UNDERSTANDING WATER QUALITY AND THE PRESENCE OF MICROCYSTIN-LR IN A SMALL DRINKING WATER SUPPLY

by

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Table of Contents

List of Tables ..... **vi**

List of Figures ..... **vii**

Abstract ..... **ix**

List of Abbreviations and Symbols Used ..... **x**

Acknowledgements ..... **xiii**

Chapter 1: Introduction **1**

1.1 Project Rationale **1**

1.2 Research Objectives **3**

Chapter 2: Literature Review **4**

2.1 Global Processes Affecting Lake Water Quality **4**

2.1.1 Climate Change 4

2.1.2 Recovery from Acidification 5

2.1.2.1 Evidence of Chemical Lake Recovery 5

2.1.2.2 Biological Responses to Chemical Recovery 6

2.2 Wastewater Discharging into Surface Waters **8**

2.2.1 Sewage Dumping on Biological Activity 8

2.2.2 Effects on Drinking Water Quality 9

2.3 Natural Organic Matter **9**

2.3.1 Concentration and Composition of NOM 10

2.3.2 Effects on Drinking Water Treatment 11

2.4 Microbial Indicators of Water Quality 12

2.4.1 Biomass ATP Analysis 12

2.4.2 Chlorophyll a Analysis 13

2.5 Cyanobacteria and the Release of Algal Toxins 14

2.5.1 Physical Characteristics of Cyanobacteria 14

2.5.2 Harmful Algal Blooms and Algal Toxins 15

2.5.3 *Microcystis* and the toxin Microcystin-LR 16

2.5.4 DNA Sequencing for Cyanobacteria Analysis 17

2.5.5 Implications on Drinking Water Treatment 18

2.6 Passive Sampling Techniques 18

2.6.1 Polar Organic Chemical Integrative Samplers and the Use for MC-LR Analysis 20

Chapter 3: Temporal Analysis of Water Quality and Natural Organic Matter within Lake Fletcher, Nova Scotia 21

3.1 Introduction 21

3.2 Materials and Methods 23

3.2.1 Study Site – Lake Fletcher, Nova Scotia 23

3.2.2 Water Collection Sites 23

3.2.3 Sample Bottle Preparation 25

3.2.4 General Water Quality Analysis 26

3.2.5 Natural Organic Matter 26

3.2.6 NOM Analysis using Fluorescence Spectroscopy 27

3.2.7 Statistical Analysis 28

3.2.7.1 Mann-Kendall Trend Analysis 28

3.2.7.2 Normalize 3D and Inner Filter Effect for Fluorescence Spectroscopy 28

3.2.7.3 Parallel Factor Analysis on Fluorescence Spectroscopy 29

3.3 Results and Discussion 29

3.3.1 Comprehensive Water Quality Analysis on Lake Fletcher 29

3.3.2 Temporal Evaluation of NOM Composition 35

3.4 Conclusions and Recommendations 39

Chapter 4: Understanding the Emergence of Microcystin-LR within Lake Fletcher, Nova Scotia 42

4.1 Introduction 42

4.2 Materials and Methods 43

4.2.1 Microbial Composition Analysis 43

4.2.1.1 Biomass ATP Analysis 43

4.2.1.2 Chlorophyll a Analysis 44

4.2.2 Surface Water Microcystin-LR Analysis 45

4.2.6 DNA Sequencing for Cyanobacteria Composition 46

4.2.6 Statistical Analysis 47

4.3 Results and Discussion 47

4.3.1 Biomass ATP and Chlorophyll a within Lake Fletcher 47

4.3.2 Microcystin-LR Emergence within Lake Fletcher 51

4.3.3 DNA Sequencing to Explore Microbial Composition within Lake Fletcher 54

4.3.4 Understanding the Occurrence of Microcystin-LR within Lake Fletcher 57

4.4 Conclusions and Recommendations 64

Chapter 5: Exploring the Detection of Microcystin-LR Using Passive Sampling within Lake Fletcher, Nova Scotia 67

5.1 Introduction **67**

5.2 Materials and Methods **68**

5.2.1 Passive Sampling for Microcystin-LR Analysis 68

5.2.2 Bench-top Passive Sampling Experiment 69

5.2.3 Stream Passive Sampling Experiment 70

5.3 Results and Discussion **70**

5.3.1 Bench-top Passive Sampling for MC-LR Calibration 70

5.3.2 Stream Passive Sampling for MC-LR Detection 72

5.4 Conclusions and Recommendations **74**

Chapter 6: Conclusion **76**

6.1 Summary of Conclusions **76**

6.2 Recommendations for Future Work **78**

References **79**

List of Tables

Table 1. Description of each Lake Fletcher sampling location. 24

Table 2. Average water quality data from 2014, 2016 and 2017 for all Lake Fletcher sampling locations (2014 n=6; 2016 n=17; 2017 n=12). 31

Table 3. Average organic water quality data from 2014, 2016 and 2017 for all Lake Fletcher sampling locations (2014 n=6; 2016 n=17; 2017 n=12). 34

Table 4. NOM composition analysis using fluorescence spectroscopy paired with PARAFAC analysis. 37

List of Figures

Figure 1. Map of all Lake Fletcher sampling locations. 25

Figure 2. SUVA results from 2014, 2016 and 2017 for all Lake Fletcher sampling locations. 36

Figure 3. Biomass ATP concentrations from 2014, 2016 and 2017 for all Lake Fletcher sampling locations. 48

Figure 4. Chlorophyll a concentrations from 2017 for all Lake Fletcher stream locations. 50

Figure 5. 2016 MC-LR concentrations within Lake Fletcher. 51

Figure 6. 2017 MC-LR concentrations within Lake Fletcher. 53

Figure 7. Proportion of cyanobacteria reads from three different sampling dates in 2016 at the FI location (Betts, 2018 - Unpublished thesis data). 55

Figure 8. Proportion of *Chroococcales* reads from three different sampling dates in 2016 at the FI location (Betts, 2018 - Unpublished thesis data). 56

Figure 9. FI results for MC-LR paired with other water quality parameters over time. 58

Figure 10. FO results for MC-LR paired with other water quality parameters over time. 59

Figure 11. DWR results for MC-LR paired with other water quality parameters over time. 60

Figure 12. Biomass ATP concentrations over time at the three locations where MC-LR was detected. 62

Figure 13. Chlorophyll a concentrations over time at the three locations where MC-LR was detected. 63

Figure 14. POCIS taken from Lake Fletcher after the 28-day exposure time. 69

Figure 15. Calibration curve from the two bench-top POCIS experiments performed. 71

Figure 16. FI POCIS analysis for MC-LR paired with stream MC-LR concentrations over time. 72

Figure 17. FO POCIS analysis for MC-LR paired with stream MC-LR concentrations over time. 73

Abstract

Over the past 15-20 years, changes in water quality have been observed in the northern hemisphere. These changes could be attributed to climate change and/or recovery from acidification due to reduced atmospheric deposition. These water quality changes are likely to alter the organic and biological composition of the water, particularly in terms of algal activity and the occurrence of algal toxins. One algal toxin of interest is microcystin-LR (MC-LR), as it is the most toxic of the microcystins and it can cause liver damage at low concentrations.

Lake Fletcher in Wellington, Nova Scotia is the source water for an integrated ultrafiltration/nanofiltration membrane filtration plant operated by Halifax Water and will be used as the study site for this thesis. Lake Fletcher is also the receiving water for discharges from a municipal wastewater treatment facility with tertiary filtration, making it an interesting site for analysis.

As part of this research, water quality data was evaluated on going sample collection from 2014 to 2017 within Lake Fletcher. Water chemistry analysis was used, including pH, dissolved oxygen, alkalinity, turbidity, true colour and dissolved and total organic carbon, in addition to more advanced natural organic matter characterization tools such as fluorescence spectroscopy. Additionally, liquid chromatography-tandem mass spectroscopy was used to quantify MC-LR concentrations in the study lake.

In 2016, MC-LR was detected above a concentration of 1.5 μg/L in lake water samples. Accordingly, the objective of this thesis was to understand this occurrence of MC-LR in the system through the analysis of general water quality and microbial indicators (e.g. biomass adenosine triphosphate (ATP) and chlorophyll a). Analysis of MC-LR was then continued throughout 2017 to understand if the 2016 occurrence was a singular event within Lake Fletcher. Detection of MC-LR did occur in 2017 during the same timeframe as previously detected in 2016, indicating that MC-LR detection in 2016 was not a singular event.

Passive sampling was also explored as an alternative MC-LR monitoring tool within Lake Fletcher. The use of passive sampling was successful at detecting MC-LR within Lake Fletcher. It was also determined that passive sampling could be a suitable method for initial MC-LR detection when concentrations are below current detection limits.

This research was found to help the local water utility understand algal toxin occurrence within the drinking water supply, as it was the first study of the sort performed on Lake Fletcher. It is recommended that monitoring for algal toxins continue within Lake Fletcher, to ensure safe drinking water and to limit any risks to public health. It is also recommended that the use of passive sampling be explored for other contaminants of interest, such as geosmin and other algal toxins.

List of Abbreviations and Symbols Used

%	Percent
°C	Degrees Celsius
®	Registered
±	Plus/Minus
ANC	Acid Neutralization Capacity
ATP	Adenosine Triphosphate
BOD	Biochemical Oxygen Demand
$CaCO_3$	Calcium Carbonate
cm	Centimeter
DBP	Disinfection By-product
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DWTP	Drinking Water Treatment Plant
ESI	Electrospray Ionization
HLB	Hydrophilic-Lipophilic-Balanced
HPLC	High Performance Liquid Chromatography
L	Litre
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
MAC	Maximum Acceptable Concentration

MC-LR	Microcystin-LR
mg	Milligram
mg/L	Milligram per Litre
min	Minute
mL	Millilitre
mm	Millimeter
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
nm	Nanometer
NOM	Natural Organic Matter
NTU	Nephelometric Turbidity Units
PARAFAC	Parallel Factor
PES	Polyethersulfone
pg	Picogram
POCIS	Polar Organic Chemical Integrative Samplers
Psi	Pounds per Square Inch
QGA	Quench-Gone Aqueous
QqQ	Triple quadrupole
RLU	Relative Light Units
rpm	Revolutions per Minute
SUVA	Specific Ultraviolet Absorbance
TCU	True Colour Units
TOC	Total Organic Carbon

UV	Ultraviolet
UV_{254}	Ultraviolet Absorbance at the 254 Nanometer Wavelength
V	Volts
WHO	World Health Organization
WWTP	Wastewater Treatment Plant
µg	Microgram
µg/L	Microgram per Litre
µL	Microliter
µm	Micrometer

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Chapter 1: Introduction

1.1 Project Rationale

Over the past 15-20 years, changes in water quality have been observed in the northern hemisphere. One potential cause is climate change, where warming atmospheric temperatures could affect equilibrium conditions within surface waters (Delpla et al., 2009). This change in equilibrium may result in increases of natural organic matter (NOM) concentration within surface waters (Delpla et al., 2009). Another potential cause for changing water quality is recovery from acidification due to reduced atmospheric deposition (Anderson et al., 2017; Evans & Monteith, 2001; Monteith et al., 2007); defined as increases in pH, alkalinity and acid neutralization capacity within surface waters. Recovery from acidification has also been found to increase NOM concentration and change NOM composition, which could lead to numerous challenges for drinking water treatment (Anderson et al., 2017; Garmo et al., 2014; Donald T Monteith et al., 2007). Along with these abiotic changes in water quality from environmental processes, there have also been observed biological changes such as shifts in species communities (Arseneau et al., 2011; Kopáček et al., 2006; Nicholls et al., 1992). These changes could increase the risk of harmful algal blooms within surface waters, which in turn could lead to the release of algal toxins (Yoo, 1995).

NOM is a concern for drinking water utilities for many reasons; notably, NOM reacts with chlorine during disinfection and results in harmful disinfection by-products (DBPs) (Reckhow & Singer, 2011). NOM has also been found to influence drinking

water treatment performance. Increasing NOM concentrations in source waters has lead to increased coagulant dosages, as well as lead to increased fouling throughout membrane treatment processes (Anderson et al., 2017; Eikebrokk et al., 2004; Lamsal et al., 2012; Nilson & DiGiano, 1996). It is therefore important for water utilities to understand the composition of NOM within surface waters used as drinking water supplies, to ensure drinking water treatment is optimized.

Algal blooms, and specifically the release of algal toxins, have become an emerging area of interest for drinking water utilities. One particular algal toxin of interest is the group of microcystins, which are produced by cyanobacteria. Microcystins are hepatotoxins and neurotoxins, which can lead to carcinogenic effects on humans and animals (Lone et al., 2015; Ziegmann et al., 2010). The major toxin of concern of the microcystins is microcystin-LR (MC-LR), which is found mainly to cause liver damage, but also affects the heart, kidney, nervous system and gastrointestinal tract (Liu et al., 2006). The main exposure route of MC-LR is through drinking water; therefore it is very important for water utilities to ensure effect removal during treatment (WHO, 1998).

Lake Fletcher in Wellington, Nova Scotia is the study site for this research. Lake Fletcher is the source water for the Collins Park Water Supply Plant, and is also the receiving body for wastewater effluent from the Lockview-MacPherson Wastewater Treatment Facility, making it an interesting site for further water quality analysis. As previously mentioned, environmental processes leading to water quality changes within source waters has become a continued area of interest within Nova Scotia (Anderson et al., 2017; Delpla et al., 2009; Donald T Monteith et al., 2007). These water quality changes have lead to changes in NOM concentration and composition, as well as changes

in microbial composition within the water, leading to problems with drinking water treatment efficiencies (Anderson et al., 2017; Eikebrokk et al., 2004; Lamsal et al., 2012; Nilson & DiGiano, 1996). Furthermore, wastewater discharge could be used as a nutrient source for microorganisms. The water quality changes due to environmental processes combined with the use of wastewater effluent as a nutrient source could lead to an increased risk of algal activity and the release of potentially harmful algal toxins within Lake Fletcher, Nova Scotia.

1.2 Research Objectives

The overall goal of this thesis is to understand algal toxins and organic carbon in Lake Fletcher as a case study of a Nova Scotia lake. The following sub-objectives were completed as part of this research:

1. Conduct a comprehensive water quality analysis on Lake Fletcher, with a focus on NOM concentration and composition.
2. Identify typical microbial indicators and understand the presence of the algal toxin MC-LR within Lake Fletcher, Nova Scotia.
3. Explore passive sampling techniques for initial MC-LR detection within Lake Fletcher, Nova Scotia.

Chapter 2: Literature Review

2.1 Global Processes Affecting Lake Water Quality

2.1.1 Climate Change

Climate change could be the cause of a number of different variations in surface water quality. Changes in atmospheric temperature and precipitation patterns have been predicted due to climate change. Hulme et al. (2002) suggests that by the 2080s, winter precipitation will increase in the UK by approximately 10-20% in low-emission areas, whereas increases could be anywhere from 15-35% in high-emission areas. Contrary to this, the summer months are predicted to be much drier with decreases of 35% or more in precipitation in low emission areas and 50% or more in high emission areas (Hulme et al., 2002). These precipitation changes were then used to predict changes in river flow. Romanowicz et al. (2006) predicted that by 2020, winter flows will increase by 4-9%, while summer river flows will decrease by on average 11%. These lower water flows in summer months could lead to less dilution of other components in the water, such as wastewater discharge (Whitehead et al., 2009).

One immediate variation in water quality from climate change, is how warming atmospheric temperatures could affect equilibrium conditions within surface waters (Delpla et al., 2009). River water temperatures are in close equilibrium with air temperatures, so as air temperatures increase, so will river water temperatures (Whitehead et al., 2009). This change in equilibrium may result in increases of turbidity and NOM concentration within surface waters (Delpla et al., 2009). Along with these

physical and chemical changes with water temperature equilibrium, increased water temperature has been found to control the growth rate of many microbial species, making surface waters sensitive to speculated temperature increases due to climate change (Wade et al., 2002).

2.1.2 Recovery from Acidification

Several years after a number of air and energy policies were put in place in North America and Europe to control air emissions, lake recovery from acidification was observed in surface waters throughout the northern hemisphere. This phenomenon of chemical recovery is defined in literature as increasing pH, acid neutralization capacity (ANC), and/or alkalinity (Evans and Monteith, 2001), and is also associated with increases in abiotic water quality parameters, including NOM as measured by dissolved organic carbon (DOC). Evidence of chemical lake recovery has been observed in the Northeastern United States, Eastern Canada and throughout parts of Europe (Arseneau et al., 2011; Garmo et al., 2014; Monteith et al., 2015; Strock et al., 2014; Waller et al., 2012).

2.1.2.1 Evidence of Chemical Lake Recovery

Driscoll et al. (2003) found that lakes in the Adirondack region of New York had decreases in sulphate concentration, which yielded increases in both pH and ANC in the surface waters. Garmo et al. (2014) analyzed trends in surface water chemistry from 173 undisturbed, (no point sources of pollution) acid-sensitive lakes in Europe and North America from 1990-2008. Of the 173 locations studied, 87% showed significant

decreases in lake sulphate concentrations due to reduced atmospheric deposition. Furthermore, all study regions with the exception of sites in the Appalachians and in East Central Europe showed an increase in DOC concentration, with a median increase of up to 0.11 mg/L/year. Similarly, Monteith et al. (2007) evaluated water chemistry from over 500 surface waters in northern Europe and North America from 1990 to 2004 and found that DOC concentrations were rising in relation to decreases in atmospheric sulphur deposition.

