Cyanobacteria north of 60º: environmental DNA approaches

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ABSTRACT

Cyanobacterial blooms, such as those recently reported in Great Slave Lake (GSL, NWT), have sparked concern over the occurrence of toxic blooms in the North. This study investigated past and present incidences of cyanobacteria in lakes above latitude 60° N. The abundance of the toxin (microcystin) gene *mcyE*, as well as genes common to all cyanobacteria (16S rRNA) and bacteria (*glnA)* were quantified from lake sediment cores using ddPCR. Individual colony isolates from a surface bloom in Yellowknife Bay (GSL) in August 2015 were amplified and identified as non-toxigenic *Dolichospermum lemmermannii*. Very low levels of microcystin genes were detected through the sediment archives (over ~100-150 yr) of GSL and other lakes, as well as in the plankton of GSL. While recent increases in *mcyE* were not observed, an increase in the cyanobacterial 16S rRNA and *glnA* genes was seen through time. In the high Arctic Meretta Lake, gene abundance profiles reflected the effects of past eutrophication and recovery.

RÉSUMÉ

Les fleurs d'eau de cyanobactéries, comme celles récemment rapportées dans le Grand lac des Esclaves (GSL, TN-O), ont suscité bien des inquiétudes sur l'apparition d'algues toxiques dans le Nord. Cette étude a examiné l'incidence des cyanobactéries dans les lacs au-dessus du 60^{e} parallèle nord. L'abondance du gène *mcyE* de la cyanotoxine microcystine, ainsi que des gènes communs à toutes les cyanobactéries (ARNr 16S) et des bactéries (*glnA*) ont été quantifiés à partir de sédiments en utilisant le ddPCR. Des colonies individuelles isolées à partir d'une fleur d'eau de cyanobactéries dans la baie de Yellowknife (GSL) en août 2015 ont été amplifiées et identifiées comme étant *Dolichospermum lemmermannii*, une souche non-toxigène. Des niveaux très faibles de gènes de microcystines ont été détectés dans les sédiments plus anciens (~ 100-150 ans) de GSL et d'autres lacs, ainsi que dans le plancton du GSL. Alors que des augmentations récentes de *mcyE* n'ont pas été observées, une augmentation des gènes cyanobactériens (ARNr 16S) et bactériens (*glnA)* ont été vus à travers le temps. Dans le lac Meretta (situé dans l'extrême arctique), les profils d'abondance de gènes reflètent les effets d'une eutrophisation et d'un rétablissement antérieur.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIC	Akaike's information criteria
CAR(1)	Continuous time autoregressive model, lag 1
Chl a	Chlorophyll a
ddPCR	Droplet digital PCR
ELISA	Enzyme linked immunosorbent assay
GAM	Generalized additive model
GCN	Gene copy number
glnA	Glutamine synthetase A gene
GSL	Great Slave Lake
HAB	Harmful Algal Bloom
MC	Microcystin
mcyE	Microcystin E gene
NWT	North West Territories
qPCR	Quantitative PCR
TP	Total Phosphorus
TN	Total Nitrogen

CHAPTER 1: GENERAL INTRODUCTION

1.1 Cyanobacteria and cyanobacterial blooms

Cyanobacteria, commonly referred to as blue-green algae, are among the most ancient, photosynthetic prokaryotes (Graham & Wilcox, 2000; Whitton & Potts, 2012). Although they inhabit a wide range of environments, cyanobacteria as a whole share several common features that allow them to dominate many aquatic ecosystems (Srivastava et al., 2012; Whitton & Potts, 2012). For example, the temperature optimum for many cyanobacteria is several degrees higher than that of other phytoplankton, allowing them to thrive in warmer climates (Kosten et al., 2012a; Taranu et al., 2012). Many cyanobacteria are known for their high salt tolerance, which allows them to avoid desiccation in high salinity environments, both in water and on land (Paerl & Paul, 2012), while several other taxa are known for their ability to fix nitrogen (N_2), providing them an advantage in environments with low concentrations of dissolved inorganic nitrogen forms (NO_3^-, NH_4^+) (O'Neil et al., 2012). Most planktonic cyanobacteria are also capable of forming gas vacuoles, which increase their buoyancy and enable some control over their position in the water column, allowing them to rise to the surface where they can obtain more light for photosynthesis and form surface blooms (Carey et al., 2012).

In general an algal bloom refers to a greater than average production of microscopic algal biomass often occurring as a response to the introduction of nutrients or increased water temperatures (Oliver & Ganf, 2000). Although algal blooms can form throughout the water column, the formation of surface blooms is restricted to those species that are buoyant and/or motile, generally members of the cyanobacteria (Oliver & Ganf, 2000). Though cyanobacterial blooms do occur naturally with reports dating back

to 1878 (Chorus & Bartram, 1999; Heisler et al., 2008; O'Neil et al., 2012; Paerl & Huisman, 2008; Paerl, 2008), human induced eutrophication and increasing global temperatures as a consequence of climate change have led to an increase in the frequency and intensity of blooms in recent years (Paerl & Paul, 2012; Paerl, 2014).

Cyanobacteria are often associated with Harmful Algal Blooms (HABs). HABs are defined as those blooms that have negative effects on humans and wildlife, (Anderson et al., 2002). HABs may produce toxins, deplete oxygen levels, and/or lead to habitat loss as a result of elevated biomass production (Anderson et al., 2002; Sellner et al., 2003).

1.2 Toxic cyanobacteria and cyanotoxins

Toxic algal blooms are quite common in marine and freshwater environments with estimates of more than 50% of blooms producing toxic compounds that can seriously harm or kill both humans and animals (Graham and Wilcox, 2000). Of all the bloom-forming algae the cyanobacteria have the highest potential of toxicity, with 50-75% of blooms generating toxins (Graham and Wilcox, 2000).

Cyanobacterial toxins or "cyanotoxins" can be broadly assigned to the classes of neurotoxins, hepatotoxins and lipopolysaccharide endotoxins (Graham and Wilcox, 2000). Toxigenic species of cyanobacteria include those observed in the genera *Microcystis, Dolichospermum, Aphanizomenon, Planktothrix (Oscillatoria), Lyngbya* and *Nodularia*, to name a few (Cronberg & Annadotter, 2006). One feature of toxigenic cyanobacteria is their ability to produce different toxins or no toxins within the same species and the same toxin throughout different genera (Rantala et al., 2006).

Of the toxin groups, the hepatotoxins tend to be the cause of most concern when it comes to the safety of drinking water supplies. Cyanobacterial hepatotoxins come in the form of nodularins and microcystins (El-Shehawy et al., 2012). Of the two, the microcystins, produced predominantly by cyanobacteria in the genera *Microcystis, Dolichospermum* and *Planktothrix*, are of the most widely reported and of greatest concern to human health (Chorus & Bartram, 1999). Though they typically affect animals and livestock to a greater degree there have been confirmed cases of human illness and death associated with the ingestion of hepatotoxins from water (Codd et al., 2005; Graham & Wilcox, 2000). As hepatotoxins have been linked to tumour promotion, cancer experts are now concerned with the health effects associated with the long-term consumption of contaminated drinking waters including increases in liver cancers (Belykh et al., 2011; Ghernaout et al., 2010; Vezie et al., 1998; Weirich & Miller, 2014). Due to their propensity to cause harm to human health, threshold levels for maximum acceptable concentrations of hepatotoxins in water have been proposed. The World Health Organization set a concentration limit of 1.0 ppb of microcystin in drinking waters and 20 ppb in recreational waters (WHO, 2011); individual countries, including Canada have adopted similar guidelines (Health Canada, 2007).

Microcystins are a family of cyclic heptapeptide hepatotoxins synthesized by the microcystin synthetase enzyme complex, which consist of a non-ribosmal peptide synthetase (NRPS) and a polyketide synthase (PKS), both of which are encoded for by *mcy* gene cluster composed of the *mcy* A-C and *mcy* D-J operons (Jungblut & Neilan, 2006; Kaebernick & Neilan, 2001). Microcystins consist of seven amino acids and share the common cyclic structure (-D-Ala-L-X-erythro-[beta]-methyl-D -isoAsp-L-Z-Adda-

DisoGlu-N-methyl dehydro-Ala), where X and Z indicate variable L-amino acids (Jaiswal et al., 2008). From this structure, the ADDA moiety is of particular importance when it comes to toxicity as it is involved in the binding of the toxin to protein phosphatases (Jaiswal et al., 2008).

Due to its harmful nature and the low threshold concentrations set for recreational and drinking waters, it is imperative that reliable and sensitive microcystin detection methods be developed. To date, a number of methods have been developed in order to detect the presence of microcystin and microcystin producing cyanobacteria in water. These include microscopic methods involving the morphological identification of known toxin producing species as well as chemical methods, such as the Enzyme Linked Immunosorbent Assay (ELISA) and High Performance Liquid Chromatography (HPLC) that are designed to measure toxin concentrations from water samples (Rantala et al., 2006; Tillmanns et al,. 2007).

However, there are limitations to these approaches, particularly in the case of microscopic identification, as it is not possible to discern toxigenic from non-toxigenic species based on morphology (Rantala et al., 2006) and hence DNA and molecular methods are now being developed. Increased knowledge of the microcystin synthetase genes has allowed for the creation of primers specific to those target genes of interest (Nishizawa, 1999; Rouhiainen et al., 2004; Tillett et al., 2000). With this it has become possible to amplify (via conventional PCR) and quantify (using techniques such as qPCR and now ddPCR) microcystin gene copies from a wide array of sources including municipal drinking waters and environmental sources, even at low concentrations (Beversdorf et al., 2015). Genes of interest include any of the functional genes required

for microcystin synthesis (genes found in the *mcy* cluster) and the 16S rRNA housekeeping gene, commonly used in studies of microbial ecology (Case et al., 2007).

1.3 Cyanobacteria at high latitudes

Typically cyanobacteria in Polar Regions are found in benthic mats or films at the bottom of streams, lakes and ponds, and are composed primarily of filamentous species within the orders *Nostocales* and *Oscillatoriales* (Kleinteich et al*.,* 2012; Vincent & Quesada, 2012). Planktonic, bloom-forming species of cyanobacteria are generally uncommon in these environments (Vincent & Quesada, 2012). However, blooms of *Dolichospermum flos-aquae* have been reported in Smith Lake, Alaska possibly as a consequence of low N:P ratios (3.0 before the onset of blooms and 6.1 during) (Gu & Alexander, 1993), while blooms containing *D. flos-aquae, Gloeocapsa turgida* and *Microcystis pulverea* have been documented in Lake Svyatoe, Northern Eurasia as a result of uncharacteristic temperature increases (Pokrovsky & Shirokova, 2013).

As bloom-forming, microcystin-producing species tend to be uncommon in polar environments (Hitzfeld et al., 2000), studies have yet to identify the toxin in northern waters. However, evidence of low levels of microcystin has been reported in Antarctic microbial mat communities on the McMurdo Ice Shelf, on Bratina Island and in the Dry Valleys (Hitzfeld et al., 2000; Jungblut et al., 2006; Wood et al., 2008). Meanwhile, there exists only a single report of microcystins in Arctic cyanobacterial mats: Kleinteich *et al.* (2012) were able to detect the toxin in very low quantities in their studies on Baffin Island, Nunavut.

It is believed that as the waters of the Arctic and Antarctic warm as a result of climate change, such regions may become more favourable to bloom-forming cyanobacteria (Vincent & Quesada, 2012). In light of ongoing climate change, increased studies into the occurrence of planktonic cyanobacteria and cyanotoxins in polar waters are warranted (Kosek et al., 2016; Smol, 2005).

1.4 Paleo-limnological approaches

Paleo-limnology is the study of the preserved physical, chemical and biological information stored in the aquatic sediments of inland waters and lakes in particular (Smol, 2008). Lake bottom sediments can be invaluable tools when assessing current impacts on a lake and in order to establish past conditions (Smol, 2008). By their nature, benthic sediments can serve as a record of the material composition of a lake, preserving and providing information on pollutants, nutrients and other particulate matter (Engstrom & Rose, 2013). Such information can be assessed both qualitatively, by noting the differences in composition over time, as well as quantitatively, by recording changes in sedimentation rates (Engstrom & Rose, 2013). These changes, when compared to present day conditions, can provide greater insight into the ecological quality of a water body, allowing for better lake management initiatives (Engstrom & Rose, 2013; Foster et al., 2011, Smol, 2008).

Sediments are repositories of several microbial, algal and invertebrate species, all of which can provide additional information on the state of a water body. For example, microalgae like diatoms as well as invertebrate species such as the chironomids and

cladocera are commonly used in paleo-limnological studies and can provide information on both historic eutrophication and climate change (Smol, 2005, Smol 2008, Antoniades, 2011 etc).

Dating sediment core subsections via techniques such as ^{210}Pb dating is common practice in paleo-limnology. Dating techniques allow for the approximate age of sediment core slices to be determined, providing better historic resolution. Following the *Law of Superposition*, undisturbed sediments will deposit with progressively younger material accumulating over older material, creating a depth-time profile which can be elucidated via dating methods (Smol, 2008). The archival material and information that can be garnered from these sediment cores can in turn help to recreate past lake and environmental conditions, providing proxy data for occurrences such as eutrophication and climate change where long term monitoring data is sparse (Smol, 2008; Smol et al., 2005, Engstrom & Rose, 2013).

The time scale of information that can possibly be gathered from a single core is dependent on the sedimentation rate (the rate at which sediments accumulate) of the water body in question (Smol, 2008). Sedimentation rates can vary from lake to lake and as such a 100 cm core obtained from one lake may contain sediments accumulated over hundreds of years while an equally long core from another lake may only cover a fraction of that time. Lakes that are subject to higher degrees of eutrophication and anthropogenic activity tend to have higher sedimentation rates, meaning sediments accumulate much faster than lakes that are not as impacted (Foster et al., 2011; Smol, 2008). Due to their remote nature, cooler temperatures and lower productivity, Arctic lakes tend to have

much lower sedimentation rates than those at more temperate latitudes (Douglas & Smol, 2000).

Recently, researchers have begun to successfully extract DNA and RNA from sediment cores allowing for the use of molecular approaches when assessing historic conditions (Coolen et al., 2006; Gregory-Eaves & Domaizon, 2014; Hurt et al., 2001; Pal et al., 2015). Cyanobacterial DNA from both surface sediments (Rinta-Kanto et al., 2009) as well as from dated sediment cores (Domaizon et al., 2013; Martinez De La Escalera et al., 2014; Pal et al., 2015; Savichtcheva et al., 2011; Ye et al., 2011) has been successfully extracted and used in downstream applications in order to assess the historic cyanobacterial assemblages of lakes from a number of different geographic locations.

1.5 Climate change impacts on northern freshwaters

Lakes and other freshwater environments are subject to several primary effects of climate change (temperature, precipitation, permafrost and lake ice cover, etc.) and also to many of their related secondary effects which can lead to changes in nutrient loadings, water column stability, residence time and water level (Mooij et al., 2005). As very few studies have been conducted on northern limnology/paleo-limnology (Antoniades et al., 2011; Douglas & Smol, 2000; John P. Smol & Douglas, 1996) much less monitoring data exists for these areas. However, several predictions about the future of northern freshwaters can be made based on existing climate trends and their known impacts.

Increases in precipitation and the warming of permafrost are known to change water-courses and alter the patterns of lakes and wetlands in the north (AMAP, 2011). The

presence of permafrost itself is important for the persistence of northern lakes, as it prevents water infiltration to the subsurface (Rouse et al., 1997; Smith et al., 2007). In their study, Smith et al. (2007) observed the role of permafrost in lake prevalence, with the presence of permafrost having a nearly two-fold effect on lake populations and densities.

Temperature increases have also led to a decrease in the winter ice coverage of high latitude oceans over the past four decades with decreases of approximately 12.5% between 1970 and 2015 (Appendix A, Fig A. 5). In addition, long-term observational records for the Arctic have shown that lake ice formation is occurring later and melt dates earlier for several lakes throughout the region with anomalies of 1-4 weeks being the norm (Duguay et al., 2014).

Warmer temperatures can also lead to increased water column stability, a condition favourable to several phytoplankton species including the cyanobacteria (Carey et al., 2012; Paerl & Paul, 2012; Paerl, 2008). As water column stability increases planktonic species are more easily able to ascend the water column in search of light, and in the case of bloom-forming cyanobacteria, to rise to the surface and form blooms (Carey et al., 2012; Paerl & Paul, 2012; Paerl, 2008).

1.6 Climate change and cyanobacterial blooms

Though cyanobacterial blooms are known to occur naturally, the majority of blooms are considered to be a consequence of eutrophication and other anthropogenic influences (Paerl, 2008). The conditions necessary for bloom formation can arise as a

direct consequence of increased nutrient inputs (e.g. nitrogen and phosphorus from wastewater treatment plants/agricultural runoff) or indirectly as a consequence of the effects of climate change. Recently, studies have begun investigating the links between the ramifications of climate change and the production of cyanobacterial blooms (Kosten et al., 2012b; Paerl & Paul, 2012).

Global temperatures are expected to rise by 1.4- 5.8 ºC until 2100 (IPCC, 2007). As several bloom forming cyanobacterial species like *Microcystis* and *Aphanizomenon* are known to have optimal growth temperatures in the range of 20- 28 ºC (Carey et al., 2012; Reynolds, 2006), it is possible that these temperature increases will cause a shift in phytoplankton biomass towards cyanobacteria as well as provide for longer growing seasons for such species (Huber et al., 2012; Kosten et al., 2012; Mehnert et al., 2010; Mooij et al., 2005; Paerl & Paul, 2012).

Studies of diatoms assemblages in some high latitude lakes have shown that with climate warming there has been an increase in the relative abundance of planktonic genera like *Cyclotella* at the expense of benthic genera such as *Fragilaria* (Smol et al., 2005; Rühhland et al., 2003), a shift that can likely happen to other phytoplankton species over time. In light of climate change, increasing global temperatures resulting in warmer water bodies will likely lead to an increase in cyanobacterial blooms as observed in a number of lakes worldwide (Huber et al., 2012; Jöhnk et al., 2008; Liu, Lu, & Chen, 2011; Michalak et al., 2013; Paerl & Huisman, 2008). Such changes can occur as a direct result of elevated temperatures, which promote the growth of several toxic species, as well as increased water column stability, which will allow buoyant bloom forming species to form surface blooms.

1.7 Thesis objectives and hypotheses

1. Investigating historic and contemporary cyanobacterial populations in planktonic and benthic assemblages in northern environments

In September of 2013 a purported cyanobacterial bloom was observed in the waters of Yellowknife Bay, Great Slave Lake (GSL), an embayment of GSL located within the city of Yellowknife; this event was considered unprecedented by local inhabitants (J. Chételat, Environment Canada, personal communication, Jan 30, 2014). In order to determine whether this represents a change in the historical cyanobacterial community, an analysis of sediment cores was conducted on GSL and some smaller adjacent lakes as well as two other lakes at much higher latitudes. DNA was extracted and amplified in order to analyze changes in cyanobacterial community structure (planktonic vs. benthic) including producers of the toxin microcystin. Furthermore, sampling in late summer of 2014 and 2015 was conducted in order to determine the composition of contemporary phytoplankton in the lake and whether there are toxin producers within the cyanobacterial assemblage.

Hypothesis:

As a result of climate change and/or eutrophication, planktonic cyanobacteria species are more abundant in northern surface waters and recent sediments in comparison to the historical assemblages.

2. Assessing the toxicity of single colonies of microcystin-producing cyanobacteria from environmental and culture sources

Though it has been established that all toxic, microcystin-producing cyanobacteria possess the microcystin synthetase genes (*mcy* genes) necessary for toxin production, it is also known that microcystin producing genera, notably *Microcystis, Dolichospermum* and *Planktothrix* (Chorus & Bartram, 1999) include both toxic non-toxic strains, which can co-exist in mixed assemblages (Vezie et al., 1998; Kurmayer et al., 2004). Current molecular methods allow for the detection of a number of *mcy* genes from water sources, however in general primers are typically developed with a single microcystin producer in mind and even when capable of targeting a range of producers such methods do not allow for the elucidation of which individuals are indeed responsible for toxin production.

Using single cell genome amplification techniques (Hamilton et al., 2015; Richlen & Barber, 2005) it should however be possible to establish which individual colonies of cyanobacteria within a mixed assemblage are responsible for toxin production. As cyanobacterial toxicity is known to vary within species, this technique may allow for the inter-species variation within the study sites to be assessed as well as the identification of specific toxin producing taxa.

This technique was used to determine if blooms in Great Slave Lake contained toxin producing species and was also used to investigate the production of microcystin by other bloom-forming cyanobacterium such as *Gloeotrichia enchinulata*, which has been purported to produce the toxin.

CHAPTER 2: CYANOBACTERIA NORTH OF 60º

2.1 Introduction

Lakes, ponds, and other freshwater systems dominate Canada's northern landscape, providing habitat to numerous plant and animal species as well as providing food and water to the human populations in the area. Though it is easy to look at these environments solely at a macroscopic level, the unseen physical, chemical and biological activities occurring in northern water bodies are arguably more important when it comes to long term ecosystem health and viability. Few studies have been conducted assessing the quality of northern lakes, likely due to their remote location and the small human populations that surround them. However, in light of the global threat of climate change, a force that affects northern regions to a much higher degree than other locations (IPCC, 2014), it is important that increased attention be dedicated to Northern ecosystems.

Northern aquatic ecosystems are known for their short growing seasons characterized by extended daylight hours. During this period primary producers such as cyanobacteria are important to the elemental cycling and aquatic food chains therein. Cyanobacteria can be found in water bodies the world over with polar lakes and oceans being no exception. In the Arctic and Antarctic, cyanobacteria are generally absent from planktonic assemblages; in contrast they are quite abundant on sediment and rock surfaces forming thick, highly pigmented benthic mats and films (Bonilla et al., 2005; Hitzfeld et al., 2000; Jungblut et al., 2010; Kosek et al., 2016). The most commonly encountered groups of cyanobacteria found in the north are the Nostocales, Oscillatoriales and Chroococcales in benthic assemblages and picocyanobacteria, typically identified as *Synechococcus* morphotypes, in the plankton (Bonilla et al., 2005; Kosek et al., 2016; Vincent et al.,

2000). Bloom-forming, planktonic taxa are rare in northern waters (Kosek et al., 2016) and thus far cyanobacterial blooms have only been reported for a few subarctic lakes (Gu & Alexander, 1993; Gu, 2012; Pokrovsky & Shirokova, 2013; Salmaso et al., 2015).

The high latitudes pose a number of physical-chemical challenges to the survival of cyanobacterial species. Such environments subject cyanobacteria to lower ambient air and water temperatures, lower nutrient supplies, varying light extremes, and a shorter growing period due to extended ice coverage. However, several of the biological properties of cyanobacteria along with their great capacity for adaptation have allowed them to thrive even under such conditions (Kosek et al., 2016). Several species of cyanobacteria are known for their cold tolerance allowing them to survive high-low temperature extremes and adapt to prolonged freezing with extracellular polymeric substances allowing for the maintenance of membrane fluidity under freezing conditions (Kosek et al., 2016; Vincent et al., 2004). Polar cyanobacteria also produce increased amounts of pigmentation for protection against high UV radiation in the summer months when daytime sunlight is prolonged (Bonilla et al., 2009; Bonilla et al., 2005; Kosek et al., 2016; Quesada et al., 1999). Some species have also adapted lower growth rates (Kosek et al., 2016; Tang et al., 1997; Vincent, 2007) possibly as a consequence of low ambient nutrient concentrations.

The low growth rates of polar cyanobacteria in and of itself may lead one to assume that the formation of cyanobacterial blooms at these latitudes is highly unlikely as, by definition, an algal bloom is a rapid and expansive accumulation of algal biomass. However, recent examples of subarctic cyanobacterial blooms (Gu & Alexander, 1993; Gu, 2012; Pokrovsky & Shirokova, 2013), coupled with first hand observational evidence

from Yellowknife Bay, Great Slave Lake (J. Chételat, Environment Canada, personal communication, Jan 30, 2014), points to the contrary. Early studies of polar lakes noted only low densities of planktonic cyanobacteria even in places experiencing the levels of nutrients that would generally promote cyanobacterial growth. In addition, further studies have indicated a general absence of many bloom-forming taxa (Kalff & Welch, 1974; Kosek et al., 2016; Lund, 1962; Rawson, 1956). Such evidence supports the theory that perhaps temperature has a larger role in bloom dynamics, at least in polar lakes, and that with increasing temperatures, as a consequence of climate change, planktonic cyanobacteria and cyanobacterial blooms will become more common in these northern systems.

One of the major concerns related to cyanobacterial blooms is their potential to produce a number of toxins known to affect humans and wildlife. One of the major toxins produced by the cyanobacteria in freshwater environments are the hepatotoxic microcystins. With the threat of toxic, microcystin-producing cyanobacterial blooms, precise and reliable methods of microcystin detection in waters is imperative. To date, several methods have been utilized including microscopic identification and molecular assays in order to identify potentially toxic cyanobacteria and to quantify microcystins directly from drinking waters. However, these methods have their limitations potentially leading to misidentifications of species/strains or incorrect measurements of toxin concentrations, which can have adverse consequences given the high potency of microcystins. As a result, researchers have turned to DNA based molecular methods in order to assess the toxicity of bloom material (Ouellette & Wilhelm, 2003). These

methods have allowed for the rapid detection and quantification of genes related to cyanobacterial microcystin production even at low concentrations, allowing for the timely implementation of protection measures.

