	
	OVERALL (%)	AVE (%)		OVERALL (%)	AVE (%)
Class Chloroplast	26.58	27.60		38.24	38.04
Ord. Gloeobacterales	0.07	0.08		0.04	0.04
Ord. Nostocales	0.45	0.47		0.70	0.66
Ord. Chroococcales	6.32	6.32		7.87	7.36
Ord. Oscillatoriales	0.00	0.00		0.13	0.15
Ord. Pseudanabaenales	1.33	1.35		0.36	0.38
Ord. Synechococcales	52.28	50.07		38.02	35.42
Other Cyanobacteria	2.48	2.58		3.34	3.54
Unassigned	10.49	11.52		11.29	14.42

Fletchers Lake - Order Composition	SEPTEMBER		OCTOBER	
	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)
Class Chloroplast	**26.58**	**27.60**	**38.24**	**38.04**
Other	0.00	0.00	1.40	3.57
Chlorophyta	2.04	3.07	1.76	2.15
Cryptophyta	47.01	40.49	66.89	48.68
Haptophyta	4.46	6.77	2.07	4.14
Rhodophyta	0.07	0.04	0.18	0.30
Stramenopiles	45.17	48.55	26.93	40.65
Streptophyta	1.25	1.09	0.77	0.50
Ord. Gloeobacterales	**0.07**	**0.08**	**0.04**	**0.04**
Fam. Gloeobacteraceae (*Gloeobacter*)	100.00	16.67	100.00	37.50
Ord. Nostocales	**0.45**	**0.47**	**0.70**	**0.66**
Fam. Nostocaceae (Other)	57.69	10.00	0.00	0.00
Fam. Nostocaceae (*Anabaena*)	0.00	0.00	53.85	28.64
Fam. Nostocaceae (*Dolichospermum*)	3.85	16.67	40.38	8.86
Fam. Nostocaceae (*Nodularia*)	38.46	6.67	0.00	0.00
Fam. Nostocaceae (*Nostoc*)	0.00	0.00	5.77	12.50
Ord. Chroococcales	**6.32**	**6.32**	**7.87**	**7.36**
Fam. Chroococcaceae	2.49	9.56	0.68	2.99
Fam. Gomphosphaeriaceae	28.45	36.64	94.71	52.20
Fam. Microcystaceae (*Microcystis*)	28.73	20.60	1.19	4.96
Fam. Xenococcaceae (Other)	38.12	32.15	3.41	14.86
Fam. Xenococcaceae (*Chroococcidiopsis*)	2.21	1.05	0.00	0.00
Ord. Oscillatoriales	**0.00**	**0.00**	**0.13**	**0.15**
Fam. Phormidiaceae (*Phormidium*)	0.00	0.00	100.00	25.00
Ord. Pseudanabaenales	**1.33**	**1.35**	**0.36**	**0.38**
Fam. Other	22.37	13.49	25.93	7.95
Fam. Pseudanabaenaceae (Other)	9.21	2.16	0.00	0.00
Fam. Pseudanabaenaceae (*Leptolyngbya*)	5.26	1.23	25.93	22.99
Fam. Pseudanabaenaceae (*Pseudanabaena*)	63.16	33.11	48.15	31.55
Ord. Synechococcales	**52.28**	**50.07**	**38.02**	**35.42**
Fam. Chamaesiphonaceae	0.03	16.67	0.04	6.25
Fam. Synechococcaceae (*Synechococcus*)	99.97	83.33	99.96	93.75
Other Cyanobacteria	**2.48**	**2.58**	**3.34**	**3.54**
Unassigned	**10.49**	**11.52**	**11.29**	**14.42**

Fletchers Lake Raw Plant Water	June	July	August	September	October
Alkalinity ($CaCO_3$ mg/L)	15.93 ± 0.06	17.06 ± 2.57	15.59	18.02 ± 0.94	16.96
pH	6.15 ± 0.15	6.27 ± 0.29	5.29	6.72 ± 0.40	6.16
TOC (mg/L)	3.53 ± 0.16	3.65 ± 0.11	3.63	3.08 ± 0.15	3.41
TN (mg/L)	0.24 ± 0.03	0.23 ± 0.04	0.15	0.13 ± 0.01	0.13
SUVA (m^{-1} abs per mg/L of DOC)	3.48 ± 0.38	3.03 ± 0.14	2.65	2.12 ± 0.01	2.92
Turbidity (NTU)	1.41 ± 0.09	1.14 ± 0.54	0.89	1.60 ± 0.54	1.95
Color (NTU)	-	-	6.50	5.67 ± 0.58	9.50