2.1.2.2 Biological Responses to Chemical Recovery

Evidence of biological responses to chemical recovery are not as widespread and understood. Many lakes have been experiencing changes in biotic water quality due to recovery from previously acidified conditions (i.e. increasing pH and DOC concentration), but in some cases information on pre-acidification biological composition is unavailable, and therefore a full recovery is unknown (Holmgren, 2014; Monteith et al., 2005; Skjelkvale et al., 2003). The changes that have been noted are in terms of species richness and diversity, as well as shifts in taxonomic composition. Yan et al. (2003) found that in some lakes and streams, biota is recovering as the water quality improves, but other surface waters in the area are not showing biological responses to recovery from acidification. Furthermore, Yan et al. (2003) noted that although evidence of recovery is promising, it appears to be too early to determine whether or not biological communities will completely recover once water quality improves after acidified damage. According to Skjelkvale et al. (2003), there is some evidence to support biological recovery in European surface waters, however, recovery to pre-industrialization chemical

and biological conditions would require further reductions in air and energy emissions, and time scales would be long.

There has been some small-scale research done to understand how phytoplankton communities respond as lakes begin to recover from acidification. Nicholls et al. (1992) studied phytoplankton of lakes in Ontario, Canada and the relationship with acidification status in order to determine if there were any patterns between phytoplankton and degree of recovery from acidification. This study found that an increase in pH from 5.6 to 6.3 resulted in an increase in phytoplankton species richness, indicating that reducing sulphur emissions and acid deposition can result in eventual restoration of aquatic communities, given the opportunity for dispersal and re-colonization of organisms (Nicholls et al., 1992). Findlay and Kasian (1996) found that once the pH was above 6, many of the biological parameters approached pre-acidification conditions, and a successful recovery of phytoplankton communities was observed. Arseneau et al. (2011) investigated biological recovery of an acid-sensitive lake in the Adirondack region of New York by looking at sediment cores. This study found that post 1995, there was a decrease in chrysophyte and diatom taxa with a low pH-optima, and increases in taxa with a higher pH-optima (Arseneau et al., 2011). This indicates evidence of biological recovery, however the current state still does not match the pre-disturbance state. Finally, Kopáček et al. (2006) studied recovery from acidification in over 91 lakes in the Tatra Mountain area in Central Europe from 1984-2004, and found that chlorophyll a concentrations were significantly higher in 2004 when compared to 1994, indicating potential changes in phytoplankton abundance.

2.2 Wastewater Discharging into Surface Waters

It is well recognized that municipal wastewater can compromise lake water quality if it is not properly treated. In Canada, municipal effluent standards require three main objectives to be met: carbonaceous biochemical oxygen demand below 25mg/L, total suspended solids below 25mg/L and total residual chlorine below 0.02mg/L (Canadian Council of Ministers of the Environment, 2009). Furthermore, proper treatment is critical for public health. For example, in North Battleford, Saskatchewan, a contamination event occurred from poor performance at the communities sewage treatment plant located upstream of the intake for the drinking water treatment facility in April 2001 (Hrudey & Hrudey, 2001). A coliform outbreak occurred, where coliform levels were found in the raw intake water to be as high as 150 000 per 100 mL (Hrudey & Hrudey, 2001).

2.2.1 Sewage Dumping on Biological Activity

In the past, sewage dumping has been linked to algal blooms from nutrient enrichment in surface waters, leading to a problem of eutrophication (Schindler, 1974; Smith et al., 1998). Discharging wastewaters into moving water bodies such as lakes and rivers is common worldwide, and has been found to alter the nitrogen and phosphorous loadings within receiving bodies (Smith et al., 1998). This nutrient load depends not only on point sources such as wastewater discharge, but also non-point sources such as runoff from the land and atmospheric inputs (Jones & Lee, 1982). However, it has been found that the divergence of wastewater effluents has led to profound water quality benefits on the receiving bodies (Smith et al., 1998).

2.2.2 Effects on Drinking Water Quality

There are multiple factors from discharging wastewater that may influence drinking water quality. As mentioned above, sewage dumping can alter the nutrient load in the lake, leading to more algal blooms and the potential release of algal toxins (Schindler, 1974; Smith et al., 1998). Another implication of discharging wastewater on drinking water quality is the potential impact on DBP formation. Galapate et al. (1999) found that discharging treated wastewater into surface waters will increase the concentration of hydrophilic dissolved organic matter in the water, which has been linked to trihalomethane formation potential when disinfected with chlorine. Finally, Watkinson et al. (2009) found that wastewater effluents in South-East Queensland, Australia contained pharmaceutical compounds that would eventually be detected in the receiving body. Wastewater treatment was found to remove greater than 80% of these pharmaceutical compounds, however these compounds were still found to pass through wastewater treatment and end up in the drinking water source in detectable concentrations (Watkinson et al., 2009).

2.3 Natural Organic Matter

NOM is a heterogeneous mixture of humic substances, proteins and other aromatic and aliphatic organic compounds containing functional groups with various combinations of oxygen, nitrogen and sulfur (Chen et al., 2003; Li et al., 2014). NOM can form in the environment through biodegradation of wastes from biological material processing, human sewage and animal feces (Spellman, 2008). NOM is a concern for

drinking water treatment, as it can cause negative aesthetic qualities to the water such as colour, taste and odour (Baghoth, Sharma, & Amy, 2011).

2.3.1 Concentration and Composition of NOM

DOC is a measure of the concentration of organic material in a water source that passes through a 0.45μm polyethersulfone (PES) filter. DOC composes 90 to 99% of the organic material found in surface waters. Specific ultraviolet absorption (SUVA) was first developed to evaluate whether total organic carbon and DBP precursor concentrations could be related to the ultraviolet absorbance at the 254nm wavelength (UV_{254}) (Edzwald et al., 1985). There are certain types of NOM that absorb UV_{254} light per unit concentration of DOC to a greater or lesser degree than other types. This allows SUVA to infer the potential composition of NOM in a water source. When SUVA values are greater than 4, this indicates the organic material in the sample has a high fraction of aquatic humic material (Edzwald & Tobiason, 1999). When SUVA is between 2 and 4, this indicates the organic material in the sample has a mixture of aquatic humic and non-humic material. When SUVA values are less than 2, this indicates the organic material in the sample has a high fraction of aquatic non-humic (or protein-like) material (Edzwald & Tobiason, 1999; Matilainen et al., 2011).

Fluorescence spectroscopy is a method commonly used for the characterization of dissolved organic matter (DOM) in water and wastewater systems (Hudson & Reynolds, 2007; Li et al., 2014). The characterization of DOM can be done using other methods, which involve the concentration and fractionation of bulk NOM (Baghoth et al., 2011). These methods were found to be laborious and time consuming, and may require pre-

treatment of the water sample which could lead to changes of the NOM character. Fluorescence spectroscopy will characterize the DOC of a water sample by identifying humic-like and protein-like signals within that water sample. The advantages of using fluorescence spectroscopy are that it is nondestructive, highly sensitive, rapid and relatively inexpensive (Hambly et al., 2010; Zhou et al., 2013). As well, fluorescence spectroscopy has been paired with the statistical technique parallel factor (PARAFAC) analysis to better understand NOM composition. This method has been found to quickly and easily give a qualitative indication of organic character within samples as operationally defined chemical groups having similar properties (Baghoth et al., 2011).

2.3.2 Effects on Drinking Water Treatment

NOM does not pose a serious risk to human health on its own, but when it reacts with a disinfectant (i.e. chlorine), it can create harmful DBPs that are a risk to human health as they are considered to be carcinogenic and/or genotoxic (Reckhow & Singer, 2011). For this reason, NOM needs to be effectively removed throughout treatment to limit the DBP formation in the finished water. Furthermore, increases in NOM and SUVA have been observed throughout Europe and North America, which has caused increased coagulant dose in water treatment plants (Anderson et al., 2017; Eikebrokk et al., 2004). This increased coagulant dose has lead to increased sludge production and challenges with filter run times. Finally, NOM has caused problems with membrane filtration processes. Hydrophobic NOM has been found to cause high-pressure membranes to foul within membrane filtration plants (Nilson & DiGiano, 1996).

2.4 Microbial Indicators of Water Quality

There is a wide range of microorganisms present within the environment and in water sources, such as bacteria, protozoa, viruses, algae and fungi (Spellman, 2008). Microbial indicators are used to help understand the biological constituents of drinking waters and source waters. This section will focus on general microbial indicators, which are used for an overall understanding of microbial character (Ashbolt et al., 2001).

2.4.1 Biomass ATP Analysis

Biomass adenosine tri-phosphate (ATP) is based off the idea that all living organisms contain a constant amount of intracellular ATP in order to maintain normal physiological activities (Keasler et al., 2013). ATP is therefore used as an estimation of viable organisms within a water source (Holm-Hansen, 1969). The benefits of using the ATP method for biological estimations are that it is rapid, robust, affordable and easy to perform (van der Kooij et al., 1995; Velten et al., 2007; Venkateswaran et al., 2003). However, one disadvantage with the ATP method is that it is not a good estimate of biomass in low concentrations, such as in finished drinking water samples (Hammes et al., 2010). Another disadvantage with this method is that the ATP within bacterial cells is not uniform; therefore conversion to bacterial cell counts can be variable (Hammes et al., 2010).

Seasonal variability within biomass ATP results are generally limited within surface waters. Helm-Hansen & Booth (1966) found that as the environmental conditions change throughout the seasons, one species may outcompete another and vice versa, causing biomass ATP concentrations within a given year to remain relatively the same.

However, there may still be spikes observed in the data due to a weather or contamination event promoting ATP production in the water at a specific instance (Tietjen & Wetzel, 2003).

2.4.2 Chlorophyll a Analysis

Chlorophyll a is a water quality measurement commonly used as an indicator of algal density (Schalles et al., 1998). More specifically, the chlorophyll a concentration is often used as a measure of phytoplankton biomass within a water source (Felip & Catalan, 2000). The concentrations of chlorophyll a are important to constantly measure, as spikes in chlorophyll a could be indicative of an algal bloom. Moreover, Kotak et al. (1993) found that seasonal variations of chlorophyll a are statistically correlated with microcystin-LR occurrence in the water. For this reason, increases in chlorophyll a concentration could be used as an initial monitoring tool for potential algal toxins within surface waters (Kotak et al., 1993).

Due to the unique light absorption pattern of chlorophyll a, detection can be performed either *in vitro* or *in vivo*. *In vitro* analysis allows for detection via an extraction method, whereas *in vivo* analysis is determined by remotely operated optical instruments (Schalles et al., 1998). The problem with the remote sensing method is the interference with other water quality parameters. Absorption by DOM and the scattering and absorption by non-algal particles (i.e turbidity) may alter the chlorophyll a signals (Morel & Prieur, 1977).

2.5 Cyanobacteria and the Release of Algal Toxins

Cyanobacteria are photosynthetic unicellular organisms that have existed for nearly three billion years (Noffke, 2010; Schirrmeister et al., 2011). Also known as blue-green algae due to their colour after bloom formation, cyanobacteria are found worldwide in many eutrophic and hypertrophic freshwater environments such as lakes, rivers, ponds and streams (Van Apeldoorn et al., 2007). Cyanobacteria have the ability to produce toxins, which can be harmful to human health and the surrounding environment (Waters, 2016).

2.5.1 Physical Characteristics of Cyanobacteria

Cyanobacteria have the ability to regulate their buoyancy within the water column, as they possess intracellular gas vacuoles (Reynolds et al., 1987). These gas vacuoles allow cyanobacteria to depth regulate within the water column in relation to many environmental factors, such as nutrients and light availability (Cullen & MacIntyre, 1998). Reynolds et al. (1987) found that size, shape and density were all related to the floatation and sinking rates of cyanobacteria. This buoyance regulation is important when light and nutrient-rich layers are stratified in the lake, and could lead to the misinterpretation of cyanobacteria when grab sampling is the only applied sampling technique.

Cyanobacteria have a unique characteristic where they can fix atmospheric nitrogen. These cyanobacteria are responsible for most planktonic nitrogen fixation in aquatic ecosystems (Howarth et al., 1988). It was determined that the rates of nitrogen

fixation by autotrophic cyanobacteria in freshwater are correlated with the biomass of nitrogen-fixing cyanobacteria in the water (Goering & Parker, 1983; Wetzel, 1983).

2.5.2 Harmful Algal Blooms and Algal Toxins

Algal blooms are defined as the temporal and spatial accumulation of phytoplankton in an aquatic environment (Pettersson & Pozdnyakov, 2013). In some cases, these blooms have been termed harmful, due to their severe economic loss and damage, problems with human health and impact on the environment (Pettersson & Pozdnyakov, 2013; Waters, 2016). However, in order for the bloom to qualify as harmful, the organisms causing the bloom must produce toxins, be in high biomass or be mucilage (Pettersson & Pozdnyakov, 2013). Cyanobacteria blooms are dependent on the following water quality conditions: total phosphorous content, high water temperatures, high water column stability, low grazing pressure and low nitrogen to phosphorous ratios (Hyenstrand et al., 1998; Paerl, 1988, 1996). It is therefore important to monitor these parameters in a drinking water source to better understand when the environmental conditions could support a harmful algal bloom.

Algal toxins are produced by cyanobacteria in surface waters and can cause serious problems to livestock and human health (Rinehart et al., 1994). There are multiple different types of algal toxins, such as microcystins, nodularins, saxitoxins, anatoxin-a, anatoxin-a(s) and cylindrospermopsin (Hitzfield et al., 2000). These different algal toxins are structurally diverse, and their effects on human health range from liver damage to neurotoxicity (Hitzfield et al., 2000). Algal toxins fall into three groups based on their chemical structure: cyclic peptides (microcystins and nodularins), alkaloids (anatoxins

and saxitoxins) and lipopolysaccharides (Hitzfield et al., 2000). In literature, the best-studied algal toxin is MC-LR, while information on the other toxins is lacking (Hitzfield et al., 2000). The most efficient method to limit algal toxin production within a water source is to prevent blooms from forming; however information on toxin production within algal blooms is not well identified (Hitzfield et al., 2000; Paerl & Millie, 1996).

2.5.3 *Microcystis* and the toxin Microcystin-LR

Microcystis aeruginosa is one of the main species responsible for freshwater cyanobacteria blooms, and is ubiquitous in water (G. Liu et al., 2018). *Microcystis* is a concern for lakes and particularly drinking water supplies, as it can produce harmful toxins called microcystins. There are multiple different families of cyanobacteria that can produce microcystins, such as *Hapalosiphonaceae, Nostocaceae and Oscillatoriaceae*, however the genus *Micocystis* is the predominant MC-LR-producing cyanobacteria (Carmichael, 2001). Microcystins are hepatotoxins and neurotoxins, which can lead to carcinogenic effects on humans and animals (Lone et al., 2015; Ziegmann et al., 2010). The major toxin of concern of the microcystins is MC-LR, which is found mainly to cause liver damage, but also affects the heart, kidney, nervous system and gastrointestinal tract (Liu et al., 2006). The main exposure route of microcystins is through drinking water and recreational water uses (WHO, 1998). For this reason, the World Health Organization (WHO) has set a drinking water guideline for MC-LR of 1µg/L, and the Guidelines for Canadian Drinking Water Quality has set a maximum acceptable concentration (MAC) for MC-LR of 1.5µg/L (Guidelines for Canadian Drinking Water Quality, 2017; Guidelines for Drinking-water Quality, 2006). There is also a limit for

total microcystins (expressed as MC-LR) within recreational waters in Canada of 20µg/L put in place by the Guidelines for Canadian Recreational Water Quality, which is important for surface water monitoring (Health Canada, 1992).

Jacoby et al. (2000) studied the environmental factors that are associated with *Microcystis aeruginosa* blooms, which is a bloom-forming cyanobacterium that can produce the toxin MC-LR. Success of *Microcystis* blooms was due to the low nitrogen to phosphorous ratios, and the low nitrate-nitrogen concentrations compared to sufficient ammonium-nitrogen concentrations (Jacoby et al., 2000). This study also found that there was no relation between *Microcystis* occurrence and the production of MC-LR, however MC-LR was found to be positively correlated with the concentration of soluble reactive phosphorous, indicating that the toxin MC-LR may be limited by phosphorous (Jacoby et al., 2000). Similarly, Wei et al. (2001) found that *Microcystis* blooms had an inverse relationship with total nitrogen concentrations, where an increase in total nitrogen lead to a decrease in *Microcystis* growth. It was also determined that a 10% increase in water pH lead to a 59.1% increase in *Microcystis* densities (Wei et al., 2001).

2.5.4 DNA Sequencing for Cyanobacteria Analysis

Deoxyribonucleic acid (DNA) sequencing is commonly performed to understand the microbial composition within surface waters. Samples can be amplified with primers specific to the organism being studied. Zwart et al. (2002) analyzed an available database of DNA sequences from North America, Europe and Asia for freshwater plankton. The majority of sequences within the database were freshwater clones or isolates, with only a few being from soils or marine habitats. This study determined that rivers and lakes have

distinct bacterial communities when compared to other environments such as soils and sediments (Zwart et al., 2002). Eiler and Bertilsson (2004) characterized the composition of cyanobacterial blooms from four different lake environments using clone libraries for DNA and RNA. It was determined that even when environmental conditions were similar, different cyanobacterial blooms may foster a different bacterial composition (Eiler & Bertilsson, 2004).