Thus far the use of quantitative PCR (qPCR) has successfully been used in order to quantify microcystin gene concentrations from both potable and environmental sources (Baxa et al., 2010; Martins et al., 2011; Rinta-Kanto et al., 2005; Te & Gin, 2011). However, the necessity of a standard curve upon which to compare replicates and estimate gene copy numbers, along with imperfect amplification efficiencies are just some of the limitations of this technique (Hindson et al., 2011). With the advent of droplet digital PCR (ddPCR), such limitations are eliminated. The combination of end-point PCR, Poisson statistic, and limiting the need for excessive dilutions have led to an approach that can provide an absolute measure of gene concentration without comparison to an external standard (Hindson et al., 2011). In ddPCR target DNA molecules are distributed over approximately 20,000 replicate reactions, which will contain either none or one or more template copies, virtually eliminating the need for technical replicates (Hindson et al., 2011). Additionally, the reliance of ddPCR on binary end-point thresholds to determine whether droplets do or do not contain template means that this method allows for wide variations in amplification efficiencies without affecting the quantification of gene copy numbers (Hindson et al., 2011).

Recently, Te et al. (2015) conducted a study comparing the efficiencies of qPCR and ddPCR in the quantification of the freshwater cyanobacteria *Cylindrospermopsis* sp. and *Microcystis* sp. from environmental sources. Their findings showed that though qPCR was more sensitive than ddPCR, provided a wider dynamic range and was more time and

cost effective ddPCR results were more precise and accurate, and not as subject to the competitive effects of other targets or inhibitors even at low copy numbers.

The present study aims to determine the historic and contemporary occurrence of cyanobacteria in northern freshwater environments, with a particular focus on toxigenic, microcystin producing taxa. DNA extracted from both planktonic water samples and benthic sediment cores was used to quantify (via ddPCR) the 16S rRNA gene specific to cyanobacterial taxa (Kaneko et al., 2007), the *mcyE* gene present in microcystin producing cyanobacteria (Conradie & Barnard, 2012; Takakazu Kaneko et al., 2007; Vaitomaa et al., 2003), and the *glnA* housekeeping gene (Stoeva et al., 2014).

The *mcyE* gene was chosen as a proxy for microcystin production. *McyE* is responsible for the synthesis and incorporation of the toxicity conferring ADDA moiety, which is required for the synthesis of all microcystin variants, making it a reliable biomarker (Ngwa, Madramootoo, & Jabaji, 2014). In addition, *mcyE* is also responsible for the incorporation of the amino acid D-Glu, another key component for toxicity (Rantala et al., 2006). The *glnA* gene responsible for coding the glutamine synthetase enzyme was chosen as it is essential for nitrogen metabolism in all bacteria and hence can be used as a biomarker for overall bacterial activity and abundance (Stoeva et al., 2014).

An assessment of surface waters was also conducted in order to resolve the taxonomic composition of the summer phytoplankton community and the presence of microcystin in the water column as well as the levels of factors considered to promote cyanobacterial bloom formation (e.g. nutrients, temperature, water column stability) (Oliver & Ganf, 2000).

2.2 Methods

2.2.1 Study sites

Although the primary focus was on Yellowknife Bay, Great Slave Lake in the Northwest Territories, a suite of lakes was chosen to cover a number of different northern regions of Canada and eutrophication states (Fig. 2.1). Two lakes in the Yellowknife area were chosen that have less human impact in their catchments than Yellowknife Bay (Madeline Lake and Pontoon Lake). In addition, Meretta Lake in the high Arctic, well studied for its history of eutrophication (Schindler et al., 1974), and Crazy Lake located just outside of Iqaluit, Nunavut not known to be directly impacted by anthropogenic activities, were compared. Coordinates for all locations are indicated in Table 2.1.

2.2.1.1 Yellowknife Bay, Great Slave Lake

Great Slave Lake (GSL), Canada's fourth largest lake, is located within the Mackenzie Basin in the North West Territories (Schertzer *et al*., 2000; Evans *et al*., 2013). The lake's location places it within an area known for the greatest year-to-year climate variability in the Northern Hemisphere (Magnuson *et al*., 2000; Sarmiento & Khan, 2010; Serreze *et al*., 2000). GSL has a surface area of 28, 568 km^2 with a mean depth of 73 m and a maximum depth of 614 m making it the deepest lake in North America (Wayland, 2004; Herdendorf, 1982). The lake can be divided into two main sections, the relatively shallow West Basin, which supports commercial fisheries, and the deeper East Arm (Evans, 2000; Evans *et al*., 2013).

Yellowknife Bay is an embayment of GSL located in the north-west portion of the lake near the City of Yellowknife, the capital and largest city in the Northwest Territories (pop. ~ 20,000) (Fig 2.2). The waters of Yellowknife Bay serve not only recreational purposes, but are also used for fishing and as a secondary drinking water source after the Yellowknife River. Historically, treated wastewater has also found its way into Yellowknife Bay by way of Back Bay. Prior to 1981 domestic waste was amassed in Niven Lake, a small lake that would subsequently discharge into Back Bay which forms part of Yellowknife Bay (Bell et al.,1976). Sewage treatment at this time was not as efficient and though the bacterial quality of Yellowknife Bay was deemed good, it was not considered safe enough for human consumption (Bell et al., 1976).

Recently, a number of studies have focused on the water quality of Yellowknife Bay because of concerns surrounding metal contamination (particularly arsenic and mercury), a legacy effect linked to the historical gold mining that occurred in the area (Galloway et al., 2012; Houben et al., 2016; Thienpont et al., 2016). While conducting such studies, Environment Canada researchers noted a potential algal bloom in Yellowknife Bay during August of 2013. Such an occurrence defies the current knowledge of cyanobacterial ecology and was not part of traditional indigenous knowledge (J. Chételat, Environment Canada, personal communication, Jan 30, 2014).

2.2.1.2 Madeline Lake and Pontoon Lake

Madeline and Pontoon Lake are two small lakes located along the Ingraham Trail (Highway 4), 25 and 30 km respectively east of Yellowknife, NWT (Falk, 1974). These

two lakes were added as additional sampling sites during the August 2015 field season and were chosen due to their small size (surface areas of 1.1 km^2 and 4.1 km^2 for Madeline and Pontoon respectively), their proximity to the city of Yellowknife, and their low levels of arsenic contamination (Falk, 1974; Galloway, 2012). As much of the Yellowknife area including Great Slave Lake has been impacted by arsenic and other loadings from local gold mines some less impacted lakes from the area were chosen to include in the study.

Both Madeline and Pontoon Lakes were chosen due to their higher phosphorus concentrations when compared to other lakes in the area (exhibiting concentrations on average approximately 10 times higher), as high levels of phosphorus are conducive to increased phytoplankton and cyanobacterial biomass and overall lake productivity. Surface water TP concentrations for Madeline lake were reported as 37 µg/L in spring (April/May) and 16 µg/L in summer (July/August) (Moore, 1980b), which represent mesotrophic to eutrophic conditions.

In addition, past studies of these lakes indicated that they might support cyanobacterial blooms. For example, Madeline Lake showed a high propensity for elevated phytoplankton biomass and high accumulations of the potentially toxic cyanobacteria *Dolichospermum flos-aquae* during the summer months, particularly in August (Moore, 1980), while Pontoon Lake had surface water temperatures of approximately 20ºC (Galloway, 2012).

2.2.1.3 Meretta Lake

Meretta Lake is a small lake (0.26 km^2) located on Cornwallis Island, Resolute Bay, Nunavut. Meretta Lake has for a long time been a lake of great interest when it comes to Arctic limnology due to its recorded history of anthropogenic eutrophication and focus during the International Biological Programme's Char Lake Project 1968-1972 (Kalff & Welch, 1974).

In the past, Meretta Lake was most likely a typical high Arctic, oligotrophic lake, however, beginning in 1949, the onset of raw sewage inputs into the lake lead to eventual eutrophication (Antoniades et al., 2011; Douglas & Smol, 2000; Michelutti, Douglas, & Smol, 2002). Direct sewage inputs into the lake continued until 1969 when the construction of berms resulted in the formation of sewage lagoons, which in turn slowed organic loadings and began the lake recovery process (Antoniades et al., 2011; Douglas & Smol, 2000).

A high resolution paleo-limnoligical study on Meretta Lake has shown increases in chlorophyll a and other phytoplankton pigments including echinenone and zeaxanthin, specific to cyanobacteria, in sediments corresponding to the years of direct sewage input, indicating an increase in algal biomass (Antoniades et al., 2011). In the years following the introduction of the sewage lagoons, pigment values decreased dramatically and in general returned to levels observed in the years prior to eutrophication (Antoniades et al., 2011).

Meretta Lake's history of eutrophication and location in the High Arctic make it an ideal study lake for the occurrence of toxic, microcystin producing, cyanobacterial

blooms. Historic pigment analysis from this lake has shown that cyanobacterial biomass can in fact reach high levels in the presence of excess nutrients even at latitudes not known for temperatures conducive to bloom formation. As such, the inclusion of Meretta Lake may provide insight on the roles of temperature and eutrophication on northern freshwater cyanobacterial communities.

2.2.1.4 Crazy Lake

Though not much physical or chemical data exist for Crazy Lake it was also included in the study as an example of a non-impacted High Arctic lake.

Located about 11 km north of Iqaluit, Nunavut Crazy Lake has a surface area of approximately 4.5 km^2 and an average depth of 10 m. As a small Arctic lake, Crazy Lake has not been extensively studied, however it was used as part of an environmental-change monitoring project by students at Nunavut Arctic College (Dyck, 2007). Students collected basic limnological data including snow and ice depths over two field seasons held between April 10-16, 2005 and 2006. Findings from this study showed relatively stable temperatures, pH and dissolved oxygen (DO) levels throughout the water column, with DO being quite high even in deep waters (8.7-14.4 mg/L). However, no major differences were observed when compared to other lakes at similar latitudes, and no changes that could be directly linked to climate change were noted, given the small data set (Dyck, 2007).

Figure 2.1 Map of study sites.

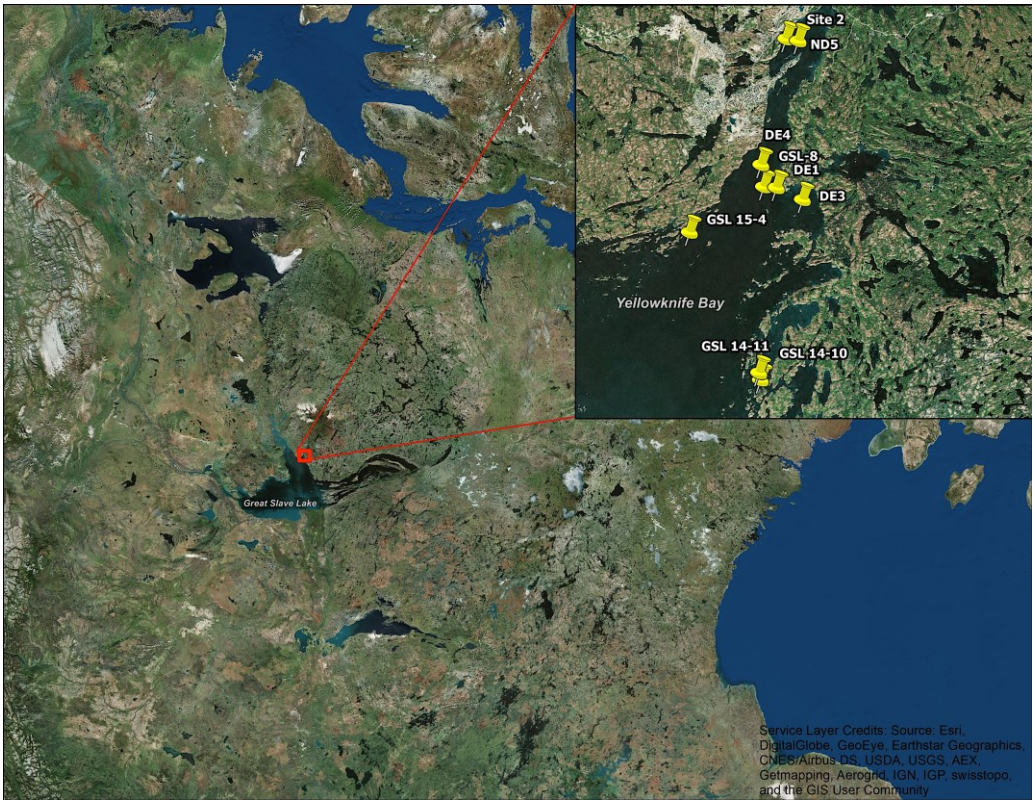

Figure 2.2 Map of Yellowknife Bay, Great Slave Lake indicating station locations.

Table 2.1 Coordinates of lakes and Yellowknife Bay (GSL) sampling stations.

Lake/ Station	Coordinates
GSL 8B*	62°25.181 N, 114°20.156 W
GSL 14-10	62°18.840 N, 114°15.910 W
GSL 14-11	62°19.037 N, 114°16.153 W
Site 2 (Yellowknife Bay)	62°29.127 N, 114°21.067 W
DE1 (Yellowknife Bay)	62°24.652 N, 114°18.722 W
DE3 (Yellowknife Bay)*	62°24.540 N, 114°16.828 W
DE4 (Yellowknife Bay)	62°24.555 N, 114°19.545 W
ND5 (Yellowknife Bay)	62°29.187 N, 114°20.250 W
GSL 15-4*	62°22.532 N, 114°23.357 W
Madeline Lake	62°32.850 N, 114°05.083 W
Pontoon Lake	62°32.517 N, 114°00.500 W
Crazy Lake	63°52.067 N, 68°28.649 W
Meretta Lake	74°41.750 N, 94°59.580 W

* GSL stations where cores were taken

2.2.2 Water Chemistry

Total nitrogen (TN) and total phosphorus (TP) concentrations for the 2015 water samples (GSL, Madeline and Pontoon lakes) were measured by the City of Ottawa Laboratory services, while 2014 samples were measured at the University of Montreal (Dr. R. Carignan laboratory). Each sample was measured in duplicate.

2.2.3 Identification of contemporary phytoplankton communities

2.2.3.1 Sample collection

Dr. John Chételat of Environment Canada collected planktonic samples from Great Slave Lake in 2014. Surface water samples were collected using an integrated tube sampler, from five sites (Yellowknife Bay Site 2, GSL-DE4, GSL 14-10, GSL 14-11, and GSL-DE3 of Fig. 2.2.) between September 5 and 9. Samples consisted of 100 mL whole water samples preserved in Lugol's iodine for phytoplankton analysis via microscopy and GF/C filters (pore size ~1.2 μm) for chlorophyll a, microcystin and DNA extraction. Whole water samples were stored in the dark at room temperatures, while filters were stored in the dark at -20° C until further analysis.

Katherine Alambo collected samples from Great Slave Lake, Pontoon Lake and Madeline Lake during the August 2015 field season with the assistance of Nicole Dion (NWT Government). Samples were collected on August 8, 2015 (GSL 15-4), August 10, 2015 (Pontoon and Madeline Lakes) and August 11, 2015 (GSL-ND5) and consisted of subsurface grabs. These were then subsampled as follows: 100 mL whole water samples

preserved in Lugol's iodine for microscopic identification and 1L filtered through GF/C filters (pore size ~1.2 μm) separately for chlorophyll a, microcystin and DNA extraction. Whole water samples were stored in the dark at room temperatures, while filters were stored in the dark at -20° C until further analysis.

2.2.3.2 Microscopic identification and phytoplankton counts

Microscopic identification and phytoplankton counts were conducted for abundance and biomass with the Utermöhl method (Utermöhl, 1931). Lugol's iodine preserved whole water samples were sub-sampled and settled overnight (24 hours) in 25mL Utermöhl settling chambers onto a 2.5 cm diameter depression slide. After the 24h-settling period the chamber was removed and replaced with a cover slip. Slides were examined using the Zeiss Observer A1 inverted microscope at low (100x), medium (200x), and high (400x) magnification. A general scan of each slide was first conducted at low power and any large phytoplankton taxa (e.g. *Ceratium* sp.) were counted. Counts of all other phytoplankton species were conducted at medium and high power over three transects or until 250 individuals were counted. Dimensions (length, width, diameter, depth) were taken for at least three individuals of each unique species in each transect within which they were seen. Photos of each species encountered were taken, particularly for those that could not be identified on sight. Subsequent species identification was conducted using a number of phytoplankton keys (Bellinger & Sigee, 2010; Cronberg & Annadotter, 2006) and consultation with various phytoplankton experts.

Calculations of total biomass and proportions of major taxonomic groups were conducted using the Algamica software developed by P. B. Hamilton (Gosselain & Hamilton, 2000; Hamilton, 1990).

2.2.4 Chlorophyll a extraction

Planktonic chlorophyll a (chl a) was measured to provide an index of phytoplankton biomass and overall lake production. Chl a was extracted from filters using ethanol. Approximately 1 L of lake water was filtered onto GF/C glass microfiber filters (~1.2 µm pore size, 47mm diameter), which were stored in the dark at -20 ºC until further processing. Filters were extracted in a 15 mL falcon tube containing 15 mL of 95% ethanol for 24 hours at 4 ºC in the dark (Sartory & Grobbelaar, 1984). Extracts had their absorbance read at 750 nm, 665 nm and 649 nm using a Cary 100 UV-Visible Spectrophotometer from Varian and a 5 cm path length cuvette. Chl a concentrations were calculated following the equation found in Bergmann & Peters (1980).

2.2.5 Microcystin analysis

2.2.5.1 Microcystin extraction

In order to determine particulate microcystin concentrations filters were extracted via Accelerated Solvent Extraction (ASE) using a Dionex ASE 350 and the method of Aranda-Rodriguez et al. (2005). Filters were placed in 10 mL stainless steel ASE cells, which were then packed with Hydromatrix. The cells were exposed to high temperature (80º C) and high pressure (1500 psi) for 20 minutes in 75% aqueous methanol. The

resulting 22 mL extracts were then evaporated to dryness using a combination of first air and then N_2 gas, at 60º C using the Reacti-Vap evaporator. Once dry the walls of the samples were rinsed with 2 mL of 100% methanol and then evaporated once again to dryness using N_2 gas at 60º C. The resulting extract of microcystin was then reconstituted in 50% aqueous methanol, transferred to HPLC vials and stored in the dark at 4º C until further analysis.

2.2.5.2 ELISA

An Enzyme Linked Immunosorbent Assay (ELISA) was carried out using the Microcystins/Nodularins (ADDA) ELISA Kit from Abraxis (Warminster, PA). In order to run this kit microcystin extracts must contain no more than 5% methanol. As microcystin extraction was conducted using 50% methanol extracts were diluted by mixing 16 µl of extract with 144 µL of sterile deionized water to a final volume of 160 µL and a finale concentration of 5% methanol. All samples were run in duplicate against a standard curve that ranged between 0 and 5 µg/L of microcystin. Additionally, a control with a concentration of 0.75 ± 0.185 µg/L, extracted from a secondary source, was run as a Quality Control Standard.

A number of samples including those obtained from sites GSL-DE4, GSL-ND5 and Pontoon Lake had to be re-run as initial readings fell outside of the detection range. They were subsequently diluted to 1.25% methanol to provide measurements that could be detected by the ELISA kit used.

2.2.5.3 DNA extraction from filter samples

DNA from whole water samples was extracted from filters following the phenol chloroform method described in Hisbergues (2003). A volume of 1 L of lake water was filtered onto GF/C glass microfiber filters (~1.2 µm pore size, 47mm diameter), which were stored in the dark at -20 ºC until further processing.

2.2.6 Historic cyanobacteria assemblages with sediment depth

2.2.6.1 Sample collection and dating

All sediment cores were collected using the UWITEC Gravity Corer (8.6 cm inner diameter). The sediment cores were sectioned into 0.5 cm segments in lab shortly after collection then stored in Whirlpak® bags at -20ºC for further analysis. Information about each core used in the study can be found in Table 2.2

Sediments were dated via ^{210}Pb. The GSL 8 core was dated at the Canada Centre for Inland Waters (Environment Canada, Burlington), while the Madeline Lake core was dated by MyCore Scientific (www.mycore.ca). Data were provided by John Chételat. Dating for the Meretta Lake core was estimated from the Antoniades et al. (2011) study.

Table 2.2 Sediment cores from sites and lakes of Figure 2.1 and Figure 2.2.

Core/Lake	Date	Length (cm)	Collected by
Great Slave Lake (GSL8)	September 7, 2013	24	J. Chételat
Yellowknife Bay (DE3)	September 9, 2014	26	J. Chételat
Meretta Lake	July 24, 2014	15	J. Chételat
Crazy Lake	August 4, 2014	9	J. Chételat
Great Slave Lake (15-4)	August 8, 2015	19.5	K. Alambo, J. Chételat
Madeline Lake	August 10, 2015	41	K.Alambo, N.Dion
Pontoon Lake	August 10, 2015	30	K.Alambo, N.Dion

2.2.6.2 DNA extraction from sediment cores

Prior to DNA extraction excess water was removed from the frozen sediment core sub-samples. Samples were thawed overnight at 4 ºC in the dark, and then approximately 1.0 g fractions were transferred to 2 mL micro-centrifuge tubes. Tubes were centrifuged at 10 000 x *g* for 30 seconds at room temperature. The resulting supernatant of water was removed and the process was repeated until all water was removed and the centrifuged tubes were filled to approximately the 1.5 mL mark. DNA was extracted from sediment core sub-samples using the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories Inc, Carlsbad, CA) following the manufacturer's protocol.

Approximately 0.25 g of each sediment sub-sample was added to the PowerBead tubes in order to prepare the sample for lysis. Following a brief vortex to disperse the sample within the PowerBead solution, 60 µL of lysis solution was added to each PowerBead tube. The tubes were then vortexed at maximum speed for 10 minutes in order to completely homogenize and lyse the cells. The tubes were then centrifuged at

10 000 x *g* for 30 seconds at room temperature. The resulting supernatant was then transferred to a clean 2 mL collection tube and 250 µL of the Inhibitor Removal Technology® reagent was added to precipitate out any non-DNA material. The tubes were then centrifuged at 10 000 x *g* for 1 minute at room temperature and 750 µL of the supernatant (free of non-DNA material) was transferred to a new 2 mL collection tube. Following this 1.2 mL of high concentration salt solution was added to each tube in order to facilitate the binding of DNA to the silica of the spin filters. Following a quick 5 second vortex, 675 µL of sample was transferred onto a spin filter and centrifuged at room temperature for 1 minute at 10 000 x *g*. The flow through was discarded and the process was repeated an additional two times. Following these three loads, 500 µL of an ethanol based wash solution was added to the spin filter tubes which were then centrifuged at 10 000 x *g* for 30 seconds at room temperature. The flow through was then discarded and the spin filter tubes were centrifuged once again at 10 000 x *g* for 1 minute at room temperature to remove any traces of the ethanol solution. The spin filters were then transferred to new 2 mL collection tubes and 50 µL of sterile elution buffer (10 mM Tris) was added to the centre of each filter to release the bound DNA from the spin filters. Tubes were then centrifuged at 10 000 x *g* for 30 seconds at room temperature. The spin filters were discarded as the resulting flow through contained the sample DNA.

The concentration of each DNA sample was measured using the NanoDrop 2000 (Thermo Scientific) and then samples were stored at -20 ºC until downstream applications.

2.2.6.3 PCR amplification of the *mcyE* gene

PCR amplification of the 186 bp fragment *mcyE* gene was conducted using the two primers mcyE F1 (5'-TAACTTTTTTGGGCATAGTCCTG-3') and mcyE R1 (5'-CGAACWGCYGCCATAATCGC-3') (N. Fortin NRC Montréal, personal communication, May 15, 2014). These primers were selected for their ability to amplify the gene in the main microcystin producing genera namely *Microcystis, Dolichospermum* and *Plankthotrix,* as well as potentially other genera of toxic cyanobacteria. Amplification was conducted beginning with an initial denaturing step for 5 minutes at 96ºC, followed by 30 cycles of 1 minute at 94ºC, 1 minute at 55ºC and 1 minute at 72ºC, finishing with a final extension step of 72ºC for 10 minutes (N. Fortin NRC Montréal, personal communication, May 15, 2014).

Amplification products were separated through a 1% agarose gel at 100V in 1X TAE buffer for 40 min. The products were visualized using the Alphaimager® EC (Alpha Innotec). The sizes of DNA fragments were then estimated via comparison to a 100 bp molecular weight ladder (New England BioLabs).

2.2.6.4 ddPCR optimization and quantification of genes

Droplet digital PCR (ddPCR) was used in order to amplify and quantify the cyanobacterial 16S rRNA, *mcyE* and *glnA* genes. Thus far the use of quantitative PCR (qPCR) has become the standard for microcystin gene quantification in water samples and the use of ddPCR has not been widely applied to environmental samples. However,

in a comparison of the two methods on cyanobacterial bloom material, Te et al. (2015), showed that ddPCR provided more accurate and precise results than qPCR and was not as prone to the effects of other targets and inhibitors even at low copy numbers. The study conducted by Te et al. (2015) was the first to quantify microcystin synthesis genes using ddPCR. Appendix D shows the efficiency of ddPCR with the target genes of interest.

Droplet digital PCR (ddPCR) requires the creation of oil suspended droplets within which target DNA is evenly distributed. In order to create such droplets 20µL of PCR product was combined with 70µL of droplet generation oil, under pressure using the QX 200 droplet generator from Bio-Rad. This process creates a 40µL solution containing on average 20 000 reaction replicate droplets which contain either no template or one or more template copies. Droplets were then transferred to a 96 well plate, which was then sealed using the PX1 Plate Sealer (Bio-Rad). Amplification was conducted using the QX 200 thermal cycler (Bio-Rad) under the following conditions: one 5 minute enzyme activation cycle at 95°C followed by 40 cycles with a 2°C/second ramp rate of 30 second denaturation at 95°C and 1 minute annealing and extension. Following this was a 5 minute cycle at 4°C and 5 minutes at 90°C for signal stabilization.

Prior to running ddPCR on final samples reaction conditions were optimized via temperature gradients to determine the optimal annealing temperature for each gene and dilution gradients in order to establish the optimal DNA concentration for each gene and sample. Primers, annealing temperatures and dilution factors can be found in Table 2.3.