Fletchers Lake Streams and Tributaries	June	July	August	September	October
Alkalinity ($CaCO_3$ mg/L)*					
Max	19.68	16.04	23.38	28.41	16.97
Min	1.71	0.03	2.16	1.86	7.06
Mean	13.39	12.33	12.97	14.92	13.30
Median	14.69	14.74	15.97	17.06	14.59
pH					
Max	6.70	6.77	5.47	7.34	6.44
Min	3.57	3.62	4.51	4.60	4.48
Mean	5.74	5.84	5.18	6.47	5.86
Median	6.26	6.10	5.28	6.56	6.03
TOC (mg/L)					
Max	21.20	12.32	8.05	12.82	15.24
Min	3.04	2.30	1.98	2.29	3.29
Mean	7.29	5.24	3.96	4.32	7.26
Median	5.05	3.67	3.55	3.40	6.61
DOC (mg/L)					
Max	20.55	11.78	8.25	13.72	15.11
Min	3.03	2.27	2.01	2.91	3.37
Mean	7.14	5.18	3.82	4.81	7.47
Median	5.03	3.70	3.35	3.32	6.97
TN (mg/L)					
Max	0.45	0.27	0.61	0.21	0.18
Min	0.07	0.06	0.09	0.06	0.08
Mean	0.24	0.19	0.19	0.14	0.12
Median	0.20	0.21	0.12	0.13	0.11

SUVA (m^{-1} abs per mg/L of DOC)					
Max	6.40	6.71	6.62	4.27	4.99
Min	3.34	2.61	2.38	1.92	2.69
Mean	4.50	4.24	3.71	2.69	3.60
Median	4.57	4.15	3.63	2.52	3.50
Turbidity (NTU)					
Max	21.93	1.29	1.66	1.67	1.18
Min	0.67	0.44	0.54	0.44	0.35
Mean	3.76	0.75	0.96	0.90	0.73
Median	1.39	0.66	0.77	0.78	0.74
Color (NTU)					
Max	-	-	146.50	102.00	134.00
Min	-	-	8.50	6.00	8.50
Mean	-	-	28.41	18.23	56.08
Median	-	-	11.50	8.00	51.75

* = Some samples were have the label "NaN," which indicated that the sample pH was too low for alkalinity to be measured.

APPENDIX B

Powder Mill Lake

Collection dates, Sample Sites, Monthly Proportion of Reads and Monthly Order Composition for Cyanobacteria Population Analysis, Along with Monthly Water Chemistry Data for In-Lake Samples

Powder Mill Lake - Collection Dates and Sample Sites													
12-Jul-16	**28-Jul-16**	**16-Aug-16**	**24-Aug-16**	**30-Aug-16**	**10-Sep-16**	**16-Sep-16**	**23-Sep-16**	**02-Oct-16**	**18-Oct-16**	**30-Oct-16**	**07-Nov-16**	**13-Nov-16**	**21-Nov-16**
1	PM-1	PM-1	PM-1*	PM-1	PM-1*	PM-1*	PM-1*	PM-1	PM-1*	PM-1	PM-1	PM-1	PM-1
2	PM-2	PM-2	PM-2*	PM-2*	PM-2	PM-2*	PM-2*	PM-2*	PM-2*	PM-2	PM-2	PM-2	PM-2
3	PM-3	PM-3	PM-3*	PM-3	PM-3	PM-3*	PM-3*	PM-3*	PM-3*	PM-3*	PM-3	PM-3	PM-3
	PM-4	PM-4	PM-4*	PM-4*	PM-4	PM-4*	PM-4*	PM-4*	PM-4*	PM-4	PM-4	PM-4	PM-4
* = Sample failed to amplify during PCR													