2.5.5 Implications on Drinking Water Treatment

Biological changes leading to algal blooms and the release of algal toxins could have implications to drinking water treatment processes. According to Meybohm and Ulrich (2007), phytoplankton exerts a high influence on the extent and cost of drinking water treatment. For example, some algae with flagellum (e.g. chrysophyceans) can actively pass through the filtration stage of treatment, which can result in taste and odour compounds in finished water (Meybohm & Ulrich, 2007). Furthermore, according to Hitzfield et al. (2000), chlorination, micro/ultrafiltration and especially ozonation are effective treatment methods for destroying cyanobacteria and removing microcystins from the water. However, during bloom conditions or high organic load, these treatment techniques may not be as effective.

2.6 Passive Sampling Techniques

Passive sampling has been accepted as a method to monitor emerging organic contaminants of concern, particularly for use in surface water monitoring (Alvarez, 2010). There are three factors that affect the rate at which chemicals are sampled via

passive sampling: water flow, temperature and biofilm buildup on the surface of the samplers (Alvarez, 2010). Samplers must be fully submerged in water for the duration of the sampling period, and it is favorable to have samplers in flowing water, as the volume of water sampled per day is proportional to water flow (Alvarez, 2010). The physical orientation of samplers is generally not important, as long as clear openings are present to allow water to pass through the samplers (Alvarez, 2010). Conventional surface water grab sampling may not accurately represent the total concentration of the contaminant of interest, as sampling generally occurs at different times and during different environmental conditions, causing the contaminant to potentially be misinterpreted (Kohoutek et al., 2008).

The main advantage with exploring passive sampling is that samplers are left in the stream constantly for a given period of time. For this reason, passive sampling is immune to accidental or extreme concentrations of the contaminant of interest, as the passive samplers will always be collecting the contaminant for the given sampling time (Namieśnik et al., 2005). Furthermore, passive sampling holds promise to provide representation of constant exposure of the contaminant of interest, since the samplers remain in the water at all times throughout the sampling period (Söderström et al., 2009). Finally, passive sampling has the ability to detect and quantify ultra-trace to trace levels of the contaminant of interest in surface waters (Arditsoglou & Voutsa, 2008).

2.6.1 Polar Organic Chemical Integrative Samplers and the Use for MC-LR Analysis

One commonly used passive sampler is the polar organic chemical integrative sampler (POCIS). POCIS are often used to analyze the water-soluble organic chemicals of interest (Alvarez, 2010). The POCIS approach to passive sampling has often been used as a screening tool to determine the presence/absence, possible sources and relative amounts of the contaminant of interest in surface waters (Arditsoglou & Voutsa, 2008). It is possible to determine water concentrations, however a calibration must be performed (Arditsoglou & Voutsa, 2008).

The use of POCIS for MC-LR detection has not been well studied in literature. Kohoutek et al. (2008) showed that microcystins were found to successfully accumulate on POCIS after an exposure time of seven days in field samples. This study looked at efficiencies in POCIS membrane and sorbent configurations, and determined that a polycarbonate membrane with an Oasis Hydrophilic-Lipophilic-Balanced (HLB) sorbent was the best configuration for MC-LR sampling (Kohoutek et al., 2008). Using the results from this 2008 study, Kohoutek et al. (2010) performed a microcystin calibration using POCIS, and determined that microcystin concentrations were linear up to a four week exposure time. This study also compared grab and passive sampling techniques for an algal bloom from 2008, and found that passive sampling gave a superior representation of the situation and allowed for a better assessment of potential microcystin risks (Kohoutek et al., 2010).

Chapter 3: Temporal Analysis of Water Quality and Natural Organic Matter within Lake Fletcher, Nova Scotia

3.1 Introduction

Over the past 15-20 years, changes in water quality have been observed in the northern hemisphere. One potential cause is climate change, where warming atmospheric temperatures could affect equilibrium conditions within surface waters (Delpla et al., 2009). This change in equilibrium may result in increases of turbidity and NOM concentration within surface waters (Delpla et al., 2009). Another potential cause for water quality undergoing change is recovery from acidification due to reduced atmospheric deposition (Anderson et al., 2017; Evans & Monteith, 2001; Monteith et al., 2007). Recovery from acidification has been found to increase NOM concentration and change NOM composition (Anderson et al., 2017; Garmo et al., 2014; Donald T Monteith et al., 2007). These changes demonstrate the importance for water utilities to further understand water quality changes in surface drinking water supplies, particularly in regions that have been exposed to chronic acid deposition.

The concentration and composition of NOM is important, as it can react with disinfectants to produce harmful DBPs (Reckhow & Singer, 2011). There are multiple different components of NOM within surface waters, such as humics, proteins and other aromatic and aliphatic organic compounds containing functional groups with various combinations of oxygen, nitrogen and sulfur (Chen et al., 2003; Li et al., 2014). In general, the main components of NOM that are present within surface water are hydrophobic acids and hydrophilic neutrals (Kent et al., 2014). Since DBP formation is

highly related to the hydrophobic component of NOM (humic substances), NOM composition within source waters is important for drinking water treatment (Reckhow & Singer, 2011).

NOM has also been found to influence drinking water treatment performance. Increases in NOM and SUVA have been observed throughout Europe and North America, which has caused increased coagulant dose in water treatment plants (Anderson et al., 2017; Eikebrokk et al., 2004). This increased coagulant dose has lead to increased sludge production and challenges with filter run times. As well, NOM has caused problems with membrane filtration processes. Hydrophobic NOM has been found to cause high-pressure membranes to foul within membrane filtration plants (Nilson & DiGiano, 1996). Additionally, Lamsal et al. (2012) found that NOM was one major cause of membrane fouling within the Collins Park Water Supply Plant on Lake Fletcher. For these reason, it is important for the local water utility to understand NOM composition within Lake Fletcher.

Lake Fletcher presents an interesting water quality environment, with wastewater effluent discharging into the drinking water source. For this reason along with the challenging water quality changes as previously mentioned, the objective of this chapter was to conduct a comprehensive water quality analysis on Lake Fletcher, with a focus on NOM. Specifically, a temporal evaluation of NOM concentration and composition was conducted via fluorescence spectroscopy, paired with conventional NOM analytes to understand any trends in NOM chemistry within Lake Fletcher.

3.2 Materials and Methods

3.2.1 Study Site – Lake Fletcher, Nova Scotia

The study site for this research was Lake Fletcher, which is in Wellington, Nova Scotia and is one of the headwaters to the Shubenacadie River watershed. Lake Fletcher has an area of 1.01 km^2 and is 4.2 km in length from inlet to outlet (Poltarowicz, 2017). Lake Fletcher has a residence time of 10.41 days (flushing rate of 35.06 times/year), and the average depth of the lake is approximately 3.72m (Hart et al., 1978). There are two main basins within the lake where the two deepest points in the lake are found: the inlet basin has a depth of 6m and the outlet basin has a depth of 11m (Poltarowicz, 2017). The trophic status of Lake Fletcher has been previously classified in literature as an oligotrophic lake, with phosphorous concentrations around 6-12µg/L and chlorophyll a concentrations around 1.2µg/L (Ginn et al., 2015; Mudroch et al., 1987). The lake also undergoes stratification, typically in mid summer between June-August (Poltarowicz, 2017).

3.2.2 Water Collection Sites

Samples were collected from eight different locations around the Lake Fletcher watershed. These locations were previously selected by the local water utilities as locations that influence the overall water quality within Lake Fletcher. Six of these locations were streams surrounding the lake, while the other two were the drinking water and wastewater treatment facilities for the community of Collins Park. A detailed description and a map of these locations are presented in Table 1 and Figure 1,

respectively. Samples were taken between May 2014 and November 2014, as well as from March 2016 until November 2017.

Table 1. Description of each Lake Fletcher sampling location.

Site Identification	Site Description
FI	Lake Thomas run, inlet to Lake Fletcher. Wide channel, fast flows and located around an urban area.
FTB	Top of Holland Brook, located in a heavily forested area with little development
FBB	Bottom of Holland Brook, located in a residential subdivision
FD	Site below the lift station that pumps wastewater into the Lockview-MacPherson wastewater treatment plant (WWTP)
FND	Control area below Lizard Lake, located in an area of new development
FO	Outlet to Lake Fletcher. Wide channel, fast flows
DWR	Raw water from the Collins Park drinking water treatment plant (DWTP)
WWT	Treated wastewater from the Lockview-MacPherson WWTP

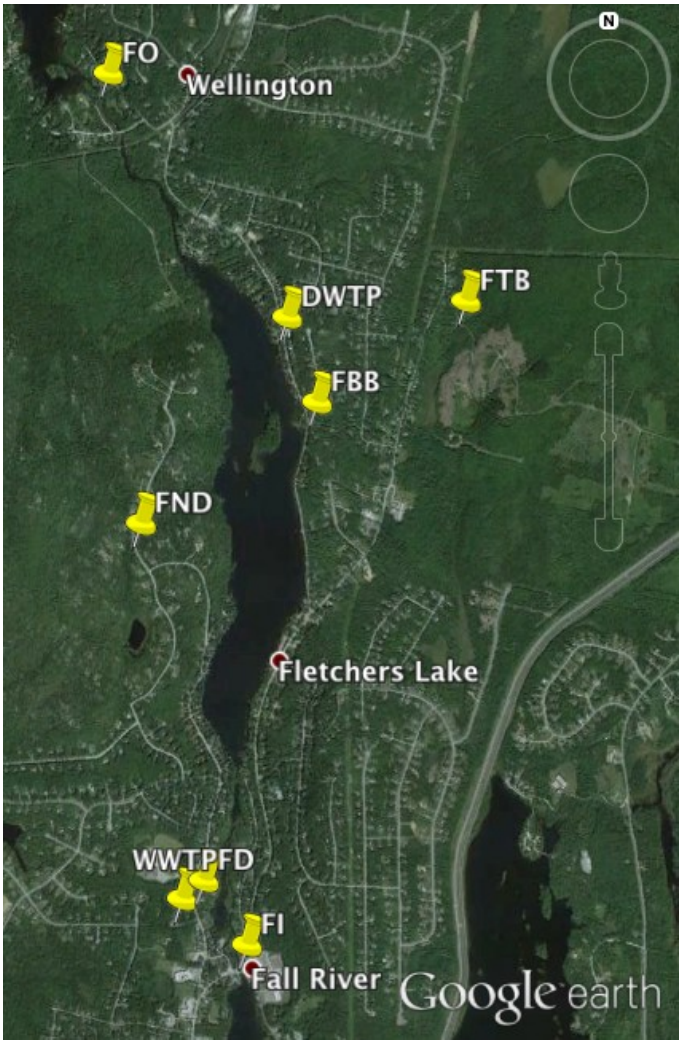


Figure 1. Map of all Lake Fletcher sampling locations.

3.2.3 Sample Bottle Preparation

Samples were taken from each sampling location using 1-L polyethylene sample bottles. Bottles were washed and sterilized using 70% ethanol before samples were taken on site. Samples were filled headspace free, capped and brought back to the laboratory. Samples were stored at 4°C for a minimum of 48 hours until further analysis could be performed.

3.2.4 General Water Quality Analysis

Multiple water quality parameters were measured either on site at the stream or back in the laboratory. Dissolved oxygen, temperature and pH were measured on site using a YSI 600R Sonde handheld device. The probe was calibrated daily for dissolved oxygen and quarterly for pH. Turbidity was measured using a HACH 2100AN Turbidimeter. Approximately 40 mL of each sample was placed in a lab Turbidimeter sample cell and placed in the Turbidimeter. Turbidity measurements were performed in triplicate and were given in units of Nephelometric Turbidity Units (NTU) (Spellman, 2008). Alkalinity was measured using a Mettler Toledo T50 auto titrator. The pH probe was initially calibrated before starting the procedure. Exactly 50 mL of sample was then placed in the auto sampler and the alkalinity was determined in duplicate in units of mg calcium carbonate ($CaCO_3$) per liter.

3.2.5 Natural Organic Matter

NOM can be quantified in multiple different ways. To obtain a general understanding of the organic carbon content in a source water, total organic carbon (TOC), DOC, UV_{254}, SUVA and true colour were used. TOC and DOC were both measured in duplicate using a TOC-V_{CPH} Total Organic Carbon Analyzer by Shimadzu. To measure TOC, raw water samples from each sampling location were put into 43 mL glass vials. Three drops of 85% phosphoric acid was added to each vial to ensure a pH of < 2 before being capped with a piece of aluminum foil and a lid. To measure DOC, samples were run through a 0.45µm PES filter before being added to the 43 mL glass vials and followed the same procedure for analysis. To measure UV_{254}, a sample cell was

pre-rinsed with ultra pure Milli-Q water. Water samples from each location were filtered through a 0.45µm PES filter and measured in triplicate using program #410 on a HACH DR 5000 ultraviolet (UV) Spectrophotometer. SUVA was determined by a calculation using the DOC concentration and the UV_{254} absorbance. The calculation was performed using the following formula:

$$SUVA = \frac{(UV_{254}\ in\ cm^{-1}) * 100\ \frac{m^{-1}}{cm^{-1}}}{DOC\ in\ {}^{mg}/_{L}}$$

True colour was measured using a sample cell pre-rinsed with ultra pure Milli-Q water. Water from each location was filtered through a 0.45µm PES filter and measured in duplicate at the 455nm wavelength using program #120 on a HACH DR 5000 UV Spectrophotometer.

3.2.6 NOM Analysis using Fluorescence Spectroscopy

In depth analysis of NOM profiles were performed using fluorescence spectroscopy. Fluorescence excitation-emission matrix analysis was performed on all water samples using a Horiba Scientific Aqualog. A glass cuvette was properly washed with ultra pure water from a Milli-Q device prior to use. A sample of ultra pure water was then run as a blank. Once the blank run was completed, the ultra pure water was removed from the cuvette, and approximately 1mL of each sample was separately injected into the cuvette. The cuvette was cleared of any access water from injection, and there was no debris or fingerprints on the cuvette, as this could interfere with the fluorescence. Once the cuvette was cleaned and placed into the instrument, the sample was run using a 3-Dimensional Absorbance Spectra and was given proper sample identification in the

software. Once completed, the injection time was changed from 0.1 seconds to 1 second, and the sample was run. Once the sample run was completed, a contour plot of fluorescent material was developed and the next sample was run following the same procedure.

3.2.7 Statistical Analysis

3.2.7.1 Mann-Kendall Trend Analysis

A Mann-Kendall trend analysis was performed on all SUVA results to determine if there were any trends in the data over time. The Mann-Kendall trend test was selected, as it is commonly used to statistically evaluate temporal trends in historical water quality data (Anderson et al., 2017). This Mann-Kendall trend analysis was performed at the 0.05 level of significance using *XL*STAT.

3.2.7.2 Normalize 3D and Inner Filter Effect for Fluorescence Spectroscopy

Once all the samples were run, samples were then normalized in comparison to the original blank. To normalize the blank, the B(Y) column was changed to X and the C(Z) column to Y and all numbers in these columns were highlighted for the 351 nm wavelength. A line was plotted that consisted of two peaks; a large peak and a small peak. Integration was performed on the smaller peak, and this number was recorded. The first sample was then selected and normalized, by inserting the blank integration value from the small peak into the software. Each sample was normalized according to the

blank area from the integration. Once all samples were normalized, the inner filter effect was performed. All samples were then exported into Matlab for further analysis.

3.2.7.3 Parallel Factor Analysis on Fluorescence Spectroscopy

All data from 2016 and 2017 were exported and loaded into Matlab before starting the PARAFAC analysis. An outlier test was performed on all samples, and it was determined that four samples were classified as outliers. WWT from March 14, 2017, October 3, 2017 and October 25, 2017, as well as FND from October 3, 2017 were all considered outliers. A test was then performed to remove these samples from the dataset in order for proper modeling to occur. Excitation wavelengths of 117-121 were also removed, to remove background noise to ensure a clear analysis. Once completed, a split half analysis was performed on all data points to determine the number of specific organic components within the watershed. Once validated, the data was exported into excel for further analysis.

3.3 Results and Discussion

3.3.1 Comprehensive Water Quality Analysis on Lake Fletcher

A characterization of water quality was performed on all Lake Fletcher water samples between May 2014 and November 2014, as well as from March 2016 until November 2017. A summary of yearly water quality data for 2014, 2016 and 2017 for each sampling location has been presented in Table 2. It is important to note that alkalinity measurements were not performed in 2014 and FO was not within the sampling

plan in 2014. As well, sample sizes were slightly different for each sampling year: n = 6 for 2014, n = 17 for 2016 and n = 12 for 2017. These changes were due to changes in project scope over time and availability.