Following amplification the 96 well plate is loaded into the QX 200 Droplet Reader. Samples are quantified provided at least 13 000 droplets were successfully created and amplified.

Quantification of gene copy numbers (GCNs) by ddPCR was calculated using the QuantaSoft Software from Bio-rad. Quantification via QuantaSoft relies on the principles of the Poisson distribution, the discrete probability distribution which determines the probability of a number of events occurring within a fixed interval of time or space, given a relatively small probability (p) and relatively large sample size (n) (Lewis, 1966). As a result, the accuracy of quantification in samples of low DNA concentration (small n value) is less than for those samples of higher concentration (large n value). In other words, at lower GCNs the margin of error in quantification is higher (BIO-Rad, 2014).

Table 2.3 Primer sequences, amplicon size and annealing temperatures used in ddPCR.

Gene	Primer	Primer Sequence	Size (bp)	Annealing Temperature (ºC)	Dilution
Microcystin (*mcyE*)	McyE F[5]	5'-TAA CTT TTT TGG GCA TAG TCC TG-3'	186	59.5	1/20
	McyE R[5]	5'-CGA ACW GCY GCC ATA ATC GC-3'			
Cyanobacterial 16S rRNA	CYAN 108 F[1,2]	5'-ACG GGT GAG TAA CRC GTR A-3'	269	54.5	1/20
	CYAN 377 R[1,3]	5'-CCA TGG CGG AAA ATT CCC C-3'			
Glutamine synthetase (*glnA*)	GlnA F[4]	5'-GAT GCC GCC GAT GTA GTA-3'	153-156	59.5	1/100
	GlnA R[4]	5'-AAG ACC GCG ACC TTP ATG CC-3'			

[1](Rinta-Kanto et al., 2005)
[2](Urbach, Robertson, & Chisholm, 1992)
[3](Nübel, Garcia-Pichel, & Muyzer, 1997)
[4](Hurt et al., 2001)
[5](N. Fortin NRC Montréal, personal communication, May 15, 2014)

2.2.6.5 Statistical analyses

Statistical analyses were conducted on the GCNs of the *mcyE*, 16S rRNA and *glnA* genes as well as the concentration of DNA from sediment samples in order to test for potential relationships between the genes of interest and the amount of sediment DNA. Sediment gene amplification data were all verified for normal distribution via the Shapiro-Wilk test. Data that did not fit a normal distribution were log-transformed and re-assessed. Correlations of normally distributed data were assessed using the Pearson Product-Moment correlation coefficient (r), while data that did not fit a normal distribution were assessed using the Spearman rank correlation coefficient (r_s). Results were considered significant when the p-value was less than 0.05.

One factor to consider when working with time-series in sediment samples is temporal autocorrelation. Temporal autocorrelation in sediment samples arises as a consequence of how sediments accumulate. Due to variations in sedimentation rates each sediment core section will encompass a variable amount of time. As it is not feasible to analyze each and every sediment subsection we are left with irregularly sampled times series (Dutilleul, Cumming, & Lontoc-Roy, 2012; Simpson & Anderson, 2009), which can be further exacerbated by inconsistency in chemical dating methods such as ^{14}C and ^{210}Pb, leading to errors in age-depth models (Blaauw & Heegaard, 2012). Using statistical methods it is possible to assess the extent to which temporal autocorrelation affects the trends seen in sediment derived time-series, as well as means (such as the continuous time autoregressive model) by which to best model these trends (Simpson & Anderson, 2009).

Sediment GCNs were assessed for temporal autocorrelation using the autocorrelation function (ACF) in R (version 3.3.2). For data that were found to be affected by temporal autocorrelation we applied a continuous-time autoregressive lag 1 (CAR(1)) using Generalized Additive Models (GAMs; mgcv package in R) (Simpson & Anderson 2009). We compared two nested models, one with and one without the CAR(1) structure, and used the Akaike Information Criterion (AIC) to identify the best model among the two.

2.3 Results

2.3.1 Surface water quality and GCN in plankton

Water quality data for the sites in GSL as well as for Madeline and Pontoon Lake can be found in Table 2.4. In general, all sites in GSL and Madeline Lake had total nitrogen (TN) and total phosphorus (TP) concentrations typical of oligotrophic lakes and TN/TP ratios were consistent with lakes exhibiting phosphorus deficiency (Wetzel, 2001). Chl a concentrations were also generally low (<6.0 µg/L) with the exception of two surface bloom samples obtained in 2015 from GSL which represented concentrated plankton samples.

Surface water microcystin concentrations in all lakes were all low, well below Health Canada guidelines of 1.5 µg/L (Health Canada, 2007). This was further corroborated by the GCNs for the *mcyE* gene in these same water samples (despite significant GCNs for the 16S rRNA cyanobacterial genes) (Table 2.5).

Table 2.4 Water quality data (chl a, microcystin, TN and TP) for sites in Great Slave Lake, Madeline Lake and Pontoon Lake. Results indicate averages of duplicates.

Sample (Year)	Chl a (µg/ L)	Microcystin (µg/L)	TN (µg/L)	TP (µg/L)	TN/TP
Site 2 (2014)	1.94	0.0003	286	9	31
GSL-DE4 (2014)	1.55	0.0003	374	8	47
GSL 14-10 (2014)	2.31	0.0008	270	9	30
GSL 14-11 (2014)	1.72	0.001	311	9	34
GSL-DE3 (2014)	1.56	0.0008	n/a	n/a	n/a
GSL- ND5 (2015)	3.08	0.180	245	6	41
GSL 15-4 (2015)	2.38	0.003	210	5	42
Madeline (2015)	5.535	0.002	680	13	52
Pontoon (2015)	1.878	0.359	1055	7	151
GSL-DE1 (2015)*	417.474	0.004	n/a	n/a	n/a
GSL-DE4 (2015)*	2154.06	0.163	n/a	n/a	n/a

* Bloom samples

Table 2.5 DNA concentrations and gene copy numbers of subsurface water samples from Great Slave Lake, Madeline Lake and Pontoon Lake.

Sample (Year)	[DNA] (ng/µL)	*mcyE* GCN/ng DNA	16S rRNA GCN/ng DNA
Site 2(2014)	8.9	487	7697
GSL-DE4 (2014)	11.9	168	49034
GSL 14-10 (2014)	10.3	453	7314
GSL 14-11 (2014)	21.6	93	44907
GSL-DE3 (2014)	9.8	92	26020
GSL- ND5 (2015)	6.5	513	513
GSL 15-4 (2015)	113.3	12	2001
Madeline (2015)	308.1	19	45
Pontoon (2015)	105.9	17	3053
GSL-DE1 (2015)*	78.5	36	49214
GSL-DE4 (2015)*	57.9	66	22798

*Bloom samples

2.3.1.1 Phytoplankton identification, concentration and biomass

Phytoplankton identification and count data for Yellowknife Bay, GSL can be found in Appendix B. The dominant taxa identified throughout all samples were species of chrysophytes and diatoms, with the diatom genera *Cyclotella* being the most frequently encountered. Of the cyanobacterial genera only *Dolichospermum* was present in all sites. A few other genera (including *Oscillatoria* sp., *Aphanizomenon* sp. and *Microcystis* sp.) and some unidentifiable coccoid and small filamentous cyanobacteria were encountered in samples obtained from site DE3 and GSL 14-10.

In addition to the whole water samples two samples of bloom material were collected and identified via light microscopy. Both blooms were composed of

Dolichospermum lemmermannii colonies, a potentially toxic species of cyanobacteria that can form surface blooms.

Concentration and biomass for each station can be found in Table 2.6. Overall the lakes had relatively low algal biomass.

Table 2.6 Phytoplankton densities, biomass and dominant taxa in Yellowknife Bay, GSL

Site	Sample Date	Concentration (#/ L)	Biomass (mg/m3)	Top 3 dominant taxa (% of total abundance)	Top 3 dominant taxa (% of total biomass)
GSL 14-10	September 6, 2014	2.215 x 10^5	564	*Cyclotella meneghiniana* (37%) *Dinobryon divergens* (13%) *Plagioselmis sp.* (12%)	*Cyclotella meneghiniana* (48%) *Dinobryon divergens* (23%) *Asterionella formosa* (13%)
GSL 14-11	September 6, 2014	3.838 x 10^5	415	*Plagioselmis sp.* (33%) *Cyclotella meneghiniana* (19%) *Dinobryon divergens* (9%)	*Cyclotella meneghiniana* (18%) *Cocconeis placentula* (18%) *Dinobryon divergens* (17%)
DE2	September 5, 2014	3.152 x 10^5	278	*Cyclotella meneghiniana* (30%) *Dinobryon divergens* (10%) *Asterionella fformosa* (9%)	*Dinobryon divergens* (27%) *Cyclotella meneghiniana* (19%) *Asterionella formosa* (14%)
DE4	September 7, 2014	6.255 x 10^5	2720	*Cyclotella meneghiniana* (27%) *Dinobryon divergens* (19%) *Lavinda sp.* (7%)	*Cyclotella sp.* (37%) *Dinobryon divergens* (17%) *Asterionella formosa* (12%)
DE3	September 9, 2014	1.327 x 10^5	206	*Cyclotella meneghiniana* (34%) *Peridinium sp.* (20%) Unknown cyanobacteria (8%)	*Peridinium sp. (*75%) *Cyclotella meneghiniana* (12%) *Dinobryon divergens* (2%)
ND5	August 11, 2015	4.431 x 10^5	645	*Cyclotella sp. (30%)* *Synedra acus (15%)* *Cyclotella meneghiniana (8%)* *Dinobryon divergens (8%)*	*Cyclotella meneghiniana* (37%) *Asterionella formosa* (25%) *Dinobryon divergens* (10%)
GSL 15-4	August 8, 2015	3.460 x 10^5	340	*Dinobryon divergens* (23%) *Dinobryon sertularia* (10%) *Cyclotella meneghiniana* (8%)	*Dinobryon divergens* (52%) *Dinobryon sertularia* (23%) *Cyclotella sp.* (8%)
2014 AVERAGE		5.184 x 10^5	738.30		

2.3.2 Sediment DNA analyses

2.3.2.1 *McyE*, 16S rRNA and *glnA* gene copy numbers with depth

Droplet digital PCR (ddPCR) was conducted on sediment core sections in order to quantify GCNs of the *mcyE* and 16S rRNA genes specific to cyanobacterial taxa and the *glnA* gene for total microbial biomass. Figures 2.3-2.7 present profiles of GCN variation with depth for each lake, along with the mean GCN for each gene over the entire profile. GCNs of the *glnA* gene were consistently the highest with on average over a million copies of the gene per gram of sediment (1.5×10^3-4.73×10^6 copy #/g sediment) in all cores except that of Pontoon Lake. The 16S rRNA gene was present at 0.14×10^3-3.5×10^6 copy #/ g sediment, while the *mcyE* gene was on average approximately 1000 times less abundant (0.4-10.5×10^3 copy #/g sediment).

The depth profile for the 2013 sediment core from Yellowknife Bay, GSL is presented herein (Fig. 2.3), while profiles for the 2014 and 2015 sediment cores from Yellowknife Bay, GSL can be found in Appendix B. All GSL cores showed fairly constant, low *mcyE* GCNs in all sections of the core, but slight increases in the 16S rRNA and *glnA* genes towards the surface sediments.

In the GSL core from 2013 (Fig 2.3) we can see that GCNs of the *mcyE* gene have not changed much over time, with the bottom most core section and top most core section both having approximately the same number of gene copies in turn corresponding to the overall mean for the gene throughout the core. Both the 16S rRNA and *glnA* genes steadily increased over time with the exception of slight declines observed at approximately 1919, 1982 and 1996. A convergence in GCNs of the 16S rRNA and *glnA* can be observed in recent years suggesting an increase in cyanobacterial dominance within the bacterial assemblage. On average, the ratio of 16S rRNA to *glnA* is 0.2 prior to the 1950s and 0.5 after 1998.

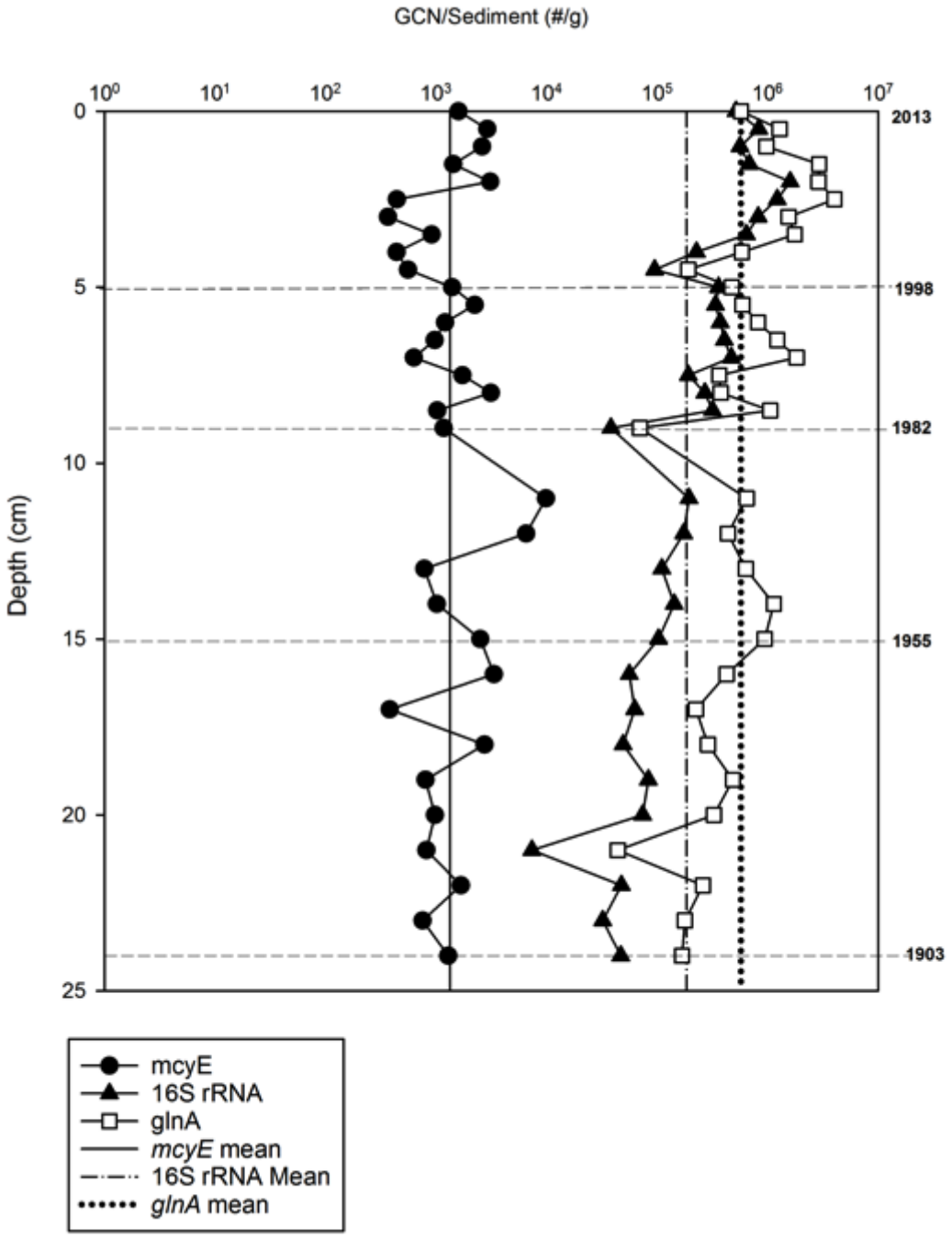

Figure 2.3 Depth profile for Yellowknife Bay, GSL 8 core (2013). GCN for target genes as a function of sediment depth (and time from ^{210}Pb dating). Note logarithmic scale.

As with the GSL core *mcyE* GCNs for the Madeline Lake core (Fig 2.4) were low and showed very little variation through the core (data close to the overall average). Once again, GCNs for the 16S rRNA and *glnA* genes appeared to increase over time, though there is much less resolution in the middle of the core, an increase in GCNs of both genes is observed at approximately 1997. However, unlike in the GSL core GCNs of the 16S rRNA and *glnA* genes do not appear to converge in more recent years in Madeline Lake.

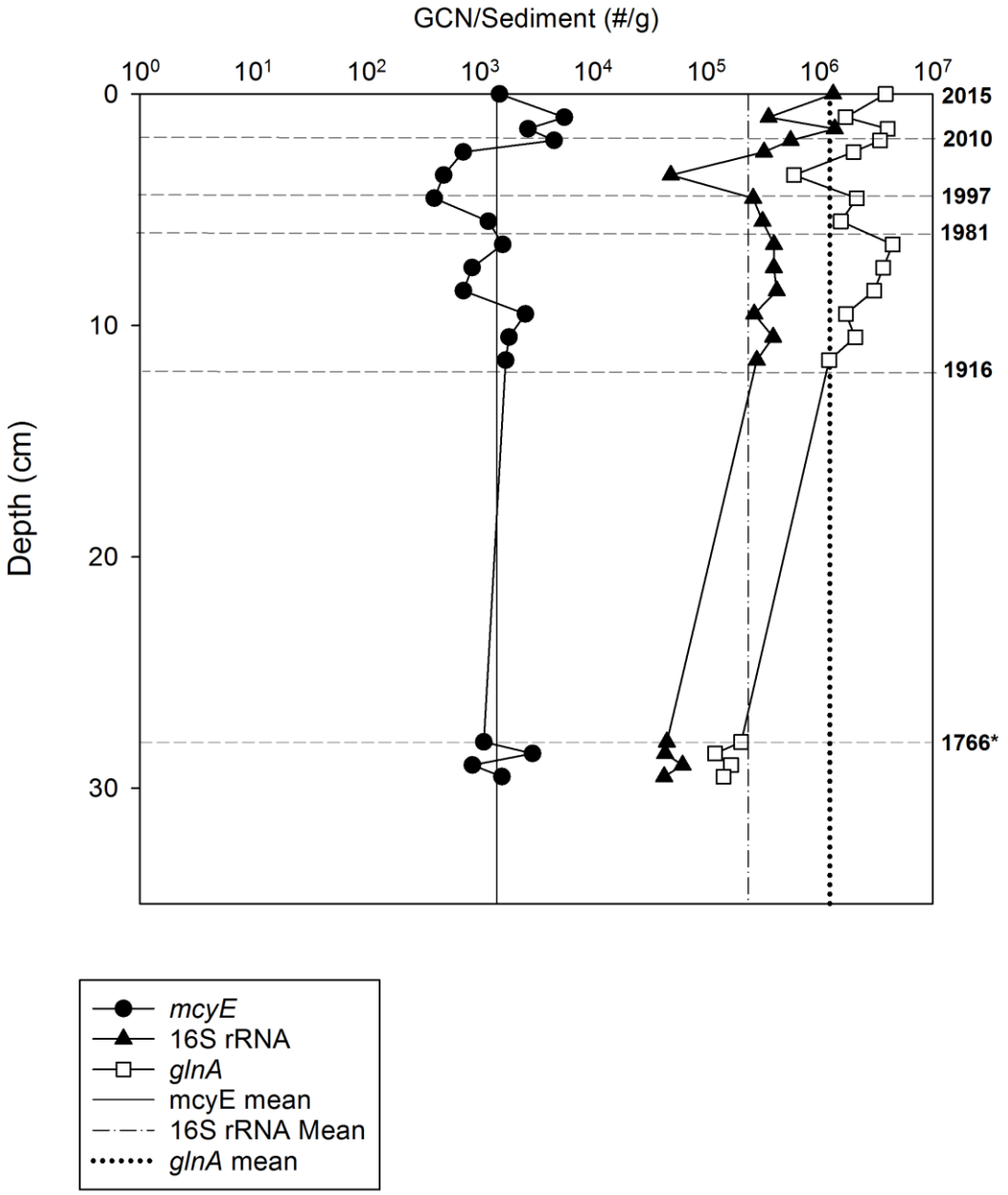

Figure 2.4 Depth profile for Madeline Lake core (2015). GCN for target genes as a function of sediment depth (and time from ^{210}Pb dating). Note logarithmic scale.
* Date estimated from sedimentation rate.

GCNs were much lower in the Pontoon Lake core (Fig 2.5) did not show any discernable pattern with depth. In addition, this sediment core did not yield high concentrations or good quality DNA (Appendix B).

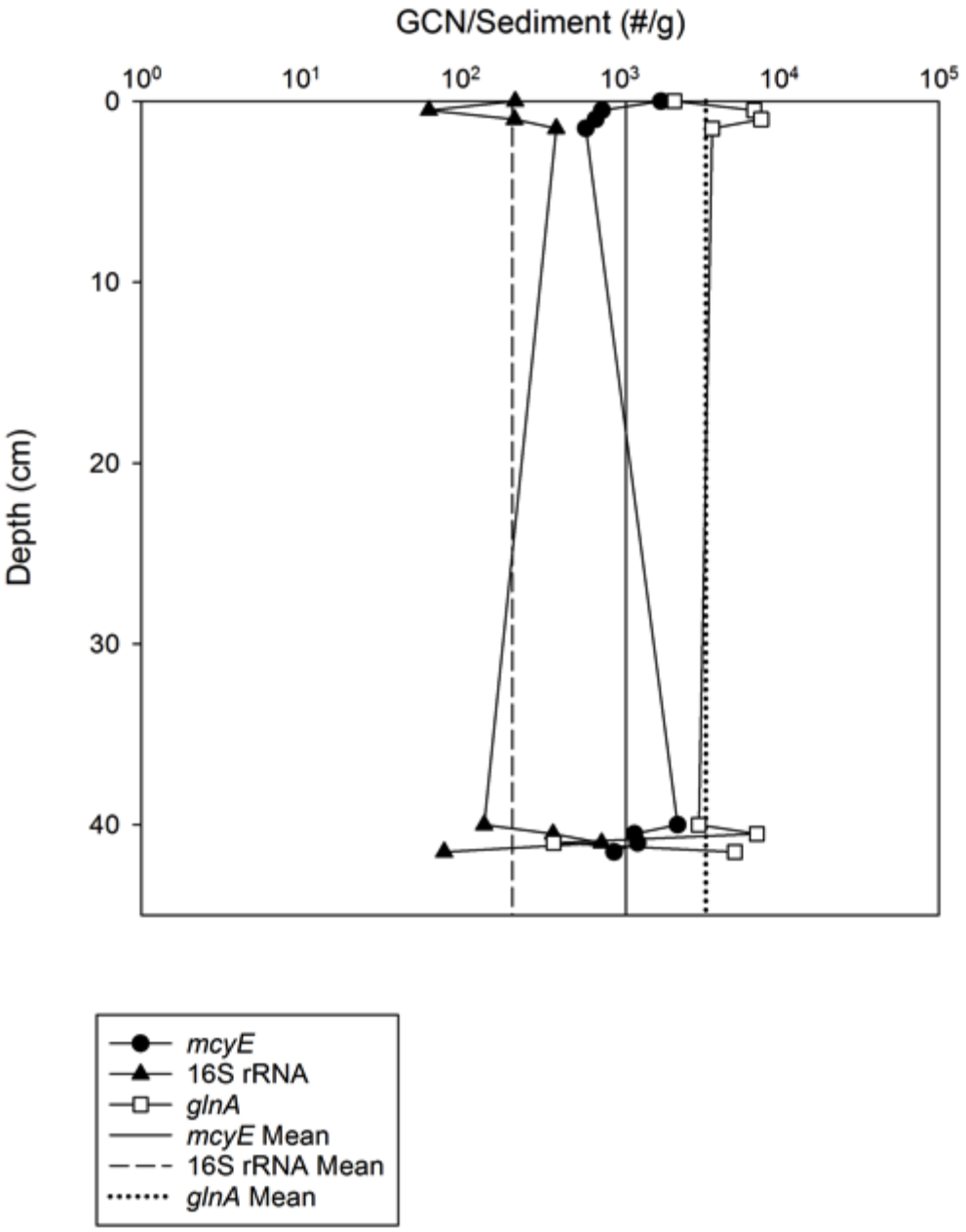

Figure 2.5 Depth profile for Pontoon Lake core (2015). GCN for target genes as a function of sediment depth. Note logarithmic scale.

The Meretta Lake core (Fig 2.6) shows similar patterns in *mcyE* GCNs as seen in the GSL and Madeline Lake cores with numbers not varying significantly from the overall mean. However, in Meretta Lake, 16S rRNA and *glnA* GCNs are seen to exhibit much more variation over time. A relatively steady increase can be seen in both genes until approximately1906 when the first major decrease is observed. Between this date and approximately 1969 a high degree of variation in the 16S rRNA and *glnA* can be observed with GCNs decreasing and increasing dramatically between core sections. After 1969 16S rRNA and *glnA* GCNs begin to stabilize until around 1998 where an approximately 10-fold increase occurs and remains relatively stable into recent years. Interestingly, in the Meretta Lake core GCNs for the 16s rRNA and *glnA* genes are very similar throughout the core, similar to what was seen in the more recent core sections of the GSL core, indicating that the cyanobacteria have likely always formed a large part of the microbial community of Meretta Lake.

