Bacterial Communities in the Glenmore Reservoir with an emphasis on the Cyanobacteria

by

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Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Lakes and reservoirs play an essential role as a source for freshwater that can be potable after proper treatment, or for irrigation to meet the water needs of the population, industry, and agriculture. However, freshwaters bodies face significant challenges (including pollutant loads and climate change) that may lead to water quality degradation; among these are taste and odour events and harmful bacterial blooms. In Calgary, Alberta, Canada, runoff from agriculture and residential development in urbanized watersheds also contributes to water quality deterioration in the Elbow River, which flows into the Glenmore Reservoir and serves as one of the primary sources of drinking water for the city. While harmful algal blooms (i.e., Cyanobacteria) have not occurred in the Glenmore Reservoir historically, increased pressures (e.g., stormwater discharges, potential erosion and runoff after wildfire) on source water quality in the city's wildfire-prone source watersheds have the potential to increase nutrient (especially bioavailable phosphorus) loading to the reservoir; this can promote algal blooms (City of Calgary, 2018). Therefore, to enable identification of source water quality shifts and treatment challenges that may impact the provision of adequate amounts of safe drinking water, it is important to monitor both water quality and bacterial and cyanobacterial communities within the reservoir.

Although bacterial communities play an important role in aquatic environments, there is typically little information about their assemblages in oligotrophic waters. Hence, the current research focused on identifying and detecting the bacterial and cyanobacterial communities that present in the oligotrophic waters of the Glenmore Reservoir. Samples were taken from four different locations representing the major compartments in the reservoir: Head Pond, Weaselhead, Heritage Cove and Mid-Lake. Open and enclosed filtration systems (i.e., glass fibre filters (GF/F) and Sterivex filter units) were used. Different pore size filters (e.g., 1.6 μm GF/F

and 0.2 μm Sterivex units) were used to ensure detection of both bacterial and cyanobacterial communities. The differences attributable to filtration approach between samples were also investigated. These samples were then sequenced using 16S rRNA gene (V4) to investigate bacterial and cyanobacterial composition in the reservoir and detect any potential taxa that may challenge drinking water treatment.

The major findings revealed the existence of a typical freshwater bacterial community (including: Proteobacteria, Actinobacteria, and Bacteroidetes). Notably, actinomycetes, a type of Actinobacteria, may be metabolically active residents of the reservoir's bacterial community. Actinomycetes bacteria are known to produce geosmin and 2-MIB; thus, they may be potential sources of taste and odor (T/O).

The observed Cyanobacteria community matched several common potential toxin-producing genera, including the picocyanobacterial genus: *Synechococcus* sp., which often dominates and proliferates in oligotrophic habitats. Open and enclosed filtration methods (i.e., GF/F and Sterivex units) both retained bacteria present in the water supply, however, GF/F were associated with greater detection of Cyanobacteria and a larger number of ASVs. This apparent advantage may be due to the size of target organism, the electrostatic charge of the glass fibres, and/or DNA extraction losses. The bacterial community in the Glenmore Reservoir was less complex than that often observed in nutrient-rich waterbodies; accordingly, the trophic status of reservoir likely direct affects bacterial community composition and diversity. Bacterial and cyanobacterial populations in oligotrophic freshwater may be largely driven nutrient availability (trophic status), which may change waterbodies because of increased erosion and runoff associated with the cumulative effects of land use and climate change-exacerbated landscape disturbances, such as wildfires and extreme storms. In this work, the diversity of the bacterial communities from

Heritage Cove significantly differed at surface and secchi depths and had significant dissimilarity from other sites which may be due to differences in flow dynamics in that location. This preliminary analysis can provide baseline information against which subsequent, more detailed investigations of reservoir bacterial and cyanobacterial communities may be compared.

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List of Abbreviations

ASV	amplicon sequence variant
BT	bootstrap
COD	chemical oxygen demand
GSM	geosmin
MC	microcystin
N	Nitrogen
NCBI	National Centre for Biotechnology Information
P	Phosphorus
PCR	polymerase chain reaction
rRNA	ribosomal RNA
TDP	Total Dissolved Phosphorus
TN	Total Nitrogen
T/O	taste and odour
TOC	Total Organic Carbon
TP	Total Phosphorus
TSS	total suspended solids
WQ	Water Quality
WQI	Water Quality Index
WTP	Water Treatment Plant

Chapter 1
Introduction

1.1 Freshwater Bacterial Communities

1.1.1 Quality of Freshwater

Surface water quality deterioration is of increasing global concern (Scanlon et al., 2007; John et al., 2014). Freshwater ecosystems are facing challenges due to high pollutant loads, climate change and other pressures from human activities, urbanization, and agriculture (Todd et al., 2012; du Plessis, 2020). Such deterioration threatens the use of water resources, in particular the provision of adequate amounts of safe drinking water. Although lakes and reservoirs play an essential role in meeting the water supply needs of communities, industry, and agriculture (Jorgensen et al., 2005), the quality of waterbodies from these supplies is at risk due to the potential for increased nutrients loads and other types of water quality deterioration that are not only expected to persist, but also continue to increase over time (Thornton et al., 2013).

Alberta holds approximately 2.2% of Canada's freshwater (Alberta Environment, 2010). Its lakes and rivers serve as the primary source of drinking water for residents and communities. Most of the water supply in Alberta originates from glaciers and high elevation snowpacks in the Rocky Mountains; hence, rainfall and snowmelt are considered the main contributors to annual river flows (Alberta Environment, 2010). As water flows downstream, its quality is cumulatively impacted by the land cover and various land uses (e.g., agriculture, wetlands, and urban areas) (i.e., cumulative watershed impacts) (Bolstad and Swank 1997; Permatasari et al.,2017; Mello et al., 2018). For example, six major river watersheds (i.e., the South Saskatchewan River basin) including the Bow River and the Elbow River, have intensive agricultural activities (Bruneau et al. 2009; Corkal et al.2011; Akbar et al. 2013). Calgary is the most populous city in Alberta.

Some of the highest risks to Calgary's drinking water source quality include stormwater pollution; widespread, high intensity wildfires as well as contamination from wastewater and industrial discharges, use of pesticides on crops, oil pipeline spills and algal blooms (City of Calgary, 2018).

Wildfires are known to impact water quality in several ways that could constitute significant challenges to water quality and treatability. The City of Calgary has indicated that wildfire is one of the key risks to the provision of safe drinking water (City of Calgary, 2018; Robinne et al., 2016; 2019). Wildfires can have significant effects on water quality as they can lead to increases in the amount of precipitation that reaches the landscape; consequently, they increase runoff of contaminants from the landscape (Williams et al., 2019; Moody & Martin, 2001), even in systems with already deteriorated source water quality (Emmerton et al., 2020). After fires, water temperature can increase (Wagner et al. 2014). Water chemistry changes in burned watersheds include higher concentrations of nutrients (Silins et al., 2014; Kunze & Stednick, 2006), suspended sediment (Silins et al., 2009; Kunze & Stednick, 2006), metals (Wolf et al., 2008), other contaminants (Crouch et al., 2006; Kalabokidis, 2000). Of important note, sediment-associated bioavailable phosphorus (P) can be released to the water column for decades following some wildfires, contributing to the proliferation of microorganisms (i.e., bacteria and algae) (Emelko et al., 2016; Stone et al., 2014), increased abundance and diversity of macroinvertebrates (Martens et al. 2019), and generally more variable water quality (Stone et al., 2011). These changes in water quality can pose significant and costly challenges for drinking water treatment (Price et al., 2017; Emelko and Sham, 2014; Emelko et al., 2011).

Runoff from the agriculture and residential developments in the urbanized portions of the watershed already contributes to some deterioration of Elbow River water quality flowing into

Calgary's Glenmore Reservoir (Sosiak and Dixon 2006; Akbar et al. 2013; City of Calgary, 2018). While algal blooms or toxin production have not historically occurred in the Glenmore Reservoir, wildfire has the potential to increasing nutrient loading to the reservoir, which can promote algal blooms (City of Calgary, 2018). Therefore, it is important to monitor both water quality and bacterial and cyanobacterial communities within the reservoir.

Freshwater bacterioplankton are important parts of freshwater ecosystems and play essential roles in the biogeochemical cycle (Newton et al., 2011). They are positively influenced by nutrient availability (Logue et al. 2011; Jankowski et al.2014). These bacterial and cyanobacterial communities are susceptible to environmental perturbations (e.g., fluctuations of water quality parameters due to severe weather events) (Jiao et al., 2018; Graves et al., 2016; Labbate et al., 2016), and may show immediate reactions to sudden water quality parameters changes (Woodhouse et al., 2016; Bergkemper and Weissie, 2017; Bergkemper et al., 2018; Ren et al.,2019). For example, water temperature, turbidity, conductivity, and bioavailable phosphorus are the most vital environmental factors that might affect the membership and distribution of bacterial communities. Exacerbated by climate change, extreme weather events (e.g., heatwaves, heavy rainfall) are expected to increase and can disturb ecosystem function (e.g., nutrient flux and alternation of bacterial community compositions) (Yang et al., 2012; Bergkemper et al., 2018). Thus, it is important to understand land disturbance impacts on bacterial communities, in which shifts may lead to changes in water quality that may be gradual or rapid (Arheimer et al., 2005; Silins et al., 2014).

1.1.2 The Glenmore Reservoir

Reservoirs are defined broadly as any human-made lakes, whether they be embedded in a river network or not (Hayes et al., 2017). They are generally built for specific purposes that

typically include municipal drinking water, agricultural and industrial supplies, flood risk reduction, and hydropower production (Yasarer and Sturm,2016; Fluet-Chouinard et al., 2017). There are two major types of reservoirs: the reservoir can be built on the line of the watercourse (i.e., online) by constructing a dam across the watercourse and the reservoir can be built offline by constructing a bunded basin adjacent to the watercourse (Patterson et al. 2016). Reservoirs receive water input from heavy rains (low flows in the winter and high flows in the spring) and runoff from surrounding lands and groundwater (Walker et al. 2007; Calgary's water security report, 2020). All reservoirs store water, though water use may vary, (e.g., irrigation reservoirs, hydroelectric reservoirs, recreational reservoirs, standard reservoirs, and flood control reservoirs) (Nilsson, 2009). Indeed, the Glenmore Reservoir provides flood protection, supplies about ~40% of the water needs, and serves as a popular site for non-motorized boating and recreation (The City of Calgary's source water protection, 2018).

The Glenmore Reservoir (3.84 km^2) is a freshwater reservoir (online reservoir) located in the southwest portion of Calgary, Alberta (**Figure 1.1**). The Glenmore Water Treatment Plant's intakes are located in the reservoir at the dam. Dam construction in 1933 resulted in the formation of an in-line reservoir. The dam was built to provide high-quality source water; ~50% of the city receives drinking water from this source after conventional treatment (Hollingshead et al. 1972, Satchwill, 2001, Watson, 2004). The depth of the reservoir ranges from 1 m at the inflow to 21 m at the dam; meanwhile, the average depth is 7.4 m. As previously mentioned, the Glenmore Reservoir provides flood protection in addition to water supply.

The Glenmore Reservoir is classified as oligotrophic (Satchwill, 2001; City of Calgary, 2018). Although periodic taste and odour events have been observed in the reservoir (Satchwill and Watson, 2007), it tends to maintain low algal productivity (i.e., low nutrients and high raw

water quality), which is preferable for drinking water treatment (City of Calgary, 2018). Stormwater and agricultural runoff may are the likely main contributors to water quality deterioration in the Elbow River, though the specific sources have not been characterized (Sosiak and Dixon, 2006). Since the reservoir is supplied by the Elbow River, it is likely that point and non-point source pollutant entry to the reservoir may increase in the future as a result of urban sprawl (Hart et al. 2016). Thus, associated increases in the abundance of problematic bacterial and cyanobacterial taxa in the source water that would require more costly water treatment could also be reasonably expected. To date, problematic taxa (e.g., toxin-producing, taste and odor compound producing) taxa have not been detected in the Glenmore Reservoir.

Dams are often required for meet human demands for water (e.g., drinking water storage, flood mitigation), however, they can cause intense environmental shifts, such as decreasing the water flow and changes in physico-chemical characteristics of the water (e.g., decreased turbulence, changes in nutrient dynamics and levels of dissolved oxygen) (Bucci et al., 2015). In addition, dam construction can cause changes in bacterial diversity and community composition (Domingues et al. 2012; Li et al. 2013; Luo et al., 2020). These changes can affect the quality of the water as well as the number and diversity of organisms that thrive at different ambient water quality conditions (e.g., river ecosystem) (Pimenta et al., 2012). Water quality degradation a to dam construction is not fully understood, however, it can cause shifts in bacterial communities in water columns and sediments (Luo et al., 2020). It is important to maintain high-quality drinking water reservoir to reduce treatment costs and to provide safe drinking water for the communities (Röske et al., 2012).

(A)

(B)

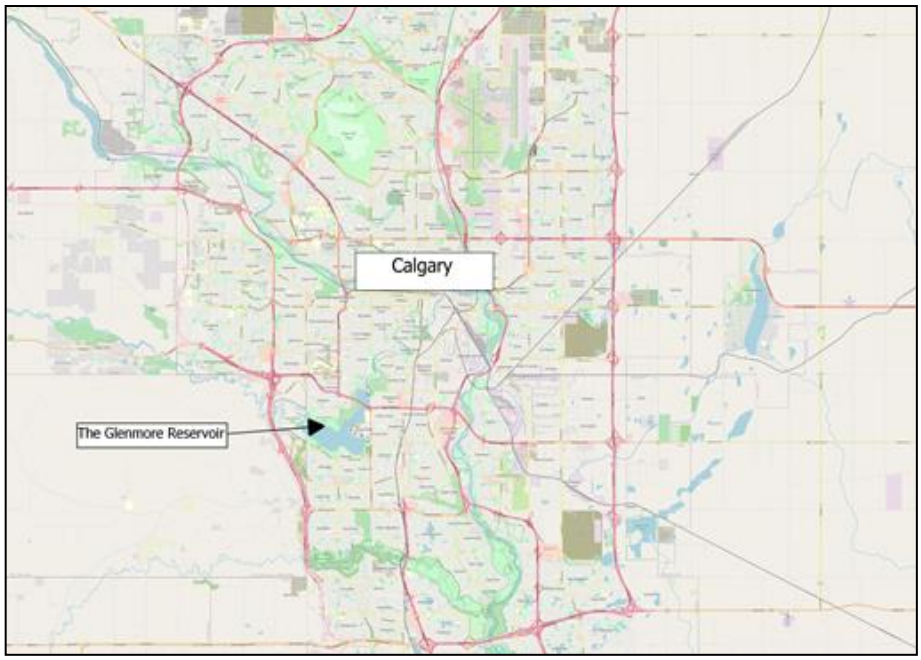


Figure 1. 1 (A) Aerial photo of the Glenmore Reservoir in Southwest Calgary, Alberta. The photo taken in 2016 -by www.stockaerialphotos.com. (B) A map shows the location of the Glenmore reservoir-map created by QGIS.org.

1.1.3 Nutrient Inputs in The Reservoir

Primary productivity in reservoirs is controlled by the quantity and density of both internal and external sources of nutrients. Nutrient limitation to primary productivity and other biological processes is widespread in freshwater ecosystems, and nitrogen (N) and phosphorus (P) are the most common limiting elements, both individually and in combination Nutrients are essential elements that are utilized for microbial growth (Merchant and Helmann, 2012). Non-point source pollutants from land-use activities (e.g., industrial, residential, and agricultural areas) may include solids, nutrients, and contaminants. Nutrient loads (nitrogen (N) and phosphorus (P)) can enter the reservoir from different sources includes bedrock, atmospheric degradation, fine sediments, and agriculture and urban activities (Carpenter et al.,1998; Walker et al., 2007; Akbar et al. 2013; Wurtsbaugh et al. 2019). Fine-grained sediment is the primary vector for P transport and delivery in aquatic systems; it can release bioavailable P to the water column (Österling et al. 2010). High concentrations of bioavailable nutrients in the water column, especially P and N, can cause excessive bacterial and cyanobacterial growth and degraded water quality (Walker et al. 2007; Wilhelm et al. 2011; Filstrup and Downing, 2017). P and N are key nutrients that facilitate bloom-forming microorganisms resulting in an apparent worsening of water quality.

Landscape disturbances (i.e., episodes that trigger distinct change in a certain environment that may affect local community) (Glasby and Underwood, 1996) may cause short-term disruption in aquatic bacterial composition (Sade et al. 2011; Shade et al. 2012; Shabarova et al. 2021). Bacterial communities underpin resistance (the ability for an ecosystem to remain unchanged when being subjected to a disturbance or disturbances) and resilience (the ability and rate of an ecosystem to recover from a disturbance and return to its pre-disturbed state) of these functions. Several studies have been conducted to assess bacterial community recovery after

disturbances (e.g., heavy rainfalls and floods). For example, Shade et al. (2012), Tseng et al. (2013) and Shabarova et al. (2021) demonstrated that the bacterial community in an aquatic environment was less resistant, but highly resilient to artificial and natural disturbances. In eutrophic aquatic ecosystems, the bacterial community can recover from washing off from extreme short-term disturbances (e.g., floods) in around two weeks period (Shabarova et al. 2021). Extreme weather events, such as heavy rainfall (i.e., floods), which Calgary prepares for annually, are recognized as such potentially impactful landscape disturbances.

