PASSIVE SAMPLERS AND QPCR ANALYSIS: A POTENTIAL DETECTION METHOD FOR CYANOBACTERIA IN DRINKING WATER SOURCES

by

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In loving memory of Amr Ghothani, the most vibrant soul I knew. His excitement for life and passion for environmental engineering were like no other and will live on in my heart forever.

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ABSTRACT

The synergistic effects of climate change and lake recovery are expected to increase the intensity and frequency of cyanobacteria harmful algal blooms (HABs) in surface waters. Many drinking water sources experience HABs but surveillance strategies lack consistency between provinces and territories. The purpose of this project was to investigate passive sampling and qPCR analysis as a monitoring strategy for cyanobacteria in drinking water sources. A proof-of-concept field-scale monitoring program was conducted at Lake Fletcher, NS that compared traditional grab sampling to a new passive sampling technique using cellulose nitrate filter membranes. Weekly passive and grab samples were paired for analysis and results showed that passive samplers were more effective at detecting *cyrA* (cylindrospermopsin gene) and *sxtA* (saxitoxin gene) at all three sampling locations. *mcyE/ndaF* (microcystin/nodularin genes) was detected more frequently in grab samples at only two of the three sampling locations. Moreover, passive samplers were able to detect *cyrA* (all locations) and *mcyE/ndaF* (one location) before grab samples, highlighting their potential for early monitoring strategies. The adsorption performance of four passive sampler materials (cellulose nitrate, acrylic copolymer, gauze and nylon) for cyanobacteria detection was assessed through bench-scale adsorption studies to determine if other materials were more suitable for passive sampling. Cellulose nitrate was the best performing adsorbent and is suspected to reach its maximum adsorption capacity between $1 \times 10^5 - 1 \times 10^8$ GU/cm^2. These results have demonstrated that with further optimization, passive sampling paired with qPCR analysis could serve as a detection method for potential toxin-producing cyanobacteria in drinking water sources.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ºC	Degrees Celsius
%	Percent
±	Plus/Minus
®	Registered
™	Trademark
AOM	Algal Organic Matter
ANC	Acid Neutralization Capacity
BSC	Biological Safety Cabinet
CO_2	Carbon Dioxide
cm^2	Square Centimeter
Ct	Cycle Threshold
cyrA	Cylindrospermopsin-producing Gene
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleside Triphosphate
DBP	Disinfection By-product
DOC	Dissolved Organic Carbon
GHG	Greenhouse Gas
GU	Gene Units
h	Hours
HAB	Harmful Algal Bloom
HPLC	High-Performance Liquid Chromatography

IAC	Internal Amplification Control
Km	Kilometer
km^2	Square Kilometer
kV	Kilovolt
L	Litre
LC-MS	Liquid Chromatography-Mass Spectrometry
LOD	Limit of Detection
m	Meter
mA	Milliampere
mcyE/ndaF	Microcystin/Nodularin-producing Gene
mg	Milligram
min	Minutes
mL	Millilitre
mm	Millimeter
NB	New Brunswick
NDIR	Non-dispersive Infrared Detector
nm	Nanometer
NOM	Natural Organic Matter
NS	Nova Scotia
NSE	Nova Scotia Environment
NTC	Non-Template Control
NTU	Nephelometric Turbidity Units
PES	Polyethersulfone
pA	Picoampere
POCIS	Polar Organic Chemical Integrative Samplers

qPCR	Quantitative Polymerase Chain Reactions
rpm	Revolutions per Minute
sec	Seconds
SEM	Scanning Electron Microscopy
SUVA	Specific Ultraviolet Absorbance
sxtA	Saxitoxin-producing Gene
TOC	Total Organic Carbon
UV	Ultraviolet
UV_{254}	Ultraviolet Absorbance at the 245 Nanometer Wavelength
µg	Microgram
µL	Microlitre
µm	Micrometer
WWTP	Wastewater Treatment Plant
WTP	Water Treatment Plant

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CHAPTER 1: INTRODUCTION

1.1. PROJECT RATIONALE

Cyanobacteria are ubiquitous organisms found in surface waters that can rapidly proliferate into harmful algal blooms (HABs) when given the right growing conditions (Paerl, 1988; Pettersson & Pozdnyakov, 2013). The cyanotoxins produced by cyanobacteria and HABs are a concern for public health and safety and are categorized as hepatotoxins (microcystins, nodularins, cylindrospermopsins), which effect the liver and kidneys, and neurotoxins (saxitoxins, anatoxin-a, anatoxin-a(s)), which effect the nervous system (Carmichael, 1992; O'Neil et al., 2012; Whitton & Potts, 2007). These toxins can be produced by many genera if the responsible toxin synthesis genes are present in their gene sequences (Pick, 2016). However, the only regulated cyanotoxins in Canada are total microcystins, with a maximum allowable concentration of 1.5 µg/L (Health Canada, 2018).

The frequency and intensity of HABs have been increasing in Canadian surface waters (Pick, 2016; Schindler et al., 2012; Winter et al., 2011) and it is anticipated that climate change and lake recovery will exacerbate this issue. Furthermore, HABs are occurring in drinking water sources but surveillance strategies lack consistency between provinces and territories. Thus, it is crucial for water treatment plants (WTPs) to adopt robust cyanobacteria monitoring strategies moving forward as additional treatment barriers may be required in the event of a toxic bloom. Traditionally, grab and composite sampling techniques are used for cyanobacteria monitoring but each technique has limitations. Grab sampling is convenient and simple but offers only a snapshot of the potential contamination in a water body. Composite sampling is more representative but can be time consuming, expensive, and less-feasible for WTPs. Therefore, researchers have been using passive

samplers as an alternative sampling approach as it creates a composite sample while maintaining the simplicity and convenience of a grab sample. Passive sampling techniques have been successful at detecting SARS-CoV-2 in wastewater (Habtewold et al., 2022; Hayes et al., 2021; Li et al., 2022; P. Liu et al., 2022; Schang et al., 2021; Vincent-Hubert et al., 2022), even when concentrations were too low to be detected in grab samples (Hayes et al., 2021; Schang et al., 2021). The technique has also been used to detect a suite of viruses and bacteria in marine water (Vincent-Hubert et al., 2021). Additionally, passive sampling methods have been used to measure cyanotoxins in surface waters but lack the ability to measure multiple toxins at once (Brophy et al., 2019; Jaša et al., 2019; Kohoutek et al., 2008, 2010). The ability to detect a suite of potentially toxic cyanobacteria at low concentrations would be valuable for WTPs, but to my knowledge, there is no literature exploring the use of passive samplers for algal cell detection.

Further, WTPs often rely on physiochemical analyses to continuously monitor cyanobacteria, which is expensive (Crawford et al., 2017; Macário et al., 2017) and why many researchers have recommended shifting to a tiered monitoring approach (Adams et al., 2018; Crawford et al., 2017; Kibuye et al., 2021; Macário et al., 2017). These strategies suggest using microscopic identification and enumeration followed by toxin potential screening before quantifying toxins (Adams et al., 2018; Crawford et al., 2017; Kibuye et al., 2021; Macário et al., 2017). Microscopic identification, enumeration and toxin quantification techniques have been researched extensively, whereas screening for toxin potential is an emerging monitoring tool. Molecular methods such as quantitative polymerase chain reaction (qPCR) can quickly, simply and effectively quantify cyanotoxin risk in source waters by targeting and quantifying many cyanotoxin genes at once (Al-

Tebrineh et al., 2012; Chiu et al., 2017; Crawford et al., 2017; McKindles et al., 2019; Ngwa et al., 2014). qPCR is specifically useful in the early monitoring season as some studies have shown its ability to detect microcystin-producing genes before microcystins were detected using chemical analyses (Fortin et al., 2010; Lu et al., 2020). It is hypothesized that pairing passive sampling techniques with qPCR analysis could enhance cyanobacteria detection, through improved resolution and reliability, in drinking water sources and serve as an additional detection method for WTPs.

1.2. RESEARCH OBJECTIVES

This work aimed to investigate the use of passive samplers and qPCR analysis as a detection method for freshwater cyanobacteria for drinking water suppliers. To conduct this research, the following sub-objectives were completed:

1. Conduct a monitoring study that compares passive sampling to traditional grab sampling through a proof-of-concept field-scale monitoring program.
2. Assess the performance of different adsorbent materials for cyanobacteria detection during suspected or known freshwater cyanobacteria blooms.

1.3. ORGANIZATION OF THESIS

Chapter 2 provides a literature review of relevant topics for this research. This includes background information on freshwater cyanobacteria, their associated toxins, harmful algal blooms, sampling techniques for algal monitoring and qPCR analysis. Chapter 3 describes the materials and methods used to complete this study. Chapter 4 presents results from sub-objective 1, which compared passive sampling to grab sampling over a monitoring period. Chapter 5 presents results from sub-objective 2, which assessed

the adsorption performance of different passive sampler materials. Chapter 7 highlights the key findings of this work and provides recommendations for future studies.

CHAPTER 2: LITERATURE REVIEW

2.1. CYANOBACTERIA

2.1.1. Traits and Characteristics

Cyanobacteria are naturally occurring, gram-negative prokaryotes (Rippka et al., 1979), commonly referred to as "blue-green algae", in fresh, brackish, and marine environments (Paerl, 1988). Many cyanobacteria species share similar traits, but they are a diverse group of organisms that have evolved different physiological adaptations (Mantzouki et al., 2016; Rippka et al., 1979). They are often found in two forms: unicellular and filamentous (Whitton & Potts, 2007). Cyanobacteria size can range from cells less than 1 μm in diameter to trichomes larger than 100 μm in diameter (Whitton, 1992). Cyanobacteria can move throughout the water column using gas vesicles (Ganf & Oliver, 1982; Rippka et al., 1979; Walsby et al., 1997), which provide buoyancy and help them retrieve nutrients and light when resources are limited (Ganf & Oliver, 1982).

There are various cell types that can be found in cyanobacteria filaments, including vegetative cells, heterocysts and akinetes (Kumar et al., 2010; Rippka et al., 1979; Whitton & Potts, 2007). Vegetative cells are photosynthetic cells that can turn into heterocysts when nitrogen is limited (Rippka et al., 1979). Heterocysts are capable of fixing atmospheric nitrogen when dissolved nitrate and/or ammonium sources are scarce or irregular (Kumar et al., 2010; Whitton & Potts, 2007). Akinetes are formed in nitrogen-fixing species when climates are changed rapidly/unfavorably or when nutrients are depleted (Paerl, 1988; Whitton & Potts, 2007). They are dormant cells (Rippka et al., 1979) which can endure cold temperatures and desiccation (Kumar et al., 2010). These cells can "seed" into nutrient rich sediments (Paerl, 1988) where they can overwinter (Preston & Stewart, 1980). As

such, akinetes can be the cause of cyanobacteria blooms in the following years (Preston & Stewart, 1980) which can be an issue for WTPs as the likeliness of bloom formation may increase.

2.1.2. Harmful Algal Blooms and Cyanotoxins

When given the right physical, chemical and biotic conditions, cyanobacteria rapidly proliferate and create algal blooms (Paerl, 1988). These blooms can be harmful if they produce toxins, high biomass and/or mucilage and in such cases are referred to as harmful algal blooms (HABs) (Pettersson & Pozdnyakov, 2013). The toxins produced by cyanobacteria are secondary metabolites known as cyanotoxins (Carmichael, 1992), and are the largest health and safety concern associated with HABs. Cyanotoxins are commonly classified by three general groups: cytotoxins, hepatotoxins, and neurotoxins (Whitton & Potts, 2007). Cytotoxins are not highly lethal to mammals and tend to carry a lower profile in comparison to hepatotoxins and neurotoxins (Carmichael, 1992; Whitton & Potts, 2007). Hepatotoxins and neurotoxins are known as "biotoxins" and can have acute and lethal effects on mammals (Carmichael, 1992). Hepatotoxins affect the liver and kidneys and neurotoxins effect the nervous system. The most common hepatotoxins include microcystins, nodularins and cylindrospermopsins, and the most common neurotoxins include anatoxin-a, anatoxin-a(s), and saxitoxins (Carmichael, 1992; O'Neil et al., 2012; Whitton & Potts, 2007). Table 1 outlines some of the main cyanotoxin producing genera (Zanchett & Oliveira-Filho, 2013).

Table 1. Main producers of common cyanotoxins (Zanchett & Oliveira-Filho, 2013)

Toxin Type	Cyanotoxin	Genera of Main Producers
Hepatotoxins	Microcystins	*Anabaena, Planktothrix, Nostoc, Anabaenopsis, Microcystis aeruginosa*
	Nodularins	*Nodularia*
	Cylindrospermopsins	*Cylindrospermopsis raciborskii, Aphanizomenon ovalisporum, Aphanizomenon flos-aquae*
Neurotoxins	Anatoxin-a	*Anabaena, Aphanizomenon, Planktothrix*
	Anatoxin-a(s)	*Anabaena*
	Saxitoxins	*Anabaena circinalis, Aphanizomenon sp., Aphanizomenon gracile, Cylindrospermopsis raciborskii, Lyngbya wollei*

Many different genera of cyanobacteria can produce cyanotoxins, but they require the presence of functional toxin synthesis genes (Pick, 2016). Most cyanotoxins remain within the cells (intracellular), but can be released from ageing cells or upon cell lysis (extracellular) (Carmichael, 1992; Whitton & Potts, 2007). Both intracellular and extracellular cyanotoxins are of major concern for WTPs as many treatment processes can cause cell lysis (He et al., 2016). Currently, microcystins are the only cyanotoxin regulated in Canada and have a maximum allowable concentration of 1.5 µg/L (Health Canada, 2018). This maximum allowable concentration is for total microcystins, which accounts for both intra- and extracellular microcystins.

2.2. FACTORS INFLUENCING BLOOM FORMATION

2.2.1. Environmental Conditions

The main drivers behind the formation of cyanobacteria HABs are hydrological changes and nutrient/light availability (Paerl, 1988). Increased nutrient availability,

especially phosphorous, will increase cyanobacteria growth (Lürling et al., 2018; Paerl, 1988; Wagner & Adrian, 2009). Because many cyanobacteria are capable of nitrogen fixation, their growth is limited by phosphorous concentrations (Paerl, 1988; Wagner & Adrian, 2009). The availability of nitrogen is the main influencer on the types of blooms that occur (Bormans et al., 2005; Elliott, 2010) and is what allows nitrogen-fixers to outcompete non nitrogen-fixers (Schindler et al., 2008).

Cyanobacteria are photosynthetic organisms (Rippka et al., 1979) that are capable of adapting to low and high light conditions (Paerl, 1988). When light is low, some species will use gas vesicles to float to the water surface for photosynthesis, but when light is high, gas vesicles are squeezed out so they can sink into the water column (Paerl, 1988). It is also believed that when nutrients are high, some cyanobacteria may be superior light competitors during turbid conditions because their photosystem and buoyancy regulation has allowed them to adapt to extreme light conditions (Downing et al., 2001).

Warm climates tend to favor cyanobacteria blooms (Elliott, 2010; Lürling et al., 2018; Paerl, 1988) because the optimal growth temperature for most species is around 20-25 °C (Bormans et al., 2005; Paerl & Huisman, 2008). Warmer water will have a lower viscosity which decreases vertical migration resistance, allowing cyanobacteria to rapidly move up and down the water column to retrieve light and nutrients (Ganf & Oliver, 1982; Paerl & Huisman, 2009). As cyanobacteria accumulate at the surface of the water, they may locally increase the water temperature; creating positive feedback and exacerbating their dominance (Paerl & Huisman, 2008).

Stratification of surface water increases the potential for cyanobacteria bloom formation (Paerl, 1988). Stratification occurs when surface water temperatures increase

and when there is little turbulence caused by wind and heavy precipitation (Jöhnk et al., 2008; Paerl & Huisman, 2009). During periods of high turbulence, mixing speeds cause cyanobacteria to disperse throughout the water column (Walsby et al., 1997). Mixing has be proven to suppress microcystis blooms (Jöhnk et al., 2008), however, it increases nutrient loading which increases the likeliness of a cyanobacteria bloom once the water column stabilizes (Elliott, 2010; Paerl & Huisman, 2008). Some studies have also shown that the formation of dense surface blooms from prolonged stratification can lead to a high accumulation of cyanotoxins at the surface (González-Piana et al., 2018; Paerl & Huisman, 2009). However, after a storm event when mixing is increased, cyanotoxins can be pushed lower in the water column, creating a concern for WTP intakes (González-Piana et al., 2018).

2.2.2. Climate Change and Water Quality

Climate change is anticipated to increase the frequency and duration of cyanobacteria blooms (Mullin et al., 2020; Paerl & Huisman, 2008, 2009; Paerl & Paul, 2012). Changes in rainfall patterns, drought severity, nutrient loading, temperature, residence times, and CO_2 are all expected to increase the proliferation of these blooms (Mullin et al., 2020; Paerl & Huisman, 2008, 2009; Paerl & Paul, 2012). It is also suspected that climate change may cause high-risk blooms in lakes that have previously experienced moderate-risk blooms (Mullin et al., 2020). The composition and timing of blooms may also shift with a warming climate (Jöhnk et al., 2008), but different physiological and morphological traits of cyanobacteria will cause different responses to these changes (Mantzouki et al., 2016).

An increasing global temperature will lead to warmer surface waters, stronger stratification and a less viscous epilimnion (Mullin et al., 2020; Paerl & Huisman, 2008, 2009; Wagner & Adrian, 2009). Most cyanobacteria have an optimal growth rate of around 20-25 °C (Bormans et al., 2005; Paerl & Huisman, 2008), which will be favored if surface water temperatures continue to increase. Increased surface water temperature may also lead to prolonged and intensified thermal stratification (O'Neil et al., 2012; Paerl et al., 2011; Paerl & Huisman, 2008; Posch et al., 2012; Wagner & Adrian, 2009). Thermal stratification often happens during the summer months, but the duration of stratification has been increasing with warming climates; creating optimal growing periods for cyanobacteria (Paerl & Huisman, 2008; Wagner & Adrian, 2009).