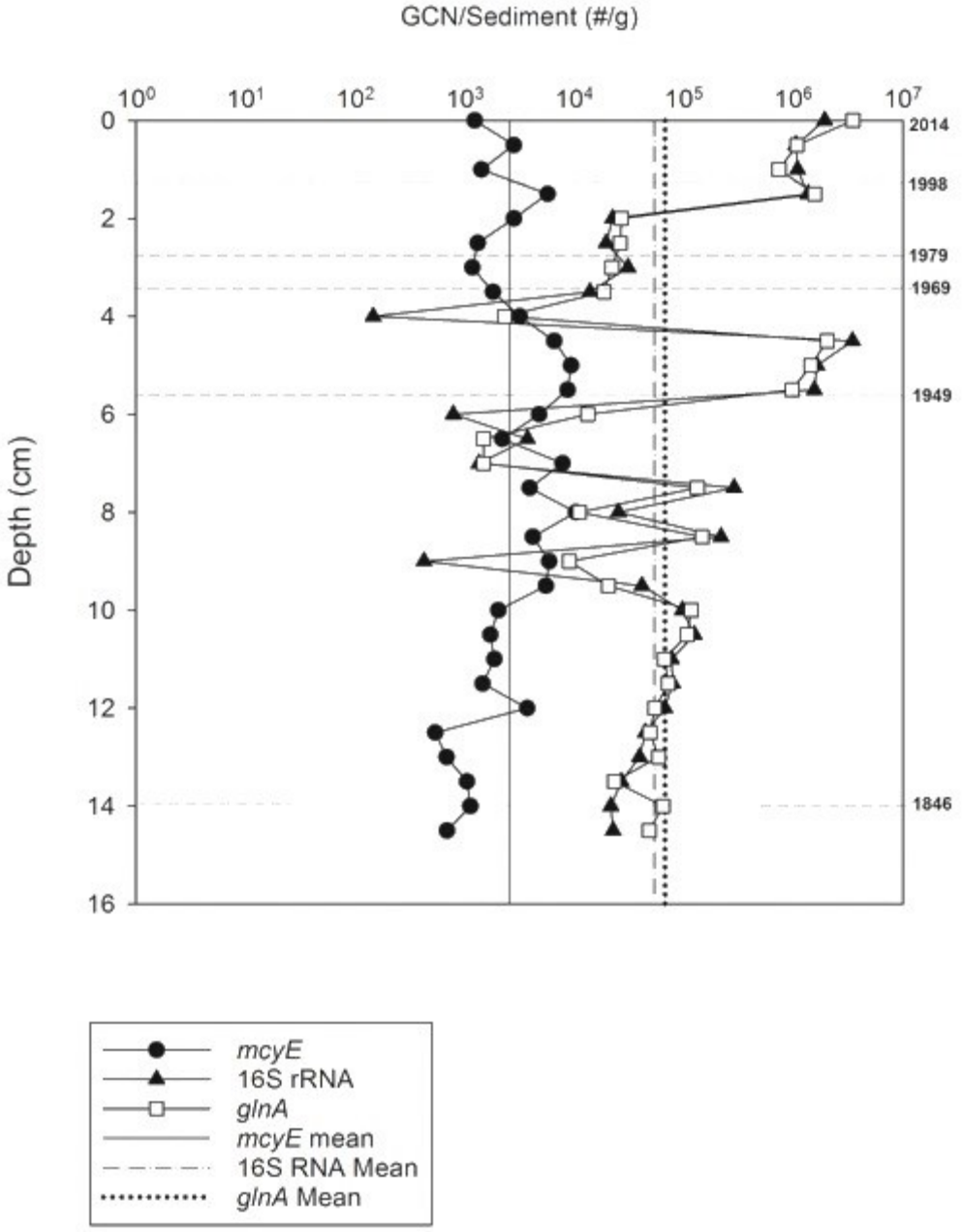

Figure 2.6 Depth profile for Meretta Lake core (2014). GCN for target genes as a function of sediment depth (and time from ^{210}Pb dating). Note logarithmic scale.

Though the resolution of the Crazy Lake core (Fig 2.7) is much less than that of the GSL, Meretta and even Madeline Lake cores similar patterns can be seen with depth. Here again *mcyE* GCNs did not vary greatly from the mean throughout the core. The 16s rRNA and *glnA* genes are also observed to increase over time and like in the GSL core, are seen to converge in more recent sediment core sections.

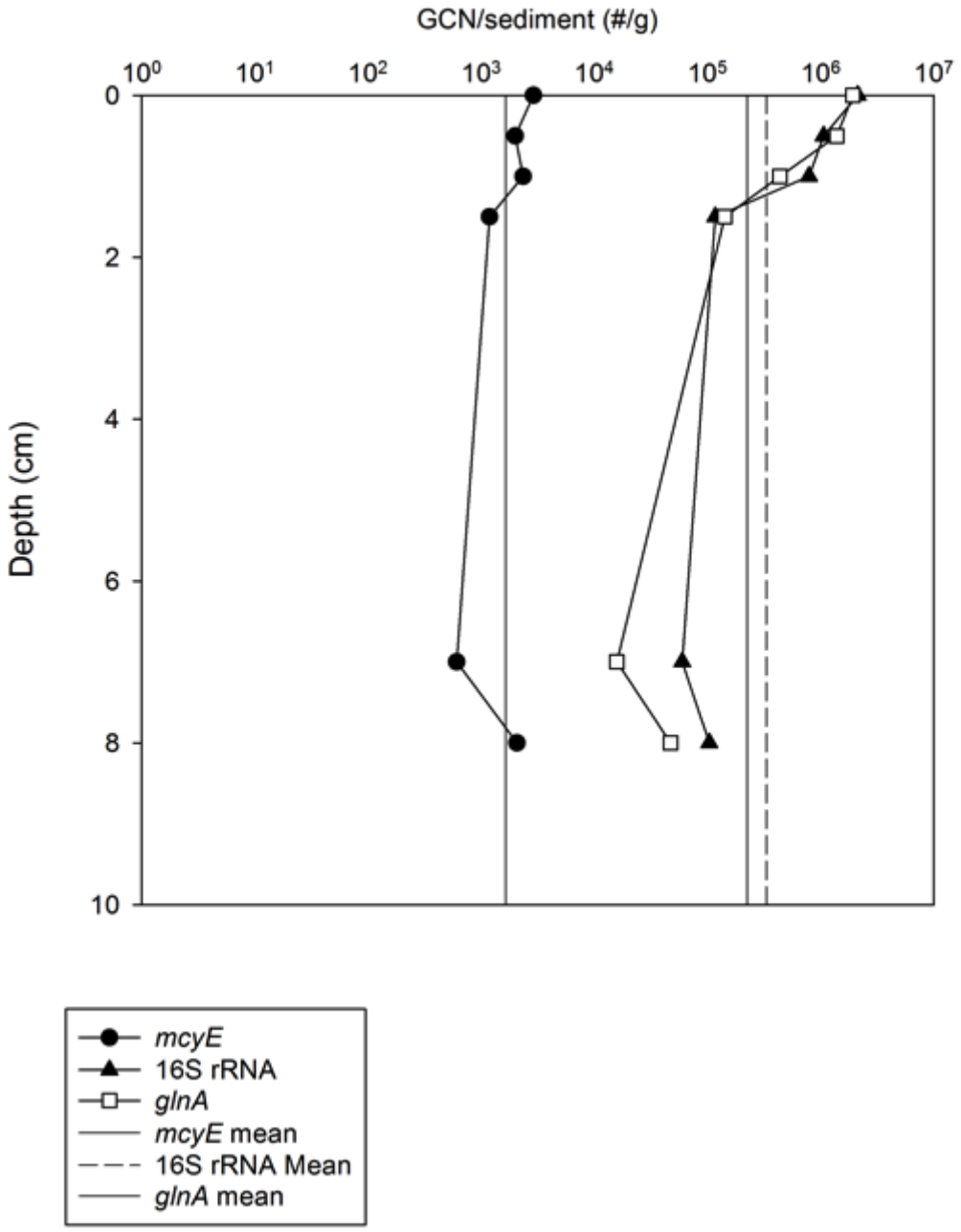

Figure 2.7 Depth profile for Crazy Lake core (2014) GCN for target genes as a function of sediment depth. Note logarithmic scale.

Additional figures were created for cores from lakes with ^{210}Pb dating information (GSL, Madeline Lake and Meretta Lake) in order to illustrate GCN variation with time since approximately 1840 (Fig. 2.8-2.10). Once again the slight increase in 16S rRNA and *glnA* GCNs is apparent over time with little change in *mcyE* GCNs. Interestingly GCNs of the 16S rRNA and *glnA* genes appear to converge in more recent years in GSL (Fig. 2.8). Conversely, GCNs of the 16S rRNA gene and *glnA* are relatively similar throughout the Meretta Lake core (Fig. 2.10) and follow the same trend of increasing through time, peaking in approximately 1950, declining and then increasing again.

As observed in Figure 2.3, Figure 2,8 shows *mcyE* GCNs remaining relatively stable over time in GSL. The 16S rRNA and *glnA* genes are observed to increase steadily over time with GCNs of the two genes becoming more similar in recent years.

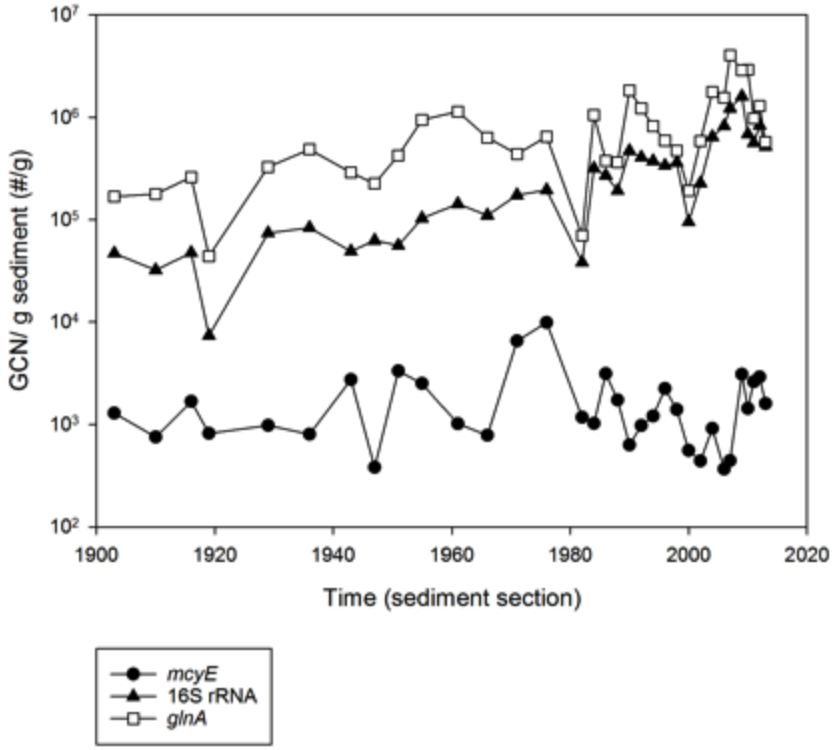

Figure 2.8 Yellowknife Bay, GSL 8 core (2013), GCN for target genes as a function of time as determined from ^{210}Pb dating. Note logarithmic scale.

Madeline Lake (Fig. 2.9) is again seen to follow a similar pattern as GSL over time with *mcyE* remaining relatively constant and 16S rRNA and *glnA* GCNs increasing steadily over time.

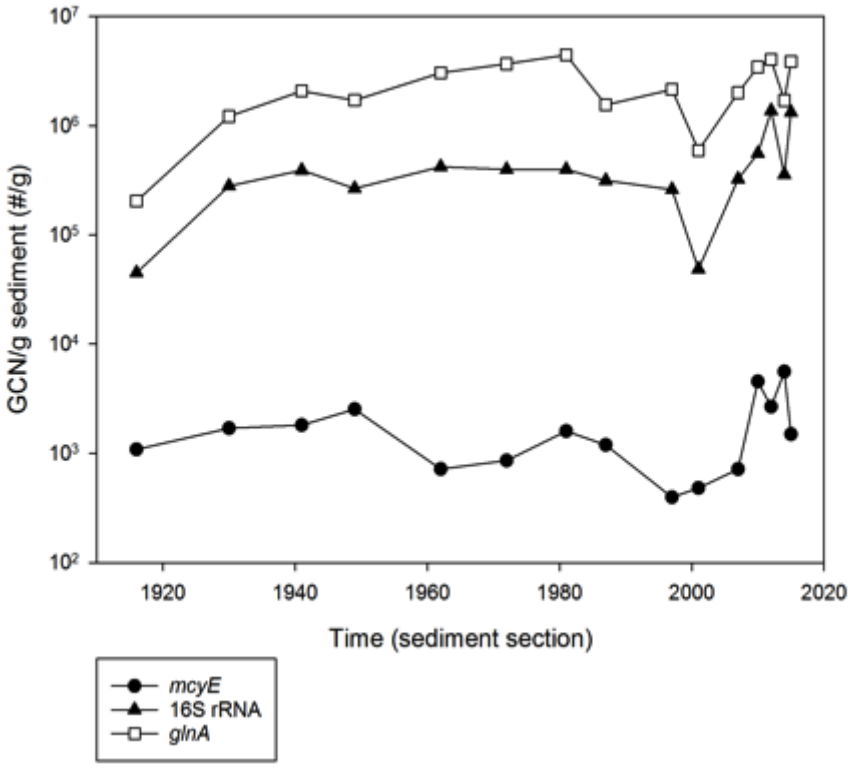

Figure 2.9 Madeline Lake core (2015) GCN for target genes as a function of time as determined from ^{210}Pb dating. Note logarithmic scale.

Figure 2.10 once again shows large variations (over three orders of magnitude) in 16S rRNA and *glnA* GCNs in Meretta Lake over time. As seen in the depth profile (Fig. 2.6), GCNs of the 16S rRNA and *glnA* genes remain similar throughout the core while GCNs of the *mcyE* gene remain relatively stable over time.

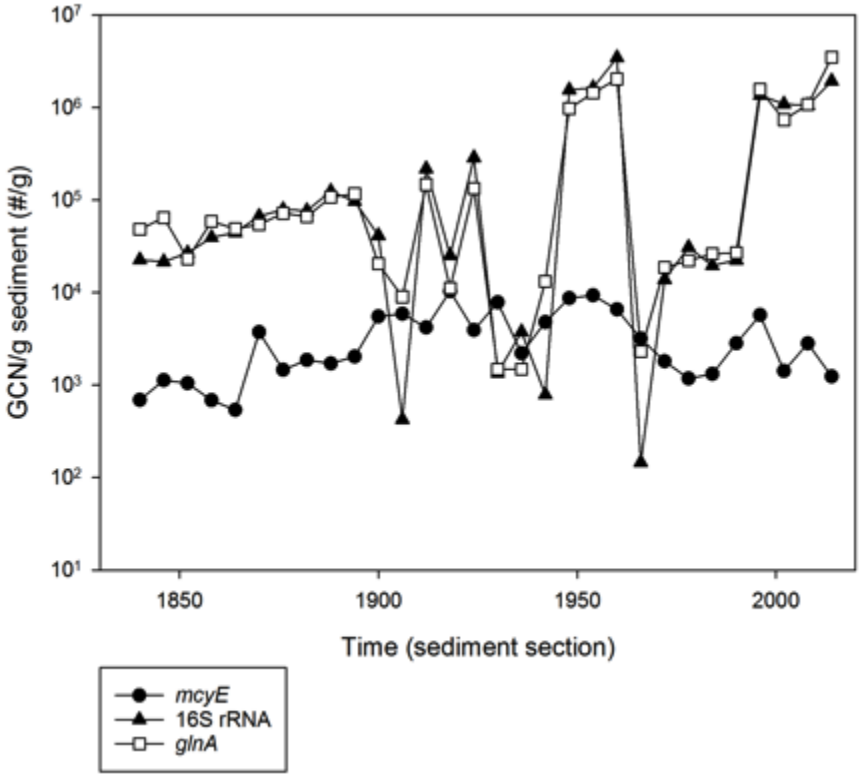

Figure 2.10 Meretta Lake core (2014) GCN for target genes as a function of time as determined from ^{210}Pb dating. Note logarithmic scale.

Comparing the 16S rRNA GCNs found in top and bottom core sections we see a marked increase from bottom to top potentially indicating greater cyanobacterial abundance in more recent years (Fig. 2.11-2.12). This was consistent in the three cores taken from GSL as well as in Madeline, Meretta and Crazy Lakes. In order to determine if these increases constitute an increase in overall cyanobacterial dominance the relative change in 16S rRNA compared to that of the *glnA* gene is shown in Figures 2.13 and 2.14. The 16S rRNA: *glnA* ratio between top and bottom core sections from GSL indicated an increase in cyanobacterial dominance in two of the three cores tested (GSL 8 and DE3), as well as in Meretta Lake and Madeline Lake, however the change in dominance in the latter two lakes was less than what was seen in GSL. A decrease was observed in the 2015 core from GSL (GSL 15-4) as well as in the core from Crazy Lake.

Such results reflect the convergence patterns seen in the depth profiles and time series graphs for each lake. For example, in the GSL 8 core we see a marked increase in the top 16S rRNA/*glnA* ratio when compared to the bottom of the core (Fig. 2.13), which is reminiscent of the convergence in GCNs of the two genes in top core sections. Similarly, in Meretta Lake (Fig. 2.14) the change in ratio between top and bottom core sections is much less dramatic reflecting the similarity in GCNs of 16S rRNA and *glnA* throughout the core.

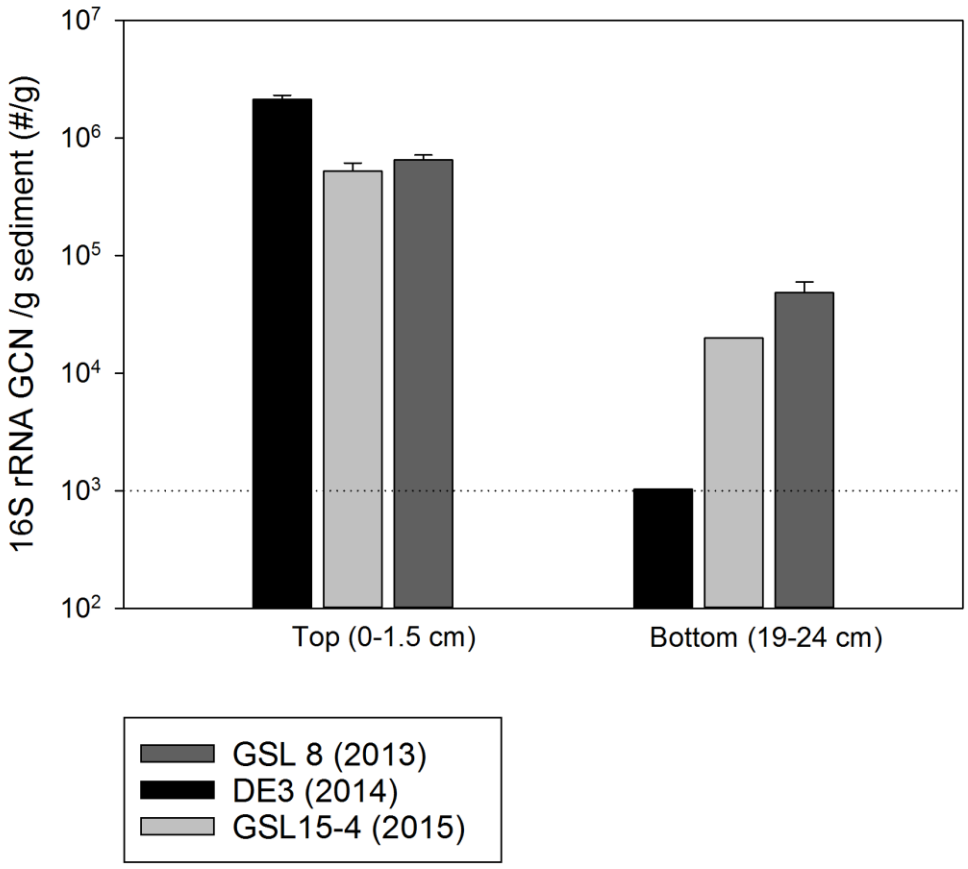

Figure 2.11 Top bottom comparison of cyanobacterial 16S rRNA GCN for GSL from three separate cores taken in Yellowknife Bay. Top core sections correspond with years 2010-2015 (n=4) while bottom core sections correspond with years 1900-1930 (n=3-4 with exception of DE3 bottom where only one sediment layer was available).

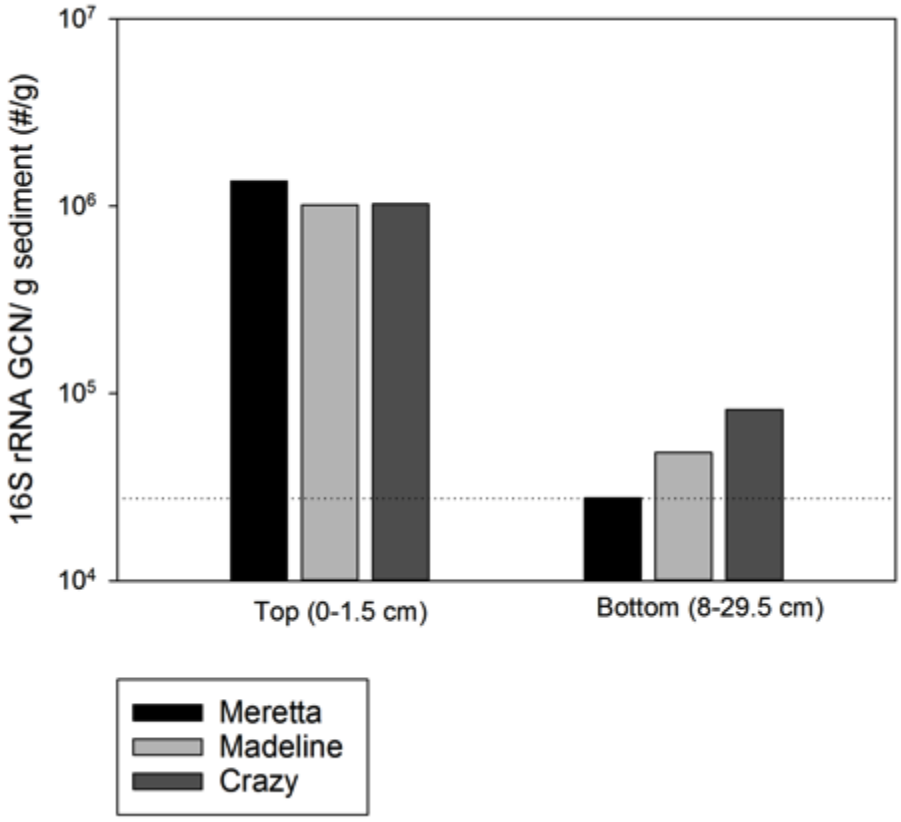

Figure 2.12 Top bottom comparison of cyanobacterial 16S rRNA GCN from Meretta Lake, Madeline Lake and Crazy Lake. Large interval in bottom cores sections as each core was from a different lake (n=3-4).

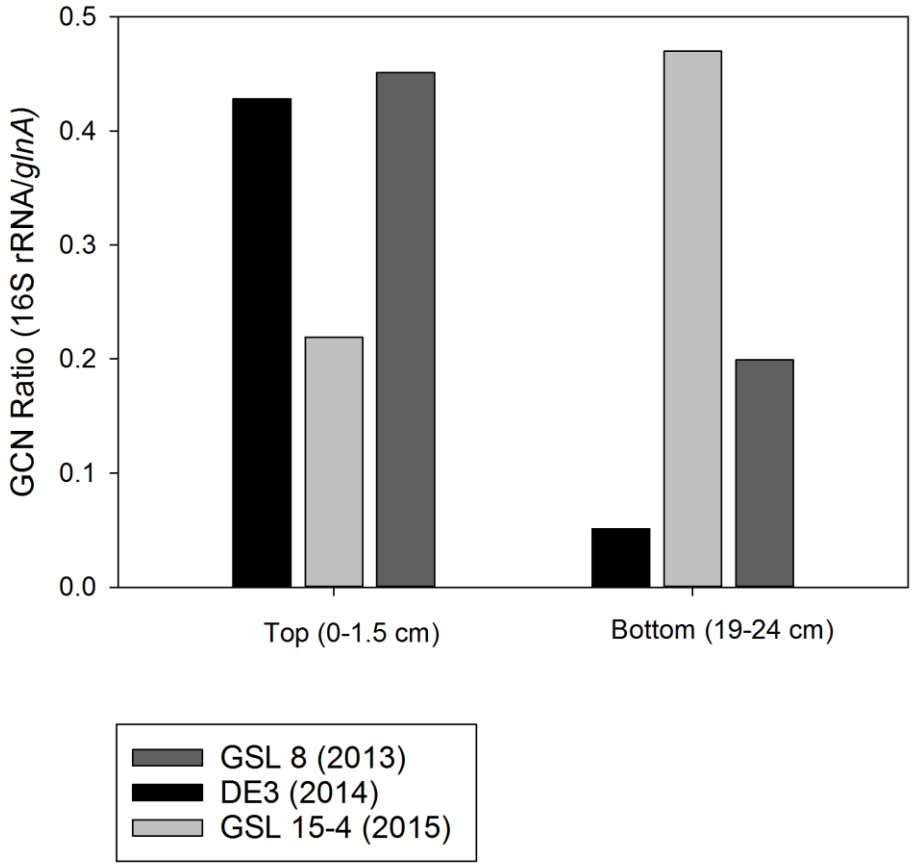

Figure 2.13 Top bottom comparison of cyanobacterial 16S rRNA and *glnA* GCN ratio for three separate cores from Yellowknife Bay, GSL (n=3-4 with exception of DE3 bottom where only one sediment layer was available)

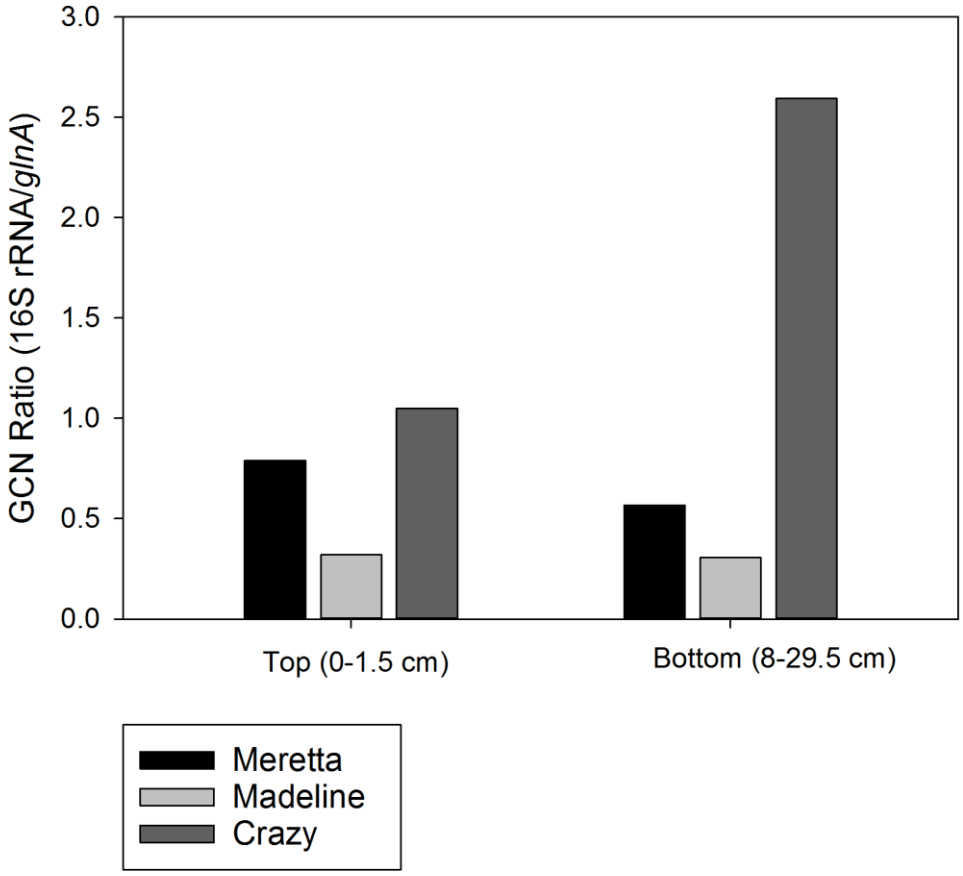

Figure 2.14 Top bottom comparison of cyanobacterial 16S rRNA and *glnA* GCN ratio for Meretta, Madeline and Crazy Lakes. Large interval in bottom cores sections as each core was from a different lake (n=3-4).