Table 2. Average water quality data from 2014, 2016 and 2017 for all Lake Fletcher sampling locations (2014 n=6; 2016 n=17; 2017 n=12).

	pH			Dissolved Oxygen (mg/L)			Turbidity (NTU)			Alkalinity (mg $CaCO_3$/L)		
	2014	2016	2017	2014	2016	2017	2014	2016	2017	2014	2016	2017
FTB	6.3±0.3	5.2±0.7	5.9±0.7	12.4±6	11.4±3	10.3±1	1.0±0.9	0.8±0.5	0.4±0.1	-	3.0±3	3.1±1
FBB	6.7±0.8	6.2±0.4	6.9±0.6	12.8±6	12.4±2	10.7±1	1.7±1.3	1.5±2.2	0.9±0.3	-	10.1±8	9.7±3
FND	5.9±0.6	4.2±0.4	4.2±0.4	6.8±3	9.3±3	6.0±1	4.3±5.8	0.8±0.4	0.5±0.2	-	1.3±1	0
FD	6.8±1.0	6.1±0.5	6.5±0.7	11.5±4	12.2±2	9.3±2	2.1±1.7	2.7±5.2	1.1±0.5	-	13.1±3	16.8±4
FI	6.6±0.6	6.3±0.5	7.1±0.5	11.5±4	12.2±3	9.7±1	1.3±0.7	1.2±0.7	0.9±0.3	-	14.0±3	15.5±1
FO	-	6.5±0.5	7.3±0.4	-	12.3±3	9.8±1	-	1.1±0.5	0.9±0.3	-	13.8±3	15.1±2
DWR	6.9±1.5	6.5±0.5	6.9±0.2	8.2±3	11.5±3	8.9±1	3.2±1.6	1.4±0.4	1.6±0.3	-	14.1±3	16.8±2
WWT	6.3±0.2	6.6±0.3	7.0±0.4	-	6.8±2	4.5±2	9.2±4.4	3.2±2.0	2.4±1.8	-	72.3±42	107.4±40

As depicted in Table 2, FTB and FBB were found to have the lowest pH in both 2016 and 2017. These locations are in heavily forested wetland areas, so can speculate a higher concentration of DOC from decaying plant material resulting in a lower pH (Cory et al., 2006). This observation was similar for 2014, however it was not as prevalent, which could be attributed to the smaller sample size. All other Lake Fletcher sampling locations had a similar pH.

Dissolved oxygen (DO) was found to be the lowest at FND and WWT. The low DO at FND was speculated to be from less biological productivity in an acidic peat bog ("T8.3 Freshwater Wetlands"). The low DO at WWT was speculated to be from the increased microbial growth throughout the aeration treatment process consuming large quantities of oxygen within the water (Templeton & Butler, 2011). All other Lake Fletcher sampling locations had similar DO concentrations.

Turbidity results varied throughout all Lake Fletcher sampling locations. The highest turbidity was found at WWT, which could be due to the particles remaining in the wastewater after treatment. An interesting point to note however was that FD had higher turbidity values when compared to other stream sampling locations. This could be due to the potential contamination or run off from the wastewater lift station located next to the stream.

Alkalinity throughout the Lake Fletcher sampling locations was found to be low to moderate at most locations. FTB and FND had very low to no alkalinity, which could be contributed to these locations being in heavily forested area containing higher amounts of humic acids and lower pH. Alkalinity was found to be much higher at WWT. This

high alkalinity was to ensure pH of the wastewater remained within the optimal range to ensure treatment effectiveness and efficiency (Templeton & Butler, 2011).

NOM water quality analysis was performed on all Lake Fletcher water samples between May 2014 and November 2014 as well as from March 2016 until November 2017. A summary of yearly organic water quality data for 2014, 2016 and 2017 for each sampling location has been presented in Table 3. It is important to note that true colour measurements were not performed in 2014 and only started in August of 2016, and FO was not within the sampling plan in 2014. As well, sample sizes were slightly different for each sampling year: n = 6 for 2014, n = 17 for 2016 and n = 12 for 2017.

Table 3. Average organic water quality data from 2014, 2016 and 2017 for all Lake Fletcher sampling locations (2014 n=6; 2016 n=17; 2017 n=12).

	TOC (mg/L)			DOC (mg/L)			True Colour (TCU)		
	2014	2016	2017	2014	2016	2017	2014	2016	2017
FTB	4.13±1.4	3.39±1.9	4.67±1.5	4.48±1.5	3.34±1.9	4.67±1.4	-	19.6±17	37.0±13
FBB	3.99±0.8	3.64±1.6	4.27±1.3	4.47±1.4	3.47±1.6	4.34±1.2	-	18.4±16	31.3±12
FND	13.01±4.8	12.17±3.2	15.24±3.4	14.85±6.6	11.89±3.0	15.62±3.9	-	127.6±19	188.0±46
FD	5.50±1.1	6.34±1.3	7.22±1.3	7.17±3.4	6.38±1.3	7.01±1.3	-	47.7±26	58.9±16
FI	3.45±0.3	3.88±0.6	3.72±0.3	3.75±0.8	3.95±0.6	3.71±0.3	-	10.1±5	17.0±5
FO	-	3.86±0.5	3.94±0.6	-	3.90±0.6	3.79±0.3	-	9.9±6	17.3±6
DWR	7.67±7.2	3.86±0.5	3.79±0.3	8.43±6.2	3.85±0.5	3.65±0.2	-	9.2±6	18.4±10
WWT	13.02±8.6	8.71±5.4	10.30±5.3	9.93±5.9	7.44±2.0	6.92±0.8	-	31.1±8	26.0±8

In terms of NOM, TOC and DOC were found to be very similar for all lake sampling locations, which is a common characteristic for surface waters in Nova Scotia that organic matter is primarily in the dissolved form (MacPhee, 1992; Stoddart & Gagnon, 2014; Waller et al., 1997). However, TOC was found to be higher than DOC for WWT, which was suspected as a result of organic particles remaining in the wastewater after treatment that would be filtered out in a DOC sample.

As depicted in Table 3, TOC/DOC was found to be the highest at the FND location. FND is located in a small peat bog environment, which causes organics to accumulate over time leading to high concentrations of organics in the water ("T8.3 Freshwater Wetlands"). TOC/DOC was also found to be moderately high at WWT and FD. The higher organic concentration at WWT could be due to the high concentration of organics found in human waste (Templeton & Butler, 2011). The cause of the high organic concentration found at FD was relatively unknown. One possibility is from runoff or contamination from the wastewater lift station located next to the stream, which could increase the organic concentration in the stream. A more detailed investigation of NOM composition and characteristics is presented in the following section.

3.3.2 Temporal Evaluation of NOM Composition

In this work, SUVA was used as a general understanding tool for NOM composition within Lake Fletcher. The SUVA results from all Lake Fletcher sampling locations are presented in Figure 2.

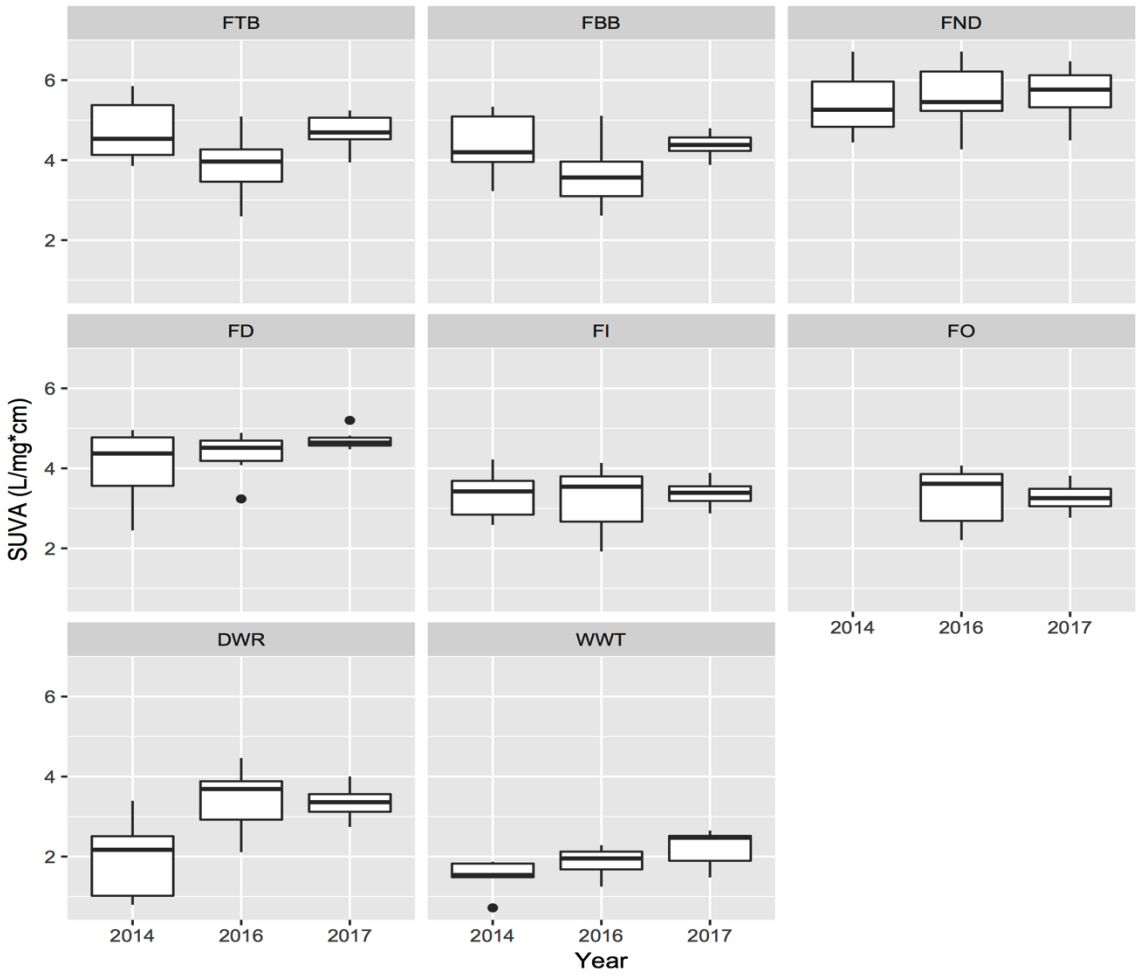


Figure 2. SUVA results from 2014, 2016 and 2017 for all Lake Fletcher sampling locations.

SUVA was found to be moderate to high (between 2 and 4) for most Lake Fletcher sampling locations, indicating a mix of humic and non-humic like sources of organic material (Edzwald & Tobiason, 2011). FND was found to have a very high SUVA value (> 4), indicating predominantly humic like material as the main constituent of organic material, which is characteristic of a peat bog environment. WWT was found to have a relatively low SUVA value (~2), indicating predominantly non-humic like or protein-like material as the main constituent of organic material, which is characteristic for organics found in human waste (Matilainen et al., 2011; Templeton & Butler, 2011).

Furthermore, SUVA results were tested using a Mann-Kendall trend analysis at the 0.05 level of significance to determine if there were any trends in the data over time. It was determined that SUVA from FO and WWT were the only sampling locations within Lake Fletcher that experienced a significant trend over time. FO had a decreasing trend over time (p-value = 0.029) whereas WWT had an increasing trend over time (p-value = < 0.0001). All other Lake Fletcher sampling locations did not have a significant trend in SUVA over time (p-values > 0.05). It was then determined whether there were significant seasonal trends for FO and WWT over time at the 0.05 level of significance. There was no seasonal trend for FO (p-value > 0.05), however there was a significant positive seasonal trend over time for WWT (p-value = 0.027).

NOM composition was assessed using fluorescence spectroscopy paired with PARAFAC analysis. The PARAFAC analysis model was validated for 5 distinct components of fluorescent organic material within the Lake Fletcher watershed. Results for this fluorescence analysis are presented in Table 4.

Table 4. NOM composition analysis using fluorescence spectroscopy paired with PARAFAC analysis.

Component	Excitation (nm)	Emission (nm)	Type of Organic
1	345	447	Humic-like
2	366	500	Humic-like
3	303	427	Humic-like
4	324	398	Humic-like
5	279	342	Protein-like

Although these fluorescent components appear to be similar, the five distinct components from PARAFAC analysis were actually quite different in terms of NOM characteristics (Table 4). Component 1 had an excitation wavelength of 345nm and an emission wavelength of 447nm. This component was found to be a humic substance corresponding with the C peak and had a terrestrial origin (Stedmon & Markager, 2005; Stedmon et al., 2003). Component 1 is commonly detected in the warmer months of the year, and is often located in a forested or wetland environment. This component is absent from wastewater organic matter. Component 2 had an excitation wavelength of 366nm and an emission wavelength of 500nm. This component was found to a be humic substance corresponding with the A peak and had a terrestrial/autochthonous origin (Stedmon & Markager, 2005; Stedmon et al., 2003). Component 2 is commonly detected in all freshwater environments and has fluorescence similar to fulvic acids. Component 3 had an excitation wavelength of 303nm and an emission wavelength of 427nm. This component was found to be a humic substance corresponding with the C peak and had a terrestrial origin (Stedmon & Markager, 2005; Stedmon et al., 2003). Component 3 is often located in a forested or wetland environment and is absent from wastewater organic matter. Component 4 had an excitation wavelength of 324nm and an emission wavelength of 398nm. This component was found to a be humic substance corresponding with the A peak and had a terrestrial/anthropogenic origin (Stedmon & Markager, 2005; Stedmon et al., 2003). Component 4 is commonly detected in all freshwater environments and may be exported through agricultural catchments. Finally, component 5 had an excitation wavelength of 279nm and an emission wavelength of 342nm. This component was found to be a protein-like substance corresponding with the C peak and was

autochthonous in origin (Stedmon & Markager, 2005; Stedmon et al., 2003). Component 5 was found to have a tryptophan-like fluorescence and is commonly found in forested environments.

3.4 Conclusions and Recommendations

The objective of this chapter was to conduct a comprehensive water quality analysis on Lake Fletcher, with a focus on organic matter. Specifically, a temporal evaluation of both NOM concentration and composition was conducted via fluorescence spectroscopy, paired with conventional NOM analytes to understand any trends in NOM chemistry within Lake Fletcher. The results for the water quality analysis within Lake Fletcher found the following:

1. The water quality varied throughout the eight Lake Fletcher sampling locations throughout the 2014, 2016 and 2017 sampling periods. The location FND was found to be the most acidic location and was also found to have the highest concentration of TOC/DOC due to its peat bog environment. FD was found to have higher levels of turbidity and TOC/DOC when compared to the other Lake Fletcher stream locations (except FND as fore mentioned), which could be attributed to leakage/run off from the adjacent wastewater lift station. Finally, WWT was found to have the lowest DO concentration due to high biological activity within the aeration treatment process, and the highest level of turbidity from particles remaining in the wastewater following treatment.

The results for the analysis of NOM composition within Lake Fletcher indicated the following:

2. SUVA results indicated that most locations had moderate to high SUVA (between 2-4 L/cm*mg). Specifically, FND was found to be the highest SUVA of 5 L/m*mg, indicating a high concentration of humic-like organic substances at this location. Additionally, WWT was found to have a low SUVA of 2 L/cm*mg, indicating the major component of organics at this location as protein-like. SUVA from FO and WWT were found to have significant trends over time at the 0.05 level of significance (p-value = 0.029 and <0.0001, respectively). Of these two locations, WWT was the only one to experience a significant seasonal trend over time (p-value = 0.027).

3. Organic profiles were developed for the Lake Fletcher watershed using fluorescence spectroscopy paired with PARAFAC analysis. Using these analyses, it was determined that there were four distinct humic-like components and one protein-like component within the Lake Fletcher watershed.

Due to natural phenomena such as climate change and recovery from acidification due to reduced atmospheric deposition, the water quality within surface waters in Nova Scotia is changing. It is therefore recommended that continued water quality and NOM monitoring over time be conducted throughout Lake Fletcher. Since the treatment plant for the community of Collins Park uses an ultrafiltration/nanofiltration membrane filtration treatment train, it is recommended that continued NOM profile development be performed as water quality continues to change within Lake Fletcher. These changes of NOM could lead to the production of more humic-like substances within the water, causing an increased potential for membrane fouling within the treatment system.

Chapter 4: Understanding the Emergence of Microcystin-LR within Lake Fletcher, Nova Scotia

4.1 Introduction

Water quality changes from climate change and/or recovery from acidification have been found to change the microbial composition within surface waters (Monteith et al., 2005; Skjelkvale et al., 2003; Yan et al., 2003). These changes could increase the risk of harmful algal blooms within surface waters, which in turn could lead to the release of algal toxins (Yoo, 1995). As well, sewage dumping has been linked to algal blooms from nutrient enrichment in surface waters (Schindler, 1974). The wastewater discharge could be used as a nutrient source for microorganisms, which could increase the risk of harmful algal blooms particularly in surface waters experiencing biological changes as a result of environmental processes (e.g. climate change, recovery from acidification).