1.1.4 Bacterial Communities

In any aquatic ecosystem, bacterial communities are the heart of all ecosystem functions, and thus play an essential role in the breaking down organic matter and nutrient cycling (Pace, 1997). Therefore, bacterial community shifts are closely related to the water chemistry, nutrient concentrations, and diversity of dominant microorganism groups (Jankowski et al., 2014; Logue et al., 2016). Alteration in bacterial community composition has been observed at different stages of harmful cyanobacterial blooms (Woodhouse et al. 2016). For example, in one study, members belonging to Bacteroidetes (heterotrophic bacteria) increased in relative abundance after the development of harmful algal blooms (Newton et al., 2011). In another study, Xu et al. (2018) showed that the occurrence of heterotrophic bacteria belong to the phyla Actinobacteria, Proteobacteria, Bacteroidetes, and Chlorobi might be associated with increased Cyanobacteria. Thus, changes in bacterial community composition could be coupled with cyanobacterial growth (i.e., cyanobacterial harmful blooms**)** in freshwater (Woodhouse et al. 2016).

Changes in source water quality such as increases in organic matter in a reservoir tend to be associated with increases in bacterial biomass, growth rate and activity (Llirós et al. 2014; Yu et al. 2014; Zhang et al. 2015; Zhou et al. 2019). Thus, it is essential to investigate bacterial and

cyanobacterial communities within reservoirs and their relationships to changing environmental conditions. For example, a study by Niu et al. (2019) investigated water quality and bacterial community composition; the results showed that physico-chemical water quality (e.g., PO_4^{3-} and COD) affected bacterial community composition. Bacterial community is one of key factors associated with source water quality change, and the dynamics and structure of these communities can help to better understand the correlation between water quality parameters and bacterial communities.

Taste and odour events can cause significant consumer complaints and decrease consumer confidence in the quality of treated drinking water; they may also require costly treatment. Some cyanobacterial and bacterial taxa may produce off-flavours; for example, geosmin (GSM) (trans-1, 10-dimethyl-trans-9-decalol) and 2-MIB (2-methylisoborneol), which are the most common odorous chemicals, though neither compound is harmful at levels present in drinking water (Watson et al., 2016; Huang et al.2018; Zhang et al. 2019). Geosmin-producing microorganisms are often affiliated with Actinomycetes and Myxococcales, as well as to the phylum Cyanobacteria *(*Dickschat et al.*,2005;* Zaitlin and Watson, 2006*;* Asquith et al.,2013; Zhang et al. *2019).* In addition, *Synechococcus* (Cyanobacteria) have positively correlated with 2-MIB in freshwater (Zhang et al. 2020), while *Planktothrix* species was identified as the main source of GSM (Clercin and Druschel, 2019). GSM and 2-MIB have been reported as musty/earthy odour compounds in drinking water, which are often related to presence of some cyanobacteria in source water (Nam-Koong et al., 2016; Lee et al., 2017) (**Table 1.1**) (**Table 1.2**). When large numbers of algae and bacteria flourish in reservoirs and other environmental factors (e.g., water temperature, nutrient availability) are also favourable for the growth of bacterial producers, T/O compound concentrations also often increase, resulting in unpleasant water taste and smell. In

the literature, there have been reported T/O events caused by members of Chrysophytes and Diatoms in the Glenmore Reservoir (Watson et al.,2001; Watson, 2004; Satchwill et al., 2007). They include the Elbow River, in which there was evidence of several isolates by Actinomycetes reported (Zaitlian wt al., 2003). Analysing the bacterial and cyanobacterial communities in the Glenmore Reservoir would provide important baseline information regarding what to monitor and how to best manage risks related to the future possible occurrence of T/O events.

1.1.5 Cyanobacteria

Cyanobacteria are prokaryotic, photosynthetic organisms that lack membrane- bound organelles and have unique physiological and morphological characteristics (Bellinger and Sigee, 2015). Cyanobacteria in the upper water column have traditionally been the only quantitatively important N_2 fixers (diazotrophs) (Zehr, 2011). Different species of cyanobacteria in lakes and reservoirs that are used as water sources and may form harmful blooms that negatively affect water quality, can be planktonic or benthic (Chapman, 2010; Pelaez et al., 2010; Herrera et al. 2014; Fadel et al. 2019). In addition, some cyanobacteria planktonic species produce undesirable T/O in freshwater ecosystems, such as *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) (*Anabaena circinalis*), *Anabaena crassa* (Lemmermann) Komárková-Legnová & Cronberg 1992, *Aphanizomenon gracile, Aphanizomenon flos-aquae, Microcystis aeruginosa, Microcystis botrys, Oscillatoria limnetica* and *Spirulina platesis*, *Synechococcus* sp.. Toxins that can harm vertebrates, including humans, may also be produced (Paerl and Trucker, 1995; Chapman, 2010; Watson et al. 2016; Zhang et al. 2020).

Table 1. 1 Common Major T/O-causing Compounds in Freshwater

Characteristics	Odorants
Earthy	Geosmin (GSM) (Wood et al., 2001; Dzialowski et al.,2009; Guttman and van Rijin, 2012).
Musty	2-methylisoborneol (2-MIB) (Wood et al., 2001; Guttman and van Rijin, 2012).
Grassy	nonanal, decanal (Guo et al., 2021)
Fishy	2,4-decadienal (Zhao et al., 2013 ; Guo et al., 2021)

1.1.6 Cyanotoxins

Numerous genera of cyanobacteria are able to produce cyanotoxins (Sivonen and Jones, 1999; Pearson et al.,2010; Rastogi et al 2014) that can be very potent and cause damage or death to animals and humans after acute exposure (Carmichael, 2001, Bownik 2010; Drobac et al., 2016). Generally, cyanotoxins are divided into hepatotoxins (e.g., microcystins, nodularins, cylindrospermopsins), neurotoxins (e.g., anatoxin-a, homoanatoxin-a, saxitoxins) and dermatotoxins (e.g., lyngbyatoxins, aplysiatoxins). The most common and well-studied producers of cyanotoxins are *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Anabaena flos-aquae*, *Cylindrospermopsis raciborskii*, *Planktothrix agardhii*, *Lyngbya majuscula*, *Nodularia*, *Synechococcus* and *Oscillatoria* (Chorus and Bartram ,1999; Rao et al.,2002; Carmichael and Li, 2006; Koker et al.,2017) (**Table 1.2**). Trophic status affects toxin-producing cyanobacterial community; specifically, cyanobacteria blooms are often associated with higher trophic status (Koker et al.,2017). In contrast, they might be absent or in low abundance in

oligotrophic reservoirs (Lie et al., 2012). Communities in these environments include often non-bloom formers (i.e., picocyanobacteria) (Callieri et al., 2012; Fukushima et al., 2017). High cell concentrations of *Microcystis* sp. in freshwater that correspond to cyanotoxin concentrations that exceed guideline or health alert levels lead to water quality degradation and decrease biodiversity (Smith, 2003; Olson et al. 2020).

There is no definitive account of the number of cyanobacteria blooms experienced in Canada each year. In 2018, nearly 150 incidences of waterbodies affected by cyanoblooms were reported on provincial government or health authority websites across Canada (O'Keeffe, 2019). The highest numbers of waterbodies affected by blooms were found in Ontario (66) and Alberta (44) (Health Canada, 2018; O'Keeffe, 2019). Most provinces apply decision protocols for responding to a bloom based on Health Canada guidance with some variations (Health Canada, 2018). The flow chart in **Figure 1.2** shows the steps for routine monitoring and bloom response in two phases: routine monitoring or during a bloom. Each province has their own approach to bloom detection and cyanotoxin monitoring and there is no standard approach to sample collection or analysis (Carmichael and Boyer, 2016).

Microcystin is widely found in Alberta waterbodies, with toxin typically occurring in 75% to 96% of waterbodies monitored annually (Health Canada, 2018). Although microcystin concentrations are typically low (i.e., up to 0.5 µg/L), in some cases, concentrations > 10 µg/L might occur (Health Canada, 2018). In 2011, microcystin was detected in source waters serving nine of 23 water treatment facilities submitting data, however, the concentrations of microcystin ranged from 0.1 to 0.6 µg/L; these were all below the Alberta guideline value of 1.5 µg/L (Alberta Environment, 2012). As indicated above, algal harmful blooms (i.e., Cyanobacteria) have not historically occurred in the Glenmore Reservoir (City of Calgary, 2018).

Table 1. 2 Summary of some Potential Toxin and Taste-and-Odour Producing Cyanobacteria Present in Blooms in Freshwater Samples. Graham et al. (2010).

Cyanobacteria genera	Potential Toxins	T/O Compound
Anabaena	Anatoxins, cylindrospermopsins, microcystins, saxitoxins	Geosmin
Aphanizomenon	Anatoxins, cylindrospermopsins, saxitoxins	Geosmin
Aphanocapsa	Microcystins	-
Cylindrospermopsis	Cylindrospermopsins, saxitoxins	-
Microcystis	Microcystins	-
Planktothrix (*Oscillatoria*)	Anatoxins, lyngbyatoxins, microcystins, saxitoxins	Geosmin, 2-methylisoborneol
Anabaenopsis	Microcystins	-
Limnothrix	Microcystins	-
Planktolyngbya	Lyngbyatoxins, saxitoxins	Geosmin, 2-methylisoborneol

1.2 Bacterial Community Analysis

Studying the bacterial and cyanobacterial communities within an oligotrophic source water can inform the dynamics of these communities in a low-nutrient freshwater environment. While surrogate parameters are used in many microbial fate and transport characterizations (e.g., Tufenkji and Emelko, 2011; Mesquita and Emelko, 2012; Zheng et al., 2019), anticipating community function requires an understanding of community composition. The vast improvements in DNA sequencing technologies over the past few decades facilitate the study of bacterial diversity (Reuter et al., 2015). Different methods and pipelines of next-generation sequencing have remarkably influenced the analysis of bacterial community composition. The 16S rRNA amplicon sequencing methods have been widespread which is used to analyze the whole set of bacterial communities present in a target environment (Lundberg et al., 2013).

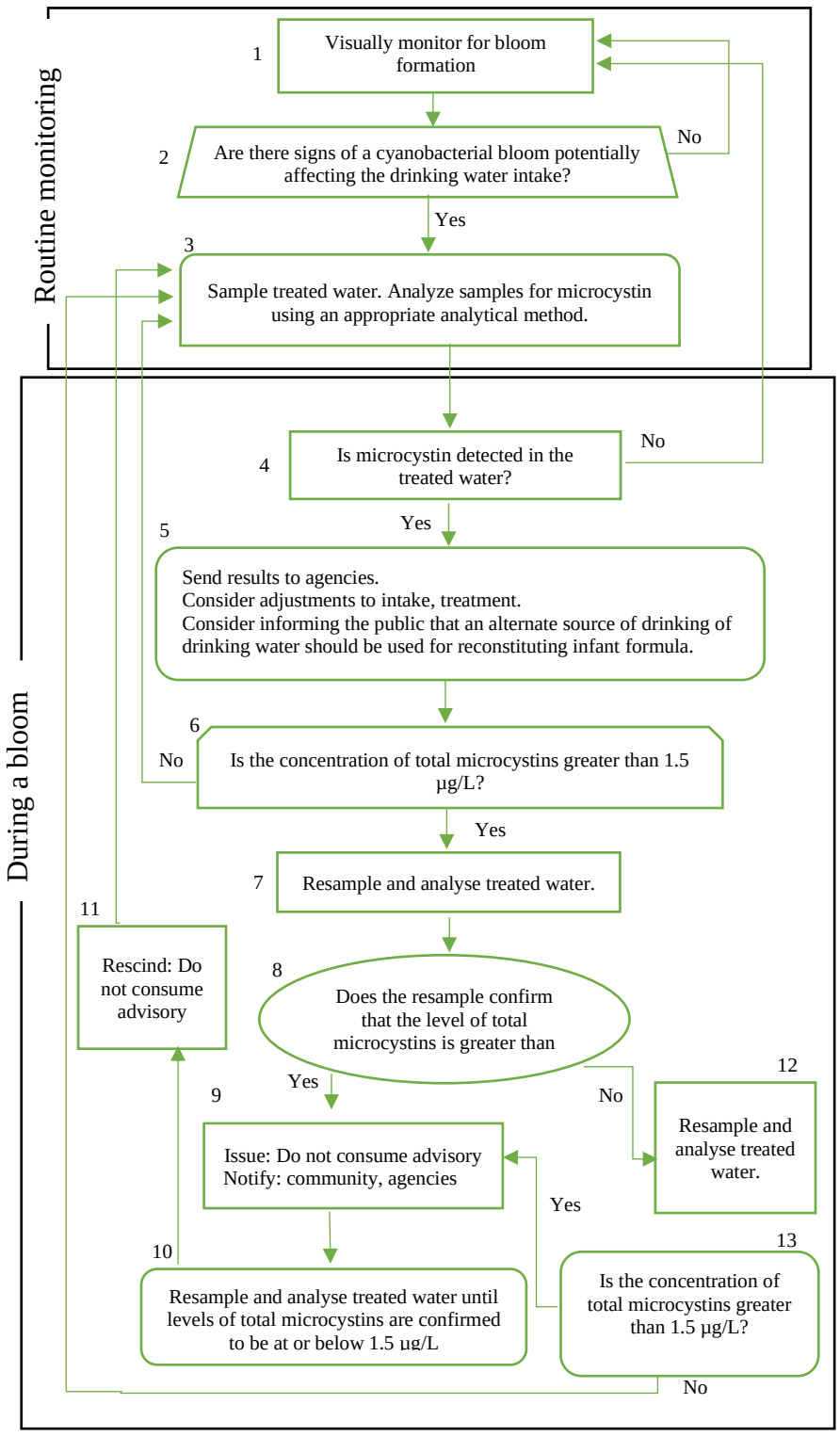


Figure 1. 2 Health Canada flow chart for routine monitoring and bloom response, modified from (Health Canada, 2018).

1.2.1 16S rRNA Gene Amplicon Sequencing

Amplification and sequencing of the 16S rRNA gene is commonplace for bacterial community characterization as well as current targets of study in phylogeny and ecology (Hugenholts et al., 1998). Because of its presence in almost all bacteria, the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and the 16S rRNA gene (1,500 bp) is large enough for informatics purposes (Hugenholts et al., 1998; Patel, 2001). The 16S rRNA gene is still subject to variation, especially in certain variable regions. Although much of the 16S rRNA gene is highly conserved, there are nine variable regions that allows sufficient diversification to provide a tool for classification and can be used to distinguish different species of bacteria and archaea (Head et al., 1998). Although the entire 16S rRNA gene can be sequenced, the presence of conserved regions allowed the design of suitable PCR primers or hybridization probes for various taxa at different taxonomic levels (Lundbeg et al., 2013). Study of the V4 (~254 bp) region has increased, as studies on estimates taxonomic assignments as well as estimates of alpha and beta diversity (Youssef et al., 2009; Liu et al., 2007; Wang et al., 2007). The associated approach, which includes variable sequencing depths, is targeted to generate meaningful information about the taxonomic structure and profiling microorganisms in communities from many samples, without sequencing entire genomes (Zaheer et al., 2018).

The amplicon post-sequencing analysis process involves sequence comparison to a database of 16S rRNA gene sequences (including NCBI database) and assignment of taxonomy based on the best database match (Balvočiūtė et al., 2017). A commonly used analytical pipeline for this task is Quantitative Insights into Bacterial Ecology version 2 (QIIME2), coupled with a de-noising and error correction tool called DADA2 (Callahan et al., 2016; Bolyen et al., 2019).

Using these tools, it is possible to organize 16S rRNA gene amplicons into amplicon sequence variants (ASVs), which are unique sequences that differ by as little as single nucleotide (Callahan et al., 2017). The relative abundance of bacterial and cyanobacterial communities can then be compared between different water column samples, locations, and depths to identify significant differences in community composition.