Climate change is predicted to alter hydrological processes, which may create heavier rainfall periods followed by more severe droughts (Paerl & Huisman, 2008). Initially, increased storm runoff and flushing will promote mixing and turbulence, distributing cyanobacteria throughout the water column. However, the increased nutrient availability from these storms will exacerbate the effects of the following drought periods (Paerl & Huisman, 2008, 2009). Many cyanobacteria species prefer stable, stratified water and profit from internal phosphorous loads (Wagner & Adrian, 2009).

Lastly, the forecasted increase of green-house gas (GHG) emissions (in the form of CO_2) is expected to support cyanobacteria proliferation (Ma & Wang, 2021; Paerl et al., 2011; Paerl & Huisman, 2008; Verspagen et al., 2014). When waters are nutrient rich, photosynthesis rates of cyanobacteria become higher and consequently demand more CO_2 (Paerl et al., 2011). High CO_2 concentrations are anticipated to acidify freshwater sources, but in nutrient-rich lakes, the high consumption of CO_2 by cyanobacteria will counteract

acidification by increasing the pH and in turn, increase their growth (Paerl & Huisman, 2009). These effects will be especially enhanced in nutrient rich, low to moderately alkaline waters (Verspagen et al., 2014). Due to the buoyancy advantage of some cyanobacteria species, when dissolved CO_2 concentrations become depleted, atmospheric CO_2 can be used and thus creates dense surface scums (Paerl et al., 2011).

2.2.3. Recovery from Acidification

Lake recovery from acidification is an ongoing phenomenon observed in the Northern Hemisphere, caused by the control of air emissions. Many researchers have provided evidence of the chemical recovery of lakes through increasing pH, acid neutralization capacity (ANC), and/or alkalinity. By measuring dissolved organic carbon (DOC) concentrations, chemical recovery has also been linked with increasing natural organic matter (NOM) in surface waters. Regions in the United States, Eastern Canada, and Northern Europe that previously experienced acid rain (sulfate deposition) have shown recovery from acidification through increasing DOC concentrations (Anderson et al., 2017; Garmo et al., 2014; Monteith et al., 2007; Redden, 2020; Skjelkvåle et al., 2001). Skjelkvåle et al., 2001 analyzed trends in water chemistry for 95 lakes in North America and Europe between 1989 and 1998 and found that declining sulfate deposition was the driving force for recovery, resulting in increasing DOC concentrations. Furthermore, time series data collected from 552 surface waters in North America and Northern Europe between 1990 and 2004 showed increasing DOC concentrations proportional to decreasing sulfate deposition (Monteith et al., 2007). Additionally, as sulfate deposition decreased in Atlantic Canada between 1990 and 2013, more surface waters experienced an increase in DOC concentrations than a decrease (Anderson et al., 2017).

Biological responses to chemical recovery from acidification have been less documented and full recovery is often unknown due to the lack of pre-acidification data (Anderson et al., 2017; Findlay et al., 1999; Monteith et al., 2005). However, changes in species diversity and richness in surface waters can indicate biological recovery (Arseneau et al., 2011; Findlay et al., 1999; Findlay & Kasian, 1996; Schindler et al., 1990; Vinebrooke & Graham, 1997). Long-term changes in water chemistry such as pH and DOC can lead to biological recovery. Many surface waters recovering from acidification have shown a positive correlation between increasing pH and increasing phytoplankton diversity (Arseneau et al., 2011; Findlay, 2003; Findlay et al., 1999; Findlay & Kasian, 1996; Nicholls et al., 1992). Through the experimental acidification of lakes, shifts in phytoplankton communities from cyanobacteria, diatoms and chrysophytes to dinoflagellates have been observed (Findlay et al., 1999; Findlay & Kasian, 1996; Schindler et al., 1990). Furthermore, as surface waters returned to their pre-acidification pH, overall species diversity increased (Findlay et al., 1999; Findlay & Kasian, 1996; Schindler et al., 1990) as well as cyanobacteria (Findlay et al., 1999). Additionally, rising DOC concentrations in some lakes have increased species abundance (Vinebrooke & Graham, 1997) and caused taxonomic shifts from dinoflagellates to cyanobacteria, chlorophytes and diatoms (Graham et al., 2007). Many surface waters in Atlantic Canada are undergoing chemical recovery from acidification (Anderson et al., 2017; Lacoul et al., 2011; Redden, 2020), therefor, biological recovery is expected and the occurrence of HABs may become more prevalent.

2.3. IMPLICATIONS FOR DRINKING WATER

2.3.1. Concerns and Challenges

Cyanobacteria have been detected in many Canadian drinking water sources, highlighting the risk of human exposure (O'Keeffe, 2019). Thus, it is imperative that WTPs adopt robust monitoring and treatment plans to ensure they are mitigating risk and above all, providing safe drinking water to the public. WTPs are faced with many treatment challenges when managing cyanobacteria and their toxins. Cyanobacteria are capable of producing taste and odor compounds that are resistant to conventional water treatment processes (Srinivasan & Sorial, 2011; Watson et al., 2008). Although some are non-toxic, inadequate removal can result in decreased consumer trust (Srinivasan & Sorial, 2011). Furthermore, the removal of cyanotoxins can be difficult as it often requires multiple disinfectants or treatment processes (He et al., 2016). Cyanobacteria are also capable of surviving in sludge from conventional treatment systems, causing an increase in cyanotoxin concentrations in the recycled supernatant (Pestana et al., 2016). Additionally, cyanobacteria can accumulate in and breakthrough WTPs (Zamyadi et al., 2012) even when the influx of cells is low (Almuhtaram et al., 2018). Breakthrough of cyanobacteria could increase disinfection by-product (DBP) formation (Westrick et al., 2010), highlighting the importance of adequate removal.

2.3.2. Monitoring Strategies and Required Adaptations

Monitoring programs are essential for assessing cyanotoxin risk in source waters, but surveillance strategies lack consistency between Canadian provinces and territories (O'Keeffe, 2019; Rashidi et al., 2021). Health Canada suggests that WTPs should visually monitor source waters for signs of bloom formation or increased cyanobacterial cell density before taking additional monitoring steps (Health Canada, 2018). If a bloom is suspected,

samples should be collected at the raw water intake for microbial (cell counts) and chemical (HPLC, LC-MS) analyses during and after the bloom (Health Canada, 2018). However, potentially toxic cyanobacteria have been detected in surface waters without any visual signs of blooms (Brown et al., 2021), which could rapidly proliferate into a toxic bloom if given the right environmental conditions. Additionally, samples representative of the entire water body are necessary to understand bloom development and exposure risks (Welker et al., 2021). Selecting the appropriate sampling approach has its own suite of challenges, due to the heterogeneous distribution of cyanobacteria in water bodies (Welker et al., 2021).

Researchers have recommended using a tiered or integrated approach for cyanobacteria monitoring (Adams et al., 2018; Crawford et al., 2017; Kibuye et al., 2021; Macário et al., 2017). Step-wise approaches can improve early detection while being more cost-effective than just using physiochemical analyses (Crawford et al., 2017; Macário et al., 2017). These strategies would include the following steps: microscopic identification and enumeration, toxin potential screening and toxin quantification (Adams et al., 2018; Crawford et al., 2017; Kibuye et al., 2021; Macário et al., 2017). Although many large WTPs in Canadian provinces are equipped with multi-barrier approaches to remove cyanotoxins, inaccessibility to rapid and cost-effective monitoring tools limit their ability to respond effectively (O'Keeffe, 2019). The development and implementation of early warning systems for cyanobacterial blooms are the key to successfully managing them (Chowdhury, 2021).

2.4. PASSIVE SAMPLING

Selecting the appropriate sampling strategy that ensures cyanobacteria and cyanotoxins are monitored effectively is imperative for WTPs. Traditionally, grab and composite sampling techniques are used, each offering their own limitations. Grab sampling is simple and convenient, but only offers a snapshot of the contaminants present at the time of sampling, which could lead to the underestimation of risk for a WTP. Increasing grab sampling frequency and locations provides a more accurate estimation of cyanobacteria abundance (Pobel et al., 2011), however, is less practical for WTPs. Furthermore, grab sampling often requires the collection of large volumes of water, especially if target contaminants are present in low concentrations (Vrana et al., 2005). Composite sampling is more representative, as it combines multiple sub-samples collected at various locations and/or times, but can be expensive, time-consuming, and less feasible for WTPs. Passive sampling techniques have been explored by many researchers to overcome these limitations. Passive sampling offers the convenience and simplicity of grab sampling, while maintaining the representativeness of composite sampling. This technique involves deploying a sampler for a predetermined amount of time, where the extracted concentration represents the time weighted average of the contaminant (Namieśnik et al., 2005). The main advantages of passive samplers are cost-effectiveness, simplicity, ability to detect low contaminant concentrations, and no requirement of large sample volumes.

Passive sampling techniques have been used to monitor various organic and inorganic compounds (Godlewska et al., 2021; Vrana et al., 2005) as well as viruses and bacteria. Polar organic chemical integrative samplers (POCIS) are one of the most commonly used passive samplers for measuring organic pollutants (Alvarez, 2010; Godlewska et al., 2021) such as pesticides (Alvarez et al., 2004, 2005), pharmaceuticals

(Alvarez et al., 2004, 2005) and cyanotoxins (Brophy et al., 2019; Jaša et al., 2019; Kohoutek et al., 2008, 2010). Recently, passive samplers using a variety of adsorbent materials have been successful in detecting SARS-CoV-2 in wastewater (Habtewold et al., 2022; Hayes et al., 2021; Li et al., 2022; P. Liu et al., 2022; Schang et al., 2021; Vincent-Hubert et al., 2022) and marine water (Vincent-Hubert et al., 2022). Some studies have shown that passive samplers were able to detect SARS-CoV-2 in wastewater communities with a low-prevalence of COVID-19 cases when grab samples were not (Hayes et al., 2021; Schang et al., 2021). Although passive sampling has been applied broadly, there was no found literature surrounding the use of passive samplers for cyanobacteria detection. The ability to detect environmental contaminants early and at low concentrations makes passive sampling appealing for drinking water monitoring. Furthermore, passive sampling is a promising tool for cyanobacteria detection in source waters; however, more research is required to investigate cellular adsorption onto membrane materials.

2.5. qPCR ANALYSIS

Sensitive physiochemical detection methods like high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) can be expensive, time consuming and laborious, especially if WTPs are consistently monitoring source water throughout the year. Thus, molecular detections methods such as quantitative polymerase chain reaction (qPCR) are becoming increasingly popular for cyanobacteria monitoring. qPCR is a fast, simple and cost-effective molecular tool that is widely used to quantify gene-types of interest in microbial samples (Al-Tebrineh et al., 2012; Meriluoto et al., 2016; Pacheco et al., 2016). Primers and probes are used to amplify toxin genes of specific species or multiple species that produce the same toxins (Al-Tebrineh et al., 2012;

McKindles et al., 2019). Both uniplex (targeting of a single toxin gene) (Al-Tebrineh et al., 2010, 2011; Lu et al., 2020; Ngwa et al., 2014; Rinta-Kanto et al., 2005; W. Zhang et al., 2014; Zupančič et al., 2021) and multiplex (targeting of multiple toxin genes) (Al-Tebrineh et al., 2012; Chiu et al., 2017; Crawford et al., 2017; McKindles et al., 2019; Ngwa et al., 2014) methods have been used to detect potentially toxic freshwater cyanobacteria. Multiplex methods however, have an added advantage of detecting multiple toxin producing genes simultaneously, making continuous monitoring more cost effective (Al-Tebrineh et al., 2012) and comprehensive (McKindles et al., 2019).

The detection of cyanotoxin producing genes using qPCR has the potential to serve as an early warning tool for cyanobacteria HABs (Al-Tebrineh et al., 2011; Fortin et al., 2010; Lu et al., 2020; Zupančič et al., 2021). The detection of microcystin-producing genes, cylindrospermopsin-producing genes, and saxitoxin-producing genes have been positively correlated to microcystin (Chiu et al., 2017; Fortin et al., 2010; Ngwa et al., 2014; Zupančič et al., 2021), cylindrospermopsin (Chiu et al., 2017), and saxitoxin (Al-Tebrineh et al., 2010) concentrations, respectively. Additionally, researchers have proven that using qPCR methods in the early monitoring season is beneficial, as microcystin-producing genes have been detected before microcystin detection by chemical analyses (Fortin et al., 2010; Lu et al., 2020). This could improve real-time risk assessment of potential toxin exposure and enable early implementation of mitigation strategies (Al-Tebrineh et al., 2010, 2011). Furthermore, qPCR methods have detected and quantified target toxin genes when conventional enumeration (Zhang et al., 2019) and microscopy (Ngwa et al., 2014; Zupančič et al., 2021) methods could not, suggesting they have higher sensitivities. There are many advantages to using qPCR for cyanobacteria monitoring; however, these methods

are not intended to replace analytical methods such as HPLC or LC-MS as they do not measure the actual toxicity of algal blooms. As such, it is recommended they are used in conjunction with these methods in a tiered monitoring approach (Adams et al., 2018; Crawford et al., 2017; Macário et al., 2017; Meriluoto et al., 2016).

CHAPTER 3: MATERIALS AND METHODS

Materials and methods included in this section explain procedures used in both sub-objectives of this work. Specific methods for experimental design and sampling are included in Chapter 4 and 5.

3.1. WATER QUALITY PROCEDURES

All water quality procedures conducted in this study were consistent with *Standard Methods for the Examination of Water and Wastewater* (APHA, AWWA, & WEF, 2012). For procedures unavailable in this text, the respective equipment manufacturer's protocols were followed. Water quality parameters measured throughout this research included turbidity, pH, total organic carbon (TOC), dissolved organic carbon (DOC), and ultraviolet (UV) absorbance at a wavelength of 254 nm. For all procedures, glassware and apparatuses were washed and rinsed three times with ultrapure water from a milli-Q® water purification system (Millipore Sigma).

3.1.1. General Water Quality Parameters

The turbidity of each sample was measured using a Hach TL2350 laboratory turbidimeter (Hach). Prior to use, the turbidimeter was zeroed with milli-Q® water. pH was measured in the laboratory using an Accumet Excel XL50 benchtop meter (Fisher Scientific). Before each use, the pH probe was calibrated using stock solutions of pH 4, 7 and 10. All samples were measured within 24 hours of collection.

3.1.2. Natural Organic Matter

TOC and DOC samples were collected in clean 40 mL glass vials without headspace and sealed with tinfoil and caps. DOC samples were filtered through 47mm,

0.45 µm polyethersulfone (PES) filter membranes (GVS North America Inc.) pre-rinsed with 500 mL of milli-Q® water to prevent any leaching of organic materials from the filter into the sample. To preserve TOC and DOC samples prior to analysis, vials were adjusted to pH < 2 using phosphoric acid (85%). TOC and DOC measurements were collected using a TOC-V CPH analyzer with a Shimadzu ASI-V autosampler and a catalytically aided combustion oxidation non-dispersive infrared detector (NDIR) with a 0.08 mg/L detection limit (Shimadzu Corporation).

UV absorbance at 254-nm wavelength (UV_{254}) was measured using a Hach DR 5000™ UV-Vis Spectrophotometer (Hach) and zeroed prior to use using milli-Q® water. Samples prepared for UV_{254} analysis were filtered through a 47mm, 0.45 µm PES filter membrane (GVS North America Inc.) pre-rinsed with 500 mL of milli-Q® water, using a vacuum pump apparatus. To calculate specific UV absorbance (SUVA), DOC and UV_{254} values were used in Equation 1.

$$SUVA = \frac{UV_{254}\ (cm^{-1})}{DOC\ (^{mg}/_{L})} \cdot 100 \qquad \text{Eq. 1}$$

3.2. NUCLEIC ACID EXTRACTIONS

The extraction of deoxyribonucleic acid (DNA) from freshwater grab samples and passive sampler filters was completed using the Phytoxigene™ extraction protocol. For aqueous samples, up to 100 mL of each sample was filtered through 25mm, 0.8 µm Versapor® acrylic copolymer membrane filters (Pall Corporation) using a sterile 50 mL syringe apparatus. Filters were placed into BioGX Bead Lysis Tubes (BioGX, Inc.) containing 500 µL of lysis buffer and 300 mg of 0.1 µm glass beads and vortexed for 15 min. Passive sampler filters were placed directly into bead lysis tubes and vortexed for 15

min. After vortexing, samples were spun down and the supernatant was transferred into sterile microcentrifuge tubes. Samples were stored at -80 ºC if they could not be processed immediately using qPCR.

3.3. qPCR ANALYSIS

3.3.1. qPCR Procedure

All DNA samples extracted for qPCR were processed using a Bio-Rad CFX Opus 96-well instrument (Bio-Rad Laboratories, Inc.). Phytoxigene™ CyanoDTec Total Cyanobacteria and Toxin Gene kits (Phytoxigene™, Inc.) were used to quantify the cyanobacteria 16S rRNA gene and toxin gene presence in each sample, respectively. In the multiplex toxin gene kit, the targeted genes were microcystin/nodularin (*mcyE/ndaF*), cylindrospermopsin (*cyrA*) and saxitoxin (*sxtA*). For the analysis of total cyanobacteria and toxin genes, 25 µL reactions were prepared using the respective kits. Each reaction contained 20 µL of Master Mix (enzymes, probe, primers and dNTP) and 5 µL of template DNA. The primers and probes sequences used for total cyanobacteria (Al-Tebrineh et al., 2010) and toxin gene (Al-Tebrineh et al., 2012) assays are shown in Table 2. Samples were prepared in clear, 96-well plates for all qPCR runs. Thermal cycling conditions included: initial denaturation at 95 ºC for 2 min followed by a denaturation step at 95 ºC for 15 sec. Denaturation was followed by 40 cycles of annealing and extension at 60 ºC for 45 sec. Positive detections were indicated when the sample cycle threshold values were less than 4 cycles above the cycle threshold of the lowest standard, corresponding to a detection limit of 45 gene copies.