Algal toxins are produced by cyanobacteria in surface waters and can cause serious problems to livestock and human health (Rinehart et al., 1994). One particular algal toxin of interest is the group of microcystins, which are produced by cyanobacteria. Microcystins are hepatotoxins and neurotoxins, which can lead to carcinogenic effects on humans and animals (Lone et al., 2015; Ziegmann et al., 2010). The major toxin of concern of the microcystins is MC-LR, which is found mainly to cause liver damage, but also affects the heart, kidney, nervous system and gastrointestinal tract (Liu et al., 2006). The main exposure route of microcystins is through drinking water and recreational water uses (WHO, 1998). For this reason, WHO has set a drinking water guideline for MC-LR of 1μg/L, and the Guidelines for Canadian Drinking Water Quality has set a MAC for

MC-LR of 1.5µg/L (Guidelines for Canadian Drinking Water Quality, 2017; Guidelines for Drinking-water Quality, 2006). There is also a limit for total microcystins (expressed as MC-LR) within recreational waters in Canada of 20µg/L put in place by the Guidelines for Canadian Recreational Water Quality, which is important for surface water monitoring (Health Canada, 1992).

Since Lake Fletcher is a drinking water supply that receives a wastewater discharge, this environment could have a higher risk/potential for algal activity, which could lead to the release of algal toxins. Accordingly, the objective of this chapter was to identify the proportions of typical microbial indicators and to understand the presence of the algal toxin MC-LR within Lake Fletcher, Nova Scotia.

4.2 Materials and Methods

4.2.1 Microbial Composition Analysis

Three different techniques were used to understand the microbial composition within Lake Fletcher: biomass ATP, chlorophyll a and DNA sequencing. Biomass ATP was measured from July 2014 to October 2017, however chlorophyll a was only measured from June 2017 to November 2017. This was to get a better understanding of phytoplankton concentration once MC-LR was detected.

4.2.1.1 Biomass ATP Analysis

The analysis of biomass ATP was measured on all samples using a LuminUltra Quench-Gone Aqueous (QGA)-100 test kit. Exactly 50mL of water from each location

was taken using a sterile syringe. A 0.45µm filter was added to the syringe, and the water was passed through the filter. The filter was then removed, and the syringe was taken apart. The filter was replaced onto the bottom of the syringe, and 1mL of UltraLyse was added into the syringe. The top portion of the syringe was replaced, and this 1mL of UltraLyse was pushed through the filter and collected in a pre-made 9mL solution of UltraLute. The syringe and filter was then discarded. The solution was inverted at least three times, and 100µL of this solution was added to a testing tube, along with 100µL of Luminase. This was mixed and placed in the Kikkoman Lumitester C-100 device, and a measure of Relative Light Units (RLU) was given. This number in units of RLU was then converted into cellular ATP (cATP) using the given formula:

$$cATP\ \left(pg\frac{ATP}{mL}\right) = \frac{RLU_{cATP}}{RLU_{ATP1}} * \frac{10\ 000\ (pg\ ATP)}{V_{sample}\ (mL)}$$

4.2.1.2 Chlorophyll a Analysis

To determine the concentration of chlorophyll a, 250mL of water from each location was measured. Approximately 2mL of magnesium carbonate was added to a 0.2µm glass fiber filter as a preservative, before the 250mL of water was passed through the filter. The filter was then labeled and stored in the freezer until further analysis. Once all samples were collected and frozen on the filters, samples were taken to the Micro-Algal Production and Evaluation Laboratory at Dalhousie University where chlorophyll a concentrations were determined.

4.2.2 Surface Water Microcystin-LR Analysis

MC-LR analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples from each location were filtered through a 0.45μm PES filter and 1440μL of sample was used for MC-LR analysis on the LC-MS/MS.

LC-MS/MS was performed by a combination of high performance liquid chromatography (HPLC) with mass spectrometry (MS). The HPLC system, from Agilent 1260 binary pump was used for MC-LR detection. An Agilent Poroshell 120 EC-C18 (4.6 X 150 mm, 2.7 μm) column was used and was maintained at 50 ^{0} C throughout the run. The mobile phase comprising of ultrapure water (A) and a mixture of acetonitrile, 30%:70%: v/v (B) at 0.6 ml min^{-1} was used for an HPLC conditioning run. 20 μL of sample was injected through the column. The gradient at 30% organic solvent (B) was initially held and the proportion of organic solvent was programmed to linearly increase to 98% for 7 minutes. A post-time of 3 minutes was added to make sure all the analytes were eluted within the time. An additional 3 minutes of post time was allowed for the column to re-equilibrate before the next injection. This resulted in a total cycle time of 10 minutes. Mass spectrometry was performed on an Agilent 6460 Triple quadrupole mass spectrometer (QqQ). Source parameter settings were as follow: Gas temperature, 350 ^{0} C; Gas Flow rate, 10 L/min; Nebulizer, 35 Psi; Sheath gas temperature, 380 ^{0} C; Sheath Gas Flow, 12 L/min; Capillary voltage, 3500V; nozzle voltage, 0V; and Delta EMV, 600V. For the analytes to be identified, data acquisition was performed in multiple reaction monitoring (MRM) mode in electrospray ionization (ESI) positive mode. Two transitions: a quantifier (most abundant product) and qualifier for the target analytes were used for most of the compounds to increase specificity for the method. Data acquisition

and analysis was performed using Agilent MassHunter software (Version Rev B.08.00). Under the method, the minimum detection limit is 0.05 µg/L and the detection limit for quantification is 0.2 µg/L.

MC-LR samples were initially taken for all locations, however to limit sampling costs and preparation time, only DWR samples were run between May and July of 2016 when MC-LR detection was not expected. Detection was not expected during these months due to colder water temperatures and less solar radiation (WHO, 1998). Sample was filtered and kept so if/when MC-LR detection was first discovered, samples could be run on all other locations.

4.2.6 DNA Sequencing for Cyanobacteria Composition

DNA sequencing was performed on samples from the inlet to Lake Fletcher (FI) on three different occasions in 2016 after algal activity was observed. Approximately 250mL of sample water was filtered through a sterile 0.22µm PES filter. Filters were then stored in the freezer until DNA extraction could be performed. Sequences were amplified using primers specific to bacteria and cyanobacteria. Further details on this method and analysis can be found in Betts, 2018 – Unpublished thesis data.

4.2.6 Statistical Analysis

A Mann-Kendall trend analysis was performed on all biomass ATP results to determine if there were any trends in the data over time. The Mann-Kendall trend test was selected, as it is commonly used to statistically evaluate temporal trends in historical water quality data (Anderson et al., 2017). This Mann-Kendall trend analysis was performed at the 0.05 level of significance using *XL*STAT.

A two-sample t-test at the 0.05 level of significance was performed on 2016 and 2017 biomass ATP concentrations and MC-LR concentrations upon detection within Lake Fletcher. These t-tests were performed in order to determine if the means from the two years were statistically different. This was performed using Mini-tab 18 ®.

4.3 Results and Discussion

4.3.1 Biomass ATP and Chlorophyll a within Lake Fletcher

Biomass ATP analysis was performed on samples from Lake Fletcher to understand the overall biological composition in the water. These results are presented in Figure 3.

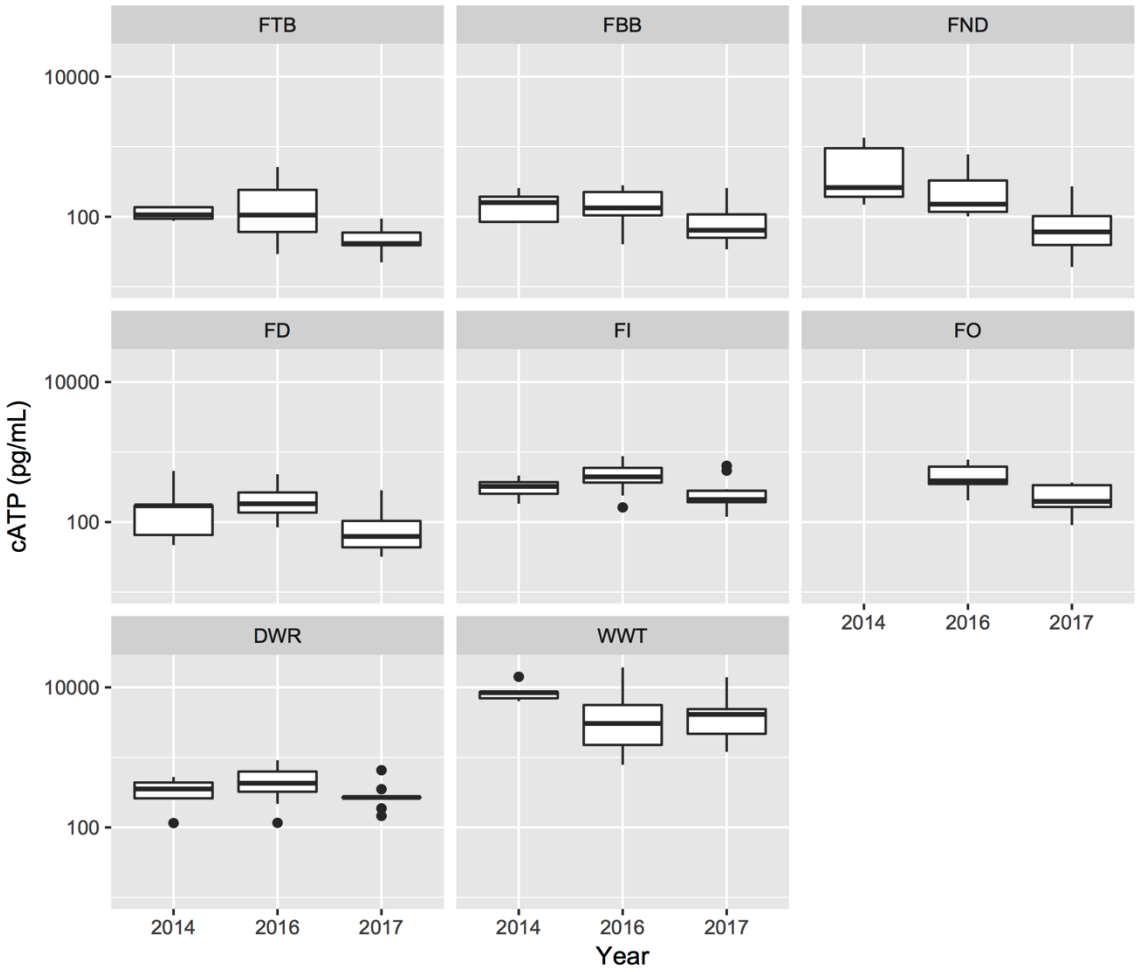


Figure 3. Biomass ATP concentrations from 2014, 2016 and 2017 for all Lake Fletcher sampling locations.

Biomass ATP concentrations in 2016 were found to be significantly higher for all Lake Fletcher sampling locations when compared to concentrations in 2017 at the 0.05 level of significance (p-value = 0.000). The only site that was not significantly different from 2016 and 2017 was WWT (p-value = 0.976 at the 0.05 level of significance), which indicates that wastewater effluent at the Lockview-MacPherson WWTP stayed relatively consistent over time. Biomass ATP concentration at all stream sampling locations was found to be relatively similar within a given year. As expected, WWT was found to have much higher concentrations of biomass ATP due to the natural characteristics of

wastewater, as well as the aeration treatment step promoting the growth of microorganisms to consume organics and other contaminants in the wastewater (Templeton & Butler, 2011).

Biomass ATP results from 2014, 2016 and 2017 were tested using a Mann-Kendall trend analysis at the 0.05 level of significance to determine if there were any trends in the data over time. It was determined that biomass ATP from FND, FI and FO were the only sampling locations within Lake Fletcher that experienced a significant trend over time. All three of these locations experienced a significant decreasing trend over time (FND: p-value = 0.006, FI: p-value = 0.018 and FO: p-value = <0.0001). All other Lake Fletcher sampling locations did not have a significant trend in biomass ATP over time (p-values > 0.05). It was then determined whether there were significant seasonal trends for FND, FI and FO over time at the 0.05 level of significance. There was no seasonal trend for FI (p-value > 0.05), however there was a significant negative seasonal trend over time for FND and FO (FND: p-value = 0.046 and FO: p-value = 0.023).

Chlorophyll a analysis was performed on stream samples starting in June of 2017 within Lake Fletcher to understand the phytoplankton concentration in the water. These results are presented in Figure 4.

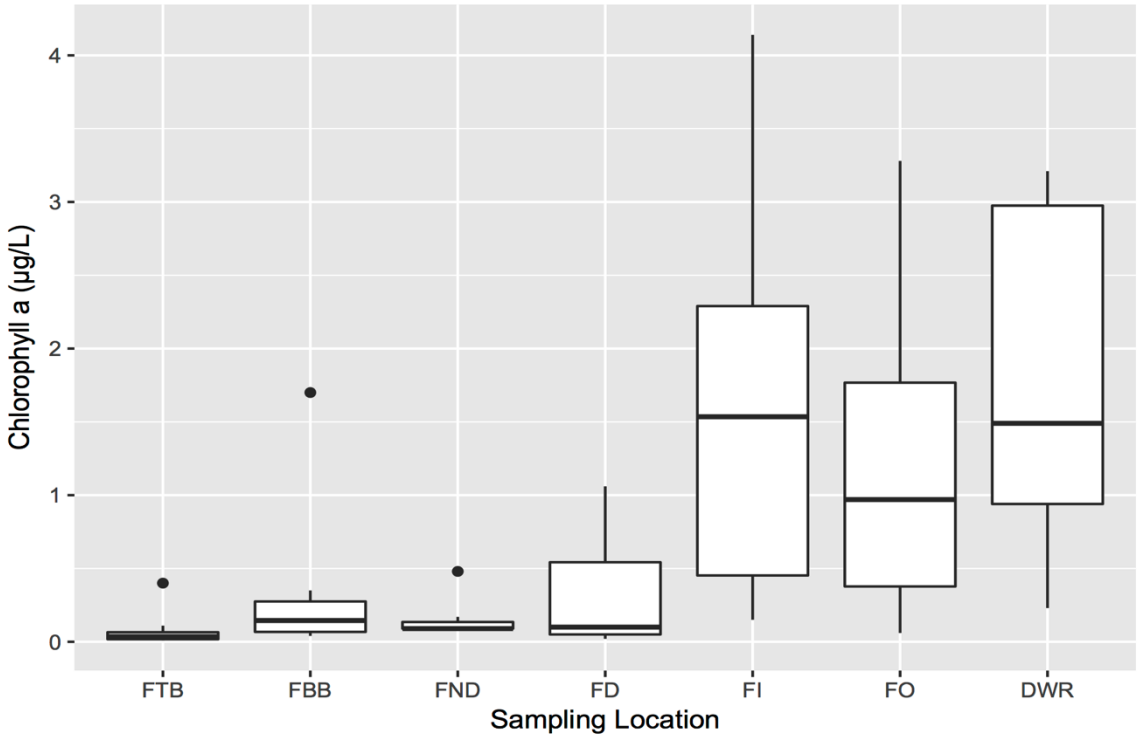


Figure 4. Chlorophyll a concentrations from 2017 for all Lake Fletcher stream locations.

The locations of FTB, FBB, FND and FD were all found to have similar and low chlorophyll a concentrations. FI, FO and DWR were also found to have similar chlorophyll a concentrations, however the concentrations were higher than the previously mentioned locations. One explanation to why FI, FO and DWR had higher chlorophyll a concentrations could be that these locations are wide channels not located in heavily forested environments, and are the best representation of chlorophyll a within Lake Fletcher as a whole. Wide channels with low tree cover tend to have more exposure to solar radiation, which would increase light levels and water temperatures to create a more favorable environment for phytoplankton growth (WHO, 1998).

4.3.2 Microcystin-LR Emergence within Lake Fletcher

MC-LR was sampled throughout Lake Fletcher from March of 2016 until November 2017. MC-LR was only detected at three locations throughout the study period: FI, FO and DWR. Results for these three locations from 2016 are presented in Figure 5.

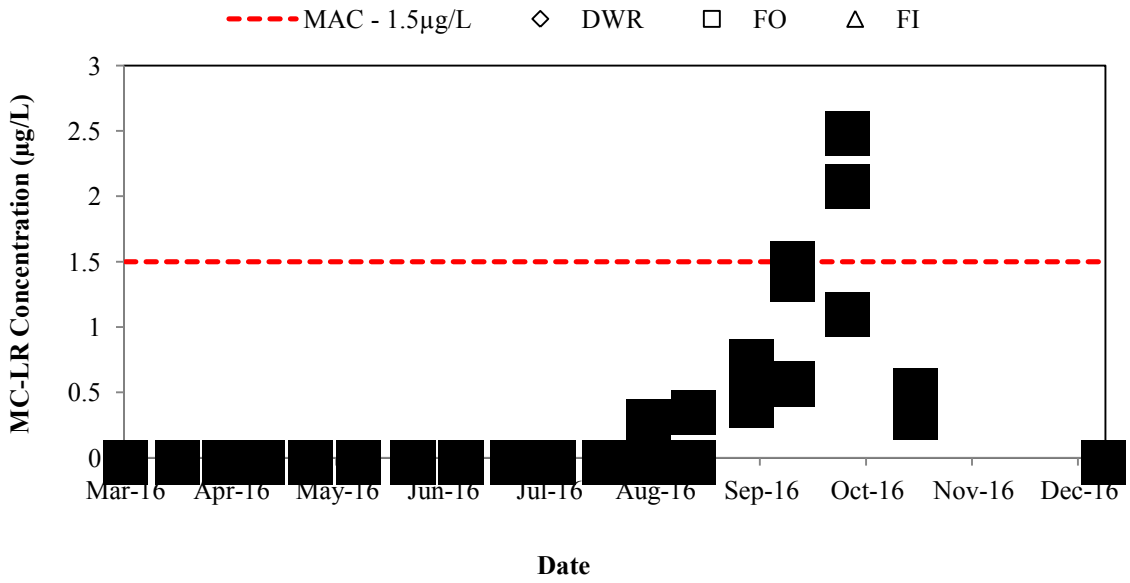


Figure 5. 2016 MC-LR concentrations within Lake Fletcher.