1.3 Thesis Objectives

This study aimed to identify the bacterial communities present in the Glenmore Reservoir in July of 2017 using 16S rRNA gene amplicon sequencing, with an emphasis on the presence of Cyanobacteria. This study serves as a baseline data set regarding the bacterial communities present in the reservoir before the water levels were lowered during the following year (2018). This baseline informs risk management and advance preparation for future source water quality-associated threats such as algal blooms and T/O events. The following questions were addressed:

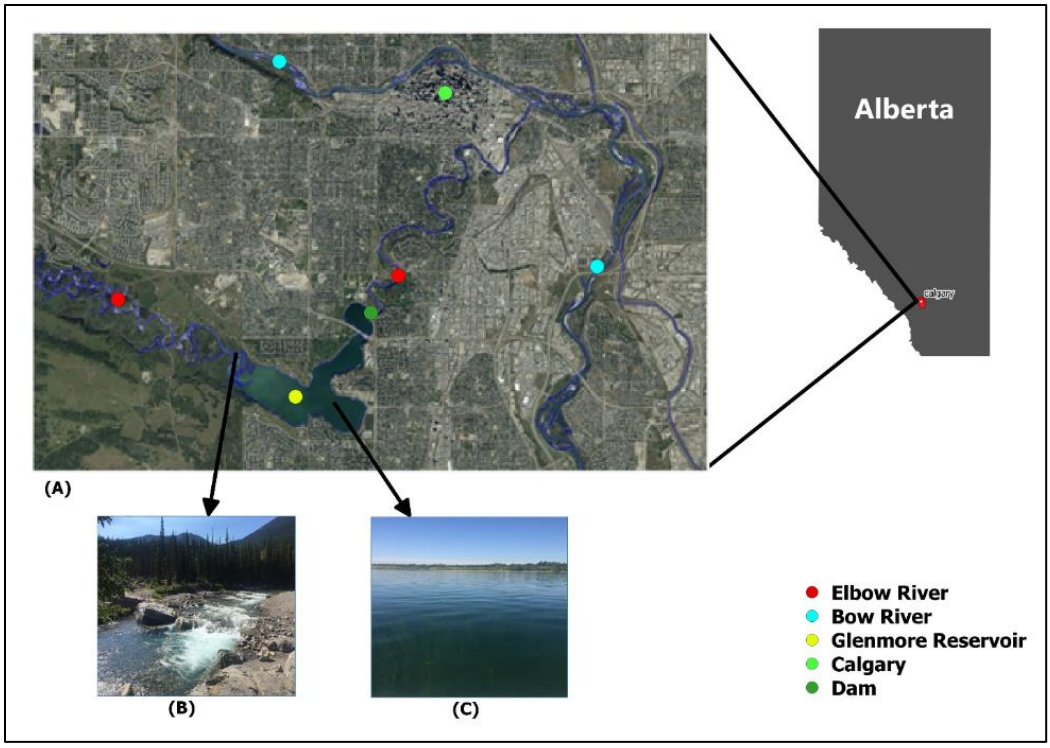
a) Are the bacterial communities in various parts of the reservoir similar or different?
b) Are there differences in the proportion of bacteria between two depths (surface and secchi) at each sampling location?
c) Are there taxa present that may lead to concerns regarding the quality or treatability of drinking water (e.g., toxin produces, taste & odour)?
d) Are there significant differences between the results obtained from samples filtered on glass fibre filters and Sterivex filter units?
e) If there are differences among the sampling locations, are they due to different environmental and water quality conditions?

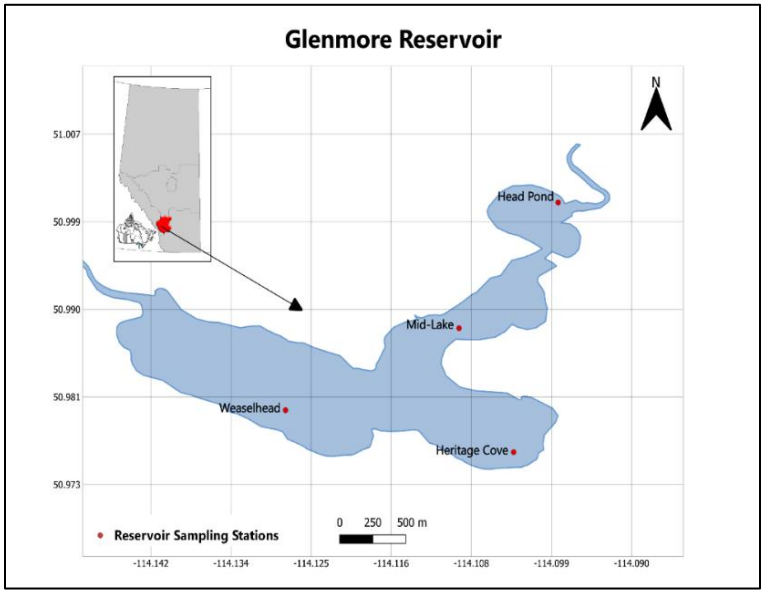
Chapter 2
Materials and Methods

2.1 Study Site

The Glenmore Reservoir

The Glenmore Reservoir (50°59′00″N to 114°08′07″W) is an artificial impoundment located on the Elbow River on the southwest portion of Calgary, Alberta, Canada (**Figure 2.1**) (Alberta Environment, 2007). It is a large reservoir with a water storage capacity of 17,642 m^3 with 75% of Total Capacity (Alberta Environment, 2008); its depth ranges from 1 m at the inflow to 21 m at the dam, with a depth of approximately 7.4 m. This reservoir (3.84 km^2) is divided into four compartments: Weaselhead, Heritage Cove, Mid-Lake, and Head Pond (**Figure 2.1 d**). Weaselhead is closest to the Elbow River inflow on the west end of the reservoir and is located near the center of the compartment. Heritage Cove is in the southeastern portion of the Glenmore Reservoir. Mid Lake is the most centered location in the Glenmore Reservoir and is close to the golf club and local hospital. Lastly, Head Pond is in the Glenmore Reservoir's northernmost location, closest to the Glenmore dam and drinking water intake.





(D)

Figure 2. 1 (A) Location of the Glenmore Reservoir, the Elbow, and the Bow Rivers southwest of Calgary in Alberta, Canada- the map was adapted from Google Earth. (B) The Glenmore Reservoir is an oligotrophic drinking water reservoir -The photo was taken in July 2017. (C) The Elbow River - the photo was taken in July 2017. (D) Geographic location of 4 different sample sites in the Glenmore Reservoir- map created using QGIS.org.

2.2 Sample Collection and Environmental Data Collection

A boat was taken offshore and anchored. Water samples were collected in each of the reservoir compartments on the 27th of July 2017 using horizontal Van Dorn sampler to obtain 1 L of water at depths (1) within 1 m of the water surface and (2) secchi depth (i.e., the depth below the surface at which the clarity disappears). The secchi disk was lowered vertically into the water at each of the four sampling locations. The secchi depth of Head Pond, Heritage Cove and Mid-Lake was ~5m, at Weaselhead it was ~4.4m. The collected water samples were vacuum filtered (1L) through a 1.6 μm glass fibre filter (Whatman GF/F; 47 mm diameter), then stored individually in polystyrene Petri dishes (50 mm plastic petri dish). After the filtration, the remaining water samples were used to filter bacterial samples using 50ml syringes and EMD Millipore filters (0.2 μm Sterivex filter columns) (EMD Millipore Corporation, MA, USA). Samples were kept in a cooler and surrounded by ice packs until they were delivered to the lab. Once received by the lab, samples were stored at -20°C until analyzed. Figure 2.2 shows the summary of the overall methodology followed in this study.

Environmental data were collected at each of the sampling sites (**Table 3.2**). Latitude and Longitude were determined using the Gaia GPS app (Trailbehind Inc.). Temperature and pH were measured using a pH/Conductivity Meter (Cole-Parmer, Montreal, Canada).

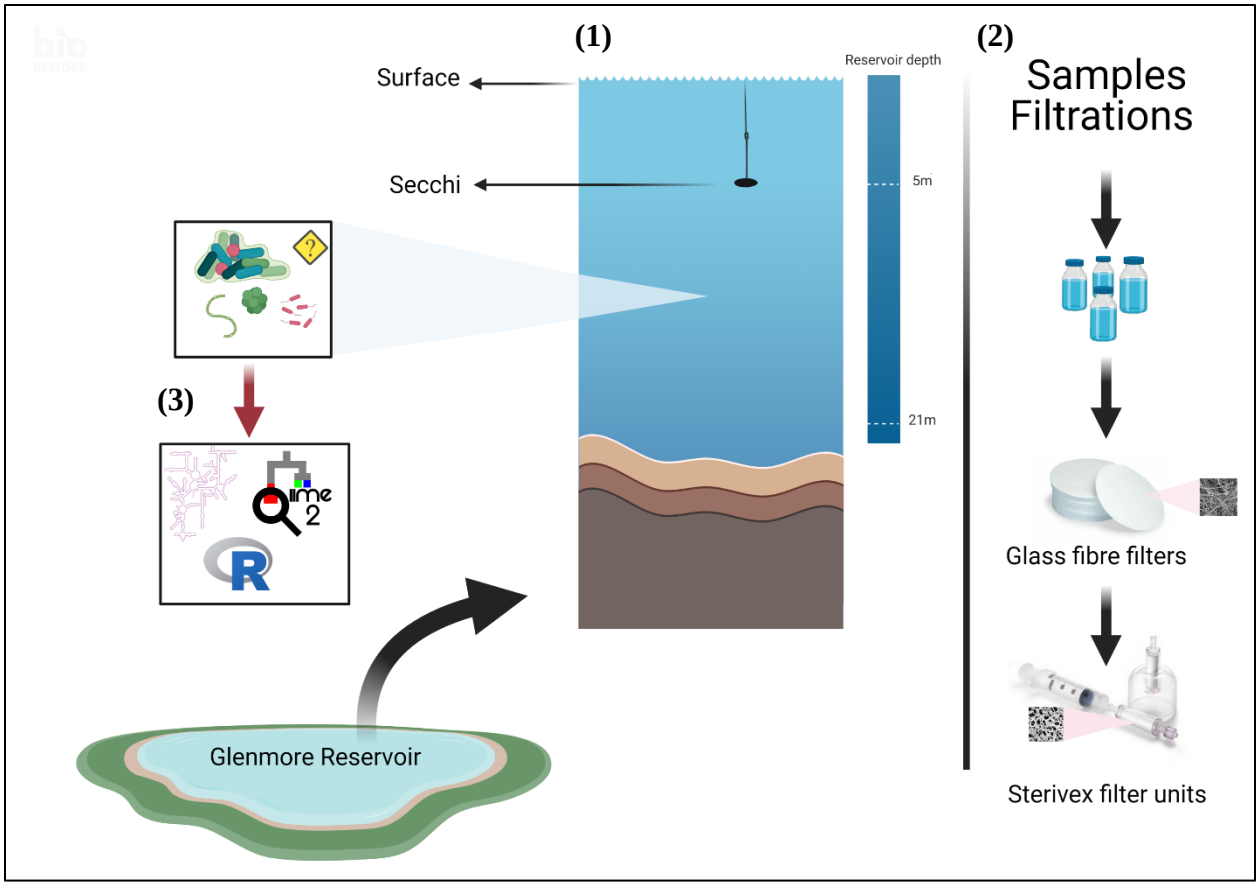


Figure 2. 2 Summary of the general methodology steps followed in this study. **(1)** Water samples collected from the four sites at the Glenmore Reservoir from the surface (1 m below the surface) and at the secchi (5 m below the surface). **(2)** Two methods of filtration were used: glass fibre filters and Sterivex filter units. **(3)** After DNA extraction, raw data were analyzed: 16S rRNA gene amplicon sequencing, analytical pipeline (QIIME2) and statistical analyses (R package). [Figure created with BioRender.com].

2.3 DNA Extraction and Sequencing of 16S rRNA Genes

For the GF/F samples, extraction was carried out according to the manufacturer's instructions. The Qiagen DNeasy® PowerSoil kit (QIAGEN Inc.,Venlo, Netherlands) was used to extract DNA from a glass fibre filter to provide high DNA yields and quality. The filters were handled aseptically. Each filter was cut in half, half stored at -20°C and the other half used for the DNA extraction. Each half used for DNA extraction was cut into smaller pieces and turned to a paste

using a mortar and pestle. Following the kit protocol for each sample, genomic DNA was quantified using a NanoDrop 2000 (Thermo Scientific, MA, USA). 20 µl of each prepared sample were submitted for amplicon sequencing using the Illumina MiSeq platform (Illumina Inc., San Diego, United States) at a commercial laboratory Metagenom Bio Inc. (Waterloo, Ontario). For the Sterivex samples, the units were also submitted to Metagenom Bio Inc. (Waterloo, Ontario) for amplicon sequencing using Illumina MiSeq platform (Illumina Inc., San Diego, United States). Using the resulting PCR products, the 16S rRNA gene hypervariable region four (V4) was sequenced. Paired-end sequencing (2 x 250 bases) was performed on a MiSeq Illumina (2000). Primers specific to the 16S V4 region used were 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Parada et al., 2016) (Apprill et al., 2015). Raw sequences are available in the Sequence Read Archive (SRA) at NCBI under accession numbers PRJNA721744 https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA721744 and PRJNA721750 https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA721750.

A phylogenetic tree was constructed for the cyanobacterial 16S rRNA gene sequences of 11 isolates derived from this study. The DNA sequences were aligned and compared with sequence data available in the NCBI database (**Table 2.1**) using the ClustalW alignment algorithm. All positions containing gaps and missing data were eliminated from the dataset. A maximum likelihood (ML) phylogenetic tree of variable region (V4) in the 16S rRNA gene sequences of cyanobacteria from the Glenmore Reservoir was constructed by MEGA X analysis software (Version 10.1.7). The ML phylogenetic tree was constructed using the General Time Reversible model. Samples compared to known cyanobacterial sequences from the NCBI database. A BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) was performed using the program "blastn".

Values above branches represent % bootstrap support using 1000 replicates. Only values above 70% were shown. *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli* and *Shigella dysenteriae* were used as outgroups and to root the phylogenetic tree.

2.4 Bioinformatics Analysis

2.4.1 QIIME2 Analysis

The raw sequence data were processed using QIIME2 software (Kuczynski et al.,2012). Subsequent FASTQ files (i.e., forward and reverse reads) were imported into QIIME2 (Bolyen et al., 2019). Reads were then denoised, dereplicated, and merged using DADA2 within QIIME2. Reads with low quality scores were excluded. This produced a feature table containing amplicon sequence variants (ASVs). Sequences were classified in QIIME 2 with a Naive Bayes classifier trained with the April 2018 SILVA release 132, 97% taxonomy classification for the 16S rRNA. The feature table was collapsed to the phylum level.

2.5 Statistical Analysis

Output files from QIIME2 were imported into R (v.3.6) for Linux (www.r-project.org) where all statistical analyses were carried out. A Venn diagram was generated to evaluate the difference of ASVs within samples and location points. A package in R called *mirlyn* (Multiple Iterations of Rarefaction for Library Normalization; Cameron and Tremblay, 2020; Schmidt et al. 2021) was applied to construct taxonomic composition bar charts and alpha and beta diversity plots. The Shannon index, an alpha diversity metric, was applied with a rarefied minimum library size and replicated with 100 iterations; moreover, principal component analysis (PCA) was conducted on the Bray-Curtis distance matrices (available at https://github.com/escamero/mirlyn).

Table 2. 1 Cyanobacterial 16S rRNA gene sequences from the NCBI database selected for phylogenetic analyses [derived from (Liyanage et al., 2016)].

Isolate	GenBank accession No.	Toxicity
Microcystis aeruginosa strain PCC 7806 *	AF139299	toxic
Raphidiopsis curvata CHAB1150 *	JN873923	toxic
Raphidiopsis curvata CHAB114	FJ890621	non-toxic
Cylindrospermopsis raciborskii CJR1	AB115485	non-toxic
Cylindrospermopsis raciborskii LEGE 051 *	HQ407326	toxic
Cylindrospermopsis raciborskii CHAB2379	FJ890634	non-toxic
Oscillatoria sp. PCC 6506 *	AY768397	toxic
Oscillatoria sp.	AJ133106	non-toxic
Phormidium autumnale CYN53 *	JX088083	toxic
Phormidium animale M8	KC768847	non-toxic
Anabaena bergii *	AF160256	toxic
Anabaena Flos-aquae 14 *	AJ133152	toxic
Cylindrospermum sp. PCC 7417	AJ133163	non-toxic
Nostoc sp. IO-102-I *	AY566855	toxic
Phormidium cf. uncinatum CYN108 *	JX088078	toxic
Nostoc sp. 152 *	AJ133161	toxic
Nostoc sp. CCAP 1453/28	HF678493	non-toxic
Hapalosiphon hibernicus BZ-3-1 *	EU151900	toxic
Hapalosiphon welwitschii M5	KC768846	non-toxic
Radiocystis sp. JJ30-3	AM710389.	non-toxic
Chroococcidiopsis sp. CCMP1489	AJ344556	non-toxic
Planktothrix agardhii *	AJ133167	toxic
Synechococcus sp. clone K1-09 *	GU784980v	toxic
Synechococcus PCC7009	AF216945	non-toxic
Leptolyngbya sp. HI09-1	GU111930	non-toxic
Leptolyngbya sp. W1	GU967417	non-toxic
Snowella litoralis 1ES42S2	DQ264220.1	non-toxic
Limnococcus sp. K71	MN621313.1	non-toxic
Shigella dysenteriae ATCC 13313	NR 026332.1	unknown
Klebsiella pneumoniae ATCC 13883	NR119278.1	unknown
Escherichia coli NBRC 102203	NR 114042.1	unknown
Proteus vulgaris	J01874.1	unknown

Chapter 3

Results

3.1 Physico-chemical Water Quality of the Glenmore Reservoir

The protection of water quality in Canadian lakes is a federal, provincial, and territorial responsibility. Consequently, lake waters in Alberta are regulated by federal and provincial guidelines and fall under the jurisdiction of Canadian Council of Ministers of the Environment (CCME), Alberta Environment and Parks (AEP), and Health Canada. The regulatory criteria selection for lake waters in Alberta are subjected to CCME's Canadian Environmental Quality Guidelines (CEQG) and AEP's Environmental Quality Guidelines for Alberta Surface Waters 2018 (EQGASW). Protection of lake water is covered under CCME's CEQG and AEP's EQGASW chapters of water quality guidelines for Protection of Aquatic Life, Protection of Agricultural Water, and protection of Recreation and Aesthetics. In addition, Health Canada's Guidelines for Canadian Recreational Water Quality for protection of lake waters have also been considered (Government of Alberta, 2018).