Table 2. Primers and TaqMan probes used in the Phytoxigene™ CyanoDTec assays

Gene Target	Sequence Type	Sequence (5' – 3')
16S rRNA	16S Forward primer	AGCCACACTGGGACTGAGACA
	16S Reverse prime	TCGCCCATTGCGGAAA
	16S Probe	FAM-CCTACGGGAGGCAGCAGTGGG-BHQ1
mcyE/ndaF	mcy Forward primer + flap	AATAAATCATAATTTAGAACSGGVGATTTAGG
	mcy Reverse primer + flap	AATAAATCATAACGRBTVADTTGRTATTCAATTTCT
	mcy Probe	CY5-AATCAAGTTAAGGTVAATGGYTATCG-BHQ1
cyrA	cyr Forward primer	GTCTGCCCACGTGATGTTATGAT
	cyr Reverse primer	CGTGACCGCCGTGACA
	cyr Probe	CY3-CCTTTGGGAACGAAATTCTCGAAGCAACT-BHQ2
sxtA	sxt Forward primer	GGAGTGGATTTCAACACCAGAA
	sxt Reverse primer	GTTTCCCAGACTCGTTTCAGG
	sxt Probe	Texas Red-TGCCGATTTAGAAGAAAGTATCCTCTCAG-BHQ2

3.3.2. Quantitative Analysis of Target Genes

Quantitative analysis of target genes was performed to assess the relative performance of passive sampling materials in comparison to traditional grab sampling methods. For aqueous samples, DNA concentrations in gene units per millilitre (GU/mL) were calculated using Equation 2. DNA concentrations in gene units per centimeter squared (GU/cm^2) for each adsorbent material were calculated using Equation 3. Example calculations for each sample type are shown in Appendix A.

$$DNA\ Concentration\ \left(\frac{GU}{mL}\right) = \frac{\text{qPCR Concentration}\left(\frac{GU}{\mu L}\right) \times Eluate\ Volume\ (500\ \mu L)}{Filtered\ Volume\ (mL)} \qquad \text{Eq. 2}$$

$$DNA\ Concentration\ \left(\frac{GU}{cm^2}\right) = \frac{\text{qPCR Concentration}\left(\frac{GU}{\mu L}\right) \times Eluate\ Volume\ (500\ \mu L)}{Membrane\ Surface\ Area\ (cm^2)} \qquad \text{Eq. 3}$$

3.4. QUALITY CONTROL

All DNA extractions were performed in a Thermo Scientific 1300 Series Class II, Type A2 Biological Safety Cabinet (BSC) and all qPCR assays were prepared in a Mystaire® MY-PCR prep station (Mystaire Inc.) to minimize contamination. To eliminate pre-contamination, all materials were sterilised using an autoclave.

For every qPCR run, a positive control and non-template control (NTC) (nuclease free water) were also run. Because the Total Cyanobacteria kits contained the Internal Amplification Control (IAC), those assays were run before, or in parallel with, Toxin Genes assays to ensure any possible inhibition with the assay was identified. For any sample where the IAC returned a cycle threshold (CT) value 1.5 cycles higher than the CT value of the NTC, the sample was diluted 1:2 or 1:10 using nuclease free water to remove inhibition (Phytoxigene, 2019).

A master standard curve was created for each assay using Phytoxigene^{TM} CyanoNAS Nucleic Acid Standards (Phytoxigene^{TM} Inc.), which are shown in Appendix B. The CyanoNAS kit included five standards 100, 1000, 10,000, 100,000 and 1,000,000 copies per microlitre for each gene target. Each point on the curves were run in duplicate. The R^2 and efficiency values for each target are shown in Table 3.

Table 3. R^2 and efficiency values for the standard curves of each target gene, where each point on the curves were run in duplicate.

Target Gene	R^2 value	Efficiency
16S rRNA	0.9993	96 %
mcyE/ndaF	0.9998	98 %
cyrA	0.9998	100 %
sxtA	0.9998	98 %

The limit of detection (LOD) for the Phytoxigene™ CyanoDTec assays is 45 gene units (GU) per reaction, with a 95% degree of confidence (Phytoxigene, 2019). For the purpose of this work, the same limit of detection (LOD) was used for passive and grab samples, however, values near the LOD in passive samples may be over or underestimated. The LOD value is not directly applicable to the passive sampling methodology as the recovery efficiency from the material is unknown. Determining a representative LOD for the sampler materials would require additional studies that investigate their adsorption kinetics, which was beyond the scope of this work.

3.5. STATISTICAL ANALYSIS

The figures for this research were generated using R (RStudio Version 1.3.1093) Software. All statistical analyses were also performed using R (RStudio Version 1.3.1093) software. Specific tests conducted for each experiment are outlined in their respective chapters. The level of significance for all tests was $p < 0.05$.

CHAPTER 4: ASSESSING THE FEASIBILITY OF PASSIVE SAMPLERS FOR CYANOBACTERIA DETECTION USING qPCR

4.1. INTRODUCTION

HAB frequency and intensity has been increasing in Canadian surface waters (Pick, 2016; Schindler et al., 2012; Winter et al., 2011), while some even persist past the expected spring/summer bloom season (Winter et al., 2011). Many Canadian surface waters experiencing HABs are used for drinking water (O'Keeffe, 2019), and it is anticipated that climate change (Mullin et al., 2020; Paerl & Huisman, 2008, 2009; Paerl & Paul, 2012) and lake recovery will exacerbate this issue. Changes in rainfall patterns, temperature, nutrient loading and pH are expected to increase phytoplankton presence (Mullin et al., 2020; Paerl & Huisman, 2008, 2009; Paerl & Paul, 2012) and diversity (Arseneau et al., 2011; Findlay, 2003; Findlay et al., 1999; Findlay & Kasian, 1996; Nicholls et al., 1992). Evidence of lake recovery from acidification has been reported in Atlantic Canada (Anderson et al., 2017; Lacoul et al., 2011; Redden, 2020), and more specifically, in Nova Scotian source waters (Anderson et al., 2017; Redden, 2020). The synergistic effects of climate change and lake recovery will likely lead to more HABs, causing more concerns for WTPs and highlighting the importance of adequate monitoring and mitigation strategies.

While Health Canada has recommended guidelines for WTPs to monitor cyanobacteria, surveillance strategies are inconsistent between provinces and territories (O'Keeffe, 2019; Rashidi et al., 2021). Health Canada suggests using visual monitoring prior to taking additional monitoring steps (Health Canada, 2018); however, potentially toxic cyanobacteria can be present in surface water without any visual signs of blooms (Brown et al., 2021). Thus, it is important for WTPs to implement early warning systems

to successfully manage HABs (Chowdhury, 2021). To understand HAB development and exposure risks, it is necessary to collect samples representative of the entire water body (Welker et al., 2021). The heterogenous distribution of cyanobacteria (Welker et al., 2021) paired with their ability to move throughout the water column using gas vesicles (Ganf & Oliver, 1982; Rippka et al., 1979; Walsby et al., 1997) makes representative sampling challenging.

Traditional sampling methods such as grab and composite sampling have their limitations. Although grab sampling is convenient and simple, it may underestimate cyanobacteria risk as it only offers a snapshot of contamination in the water body. Composite sampling combines multiple sub-samples from different locations and times, making it more representative; however, this technique can be time-consuming, expensive, and less-feasible for WTPs. Passive sampling has been adopted by many researchers as an alternative approach as it creates a composite sample while maintaining the simplicity and convenience of a grab sample. In this method, a sampler containing adsorbent material is deployed for a pre-determined amount of time and the extracted concentrations represents the time-weighted average of the contaminant (Namieśnik et al., 2005). Passive sampling methods have been used for cyanotoxin monitoring in surface waters but lack the ability to measure multiple toxins at once (Brophy et al., 2019; Jaša et al., 2019; Kohoutek et al., 2008, 2010). Recently, passive sampling techniques have detected SARS-CoV-2 in wastewater (Habtewold et al., 2022; Hayes et al., 2021; Li et al., 2022; P. Liu et al., 2022; Schang et al., 2021; Vincent-Hubert et al., 2022), even when concentrations were too low to be detected in grab samples (Hayes et al., 2021; Schang et al., 2021), and in marine water (Vincent-Hubert et al., 2022). The ability to detect contaminants at low concentrations

could be beneficial for early cyanobacteria monitoring; however, there was no found literature exploring the use of passive samplers for detecting algal cells. Thus, the applicability of passive samplers for cyanobacteria detection should be investigated.

The continuous monitoring of cyanobacteria can be expensive when relying solely on physiochemical analyses (Crawford et al., 2017; Macário et al., 2017) which is why many researchers have recommended using a tiered or integrated monitoring approach (Adams et al., 2018; Crawford et al., 2017; Kibuye et al., 2021; Macário et al., 2017). The suggested steps in these strategies include: microscopic identification and enumeration, toxin potential screening and toxin quantification Adams et al., 2018; Crawford et al., 2017; Kibuye et al., 2021; Macário et al., 2017). Steps one and three have been researched extensively while screening for toxin potential has been an emerging monitoring tool. qPCR is a fast, simple and effective molecular method for quantifying cyanotoxin risk, as it can target and quantify many cyanotoxin genes at once in environmental samples (Al-Tebrineh et al., 2012; Chiu et al., 2017; Crawford et al., 2017; McKindles et al., 2019; Ngwa et al., 2014). Studies have detected microcystin-producing genes using qPCR before detecting microcystin using chemical analyses, demonstrating the benefit of its use in the early monitoring season (Fortin et al., 2010; Lu et al., 2020). Furthermore, qPCR methods have quantified cyanotoxin genes when conventional enumeration (S. Zhang et al., 2019) and microscopy (Ngwa et al., 2014; Zupančič et al., 2021) methods did not, suggesting they have higher sensitivities.

This work aimed to investigate the use of passive samplers and qPCR analysis as a detection method for freshwater cyanobacteria for drinking water suppliers. To complete this research, a proof-of-concept field-scale monitoring program comparing a passive

sampling technique to traditional grab sampling was conducted. This study was carried out at Lake Fletcher, in Wellington, Nova Scotia (NS) due to its history of cyanobacteria presence (Betts, 2018; Brophy et al., 2019) and previous monitoring programs (Brophy, 2019; Poltarowicz, 2017).

4.2. MATERIAL AND METHODS

4.2.1. Reagents and Materials

Cellulose nitrate filter membranes (47 mm, 0.1 μm) used in the passive samplers were purchased from Millipore Sigma. Commercially available kits for DNA extractions, 16S rRNA assays and toxin gene assays were obtained from Phytoxigene™, Inc. Nuclease free water was obtained from LuminUltra Technologies (LuminUltra Technologies Ltd) and Millipore Sigma to rehydrate reagents.

4.2.2. Comparing the Performance of Passive Samplers to Grab Samples at Three Watershed Locations

To assess the feasibility of passive sampling for the detection of cyanotoxin producing genes in freshwater, a monitoring program was conducted from July 13 to November 30, 2021. During this period, a novel passive sampling method was compared to a traditional grab sampling technique.

4.2.2.1. Experimental Design

A total of 50 grab sampling events, 52 weekly passive sampling events and 15 bi-weekly passive sampling events took place over the monitoring program. The number of sampling events that occurred at each location are shown in Table 4. This study investigated how the detection frequency of target genes was impacted by sample type and exposure time. To evaluate detection frequency by sample type, weekly passive and grab

samples were simultaneously collected at each location and paired for analysis. To assess how exposure time affected detection frequency in passive samples, weekly and bi-weekly samplers were collected on the same days and paired for analysis.

Table 4. Total number of sampling events that occurred at each location for all sample types from July 13 to November 30, 2021.

Location	Grab Samples	Weekly Passive Samples	Bi-weekly Passive Samples
Inlet	20	19	9
Outlet	20	17	6
Intake	10	16	NA
Total	50	52	15

4.2.2.2. Study Site

Lake Fletcher in Wellington, NS was selected as the study site for this research due to its history of microcystin-LR detections (Brophy et al., 2019). It is a part of a larger urban watershed where it receives water from Thomas Lake and flows into Grand Lake. From inlet to outlet, Lake Fletcher is 4.2 km long with an area of 1.01 km^2 (Poltarowicz, 2017). The average depth of the lake is 3.72 m and its residence time is 10.41 days (flushing rate of 35.06 times/year) (Hart, 1978). There are two basins within Lake Fletcher where its two deepest points are located. The inlet basin is 6 m deep and the outlet basin is 11 m deep (Poltarowicz, 2017). The lake has historically had phosphorus concentrations between 3.7 and 9.3 µg/L (Hart, 1978; Mudroch et al., 1987; Poltarowicz, 2017), and has been previously classified as oligotrophic (Poltarowicz, 2017). Lake Fletcher typically stratifies between June-August and turns over in the early spring and again in the early fall each year (Poltarowicz, 2017). There is also a WTP and a wastewater treatment plant (WWTP) located within Lake Fletcher.

Three sampling sites within Lake Fletcher were selected for the monitoring program. The sampling locations included the inlet and outlet streams to the lake, and the raw water intake to the Collin's Park WTP. These locations are displayed in Figure 1 and were selected based on their accessibility and previous monitoring programs (Brophy, 2019; Poltarowicz, 2017). Both streams have wide channels and fast flows, and the WTP intake is submerged in the water column. Passive and grab samples were collected at each location from July 13 to November 30, 2021.

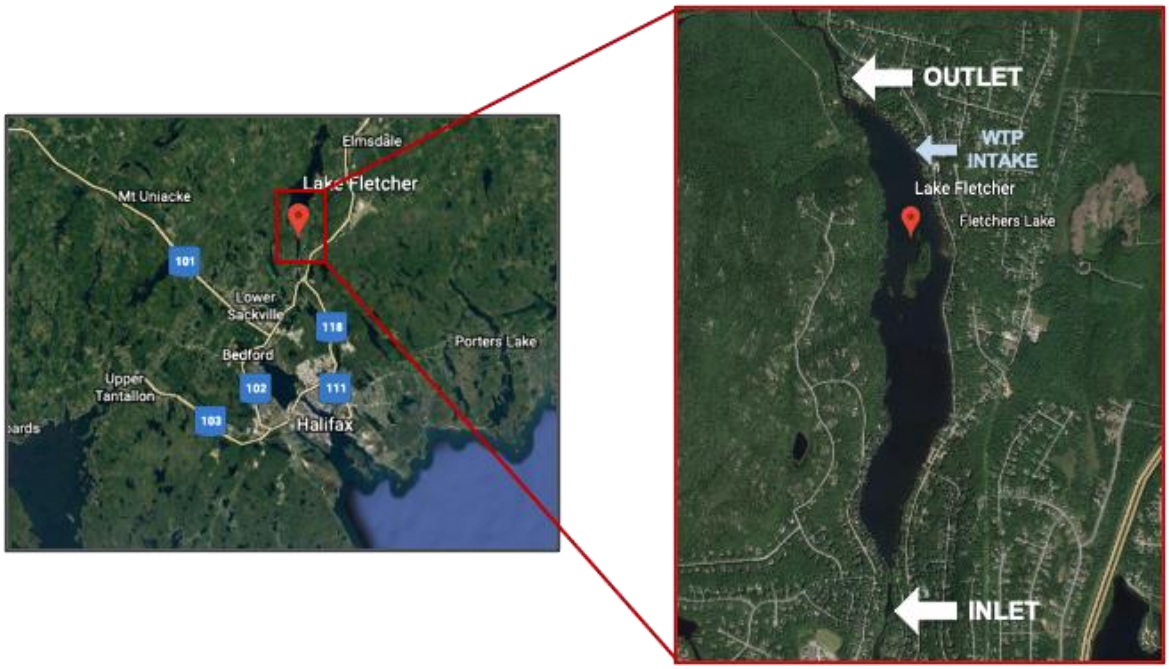

Figure 1. Sampling sites for the monitoring program at Lake Fletcher in Fletcher's Lake, NS.

4.2.2.3. Grab Sampling

For weekly inlet/outlet monitoring, freshwater samples were collected in 1 L volumes at each sample site. A sampling stick with an attached bottle was used at the inlet and outlet sites to collect water further away from the shoreline and close to where the passive samplers were deployed. Collected samples were poured into sterile Nalgene bottles and transported to the laboratory in a cooler on ice. Upon arrival to the laboratory,

DNA was extracted within 24 hours and stored at -80 ºC until qPCR analysis. The remainder of each sample was stored at 4 ºC for up to 24 hours prior to any water quality characterization. Grab samples from the raw water intake were collected and analyzed by Halifax Water staff. Throughout the monitoring program, a total of 50 grab sampling events occurred.

4.2.2.4. Passive Sampling

The passive sampling devices used in this study were adapted COVID-19 sewer cages (COSCas) (Hayes et al., 2021), shown in Figure 2. Two passive samplers were deployed at the inlet and outlet streams of Lake Fletcher and one was deployed at the WTP raw water intake in July, 2021. All passive samplers contained inserts which held an electronegative cellulose nitrate filter (47mm, 0.1 μm) in place during deployment periods. Cellulose nitrate filter membranes were chosen as the adsorbent material due to their success at capturing SARS-CoV-2 in wastewater (Hayes et al., 2021; Schang et al., 2021). At stream locations, the samplers were tied to nylon rope and attached to trees on the shoreline to ensure they could be easily deployed and removed. A total of 52 and 15 weekly and bi-weekly passive samples, respectively, were collected throughout the sampling period. During each sampling event, passive samplers were retrieved from the streams, and the filter inserts were removed and sealed in separate plastic bags. New filter inserts were replaced in each passive sampler prior to redeployment. As the passive samplers were deployed in publicly accessible locations, there were a few sampling events where, upon arrival to the site, they were found removed from the streams. Those samples were omitted from analysis. For the raw water intake, only a weekly deployment period was investigated. These samplers were deployed, collected and redeployed by the Halifax Water operators

at the WTP for a total of 16 times. Collected samples were transported back to the laboratory in a cooler on ice. Upon arrival to the laboratory, DNA was extracted from the filters and stored at -80 ºC until qPCR analysis.