2.3.2.2 Relationship between sediment gene proxies (*mcyE*, 16S rRNA, *glnA*), and DNA concentration

Statistical analysis of lake sediment molecular data (Table 2.7-2.8, Appendix B Table B.2-B.4) was conducted on sediment cores from all five lakes in order to test for significant relationships between the genes analyzed as well as between the GCNs and the concentration of DNA in each sediment core subsection. In general data were normally distributed either as they were or once log transformed. However, in the case of Meretta and Madeline Lakes some data sets were not normally distributed even after transformation. All lakes (save Pontoon Lake) showed positive and significant correlations (see Appendix B) of GCN pairings of 16S rRNA with *glnA* and DNA, and DNA with *glnA* though the strength of these correlations varied between lakes. No relationships were observed between *mcyE* and the other genes or DNA.

Table 2.7 Pearson Product-Moment correlations (*r*) of normalized molecular data (per wet gram of sediment) in the 2013 Great Slave Lake core (d.f.= 31).

	mcyE	**16S RNA**	**glnA**	**DNA**
mcyE	1.00			
16S rRNA	0.0612	1.00		
glnA	0.0141	0.885***	1.00	
DNA	0.125	0.641***	0.461*	1.00

* p< 0.01
***p<0.0001

Table 2.8 Pearson Product Moment correlations (*r*) and Spearman rank correlations (r_s) of normalized molecular data (per wet gram of sediment) in the 2014 Meretta Lake core. Spearman rank correlations indicated in bold (d.f.= 28).

	mcyE	**16S RNA**	**glnA**	**DNA**
mcyE	1.00			
16S rRNA	0.0544	1.00		
glnA	0.0514	0.926 ***	1.00	
DNA	**0.306**	**0.658*** **	**0.645*** **	1.00

***p<0.0001

2.3.2.3 Assessment of temporal autocorrelation in sediment samples

GCNs of all three genes were assessed for temporal autocorrelation using the autocorrelation function (acf) in R. Temporal autocorrelation was found to be absent in in all cores save for the Meretta Lake core and the 2013 GSL8 core. Therefore, GAMs both with and without the CAR(1) structure were run for these two lakes and model likelihood was assessed using the AIC. It was found that for Meretta Lake, the model without the CAR(1) structure best described the data (i.e. temporal autocorrelation was not significant; AIC=2618.754 without CAR(1), AIC=2651.505 with CAR(1)). In the case of the GSL8 core the GAM model with the CAR(1) structure best described the data (AIC=2877.537 with CAR(1), AIC=2920.746 without CAR(1)), however the significance of both models was approximately the same ($p<0.05$) and the omittance of the CAR(1) structure did not alter the trends seen.

2.4 Discussion

2.4.1 Great Slave Lake phytoplankton and water chemistry

In general the summer phytoplankton composition (at least at the division level) was similar to what was reported in the early studies on the net plankton of Great Slave Lake and Yellowknife Bay by Rawson (1956), Moore (1980) and Fee et al. (1985). Though decades separate the studies, a number of commonalities exist between the three and the study present herein. In the past the most frequently encountered taxa belonged to the algal classes of chrysophytes, cryptophytes and diatoms with the species *Aulacoseira islandica* (formerly *Melosira islandica*)*, Asterionella formosa* (diatoms) and *Dinobryon divergens* (chrysophyte) being most abundant during the summer months of June to August (Fee et al., 1985; Moore, 1980; Rawson, 1956). Similar patterns were seen in the samples obtained in summer of 2014 and 2015 where once again the dominant taxa were diatoms, chrysophytes and cryptophytes (Appendix B, Table B.1). Though *A. formosa* and *D. divergens* remained among the top three most frequently encountered species in more recent years, the species *A. islandica* and all species of *Aulacoseira* were much less common. In fact, *Aulacoseira* was only identified in samples from three sites: Site 2, DE4 and GSL 14-11, and instead the most frequently identified species in August of 2014 and 2015 was the centric diatom species *Cyclotella meneghiniana* and other unidentified *Cyclotella* species (size range 5-30 µm).

Though Rawson did list a number of *Cylotella* species in the waters of GSL and Yellowknife Bay (Rawson, 1956), the genera were not listed as being a major contributor to the phytoplankton biomass until Moore's study (1980). A shift in diatom species leading to an increase in planktonic species, particularly *Cyclotella,* but also *Asterionella*

and *Tabellaria* has been interpreted as a sign of climate warming in temperate latitudes as well as in northern water bodies (Rühhland et al., 2003; Smol et al., 2005).

Diatoms are widely used as bio-indicators in environmental studies due to their specific ecological preferences, diversity and sensitivity to environmental change. A number of paleo-ecological studies of sediment core from lakes spanning latitudes in the subarctic to the high arctic have shown similar trends of an increased abundance of *Cyclotella* species in upper sediment layers, indicating changes in diatom assemblages from benthic to planktonic species since preindustrial times (Stoermer et al., 1990; Korhola et al., 2002; Rühhland et al., 2003; Smol et al., 2005).

Such trends were also observed in cores obtained from lakes surrounding the Yellowknife region (Pienitz & Smol, 1993; Rühhland et al., 2003) and from parts of GSL itself (Stoermer et al., 1990). A 52 cm core obtained from McLeod Bay, a north-eastern embayment of GSL, showed consistent increases in the abundance of *Cyclotella* species in layers ranging from 23 cm in depth to the surface (Stoermer et al., 1990). Consistent to all studies is that the more recent success of *Cyclotella* appears to be induced by warming waters, typically during the later part of the thermal stratification period, and that the trend is supported by lakes known to be experiencing warming (Rühhland et al., 2003). As such, the abundance of *Cyclotella* in the waters of Yellowknife Bay is likely an indication of the climate change effects on GSL.

Surface waters in GSL provided results consistent with oligotrophic lakes, characterized by low nutrient levels and productivity, which are typical of Northern regions. Chl a, total nitrogen and total phosphorus values were all relatively low as were microcystin concentrations (Table 2.4). The microcystin results from the ELISA were

consistent with the ddPCR data, which showed very low GCNs for *mcyE* (Table 2.5). All samples, including those obtained from the two blooms encountered in August 2015 and Pontoon Lake, which exhibited the highest level, had microcystin concentrations well below the Health Canada guidelines of 1.5 µg/ L and thus are not deemed to pose any threat to humans or animals. In fact, the Ontario Ministry of the Environment does not even report on concentrations that are below said guideline (Koene & Toner, 2010) and as ELISA testing can occasionally lead to false positive results (particularly in the case of excess methanol or increased salinity) (Metcalf et al., 2000; Rivasseau et al., 1999) it would appear that the levels obtained in our Yellowknife lakes are not a cause of concern.

2.4.1.1 Late summer blooms in Yellowknife bay, Great Slave Lake

A number of cyanobacterial blooms were observed in the waters of Yellowknife Bay over the course of this study. One was observed in early September during the 2014 field season, while two were observed during the 2015 field season on August 8th and 13th at sites DE4 and DE1 respectively. Bloom material from the 2015 blooms was examined via light microscopy as well as sequenced (Chapter 3) and was identified as a bloom of *Dolichospermum lemmermannii*, a potential microcystin-producing cyanobacterium that forms blooms in cold-temperate regions between the 40th and 60th parallels (Salmaso et al., 2015). Blooms of *D. lemmermannii* have frequently been reported in a number of subalpine lakes, in countries such as Norway, Sweden, Finland and Italy (Lepistö & Holopainen, 2008; Salmaso et al., 2015; Willén, 2003; Zapomělová et al., 2009), where they are the most abundant and dominating species and account for at least half of the toxic blooms reported (Salmaso et al., 2015).

The presence of *D. lemmermannii* in Yellowknife Bay was reported by Rawson (1956) in the mid-1950s. At the time of his study *D. lemmermannii* was frequently encountered in the main part of Great Slave Lake and in Yellowknife Bay, however no surface blooms were reported suggesting that the recent reports of blooms are likely a modern occurrence.

Material from both 2015 blooms was assessed for microcystin content (Table 2.4). Though the bloom occurring at site DE4 on August 8th, 2015 did have a microcystin concentration forty-fold higher than what was measured for the later bloom at site DE1, it was still not the highest recorded concentration of microcystin in Yellowknife Bay during the sampling season (this occurred at site ND5) and was still well below the health and safety guidelines of 1.5 μg/L for microcystins in drinking waters set by Health Canada (Health Canada, 2007).

2.4.2 History of cyanobacteria from lake sediment DNA

Using ddPCR we were able to successfully quantify a number of genes representative of the historic cyanobacterial and microbial assemblages from sediment samples representing the past 100-170 years. GCNs of the 16S rRNA gene specific to all cyanobacteria, and the bacterial *glnA* genes showed an increase from the bottom to the top of cores from all lakes (with the exception of Pontoon lake) suggesting an increase in overall microbial and cyanobacterial biomass over time (Fig. 2.3-2.7). In addition, the relative change in the 16S rRNA gene when compared to the *glnA* gene appear to indicate an increase in the overall cyanobacterial dominance in most of the lakes tested, save for

GSL 15-4 and Crazy Lake (Fig. 2.13-2.14). The trends observed were quite robust and evident even when temporal autocorrelation was taken into consideration.

Though our results did indicate an increase in the overall productivity and cyanobacterial abundance of the lakes there did not however appear to be any significant change in the toxicity conferring *mcyE* gene indicating that there has been little change in the amount of toxigenic cyanobacteria over time. In addition, *mcyE* GCNs were all exceptionally low with levels that can be considered background, or that would be at detection when using other methods like qPCR.

There are a number of considerations to take into account when using molecular markers as proxies of gene abundance in paleo-limnological studies. For example, degradation is an important factor to consider when examining DNA extracted from sediment cores as DNA is known to degrade over time and hence with depth (Fernandez-Carazo et al., 2013). The extent of degradation however, is often mitigated by cold temperatures and the anoxic conditions found in the sediments of deep lakes (Anderson-Carpenter et al., 2011; Coolen et al., 2004). In addition, the quality of data that can be derived from the DNA extracted from sediment in downstream applications is dependant on the size of the gene fragment to be amplified. Smaller gene fragments are better preserved in sediment and as such, are the best choice for analysis if one wants to minimize PCR bias (Martinez De La Escalera et al., 2014). The genes used in this study were all relatively small (<300 bp) and are thus less likely to be affected by degradation, therefore the relative changes seen in GCN between genes, particularly in the case of the 16S rRNA and *glnA* genes are likely not complicated due to DNA degradation with

increasing core length. An examination of the relative change in these two genes can in turn inform on the relative dominance of cyanobacteria (Fig. 2.13, 2.14).

Another factor to consider when assessing the relative change in GCN between genes is the number of copies of each gene each cell possesses. The cyanobacterial 16S rRNA gene is present in multiple copies in cyanobacterial cells (Kaneko et al., 2007) while the microbial *glnA* gene is present as a single copy (Stoeva et al., 2014). This is particularly relevant when assessing the relative increase in the 16S rRNA gene when compared to *glnA*.

As there is a significant positive correlation observed between the genes (Table 2.7-2.8, Appendix B Table B.2-B.4) it is unlikely that this observed change is a result of a change in copy number per cell and hence this difference is more likely attributable to changes in the overall abundance of each gene.

Though a number of commonalities exist in the data obtained for all of the lakes included in this study, each lake is its own distinct system with different physical and chemical properties as well as different stressors. In order to best explain the trends seen we must look at each lake individually taking into account their distinct histories.

Yellowknife Bay, Great Slave Lake

As an embayment of GSL located off the coast of the City of Yellowknife, Yellowknife Bay has a history of moderate eutrophication. The historic gold mining that

occurred in the area left high levels of arsenic and other heavy metals in the surrounding environment. In addition, the bay has also been subject to domestic waste/runoff as a result of general recreational usage and the now (since 1981) decommissioned Niven Lake sewage lagoon. Study into the legacy affects of arsenic in Yellowknife Bay lead to the discovery of the 2013 bloom, which in turn sparked this research. However, arsenic is not known to stimulate cyanobacterial growth (Wang et al., 2012) and thus cannot be the cause of the 2013 bloom or the others that occurred the following two summers along the coastline of Yellowknife Bay. As such, this study aimed to examine the historic cyanobacterial presence in the lake, with a focus on the presence of toxic microcystin producing taxa. As microcystin is a potent hepatotoxin and Yellowknife Bay is known for its recreational usage and as a secondary water source for the people of Yellowknife, information on the toxicity of any current and future blooms is quite pertinent.

Subsurface sampling of the epilimnion in the summers of 2014 and 2015 indicated that levels of microcystin in the waters of Yellowknife Bay were well below those deemed to pose a threat to humans and wildlife and as such do not appear to pose any immediate concern. The sediment record for Yellowknife Bay has shown an increase in both cyanobacterial 16S rRNA and bacterial *glnA* in recent years (Fig. 2.3 and 2.8), which is a potential indication of an increase in overall bacterial and cyanobacterial abundance. Examining this further, cyanobacterial dominance in Yellowknife Bay also appears to have increased over time. In Figure 2.8 we can see that in more recent years GCNs of the 16S rRNA and *glnA* genes have begun to converge whereas Figure 2.13 shows that the proportion of 16S rRNA to *glnA* is higher in top core sections of the 2014 (DE3) and 2013 (GSL 8) cores than in bottom sections indicating that the cyanobacteria

may be contributing more to the microbial community. Discrepancies with the 2015 core from site GSL 15-4 can likely be attributed to core length. The 2015 core was shorter than the other two GSL cores and hence likely did not cover as long a time frame.

Changes in the phytoplanktonic composition of Yellowknife Bay are likely a consequence of a changing climate. The City of Yellowknife has experienced an increase in mean annual temperatures of approximately 2.5 ºC since 1942 (Appendix A, Fig. A.1) with similar increases being observed over the summer months (Appendix A, Fig. A.3) when lake productivity and cyanobacterial bloom formation is generally known to increase. Temperature increases have also lead to changes in the winter sea ice coverage in Northern Canada with decreases of approximately 12.5% being observed over the last four decades (Appendix A, Fig. A.6). Similar changes have likely influenced Arctic lake ice formation, which has been observed to be occurring 1-4 weeks later along with equivalently earlier melt dates for several lakes within the Yellowknife region (Duguay et al., 2014). Such changes can have a dramatic effect on lake productivity, providing a longer growing season along warmer water temperatures and increased water column stability for those species that benefit from such. It is likely that such effects, coupled with the human induced impacts related to the growing city of Yellowknife have lead to the recently observed changes in the lake.

Madeline Lake and Pontoon Lake

Madeline and Pontoon Lakes were included in this study due to their proximity to Yellowknife and their potential to support cyanobacterial blooms due to their historic

phosphorus content. Much like Great Slave Lake, Madeline Lake showed a general trend of increasing bacterial and cyanobacterial biomass over time (Fig. 2.9). Madeline Lake is extensively used for recreational purposes and has a park with a day use area. Therefore, it is possible that the observed increase is a result of anthropogenic influences on the lake.

Meanwhile, DNA extracted from Pontoon Lake proved to be inconclusive, likely as a result of poor sample quality. The core obtained from Pontoon Lake was very inconsistent in texture and had a strong smell of sulphur when compared to the other cores obtained during the same sampling period. In addition, DNA extraction from this core also proved challenging and two separate extractions were required before usable DNA was acquired, which overall was of poor quality and low concentration. It has been reported that not all lake sediments provide quality DNA with coloured lakes, high in organic acid concentrations, often being problematic (Pal et al., 2015).

Meretta Lake

The data obtained from Meretta Lake showed a general increase in cyanobacterial and bacterial biomass from bottom to top and was in part consistent with earlier paleo-limnological studies conducted on sediments obtained from the lake (Antoniades et al., 2011; Douglas & Smol, 2000; Michelutti et al., 2002). ^{210}Pb dating was not conducted on the core used in this study, however dates were inferred from the sedimentation rates of the dated core from the Antoniades et al. study (2011).

Meretta Lake was clearly impacted by direct sewage inputs from the hamlet of Resolute Bay, which began in 1949, were reduced in 1969 and ceased in 1998 (Douglas

& Smol, 2000; Antoniades et al., 2011). Antoniades et al. (2011) examined a number of algal pigments including chl a and two cyanobacterial pigments (echinenone and zeaxanthin) and produced depth profiles to show the change in pigment abundance with depth/over time. Their results indicated an increase in chl a beginning shortly after the introduction of raw sewage, which continued steadily until the mid-70s, tapered off and then began increasing again. Cyanobacterial pigments remained relatively stable during the period of direct sewage input but spiked during the early to mid-70s, continuing to increase until approximately 1998 (when sewage inputs ceased) when they began to return to their original concentrations Such results indicate the profound effects that eutrophication played on the phytoplankton community of Meretta Lake and how the subsequent reduction and cessation of which led to recovery, however the more recent increases in said pigments appear to indicate the effects of climate change (Antoniades et al., 2011).

The depth profile presented in this study (Fig. 2.6) also illustrates similar trends, represented in the GCNs of bacterial and cyanobacterial genes. Here we see a sharp increase in both the 16S rRNA and *glnA* genes shortly after 1949, the onset of direct sewage inputs, which persisted until just before 1969 (sewage reduction period). GCNs for these genes then stabilized during the 70s, 80s and 90s until interestingly, at approximately 1998 we observe another sharp increase in GCN. Since 1998 was the year of sewage cessation it would follow that if eutrophication were the only factor promoting cyanobacterial and microbial growth we would likely not see such an increase. Much like in the Antoniades et al. study this spike can likely be attributed to temperature increases

as a consequence of climate change, however there are likely anthropogenic impacts from Resolute Bay that are contributing to said increases.

Meretta Lake's history of eutrophication has likely contributed to the high degree of variability seen in the sediment record. For example, comparing the profile created for GSL (Fig. 2.3) to that created for Meretta Lake we can see that the GCNs of the genes tested (and for the 16S rRNA and *glnA* genes in particular) deviate from the mean to a much greater degree. However, as previously mentioned, the most extreme deviations seem to correspond with the history of sewage addition, reduction and cessation.

Shifting focus to the *mcyE* gene, there appears to be a slight increase in GCNs in the years following 1949. However, unlike the 16S rRNA and *glnA* genes, the *mcyE* gene does not show the same kind of dramatic increase in the mid-90s, remaining instead near the overall average. It therefore appears that the increase in the late 1940s is likely just a result of the overall increase in cyanobacteria, however it may potentially be an indication that eutrophication plays a larger role in the production of microcystin than temperature increases, at least in the case of Meretta Lake.

Crazy Lake

As our example of a non-impacted lake, Crazy Lake may provide the most information about the direct impacts of climate change on the phytoplankton communities of Northern lakes. As was seen in the all of the other lakes tested, Crazy Lake also showed an increasing trend in 16S rRNA and *glnA* GCNs in top core sediments and little change in *mcyE* GCN throughout the core (Fig. 2.7). Such results appear to

mirror those seen in our other lakes with apparent increases in the total amount of microbial and cyanobacterial biomass. However it is not as clear here if cyanobacterial dominance has in fact changed. Examining Figure 2.14 it appears that cyanobacterial dominance in Crazy Lake has in fact decreased over time, however these results may be complicated due to low number of samples, particularly in the bottom of the core. From these findings it is not possible to state with certainty if climate change has in fact lead to the increase in productivity.

2.5 Conclusions

The results of this study suggest that cyanobacterial dominance in Northern lakes appears to be increasing over time, possibly as a consequence of climate change as evidenced in the GSL core. All lakes included in the study exhibited increasing GCNs of cyanobacterial 16S rRNA and *glnA*, suggesting perhaps an increase in overall lake productivity at the microbial level as described in other paleo-limnological studies of northern lakes. This assumes however no significant degradation of DNA through time in the sediment cores. Regardless of any degradation, the relative importance of cyanobacteria within the total bacterial community appears to have increased. No increasing trend was seen in GCNs of the *mcyE* gene indicating that there has not been an increase in the overall amount of toxigenic, microcystin-producing cyanobacteria in these lakes. These findings were supported by present day water column data, which showed very low levels of microcystin and the *mcyE* gene.

It is important to note that though all lakes presented in this study appear to be responding to climate change, its effects can have varying impacts throughout the North. As such, we cannot make any sweeping conclusions on the magnitude of the effects of climate change on each lake without more monitoring data, thus highlighting the importance of research in the North. In addition, the paleo-limnological DNA methods used cannot discern between benthic and planktonic species of cyanobacteria. Thus, it is not possible to conclude that the increases in cyanobacterial dominance were necessarily due to planktonic cyanobacterial taxa, although recent blooms of Nostocales in Yellowknife Bay point to an increase in planktonic forms. Future studies should aim to develop a means by which to distinguish planktonic from benthic cyanobacteria DNA in sediments. Such methodology would allow us to determine more specifically if planktonic, bloom-forming species are in fact increasing and determine whether the recent blooms reported by the communities surrounding Yellowknife Bay are a reflection of this.

CHAPTER 3: SINGLE COLONY DNA EXTRACTION AND GENE AMPLIFICATION

3.1 Introduction

As toxin-producing cyanobacterial blooms become more of a threat to inland waters, toxigenic cyanobacterial species have become the focus of increasing research efforts (Pick, 2016). In regional lake surveys the genera *Microcystis, Dolichospermum* and *Planktothrix* appear largely responsible for the variation in concentrations of microcystins (e.g. Rolland et al., 2005; Dolman et al., 2012) which are the most commonly encountered freshwater cyanotoxins. Other microcystin producing genera may also include *Aphanizomenon* (Pick, 2016), *Gloeotrichia* (Carey et al., 2012) and some others (Metcalf & Codd, 2012). However, within each of these genera exist both toxin producers and non-producer species, and beyond this, even certain strains of known producer species may not contain all the genes required for producing cyanotoxins (Rantala et al., 2004).

Toxin producing taxa are still being discovered as more blooms arise (Rastogi et al., 2015). For example, the cyanobacterium *Gloeotrichia*, a nitrogen fixing genus in the order Nostocales which is characterized by large (1- 3 mm in diameter), macroscopic colonies comprising radially arranged trichomes (Fey et al., 2010; Carey et al., 2012). Known to form blooms in meso-eutrophic and eutrophic lakes, *Gloeotrichia* has been observed producing late summer blooms in some oligotrophic and mesotrophic lakes in the northeastern US and Canada, not previously known for *Gloeotrichia* bloom production (Carey et al., 2008; Carey & Rengefors, 2010; Fey et al., 2010). Generally such blooms tend to occur after winter (though in Ontario they are typically not

encountered until the late summer months) when *Gloeotrichia* cells, which overwinter as akinetes in the sediments, begin to germinate and rise in the water column. *Gloeotrichia* will then spend anywhere between 2-4 weeks in the water column, growing and dividing before forming akinetes and settling back within the sediments (Karlsson-Elfgren et al., 2003; Fey et al., 2010). As a result, monitoring efforts for *Gloeotrichia* are limited to a short season. Additionally, the large size of *Gloeotrichia* colonies often preclude them from biomass counts that generally focus on a much smaller volume of water than would be necessary for estimating their importance.