MC-LR was found to be below the LC-MS/MS detection limit from March until July of 2016. During the month of August, the first detection of MC-LR occurred at FO. After this first detection, MC-LR was also detected at FI and DWR within Lake Fletcher. Concentrations of MC-LR throughout August and the beginning of September were relatively low, however began to increase over time. Concentrations increased to a maximum peak of above 2.5µg/L in September, which was well above the MAC set by the Guidelines for Canadian Drinking Water Quality of 1.5µg/L, but was still less than

the Guidelines for Canadian Recreational Water Quality of 20µg/L. MC-LR concentrations decreased in October and were again below detection limit as of December 2016. It is well documented in the literature that cyanobacteria need three conditions in order to bloom: 1) adequate concentrations of nitrogen and phosphorous, 2) water temperatures between 15°C and 30°C and 3) a pH between 6 and 9 (Yoo, 1995). For these reasons, MC-LR detection could only occur in Lake Fletcher during the summer and early fall months when these water quality conditions were met in the lake, which is exactly what was found in 2016. These results were also interesting to find, as these concentrations of MC-LR were quite high for a lake classified as oligotrophic (Mudroch et al., 1987).

One challenge faced with this initial detection, was whether or not MC-LR was present in the past. This study was the first to be performed on algal toxins within Lake Fletcher, meaning there is no background data on MC-LR occurrence within the water supply. This idea brought up numerous different questions: was this the first time MC-LR was present within Lake Fletcher, or is the toxin present every summer and has not been reported? August and September of 2016 were relatively dry for Nova Scotia with 129mm of precipitation compared to the average of 205mm, so was this the cause of such high concentrations of MC-LR? For these reasons, sampling for MC-LR within Lake Fletcher was continued for the 2017 sampling season. Results from the 2017 MC-LR sampling season are presented in Figure 6.

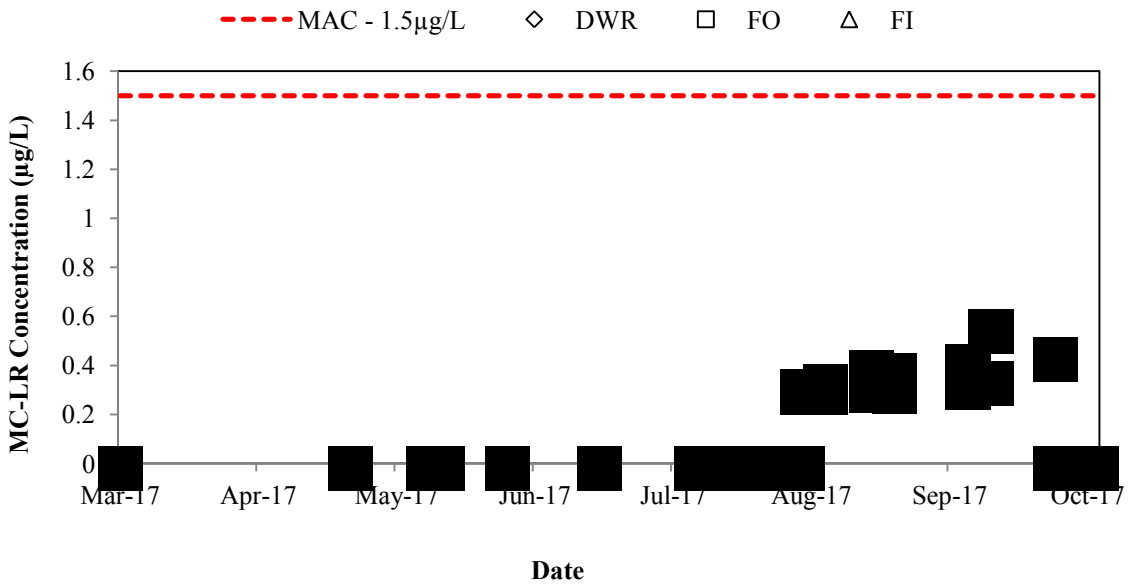


Figure 6. 2017 MC-LR concentrations within Lake Fletcher.

MC-LR was again found to be below the LC-MS/MS detection limit from March until July of 2017. However, MC-LR was first detected again at FO at the beginning of August around the same time as previously discovered in 2016. MC-LR was then detected at both FI and DWR at the end of August. MC-LR concentrations in 2017 began to increase throughout August and September before a decrease occurred at the beginning of October and was below the detection limit at the end of October.

A t-test was performed on MC-LR data from 2016 and 2017 once the toxin was detected within Lake Fletcher. It was determined that there was a statistical difference between MC-LR concentrations at FI, FO and DWR between 2016 and 2017 at the 0.05 level of significance (p-value = 0.017). This indicates that the MC-LR concentrations from FI, FO and DWR were significantly higher in 2016 when compared to 2017.

In 2017, MC-LR concentrations did not reach the MAC of 1.5µg/L set by the Guidelines for Canadian Drinking Water Quality. The maximum concentration of MC-LR detected within Lake Fletcher in 2017 was at FO at approximately 0.56µg/L. This lower concentration demonstrates that the occurrence of MC-LR was not a singular event. It also shows that the dry weather conditions in 2016 did not cause the MC-LR to occur within Lake Fletcher. The precipitation in August and September of 2017 was higher at 236mm compared to 129mm in 2016, and although concentrations of MC-LR were lower, the toxin was still detected.

4.3.3 DNA Sequencing to Explore Microbial Composition within Lake Fletcher

DNA sequencing of cyanobacteria was performed in order to better understand the microbial composition of cyanobacteria present within Lake Fletcher (Betts, 2018 – Unpublished thesis data). DNA sequencing was performed on samples from the inlet to Lake Fletcher (FI) on three different occasions in 2016 after algal activity was observed. These results are presented in Figure 7.

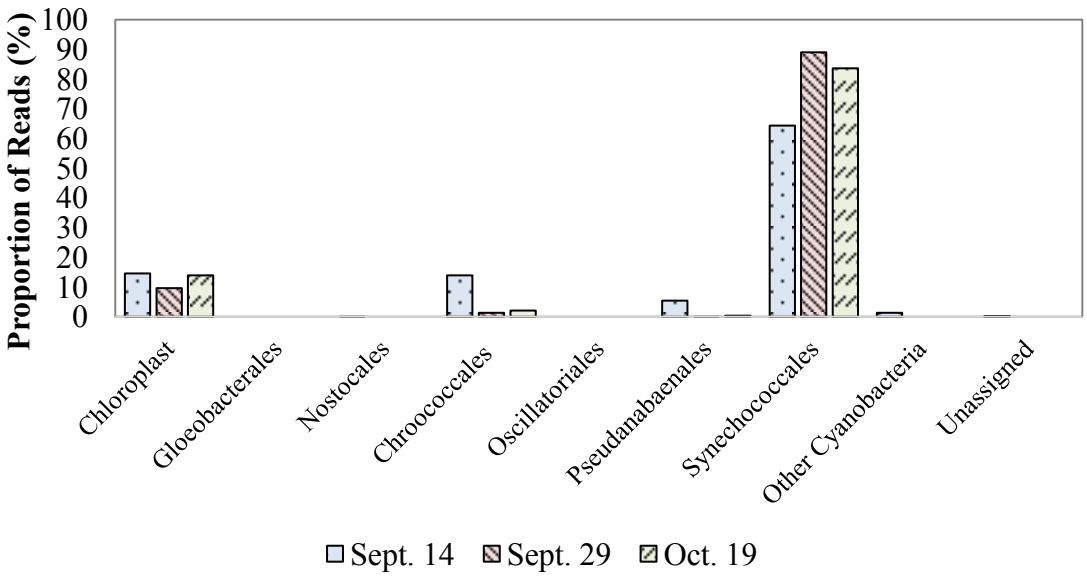
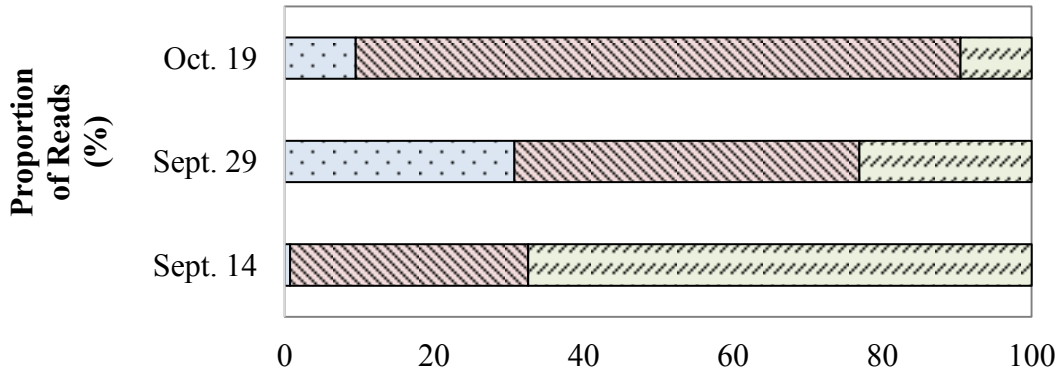


Figure 7. Proportion of cyanobacteria reads from three different sampling dates in 2016 at the FI location (Betts, 2018 - Unpublished thesis data).

The order *Synechococcales* was found to have the highest proportion of reads in all three sampling days. September 14th, 2016 had 64% *Synechococcales*, whereas September 29th, 2016 and October 19th, 2016 had greater than 80% *Synechococcales*. The order *Chloroplasts* made up the next highest proportion of reads, which had greater than 10% of total reads for all three sampling days. Finally, an important order to explore was the order *Chroococcales*, which had a proportion of around 14% on September 14th, 2016 and around 2% for September 29th, 2016 and October 19th, 2016. This order is important, as it contains the family *Microcystacceae*, which is the family that could contain the MC-LR producing genus *Microcystis*. A more detailed look into the proportion of the order *Chroococcales* within the Lake Fletcher inlet is presented in Figure 8.



	Sept. 14	Sept. 29	Oct. 19
Fam. Chroococcaceae	0.72	30.77	9.52
Fam. Gomphosphaeriaceae	31.88	46.15	80.95
Fam. Microcystaceae (Gen. Microcystis)	67.39	23.08	9.52

Figure 8. Proportion of *Chroococcales* reads from three different sampling dates in 2016 at the FI location (Betts, 2018 - Unpublished thesis data).

It was determined that the family *Microcystaceae* was found in all three of the sampling days that DNA sequencing was performed. The highest proportion of reads for *Microcystaceae* occurred on September 14th, 2016 with around 67% of the total *Chroococcales* proportion, and then decreased for each subsequent sampling day until the lowest proportion of 9.5% occurred on October 19th, 2016. These results indicated that the MC-LR producing family *Microcystaceae* was present within Lake Fletcher, however it does not necessarily indicate that *Microcystaceae* was the cause of MC-LR detection. There are multiple different families of cyanobacteria that can produce microcystins, such as *Hapalosiphonaceae, Nostocaceae and Oscillatoriaceae*, however the genus *Micocystis*

is the predominant MC-LR-producing cyanobacteria (Carmichael, 2001). These results gave an understanding of the different types of cyanobacteria present within Lake Fletcher, and gave an idea of what families could be producing the MC-LR detected within the lake.

4.3.4 Understanding the Occurrence of Microcystin-LR within Lake Fletcher

In order to understand the occurrence of MC-LR within Lake Fletcher, a more detailed investigation of MC-LR paired with other water quality parameters was explored. The results for FI, FO and DWR are presented in Figures 9, 10 and 11, respectively.

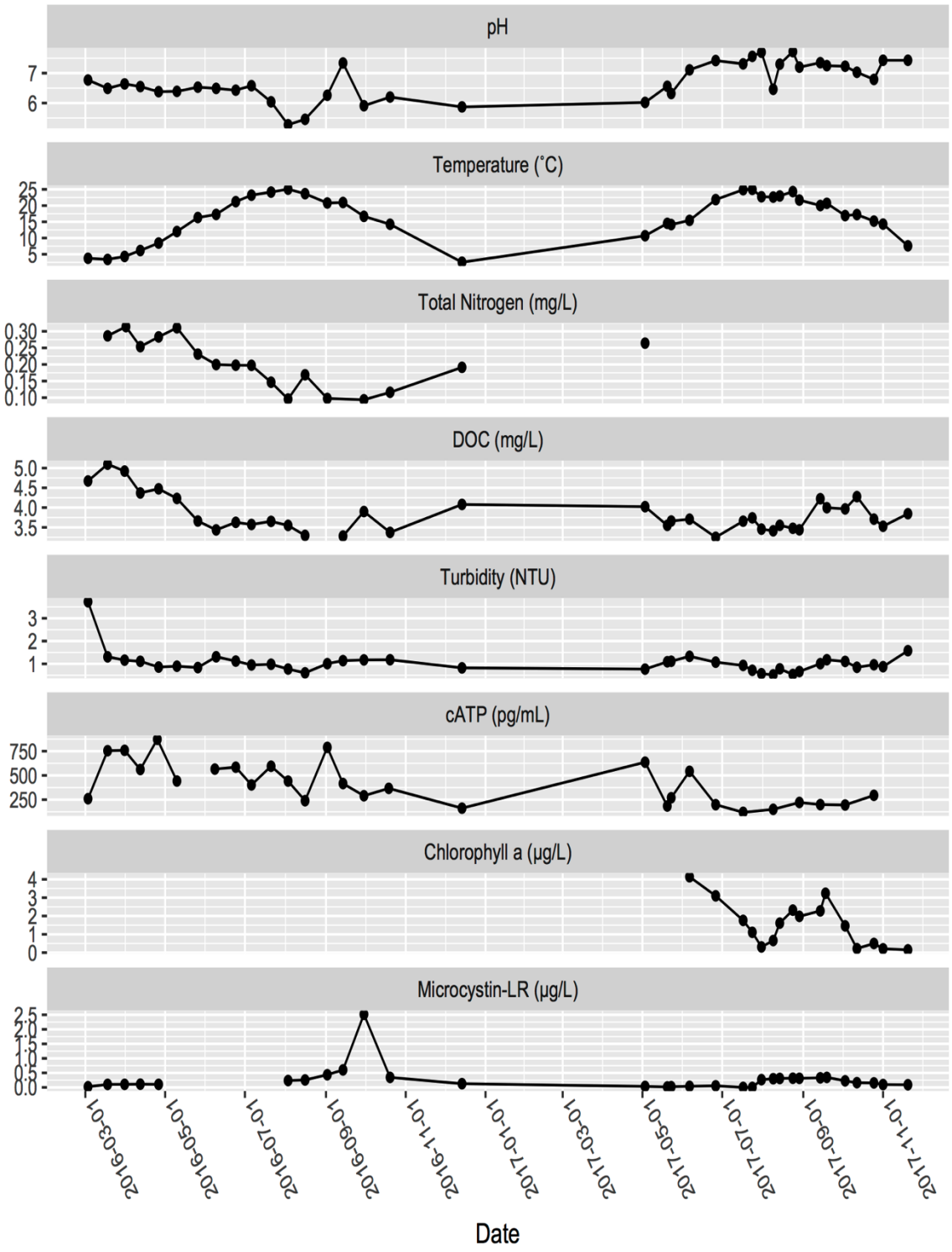


Figure 9. FI results for MC-LR paired with other water quality parameters over time.