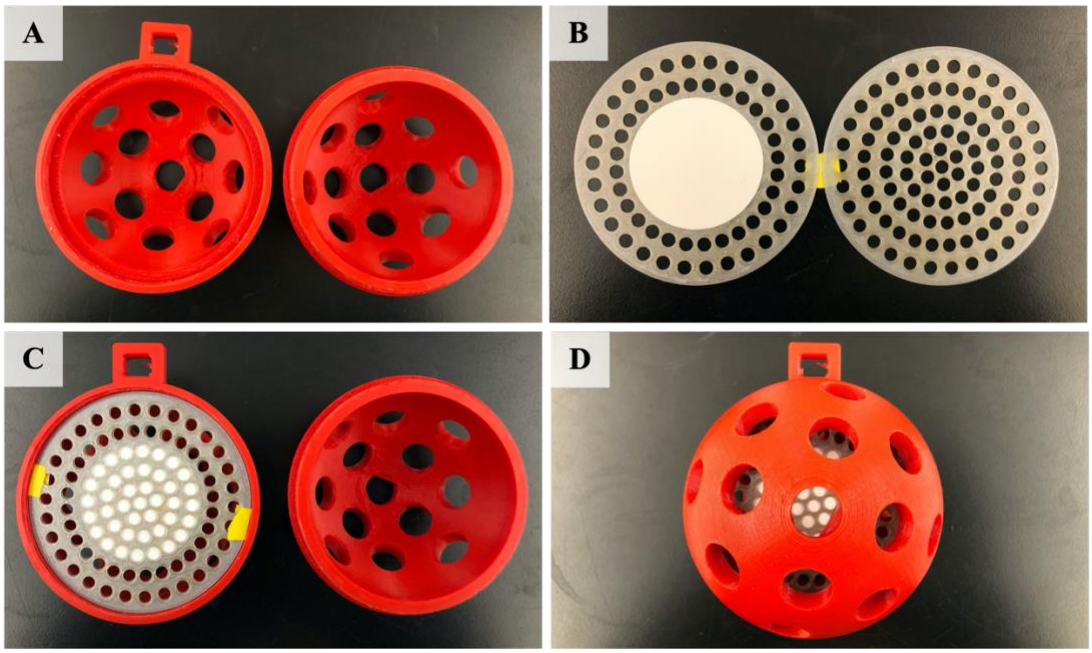

Figure 2. Passive sampling device used for the monitoring program. Photos show A) an empty and opened sampler B) filter inserts with a clean cellulose nitrate filter membrane (47mm, 0.1 µm) C) inserts loaded in a passive sampler D) passive sampler ready for deployment.

4.2.3. Environment Data

Daily environment data for temperature and precipitation was collected from the Shearwater RCS weather station (Station 8205092) in Halifax, NS. Data was acquired for the beginning of July to early December and downloaded through the Environmental Canada website (E. and C. C. Canada, 2011).

4.2.4. Nucleic Acid Extraction

For inlet and outlet grab samples, 100 mL of each sample was filtered through 25 mm, 0.8 µm Versapor® acrylic copolymer membrane filters (Pall Corporation) using a

sterile 50 mL syringe apparatus. For the intake grab samples, 25 mL of each sample was filtered through the same membrane filter type. All grab and passive sampling filters were extracted using the Phytoxigene™ extraction protocol outlined in Section 3.2.1. Samples were stored at -80 ºC if they could not be processed immediately using qPCR.

4.2.5. qPCR Analysis

Phytoxigene™ CyanoDTec Total Cyanobacteria and Toxin Gene kits (Phytoxigene™ Inc.) were used to quantify 16S rRNA and toxin gene presence in all grab and passive samples. The assays run in this study had the same targets and parameters as outlined in Section 3.3.1.

4.2.6. Quantitative Analysis of Target Genes

To assess the relative performance of passive sampling to grab sampling, a quantitative analysis of each target gene was conducted. For grab samples, DNA concentrations were calculated in gene units per millilitre (GU/mL) using Equation 2. DNA concentrations for passive samples were calculated in gene units per centimeter (GU/cm^2) squared using Equation 3. Both equations are shown in Section 3.3.2.

4.2.7. Statistical Analysis

The frequency of positive detections for each target gene (%) was calculated for paired weekly passive and grab samples at each location. The number of positive samples for each target was divided by the total number of samples collected at each location. Furthermore, a two-sided proportion test was conducted to determine if proportions of positive detections were the same in both groups overall. Statistical analyses included gene concentrations above the LOD only, with a significance level of $p < 0.05$.

4.3. RESULTS AND DISCUSSION

4.3.1. Source Water Characterization

To better understand the water quality of Lake Fletcher, basic water quality parameters such as pH, turbidity, TOC/DOC and UV_{254} were measured on a couple Lake Fletcher water samples. A summary of the results paired is shown in Table 5 and paired with water quality data collected in 2016 and 2017 (Brophy, 2019). Samples for the intake were collected in spring of 2021, and samples from the inlet and outlet streams were collected in autumn of 2021. Turbidity, pH and UV_{254} values were consistent between locations. Organics were highest at the intake, followed by the outlet stream and then the inlet stream. Mean SUVA values for the inlet and outlet streams were 3.74 and 4.37, respectively in 2021. There was insufficicent data to calculate SUVA for the intake, but in 2017, SUVA values were between 3 and 4. For all locations, it is suggested that DOC is largely composed of aquatic humics and is relatively hydrophobic in nature (Edzwald, 1993). However, Lake Fletcher has likely been experiencing temporal changes in NOM like many surface waters in Nova Scotia (Anderson et al., 2017). In 2016 and 2017, both the inlet and outlet streams had mean SUVA values below 4. The mean SUVA has increased at the outlet stream since 2017, and has remained constant at the inlet stream. Thus, changes in NOM concentration and composition should be considered in passive sampler design and optimization.

Table 5. Water characterization for each Lake Fletcher sampling location (2016 n=17; 2017 n=12; 2021 n=2)

Location	pH			Turbidity (NTU)		
	2016	2017	2021	2016	2017	2021
Inlet	6.3±0.5	7.1±0.5	6.6±0.0	1.2±0.7	0.9±0.3	1.0±0.0
Outlet	6.5±0.5	7.3±0.4	6.7±0.1	1.1±0.5	0.9±0.3	1.0±0.4
Intake	6.5±0.5	7.3±0.4	7.2±0.1	1.4±0.4	1.6±0.3	1.3±0.1

Location	TOC (mg/L)			DOC (mg/L)			UV_{254} (cm^{-1})		
	2016	2017	2021	2016	2017	2021	2016	2017	2021
Inlet	3.9±0.6	3.7±0.3	3.1±0.0	4.0±0.6	3.7±0.3	3.5±0.1	-	-	0.1±0.7
Outlet	3.9±0.5	3.9±0.6	4.0±0.0	3.9±0.6	3.8±0.3	4.1±0.6	-	-	0.2±0.9
Intake	3.9±0.5	3.8±0.3	4.6±0.4	3.9±0.5	3.7±0.2	-	-	-	-

All values are reported as mean ± standard deviation

4.3.2. Target Gene Detections in Grab and Passive Samples Over Monitoring Period

4.3.2.1. 16S rRNA

Of the total (passive and grab) 117 sampling events, 116 samples were analyzed for 16S rRNA. All of the samples were positive and above the LOD (Figure 3), indicating that cyanoabcteria cells were present in all samples. For grab samples, concentrations at each location remained constant over the entire monitoring period. The inlet and outlet streams followed nearly the same pattern in concentration, with mean concentrations (± standard deviation) of $1.6 \times 10^5 \pm 8.5 \times 10^4$ and $1.5 \times 10^5 \pm 9.9 \times 10^4$ GU/mL, respectively. The concentrations were highest at the intake to the WTP, with a mean concentration of $1.7 \times 10^7 \pm 1.2 \times 10^7$ GU/mL. Although 16S rRNA concentrations were higher at the WTP, all locations had concentrations within the detection range of 10^1 and 10^7 GU per reaction for

this assay (Al-Tebrineh et al., 2012). Furthermore, the concentration of 16S rRNA detected at each location was comparable to those detected in a lake with known algal blooms in Lebanon ($5.1 \times 10^3 - 9.8 \times 10^6$ GU/mL) (Hammoud et al., 2021). The inlet and outlet stream locations experience a much higher flow rates than the WTP intake which causes cyanobacteria to disperse throughout the water column (Walsby et al., 1997). This, paired with samples being collected near the water surface may be why lower 16S rRNA concentrations were observed in stream grab samples.

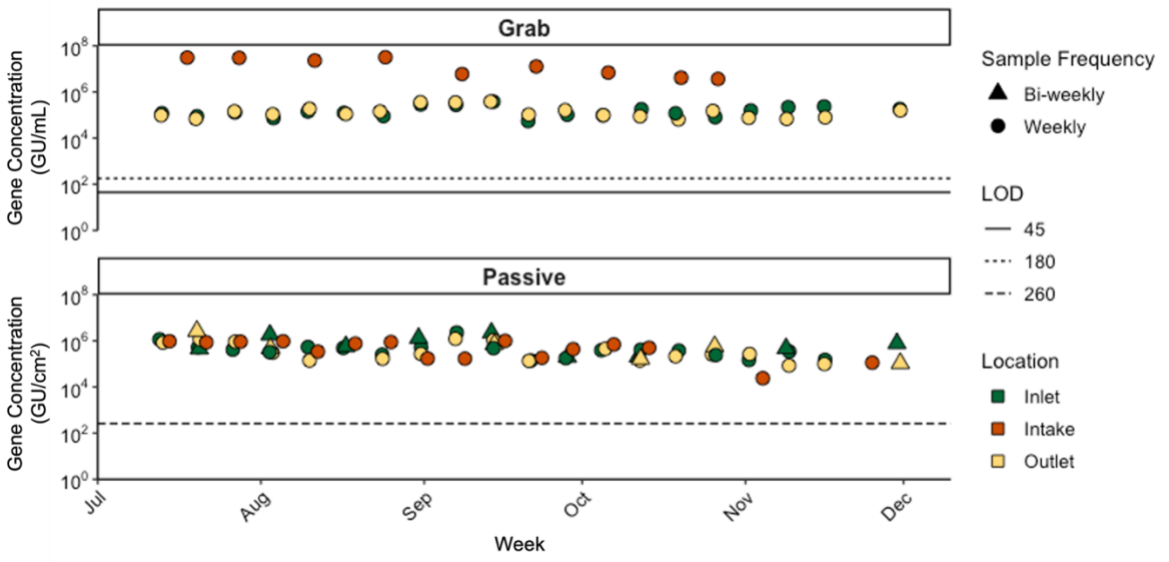

Figure 3. 16S rRNA concentrations detected in grab samples (GU/mL) and passive samples (GU/cm^2) at three watershed locations over 116 sampling events.

Passive samplers followed similar patterns between locations for both sample frequencies. The mean concentrations ($\pm$ standard deviation) of 16S rRNA from passive samples at the inlet stream, outlet stream and intake were $6.2 \times 10^5 \pm 5.9 \times 10^5$, $5.4 \times 10^5 \pm 5.9 \times 10^5$ and $5.6 \times 10^5 \pm 3.6 \times 10^5$ GU/cm^2, respectively. Between sample locations, the maximum concentrations of adsorbed 16S rRNA ranged from 1.0×10^6 to 2.7×10^6 GU/cm^2. This suggests that the filter membranes may reach adsorption capacity around 3

x 10^6 GU/cm^2. In other passive sampling studies, adsorption capacities have been observed for the detection of SARS-CoV-2 in wastewater on electronegative membranes (Habtewold et al., 2022; Hayes et al., 2022) and general *Bacteroidales* markers (AllBac) in seawater on electropositive membranes (Vincent-Hubert et al., 2021). Additionally, passive samplers deployed for 15 day periods detected AllBac at concentrations between 1 x 10^1 and 1 x 10^4 GU/cm^2 (Vincent-Hubert et al., 2021). Based on these data, it is unclear when the filter membranes reach their adsorption capacity. Furthermore, cyanobacteria are likely competing with NOM and sediment for adsorption sites, which would prevent higher maximum adsorption capacities. NOM is known for fouling membranes (Zularisam et al., 2006) and many viral adsorption and elution studies have shown that it reduces the recovery of viruses from electronegative and electropositive filters (Shi et al., 2017). As lake recovery changes NOM composition and concentration in surface waters, its impacts on cyanobacteria adsorption should be considered in passive sampler design. Additionally, passive sampling devices have been shown to adsorb sediment from freshwater (Sbodio et al., 2013) and suspended solids from wastewater (Hayes et al., 2022). Further kinetics studies should be conducted to determine the point at which the samplers reach their adsorption capacity and how adsorption is affected by NOM and sediment.

4.3.2.2. Toxins

Over 117 sampling events, *mcyE/ndaF, cyrA* and *sxtA* were detected above the LOD 74, 20 and 4 times, respectively (Figure 4). Only *mcyE/ndaF* and *cyrA* were detected in grab samples whereas all three target genes were detected in passive samples. Overall, there were more positive detections of *mcyE/ndaF* in grab samples at the inlet (20 >LOD) and outlet (20 >LOD) streams than at the WTP intake (2 >LOD). The inlet and outlet

stream grab samples had similar trends in concentration over time, with maximum concentrations of 5.9 x 10^3 and 3.8 x 10^4 GU/mL, respectively, occurring at the beginning of September. Consistently, the detected levels of *mcyE/ndaF* at the outlet stream were higher than at the inlet stream over the monitoring program. There were only two sampling events where *mcyE/ndaF* was detected at the WTP intake. Both samples had concentrations around 1.4 x 10^5 GU/mL, which were higher than the peak concentrations observed at the streams. Where smaller volumes of water were filtered from the intake (25 mL) than the streams (100 mL), there may not have been enough sample to detect low concentrations of *mcyE/ndaF.* The LOD for the assay is 45 gene copies per reaction, which corresponds to 45 GU/mL and 180 GU/mL for the stream and intake samples, respectively. Increasing the filtered volume for the intake samples may have resulted in more detections, however, it could have also increased the concentration of qPCR inhibitors. Previous studies have shown that humic substances (NOM) cause inhibition in qPCR analysis (Dalecka & Mezule, 2018; Gentry-Shields et al., 2013). Despite the difference in detection frequencies, each location had *mcyE/ndaF* concentrations similar to those observed in other monitoring studies (Hammoud et al., 2021; Kramer et al., 2018) and within the detection range of this assay (Al-Tebrineh et al., 2012). The maximum concentrations of *mcyE/ndaF* at each location fell within the range of concentrations (2.8 $\pm$ 1.8 x 10^3 to 3.8 $\pm$ 1.0 x 10^5 GU/mL) detected during a bloom in Florida surface waters (Kramer et al., 2018). These results indicate that Lake Fletcher could have been experiencing an algal bloom even though there were no visible signs of one, highlighting the importance of continuous monitoring for WTPs. However, toxin gene concentrations may not always correlate to cyanobacteria cell concentrations. In some cases, studies have shown higher target gene concentrations than

cell counts, which may be caused by variations in cellular copy numbers of target genes or the presence of DNA from dead cells (Pacheco et al., 2016). Thus, the high concentrations of *mcyE/ndaF* may or may not be representative of high cell densities.

mcyE/ndaF was detected in passive samples over the monitoring period (Figure 4), however, these data show less of a trend in *mcyE/ndaF* concentration over time. Contrary to grab samples, there were more detections in passive samples at the intake (13 >LOD) than at the inlet (9 >LOD) and outlet (10 >LOD) streams. Moreover, *mcyE/ndaF* concentrations were highest at the WTP intake. The passive samplers deployed at the WTP intake experienced a lower, more controlled flow rate than those in the streams, which may explain why higher concentrations of *mcyE/ndaF* were observed at this location. These data also indicate that *mcyE/ndaF* was detected in passive samplers before grab samples. Bacterial adsorption is influenced by physical (pores, organic matter, water flow, etc.), chemical (species, ionic strength, pH, etc.) and microbiological (bacterial concentration, hydrophobicity, cell surface characteristics) factors (Kristian Stevik et al., 2004). If bacteria hasn't had enough contact time with an adsorbent, or if the material is not the best suited for the target species, adsorption can be reversible (Kristian Stevik et al., 2004). Although there were no found studies investigating the effects of turbulence on cyanobacteria adsorption to filter membranes, increased shear stress has been proven to significantly reduce cyanobacteria biofilm growth (Faria et al., 2020). A similar phenomenon may be happening with the passive samplers in the stream locations causing reversible adsorption of cyanobacteria cells. Nonetheless, the passive and grab sampling data indicate there were microcystin/nodularin producing cyanobacteria present upstream and within Lake Fletcher throughout the monitoring period.

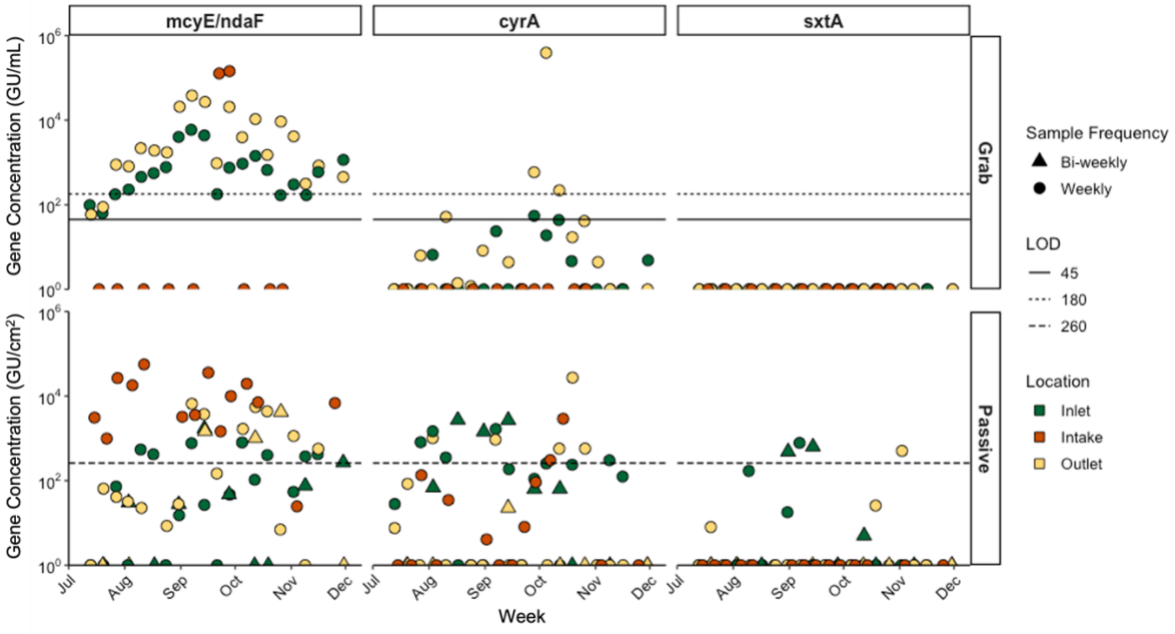

Figure 4. *mcyE/ndaF, cyrA* and *sxtA* concentrations detected in grab samples (GU/mL) and passive samples (GU/cm^2) at three watershed locations over 117 sampling events. Non-detects are shown on the x-axis.