Though blooms of *Gloeotrichia* may be common in Ontario (Winter et al., 2011), they are generally only regarded as nuisance blooms in the sense that their high amounts of biomass can have negative impacts on the ecology of lakes leading to a number of lake management problems (Carey et al, 2008). However, *Gloeotrichia* has been known to produce both neurotoxins and hepatotoxins in some lakes in the northern United States (Ingram & Prescott, 1954). Only two reports of microcystin production by *Gloeotrichia* currently exist. In their 2007 study, Carey et al. were able to measure very low concentrations of the hepatotoxin microcystin (MC) in colonies of *Gloeotrichia echinulata* collected over two days from Lake Sunapee in central New Hampshire, USA. In their study four samples (two from each sampling day) consisting of one hundred *G. echinulata* colonies were isolated using an Olympus SZH10 dissecting microscope and subjected to a number of freeze-thaw cycles and sonification in order to release any toxins from the cells. The extracts were then tested on an ELISA in order to quantify microcystin (Carey et al., 2007). Results showed low microcystin content for both of the sampling days. The study was repeated in 2012 with samples from 18 northeastern US

lakes with *G. echinulata* colonies exhibiting between 58.5 ± 4.2 ng MC g^{-1} dry weight and 7148.1 ± 1521.5 ng MC g^{-1} dry weight (Carey et al., 2012). Overall, the microcystin content of these lakes were considered low as they fall significantly below the World Health Organization (WHO) guidelines for microcystins in drinking waters, which allow for a maximum of 1 μg MC-LR L^{-1} (WHO, 1998).

In light of these findings, and coupled with the fact that cyanobacterial blooms often arise in mixed species assemblages, it is often difficult to discern using current molecular methods, which specific taxa in a particular bloom are in fact responsible for toxin production. Aside from the use of chemical tests (e.g. ELISA, HPLC) bloom toxicity is currently established by extracting and analysing DNA obtained from whole water samples. Lake water obtained during a bloom event is passed through a glass-fibre filter to retain planktonic populations; the filter is then processed to extract total DNA. The extracted DNA is subsequently characterized using a number of techniques and genes including various microcystin genes found within the microcystin synthetase enzyme complex, in particular the *mcyD* gene (Fortin et al., 2010) and the *mcyE* gene (Rantala et al., 2006). Though these methods are effective and allow for the detection and quantification of microcystin genes (Fortin et al., 2010), they have their own limitations and cannot be used to pinpoint the precise individual species or strains responsible for toxin production. Typically, this is inferred by comparing microcystin gene concentrations against the abundance of potential toxigenic taxa obtained through microscopic enumerations and assessing the strength of this relationship by examining statistical correlations. Alternatively, if a species or strain is a suspected producer this can

only be demonstrated by isolating and bringing the specific taxon into culture. This can be very time consuming and is not necessarily successful given that the specific growth conditions for most microbes are poorly known. Additionally, it has been observed that some cyanobacteria lose their ability to produce toxins when grown in culture over extended periods of time (Heisler et al., 2008). Therefore, more direct methods need to be developed in order to better characterize these taxa in environmental samples.

With the advent of single cell genome sequencing techniques it is now possible to perform sequencing and whole genome amplification for those microorganisms that are difficult to assess, thus allowing for their genetic characterization and the analysis of intra-species variability (Rodrigue et al., 2009). To date, such techniques have been successfully used in the characterization of *Escherichia coli* and the cyanobacterium *Prochlorococcus marinus* via flow cytometry sorting an polymerase cloning, a form of multiple displacement amplification (Zhang et al., 2006). Methods of single-cell DNA extraction for algal taxa such as dinoflagellates and diatoms have also been developed to extract DNA from single, preserved and unpreserved cells. Techniques first established by Marín et al. (2001) were further improved upon by Ki *et al.* (2005) for use in the species identification of single dinoflagellate cells from environmental samples and can likely be used for other algal taxa (Ki et al., 2005). In fact, single cell isolation techniques have recently been used in order to resolve the taxonomy and the phylogeny of the benthic diatom genus *Neidium* (Hamilton et al., 2015). Similarly, single cell DNA extraction and amplification may also prove useful when attempting to identify toxin producers within mixed natural assemblages of cyanobacteria.

However, unlike the dinoflagellates, which are large (ranging between 20-200µm) and typically found as solitary cells, single cyanobacterial cells are relatively small (ranging anywhere from 0.7-20µm) or are aggregated in larger colonies or filaments (Bellinger & Sigee, 2010). As such, the isolation of a single cyanobacterial cell is virtually impossible. In order to overcome this challenge, single colonies of cyanobacteria can be isolated in place of a single cell. As with other prokaryotes, all cells within a cyanobacterial colony or filament are derived from a single mother cell that multiples and grows asexually leading to the formation of a colony of genetically identical cells. As a result it is possible to garner information on the initial ancestral cell by isolating a single colony.

The present study aims to test the use of single cell isolation techniques on single colonies of cyanobacteria in order to identify specific toxigenic taxa within environmental samples. Using a modified methodology (Hamilton et al., 2015) single colonies of potentially toxigenic cyanobacteria, including species of *Microcystis, Dolichospermum, Nostoc* and *Gloeotrichia* were isolated from environmental samples and assessed for the presence of 16S rRNA specific to the cyanobacteria as well as the microcystin synthetase gene *mcyE*. These genes were chosen for their highly conserved nature within the cyanobacteria with the 16S rRNA gene being present in all cyanobacterial taxa and the *mcyE* gene being highly conserved in microcystin producing taxa due to its role in the synthesis of the toxicity conferring ADDA moiety (Rantala et al., 2006).

3.2 Methods

3.2.1 Sample collection

A number of environmental samples were obtained from various lakes between the summer of 2014 and fall of 2015 in Ontario, Québec and the Northwest Territories, when surface cyanobacterial blooms were observed or when cyanobacterial colonies were observed in the water column (Table 3.1). Each sample was concentrated using a 65 µm sieve and species present were identified using light microscopy and taxonomic keys (Bellinger & Sigee, 2010; Cronberg & Annadotter, 2006). Additionally a number of cultured species from the Canadian Phycological Culture Collection (CPCC) and the University of Texas Culture Collection of Algae (UTEX) including known toxin producer strains of *Microcystis aeruginosa* (CPCC 299 and CPCC 300) were analyzed to determine if the *mcyE* gene could successfully be amplified in a single colony from a known producer.

Screened whole water samples were preserved for future analyses by adding 0.5 mL of sample to 1.5 mL of RNALater solution (http://www.protocol-online.org/prot/Protocols/ RNAlater-3999.html). Samples were hand shaken and left at room temperature for 24 hours then stored in the dark at 4°C until needed for colony isolations.

Table 3.1 Single Colony Isolates of Cyanobacteria

Sample ID (Number)*	Sample Source	Sample Source Coordinates	Sampling Date**	Taxon	Number of Colony Isolates
PB	Picton Bay, Lake Ontario	44º 1.614 N 77º 7.686 W	August 18, 2014	*Microcystis* sp	2
AL	Lake Heney	46º 1.686 N 75º 55.368 W	June 21, 2015	*Dolichospermum lemmermannii*	4
AF	Bob's Lake	44º 42.216 N 76º 33.522 W	July 5, 2015	*Dolichospermum flos-aquae*	2
G	Bob's Lake	44º 42.216 N 76º 33.522 W	July 5, 2015	*Gloeotrichia echinulata*	18
DE1	Great Slave Lake (Bloom)	62°24.652 N 114°18.722 W	August 13, 2015	*Dolichospermum lemmermannii*	3
GJ	Jack's Lake	44º 41.286 N 78° 2.946 W	September 19, 2015	*Gloeotrichia echinulata*	1
NJ	Jack's Lake	44º 41.286 N 78° 2.946 W	September 19, 2015	*Nostoc* sp.	1
LB2383	Culture UTEX LB 2383	n/a	January 14, 2015	*Dolichospermum lemmermannii*	1
300	Culture CPCC 300	n/a	July 8, 2015	*Microcystis aeurginosa*	1
299	Culture CPCC 299	n/a	July 9, 2015	*Microcystis aeurginosa*	4
LL13-3	Lake Land Culture, 2013	45º 14.503 N 75º 34.943 W	December 8, 2015	*Microcystis aeurginosa*	

*Numbers indicate replicates/ other colonies extracted from same source. ** Date corresponds to when the sample was obtained from the lake in the case of environmental samples and the day the colony was isolated for culture sample

3.2.2 Cyanobacteria colony isolation and DNA extraction

Single cell isolation and DNA extraction techniques originally described for the microalgal group comprised of diatoms (Richlen & Barber, 2005; Hamilton et al., 2015) were followed for the isolation of single colonies of cyanobacteria. Single colonies were isolated using micro-pipetting, washed a number of time in sterile MilliQ water to remove debris and other cells, and then transferred to a PCR tube containing 200 µL of 10% Chelex® 100 solution (Richlen & Barber, 2005). For larger, macroscopic colonies, sterile fine tipped forceps and Pasteur pipettes were used for isolation.

The PCR tubes were vortexed at high speed for 15 seconds then centrifuged at 13 000 rpm for 10 seconds in a Heraeus Pico microcentrifuge (Biofuge/ Thermo Scientific). A freeze thaw cycle was then conducted in order to aid in the lysis of cells and to increase DNA yield. Isolates were placed in a Bio-Rad S1000 thermo-cycler and subjected to 20 minutes at 95° C, followed by 10 minutes in a freezer set at -80° C. This freeze thaw cycle was repeated three times. Isolates were subjected to an additional vortex and centrifuge cycle prior to amplification.

3.2.3 PCR amplification of single colony isolates

PCR was conducted on all single colony isolates in order to amplify the 16S rRNA gene and the *McyE* gene found in toxigenic, microcystin producing taxa. The amplification protocol used herein deviates from that found in Hamilton et al. (2015) as nested PCR was not necessary for our single colony isolates which contained ample genetic material when compared to what would be found in a single diatom cell. The average genome size of a single diatom is 30Mbp

(Vardi et al., 2008), while that of a single cyanobacterial cell is approximately 5-7Mbp (Kaneko et al., 2001;Kaneko et al., 2007).

3.2.3.1 PCR amplification of the cyanobacteria specific 16S rRNA from single colony isolates

PCR amplification of the cyanobacterial 16S rRNA fragment was conducted using the two primers CYA 108 F (5'- ACGGGTGAGTAACRCGTRA -3') and CYA 377R (5'- CCATGGCGGAAAATTCCCC -3') (Urbach, Robertson, & Chisholm, 1992; Nübel, Garcia-Pichel, & Muyzer, 1997; Rinta-Kanto et al., 2005). Amplification was conducted using the Bio-Rad S1000 Thermal Cycler, starting with an initial denaturing step for 5 minutes at 95°C, followed by 50 cycles of 30 seconds at 94°C, 1 minute at 56°C and 30 seconds at 72°C, finishing with a final extension step of 72°C for 15 minutes (Rinta-Kanto et al., 2005).

Amplification was also carried out on DNA extracted from the CPCC 300 culture of toxic *Microcystis aeruginosa* as a positive control and sterile water as a negative control. CPCC 300 is a toxigenic strain, producing two types of microcystin congeners (microcystin-LR and [Dha7] desmethylmicrocystin- LR) (LeBlanc et al. 2011).

3.2.3.2 PCR amplification of the *mcyE* gene from single colony isolates

PCR was also conducted on isolates in order to amplify the *mcyE* gene, a highly conserved gene located within the microcystin synthetase complex found in all toxic,

microcystin-producing cyanobacteria (Tillett et al., 2000; Rantala et al., 2006). PCR amplification of the 186bp fragment *mcyE* gene was conducted using the two primers mcyE F1 (5'-TAACTTTTTTGGGCATAGTCCTG-3') and mcyE R1 (5'-CGAACWGCYGCCATAATCGC-3') created to be specific to the common microcystin producing genera *Microcystis*, *Dolichospermum* and *Planktothrix* (N. Fortin, NRC Montreal, personal communication, May 14, 2014). Amplification was conducted starting with an initial denaturing step for 5 minutes at 96°C, followed by 30 cycles of 1 minute at 94°C, 1 minute at 60°C and 1 minute at 72°C, finishing with a final extension step of 72°C for 10 minutes (N. Fortin, NRC Montreal, personal communication, May 14, 2014). Controls used for the amplification of *mcyE* were the same as those used for the amplification of 16S rRNA.

3.2.4 Gel electrophoresis of PCR products

Amplification products were separated through a 1% agarose gel at 100V in 1X TAE buffer for 30 min. The sizes of DNA fragments were estimated via comparison to a 100 bp DNA ladder (New England BioLabs).

3.2.5 Single colony sequencing

Partial sequencing of all single colony isolates was conducted by K. Lefebvre at the Canadian Museum of Nature using the 16S rRNA primers described above (Nübel et al., 1997; Rinta-Kanto et al., 2005; Urbach et al., 1992) and methods detailed in Hamilton et al. (2015). Nucleotide sequences were generated using the Applied Biosystems 3130xl automated sequencer. Resultant sequences were then compared to the GenBank database of existing

sequences using the nucleotide Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology (NCBI) website (Zhang et al., 2000). Additionally, sequences from the three Great Slave Lake bloom isolates, identified as *D. lemmermannii* via microscopic identification were compared to sequences of *D. lemmermannii* from blooms in a number of subalpine lakes including Lake Garda, Lake Como, Lake Iseo and Lake Maggiore (Salmaso et al.,2015).

3.3 Results

3.3.1 Single colony isolation

A total of 41 single colony isolates were obtained from culture and environmental samples covering four different cyanobacterial genera including *Microcystis, Dolichospermum, Gloeotrichia* and *Nostoc* (Fig. 3.1).

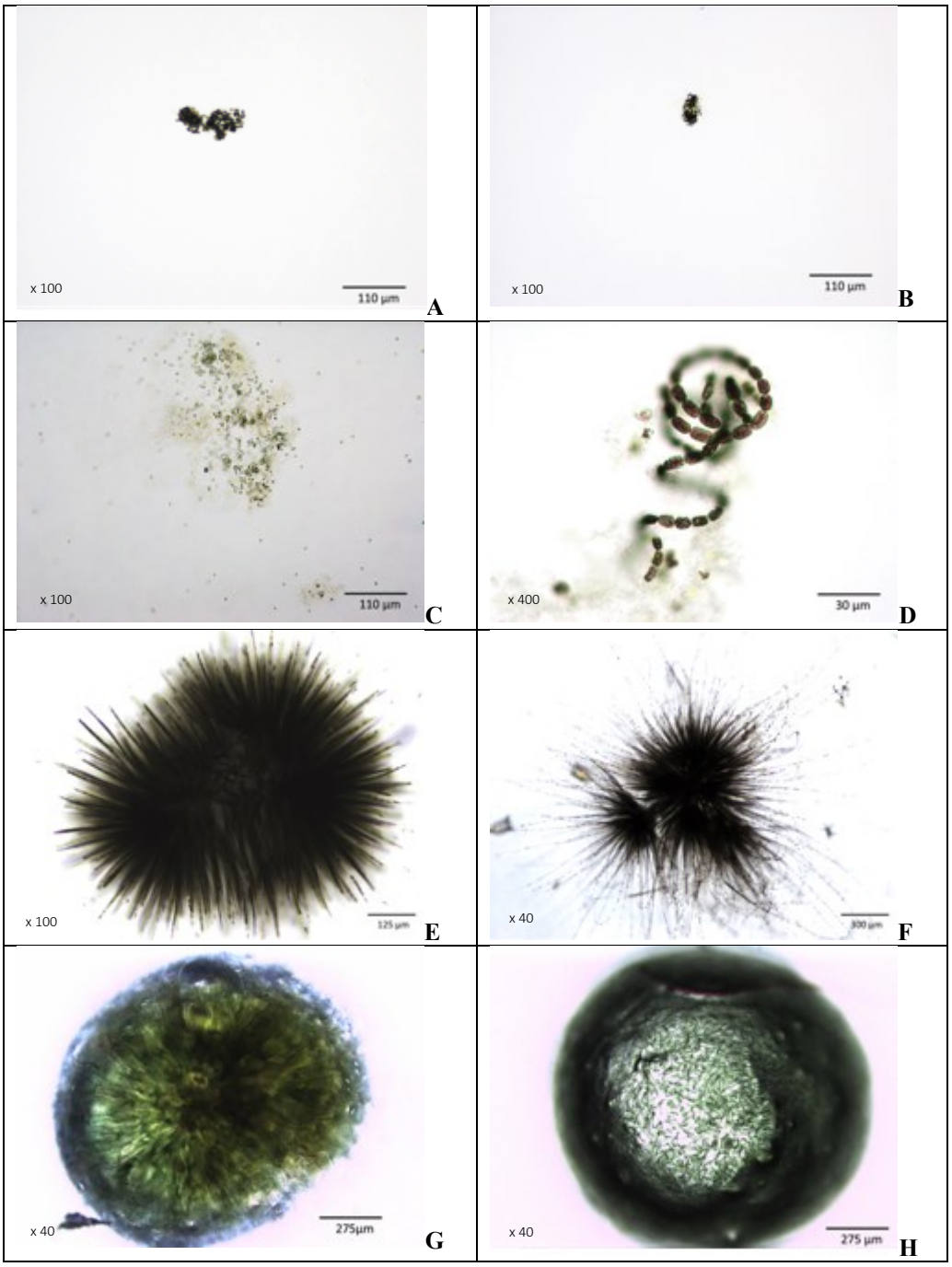

Figure 3.1 Single colony isolates. A and B: *Microcystis sp.* from Picton Bay; C: *Microcystis aeruginosa* (CPCC 300); D: *Dolichospermum lemmermannii* from Great Slave Lake site DE1; E and F*: Gloeotrichia sp.* from Bob's Lake; G *Gloeotrichia sp.* from Jack's Lake; H: *Nostoc sp.* from Jack's Lake. Scales bars indicated in lower right hand corner and magnification in lower left hand corner of each panel.

3.3.2 PCR amplification of 16S rRNA specific to cyanobacteria

PCR amplification of the 16S rRNA gene was conducted on all 41 isolates. As the 16S rRNA gene is conserved throughout all cyanobacterial genera it was used to establish if the DNA extraction was successful. Additionally, these PCR products were used to sequence isolates.

Gel electrophoresis was conducted using the amplification products (Fig. 3.2-3.4). Results produced bands for all isolates though some were fainter than others, potentially indicating lower DNA yield as a result of incomplete DNA extraction or low DNA content from the single colony.

3.3.3 PCR amplification of the mcyE gene

Results from the PCR amplification of the *mcyE* gene show that the gene was successfully amplified in all culture isolates that were known to produce microcystin (*Microcystis aeruginosa* strains CPCC 299 and CPCC 300) as well as faintly in one *Dolichospermum lemmermannii* isolate (AL 3) from Heney Lake (Fig. 3.5) and one *Gloeotrichia sp.* isolate (G3) from Bob's Lake (Fig. 3.6).

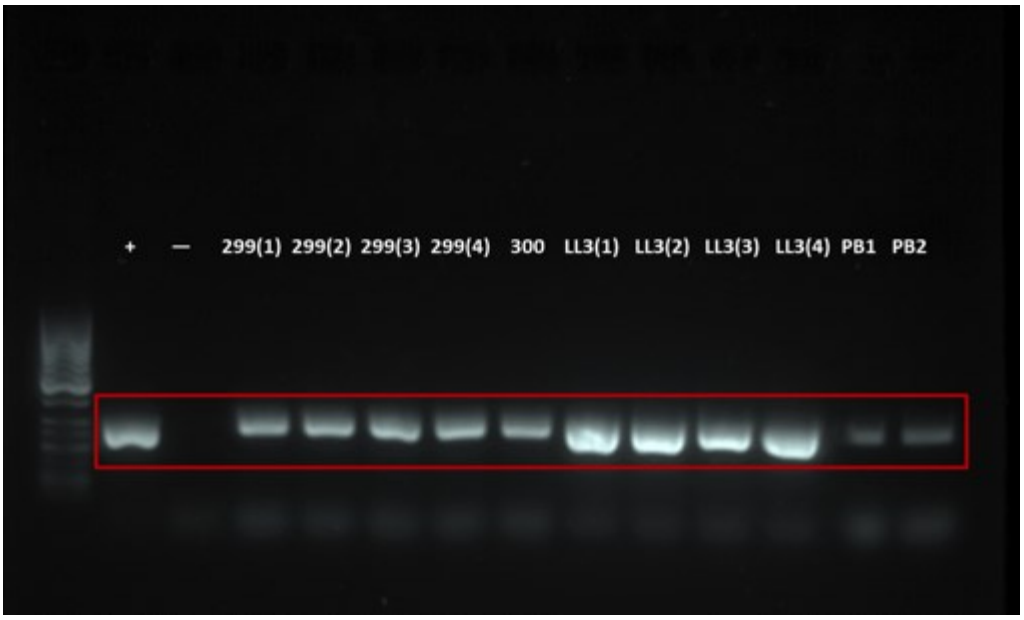

Figure 3.2 1% agarose gel electrophoresis of the 16S rRNA gene specific to cyanobacterial species. 299 refers to *Microcystis aeruginosa* isolates from CPCC 299 culture; **300** refers to *Microcystis aeruginosa* isolates from culture CPCC 300; **LL3** refers to *Microcystis aeruginosa* isolates Lake Land culture 3; **PB** refers to *Microcystis sp.* isolates from Picton Bay, Lake Ontario.

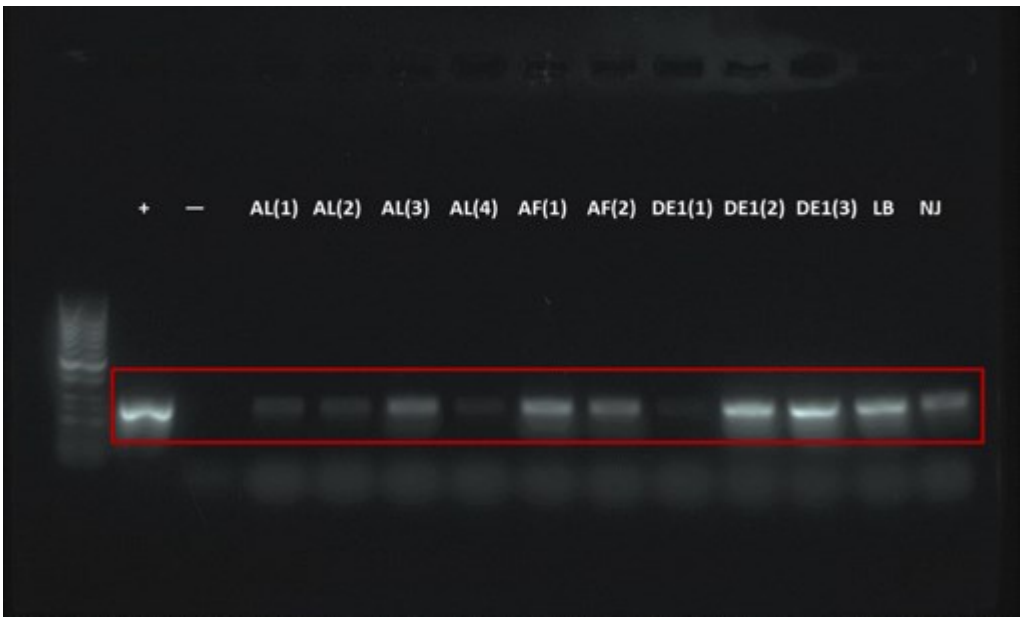

Figure 3.3 1% agarose gel electrophoresis of the 16S rRNA gene specific to cyanobacterial species. AL refers to *Dolichospermum lemmermannii* isolates from Heney Lake; **AF** refers to *Dolichospermum flos-aquae* isolates from Bob's Lake; **DE1** refers to *Dolichospermum lemmermannii* isolates from Great Slave Lake; **LB** refers to culture LB 2383 (*Dolichospermum flos-aquae*); **NJ** refers to *Nostoc sp.* isolate from Jack's Lake.

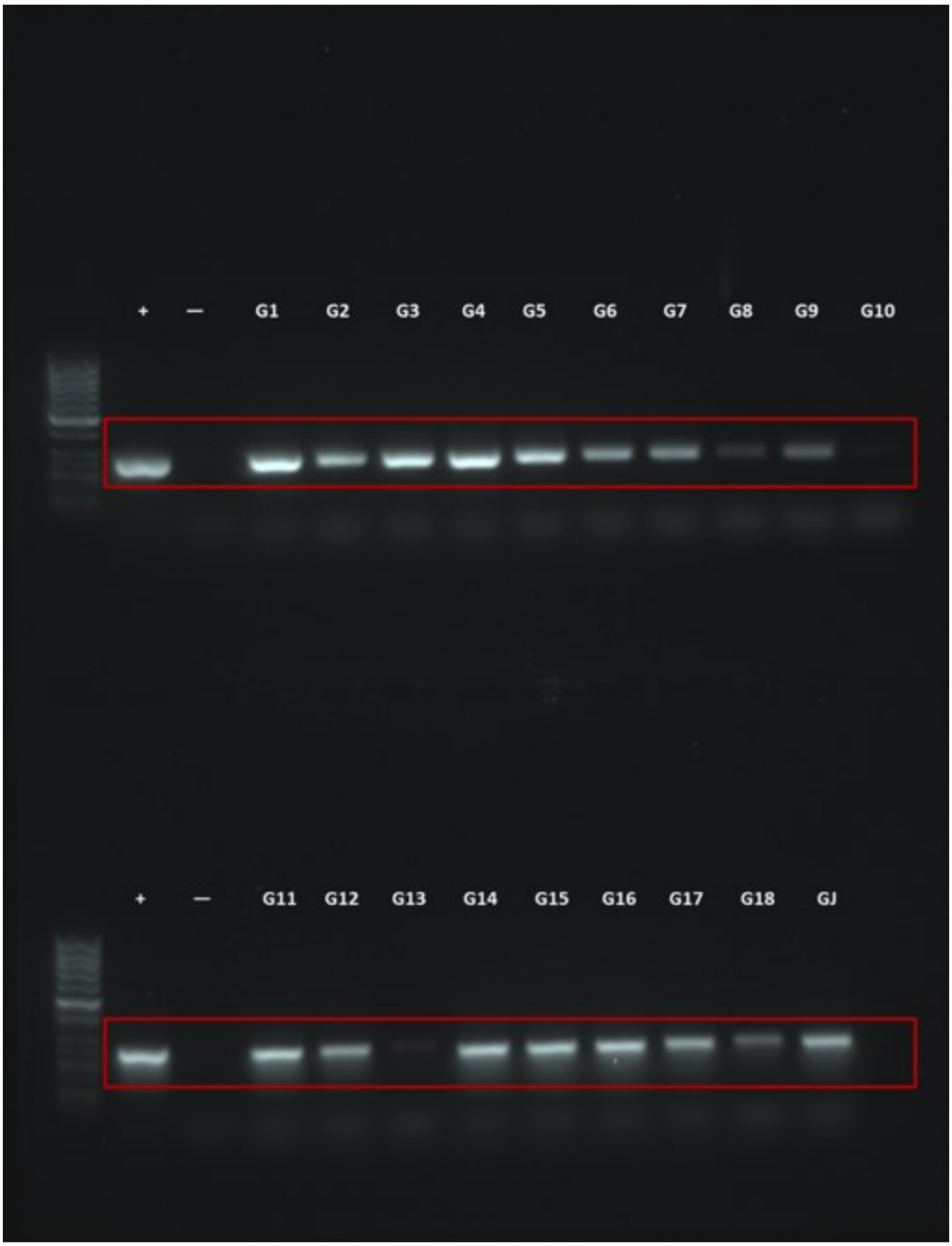

Figure 3.4 1% agarose gel electrophoresis of the 16S rRNA gene specific to cyanobacterial taxa. G1-18 refers to *Gloeotrichia sp.* isolates from Bob's Lake while **GJ** refers to the *Gloeotrichia sp.* isolate from Jack's Lake.