The physico-chemical quality of water in the Glenmore Reservoir on July 27, 2017 is listed in Table 3.2 (City of Calgary, 2017); May, June, August, and September 2017 geochemical data that are not presented in Table 1 are available in Appendix 1. A high secchi depth (i.e., >4 m) is generally characteristic of an oligotrophic waterbody (**Table 3.1**) with high quality, clear water. This was observed in measured secchi depths at the Head Pond, Mid-Lake and Heritage Cove and Weaselhead sites. Dissolved oxygen levels for all sampling events across the four study sites ranged from 8.60 mg/L to 9.40 mg/L. These levels are within the regulatory criteria for dissolved oxygen (9.5 mg/L for early life stages and 6.5 mg/L for all other life stages) (Canadian Council of Ministers of the Environment, 2011). In addition, water temperatures were recorded at the four

sites in July 2017; the average temperature was 19.2° C (standard deviation: ± 0.79) in the Glenmore Reservoir. Nitrogen concentrations in the samples collected from the reservoir in 2017 had an average of 0.147 mg/L of total nitrogen, which does not exceed the applicable environmental quality guidelines for Alberta surface waters for protection of agricultural water and freshwater aquatic life (Government of Alberta, 2018). Furthermore, total phosphorous had an average of 0.005 mg/L in samples collected in July 2017. Total phosphorous concentrations of all samples collected during 2017 also did not exceeded the applicable environmental quality guidelines for Alberta surface waters for protection of agricultural water and freshwater aquatic life (0.050 mg/L) (Government of Alberta, 2018). Accordingly, multiple lines of evidence (i.e., secchi depth, total nitrogen, and total phosphorus) suggest that the Glenmore Reservoir is oligotrophic (City of Calgary,2018) (**Table 3.1**).

Table 3. 1 General classification of trophic status based on lake water physico-chemical quality (Nurnberg, 1996).

Trophic State	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Secchi Depth (m)
Oligotrophic	<0.01	<0.35	>4
Mesotrophic	0.01 – 0.03	0.35 – 0.65	4 - 2
Eutrophic	0.03 – 0.10	0.65 – 1.20	2 - 1
Hypereutrophic	>0.1	>1.20	<1

3.2 Abundance and Diversity of the Bacterial Communities

3.2.1 Bacterioplankton Community Structure

16S rRNA gene sequencing across the eight samples (four locations at two depths: surface and secchi) filtered on glass fibre filters generated 37,183 reads that were classified into 780 amplicon

sequence variants (ASVs) (**Table 3.2**). Additionally, 576 amplicon sequence variants (ASVs) with total a read count of 132,314 taken from eight samples (four locations at two depths: surface and secchi) filtered on Sterivex filters (**Table 3.2**). There was a difference in frequencies between samples filtered on glass fibre filters and Sterivex units. Unexpectedly, there were no ASVs shared between the samples filtered on glass fibre filters and samples filtered on Sterivex filter units (**Figure3.1A**).

Samples Filtered on Glass Fibre Filter:

For all the samples filtered on glass fibre filters, all of the reads (i.e., 90%), were assigned to four phyla (**Table 3.3**). Proteobacteria, Bacteroidetes, Actinobacteria and Cyanobacteria accounted for 39.2%, 23.6%, 20.6%, 6.6% of the total reads, respectively. A taxonomic barplot of bacterial phyla that consist of 90% of the community composition within each sample and at each depth demonstrates the relative abundance of these phyla (**Figure 3.2**). The three most abundant phyla across all sites were Proteobacteria, Bacteroidetes, Actinobacteria (**Figure 3.3**). An additional taxa heatmap of relative abundance is provided in Appendix 2. Cyanobacterial ASVs were observed in Weaselhead and Mid-Lake; however, they were only present in Head Pond samples at less than 1% abundance. In addition, Cyanobacteria were only observed in Heritage Cove at secchi depth but were absent at the surface. Despite this, Heritage Cove had a variety of different phyla at both depths. The total observed unique ASVs near the water surface at the four study locations was 367, while at secchi depth there were 445. Notably, there were only 32 common ASVs; the majority of ASVs were Proteobacteria and Actinobacteria with only one Cyanobacteria ASV detected between surface and secchi depths (**Figure3.1 B**). It is apparent that more unique ASVs were found at secchi depth than at the other depths, across all four locations.

Thus, variation in bacterial community composition in the reservoir was evident, especially with the contribution of depth.

Samples Filtered on Sterivex Filter Units:

For all of the samples filtered on Sterivex filter units, all of the reads (i.e., 97.6%), were assigned to three phyla (**Table 3.4**). Here, the variation in bacterial community composition over water depth (i.e., between surface and secchi) was not substantial. Actinobacteria, Proteobacteria and Bacteroidetes accounted for 48.6%, 42.4%, and 6.6% of the total reads, respectively. **Figure 3.4** shows the proportion of phyla in the bacterial community according to the rarefied 16S rRNA amplicon sequencing dataset where the phyla Actinobacteria, Proteobacteria and Bacteroidetes contribute to approximately 97.6% of all the ASVs in the dataset. However, Cyanobacteria were not detected using Sterivex filter units. Head Pond at secchi depth had the most unique ASVs (375) where the majority assigned to Proteobacteria. In addition, at secchi depth, Head Pond had a variety of bacterial groups. The total observed unique ASVs on the surface of the four location points were 370, and on the secchi were 558 with 352 shared ASVs**.** (**Figure 3.1 C**).

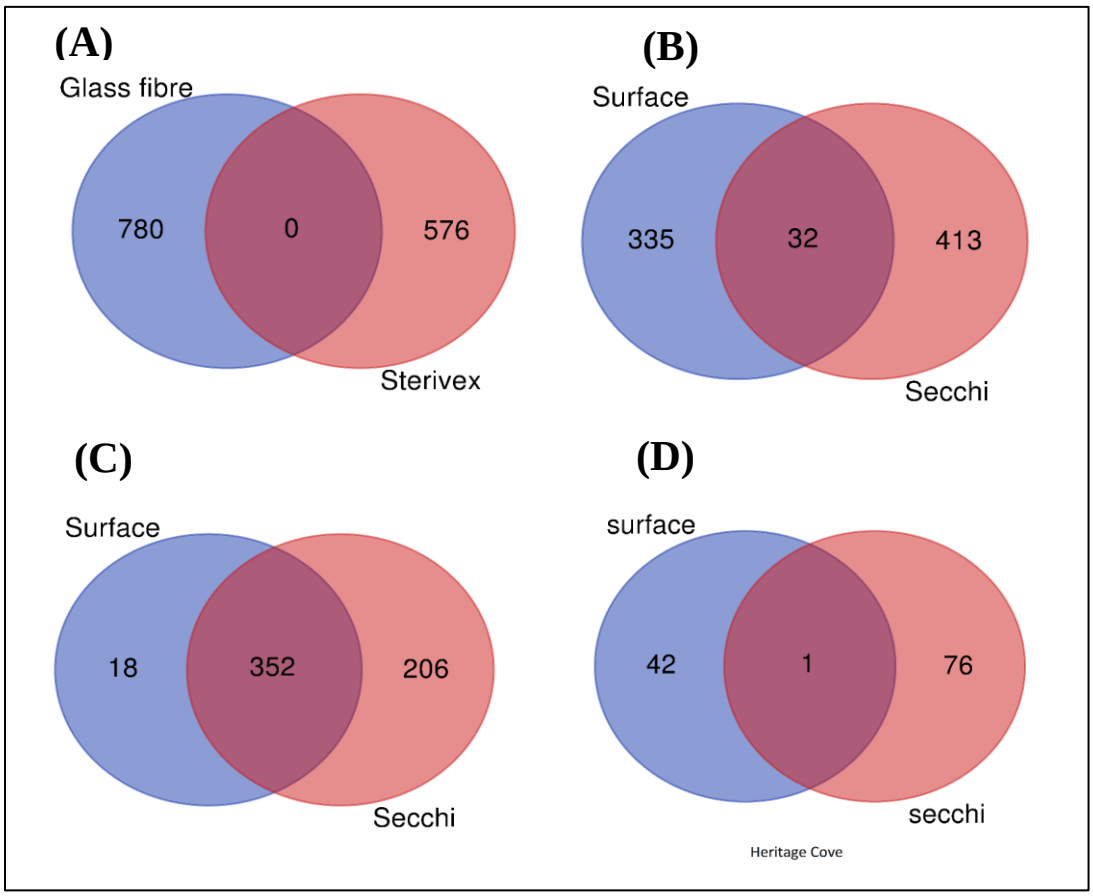


Figure 3. 1 A Venn diagram with shared ASVs between **(A)** the ASVs from the samples filtered on glass fibre filters and the samples filtered on Sterivex units, **(B)** the ASVs from the surface and secchi from the samples filtered on glass fibre filters from the 4 sites, **(C)** the ASVs from the surface and secchi from the samples filtered on Sterivex units from the 4 sites, **(D)** the ASVs from the surface and secchi from the samples filtered on glass fibre filter from the Heritage Cove site.

Table 3. 2 Physico-chemical water quality parameters obtained in the water column from the four sites in the Glenmore Reservoir in July 2017 (City of Calgary, 2017).

Characteristics	Head Pond	Mid-Lake	Heritage Cove	Weaselhead
Coordinates	Latitude: 51.00028300 Longitude: -114.09816000	Latitude: 50.98835500 Longitude: -114.109019000	Latitude: 50.97808200 Longitude: -114.10314000	Latitude: 50.98348100 Longitude: -114.12763400
Secchi Depth (m)	5.0	5.0	5.0	4.4
Depth (m)	14.5	8.7	9.2	4.4
Temperature (°C)	20.22	19.35	18.9	18.33
PH	8.3	8.2	7.8	8.0
Conductivity (µS/cm)	382.6	380.0	378.8	382.0
Diss Oxygen (µg/l)	8.60	9.19	9.22	9.40
Total Phosphorus (µg/l)	0.005	0.005	0.006	0.006
Total Nitrogen (µg/l) calc	0.154	0.146	0.150	0.138
Nitrate+Nitrite (µg/l)	0.034	0.016	0.01	0.008
Total Organic Carbon (µg/l)	1.6	1.7	1.7	1.8
Silica (µg/l)	4.13	3.84	3.69	3.5
Extracted Chlorophyll-*a* (µg/l)	1.0	0.88	0.9	0.73

Table 3. 3 16S rRNA gene sequence read counts from the samples filtered on GF/F from the surface and secchi and samples filtered on Sterivex filter units from the surface and secchi.

Filtration type	Depth	Sample	Read counts	No. of ASVs
Glass fibre filters				
	surface	Heritage Cove	2234	43
		Weaselhead	4669	101
		Mid-Lake	5978	145
		Head Pond	4128	104
	Secchi	Heritage Cove	4285	77
		Weaselhead	5150	126
		Mid-Lake	5261	125
		Head Pond	5478	149
Sterivex filters				
	surface	Heritage Cove	16704	266
		Weaselhead	12739	231
		Mid-Lake	17197	225
		Head Pond	14434	224
	Secchi	Heritage Cove	9667	219
		Weaselhead	16997	225
		Mid-Lake	18318	249
		Head Pond	26258	375

Table 3. 4 Dominant phyla and relative abundance % of bacterial sequences from samples filtered on GF/F from the four sites in the Glenmore Reservoir [Heritage Cove, Head Pond, Mid-Lake, Weaselhead] from the surface and the secchi.

Depth	Site Name	Proteobacteria (%)	Bacteroidetes (%)	Actinobacteria (%)	Cyanobacteria (%)
Surface	Heritage Cove	58.1	3.9	6.1	--
	Weaselhead	46.7	20.3	23.5	<1
	Mid-Lake	49.3	14.4	30.6	<1
	Head Pond	42.4	25.8	28.7	<1
Secchi	Heritage Cove	34.9	22.1	5.3	<1
	Weaselhead	41	22.2	25.5	1.2
	Mid-Lake	40.7	24	26.6	<1
	Head Pond	40.2	26.8	29.7	<1

Table 3. 5 Dominant phyla and relative abundance % of bacterial sequences from samples filtered on Sterivex filter units from the four sites in the Glenmore Reservoir [Heritage Cove, Head Pond, Mid-Lake, Weaselhead] from the surface and the secchi.

Depth	Site Name	Proteobacteria *(%)*	Bacteroidetes *(%)*	Actinobacteria *(%)*
Surface	Heritage Cove	58.8	2.3	37.6
	Weaselhead	44.2	4.8	49.7
	Mid-Lake	46.5	3.4	49.3
	Head Pond	46.4	9.7	43.59
Secchi	Heritage Cove	55.1	6.8	37.7
	Weaselhead	36.8	3.3	59.3
	Mid-Lake	45	5.6	48.6
	Head Pond	58.7	7	22.6

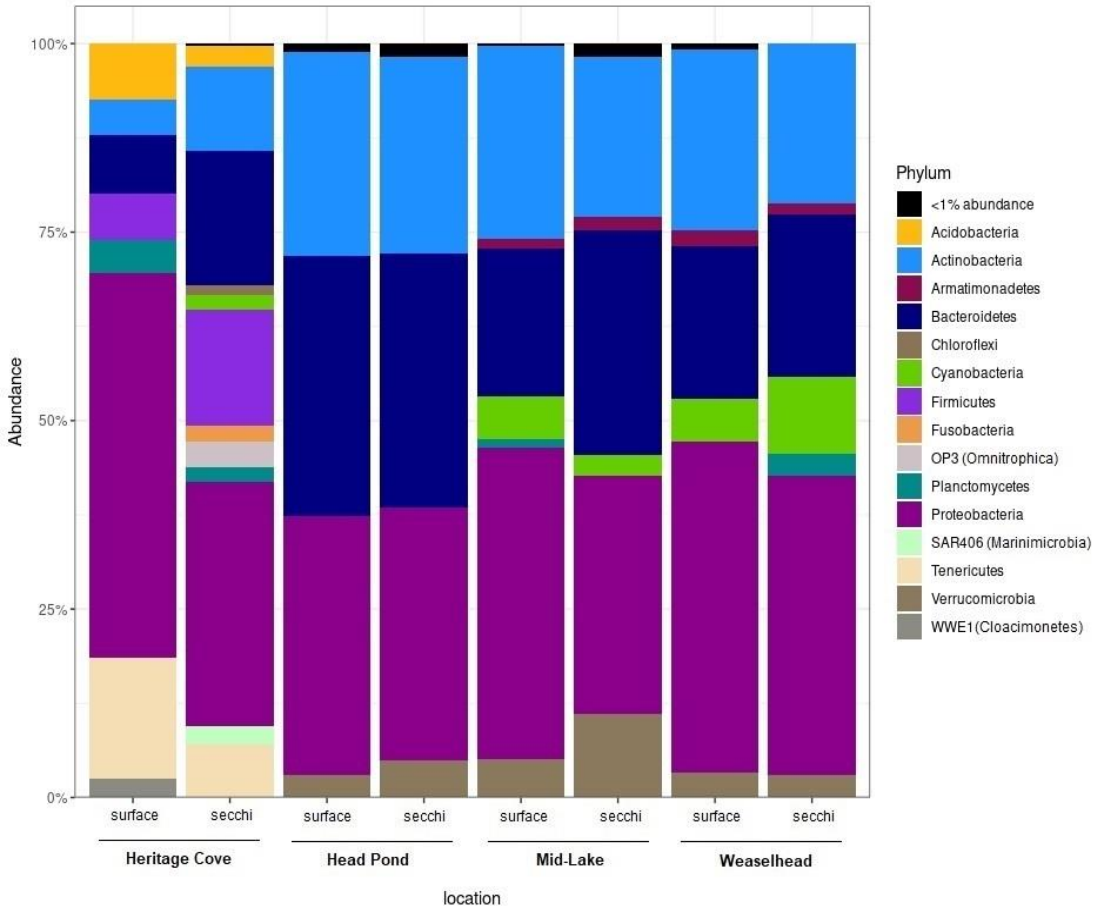


Figure 3. 2 Stacked barplot depicting the proportion of phyla in the bacterial community for water samples filtered on glass fibre filters (GF/F) from the four sites and two depths (i.e., surface and secchi) according to the rarefied 16S rRNA amplicon sequencing dataset. The phyla Proteobacteria, Bacteroidetes and Actinobacteria, contribute to approximately 90% of all the ASVs in the dataset.