Throughout the sampling period, passive samplers (15 >LOD) had more *cyrA* detections than grab samples (5 >LOD). In passive samples, *cyrA* was detected at all three watershed locations, whereas grab samples detected *cyrA* at the inlet and outlet streams only. There were more *cyrA* detections at the outlet stream (4 >LOD) than the inlet stream (1 >LOD) in grab samples. Furthermore, the concentration of *cyrA* in grab samples was consistently higher at the inlet than at the outlet. Conversely, there were more positive detections of *cyrA* in passive samplers deployed at the inlet stream (8 >LOD) than at the outlet stream (5 >LOD). Trends in adsorbed *cyrA* concentration were less clear between sampling locations, however, *cyrA* was detected at the inlet and outlet streams in passive samples before grab samples. This may be caused by low cell concentrations accumulating on the filter over time, highlighting that grab sampling can lack sensitivity as demonstrated

in similar studies (Hayes et al., 2021; Schang et al., 2021; Vincent-Hubert et al., 2021). Moreover, *sxtA* was not detected in any grab samples over the monitoring period, but was detected in four passive samples. Three of the four positive detections were at the inlet stream from the beginning of August to mid-September. The other *sxtA* detection was at the outlet stream at the beginning of November. These data show that cylindrospermopsin and saxitoxin producing cyanobacteria were present upstream of and within Lake Fletcher. There were no observed *sxtA* detections at the intake to the WTP.

4.3.3. Environmental Inferences for Target Gene Detection

To better understand the detection of cyanobacteria in Lake Fletcher, weekly *mcyE/ndaF* concentrations were paired with historical weather data as shown in Figure 5. There was insufficient data for *cyrA* and *sxtA*, thus, they were excluded from analysis. Average daily precipitation and temperature data were investigated as the formation of cyanobacteria HABs are largely impacted by temperature and hydrological changes (Paerl, 1988).

Figure 5 shows a similar pattern in *mcyE/ndaF* concentrations detected in grab samples at the inlet and outlet streams. As the summer progressed, the concentration of *mcyE/ndaF* increased steadily, peaking in September, before declining again throughout autumn. Simultaneously, air temperatures also increased over the monitoring period, peaking in August. It is expected to see cyanobacteria proliferation as a result of increasing air temperatures because warm climates favor cyanobacteria blooms (Bormans et al., 2005; Paerl & Huisman, 2008). Additionally, there was little precipitation in July and August, reducing the amount of mixing occurring in the water column and exacerbating stratification that occurs in Lake Fletcher between June-August (Poltarowicz, 2017). The

observed increase in *mcyE/ndaF* concentrations in July and August may also be a result of stratification, as it is known to increase the formation potential of cyanobacteria blooms (Paerl, 1988). Furthermore, grab samples from both streams showed small drops in *mcyE/ndaF* concentration in August and late September. Both of these concentration decreases were observed after rainfall events occurring at the end-of-July/early August and early to mid-September. Heavy precipitation can create turbulence in the water column (Jöhnk et al., 2008; Paerl & Huisman, 2009) and cause cyanobacteria to disperse (Walsby et al., 1997). This, along increased shear stress, may have caused the drops in *mcyE/ndaF* concentrations.

The changes in *mcyE/ndaF* concentrations from passive samples are also shown in Figure 5. The detection levels of *mcyE/ndaF* at the WTP intake were consistently higher than at the inlet and outlet streams. A drop in *mcyE/ndaF* concentration was observed at all locations in mid- to late September, following a heavy rainfall event. Turbulence may have been increased in the water column, causing cell desorption from the inlet and outlet samplers. The intake samplers would be less impacted because they are located in the WTP and experience a lower, more controlled flow.

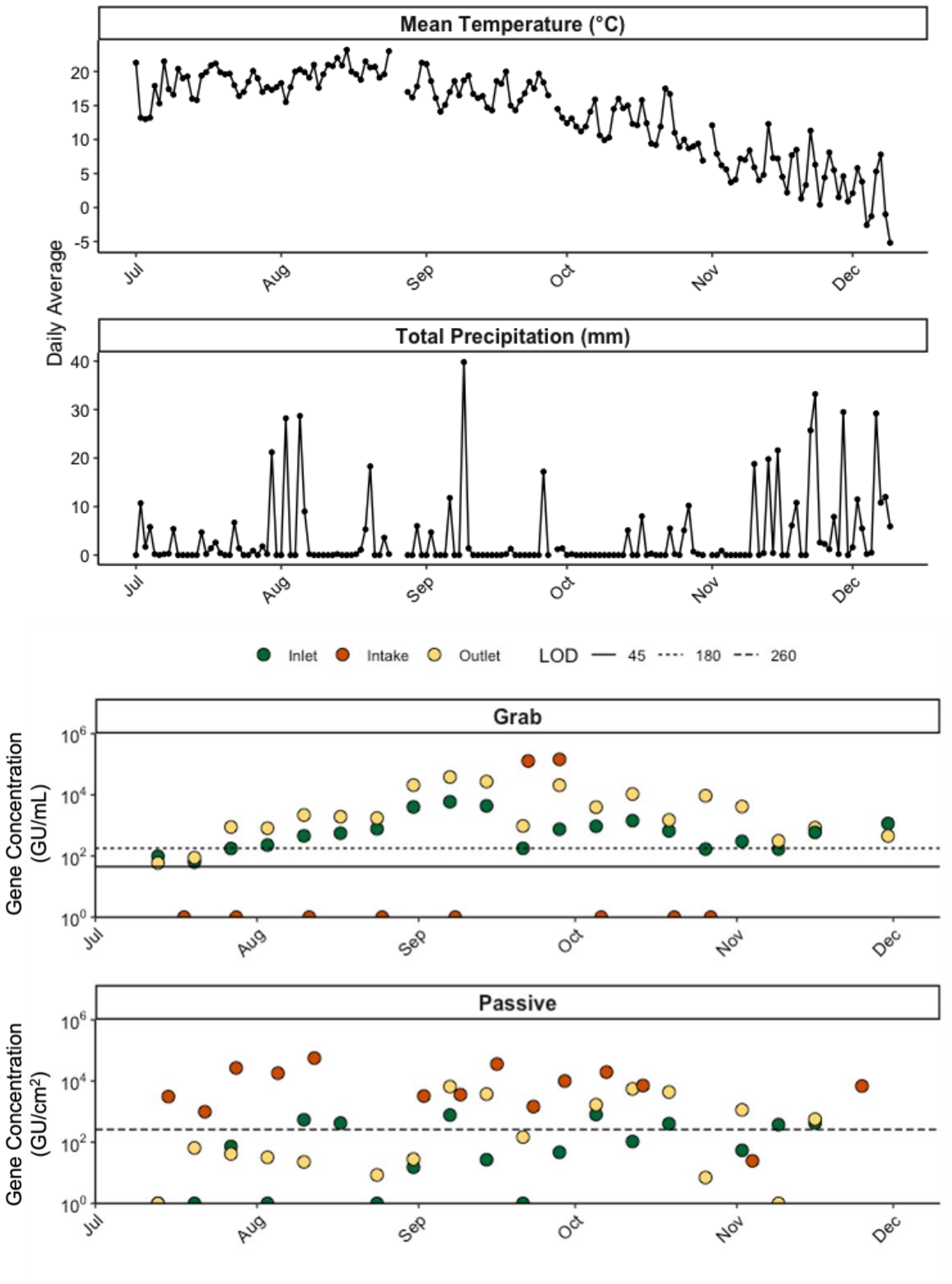

Figure 5. Concentration of *mcyE/ndaF* in grab and passive samples paired with climate data over 2021 monitoring period.

4.3.4. Comparison of Sample Types for Target Gene Detections

4.3.4.1. Paired Weekly and Bi-weekly Passive Samples

To compare the detection frequency of target genes on passive samplers based on exposure time, samplers were deployed at the inlet and outlet streams to Lake Fletcher for weekly and bi-weekly periods. Two-week periods that had both a bi-weekly sample and two weekly samples were paired for analysis. The results are shown in Figure 6, where each tile represents one week. For bi-weekly passive samples, if a target gene was detected, both weeks during that period were marked as a detection. In total, there were 8 bi-weekly periods with paired samples at the inlet and 5 bi-weekly periods with paired samples at the outlet. Overall, there were more positive detections of *mcyE/ndaF* in weekly samples (7 periods) than bi-weekly samples (4 periods). All periods with bi-weekly detections also had at least one weekly detection, whereas there were 3 periods where only weekly samples had detections of *mcyE/ndaF*. Furthermore, *cyrA* was detected more frequently in weekly samples (8 periods) than bi-weekly samples (3 periods). There were 6 periods where *cyrA* was detected in weekly samples only, and 1 period where *cyrA* was detected in bi-weekly samples only. Lastly, *sxtA* was detected in bi-weekly samples (2 periods) once more than weekly (1 periods). There was only one period where *sxtA* was detected in bi-weekly samples only.

Based on these results, weekly samplers were more effective at capturing *mcyE/ndaF* and *cyrA* than bi-weekly samplers. A longer exposure period may cause cells to degrade or lyse on the filter membrane, resulting in lower concentrations. Studies assessing the effects of ultrafiltration on cell integrity indicated that cell damage and lysis can occur, but isn't observed frequently (Campinas & Rosa, 2010; B. Liu et al., 2017). As there was no found research investigating cell degradation on passive samplers, further

studies should explore this idea. Weekly sampling data may be more informative for water utilities as gene detections occur in a shorter exposure period. Furthermore, shorter exposure periods would allow WTPs to make timely treatment adaptations for cyanobacteria and cyanotoxin removal. There were less conclusive results for *sxtA*, as both exposure periods had very low detection frequencies.

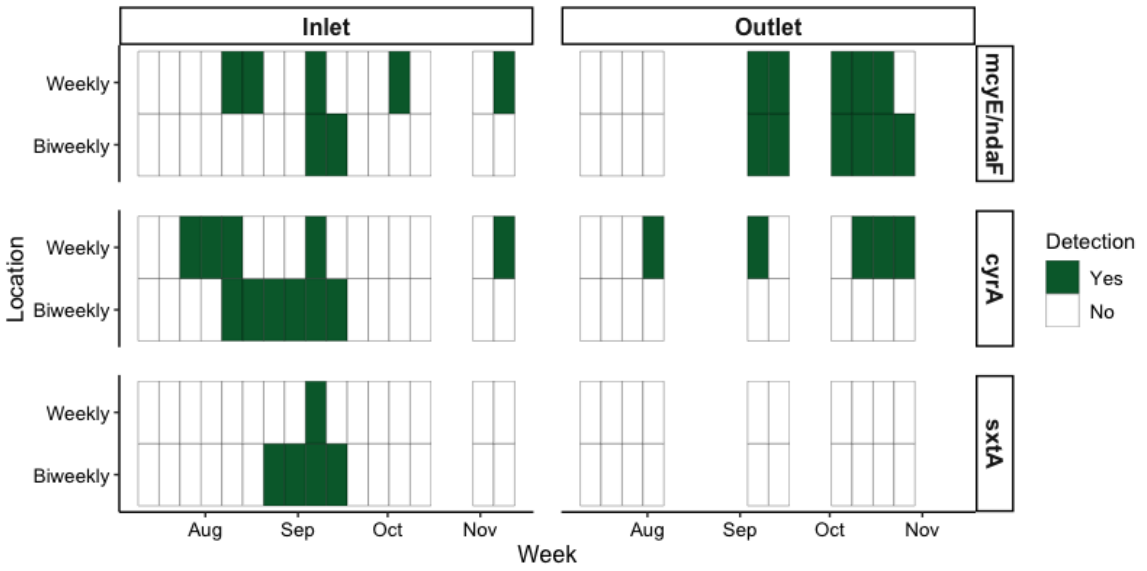

Figure 6. Comparison of mcyE/ndaF, cyrA and sxtA detections in paired weekly and bi-weekly passive samples at each stream location.

4.3.4.2. Paired Weekly Grab and Passive Samples

To compare the performance of sampling techniques at each location, passive and grab samples that were collected within the same week were paired for analysis. In total, there were 43 paired samples: 8 at the WTP intake, 18 at the inlet stream and 17 at the outlet stream. In Figure 7, the detection frequency of *mcyE/ndaF, cyrA* and *sxtA* at each watershed location is displayed based on sampling type. Overall, there were significantly more positive detections of *mcyE/ndaF* in grab samples (37 detections) than passive

samples (21 detections) (p = 0.0002). Conversely, passive samples had significantly more positive detections of *cyrA* (11 detections) than grab samples (4 detections) (p = 0.047). There were more positive detections of *sxtA* in passive samples (2 detections) than grab samples (0 detections), but the difference between sample types was not significant.

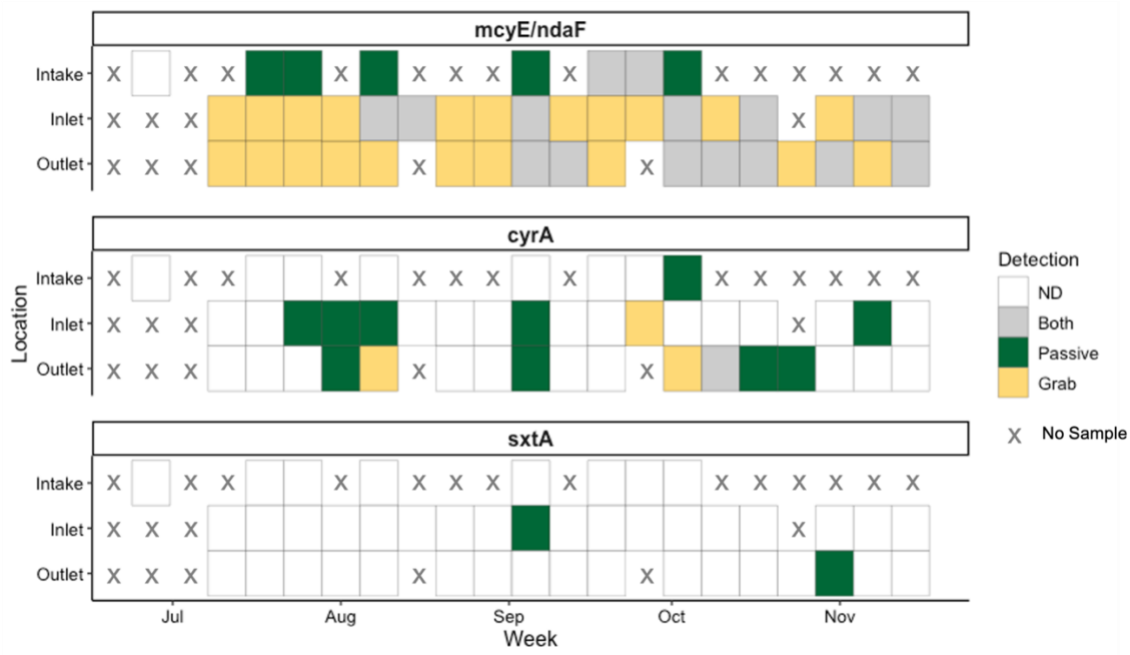

Figure 7. Comparison of *mcyE/ndaF, cyrA* and *sxtA* detections in paired passive and grab samples at each sampling location.

The detection frequency of each target gene at all sample locations for paired weekly data is summarized in Table 6. At the intake, *mcyE/ndaF* was detected more frequently in passive samples (88%) than in grab samples (25%). Conversely, the detection frequency of *mcyE/ndaF* was higher in grab samples at both the inlet (100%) and outlet streams (100%) than in passive samples (39% and 41%, respectively). As previously discussed, the passive samplers deployed in the streams experience more dynamic flows that those at the intake and water flow affects adsorption (Kristian Stevik et al., 2004), cells may have more difficulty staying adsorbed onto the filter membranes at these locations.

Furthermore, week-long deployment periods may cause adsorbed cells to degrade or lyse before the samplers are collected and analysed. Dynamic flows may also increase the dispersion of sediment particles, causing the membranes to reach their capacity sooner with other organic material. Additionally, the higher frequency of *mcyE/ndaF* detections in passive samples at the intake may be caused by cell densities that are too low to be detected in grab samples but are accumulating on the filter membranes over time. A recent study showed that passive samplers were successful at detecting low concentrations of Norovirus in seawater while grab samples were not (Vincent-Hubert et al., 2021).

Table 6. Detection frequency of *mcyE/ndaF, cyrA* and *sxtA* at all sample locations for paired weekly data.