Powder Mill Lake - Monthly Proportion of Reads	JULY		AUGUST		SEPTEMBER		OCTOBER		NOVEMBER	
	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)
Class Chloroplast	33.06	33.07	42.03	42.03	41.28	41.28	42.80	42.80	43.01	43.01
Ord. Gloeobacterales	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Ord. Nostocales	0.95	0.95	0.02	0.02	0.27	0.27	0.00	0.00	0.04	0.04
Ord. Chroococcales	5.56	5.56	2.09	2.09	4.71	4.71	1.05	1.05	0.51	0.51
Ord. Oscillatoriales	0.09	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Ord. Pseudanabaenales	7.64	7.63	0.72	0.73	0.33	0.33	0.25	0.25	0.34	0.34
Ord. Synechococcales	51.64	51.63	54.27	54.27	53.01	53.01	55.32	55.32	55.34	55.34
Other Cyanobacteria	0.17	0.17	0.02	0.02	0.13	0.13	0.08	0.08	0.08	0.08
Unassigned	0.88	0.88	0.86	0.86	0.27	0.27	0.50	0.50	0.67	0.67

Powder Mill Lake - Order Composition	JULY		AUGUST		SEPTEMBER		OCTOBER		NOVEMBER	
	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)
Class Chloroplast	**33.06**	**33.07**	**42.03**	**42.03**	**41.28**	**41.28**	**42.80**	**42.80**	**43.01**	**43.01**
Other	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Chlorophyta	3.65	4.20	3.39	3.42	2.67	2.67	1.12	1.14	0.45	0.45
Cryptophyta	59.15	51.58	29.16	29.38	36.49	37.12	61.30	61.56	60.27	60.50
Euglenozoa	0.00	0.00	0.50	0.54	0.00	0.00	0.00	0.00	0.07	0.08
Haptophyta	3.69	4.41	5.27	5.42	13.19	13.22	13.78	13.66	14.90	14.78
Stramenopiles	31.42	37.63	61.56	61.14	47.65	47.00	23.22	23.06	23.72	23.59
Streptophyta	2.09	2.18	0.08	0.07	0.00	0.00	0.59	0.59	0.61	0.61
Ord. Gloeobacterales	**0.01**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.01**
Fam. Gloeobacteraceae (*Gloeobacter*)	100.00	14.29	0.00	0.00	0.00	0.00	0.00	0.00	100.00	8.33
Ord. Nostocales	**0.95**	**0.95**	**0.02**	**0.02**	**0.27**	**0.27**	**0.00**	**0.00**	**0.04**	**0.04**
Fam. Nostocaceae (Other)	0.00	0.00	100.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00
Fam. Nostocaceae (*Anabaena*)	69.70	47.91	0.00	0.00	75.00	77.78	0.00	0.00	40.00	12.50
Fam. Nostocaceae (*Dolichospermum*)	30.30	23.52	0.00	0.00	25.00	22.22	0.00	0.00	60.00	20.83
Ord. Chroococcales	**5.56**	**5.56**	**2.09**	**2.09**	**4.71**	**4.71**	**1.05**	**1.05**	**0.51**	**0.51**
Fam. Chroococcaceae	7.24	7.14	12.42	13.20	4.26	4.64	4.78	5.01	0.00	0.00
Fam. Cyanobacteriaceae	4.13	5.16	0.00	0.00	0.71	0.51	0.00	0.00	1.65	0.76
Fam. Gomphosphaeriaceae	88.11	87.01	85.98	85.13	92.20	91.49	90.46	89.43	84.50	73.37
Fam. Microcystaceae (*Microcystis*)	0.52	0.69	0.80	0.83	2.84	3.36	0.00	0.00	0.00	0.00
Fam. Xenococcaceae	0.00	0.00	0.80	0.83	0.00	0.00	4.76	5.56	7.75	8.61
Fam. Spirulinaceae (*Spirulina*)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.10	8.93
Ord. Oscillatoriales	**0.09**	**0.09**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.01**
Fam. Phormidiaceae (*Phormidium*)	100.00	14.29	0.00	0.00	0.00	0.00	0.00	0.00	100.00	8.33
Ord. Pseudanabaenales	**7.64**	**7.63**	**0.72**	**0.73**	**0.33**	**0.33**	**0.25**	**0.25**	**0.34**	**0.34**
Fam. Other	0.19	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fam. Pseudanabaenaceae (Other)	0.19	0.14	0.00	0.00	0.00	0.00	0.00	0.00	2.45	8.33
Fam. Pseudanabaenaceae (*Leptolyngbya*)	0.94	5.14	0.00	0.00	10.00	5.56	0.00	0.00	0.00	0.00
Fam. Pseudanabaenaceae (*Pseudanabaena*)	98.68	94.52	100.00	100.00	90.00	94.44	100.00	75.00	97.55	75.00
Ord. Synechococcales	**51.64**	**51.63**	**54.27**	**54.27**	**53.01**	**53.01**	**55.32**	**55.32**	**55.34**	**55.34**
Fam. Synechococcaceae (Other)	0.06	0.05	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01
Fam. Synechococcaceae (*Prochlorococcus*)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04
Fam. Synechococcaceae (*Synechococcus*)	99.94	99.95	99.97	99.97	100.00	100.00	100.00	100.00	99.95	99.95
Other Cyanobacteria	**0.17**	**0.17**	**0.02**	**0.02**	**0.13**	**0.13**	**0.08**	**0.08**	**0.08**	**0.08**
Unassigned	**0.88**	**0.88**	**0.86**	**0.86**	**0.27**	**0.27**	**0.50**	**0.50**	**0.67**	**0.67**