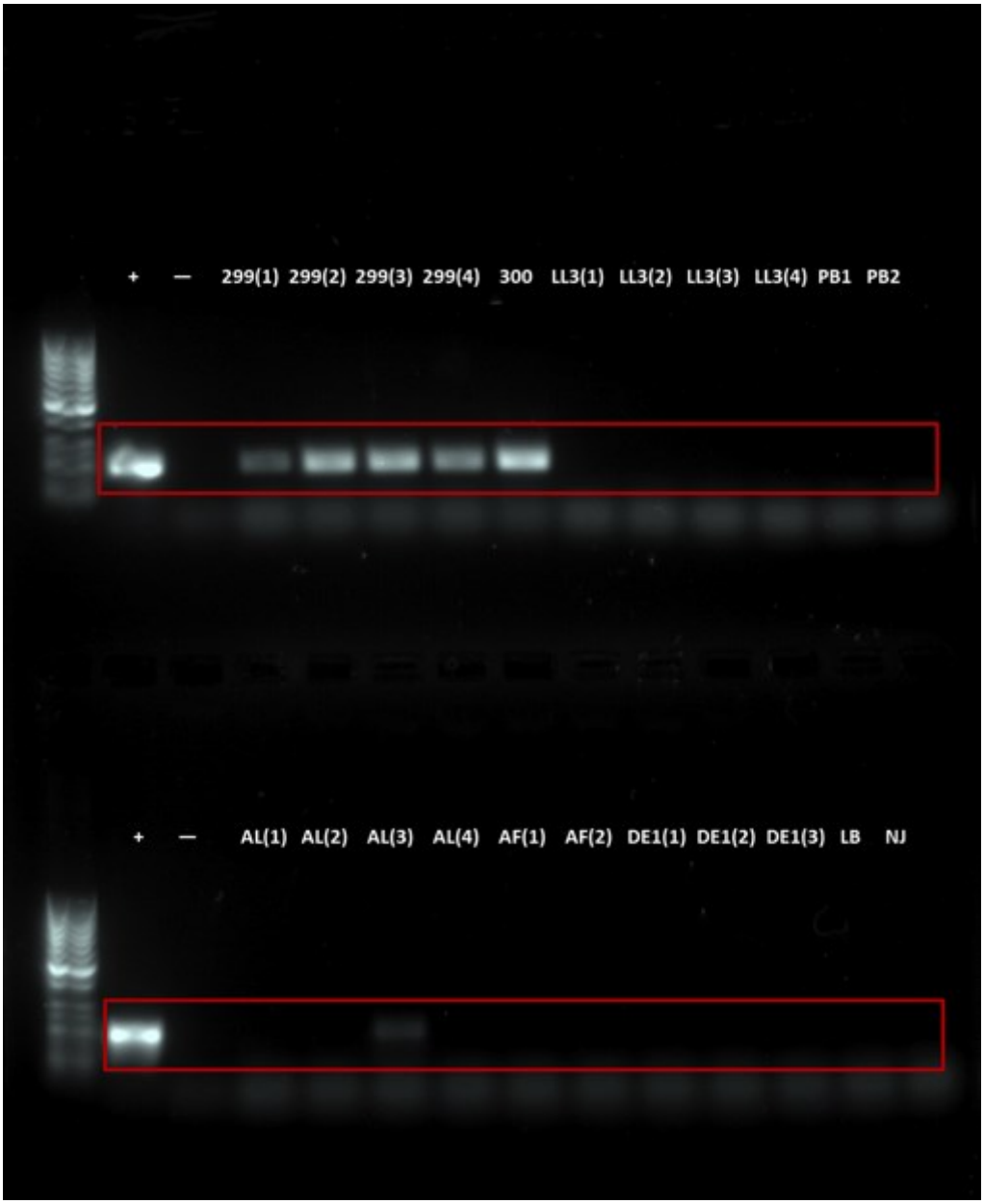

Figure 3.5 1% agarose gel electrophoresis of the *McyE* gene. 299 refers to *Microcystis aeruginosa* isolates from CPCC 299 culture; **300** refers to *Microcystis aeruginosa* isolates from culture CPCC 300; **LL3** refers to *Microcystis aeruginosa* isolates Lake Land culture 3; **PB** refers to *Microcystis sp.* isolates from Picton Bay, Lake Ontario; **AL** refers to *Dolichospermum lemmermannii* isolates from Heney Lake; **AF** refers to *Dolichospermum flos-aquae* isolates from Bob's Lake; **DE1** refers to *Dolichospermum lemmermannii* isolates from Great Slave Lake; **LB** refers to culture LB 2383 (*Dolichospermum flos-aquae*); **NJ** refers to *Nostoc sp.* isolate from Jack's Lake.

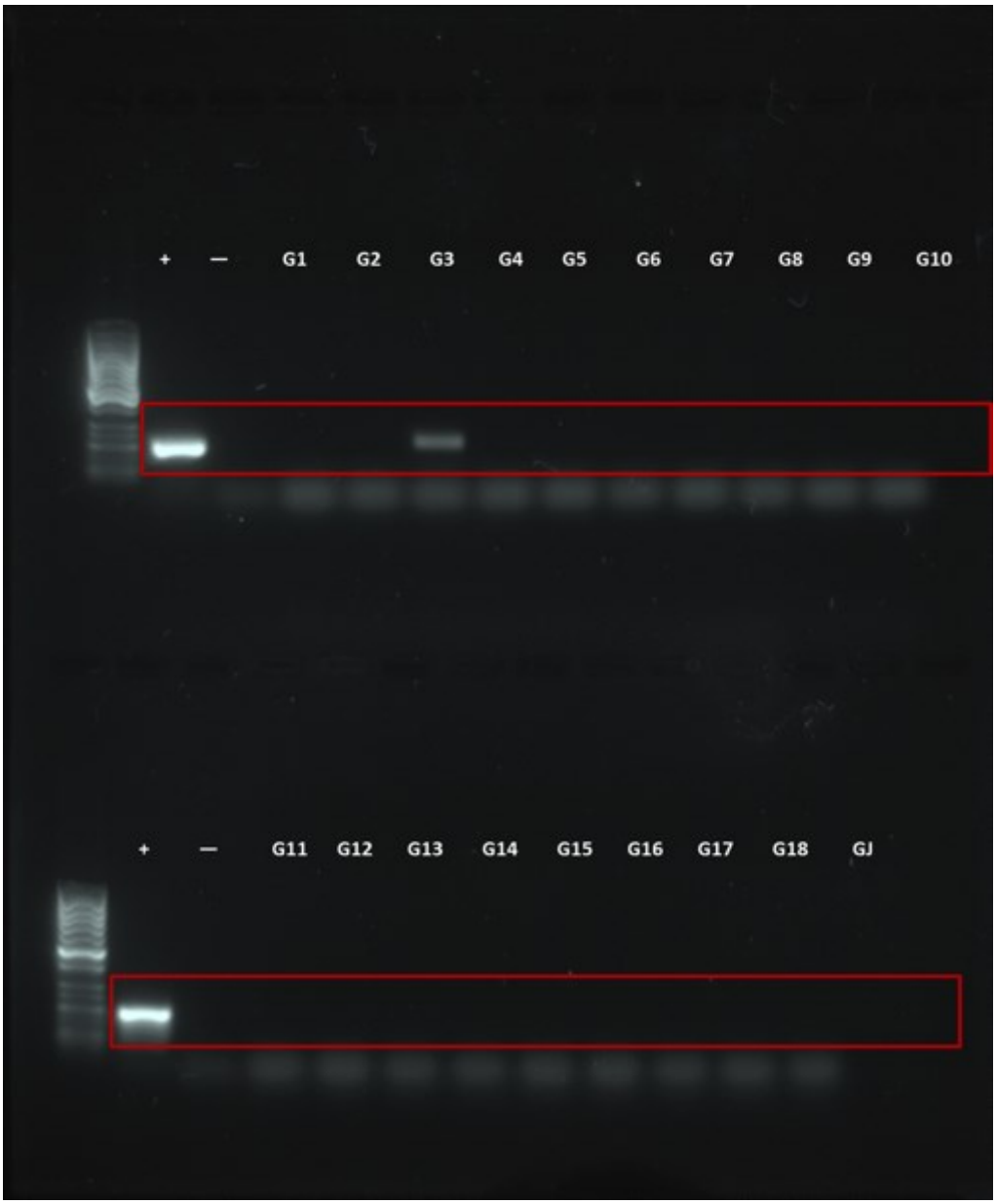

Figure 3.6 1% agarose gel electrophoresis of the *McyE* gene. G1-18 refers to *Gloeotrichia sp.* isolates from Bob's Lake while **GJ** refers to the *Gloeotrichia sp.* isolate from Jack's Lake.

3.3.4 Single colony sequencing

Sequencing results were obtained for 34 of the 41 isolates however sequencing was not successful for samples AL 1-4, G10, G13 and GJ, likely as a result of human error when transferring the 0.6 µL amount of sample to the sequencing reaction or if amplification did not occur.

Sequences can be found in Appendix B while sequence identifications are indicated in a phylogenetic tree demonstrating sequence identity matches (Fig. 3.7). Results correspond with the genus expected based on microscopic identification and culture strain in the case of isolates from culture.

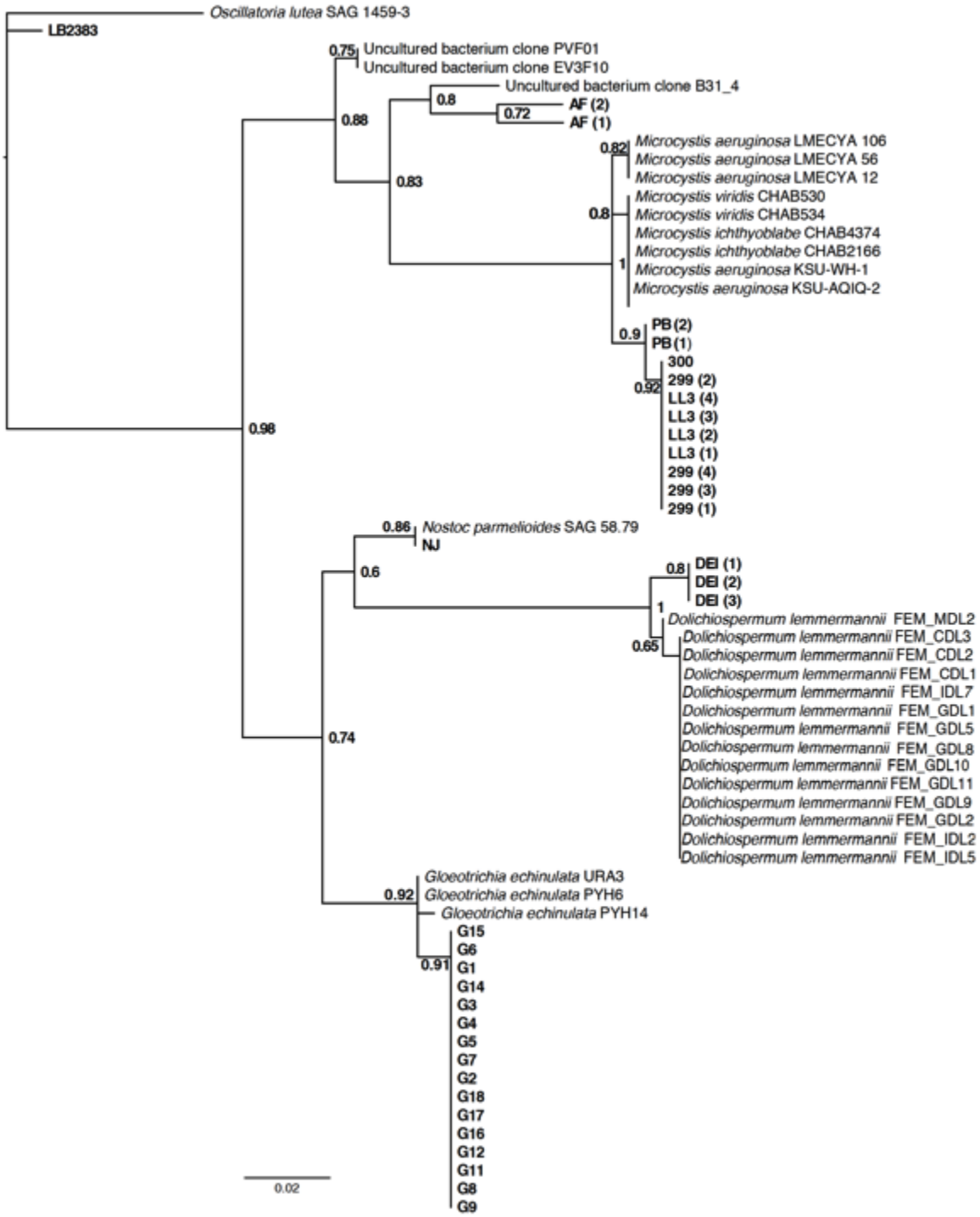

Figure 3.7 Phylogenetic tree of single colony isolates sequences. Single colony isolates are represented by sample ID. Species and strains were obtained through GenBank with *D. lemmermannii* strains obtained from Salmaso et al., 2015. Bootstrapping statistics support shown (with 1 indicating maximal support).

3.4 Discussion

Novel application of single cell genome amplification techniques on cyanobacteria

All isolates save that of G10 (*Gloeotrichia echinulata* isolate from Bob's Lake) provided amplification results for the 16S rRNA gene when run on gel electrophoresis (Fig. 3.2-3.4). Though it is possible that G10 provided no amplification results as a consequence of low DNA yield and purity (Appendix D), other samples with similar and even lower concentrations and A260/A280 ratios did so this is likely not the case. Such results indicate that cyanobacterial DNA was in fact obtained from the isolate as only members of the Cyanobacteria carry this specific gene. Differences in band intensity are likely a result of differences in the amount of starting material and the efficiency of the extraction procedure. Colony sizes between isolates varied as a result of differences in species and individuals thus providing samples covering a range of, albeit, low DNA concentrations. Said differences are likely attributable to differences in the genome size of each species. For example genome sizes for *Microcystis* and *Dolichospermum* species are approximately 5,842,795 base pairs (Kaneko et al., 2007) and 6,413,771 base pairs (Kaneko et al., 2001) (5.974×10^{-6} ng and 6.558×10^{-6} ng of DNA per cell) respectively.

Additionally, some isolates, particularly those of *Gloeotrichia echinulata* or *Nostoc sp*. were perhaps more resilient to lysis than that of other genera as a result of the copious mucilage within which the colonies are embedded (Bellinger & Sigee, 2010). This could explain the fainter bands observed with these samples.

Amplification of the *mcyE* gene was successful in far fewer samples, but was successful in all reference cultures (Fig. 3.5). As previously stated, colonies from known microcystin producing cyanobacterial strains were subjected to the same isolation and extraction procedure as

environmental samples to serve as secondary positive controls. As it was known that these isolates should in fact contain the *mcyE* gene of interest successful amplification of these isolates serve as verification of this technique.

The *mcyE* gene was only amplified in two of the environmental isolates included: one isolate of *Dolichospermum lemmermannii* from Lake Heney (sample AL3, Fig. 3.5) and a *Gloeotrichia echinulata* isolate from Bob's Lake (sample G3, Fig. 3.6). It is of interest to note that both of these isolates were obtained from samples from which more than one colony of the same species was isolated, yet only one isolate tested positive. Additionally, the fact *mcyE* was not amplified in any of the samples of *D. lemmermannii* from the Great Slave Lake bloom is corroborated by the low *mcyE* copy numbers measured via ddPCR from whole water samples (Chapter 2).

Both toxigenic and non-toxigenic species of cyanobacteria can co-exist within a population, therefore it is possible that only some of the given isolates were in fact toxin producers (Vezie et al., 1998; Kurmayer et al., 2002; Kurmayer et al., 2004; Via-Ordorika et al., 2004). Though toxin production is often observed across multiple species and genera (for example a number of species of *Dolichospermum* and *Microcystis* produce microcystin), not all species within a genus will necessarily produce toxins. Similarly, not all strains within a species will necessarily be toxic (Christiansen et al., 2008). Therefore, though only one of a given isolate from the same sample was deemed toxic, it is possible that the population was comprised of both toxigenic and non-toxigenic individuals, however this could only be confirmed given a larger sample size with which to establish gene frequency.

Sequencing of single colony isolates

In order to verify the amplification results obtained with single colony isolates, all samples were sequenced in order to ensure that only the target cyanobacteria were present. Results for the 34 isolates that were successfully sequenced (Fig. 3.7) were consistent with what were expected based on morphologic identification and the taxa of isolates from culture for most isolates. Additionally, sequences from the *Dolichospermum lemmermannii* isolates from Great Slave Lake were also aligned using partial 16S RNA sequences of *D. lemmermannii* isolated from a number of deep southern subalpine lakes (Salmaso et al., 2015), resulting in a greater than 99% identities match. Sequencing for sample G10 was unsuccessful. Coupled with the fact that this sample also provided no amplification products for the 16S RNA gene it is likely that amplification did not occur for this sample.

Sequences of isolates AF1, AF2 and LB2383 did not result in conclusive identifications when using NCBI BLAST and were identified as uncultured bacterium/cyanobacterium clones. Interestingly, all three of these samples were either known to be *Dolichospermum flos-aquae* (LB2383 was a culture of *D. flos-aquae*) or was identified as such via light microscopy. Sequences were also consistent between isolates of the same species and from the same sample site such that all *Gloeotrichia echinulata* provided nearly identical sequences as did all *Gloeotrichia echinulata* from Bob's Lake. Such results further confirm the efficacy of this methodology.

Gloeotrichia as a novel microcystin producer

Though the sample size was small and consisted of colonies from only two lakes, it was possible to amplify the *mcyE* gene in a single *Gloeotrichia echinulata* isolate (G3). Interestingly, this colony was obtained from Bob's Lake and was the only colony in the 18 isolated from this lake to test positive for microcystin synthetase genes. The presence of the *mcyE* gene in at least one isolate of *Gloeotrichia echinulata* helps support past studies on *Gloeotrichia* toxicity and microcystin production. In their 2008 and 2012 studies Carey et al.(20..) measured the concentration of microcystin obtained from environmental samples of *Gloeotrichia echinulata.* The microcystin concentrations measured in that study were very low and may possibly have come from secondary sources such as picocyanobacteria, some of which have been found to produce low levels of microcystin (Oudra et al., 2002). The current study shows that *Gloeotrichia spp.* may in fact have the genes required for the production of microcystin, however this requires further investigation with a larger sample size obtained from a greater number of sources. We cannot therefore, draw any broad conclusions on the toxic potential of *Gloeotrichia* from this study.

Future applications

The possibility of single colony gene amplification opens the door to a number of prospects when it comes to the study of cyanobacteria, cyanotoxins and cyanobacterial bloom dynamics. Though much research has been done on microcystin-producing cyanobacteria there are still knowledge gaps regarding the role of the toxin, the factors that promote its production and the factors that promote bloom formation. To date the major consensus is that cyanobacterial blooms are brought on by excessive nutrient inputs and a number of climate change events,

including elevated temperatures; however it is still not known exactly how or to what extent each of these influences the formation of toxigenic blooms (Kaebernick & Neilan, 2001; Paerl & Huisman, 2008; Kleinteich et al., 2012; O'Neil et al., 2012; Paerl & Paul, 2012; Rastogi et al., 2015). Similarly, though many laboratory studies have investigated the role of microcystin and the effect of a number of conditions on its production (Long et al., 2001; Tonk et al., 2007; Jiang et al., 2008; Wu et al., 2010; Dziallas & Grossart, 2011; Van de Waal et al., 2011; Zamyadi et al., 2015) there is still no consensus as to its major purpose. Additionally, such studies are often performed on cyanobacterial cultures, which have been known to change over time in toxin production and life history stage expression, and to show different responses to environmental stimuli when kept for extended periods of time (Heisler et al., 2008). Using the techniques described in this study may assist in gaining better insight into the nature of microcystin-producing blooms. By isolating individual colonies from bloom material and comparing a number of proxies between colonies (especially between toxigenic and non-toxigenic colonies from the same population) it may be possible to quantify any differences in gene copy numbers or gene expression that may be linked to nutrient metabolism and temperature responses or other key processes.

3.5 Conclusions

The results of this study show promise for future research on natural populations of cyanobacteria and other microalgae groups, and open up a number of new possibilities when investigating bloom dynamics and the role of toxins. The focus of this work has established that it is possible to extract DNA from single colonies of cyanobacteria and that this DNA can be amplified and used in downstream applications.

Sequencing results showed that all isolates of the same genus in a given sample were in fact the same species. Nevertheless, only a single colony of *Gloeotrichia echinulata* and *Dolichospermum lemmermannii* were deemed toxic, begging the question as to why the gene for microcystin production is maintained within populations.

CHAPTER 4: CONCLUSIONS

The overall objective of this thesis was to investigate the past and present occurrence of cyanobacteria in lakes above 60º N in latitude in order to establish whether or not the recent blooms reported in northern waterbodies such as Yellowknife Bay, GSL represent a change in the historical cyanobacterial community (Chapter 2). In addition, using modified single cell genome amplification techniques, we were able to amplify the toxic *mcyE* gene in single colony isolates from temperate lakes in the Ottawa area, and used these same methods to assess the toxicity of colonies isolated from bloom material from Yellowknife Bay (Chapter 3).

The cyanobacteria are ubiquitous to all water bodies and Northern lakes are no exception. In general cyanobacteria in the north are known to primarily form benthic mats and films, however recently they have been observed forming surface blooms. Though planktonic potentially bloom-forming species have been reported in the waters of Yellowknife Bay since the early 1950s, no reports of blooms had been made until recent years leading one to attribute their occurrence to more recent climate change. As several bloom-forming species are known to produce the c hepatotoxic microcystins it is of utmost importance that more information on the occurrence of such blooms and their toxicity be obtained.

After extracting DNA from a number of sediment cores from northern lakes, as well as surface waters from Great Slave Lake and two nearby lakes (Madeline Lake and Pontoon Lake) we tested for the presence of a number of genes including the *mcyE* gene found exclusively in microcystin producing cyanobacteria, as well as the cyanobacterial 16S rRNA gene and the bacterial *glnA* gene using ddPCR. Much like the more commonly used qPCR, ddPCR can be used to quantify copy numbers for a number of genes, however unlike qPCR it is capable of absolute quantification and thus can give a more accurate idea of the presence of genes in

samples. To date, ddPCR had only been used to quantify microcystin genes in one other study (Te et al., 2015) and this is the first study to use the approach to quantify microcystin genes from sediment-extracted samples.

Our findings showed that while the *mcyE* gene was present throughout the sediment archives and within the plankton of all lakes studied, it was only in very low quantities, far below those that are considered to be a threat to humans and wildlife. In addition, there did not appear to be any significant change in the *mcyE* GCN over time indicating that there has been no real increase in toxicity. Conversely, increases in both the 16S rRNA and *glnA* genes were seen in all samples indicating a potential increase in overall cyanobacterial and bacterial abundance (however, given the possibility of DNA degradation with depth, the amount of this increase cannot be precisely determined). This was seen in all lakes assessed and appears to reflect recent climate warming, and in the case of Meretta Lake, historic eutrophication and recovery.

The single colony isolation and amplification techniques described in Chapter 3 allowed for the amplification of the *mcyE* gene in a number of single colonies of cyanobacteria, most notably in colonies of *Gloeotrichia echinulata* a species whose ability to produce microcystin is still being assessed by researchers. In addition, the technique was used on single colony isolates from an August 2015 bloom in Yellowknife Bay, GSL. Though said samples provided no amplification results for the *mcyE* gene, sequencing allowed us to establish that the bloom was comprised of the filamentous, nitrogen-fixing species *Dolichospermum lemmermannii*, which has been known to form toxic blooms in a number of subalpine lakes in Europe.