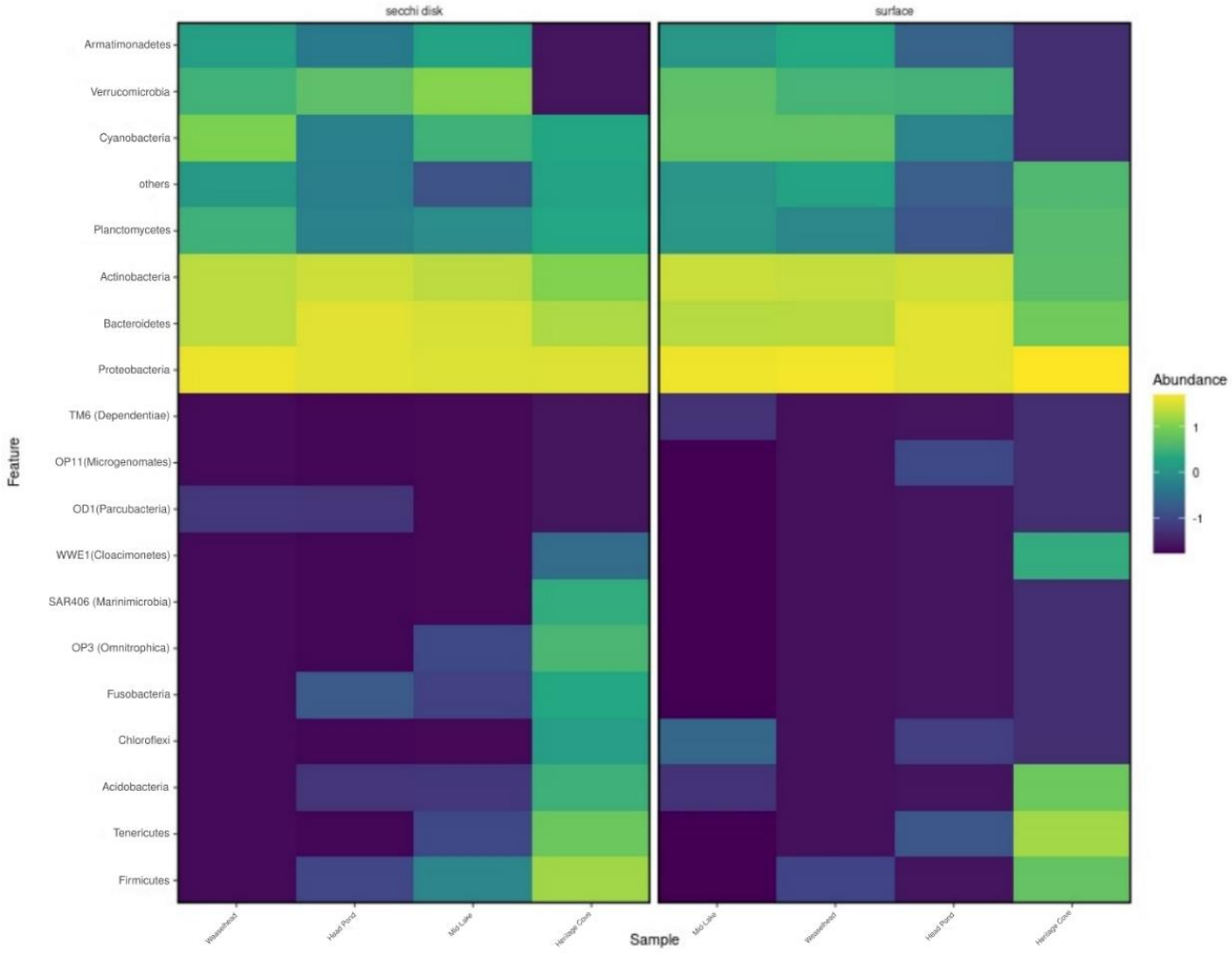


Figure 3. 3 Taxa heatmap of the abundance of bacterial communities at the phylum level from samples filtered on GF/Fat from four location points at two depths (i.e., at the surface and at the secchi) in the Glenmore Reservoir. Heatmap color (yellow to dark blue) displays the scaled abundance of each phylum across all locations.

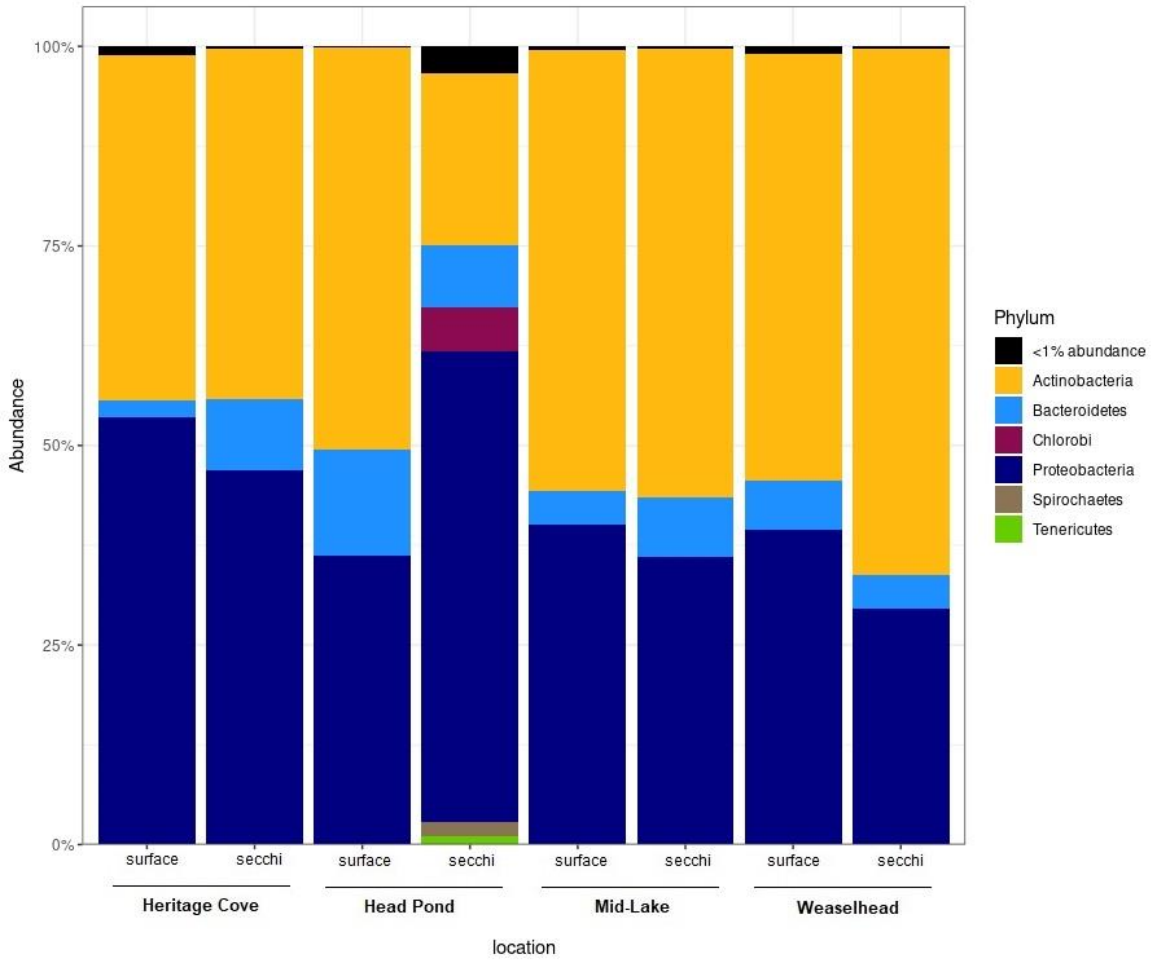


Figure 3. 4 Stacked barplot depicting the proportion of phyla in the bacterial community for water samples filtered on Sterivex filter units from the four sites and two depths (Surface and secchi) according to the rarefied 16S rRNA amplicon sequencing dataset. The phyla Actinobacteria, Proteobacteria and Bacteroidetes contribute to approximately 97.6% of all the ASVs in the dataset.

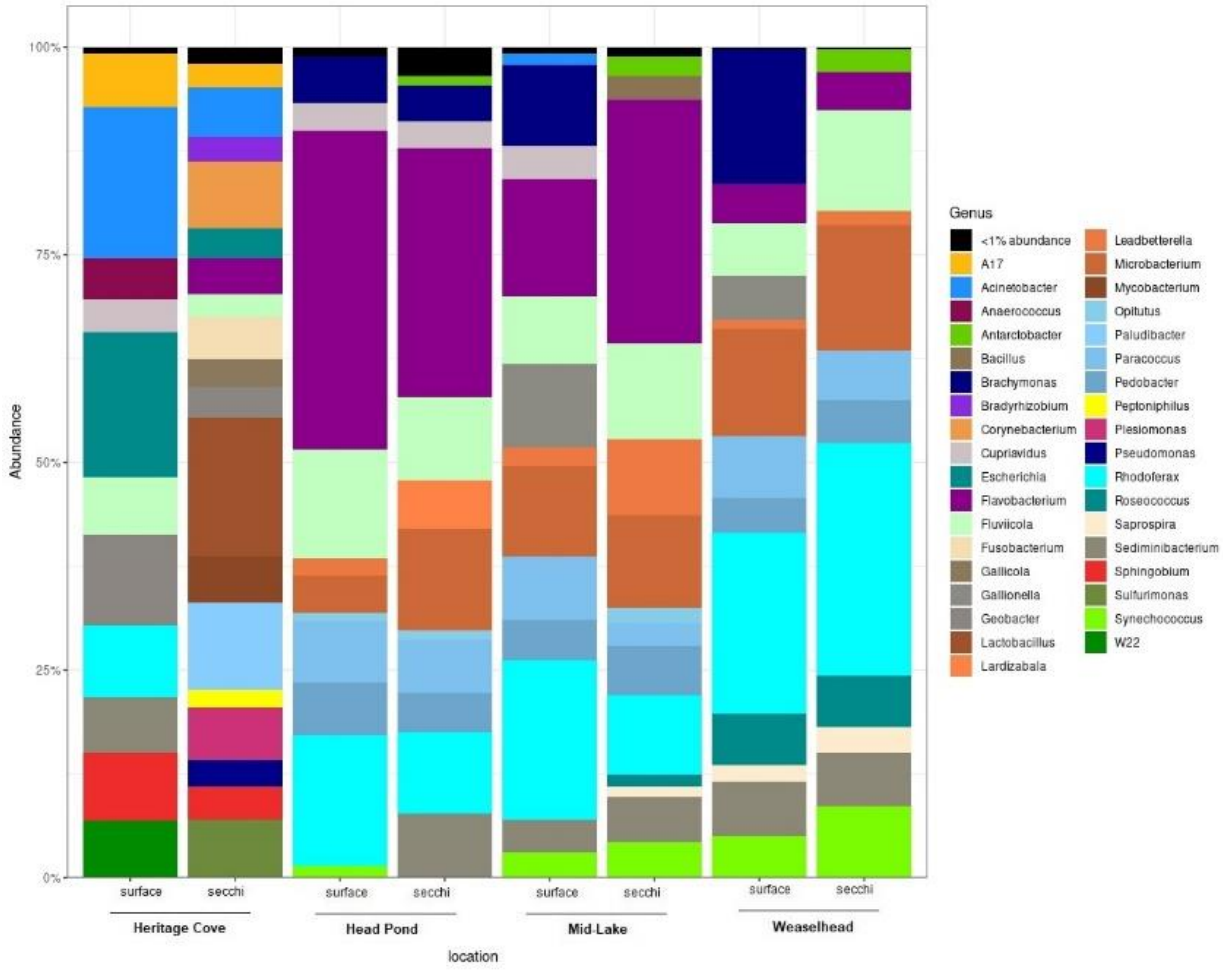


Figure 3. 5 Stacked barplot depicting the proportion of genera in the bacterial community for water samples filtered on glass fibre filters (GF/F) from the four sites and two depths (i.e., surface and secchi) according to the rarefied 16S rRNA amplicon sequencing dataset.

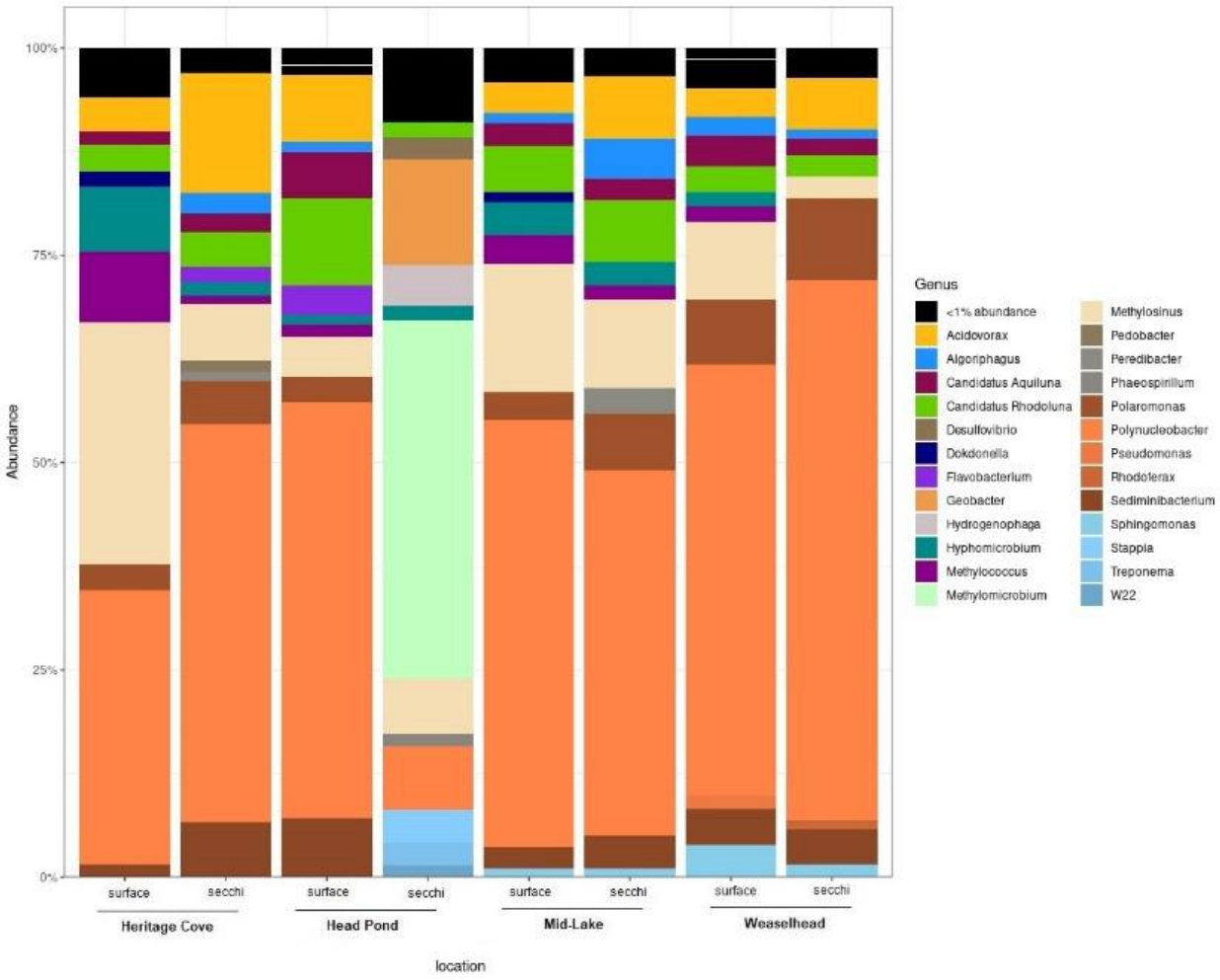


Figure 3. 6 Stacked barplot depicting the proportion of genera in the bacterial community for water samples filtered on Sterivex filter units from the four sites and two depths (Surface and secchi) according to the rarefied 16S rRNA amplicon sequencing dataset.