Powder Mill Lake In Lake Samples	July	August	September	October	November
Alkalinity ($CaCO_3$ mg/L)	-	-	-	24.38 ± 2.87	21.47 ± 0.72
pH	7.06 ± 0.27	7.32 ± 0.30	7.23 ± 0.18	7.10 ± 0.28	7.48 ± 0.15
TOC (mg/L)	-	-	-	3.10 ± 0.36	3.53 ± 1.41
TN (mg/L)	-	-	-	0.13 ± 0.01	0.14 ± 0.01
TP (µg/L)	-	-	-	< 10	< 10
UV_{254} (cm^{-1})	-	-	-	0.07 ± 0.01	0.08 ± 0.001
SUVA (m^{-1} abs per mg/L of DOC)	-	-	-	2.11 ± 0.66	2.47 ± 0.34
Turbidity (NTU)	-	-	-	0.67 ± 0.11	0.93 ± 0.27
Color (NTU)	-	-	-	6.25 ± 4.41	9.64 ± 1.22

APPENDIX C

POCKWOCK LAKE

Collection dates, Sample Sites, Monthly Proportion of Reads and Monthly Order Composition for Cyanobacteria Population Analysis, Along with Monthly Water Chemistry Data for In-Lake and Tributary Samples

Pockwock Lake - Collection Dates and Sample Sites											
19-Jul-16	**21-Jul-16**	**02-Aug-16**	**04-Aug-16**	**16-Aug-16**	**18-Aug-16**	**29-Aug-16**	**30-Aug-16**	**13-Sep-16**	**14-Sep-16**	**05-Oct-16**	**12-Oct-16**
PG-1A*	PG-3	PG-1A*	PG-6*	PG-1A	PG-3*	PG-1A*	PG-3*	PG-10*	PG-1A*	PG-3*	PG-1A
PG-2A*	PG-10*	PG-2A*	PG-10*	PG-2A	PG-10*	PG-2A	PG-10*	PG-11*	PG-3	PG-10*	PG-3A
PG-3C*	PG-11	PG-3A*	PG-11*	PG-3A	PG-11*	PG-3A*	PG-11*	PG-12S*	PG-3A*	PG-11*	PG-3C*
PG-6	PG-12S*	PG-3C	PG-12S*	PG-3C	PG-12S	PG-3C	PG-12S*	PG-13S*	PG-3A-US*	PG-12S*	PG-8*
PG-8*	PG-13S*	PG-5	PG-13S*	PG-5	PG-13S*	PG-5*	PG-13S*	PG-14S*	PG-3C*	PG-13S*	PG-9A*
PG-9A	PG-14 *	PG-6*	PG-14S*	PG-6	PG-14S*	PG-6*	PG-14S*	PG-17S*	PG-6	PG-14S*	PG-9B*
PG-9B	PG-14S*	PG-8	PG-15	PG-8	PG-15*	PG-8	PG-15*	PLDL-21*	PG-8*	PG-15*	
PG-9C*	PG-15	PG-9A	PG-16S	PG-9A	PG-16S*	PG-9A*	PG-16S*	PLDL-3CS*	PG-9A*	PG-17S*	
	PG-16*	PG-9B*	PG-17S*	PG-9B	PG-17S*	PG-9B	PG-17S*		PG-9B*		
	PG-17S*	PG-9C*		PG-9C		PG-9C*			PG-9C*		
									PG-10		
									PG-11		
									PG-12S*		
									PG-13S*		
									PG-14S		
									PG-15*		
									PG-17S		
* = Sample failed to amplify during PCR											