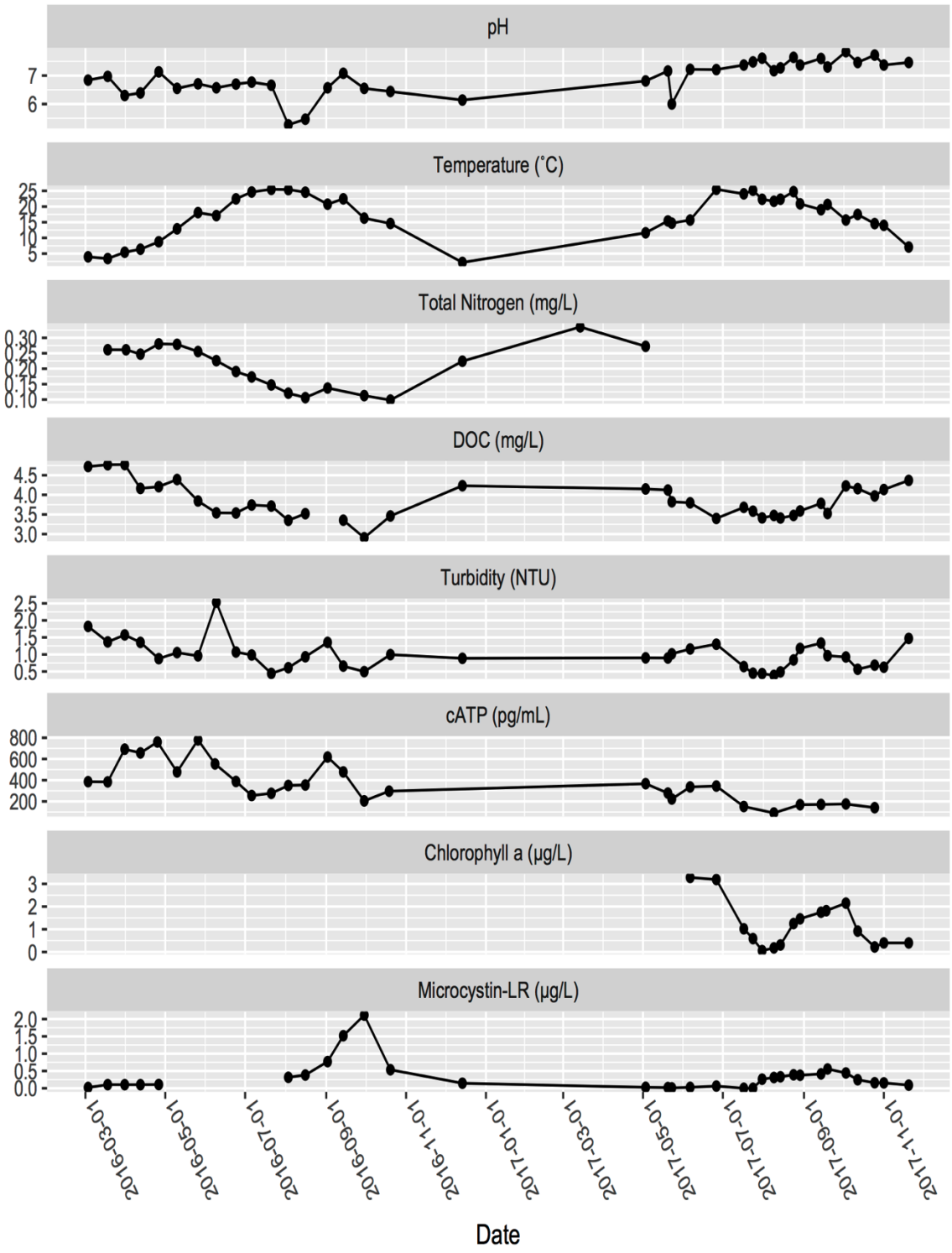


Figure 10. FO results for MC-LR paired with other water quality parameters over time.

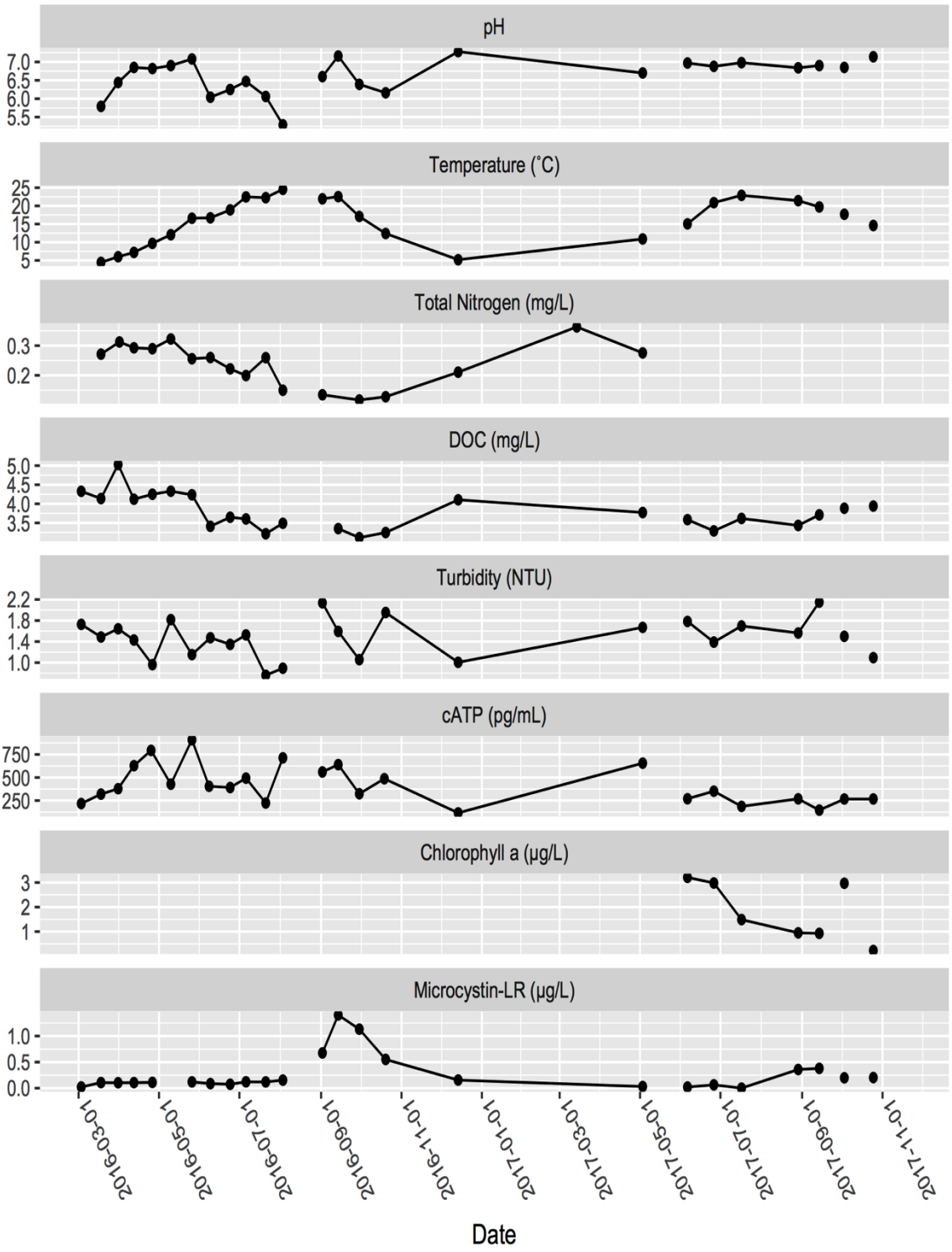


Figure 11. DWR results for MC-LR paired with other water quality parameters over time.

Based on Figures 9, 10 and 11, it was determined that the MC-LR spikes observed in 2016 and 2017 at FI, FO and DWR appeared to have similar water quality at all sampling locations when MC-LR was detected. The MC-LR spike that occurred in 2016 at the three locations had observed turbidity of ~1.1 NTU, DOC of ~3.3mg/L, pH of ~6.5, total nitrogen of ~0.1mg/L and temperatures that were decreasing from the high water temperatures found in the summer (e.g. ~20˚C). The MC-LR spike that occurred in 2017 at the three locations had observed turbidity of ~1.3 NTU, DOC of ~3.7mg/L, pH of ~7.2 and temperatures that were decreasing from the high water temperatures found in the summer (e.g. ~20˚C). As previously noted in literature, cyanobacteria blooms need numerous water quality conditions to be met in order for a bloom to occur, such as adequate sources of nutrients, high water temperatures and a pH between 6 and 9 (Yoo, 1995). These conditions appeared to be met for temperature and pH, however there are no clear indications in literature as to the affect of turbidity and organic carbon on MC-LR occurrence. Furthermore, nutrient data was not sufficient or reliable to perform an adequate analysis.

Along with the observed physical and chemical water quality data stated above, there were also trends in microbial indicators once MC-LR was detected within Lake Fletcher. A more detailed look at biomass ATP results over time for the three locations where MC-LR was detected within Lake Fletcher are presented in Figure 12.

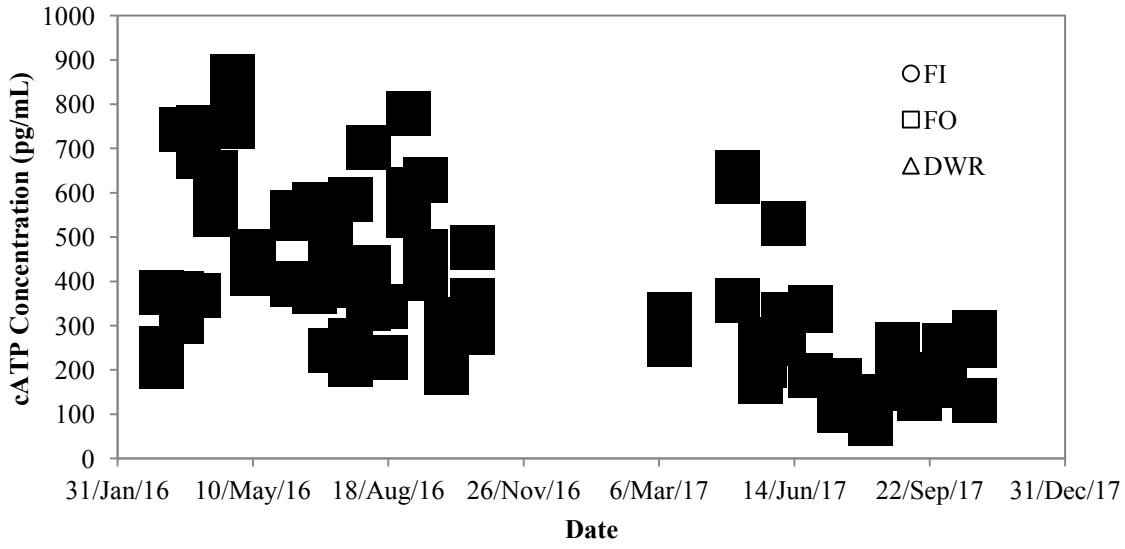


Figure 12. Biomass ATP concentrations over time at the three locations where MC-LR was detected.

A t-test was performed on biomass ATP results for 2016 and 2017 from FI, FO and DWR. It was confirmed that there was a statistical difference between biomass ATP concentrations at these locations in 2016 and 2017 at the 0.05 level of significance (p-value = 0.000). This indicates that the results from FI, FO and DWR were all significantly higher in 2016 when compared to 2017. This could be one reason why MC-LR concentrations were lower in 2017 than 2016, since in general there was less biomass present within the water in 2017 (Kotak et al., 2000).

All organisms producing ATP are accounted for when analyzing total biomass. As environmental conditions change temporally (e.g. due to seasonality), one species may outcompete another and vice versa, causing biomass ATP concentrations within a given year to remain relatively the same (Helm-Hansen & Booth, 1966). Any substantial changes in biomass ATP could be due to a weather or contamination event promoting

ATP production in the stream at a specific instance (Tietjen & Wetzel, 2003). Therefore, in this work, no seasonal trend in biomass ATP for FI and DWR was expected; however the observed statistically significant ($p < 0.05$) seasonal trends stated above from FO may be an anomaly due to a contamination or weather event within Lake Fletcher.

Chlorophyll a results over time for the three locations where MC-LR was detected within Lake Fletcher are presented in Figure 13.

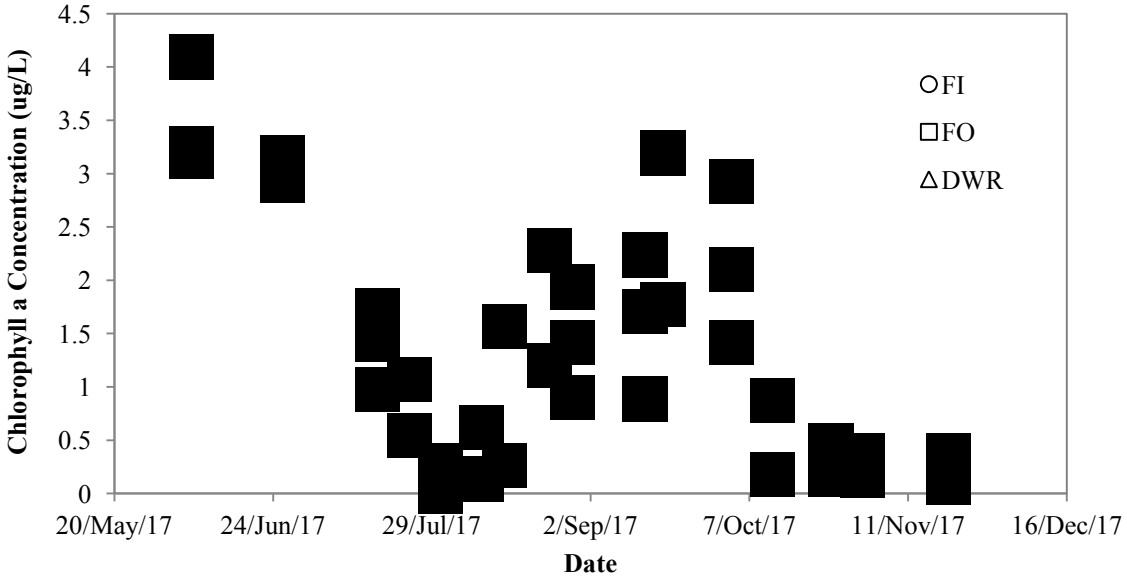


Figure 13. Chlorophyll a concentrations over time at the three locations where MC-LR was detected.

The concentration of chlorophyll a within FI, FO and DWR did vary over time. Unlike the biomass ATP concentration where no seasonal changes were observed, there was an increase in chlorophyll a during warmer months, which coincided with the detection of MC-LR. Specifically, concentrations of chlorophyll a began to rise when MC-LR was first detected in mid-August, and continued to increase until mid-September

when MC-LR was the highest, and then decreased as MC-LR began to decrease in October. A similar result was found by Kotak et al. (1993), where seasonal concentrations of MC-LR were statistically correlated with chlorophyll concentrations. Although no seasonal change was observed for the biomass ATP concentration within Lake Fletcher, there is evidence to support the microbial composition within the water did change. The observed spike in chlorophyll a from August to October of 2017 indicates an increase in the concentration of phytoplankton within the water, which could include the microcystin-producing cyanobacteria of interest.

4.4 Conclusions and Recommendations

The objective of this chapter was to identify the proportions of typical microbial indicators and to understand the presence of the algal toxin MC-LR within Lake Fletcher, Nova Scotia. The results of this study indicated the following:

1. Biomass ATP concentrations were found to be similar for all Lake Fletcher sampling locations except WWT, which was found to be higher. The high biomass at WWT was due to the natural characteristics of wastewater and the promotion of biological growth throughout the aeration treatment process. Biomass ATP from FND, FI and FO were found to have significant decreasing trends over time at the 0.05 level of significance (p-value = 0.006, 0.018 and <0.0001, respectively). Of these three locations, FND and FO experienced significant seasonal trends over time (p-value = 0.046 and 0.023, respectively).

2. Chlorophyll a concentrations were found to be similar and low for FTB, FBB, FND and FD (around 0.2µg/L). The other locations, FI, FO and DWR were also found to be similar, but had a higher concentration (around 1.5µg/L). This higher concentration of chlorophyll a at FI, FO and DWR could be because these locations are wide channels and not located in heavily forested environments, which would provide lots of light to the stream promoting the growth of cyanobacteria.

3. The occurrence of MC-LR in Lake Fletcher was not a singular event, as concentrations were detected in both 2016 and 2017. MC-LR was found in three different locations within Lake Fletcher: FI, FO and DWR. MC-LR was first detected during the month of August, and was no longer present within the water in October. The concentration of MC-LR was found to be significantly higher in 2016 than in 2017 at the 0.05 level of significance (p-value = 0.017), which could be due to the increased precipitation found between August and September of 2017.

4. The MC-LR spikes that occurred in both 2016 and 2017 at FI, FO and DWR all experienced similar water quality (turbidity, DOC, pH and water temperatures) when MC-LR was detected from year to year.

5. As the environmental conditions change throughout the seasons, one species should outcompete another and cause biomass ATP concentrations within a given year to remain relatively consistent. However, there was a seasonal change in chlorophyll a concentration. This indicates that although the total biomass did not change, the microbial composition shifted towards more phytoplankton during the summer months, which could be responsible for the MC-LR production during this time.

As previously mentioned, water quality changes from climate change and/or recovery from acidification have been found to change the microbial composition within surface waters. It is therefore recommended that the local water utility continue monitoring microbial composition throughout Lake Fletcher to help understand how biological activity is influenced by environmental changes in water quality. It is also recommended that the local water utility monitor for other algal toxins within Lake Fletcher. Since MC-LR was detected in the lake, other algal toxins such as saxitoxins, anatoxins and/or cylindrospermopsin could also be present, however was not within the scope of this project. This may be important for future consideration, as the wastewater discharging into Lake Fletcher could promote the growth of other cyanobacteria and release other algal toxins of concern.

Chapter 5: Exploring the Detection of Microcystin-LR Using Passive Sampling within Lake Fletcher, Nova Scotia

5.1 Introduction

Cyanobacteria have the ability to regulate their buoyancy within the water column, as they possess intracellular gas vacuoles (Reynolds et al., 1987). These gas vacuoles allow cyanobacteria to depth regulate within the water column in relation to many environmental factors, such as nutrients and light availability (Cullen & MacIntyre, 1998). Accordingly, surface water grab sampling alone may not accurately represent the total concentration of MC-LR within lakes (Kohoutek et al., 2008). Surface grab sampling generally occurs at different times and during different environmental conditions, therefore toxins within a lake may be misinterpreted.