3.2.2 Cyanobacterial Community Composition

The number of hits of each cyanobacterial 16S rRNA gene sequence in water column samples was determined using QIIME2. Cyanobacteria were only observed in samples collected from Weaselhead, Mid-Lake and Head Pond and Heritage Cove at secchi depth and filtered using the glass fibre filters. The sample from Heritage Cove at the surface was excluded as no cyanobacteria hits were observed (**Table 3.5**). However, 284 reads were identified as being *Synechococcus* sp, and of the 284 reads, most were found at Weselhead at secchi depth. As expected *Planktothrix agardhii* was observed in the water column at secchi depth in Mid-Lake, as well as in the top layer of sediment (Shardlow, unpublished). Only 11 cyanobacterial (Cyano1-Cyano11) ASVs were observed, some assigned to potential toxin-producing genera (including: *Planktothrix agardhii*, *Synechococcus* sp.) Each unique ASV was given a specific ID (Cyano1 – Cyano11) (**Table 3.6**). Cyano9, Cyano10 and Cyano11 were mainly found in Weaselhead at secchi depth. Cyano3 and Cyano4 were observed in the water column at secchi depth in Mid-Lake.

Table 3. 6 Summary of the number of cyanobacterial reads and ASVs for water samples filtered on glass fibre filters (GF/F) from the four sites and two depths (i.e., surface and secchi) according to the rarefied 16S rRNA amplicon sequencing dataset.

Depth	Site Name	Cyanobacterial hits	No. ASVs
Surface	Heritage Cove	-	-
	Weaselhead	139	4
	Mid-Lake	117	7
	Head Pond	17	4
Secchi	Heritage Cove	80	1
	Weaselhead	354	4
	Mid-Lake	133	6
	Head Pond	54	9

Table 3. 7 Taxonomic assignment to Cyanobacteria ASVs from samples filtered on GF/F by BLAST.

ASV ID	Closest hit(s)	GenBank accession no.	% Identity
Cyano1	*Cyanobium* sp. JJ9-A3	AM710378.1	98.9%
Cyano2	*Limnococcus limneticus* Svet06	GQ375048.1	98.9%
Cyano3	*Planktothrix agardhii* NIES-905	LC455659.1	98.9%
Cyano4	*Planktothrix* sp. Plank-SS-01	MG762092.1	99.2%
Cyano5	*Radiocystis* sp. JJ30-12	AM710388.1	100%
Cyano6	uncultured cyanobacterium OTU_735	KR923314.1	98.3%
Cyano7	*Snowella litoralis* 0TU35S07	AJ781039.1	98.6%
Cyano8	uncultured cyanobacterium 15WD7	MT772222.1	99.6%
Cyano9	*Synechococcus* sp. 1tu14s11	AM259272.1	99.6%
Cyano10	uncultured *Synechococcus* sp. Waahi-22	EU015871.1	99.2%
Cyano11	uncultured *Synechococcus* sp. XZNMC83	EU703265.1	98.9%

Table 3. 8 Closest Cyanobacterial hits from the NCBI database in water column samples.

Depth	Site Name	ASV ID	Closest hit(s)
Surface	Weaselhead	Cyano1	*Cyanobium* sp.
		Cyano9	*Synechococcus* sp.
		Cyano10	uncultured *Synechococcus* sp.
		Cyano11	uncultured *Synechococcus* sp.
	Mid-Lake	Cyano1	*Cyanobium* sp.
		Cyano4	*Planktothrix* sp.
		Cyano5	*Radiocystis* sp.
		Cyano6	uncultured cyanobacterium
		Cyano9	*Synechococcus* sp.
		Cyano10	uncultured *Synechococcus* sp.
		Cyano11	uncultured *Synechococcus* sp.
	Head Pond	Cyano1	*Cyanobium* sp.
		Cyano2	*Limnococcus limneticus*
		Cyano5	*Radiocystis* sp.
		Cyano9	*Synechococcus* sp.
Secchi Depth	Heritage Cove	Cyano7	*Snowella litoralis*
	Weaselhead	Cyano1	*Cyanobium* sp.
		Cyano9	*Synechococcus* sp.
		Cyano10	uncultured *Synechococcus* sp.
		Cyano11	uncultured *Synechococcus* sp.
	Mid-Lake	Cyano1	*Cyanobium* sp.
		Cyano4	*Planktothrix* sp.
		Cyano3	*Planktothrix agardhii*
		Cyano6	Uncultured cyanobacterium
		Cyano5	*Radiocystis* sp.
		Cyano9	*Synechococcus* sp.
	Head Pond	Cyano1	*Cyanobium* sp.
		Cyano2	*Limnococcus limneticus*
		Cyano3	*Planktothrix agardhii*
		Cyano5	*Radiocystis* sp.
		Cyano6	uncultured cyanobacterium
		Cyano8	uncultured cyanobacterium
		Cyano9	*Synechococcus* sp.
		Cyano10	uncultured *Synechococcus* sp.
		Cyano11	uncultured *Synechococcus* sp.

Cyanobacteria phylogenetic tree:

The phylogenetic tree demonstrated 11 cyanobacterial ASVs within five family groups irrespective of their toxicity (green colored) (**Figure 3.5**). The tree showed lineage to major taxonomic family levels in cyanobacteria, *Microcoleaceae* (Cyano3 and Cyano4), *Merismopediaceae* (Cyano2), *Microcystaceae* (Cyano5 and Cyano6), *Coelosphaeriaceae* (Cyano7), *Synechococcaceae* (Cyano1, Cyano8, Cyano9, Cyano10 and Cyano11). The family *Microcoleaceae* contained ASVs Cyano3 and Cyano4. They were assigned to *Planktothrix agardhii*, which matches their 16S rRNA gene alignments. In addition, the family *Merismopediaceae* contained only the Cyano2 ASV, which was assigned to *Limnococcus limneticus* . The family *Microcystaceae* contained the Cyano5 ASV assigned to *Radiocystis sp.*; a separate single branch ASV, Cyano6, was assigned to uncultured cyanobacterium. The Cyano7 ASV was assigned to *Snowella litoralis* in the family *Coelosphaeriaceae.* The most diverse family contained five ASVs (Cyano1, Cyano8, Cyano9, Cyano10 and Cyano11) which had close 16S rRNA gene alignments with species in Synechococcaceae family group. Cyano10 and Cyano11 were assigned to uncultured *Synechococcus* sp., while Cyano1 t had close 16S rRNA gene alignment with *Cyanobium* sp. Cyano8 and Cyano9 had close 16S rRNA gene alignments with uncultured cyanobacterium and *Synechococcus* sp., respectively.

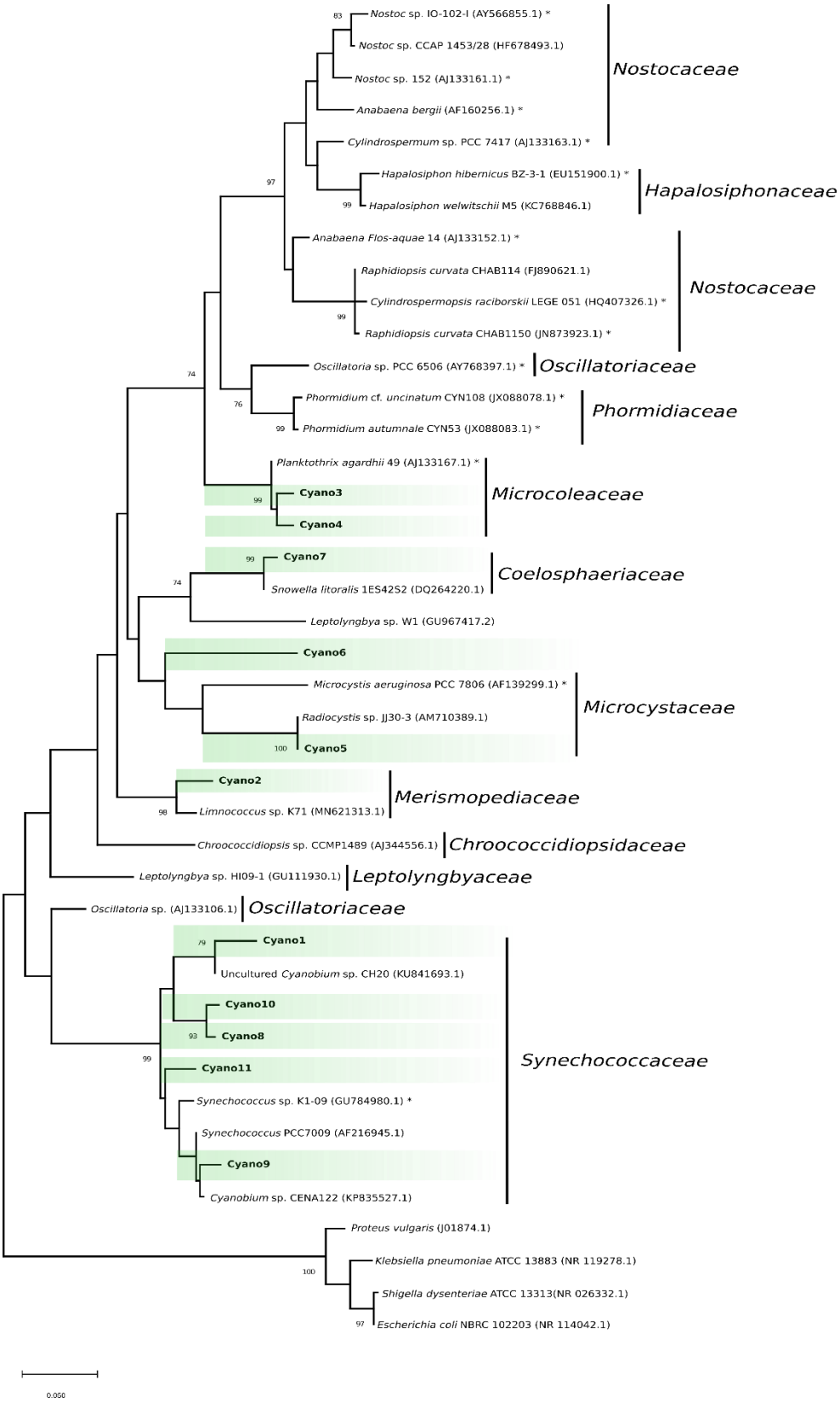


Figure 3. 7 A depiction of a maximum likelihood phylogenetic tree of variable region (V4) in the 16S rRNA gene sequences of cyanobacteria from the Glenmore Reservoir. Samples compared to known cyanobacterial sequences from the NCBI database. Values above branches represent % bootstrap support using 1000 replicates. Only values above 70% were shown. family level taxonomy is also noted.

3.2.3 Diversity of the Bacterial Communities

Alpha and Beta diversity

Alpha and beta diversity indices were used to examine differences in the bacterial communities in the Glenmore Reservoir (**Figure 3.6**). For samples filtered on the glass fibre filters, it was found that alpha diversity index for samples collected near the water surface in Heritage Cove had lower Shannon diversity. The next lowest diversity was at the same location for samples collected at secchi depth. The most diverse samples were collected from Mid-Lake at surface and secchi, Head Pond at secchi and Weaselhead at secchi. The samples collected from these locations had a similar amount of diversity. Head Pond and Weaselhead at surface contained similar amount of diversity. Higher diversity index was observed at secchi depth at each sampling location except Mid-Lake, where the diversity indices at surface and secchi depths were relatively similar **(Figure 3.6 A**). In contrast, for the samples filtered on the Sterivex filters, bacterial communities from Heritage Cove at the surface and Head Pond at secchi depth had higher Shannon diversity. Weaselhead at both depths had a lower diversity index. Head Pond at surface and Mid-Lake at both depths was similarly diverse (**Figure 3.6 B**). The Shannon diversity index decreased compared to the samples first filtered on glass fibre filters except for the Heritage Cove.

(A) **(B)**

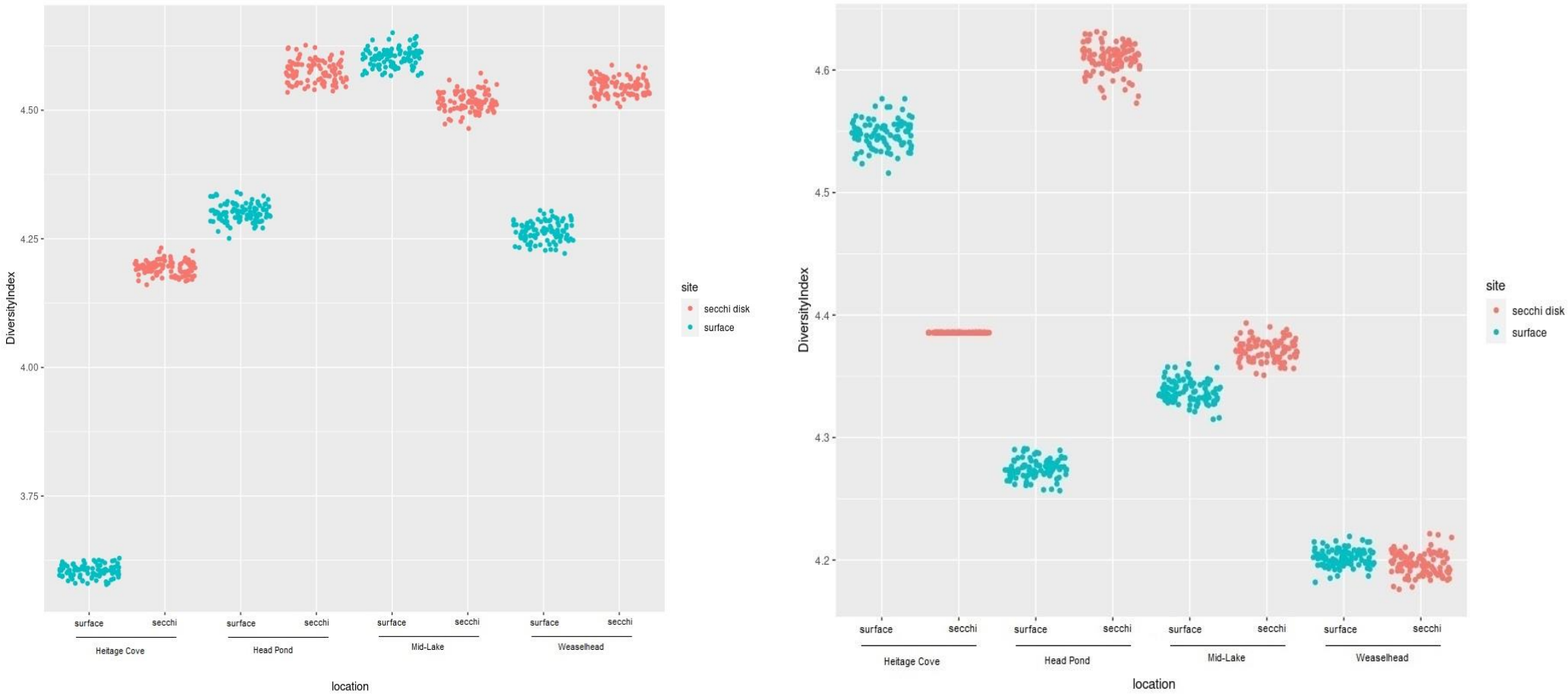


Figure 3. 8 Alpha diversity of the bacterial communities from the four location points at two depths (surface and secchi). (A) Alpha diversity of the bacterial communities from the samples filtered on the glass fibre filters. (B) Alpha diversity of the bacterial communities from the samples filtered on the Sterivex filters.

The beta diversity analysis based on Bray-Curtis distances further illustrated the difference between samples collected at the two depths at each site. Notably, samples filtered on glass fibre filters showed highly similar communities (grouped together) in each sample for all study locations except Heritage Cove. Bacterial communities from Heritage Cove significantly differed at surface and secchi (**Figure 3.7 A)**. In contrast, samples filtered on Sterivex filter units showed two groups: (1) Weaselhead, Head Pond (at two depths) and Mid-Lake (at surface) and (2) Heritage Cove (at surface) and Mid-Lake (at secchi). The bacterial groups in Heritage Cove at secchi depth observed distinct. Bacterial communities from Heritage Cove significantly differed at secchi and surface depths **(Figure 3.7 B)**. Thus, two slightly different results from samples filtered on glass fibre and on Sterivex units were observed.