Pockwock Lake - Monthly Proportion of Reads	JULY		AUGUST		SEPTEMBER		OCTOBER	
	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)
Class Chloroplast	62.14	62.14	25.38	25.38	37.51	37.51	54.31	54.31
Ord. Gloeobacterales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ord. Nostocales	0.47	0.47	1.88	1.88	14.00	14.00	1.11	1.11
Ord. Chroococcales	25.48	25.48	3.71	3.71	31.25	31.25	0.00	0.00
Ord. Oscillatoriales	0.00	0.00	0.70	0.70	0.01	0.01	0.00	0.00
Ord. Pseudanabaenales	0.53	0.53	1.94	1.94	0.38	0.38	0.00	0.00
Ord. Synechococcales	4.37	4.37	0.87	0.87	12.43	12.43	0.00	0.00
Other Cyanobacteria	0.12	0.12	1.82	1.82	0.13	0.13	32.44	32.44
Unassigned	6.88	6.88	63.70	63.70	4.29	4.29	12.13	12.13

Pockwock Lake - Order Composition	JULY		AUGUST		SEPTEMBER		OCTOBER	
	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)	OVERALL (%)	AVE (%)
Class Chloroplast	**62.14**	**62.14**	**25.38**	**25.38**	**37.51**	**37.51**	**54.31**	**54.31**
Other	0.00	0.00	0.01	0.02	0.31	0.43	0.00	0.00
Chlorophyta	6.80	7.03	8.99	5.52	3.18	4.35	1.72	1.30
Cryptophyta	31.34	36.57	18.27	14.62	50.36	43.80	41.43	47.62
Euglenozoa	0.00	0.00	11.47	5.37	0.27	0.41	0.00	0.00
Haptophyta	0.48	0.45	0.14	0.12	5.68	7.91	1.72	1.30
Rhodophyta	0.00	0.00	0.11	0.04	0.00	0.00	0.00	0.00
Stramenopiles	55.74	52.25	46.29	32.44	37.20	41.41	55.13	49.78
Streptophyta	5.65	3.70	14.72	8.54	3.00	1.70	0.00	0.00
Ord. Gloeobacterales	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Ord. Nostocales	**0.47**	**0.47**	**1.88**	**1.88**	**14.00**	**14.00**	**1.11**	**1.11**
Fam. Nostocaceae (*Dolichospermum*)	100.00	33.33	100.00	14.29	99.73	83.12	0.00	0.00
Fam. Nostocaceae (*Nostoc*)	0.00	0.00	0.00	0.00	0.27	0.21	100.00	50.00
Ord. Chroococcales	**25.48**	**25.48**	**3.71**	**3.71**	**31.25**	**31.25**	**0.00**	**0.00**
Fam. Other	34.94	33.32	56.04	17.12	32.21	28.72	0.00	0.00
Fam. Gomphosphaeriaceae	0.00	0.00	1.28	4.56	8.69	7.45	0.00	0.00
Fam. Microcystaceae (*Microcystis*)	0.00	0.00	0.00	0.00	0.07	0.10	0.00	0.00
Fam. Xenococcaceae	65.06	33.35	42.68	6.89	59.03	63.74	0.00	0.00
Ord. Oscillatoriales	**0.00**	**0.00**	**0.70**	**0.70**	**0.01**	**0.01**	**0.00**	**0.00**
Fam. Phormidiaceae (Other)	0.00	0.00	0.28	0.01	0.00	0.00	0.00	0.00
Fam. Phormidiaceae (*Phormidium*)	0.00	0.00	99.72	19.03	100.00	16.67	0.00	0.00
Ord. Pseudanabaenales	**0.53**	**0.53**	**1.94**	**1.94**	**0.38**	**0.38**	**0.00**	**0.00**
Fam. Other	0.00	0.00	1.50	0.26	11.92	19.44	0.00	0.00
Fam. Pseudanabaenaceae (Other)	22.22	3.70	0.00	0.00	14.93	16.67	0.00	0.00
Fam. Pseudanabaenaceae (*Leptolyngbya*)	0.00	0.00	0.10	0.02	0.00	0.00	0.00	0.00
Fam. Pseudanabaenaceae (*Pseudanabaena*)	77.78	12.96	98.40	28.29	73.15	47.22	0.00	0.00
Ord. Synechococcales	**4.37**	**4.37**	**0.87**	**0.87**	**12.43**	**12.43**	**0.00**	**0.00**
Fam. Synechococcaceae (*Paulinella*)	0.00	0.00	0.23	0.08	0.00	0.00	0.00	0.00
Fam. Synechococcaceae (*Synechococcus*)	100.00	50.00	99.77	23.73	100.00	100.00	0.00	0.00
Other Cyanobacteria	**0.12**	**0.12**	**1.82**	**1.82**	**0.13**	**0.13**	**32.44**	**32.44**
Unassigned	**6.88**	**6.88**	**63.70**	**63.70**	**4.29**	**4.29**	**12.13**	**12.13**