Passive sampling has been accepted as a method to monitor emerging organic contaminants of concern, particularly for use in surface water monitoring (Alvarez, 2010). The main advantage with exploring passive sampling is that samplers are left in the stream constantly for a given period of time. For this reason, passive sampling is immune to accidental or extreme concentrations of the contaminant of interest, as the passive samplers will always be collecting the contaminant for the given sampling time (Namieśnik et al., 2005). As well, passive sampling is a good representation of constant exposure of the contaminant of interest, since the samplers remain in the water at all times throughout the sampling period (Söderström et al., 2009).

MC-LR representation within surface waters can be variable due to the microbial properties of cyanobacteria. The objective of this chapter was to explore the use of passive sampling as an alternative MC-LR monitoring tool within Lake Fletcher, Nova Scotia.

5.2 Materials and Methods

5.2.1 Passive Sampling for Microcystin-LR Analysis

Passive sampling for MC-LR detection was performed using POCIS from Environmental Sampling Technologies. The POCIS contained two pieces of 0.2μm PES filter membranes, with an Oasis HLB sorbent between the filters (Figure 14). Three POCIS were placed in a metal holder and installed into the FI and FO locations. POCIS were kept in the stream for a minimum of 28 days. After this time, POCIS were taken back to the lab and stored in the freezer at -4°C for a maximum of one week before an extraction for MC-LR was performed. Concentrations from POCIS were expressed in units of μg MC-LR/POCIS/day as opposed to μg/L, as flow measurements were not taken due to difficulties getting an accurate representation of flow at the exact location of samplers and equipment availability.

Figure 14. POCIS taken from Lake Fletcher after the 28-day exposure time.

5.2.2 Bench-top Passive Sampling Experiment

Before samplers were placed in the streams, POCIS were tested using a bench top method to understand uptake of MC-LR and to ensure an effective extraction procedure. 15L of water from Lake Fletcher was added to a container and spiked with a specific concentration of MC-LR. Two different tests were performed with two different MC-LR concentrations: 0.1µg/L and 1µg/L. These concentrations were selected, as they are similar to what would be expected within Lake Fletcher. Water was then recirculated throughout the system, and POCIS were left in the container for 7 days. After the 7 days, extraction for MC-LR was performed using the procedure stated below in section 5.2.3.

5.2.3 Stream Passive Sampling Experiment

Extraction for MC-LR was performed following a similar procedure adapted from Kohoutek et al. (2008) and Kohoutek et al. (2010). POCIS were taken from the freezer and disassembled. One at a time, each POCIS was opened, and sorbent was washed into a 50mL centrifuge tube with 10mL of 90% methanol. Once this was completed for all three POCIS, centrifuge tubes were placed in an ultrasonic bath for 15 minutes. After sonication, samples were then centrifuged for 10 minutes at 2500 revolutions per minute (rpm). After being centrifuged, supernatant was carefully removed and placed in a new centrifuge tube, and centrifuged again for 5 minutes at 2500 rpm. Once completed, exactly 1440μL of sample was taken from each centrifuge tube and analyzed for MC-LR on the LC-MS/MS. This procedure was completed four times, to ensure all MC-LR was washed off the sorbent. The only change was for washes three and four, samples were only centrifuged the first round for 5 minutes instead of 10 minutes.

5.3 Results and Discussion

5.3.1 Bench-top Passive Sampling for MC-LR Calibration

Two MC-LR bench-top passive sampling experiments were performed using Lake Fletcher water in order to understand MC-LR uptake by POCIS. The results are presented in Figure 15.

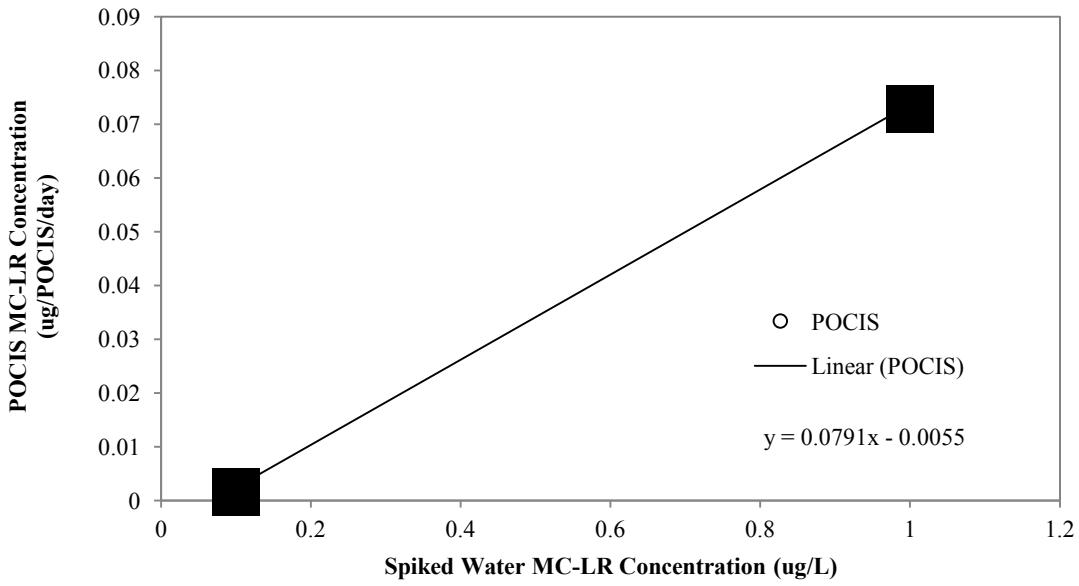


Figure 15. Calibration curve from the two bench-top POCIS experiments performed.

The two MC-LR concentrations of 0.1µg/L and 1µg/L were selected to represent concentrations that could be expected within Lake Fletcher. Measured MC-LR concentrations from these bench top experiments were then used to make a two-point calibration curve for POCIS MC-LR detection. This calibration curve could then be used to estimate average surface water MC-LR concentration within Lake Fletcher once stream POCIS experiments were performed.

An example of using this calibration curve is as follows. Assume the concentration from the POCIS was found to be 0.03 µg MC-LR/POCIS/day. This number can then be plugged into the regression equation of $y = 0.0791x - 0.0055$ to estimate the average concentration of MC-LR in the water over the time period the POCIS were in the water. This would give an average MC-LR concentration in the water of 0.38µg/L. An application of this method will be discussed for Lake Fletcher samples in the following sections.

5.3.2 Stream Passive Sampling for MC-LR Detection

Stream passive sampling experiments were performed from July to November 2017 at the FI and FO sampling locations. Results from the FI POCIS experiments paired with observed MC-LR concentrations in the water were presented in Figure 16.

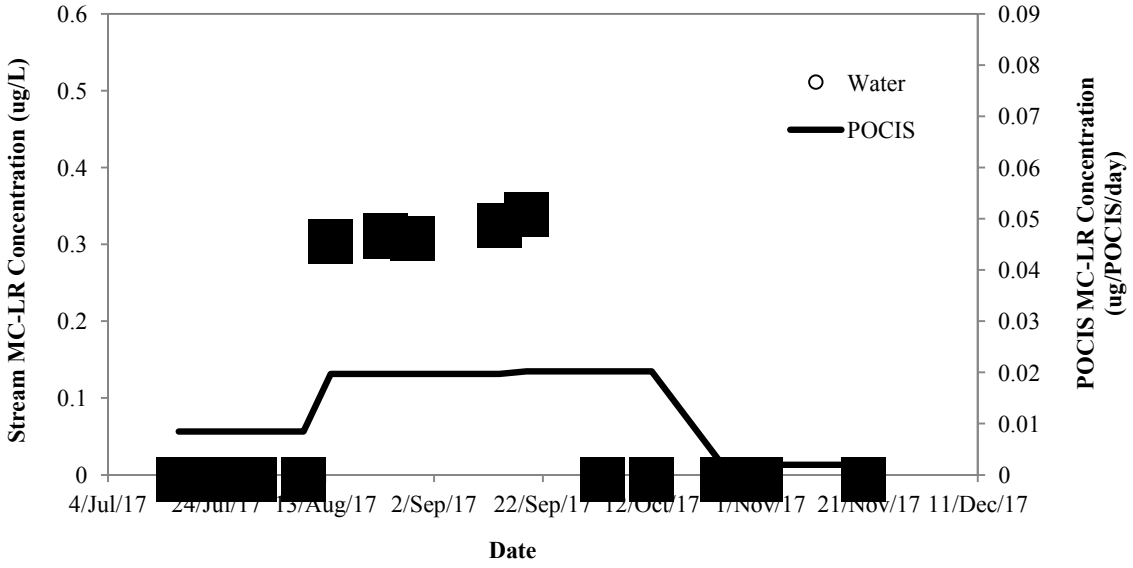


Figure 16. FI POCIS analysis for MC-LR paired with stream MC-LR concentrations over time.

The POCIS MC-LR concentrations found at FI followed a similar trend as the MC-LR concentrations in the water. The concentration found using POCIS was low throughout July and early August, increased throughout the end of August and stayed relatively the same until early October and decreased again between end of October and beginning of November.

Throughout July and early August, there was no detection of MC-LR within the water of FI, however there was detection during this time using POCIS analysis. This indicates that although the MC-LR concentration in the water was below the detection limit for the LC-MS/MS method, it does not mean the toxin was not present in the water. If the concentration from the POCIS analysis throughout July and early August was assessed using the calibration curve from the bench-top experiments, it could be predicted that the average concentration in the water throughout this time was around 0.11µg/L.

Results from the FO POCIS experiments paired with observed MC-LR concentrations in the water were presented in Figure 17.

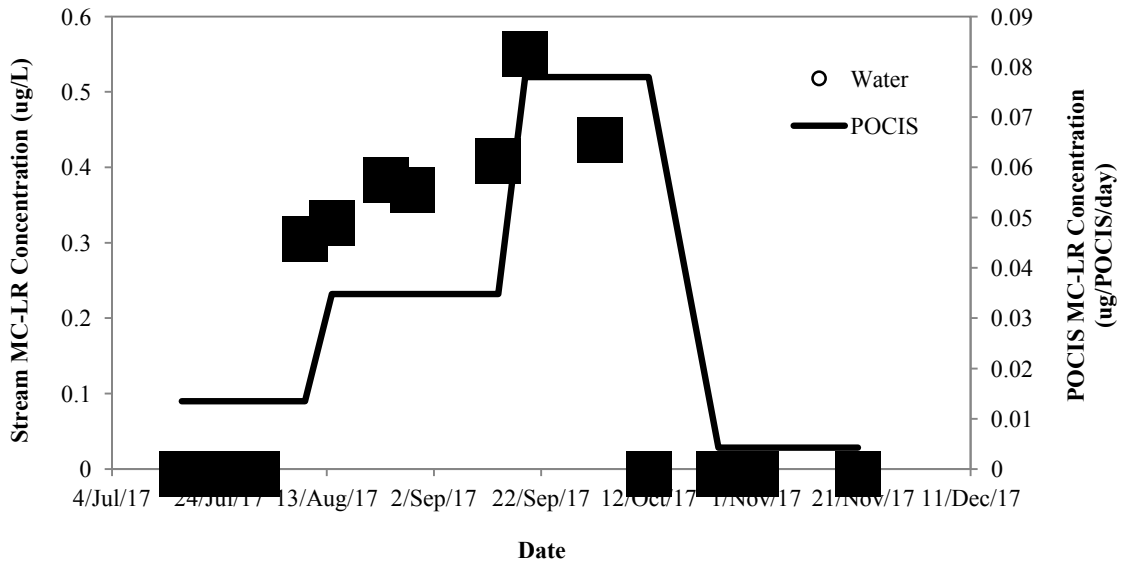


Figure 17. FO POCIS analysis for MC-LR paired with stream MC-LR concentrations over time.

Again, the POCIS MC-LR concentrations found at FO followed a similar trend as the MC-LR concentrations in the water. The concentration found using POCIS was low throughout July and early August, increased throughout the end of August and early September, increased again throughout the end of September and early October before finally decreasing between the end of October and beginning of November.

A similar analysis was performed using the calibration curve from the bench-top experiments to estimate MC-LR concentration in the water from the end of October to early November using stream POCIS results when MC-LR was below the detection limit in the water. It was predicted that the average concentration of MC-LR in the water at FO from the end of October to the beginning of November was around 0.059µg/L.

The use of POCIS for MC-LR detection within Lake Fletcher was found to be successful and a useful monitoring tool. The POCIS method has many different applications within the drinking water industry, particularly to lower method detection limits for MC-LR in drinking water supplies, allowing for initial low concentrations of MC-LR to be analyzed.

5.4 Conclusions and Recommendations

The objective of this chapter was to explore the use of passive sampling as an alternative MC-LR monitoring tool within Lake Fletcher, Nova Scotia. The results of this study indicated the following:

1. Bench-top POCIS analysis was found to be successful in creating a two-point calibration curve, which was then employed to predict low concentrations of MC-LR within Lake Fletcher.

2. The POCIS analysis was found to be a suitable monitoring tool for initial MC-LR detection within Lake Fletcher. Detection of MC-LR using POCIS was successful at both the FI and FO locations and followed a similar trend as observed MC-LR grab sampling. This sampling method was found to be best suited for occasion when MC-LR concentrations in the lake were below the method detection limit for LC-MS/MS.

As previously mentioned, MC-LR representation within surface waters can be variable due to the microbial properties of cyanobacteria. It is therefore recommended that passive samplers be used in other drinking water supplies to determine if MC-LR is present but in non-detectable concentrations within the water. It is also recommended that the local water utilities employ the use of passive sampling techniques for other contaminants of interest. For example, the city of Halifax has a seasonal problem of geosmin within their water supplies, so the use of passive samplers to identify initial geosmin detection may be an area for future research.

Chapter 6: Conclusion

6.1 Summary of Conclusions

A comprehensive analysis of water quality was conducted throughout Lake Fletcher, Nova Scotia with the focus on NOM concentration and composition. The results from the water quality analysis found that water quality varied throughout the eight Lake Fletcher sampling locations throughout the 2014, 2016 and 2017 sampling periods, with particular locations of interest being FND, FD and WWT due to their higher levels of organics. The high organics at FND could be attributed to the peat bog environment, where as FD could be from run off/contamination from the wastewater lift station located adjacent to the stream. It was also determined via fluorescence spectroscopy that there were five distinct components of organic material with the Lake Fletcher watershed: four humic-like components and one protein-like component. It was important to understand the composition of organics within Lake Fletcher, because NOM effects drinking water treatment as it reaction with chlorine to create DBPs as well as causes membrane fouling within the Collins Park Water Supply Plant.

Next, an analysis of microbial indicators and understanding the presence of the algal toxin MC-LR within Lake Fletcher was performed. Three microbial indicators were assessed: biomass ATP, chlorophyll a and DNA sequencing. Biomass ATP concentrations were found to be similar for all Lake Fletcher sampling locations except WWT due to the natural characteristics of wastewater containing more biomass. A Mann-Kendall trend analysis was performed on biomass ATP results, and it was determined that FND, FI and FO all experienced a significant decreasing trend over time. It was then

determined that of these locations, FND and FO had significant seasonal trends in biomass ATP concentrations over time. Chlorophyll a concentrations were found to be highest for FI, FO and DWR, due to these stream environments being more susceptible to light, increasing the likelihood of cyanobacteria growth. MC-LR was found to be present in both 2016 and 2017 at the FI FO and DWR locations. The concentrations in 2016 were found to be significantly higher than 2017 at the 0.05 level of significance (p-value = 0.017), which could be attributed to the increased precipitation found in 2017 when compared to 2016. The MC-LR spikes that occurred in both 2016 and 2017 at FI, FO and DWR all experienced similar water quality (turbidity, DOC, pH and water temperatures) when MC-LR was detected. It was also determined that the total biomass remained relatively consistent over the study period, however the concentration of chlorophyll a increased throughout the summer months, which could be responsible for the MC-LR occurrence during this time.

Finally, passive sampling was assessed as an alternative monitoring tool for initial MC-LR detection within Lake Fletcher. Bench-top experiments were conducted to create a two-point calibration curve, which was then used to interpret some of the stream POCIS results. The two POCIS devices that were installed at FI and FO were found to be successful for MC-LR monitoring when compared to stream MC-LR concentrations within Lake Fletcher. It was determined that this sampling method was best suited for occasions when MC-LR concentrations in the lake were below the method detection limit for LC-MS/MS.

6.2 Recommendations for Future Work

Due to anthropogenic phenomena such as climate change and recovery from acidification, it is therefore recommended that continued monitoring of all water quality parameters be performed. Monitoring of NOM concentration and composition within Lake Fletcher would be important, as changes in NOM could lead to the production of more humic-like substances, increasing the potential for membrane fouling within the treatment system. As well, continued monitoring of MC-LR and potentially other algal toxins such as anatoxin, saxitoxin and/or cylindrospermopsin within Lake Fletcher would be recommended, as these toxins could be a risk to public health if detected at high concentrations in the drinking water.

MC-LR representation within surface waters can be variable due to the microbial properties of cyanobacteria. It is therefore recommended that passive samplers be used in other drinking water supplies to determine if MC-LR is present but in non-detectable concentrations within the water. It would also be recommended that passive samplers be used for other contaminants of interest. The city of Halifax has a seasonal problem of geosmin within their water supplies, so the use of passive samplers to identify initial geosmin detection may be an area for future research.

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