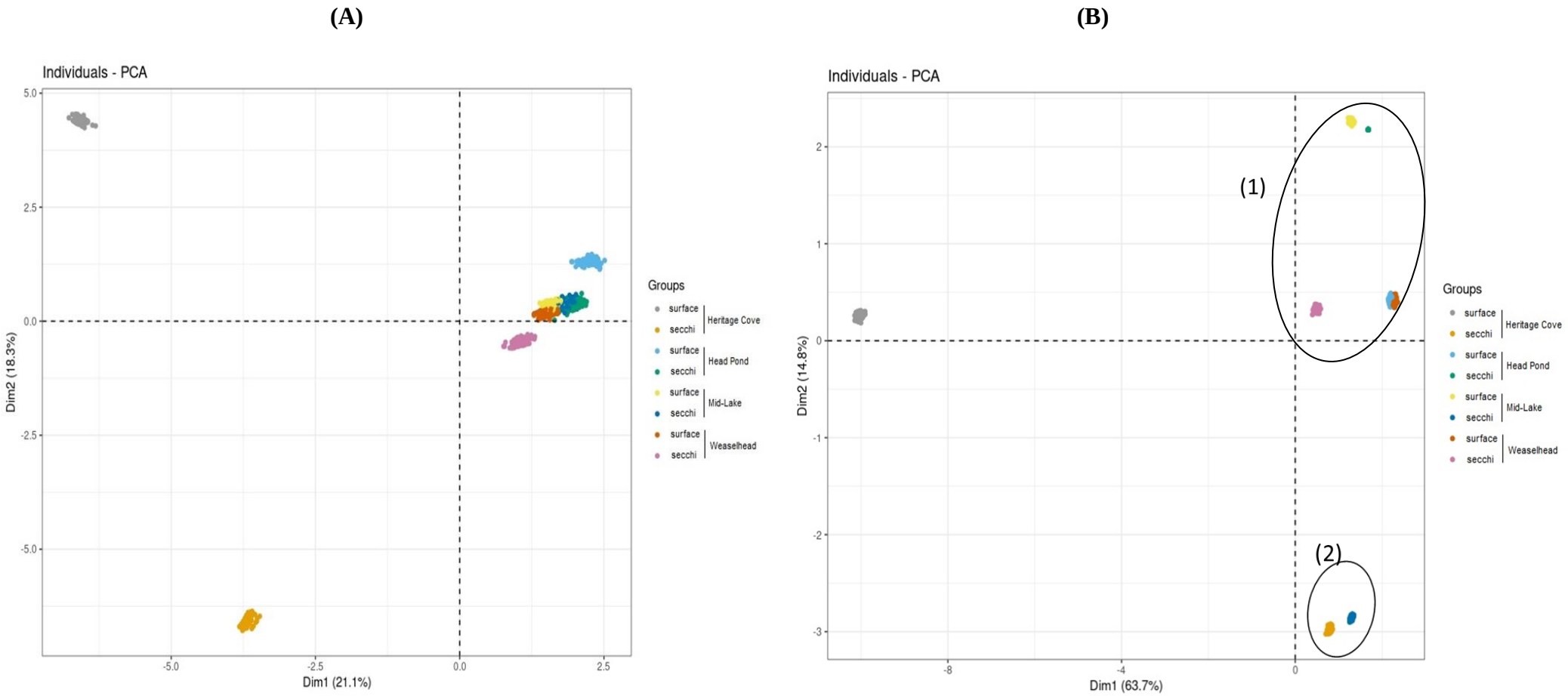


Figure 3. 9 PCA plots show the Bray-Curtis distance of the bacterial community beta diversity from the four locations points at two depths (surface and secchi) (A) samples filtered on glass fibre filters and (B) samples filtered on the Sterivex filter units.

Chapter 4
Discussion

There is no end to the effects that the transition from rural to urban land uses has on waterbodies. Although studies have shown a gradual deterioration of water quality in the Elbow River over time (Sosiak, 1999; Sosiak and Dixon 2006; City of Calgary, 2018), historical records show that water quality deterioration in the Glenmore Reservoir has not been of concern previously. (City of Calgary, 2018). The present study aimed to provide a snapshot and insights into the bacterial communities present in Glenmore Reservoir, prior to the water levels being lowered the following year (2018). In addition, this preliminary analysis can provide baseline information against which subsequent, more detailed investigations of reservoir bacterial and cyanobacterial communities may be compared. To determine the major taxa that are present within the Glenmore in the four locations, the 16S rRNA gene amplicons (V4) were sequenced and taxonomy was assigned. Alpha and beta diversity indices were also quantified from metagenome sequences which were analyzed in R packages (*mirlyn*). Some of these procedures are most used for microbiome analysis where there is particular emphasis on the compositional structure of microbiome data. In addition, the two filtration methods (i.e., GF/F and Sterivex units) yielded different species composition. Overall, the research presented here will give a general idea of the bacterial and cyanobacterial communities that are present in the Glenmore Reservoir in July of 2017 and give insight into future recommendations for spatial and temporal sampling and bacterial and cyanobacterial analyses of the water column.

4.1 Taxonomy and distribution of the Bacterial and Cyanobacterial Communities from the Four-Sampling Locations in the Glenmore Reservoir

Next generation Illumina MiSeq sequencing analysis revealed the presence of a typical freshwater bacterial community (including: Actinobacteria, Proteobacteria and Bacteroidetes), as described from numerous other freshwater systems (Llirós et al., 2014; Zhang et al., 2015; Avila et al., 2017; Li et al., 2020). Members of the Actinobacteria, mainly belonging to the ACK-M1 family, also known as the acI group (hgcI clade) (Warnecke et al., 2004) represented most of the total sequences in samples filtered on glass fibre filters (9%) and samples filtered on Sterivex units (42.1%). The dominance of Actinobacteria in freshwater systems, besides the contribution to T&O, presumably generate beneficial impacts on lake water quality, for example, they have been demonstrated to degrade complex mixtures of organic matter (Ghai et al., 2014), and they are also capable of adapting to oligotrophic conditions (Lauro et al., 2009; Avila et al., 2017). Members of the hgcI clade are characterized by slower growth rates which are indicative of oligotrophic systems. Members of this clade are also known to have several genes that likely provide a competitive advantage when searching for nutrients in freshwater habitats (Ghylin et al., 2014). Moreover, the large presence of acI group in Glenmore Reservoir and elsewhere could be related to the fact that they have have a defense mechanism due to their small cell size and cell wall structure, which protects them from grazing by other organisms and helps them to survive in an oligotrophic environment (Jezbera et al., 2006).

Among the Proteobacteria, the class Betaproteobacteria containing mainly the genera *Polynucleobacter* (14.8%) which were observed in Sterivex-filtered samples (**Figure 3.6**), and *Rhodoferax* (4.1%) were mainly observed in samples filtered on GF/F (**Figure 3.5**) and were the most abundant followed by class Alphaproteobacteria. The adaptation of Betaproteobacteria to

outgrow other bacterial groups under low-nutrient level conditions make this class one of the most dominant and important groups in many freshwater environments (Keshri et al., 2017). The presence of the free-living bacteria *Polynucleobacter* (family *Burkholderiaceae*) in the reservoir might be due to their adaptation to heterogeneous combination of aquatic organic carbon compounds that are likely to be present in these waters (Jezbera et al., 2012; Llirós et al., 2014). The members of anoxygenic phototrophs (genus: *Rhodoferax*) and methylotrophs (family: *Methylobacteriaceae*) observed in the reservoir also could be due to their important role for carbon and nutrient cycling (Ram et al., 2019).

It was observed that *Flavobacterium* (4.9%) affiliated with the Bacteroidetes, was primarily observed from GF/F-filtered samples. *Flavobacterium* are well known to inhabit a wide range of temperate and cold waters and their presence depends on the concentration of organic carbon and inorganic nutrients (Kirchman, 2002; Eiler and Bertilsson, 2007; Battin et al., 2016). The genus: *Flavobacterium* was mainly detected in Head Pond (surface: 10.9% and secchi: 10.2%) and this could be due to its depth and location. Head Pond is the reservoir compartment located closest to the Glenmore Water Treatment Plant and the dam, where the depth is ~14 m deep. It is worth mentioning that high cell concentrations of certain pathogenic strains of *Flavobacterium* (e.g., *Flavobacterium psychrophilum* and *F. columnare*) can infect fish such as trout (Laanto et al., 2011; Wahli and Madsen, 2018; Mühle et al., 2021).

Cyanobacteria are commonly used as a bioindicators of degraded water quality in freshwater ecosystems (Benayache et al., 2019; Tokodi et al., 2020). Typically, *Synechococcus* is the most abundant genus of picocyanobacteria (0.2−2.0 μm cell) in freshwater environments (Callieri, 2008). Members of this genus are well suited to oligotrophic waters and may prefer ammonium as a nitrogen source. They are also inhibited at low pH and adapted to low light (Callieri, 2010;

Fukushima et al., 2017). In this study, cyanobacterial ASVs were only detected from samples filtered on GF/F. Only five ASVs (i.e., Cyano1, 8, 9,10 and 11) were classified at the genus level (*Synechococcus* or *Cyanobium*). In addition, cyanobacterial ASVs were only observed in Weaselhead, Mild-Lake, Head Pond and Heritage Cove, at secchi depth. This could be due to the secchi depth being closer to the fine sediment of the reservoir, which is a source of bioavailable P, as supported by the research findings by Yang (2018), which demonstrated that the presence of potential toxin-forming cyanobacteria (e.g., *M. aeruginosa*) proliferation can be enhanced by fine sediment in the Glenmore Reservoir. Members of the *Synechococcaceae* were primarily observed at the Weaselhead location at both depths, which could be due to its depth 4.4 m, and its location in the west portion of the Glenmore Reservoir, near to the Elbow River inflow and the Weaselhead flats (natural park). Finally, Cyano3 and 4 are affiliated with the *Microcoleaceae* family (species: *Planktothrix agardhii*). They were observed in the water columns at secchi depth in Mid-Lake, as well as in the top layer of the Glenmore sediment (Shardlow, unpublished).

4.2 Bacterial Communities Diversity

Alpha diversity is the variance within a particular sample or ecosystem which is a commonly used component in characterization of communities (Thukral, 2007). Shannon index is an indicator of species evenness (proportional distribution of the number of each species in a sample) that exhibit values greater than zero (Thukral, 2007; Magurran,2013). Beta diversity is generally the thinking behind clustering algorithms that try to show differences or similarities among samples (Wagner et al., 2018). In this study, the differences, and the distribution in the proportion of the bacterial communities between two depths (surface and secchi) for the four different locations at the reservoir using alpha and beta diversity were applied. The Glenmore

Reservoir is considered oligotrophic which represents low nutrients status, with low inorganic and organic content at the two depths. Low nutrient availability can increase bacterial community alpha diversity (Li et al., 2020). The higher diversity observed mostly at secchi depth (5m below surface), may be due to community processes involving metabolic activities and syntrophic interactions closer to the bottom, as well as bacteria released from the sediment (Avila et al., 2017; Kurniawan et al., 2020), leading to a high proportion of unique bacteria important for biogeochemical cycles in the deeper depths. From the samples that filtered on GF/F, Heritage Cove at the surface had a lower Shannon diversity followed by at secchi compared to other locations, this might be due to the lower number of ASVs (community richness) observed at surface (43 ASVs) and secchi (77 ASVs) depths. However, the Shannon diversity index increased in Heritage Cove in the samples filtered on Sterivex units. This was likely caused by species differences due to filtration methods, which will be explained in the next section.

The Beta diversity analysis based on Bray-Curtis distances shows the difference among bacterial communities from different environments (Wang et al., 2016), where the main focus is on the difference in taxonomic abundance profiles from different samples. Considering that beta diversity often produces a beta with a hidden dependence on alpha (Jost, Lou, 2007). As expected, the bacterial communities from Heritage Cove differed at surface and secchi depths, from both types of filtered samples (GF/F and Sterivex) with unique ASVs at surface depths that differed from the unique ASVs at secchi depth. There was only one shared ASV by the samples from the two depths (**Figure 3.1 D**). This could possibly be because of the differences in flow dynamics, where there is no mixing of water (i.e., there are dead zones) between the reservoir and Heritage Cove at higher flow rates (Stone, 2021).

4.3 Comparison of Bacterial Communities between an Open (GF/F) and enclosed (Sterivex units) Filtration Methods

This study demonstrated that the method of water sample filtration can have considerable impact on the detection and the composition of the bacterial and cyanobacterial communities. Studies of diverse aquatic ecosystems consistently show that bacterial community composition in the prefilter fraction (glass fibre filters) differed from that of the smaller size fraction (Sterivex filter units) (Simon et al., 2002; Simon et al., 2014; Rieck et al., 2015; Takahashi et al., 2020). Both filtration methods are effective in providing good outcomes of the (particle-associated and free-living) bacterial communities of target freshwater location, however, GF/F provide an advantage in detecting cyanobacteria and yielded a larger number of ASVs. There are a few possible explanations for the observed compositional differences. It is possible that the amount of DNA detected depends on how one applies pressure during the filtration process. The GF/F filtration was conducted with negative pressure using an aspirator, while Sterivex filtration was conducted with positive pressure using a syringe (Li et al., 2018; Takahashi et al., 2020). In addition, finer filters tend to clog, and therefore either require a longer time or are unable to filter a sufficient volume of water (Padilla et al., 2015). It is possible that increased retention of cells <0.2 μm through Sterivex filters, as few bacteria likely pass through the 0.2 μm filter (i.e., ultramicrobacteria) (Wang et al.,2008).

The community shifts in the two fractions could also be driven by complexity of the bacterial community between free-living (FL) and particle-associated (PA) bacteria. PA bacteria are those found in fractions >0.8 μm pore-size filter membrane (Oris et al.,2015), while FL bacteria are those that can pass through larger filter membrane (i.e., 1.6 μm), but are intercepted on 0.22 μm pore-size filter membranes (Jiao et al., 2010), however, this is not always the case. Most bacteria

are negatively charged in natural water, and this is an important feature that leads to their attachment to positively charged surfaces (Gottenbos et al.,2001). There are electrostatic differences between the GF/F and Sterivex unit materials. The Sterivex filter membrane material is polyethersulfone (PES), which remain positively charged at neutral pH in natural water (Hilal and Johnson, 2010). In contrast, the glass fibre filters are made of borosilicate. Silica and silicate glass surfaces are known to have negative surface charge at neutral pH in natural water (Ojovan et al, 2019). This result can be attributed to the presence of electrostatic repulsion, which may be prevented bacteria from passing through membrane (Nnadozie et al., 2015). Unexpected results observed during the analysis lead to the discussion of these results as evidence that samples filtered using glass fibre filter as prefilters and subsequently filtering throughSterivex filter units should be critically examined in comparing estimates of aquatic bacterial composition and diversity.

4.4 Potential Problematic Bacterial and Cyanobacterial Taxa in the Glenmore Reservoir

The City of Calgary is committed to delivering high quality, safe drinking water to the community. The extensive water quality monitoring shows that the Bow River generally provides very high-quality source water to the city (City of Calgary-The Water Source Protection Plan, 2018). Some deterioration of water quality in the Elbow River has been observed over time (Sosiak, 1999; Sosiak and Dixon 2006) through the city's Watershed Monitoring Program which informs and helps in protecting source water quality (**Table 5.1**). Although, some increases in total suspended solids (TSS), TOC, metals, *E. coli* and protozoa) have been observed over time, the Elbow River system is considered oligotrophic (**Table 5.1**) and currently provides high quality source water; notably, cyanobacterial blooms or toxins have not been observed in the

system (City of Calgary, 2018). There are few studies on the Glenmore Reservoir regarding water and sediments quality (e.g., Yang, 20018; Shardlow, unpublished; Glasbergen et al., 2015; Blakely et al., 2014; Watson, 2004; Zaitlin et al., 2003; Satchwill, 2002). Moreover, the City of Calgary conducts monthly monitoring (May to September) of the correlation of various water quality parameters, WQI, phytoplankton (algal blooms) T/O compounds concentrations. This study provides a baseline data set on the bacterial community that observed in the Glenmore Reservoir water column with an emphasis of potential problematic taxa in July 2017.

Actinomycetes have long been involved in the production of taste and odour in water, as they are frequently discovered in waterbodies (Zaitlin and Watson 2006). Currently, several genera affiliated with Order Actinomycetales (e.g., *Streptomyces*, *Microbispora*, *Nocardia*, *Actinomadura*, *Thermoactinomyces*, etc. (Zaitlin and Watson 2006)) have been proven to have the ability to produce odour -causing compounds gesomin and 2-MIB. Actinomycetales (family: ACK-M1), which were observed in high relative abundance in the reservoir particularly at secchi depth at the Weaselhead location (60.2%) and at all of the other study locations ranging from (18%-47%) from Sterivex samples where presumably have the ability to produce taste and odour -causing compounds. Myxobacteria (Order: Myxococcales) are also an important source for earthy and musty odour yet observed in low abundance in Weaselhead from GF/F samples.