Pockwock Lake In-Lake	June	July	August	September	October
Alkalinity ($CaCO_3$ mg/L)	1.04 ± 0.50	1.79 ± 0.56	1.25 ± 0.28	-	1.85 ± 0.60
pH	5.24 ± 0.59	5.16 ± 0.21	5.12 ± 0.22	-	5.25 ± 0.04
TOC (mg/L)	3.21 ± 0.36	3.60 ± 0.27	3.16 ± 0.18	-	2.88 ± 0.36
TN (mg/L)	0.09 ± 0.03	0.06 ± 0.01	-	2.83*	0.09 ± 0.01
UV_{254} (cm^{-1})	0.11 ± 0.002	0.096 ± 0.003	0.10 ± 0.05	0.09 ± 0.05	0.068 ± 0.0008
SUVA (m^{-1} abs per mg/L of DOC)	3.43 ± 0.36	2.76 ± 0.20	2.80 ± 0.69	2.81 ± 0.60	2.61 ± 0.11
Turbidity (NTU)	0.60 ± 0.20	0.64 ± 0.36	0.71 ± 0.45	-	0.45 ± 0.08
Color (NTU)	22.36 ± 1.82	19.56 ± 3.48	14.38 ± 4.55	37.89 ± 21.69	10.63 ± 0.72

* = At depth sample may have been mislabelled as a surface sample.

Pockwock Lake Streams and Tributaries	June	July	August	September	October	November
Alkalinity ($CaCO_3$ mg/L)*						
Max	16.56	18.52	53.08	-	-	-
Min	0.48	0.03	0.30	-	-	-
Mean	4.02	5.06	10.00	-	-	-
Median	1.17	1.61	3.47	-	-	-
pH						
Max	5.89	-	-	-	-	-
Min	3.90	-	-	-	-	-
Mean	4.92	-	-	-	-	-
Median	4.96	-	-	-	-	-
TOC (mg/L)						
Max	21.18	32.84	23.37	-	32.67	20.39
Min	2.55	2.40	2.92	-	3.62	4.03
Mean	8.80	11.67	9.67	-	14.15	11.88
Median	3.99	8.70	6.73	-	14.03	11.12
DOC (mg/L)						
Max	-	19.81	18.43	15.88	44.74	-
Min	-	2.38	2.35	2.30	4.89	-
Mean	-	9.63	8.50	6.80	22.76	-
Median	-	8.32	5.18	3.73	21.71	-

TN (mg/L)						
Max	1.06	1.34	3.65	-	0.30	0.23
Min	0.08	0.07	0.09	-	0.12	0.09
Mean	0.29	0.36	0.61	-	0.20	0.16
Median	0.20	0.26	0.29	-	0.19	0.15
UV_{254} (cm^{-1})						
Max	0.87	1.18	1.09	-	-	-
Min	0.11	0.07	0.07	-	-	-
Mean	0.37	0.53	0.41	-	-	-
Median	0.19	0.42	0.15	-	-	-
SUVA (m^{-1} abs per mg/L of DOC)						
Max	-	6.92	6.01	-	-	-
Min	-	2.85	2.38	-	-	-
Mean	-	4.66	4.23	-	-	-
Median	-	4.61	3.87	-	-	-
Turbidity (NTU)						
Max	34.50	36.92	17.00	-	-	-
Min	0.46	0.46	0.74	-	-	-
Mean	4.92	6.40	3.89	-	-	-
Median	1.23	0.98	2.02	-	-	-
Color (NTU)						
Max	475.00	494.00	688.00	421.00	-	-
Min	19.00	21.00	3.00	63.00	-	-
Mean	137.05	153.78	150.27	186.63	-	-
Median	81.00	47.50	66.00	158.50	-	-

* = Some samples were have the label "NaN," which indicated that the sample pH was too low for alkalinity to be measured.