Target	**Intake Detections (n = 8)**		**Inlet Detections (n = 18)**		**Outlet Detections (n = 17)**	
	Grab (%)	Passive (%)	Grab (%)	Passive (%)	Grab (%)	Passive (%)
mcyE/ndaF	2 (25 %)	7 (88 %)	18 (100 %)	7 (39 %)	17 (100 %)	7 (41 %)
cyrA	0 (0 %)	1 (13 %)	1 (5 %)	5 (28 %)	3 (18 %)	5 (29 %)
sxtA	0 (0 %)	0 (0 %)	0 (0 %)	1 (5 %)	0 (0 %)	1 (6 %)

The detection frequency for *cyrA* was higher in passive samples (intake = 13%, inlet = 28%, outlet = 29%) than in grab samples (intake = 0%, inlet = 5%, outlet = 18%) at all three locations. The lower frequency of *cyrA* detections in grab samples may be caused by cell densities that are too low to be detected in the volume that was filtered. Additionally, a higher *cyrA* detection frequency in passive samples may be the result of low cell concentrations accumulating on the filter membranes over time. Lastly, *sxtA* was only detected in passive samples at each of the stream locations. This may also be a result of cell concentrations too low to detect in grab samples and accumulating over time on the filter membranes. Furthermore, benthic cyanobacteria species like *Lyngbya wollei* can

carry the *sxtA* gene (Mihali et al., 2011), and have been found in Canadian freshwater (Lajeunesse et al., 2012). Because the samplers were deployed in streams with more dynamic flows, the detection of *sxtA* could have been from benthic species.

As adsorption is influenced by physical, chemical and microbiological factors (Kristian Stevik et al., 2004), the impacts of cell morphology on adsorption should be considered. Cyanobacteria are often characterized by being either unicellular (coccoidal) or filamentous (Whitton & Potts, 2007). To my knowledge, there are no published studies that investigate the impacts of cell morphology on the passive adsorption of cyanobacteria onto filter papers; however, some studies have shown the effects of cell morphology on cyanobacteria removal through filtration. Coccoidal cells tend to have lower removal rates than filamentous species in filters (Dugan & Williams, 2006; Lawton et al., 1998), which may indicate that they have lower adsorption rates to filter papers. In 2016, 16S sequencing results (unpublished data from Betts, 2018) from the inlet to Lake Fletcher indicated that *Synechococcales* had the highest relative abundance, followed by *Chloroplasts* and *Chroococcales* (Brophy, 2019). Furthermore, the cyanobacteria genera detected at the inlet to Lake Fletcher in 2016 that are capable of producing cyanotoxins are summarized in Table 7. *Microcystis, Synechococcus* and *Nostoc* are all known to produce microcystin and are coccoidal in nature (Komárek, 2003), whereas *Anabaena* and *Dolichospermum* produce microcystin and cylindrospermopsin but are filamentous in nature (Komárek et al., 2003). If *mcyE/ndaF* and *cyrA* genes were detected from coccoidal and filamentous species, respectively, passive samplers may have been more effective at detecting *cyrA* due to the difference in cell morphology. However, there is insufficient data to conclude what species were adsorbed onto the passive samplers. Further studies should be conducted to

investigate what species are adsorbed onto filter membranes and what role cell morphology plays on adsorption.

Table 7. Summary table of cyanobacteria genera detected in Lake Fletcher's inlet in 2016 and their associated toxins (Betts, 2018).

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

4.4. CONCLUSIONS

The purpose of this study was to investigate the use of passive samplers and qPCR analysis as a detection method for freshwater cyanobacteria for drinking water suppliers. This research was completed by conducting a proof-of-concept field-scale monitoring program at Lake Fletcher, NS, comparing a passive sampling technique to traditional grab samples. The results from this study indicated that passive samplers could detect 16S rRNA, *mcyE/ndaF, cyrA* and *sxtA* genes in freshwater. Overall, grab samples had significantly more *mcyE/ndaF* detections than passive samples. However, when analyzed by location, grab sampling was only more effective at detecting *mcyE/ndaF* at the inlet and outlet streams and passive samplers were more effective at detecting *mcyE/ndaF* at the intake to the WTP. It is suspected that the difference in flows between the streams and intake may have impacted cyanobacteria adsorption onto the filter membranes and thus,

the hydrological conditions of sampler location should be considered in future designs and monitoring programs.

Conversely, passive samples had significantly more *cyrA* detections than grab samples and were more effective at all locations. Additionally, passive samplers were able to detect *cyrA* (all locations) and *mcyE/ndaF* (intake only) before they were detected in passive samples. This may be caused by cell concentrations being too low to detect in grab samples but are accumulating on the filters over time, highlighting their potential as an early warning detection method. Moreover, because grab samples are a snapshot in time they may be missing when cyanobacteria pass through each location. There was insufficient data to conclude whether passive samplers were more effective at detecting *sxtA* genes than grab sampling.

Weekly deployment periods were more effective than bi-weekly periods for the detection of *mcyE/ndaF* and *cyrA*, and exposure time had no conclusive effect on *sxtA* detection. Shorter deployment periods may be more informative for water utilities as longer exposure times could cause cell degradation and/or lysis. Furthermore, it was suggested that the passive samplers reach their adsorption capacity around 3×10^6 GU/cm^2 in surface water, which may be due to the competition between cyanobacteria and NOM and/or sediment for adsorption sites. Bacterial adsorption is influenced by physical, chemical and microbiological factors (Kristian Stevik et al., 2004), thus, the impacts of cell morphology, material surface charge and water quality should be considered.

Further research should be conducted to investigate the adsorption characteristics of cyanobacteria onto passive sampling material. Various adsorbents should be explored as species may have greater affinities to different materials based on their surface charge

and pore size. Kinetic and equilibrium isotherm characteristics should be assessed over a range of NOM and sediment concentrations for coccoidal and filamentous species. Lastly, the extraction protocol used in this study was adapted for passive sampling and may have resulted in lower gene target recoveries. Thus, future research should also be conducted to optimize the extraction of these target genes from adsorbent material.

CHAPTER 5: BENCH-SCALE EVALUATION OF PASSIVE SAMPLER MATERIALS FOR CYANOBACTERIA DETECTION USING qPCR

5.1. INTRODUCTION

Under the right growing conditions, freshwater cyanobacteria can proliferate into harmful algal blooms (HABs) and release cyanotoxins. HABs have been detected in Canadian drinking water sources and are expected to increase in frequency and intensity in future years (O'Keeffe, 2019; Pick, 2016). Although HABs pose public health and safety concerns, surveillance strategies are still inconsistent across the country (O'Keeffe, 2019). To mitigate HABs, water treatment plants (WTPs) need to implement early warning monitoring strategies that detect cyanobacteria before the onset of HABs. Currently, monitoring strategies involve grab and composite sampling techniques, each offering their own limitations. Grab sampling, although simple and convenient, can underestimate HAB risk as it only represents a snapshot of the potential cyanobacteria presence in a water body. Composite sampling is more representative but is less feasible for WTPs because it can be time consuming and expensive. Many researchers have explored passive sampling as an alternative method due to its ability to create a composite sample while maintaining the convenience and simplicity of a grab sample.

Passive samplers have been used to detect many organic and inorganic contaminants in freshwater, seawater and wastewater (Godlewska et al., 2021; Vrana et al., 2005). This method involves deploying a sampler containing an adsorbent material for a pre-determined amount of time and the extracted concentrations represent the time-weighted average of the contaminant (Namieśnik et al., 2005). Recently, passive sampling techniques have been successful at detecting SARS-CoV-2 in wastewater (Habtewold et al., 2022; Hayes et al., 2021; Li et al., 2022; P. Liu et al., 2022; Schang et al., 2021; Vincent-

Hubert et al., 2022) and in marine water (Vincent-Hubert et al., 2022). Additionally, some studies have investigated the relative adsorption performance of different sampler materials for SARS-CoVo2 (Hayes et al., 2021; Schang et al., 2021) and seawater pathogens and viruses (Vincent-Hubert et al., 2017). The results of these studies demonstrated that material plays a role in bacterial and viral adsorption as they each have their own maximum adsorption capacities. To my knowledge, there are no studies that investigate the passive adsorption of cyanobacteria onto different adsorbent materials. Thus, it is hypothesized that material will impact the adsorption of freshwater cyanobacteria, as seen in other viral and bacterial adsorption studies.

The purpose of this study was to investigate the adsorption performance of four sampler materials: cellulose nitrate, gauze, nylon and acrylic copolymer. TCellulose nitrate filter membranes have been successful at detecting SARS-CoV-2 in wastewater (Hayes et al., 2021; Schang et al., 2021) and have higher viral recoveries than other adsorbent materials (Hayes et al., 2021). Gauze has been proven to be an effective adsorbent material for detecting viruses and bacteria in various water matrices (Hayes et al., 2021; Sbodio et al., 2013; Schang et al., 2021; Tian et al., 2017; Vincent-Hubert et al., 2017) and it is easily accessible due to its low cost and availability at local pharmacies. Vincent-Hubert et al. (2017) demonstrated that nylon membranes could detect two viruses that are harmful to coastal oyster farms. Lastly, acryclic copolymer filter membranes were not found in any bacterial adsorption studies but are recommended for the extraction and recovery of cyanobacteria DNA in freshwater samples (Phytoxigene, 2019). Two bench-scale studies were conducted to investigate the effects of sampler material on cyanobacteria adsorption.

5.2. MATERIALS AND METHODS

5.2.1. Reagents and Materials

Cotton gauze was acquired from a local pharmacy, Versapor® acrylic copolymer membrane filters (25 mm, 0.8 µm) were purchased from VWR™ International, LLC., and cellulose nitrate (47 mm, 0.1 µm) and nylon (25 mm, 1.2 µm) membrane filters were purchased from MilliporeSigma. Commercially available kits for DNA extractions, 16S rRNA assays and toxin gene assays were obtained from Phytoxigene™, Inc. To rehydrate reagents, nuclease free water was obtained from LuminUltra Technologies (LuminUltra Technologies Ltd) and MilliporeSigma (MillipreSigma). In both studies, a New Brunswick™ Innova® 40/40R benchtop orbital shaker table (Eppendorf) was used to continuously mix samples and maintain a consistent air temperature for the duration of each experiment.

5.2.2. Experimental Setup for Bench-scale Batch-adsorption Studies

The relative adsorption performance of gauze and three filter membranes: cellulose nitrate, acrylic copolymer and nylon was assessed through batch-adsorption studies. Gauze was chosen as a potential passive sampling material due to its low cost, availability at local pharmacies and ability to detect viruses and bacterial pathogens in seawater (Tian et al., 2017; Vincent-Hubert et al., 2021). Cellulose nitrate filter membranes were included in this study because of their success at adsorbing SARS-CoV-2 RNA from wastewater (Hayes et al., 2021; Schang et al., 2021). Acrylic copolymer filter membranes were investigated as they were recommended for DNA extractions in the Phytoxigene™ protocol. Lastly, nylon was chosen due to its ability to passively adsorb two coastal water viruses; NoV GII and OshV-1 (Vincent-Hubert et al., 2017). These materials were evaluated for DNA concentrations of 16S rRNA (total cyanobacteria).

For each batch-adsorption study, freshwater samples were mixed and divided into sterile 250 mL Erlenmeyer flasks. Assuming the sample was homogeneous, each flask was prepared with 200 mL of sample while 100 mL was set aside for initial DNA extraction. The remaining sample was stored at 4 ºC for up to 24 hours prior to being characterized using basic water quality parameters. Each adsorbent material was placed into its respective flask before the flask was sealed with Bemis™ Parafilm™ M Laboratory wrapping film. The flasks were placed on the orbital shaker table and continuously stirred at 120 rpm for 8- or 24- hour periods. The air temperature of the shaker table was set to 25 ºC as it is an optimal growing temperature for cyanobacteria (Bormans et al., 2005; Paerl & Huisman, 2008). Figures in Appendix C show the experimental setup for each batch-adsorption study. For each experiment, a Parafilm® sealed Erlenmeyer flask was left with sample only to serve as a control. At the end of each experiment, the DNA concentrations of the control were measured to determine any changes in gene concentrations over the exposure period.

5.2.3. Scanning Electron Microscopy

A Zeiss SIGMA 300 VP (Jena) scanning electron microscope (SEM) was used to characterize the surface of each adsorbent material (nylon, gauze, cellulose nitrate, acrylic copolymer). Each material was cut into sections, mounted onto aluminum studs and sputter-coated with gold/palladium (80/20) in argon. Samples were coated until a thickness of 15 nm was achieved using a Leica EM ACE600 high vacuum sputter coater with a 30-mA current. The SEM had working distances of 12 and 15 mm with an acceleration voltage of 5 kV and a current probe of 220 pA. All samples were analyzed in duplicate.

5.2.4. Sample Collection

5.2.4.1. Collection Sites

At the time of this study, pure cyanobacteria cultures were inaccessible. For this reason, environmental samples were used for water matrices in all batch adsorption experiments instead of water spiked with pure cultures. For the 8-h adsorption studies, water was collected from four Nova Scotian lakes: Chocolate Lake (Halifax, NS), Grand Lake (Shubenacadie, NS), Lumsden Pond (Wolfville, NS) and William's Lake (Spryfield, NS). These lakes were chosen based on swimming advisories put out by Nova Scotia Environment (NSE) due to potential HAB presence. For the 24-h adsorption studies, water was collected from the McLaughlin Road Reservoir in Moncton, New Brunswick (NB). At the time of sample collection, there was a known HAB present in the reservoir.

5.2.4.2. Grab Sampling

For 8-h adsorption experiments, two 1 L volumes were collected at each site using public beach access points. A sampling stick with an attached bottle was used at each site to collect water further away from the shoreline. Collected samples were poured into sterile Nalgene bottles and transported to the laboratory in a cooler on ice. For 24-h adsorption experiments, clean, 8 L buckets were used to collect water near the shoreline of the McLaughlin Road Reservoir. Samples were shipped to the laboratory within a day on ice. Upon arrival to the laboratory, DNA was extracted from all samples and analyzed immediately using qPCR. Samples that tested positive for cyanobacteria were used for experiments and the remaining sample volumes were stored at 4 ºC for up to 24 hours prior to basic water quality characterization.

5.2.5. Source Water Characterization

5.2.5.1. Basic Water Quality Parameters

Following the procedures described in Sections 3.1.1 and 3.1.2, the water used in each experiment was characterized by measuring pH, turbidity, UV_{254}, TOC, and DOC. Due to the high concentration of algal material in the 24-h adsorption study source water, TOC was not measured as there was a risk of clogging the TOC analyzer. All samples were stored at 4 ºC and analyzed within 24 hours of collection.

5.2.5.2. Nucleic Acid Extraction

To determine the quantity of cyanobacteria in each batch adsorption experiment, the Phytoxigene™ extraction protocol outlined in Section 3.2.1 was followed. For the 8-h adsorption studies, 100 mL of each sample was filtered through 25 mm, 0.8 µm Versapor® acrylic copolymer membrane filters (Pall Corporation) using a sterile 50 mL syringe apparatus. For the 24-h adsorption studies, 8-10 mL of each sample was filtered through the same type of membrane filter. Less volume was required for the 24-h study due to its visibly high concentration of algal material. Samples were stored at -80 ºC if they could not be processed immediately using qPCR.

5.2.6. Nucleic Acid Extraction from Adsorbent Materials

At the end of each experiment, the adsorbent materials were retrieved from each Erlenmeyer flask using sterile tweezers and stir sticks. Each material was folded and placed directly into a lysis tube and extracted following the Phytoxigene™ protocol outlined in Section 3.2.1. If samples could not be processed immediately using qPCR, they were stored at -80 ºC.

5.2.7. qPCR Analysis

To assess the adsorption performance of each material, Phytoxigene™ CyanoDTec Total Cyanobacteria kits were used to quantify 16S rRNA presence in each sample. The assays run in this study had the same targets and parameters as outlined in Section 3.3.1.

5.2.8. Quantitative Analysis of Material Performance

To assess the relative performance of each adsorbent material, a quantitative analysis of each target gene was conducted. DNA concentrations were calculated in gene units per centimeter squared using Equation 3, shown in Section 3.3.2.

5.2.9. Statistical Analysis

A Shapiro-Wilk test was performed to check for normality. Based on its results, a non-parametric Kruskal-Wallis and pairwise Wilcoxon rank sum tests were performed to determine whether the amount of adsorbed total cyanobacteria varied according to material type. No correction factors were used due to the small sample size. Each test had a significance level of $p < 0.05$. All statistical analyses were conducted using R (RStudio Version 1.3.1093) software.

5.2.10. Quality Control

All quality control measures and parameters outlined in Section 3.4 were followed in this study. Additionally, all batch-adsorption experiments were prepared in the BSC, where each Erlenmeyer flask was sealed using Bemis™ Parafilm™ M Laboratory wrapping film prior to being placed in the orbital shaker table. Because different environmental samples were used in each experiment, biological replicates were not feasible. Additionally, the 8-h adsorption study served as a preliminary investigation into the adsorption performance of various materials, and therefore samples were not run on the

qPCR in technical replicates to conserve reagents. Conversely, the 24-h adsorption studies were conducted to further investigate material performance and were run in technical triplicates.

5.3. RESULTS AND DISCUSSION

5.3.1. 8-hour Batch-Adsorption Study

5.3.1.1. Water Characterization

For each bench-scale adsorption study, basic water quality parameters such as pH, turbidity, TOC/DOC and UV_{254} were measured. The source water characterization of each water matrices is shown in Table 8. These data show similar NOM characteristics between Grand Lake and Lumsden Pond, and between Chocolate Lake and William's Lake. Grand Lake and Lumsden Pond had higher TOC/DOC and UV_{254} values than Chocolate Lake and William's Lake. Furthermore, Grand Lake and Lumsden Pond had SUVA values of 5.36 and 4.41, respectively, suggesting that the DOC of these lakes is largely composed of aquatic humics and is relatively hydrophobic in nature (Edzwald, 1993). Conversely, Chocolate Lake and William's Lake had lower SUVA values (1.11 and 2.03, respectively), suggesting that the DOC of these lakes is largely composed of non-humic materials and is relatively hydrophilic in nature (Edzwald, 1993).

Table 8. Source water characteristics of 8-hr bench-scale adsorption studies water matrices. For each parameter n = 3, except TOC/DOC where n = 2.