Cyanobacteria cause a multitude of water-quality concerns, including the potential to produce toxins and taste-and-odour compounds (Graham et al., 2012; Noh et al., 2014; Zastepa, 2014). Picocyanobacteria are found across oligotrophic and hypereutrophic freshwater environments (Callieri et al., 2012; Fukushima et al., 2017) as observed in several isolates that belong to *Synechococcaceae* in the oligotrophic Glenmore Reservoir. *Synechococcus* sp. are not known to cause toxic blooms events (Stockner et al., 2000; Fukushima et al., 2017), although some strains

can produce β-N-methylamino-L-alanine (BMAA), a neurotoxic non-protein amino acid (Cox et al., 2005), and microcystin (MC) (Bláha and Maršálek, 1999; Furtado et al., 2009). Within *Microcoleaceae,* the genus *Planktothrix* is a dominant cyanobacterial genus that forms toxic blooms in temperate freshwater ecosystems (Beard et al., 2000; Walsby, 2005; Wood et al., 2010; Pancrace et al., 2017). *Planktothrix agardhii* was observed in the upper layer of the Glenmore Reservoir sediments (Shardlow, unpublished) as well as observed in low abundance in the water column at secchi depth in Mid-lake and Head Pond. Additionally, *Radiocystis* species were also known to produce MC (Vieira et al., 2003; Lombardo et al., 2006), which observed in Head Pond, Mid-Lake and Weaselhead at both depths.

4.5 Bacterial Communities and Water Treatment Process

Calgary has two water treatment plants (WTPs) that treat water from the Bow and Elbow Rivers. The Bearspaw WTP draws water directly from the Bearspaw reservoir on the Bow River, while the Glenmore WTP draws water from the Glenmore Reservoir, which is fed by the Elbow River. Glenmore WTP comprised eight elements: coarse screens raw waters, pre-treatment, and clarification (flocculation), residuals treatment, disinfection, filtration, onsite storage reservoir, high-lift pumping and distribution, and water quality services (The City of Calgary's- Homeowner Water Guide, Water Treatment System, n.d.). This level of monitoring ensures that the drinking water consistently meets all federal Health Canada guidelines and provincial standards set by Alberta Environment and Parks (The City of Calgary's- Homeowner Water Guide, Water Treatment System, n.d.). Disinfection is critical to control the microbiome released into the treated water and inhibits bacterial growth during distribution (Li et al., 2017). Disinfection may be achieved using chlorine, ultraviolet light, chlorine dioxide, or ozone. Disinfection using chlorine and chloramines is highly effective to eliminate the microbial

community including bacterial pathogens (Al-Abri et al., 2019). Chlorine is highly effective for inactivating bacteria and viruses; however, it is less effective for protozoa (Health Canada, 2013) Even though protozoa can be inactivated through chemical disinfection, they are much more resistant than bacteria or viruses (Health Canada, 2013).

In some cases, the T/O problems may be caused by contaminants produced in the source water, which can subsequently be transferred to the distribution system. Such chemicals can be removed by applying advanced treatment not typically found in conventional treatment plants (e.g., activated carbon and advanced oxidation processes (Chestnutt et al., 2007; Comninellis et al., 2008)).

Table 4. 1 Summary of Calgary's watershed monitoring program: Elbow River source watershed (2014-2016) derived from (The City of Calgary, 2018)

Bow River Source Watershed		
Monitoring Site	Sampling frequency	Summary of water quality
Elbow River above Cobble Flats	Monthly, May to October	Excellent (100)
Elbow River above Bragg Creek	Monthly, year-round	Good (90)
Elbow River at Highway 22 bridge		Good (92)
Elbow River at Twin Bridges		Good (87)
Elbow River at Sarcee Bridge		Good (86)
Elbow River at Weaselhead foot bridge		Good (87)
Tributaries		
Prairie Creek near mouth	Monthly, May to October	Excellent (100)
McLean Creek near mouth		Good (88)
Lott Creek near the mouth		Good (88)
Bragg Creek at the mouth	Monthly, year-round	Good (88)
Bearspaw Reservoir		
Glenmore Reservoir – Head Pond	Monthly, May to September	Oligotrophic*
Glenmore Reservoir – Mid-lake		
Glenmore Reservoir – Heritage Cove		
Glenmore Reservoir – Weaselhead		

*River values based on WQI; reservoir value based on trophic status, where oligotrophic represents a desirable low nutrient status.

Previous studies have demonstrated that cyanobacteria can accumulate inside drinking water treatment plants and cause operational problems such as filter clogging. This may lead to service disruptions or outages, or toxin accumulation and release if it is present (Emelko et al., 2011; Kommineni, 2009; Zamyadi et al., 2012). There is little information, however, on the potential for such toxic cell accumulation in plants drawing from water sources that do not experience obvious blooms at the water surface. Monitoring for cyanobacteria or related toxins in source or treated water is normally triggered by observations of blooms in the source (Health Canada, 2018). In the absence of a bloom, water quality in WTPs will typically not be monitored for cyanobacteria or toxins (Almuhtaram et al., 2018). Although, microcystins and anatoxin-a might be detected across treatment plants, it has not correlated to cell concentrations as reported by Almuhtaram et al. (2018). Commonly used oxidants such as chlorine and permanganate effectively degrade some cyanotoxins (Westrick et al., 2010; Newcombe, 2012), however, chlorination can produce halogenated disinfection byproducts formed from the reaction of chlorine with organic matter (Rodríguez et al., 2007). More studies are thus needed on the degradation of environmentally cyanotoxin mixtures, which are occur in the environment, in actual surface water or water withdrawn from a drinking water treatment process prior to the oxidation step (Schneider and Bláha, 2020).

Chapter 5

Conclusions and Future Directions

The City of Calgary draws water from the Glenmore Reservoir to provide an adequate amounts of safe drinking water. There are several negative effects of the transition from rural to urban land uses on source water in Calgary. Although studies have shown a gradual deterioration of water quality in the Elbow River over time (Sosiak, 1999; Sosiak and Dixon 2006; City of Calgary, 2018), historical records show that the water quality in the Glenmore Reservoir has not been the subject of concern to water deterioration (City of Calgary, 2018). This study provides important baseline data on the bacterial and cyanobacterial communities to manage and prepare in advance of any changes or risks in the Glenmore Reservoir. The 16S rRNA gene next-generation sequencing (V4) was used to explore the bacterial and cyanobacterial assemblages in the Glenmore Reservoir in Calgary, AB, in July 2017. The results revealed that oligotrophic reservoir can contain diverse bacterial communities from each depth (i.e., surface and secchi), whose structure is influenced by trophic status. Both filtration methods (i.e., GF/F and Sterivex) were used and both were effective in providing good outcomes of the bacterial communities of target freshwater location. The reservoir was dominated by typical freshwater groups mostly Proteobacteria, Actinobacteria, Bacteroidetes, with relative abundance differences from GF/F and Sterivex. The dominant Phyla identified within the reservoir were Proteobacteria and Actinobacteria (i.e., core communities) (**Figure 3.2-3.4**) which was consistent across all four locations observed in this study, with high abundances of ASVs associated with the family *Comamonadaceae* followed by family ACK.M1 between filtered-samples on GF/F and Sterivex. Despite these similarities across the different locations, the bacterial communities were found to be quite different from both filtered samples. The second dominant phylum from GF/F samples

was Bacteroidetes, which was detected at 23.6% relative abundance, whereas Proteobacteria was the second dominant phylum in Sterivex samples, at 42.4% relative abundance. Additionally, GF/F fractions provide an advantage in detecting Cyanobacteria and a larger number of ASVs. This research demonstrated that Heritage Cove contained common groups of bacteria that are distinct from other sites. While Calgary has a high-quality water supply and has not had a history of any concerns regarding algal blooms or toxin producers, presence of potential problematic taxa (e.g., toxin produces, taste & odour) were detected and observed in the reservoir in the four location points. Further research including seasonal sampling and analyzing of the water column are required to be able to fully understand the dynamics of microorganisms in the Glenmore Reservoir after short-term disturbances (e.g., heavy rainfalls and runoffs). It is recommended to consider filtering the samples using an open and enclosed filtration systems in case of differences as shown in this study.

The bacterial communities present in the Glenmore Reservoir water column were evaluated; here, we focused on the other important objective of this research, which was detecting the presence of any potentially problematic taxa (i.e., Cyanobacteria) that may preclude the provision of adequate amounts of safe drinking water (e.g., toxin produces, taste & odour). Cyanobacteria were only detected in the samples that were filtered on the GF/F (at 6.6%), but not the Sterivex filters (**Figure 3.2**). Cyanobacteria were only observed in Weaselhead, Mid-Lake and Head Pond and in Heritage Cove at secchi depth. Only 11 cyanobacterial ASVs observed were assigned to potential toxin-producing genera (including: *Planktothrix agardhii*, *Synechococcus* sp.). Picocyanobacteria are often dominated by *Synechococcus* sp., which are known to have some toxic strains (Gin et al., 2021). Even though Cyanobacteria found at much lower abundances, one detected isolate *Synechococcus* sp. strain was grouped with toxic

Synechococcus strain in the phylogenetic tree (**Figure 3.7**). Future research should ideally investigate cyanobacterial community in the Glenmore Reservoir even if there are no visible signs of a bloom, as well as identify the presence of microcystin and anatoxin-a genes to confirm that a particular cyanobacterial strain is a toxin-producer.

Besides potential toxin-producing cyanobacteria, Actinomycetes have long been involved in the production of taste and odour in water. Actinomycetales (family: ACK-M1), which were observed in high relative abundance (i.e., >50%) in the reservoir presumably have the ability to produce taste- and odour-causing compounds. Further studies are needed to understand the dynamics of the bacterial community in the Glenmore Reservoir and how they may change in response to watershed disturbances such as wildfires, especially taxa that can be potentially problematic and gradually degrade the water quality. This study has provided a baseline data set of bacterial and cyanobacterial communities which will aid the future understanding of bacterial communities present in the Glenmore Reservoir as well as manage and prepare in advance for any events and/or changes in the watershed that could threaten drinking water source quality and treatability in Calgary.

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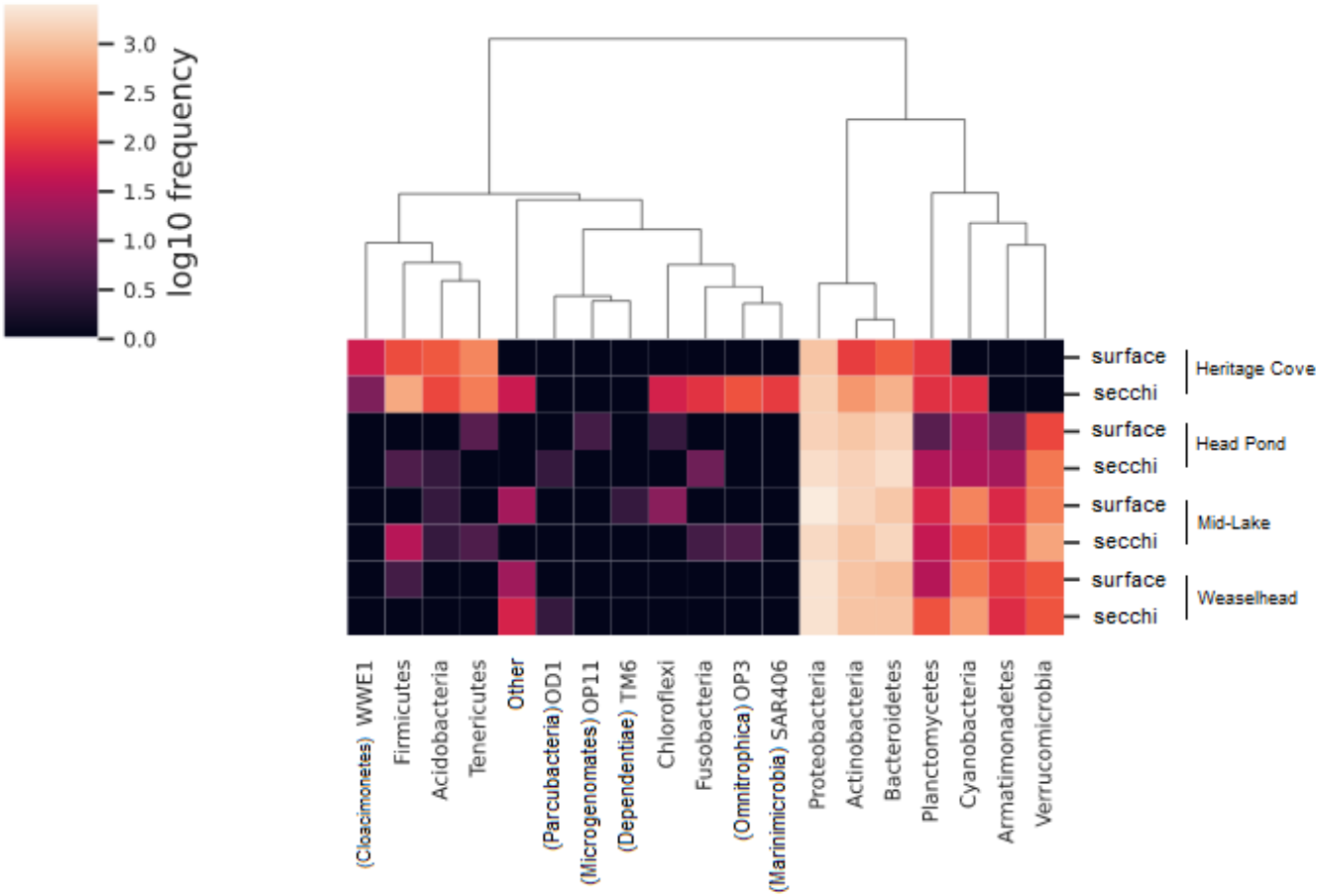
Appendices

Appendix 1

Site	Date	Secchi Depth(m)	Temperature (deg C)	PH	Conductivity (uS/cm)	Diss Oxygen (mg/L)	Turbidity (NTU)	TP (mg/L)	TDP (mg/L)	TN (mg/L)	Nitrate+ Nitrite	TOC (mg/L)	Silica (mg/L)	Extracted Chlorophyll-a (µg/l)	TN:TP
Head Pond	23-May-17	3.4	12.97	8.2	416.5	9.31	1.52	0.007	0.003	0.315	0.095	2.9	3.77	1.1	45.0
Mid-Lake	23-May-17	1.8	13.04	8.1	411.0	9.24	4.09	0.009	0.004	0.365	0.105	3.0	3.96	1.1	40.6
Heritage Cove	23-May-17	1.7	12.48	7.9	407.3	9.47	7.38	0.009	0.003	0.340	0.1	3.1	4.05	0.86	37.8
Weaselhead	23-May-17	0.3	14.21	8.0	384.0	9.76	7.86	0.012	0.002	0.254	0.074	2.8	3.66	1.1	21.2
Head Pond	26-Jun-17	3.6	16.31	8.3	367.9	8.79	1.24	0.004	0.002	0.248	0.108	2.1	3.97	0.7	62.0
Mid-Lake	26-Jun-17	3.8	16.30	8.1	371.6	9.21	1.75	0.005	0.001	0.224	0.094	1.8	4.20	0.71	44.8
Heritage Cove	26-Jun-17	3.7	16.23	7.8	371..3	9.30	1.95	0.005	0.002	0.219	0.089	2.0	4.22	0.63	43.8
Head Pond	28-Aug-17	7.0	18.82	8.5	373.1	8.71	0.68	0.005	0.003	0.350	0.003	1.6	3.81	0.79	70.0
Mid-Lake	28-Aug-17	6.5	18.44	8.5	372.0	9.18	0.74	0.004	0.002	0.290	0.003	1.8	3.88	0.74	72.5
Heritage Cove	28-Aug-17	8.0	17.71	8.4	370.0	9.62	0.71	0.006	0.002	0.370	0.003	2.0	3.95	0.64	61.7
Weaselhead	28-Aug-17	3.1	17.58	8.5	375.3	10.26	1.45	0.009	0.002	0.460	0.003	2.0	4.02	0.82	51.1
Head Pond	25-Sep-17	7.0	13.51	8.4	380.4	9.06	0.70	0.005	0.003	0.110	0.003	1.6	4.22	0.84	22.0
Mid-Lake	25-Sep-17	6.5	11.75	8.5	386.6	9.27	0.75	0.005	0.002	0.120	0.003	1.7	4.18	0.85	24.0
Heritage Cove	25-Sep-17	6.5	10.62	8.7	388.8	9.75	0.58	0.008	0.003	0.190	0.003	2.1	4.31	0.59	23.8
Weaselhead	25-Sep-17	2.78	10.47	8.6	391.8	10.49	0.69	0.005	0.001	0.120	0.003	1.9	4.11	0.63	24.0

Appendix 2



Heatmap at the phylum level of the 16Sr RNA gene V4 sequencing dataset. The logarithmic scale in which colour intensity determines the abundance of the taxa can be seen in the top left-hand corner. The depths and locations names of the samples are given on the right, while the names of the phylum are stated below. On the top the dendrogram of similarity between all the samples is presented.