APPENDIX D

LAKE MAJOR

Collection dates, Sample Sites, Monthly Proportion of Reads and Monthly Order Composition for Cyanobacteria Population Analysis, Monthly Water Chemistry Data for Pump House and Tributary Samples

Lake Major - Collection Dates and Sample Sites		
23-Jun-16	**28-Jul-16**	**20-Oct-16**
3*	LMG-1*	LM Raw 1*
	LMG-3*	LM Raw 2*
	LMG-6A	
	LMG-7	
* = Sample failed to amplify during PCR		

Lake Major - Monthly Proportion of Reads	JULY	
	OVERALL (%)	AVE (%)
Class Chloroplast	41.56	34.11
Ord. Gloeobacterales	0.00	0.00
Ord. Nostocales	0.25	0.23
Ord. Chroococcales	2.23	1.82
Ord. Oscillatoriales	2.78	2.27
Ord. Pseudanabaenales	13.30	10.88
Ord. Synechococcales	0.12	0.16
Other Cyanobacteria	1.48	1.92
Unassigned	38.28	48.62

Lake Major - Order Composition	**JULY**	
	OVERALL (%)	AVE (%)
Class Chloroplast	**41.56**	**34.11**
Other	0.15	0.08
Chlorophyta	1.34	0.68
Cryptophyta	30.21	29.40
Stramenopiles	60.57	65.94
Streptophyta	7.74	3.91
Ord. Gloeobacterales	**0.00**	**0.00**
Ord. Nostocales	**0.25**	**0.23**
Fam. Nostocaceae (*Anabaena*)	50.00	66.67
Fam. Nostocaceae (*Dolichospermum*)	50.00	33.33
Ord. Chroococcales	**2.23**	**1.82**
Fam. Xenococcaceae	100.00	50.00
Ord. Oscillatoriales	**2.78**	**2.27**
Fam. Phormidiaceae (*Phormidium*)	100.00	50.00
Ord. Pseudanabaenales	**13.30**	**10.88**
Fam. Pseudanabaenaceae (*Arthronema*)	0.47	50.00
Fam. Pseudanabaenaceae (*Pseudanabaena*)	99.53	50.00
Ord. Synechococcales	**0.12**	**0.16**
Fam. Synechococcaceae (*Synechococcus*)	100.00	50.00
Other Cyanobacteria	**1.48**	**1.92**
Unassigned	**38.28**	**48.62**

Lake Major Pump House Water	June	July	August	September	October	November
pH	5.48	5.51 ± 0.07	5.59 ± 0.06	5.56 ± 0.23	5.27 ± 0.15	5.25
TOC (mg/L)	4.86 ± 0.04	4.76 ± 0.24	4.47 ± 0.14	3.98 ± 0.27	4.90 ± 1.18	-
UV_{254} (cm^{-1})	0.24 ± 0.01	0.21 ± 0.01	0.18 ± 0.002	0.18 ± 0.01	0.19 ± 0.01	0.20
SUVA (m^{-1} abs per mg/L of DOC)	5.11 ± 0.16	4.58 ± 0.26	3.93 ± 0.15	4.60 ± 0.32	4.20 ± 0.43	-
Turbidity (NTU)	0.40 ± 0.02	0.43 ± 0.08	0.31 ± 0.03	0.29 ± 0.08	0.33 ± 0.04	0.32
Color (NTU)	44.38 ± 1.94	35.33 ± 3.37	27.50 ± 1.91	26.33 ± 0.58	27.67 ± 3.06	32

Lake Major Streams and Tributaries	July
pH	
Max	6.20
Min	4.50
Mean	5.57
Median	5.79
TOC (mg/L)	
Max	16.07
Min	3.09
Mean	7.26
Median	6.96
DOC (mg/L)	
Max	13.62
Min	2.76
Mean	6.56
Median	5.30
TP (µ/L)	
Max	27.00
Min	5.00
Mean	15.25
Median	14.50
Turbidity (NTU)	
Max	22.60
Min	0.68
Mean	6.54
Median	1.43

Color (NTU)	
Max	50.00
Min	12.00
Mean	33.50
Median	36.00