Lake	pH	Turbidity (NTU)	TOC (mg/L)	DOC (mg/L)	UV_{254} (cm^{-1})	SUVA
Chocolate	5.42 +/- 0.0	0.63 +/- 0.0	0.38 +/- 0.0	0.42 +/- 0.1	0.01 +/- 0.0	1.11
Grand	6.76 +/- 0.1	8.17 +/- 0.8	5.67 +/- 0.3	5.41 +/- 0.4	0.29 +/- 0.0	5.36
Lumsden	6.40 +/- 0.0	1.79 +/- 0.0	5.72 +/- 0.1	6.12 +/- 0.0	0.27 +/- 0.0	4.41
William's	6.72 +/- 0.0	0.78 +/- 0.0	3.16 +/- 0.1	2.98 +/- 0.0	0.06 +/- 0.0	2.03

All values are reported as mean +/- standard deviation

The similarities between lakes in terms of pH and turbidity measurements were less consistent. The pH of Grand Lake, William's Lake and Lumsden Pond were similar (~ 6.6), while Chocolate Lake was slightly more acidic (~ 5.4). Additionally, Chocolate Lake and William's Lake had low turbidity measurements (< 1 NTU) with values very close to one another. The turbidity of Lumsden Pond was slightly higher (< 2 NTU) and the turbidity of Grand Lake was the highest (> 8 NTU).

5.3.1.2. Target Gene Detections of Each Material

To evaluate the detection of total cyanobacteria (16S rRNA), four adsorbent materials were tested in a controlled bench-scale experiment. Gauze, nylon, acrylic copolymer and cellulose nitrate filter membranes were exposed to freshwater with suspected cyanobacteria blooms for 8 hours. The adsorbed DNA concentrations (GU/cm^2) for each material are shown in Figure 8. In each experiment, 16S rRNA was recovered from all materials. Acrylic copolymer had the highest mean concentration ($\pm$ standard deviation) of adsorbed total cyanobacteria ($5.1 \times 10^5 \pm 8.5 \times 10^5$ GU/cm^2), followed by cellulose nitrate ($2.5 \times 10^5 \pm 2.5 \times 10^5$ GU/cm^2) and gauze ($2.1 \times 10^5 \pm 2.1 \times 10^5$ GU/cm^2). Nylon had the lowest adsorption ($3.1 \times 10^3 \pm 2.0 \times 10^3$ GU/cm^2) of all the materials.

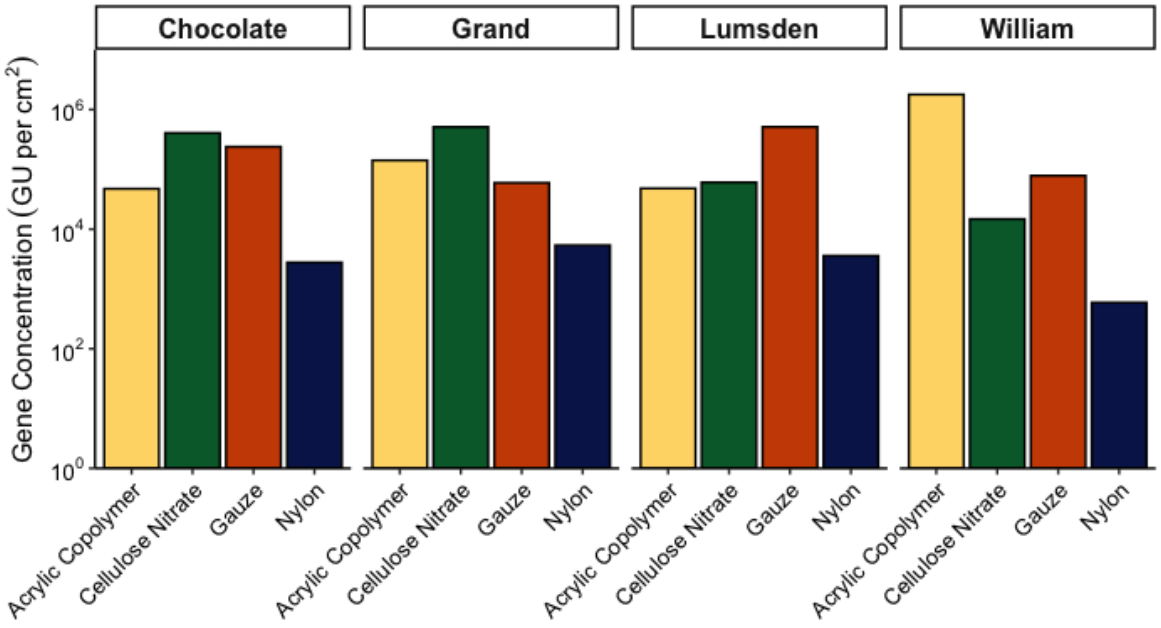

Figure 8. Concentrations of 16S rRNA adsorbed onto four materials: acrylic copolymer, cellulose nitrate, gauze and nylon, during bench-scale batch adsorption experiments. Materials were placed in water collected from freshwater sources with suspected cyanobacteria blooms for 8-hour periods.

Although acrylic copolymer yielded the highest mean concentration, when looking at the median adsorbed concentrations of each material (Figure 9), cellulose nitrate had the best overall adsorption (2.3 x 10^5 GU/cm^2), followed by gauze (1.6 x 10^5 GU/cm^2). The mean concentration for acrylic copolymer was likely skewed due to its high concentration from the experiment using water from William's Lake, as its median concentration was 9.5 x 10^4 GU/cm^2. Again, nylon performed the worst out of all the materials with a median concentration of 3.2 x 10^3 GU/cm^2. There was no significant difference in adsorption between cellulose nitrate, acrylic copolymer and gauze, but nylon performed significantly worse than all three materials ($p = 0.029$ each). There were no clear trends in water quality parameters and adsorbed 16S rRNA concentrations.

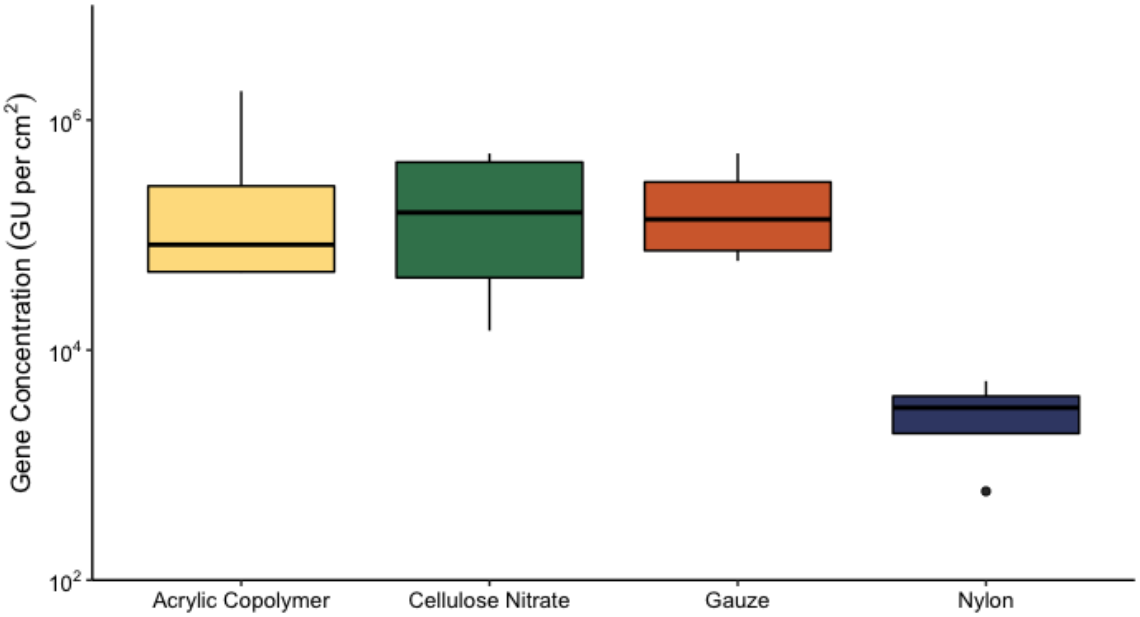

Figure 9. 16S rRNA concentrations extracted from adsorbent materials exposed for 8 h. Boxplots show the minimum, 25th percentile, median, 75th percentile and maximum concentrations for each material. LOD is 260 GU/cm^2.

Bacterial adsorption is influenced by physical (pores, organic matter, water flow, etc.), chemical (species, ionic strength, pH, etc.) and microbiological (bacterial concentration, hydrophobicity, cell surface characteristics) factors (Kristian Stevik et al., 2004). To better understand the effects of pore size on cyanobacteria adsorption, the surface morphology of each adsorbent material was characterized by a scanning electron microscope (SEM). Appendix D shows the SEM images for acrylic copolymer, cellulose nitrate, gauze and nylon. Notably, gauze had the largest pore sizes, but its weaving threads had smaller pore spaces within them. Acrylic copolymer and cellulose nitrate membranes had the most uniform pores, whereas nylon was less uniform and gauze was fairly nonuniform. As cellulose nitrate and gauze had similar adsorption performances but dissimilar surface morphologies, pore size may not have been a driving factor for

adsorption. Furthermore, the surfrance morphology of nylon was similar to cellulose nitrate and acrylic copolymer but had the worst adsorption performance.

Surface water characteristics such as pH and ionic strength can affect bacterial adsorption onto surfaces by changing the surface charge of cells (Kristian Stevik et al., 2004; Martinez et al., 2008). Martinez et al. (2008) showed that when pH was below 7, two cyanobacteria species had a negative overall zeta potential, and when pH was between 7 and 10, the overall zeta potential became positive. If cyanobacteria in Lake Fletcher have negative surfaces charges, they may have a higher affinity to electropositive membranes. However, the cellulose nitrate filter membranes used in this study were negatively charged and had the greatest adsorption. Hydrophobicity is another factor that impacts bacterial adsorption (Fattom & Shilo, 1984; Kristian Stevik et al., 2004). Because many planktonic cyanobacteria are hydrophilic (Fattom & Shilo, 1984), the hydrophobic nature of the nylon membranes may have contributed to it being the worst adsorbent of the four materials.

5.3.2. 24-hour Batch-Adsorption Study

5.3.2.1. Water Characteristics

Basic water quality parameters such as such as pH, turbidity, TOC/DOC and UV_{254} were measured for each bench-scale adsorption experiment. The source water characterization of each water matrices is shown in Table 9. In each set of experiments, samples were collected from the reservoir, but were collected on different days. These data show that the concentration of organics increased between Run 1 and Run 2. Both the DOC and UV_{254} values for Run 2 were double those of Run 1. The SUVA values for each run were consistent (~ 5.6), indicating that NOM composition remained the same. It is suggested that the DOC of this reservoirs is largely composed of aquatic humics and is

relatively hydrophobic in nature (Edzwald, 1993). Similarly, the turbidity values were much higher for Run 2 than in Run 1. The pH remained constant between each run with values around 6.6.

Table 9. Source water characteristics of 24-hour bench-scale adsorption studies water matrices.

Run	pH	Turbidity (NTU)	DOC (mg/L)	UV_{254} (cm^{-1})	SUVA
1	6.61 +/- 0.6	777 +/- 455	5.12 +/- 3.6	0.29 +/- 0.0	5.66
2	6.68 +/- 0.1	1658 +/- 465	10.18 +/- 1.2	0.56 +/- 0.0	5.50

All values are reported as mean +/- standard deviation

5.3.2.2. Detection of 16S rRNA from Each Material

To further evaluate the detection of total cyanobacteria (16S rRNA), gauze, acrylic copolymer and cellulose nitrate membranes were tested in a controlled bench-scale experiment. Each material was exposed to freshwater with known cyanobacteria blooms for 24 hours. The adsorbed DNA concentrations (GU/cm^2) for each material are shown in Figure 10. In each experiment, 16S RNA was recovered from all materials. Cellulose nitrate had the highest mean concentration ($\pm$ standard deviation) of adsorbed total cyanobacteria ($3.9 \times 10^7 \pm 3.9 \times 10^7$ GU/cm^2), followed by acrylic copolymer ($2.2 \times 10^7 \pm 1.6 \times 10^7$ GU/cm^2) and gauze ($1.4 \times 10^7 \pm 1.4 \times 10^7$ GU/cm^2). The only significant difference in overall adsorption performance was between cellulose nitrate and gauze, where cellulose nitrate yielded higher DNA concentrations ($p = 0.04$). However, when NOM was low (Run 1a and 1b), cellulose nitrate and acrylic copolymer had significantly better adsorption than gauze ($p = 0.002$ each).

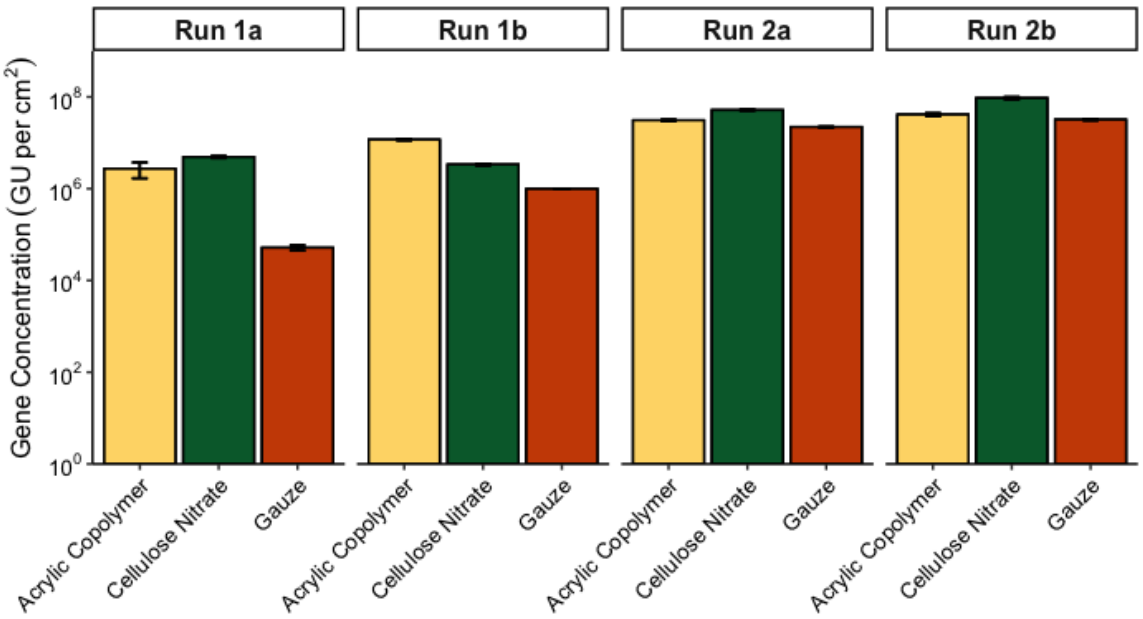

Figure 10. Concentrations of 16S rRNA adsorbed onto three materials: acrylic copolymer, cellulose nitrate, gauze and nylon, during bench-scale batch adsorption experiments. Materials were placed in water collected from freshwater sources with known cyanobacteria blooms for 24-hour periods.

The median concentrations of adsorbed 16S rRNA for each material were consistent with their mean values. Cellulose nitrate had the highest concentration (2.8 x 10^7 GU/cm^2), followed by acrylic copolymer (2.1 x 10^7 GU/cm^2) and gauze (1.1 x 10^7 GU/cm^2). These values are shown in Figure 11, where the variability in adsorbed 16S rRNA concentrations for gauze is highlighted. Based on these data, adsorption performance seems to be affected by NOM concentration and turbidity. As the samples used in this study were collected from a dense HAB, the increased NOM concentration between runs may have been caused by increased algal biomass. Increased cell concentrations can increase adsorption rates until adsorbents become saturated (Kristian Stevik et al., 2004). This could be why the materials in Run 2 have more similar 16S rRNA concentrations, suggesting they are reaching the same maximum adsorption capacities. Additionally, the concentration of 16S rRNA decreased in the control sample (Appendix E) during Run 1 but increased in

Run 2. Lower adsorbed concentrations in Run 2 may be caused by less whole cells adsorbing onto the materials due to cell lysis. In future studies, pure cultures should be used to maintain cell integrity during bench-scale experiments and to more accurately determine the recovery of each material.

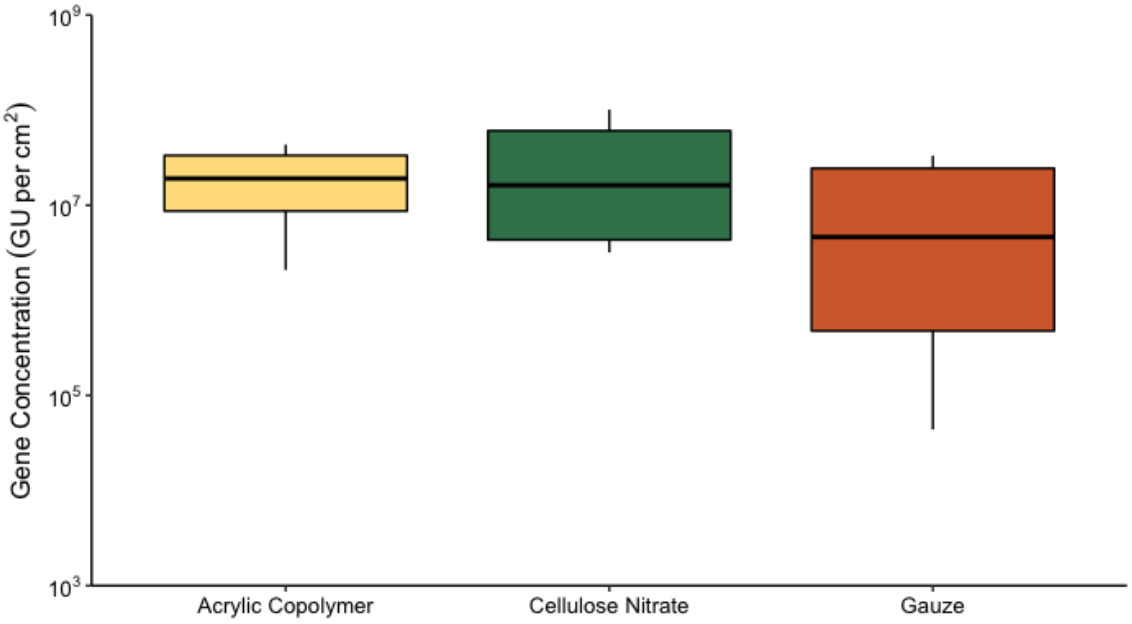

Figure 11. 16S rRNA concentrations extracted from adsorbent materials exposed for 24 h. Boxplots show the minimum, 25th percentile, median, 75th percentile and maximum concentrations for each material. LOD is 260 GU/cm^2.

5.4. CONCLUSIONS

The purpose of this study was to assess the performance of four adsorbent materials for cyanobacteria detection using qPCR analysis. This research was completed by running bench-scale adsorption studies over 8- and 24-hour periods using water collected from surface waters with known or expected HABs. The results from the 8-h study showed that cyanobacteria were able to passively adsorb onto all four materials. However, nylon performed significantly worse than cellulose nitrate, gauze and acrylic copolymer membranes. Because the other three materials had similar adsorption performances, they

were investigated further in the 24-hr adsorption study. The results from the 24-h study indicated that cellulose nitrate was the best adsorbent material. This study also showed that NOM concentration may influence cellular adsorption to gauze. Moreover, it is hypothesized that the adsorption capacities of materials in the 8-h and 24-h studies were between $1 \times 10^5 - 1 \times 10^6$ GU/cm^2 and $1 \times 10^7 - 1 \times 10^8$ GU/cm^2, respectively. A lower adsorption capacity in the 8-h study may have been from the surface waters having much lower concentrations of algal material than those used in the 24-h study, allowing more opportunities for NOM to use up adsorption sites.

Further studies should be completed to investigate the adsorption characteristics of cyanobacteria onto passive sampler material. This work should be conducted using pure cultures of coccoidal and filamentous species to determine the recovery of each adsorbent material. Other materials that may have higher adsorption capacities than cellulose nitrate filter membranes should be assessed. Furthermore, extraction protocols should be optimized to ensure maximum gene target recoveries. Using pure cultures of coccoidal and filamentous cyanobacteria, kinetic and equilibrium characteristics of potential sampler materials should be assessed over a range of NOM and sediment concentrations. This work would help inform WTPs on what sampler materials and deployment periods are best suited for passive sampling monitoring based on source water characteristics. Lastly, future studies should also factor in the challenges associated with running laboratory experiments using cyanobacteria, such as their photosynthetic nature. Having the appropriate growing conditions (e.g., light) should be considered.

CHAPTER 6: CONCLUSION

6.1. SUMMARY OF CONCLUSIONS

The ongoing and anticipated impacts of climate change and lake recovery highlight the need for effective source water monitoring strategies. Traditional sampling techniques have limitations, thus, alternative sampling strategies should be considered. The purpose of this research was to investigate if passive samplers paired with qPCR analysis could serve as a detection method for cyanobacteria in drinking water sources Through a proof-of-concept field-scale monitoring program at Lake Fletcher, NS, passive samplers were proven to be more effective at detecting *cyrA* and *sxtA* gene targets than passive sampling at all watershed locations. Although grab samples had more detections for *mcyE/ndaF* at stream locations, passive samplers were more effective at detecting *mcyE/ndaF* at the WTP intake. Additionally, passive samplers were able to detect *mcyE/ndaF* and *cyrA* before grab samples, highlighting their potential for early warning monitoring. Bench-scale adsorption studies were conducted as a preliminary assessment of the relative performance of different adsorbent materials for cyanobacteria detection. The results from this study indicated that cellulose nitrate filter membranes were the best suited material for cyanobacteria adsorption. This research will serve as a foundation for the optimization and implementation of passive samplers for cyanobacteria detection.

6.2. RECOMMENDATIONS FOR FUTURE WORK

Further research should be conducted to investigate the adsorption characteristics of cyanobacteria onto passive sampling material. Passive sampling coupled with qPCR analysis has the potential to be a quick, detection strategy for drinking water suppliers.

However, more research is required to optimize passive sampler adsorption and recovery. The following work is recommended:

- Bench-scale adsorption studies should be conducted using pure cultures of coccoidal and filamentous cyanobacteria species to determine the recovery of each adsorbent material tested. This work should be expanded to new materials to improve upon the capacity limitations of cellulose nitrate filter membranes outlined in this work. Additionally, this research should optimize the extraction protocol for adsorbent materials to aid in target gene recoveries.
- Kinetic and equilibrium isotherm characteristics of adsorbent materials should be assessed over a range of NOM and sediment concentrations using pure cultures of coccoidal and filamentous cyanobacteria species. This work would inform WTPs on what material is best suited for passive sampling monitoring based on source water characteristics.
- Monitoring studies should be continued using adsorbent materials and deployment times informed by adsorption, kinetics and equilibrium studies. Cell enumeration, DNA sequencing and bloom forming parameters (organics, light, temperature, nutrients, etc.) should be paired with passive sampling and qPCR analysis to further investigate cyanobacterial adsorption mechanisms.
- Should qPCR be implemented as part of a monitoring strategy, toxin genes of cyanobacteria native to Atlantic Canadian source waters should be investigated to ensure qPCR assays are relevant. Furthermore, the LOD for toxin gene assays should be determined for passive sampling methods.

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APPENDIX A – EXAMPLE CALCULATIONS FOR DNA CONCENTRATION

Examples for calculating DNA concentrations in grab samples (Eq. 2) and passive samples (Eq. 3) are shown below.

$$DNA\ Concentration\ \left(\frac{GU}{mL}\right) = \frac{\text{qPCR Concentration}\left(\frac{GU}{\mu L}\right) \times Eluate\ Volume\ (500\ \mu L)}{Filtered\ Volume\ (mL)} \quad \text{Eq. 2}$$

3

$$DNA\ Concentration\ \left(\frac{GU}{mL}\right) = \frac{9\frac{GU}{\mu L} \times\ 500\ \mu L}{100\ \text{mL}}$$

$$DNA\ Concentration\ \left(\frac{GU}{mL}\right) = 45\ \frac{GU}{mL}$$

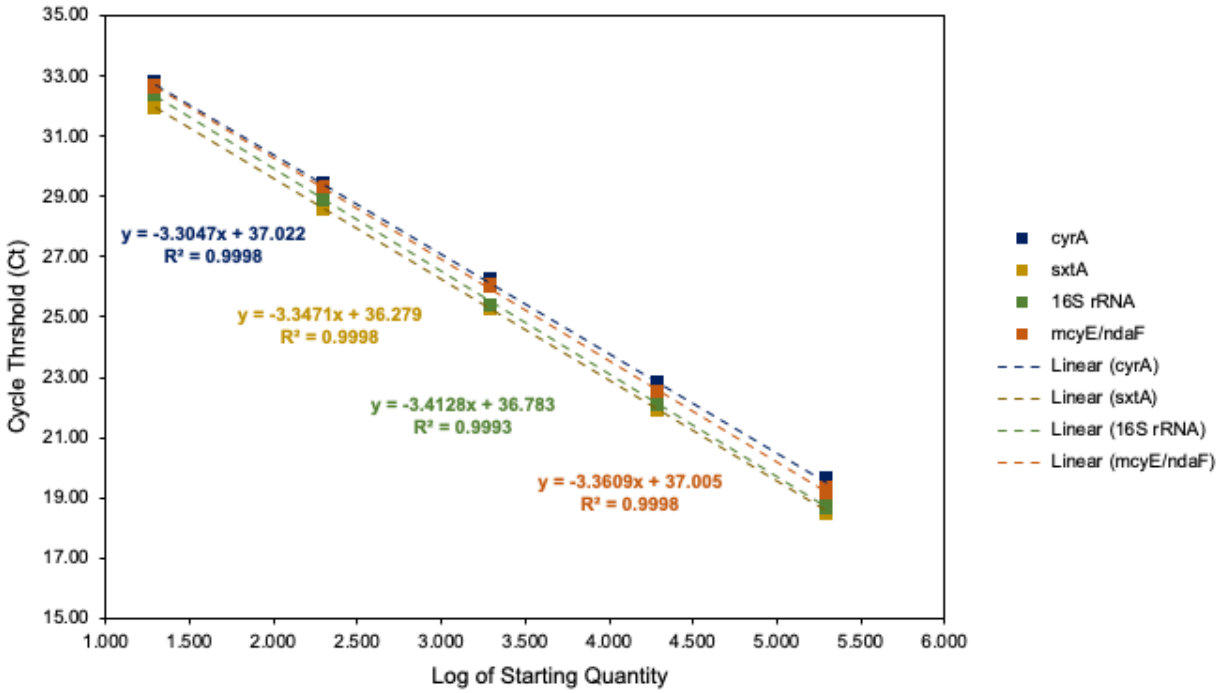
$$DNA\ Concentration\ \left(\frac{GU}{cm^2}\right) = \frac{\text{qPCR Concentration}\left(\frac{GU}{\mu L}\right) \times Eluate\ Volume\ (500\ \mu L)}{Membrane\ Surface\ Area\ (cm^2)} \quad \text{Eq. 3}$$

$$DNA\ Concentration\ \left(\frac{GU}{cm^2}\right) = \frac{9\frac{GU}{\mu L} \times\ 500\ \mu L}{\pi(2.35\ cm)^2}$$

$$DNA\ Concentration\ \left(\frac{GU}{cm^2}\right) = 260\ \frac{GU}{cm^2}$$

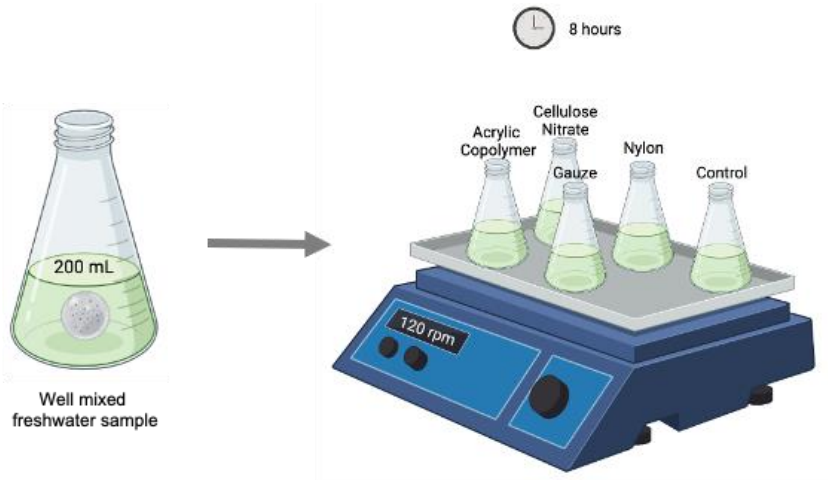
APPENDIX B – qPCR STANDARD CURVES

Standard curves for 16S rRNA, *mcyE/ndaF, cyrA* and *sxtA* gene targets are shown below.

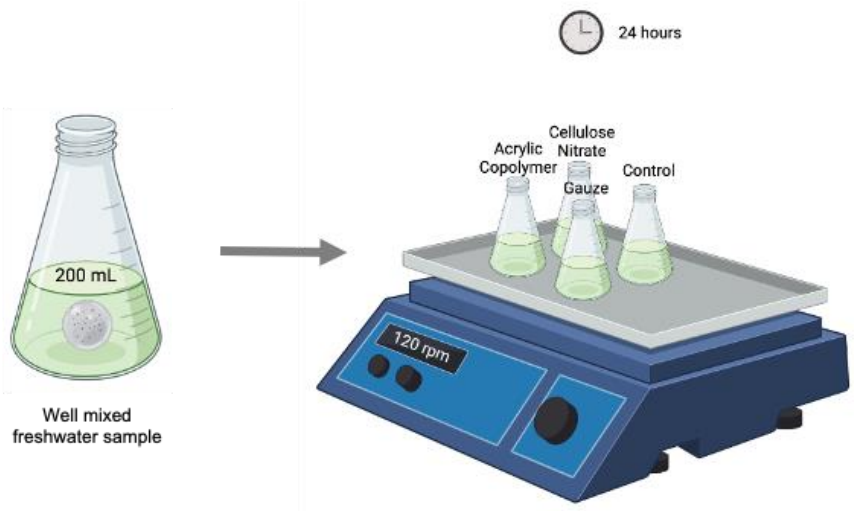


APPENDIX C – EXPERIMENTAL DESIGN FOR ADSORPTION STUDIES

The experimental setup for the 8-h bench-scale adsorption study is shown below.

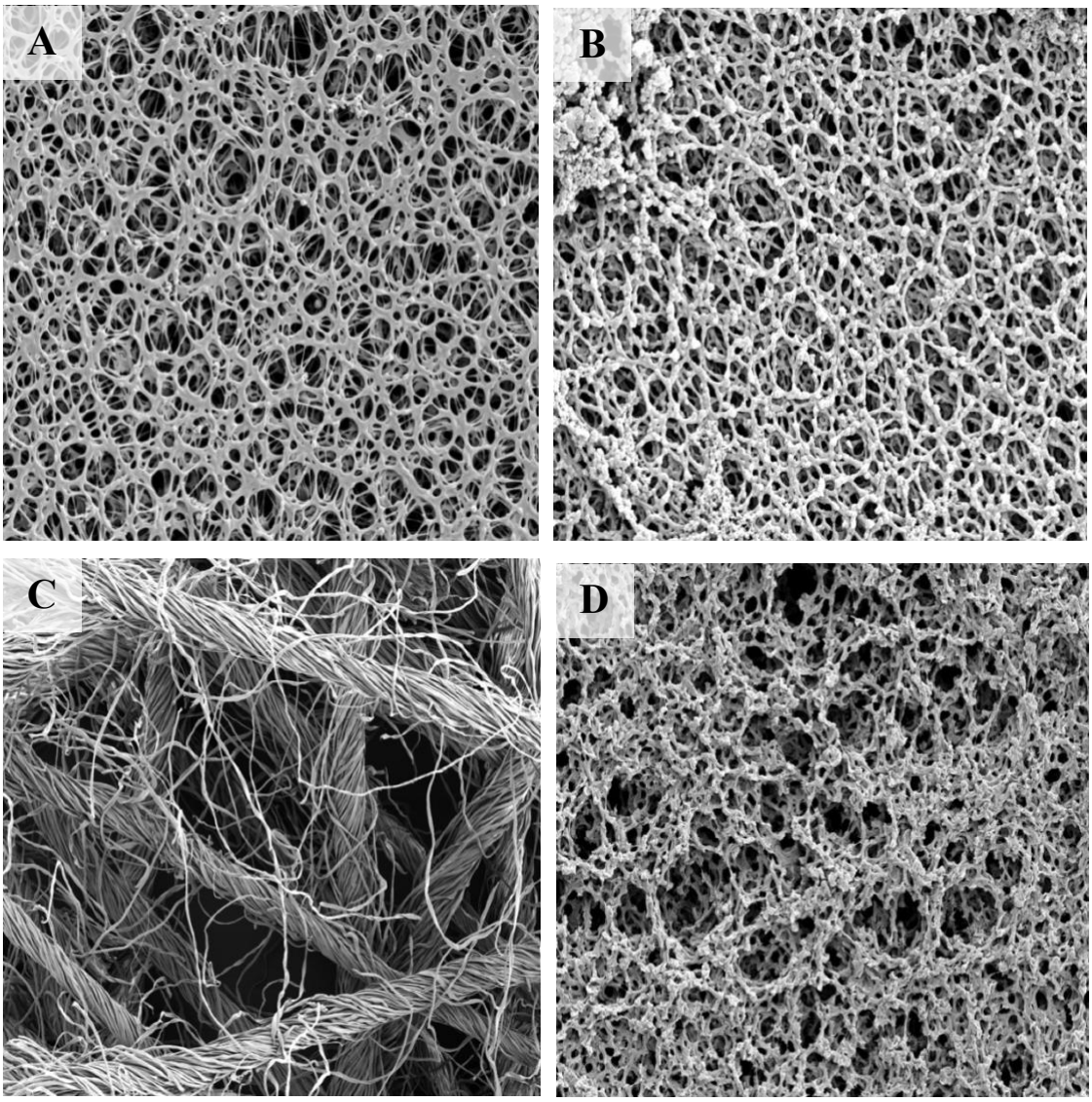


The experimental setup for the 24-h bench-scale adsorption study is shown below.



APPENDIX D – SEM IMAGES

SEM images of A) acrylic copolymer (Mag: 1.2KX, 10 μm), B) cellulose nitrate (Mag: 3.8KX, 2 μm), C) gauze (Mag: 31X, 300 μm) and D) nylon filter membranes (Mag: 1.2KX, 10 μm) are displayed below.



APPENDIX E – 16S rRNA CONCENTRATIONS IN CONTROL SAMPLES

Below are the gene concentrations in GU/mL of water matrices at t = 0 hour and t = 8 hours for each 8-hour batch adsorption experiment.

Lake	Concentration (GU/mL)		Change in Concentration (GU/mL)
	0 hours	8 hours	
Chocolate	2.7×10^4	1.3×10^5	1.0×10^5
Grand	2.5×10^4	2.0×10^4	-5.5×10^3
Lumsden	2.1×10^5	2.9×10^5	8.0×10^4
William	9.2×10^4	7.9×10^2	-9.1×10^4

Below are the gene concentrations in GU/mL of water matrices at t = 0 hour and t = 24 hours for each 24-hour batch adsorption experiment.

Run	Concentration (GU/mL)		Change in Concentration (GU/mL)
	0 hours	24 hours	
1a	1.0×10^6	1.6×10^5	-8.8×10^5
1b	1.5×10^7	3.4×10^5	-1.5×10^7
2a	2.1×10^6	8.6×10^6	6.5×10^6
2b	7.2×10^6	1.3×10^7	5.8×10^6