The use of two novel techniques in the study of cyanobacteria were illustrated in this thesis; ddPCR for the quantification of cyanobacterial GCN from environmental sources and single colony isolation, DNA extraction and amplification. Future applications of these

approaches in the study of cyanobacteria will likely provide much more insight into the nature of blooms and the dynamics of their toxicity. This work also highlights the importance of limnological and environmental studies in the North. As global temperatures continue to increase in light of climate change it is the polar latitudes that are most greatly affected. It is imperative that the current data gaps for these northern environments be filled, not only for the sake of the scientific community, but also for residents of northern communities already facing the myriad challenges of climate change.

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APPENDIX A: Climate Data for Yellowknife, NWT

It is common knowledge within the scientific community that the effects of climate change are greatest at high latitudes (Smol et al., 2005; Korhola et al., 2002). The Arctic and Antarctic have been and are continuing to warm at a much faster rate than any other place on Earth (IPCC, 2014). In fact, a review of climate data for the North West Territories (NWT) has shown an overall average increase of 2.4°C in temperature between 1958 and 2012 with winter and spring temperatures increasing more than summer and fall (NWT, 2015)

The effects of climate change in the North and in the Yellowknife (NWT) area, which is the subject of this thesis, are evidenced by climate and ice coverage data for Northern Canadian waters. Historic temperature trends have shown an increase of approximately 2.5 º C since 1942 in the city of Yellowknife (Fig. A.1**)**. Temperature increases for Yellowknife are also evidenced in the number of heating and cooling degree-days. Heating degree days (HDD) are a measure of how much (in degrees) and for how long the outside air temperature is below a given temperature, while cooling degree days (CDD) are a similar measurement for days that are above a said temperature. Such information can be useful when investigating energy consumption as HDD and CDD can be used as an indication of when heating and cooling systems are most likely in use. With increased ambient temperatures as a result of climate change it is likely that the number of CDD will increase. The HDD and CDD provided by Environment and Climate Change Canada use 18ºC as their baseline external temperature. From 1991 to 2015 HDD have remained consistent, however CDD have increased by an average of 4 degree-days (Fig. A.2). Summer (June-September) temperatures have also increased between 1942 and 2015 with average increases ranging between 0.5-2.5 ºC (Fig A.3).

Climate change has also led to changes in precipitation patterns. In general, there has been an increasing trend in both the amount of snowfall and rainfall in the Yellowknife area (*NWT Climate Observations-Temperature, precipitation observations in the NWT*, 2015). Increases in summer (June-September) precipitation have shown an average increase of about 30 mm (Fig. A.4).

Increases in temperature and precipitation are two of the more visible climate change effects, however they can also lead to a cascade of secondary effects that can be equally detrimental to Northern ecosystems. Canada's north is made up extensively by lakes and other freshwaters all of which are impacted by such changes. Due to the unique characteristics and organisms that live within such environments, climate change has the potential to drastically change the ecology of Northern lakes and the food webs therein potentially making these waters less safe for human and animal use.

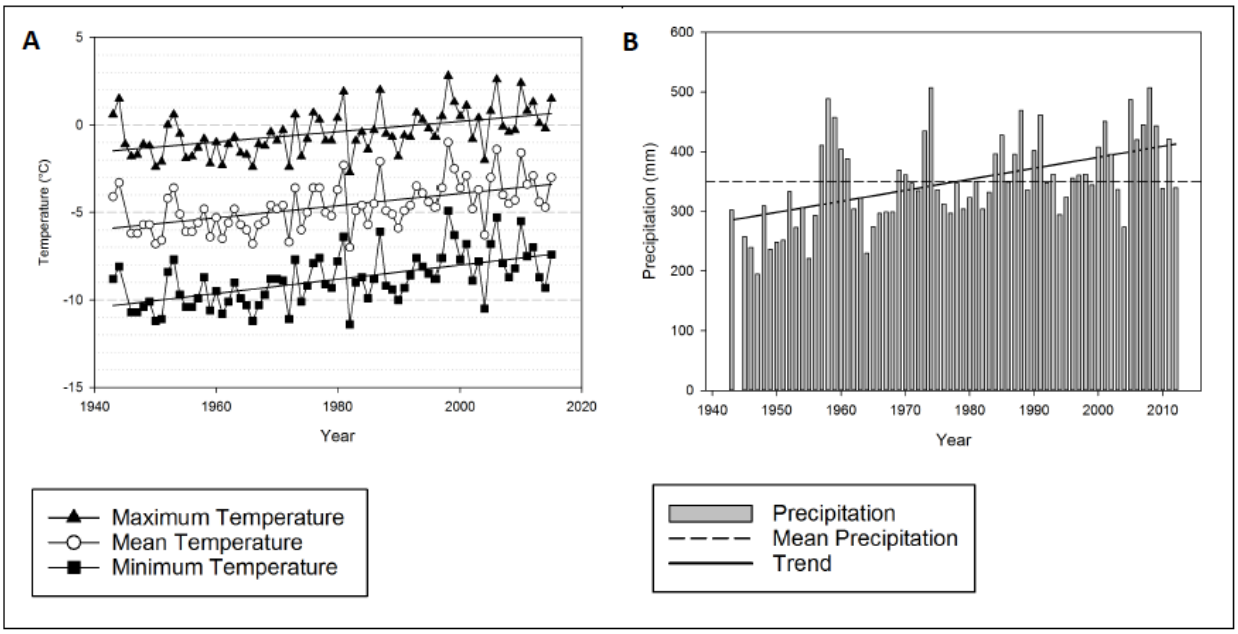

Figure A.1 Average annual temperature (A) and precipitation (B) changes for the city of Yellowknife, NWT (1942-2015). Mean precipitation of 350 mm. Data obtained from Environment and Climate Change Canada.

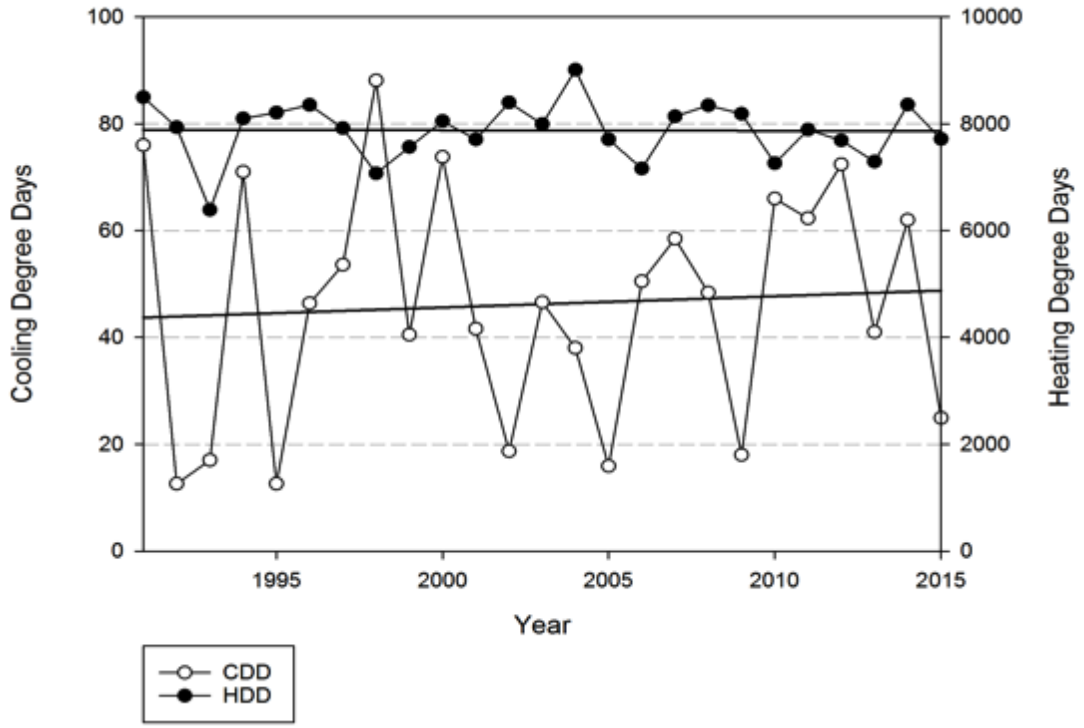

Figure A.2 Cooling (CDD) and heating (HDD) degree days for the city of Yellowknife, NWT (1991-2015). HDD have remained over the past two decades while CDD have increased by approximately 4, indicating an increased use of energy for cooling buildings and dwellings. (Environment and Climate Change Canada)

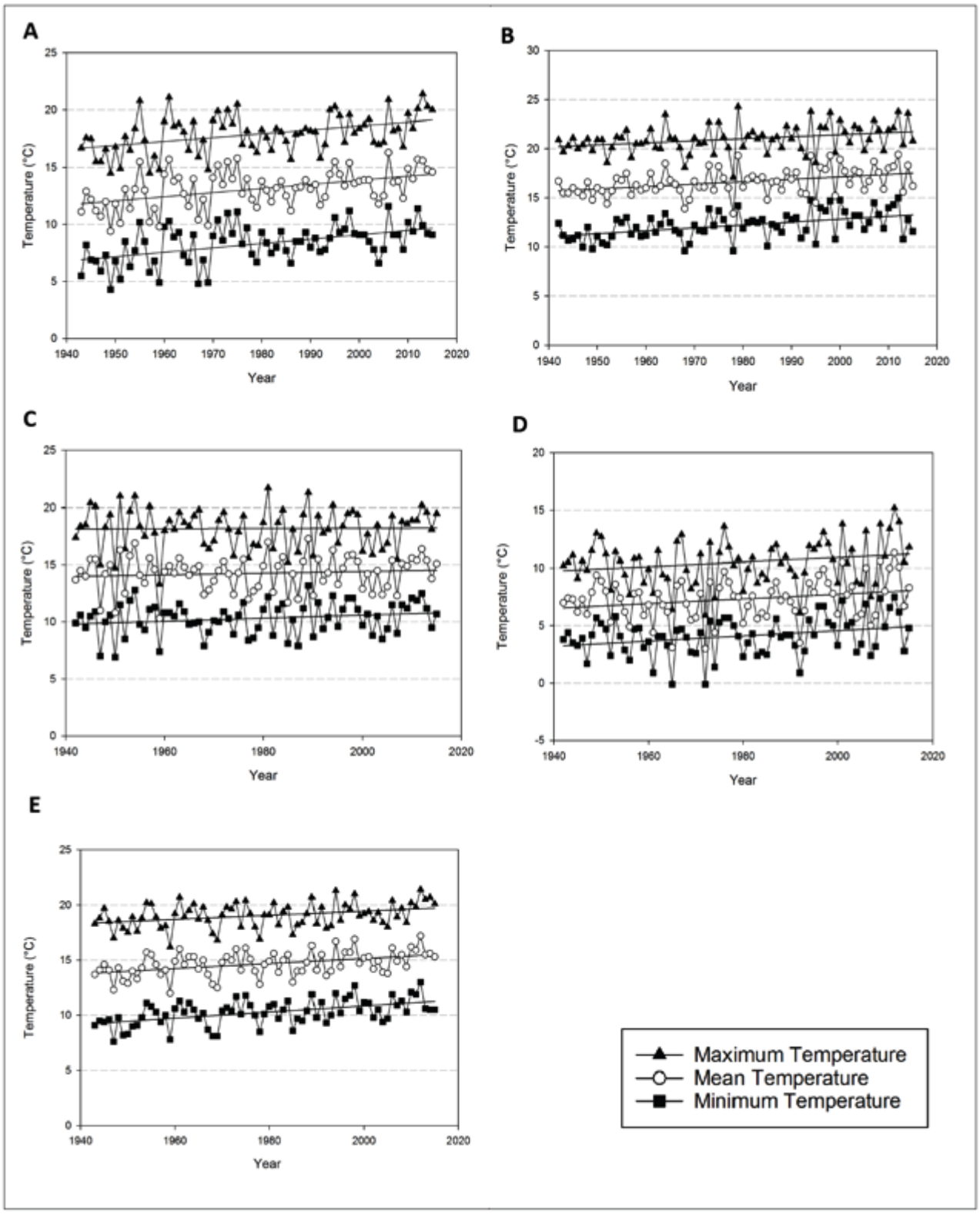

Figure A.3 Average summer temperature changes for the city of Yellowknife, NWT (1942-2015). A June (average increase of 2.5 ºC) **B** July (average increase of 1.5 ºC) **C** August (average increase of 0.5 ºC) **D** September (average increase of 1.5 ºC) **E** Average of summer months (June-September). Data from Environment and Climate Change Canada.

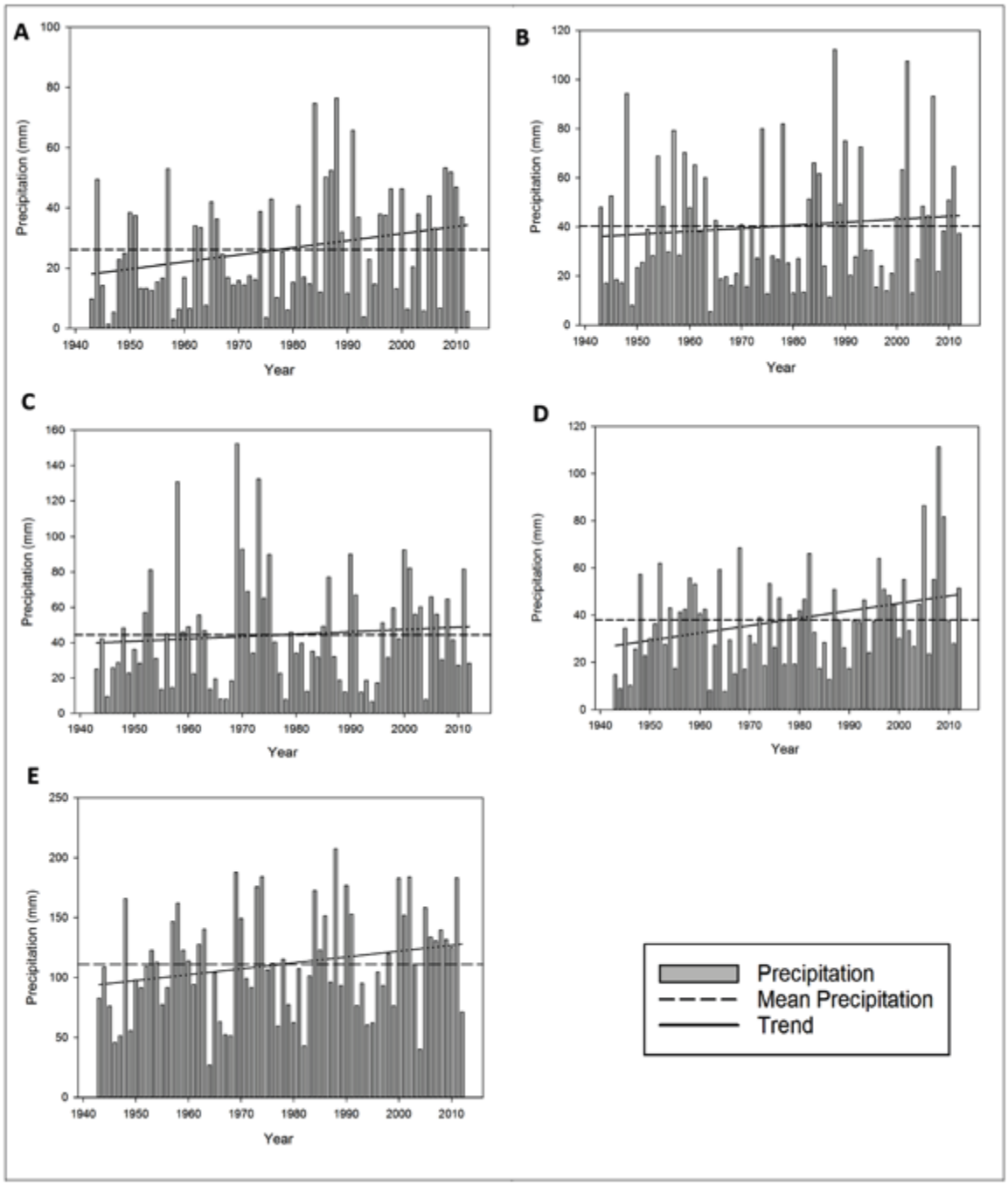

Figure A.4 Average summer precipitation changes for the city of Yellowknife, NWT (1942-2015). **A** June (mean precipitation 27 mm) **B** July (mean precipitation 40 mm) **C** August (mean precipitation 44 mm) **D** September (mean precipitation 38 mm) **E** Average of summer months (June-September) (mean precipitation 110 mm). Data obtained from Environment and Climate Change Canada.

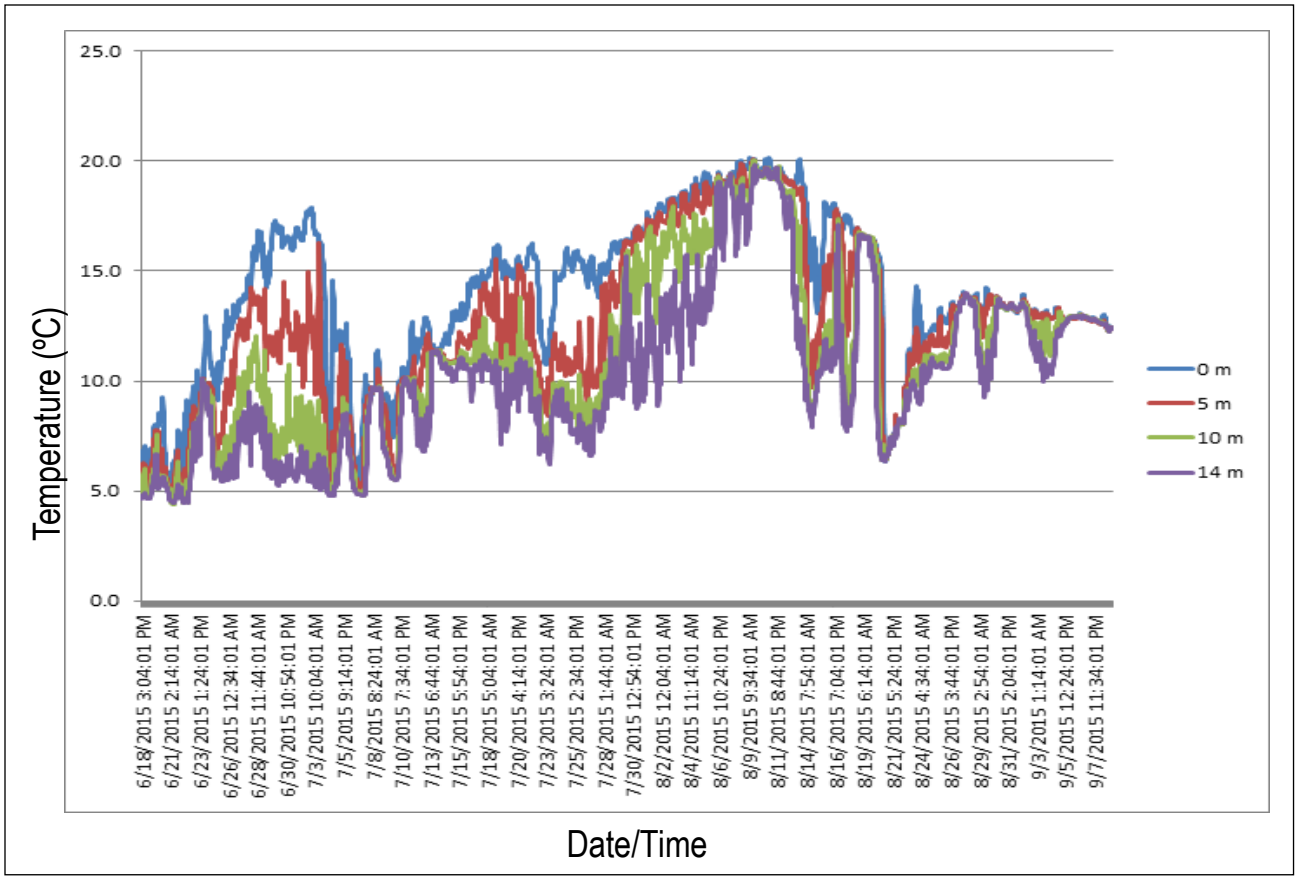

Figure A.5 Water column depth profile for Yellowknife Bay, GSL, Summer 2015. Thermistor set at mouth of Yellowknife Bay, near Dettah. **Environment Canada data collected (Dr. John Chetelat, NWRC)**

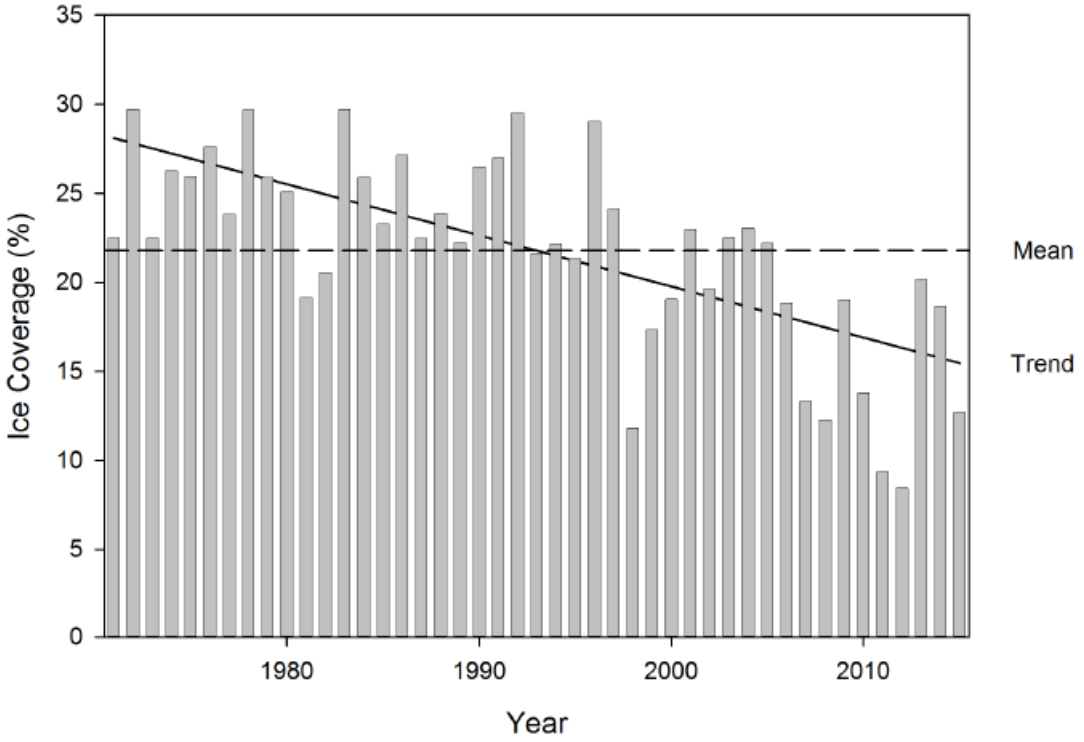

Figure A.6 Change in sea ice coverage of Northern Canadian Waters (1970-2015). Measurements were taken on approximately September 17th of each year. Mean ice coverage was 22%. Northern Canadian waters refer to seas. Data obtained from Environment and Climate Change Canada.

APPENDIX B: CHAPTER 2 SUPPLEMENTAL MATERIAL

Table B.1 Phytoplankton taxa identified in Great Slave Lake surface water samples

SITE	DIVISION	TAXON
Yellowknife Bay Site 2 (DE2)	**Diatoms**	*Asterionella formosa*
		Cocconeis sp.
		Cyclotella sp.
		Cyclotella meneghiniana
		Gomphonema sp.
		Navicula sp.
		Psammothidium sp.
		Synedra acus
		Synedra ulna
		Melosira sp.
		Lindavia sp.
	Chrysophytes	*Dinobryon divergens*
		Chromulina sp.
	Chlorophytes	*Oocystis elliptica*
		Oocystis sp.
		Kirchneriella sp.
		Scenedesmus sp.
		Gloeocystis sp.
		Gloeocapsa sp.
	Cyanobacteria	*Dolichospermum sp.*
	Cryptophytes	*Plagioselmis sp.*
Yellowknife Bay Site 4 (DE4)	**Diatoms**	*Asterionella formosa*
		Aulacodiscus sp.
		Cocconeis sp.
		Cocconeis pediculus
		Cyclotella sp.
		Cyclotella meneghiniana
		Fragilaria sp.
		Lavinda sp.
		Synedra acus
		Synedra ulna
		Tabellaria sp.
		Rhizolsolenia sp.
		Navicula sp.
		Melosira sp.
	Dinoflagellates	*Ceratium hirundella*

SITE	DIVISION	TAXON
	Chrysophytes	*Dinobryon divergens*
	Chlorophytes	*Oocystis sp.*
		Kirchneriella sp.
		Oocystis borgei
		Monoraphoridium sp.
	Cryptophytes	*Plagioselmis sp.*
	Cyanobacteria	*Dolichospermum sp.*
GSL 14-10	**Diatoms**	*Asterionella formosa*
		Brachysira sp.
		Cocconeis sp.
		Cyclotella sp.
		Cyclotella meneghiniana
		Gomphonema sp.
		Fragilaria sp.
		Gomphonema geminatum
		Navicula sp.
		Synedra acus
		Synedra ulna
	Dinoflagellates	*Peridinium sp.*
		Ceratium hirundella
	Chrysophytes	*Dinobryon divergens*
	Chlorophytes	*Oocystis elliptica*
		Kirchneriella sp.
		Gloeocystis sp.
		Crucigenia sp.
		Scenedesmus sp.
	Cryptophytes	*Plagioselmis sp.*
	Cyanobacteria	*Oscillatoria sp.*
		Dolichospermum sp.
GSL 14-11	**Diatoms**	*Achananthidium minutissimum*
		Asterionella sp.
		Asterionella formosa
		Aulacodiscus sp.
		Cocconeis sp.
		Cocconeis placentula
		Cyclotella comta
		Cyclotella meneghiniana
		Fragilaria sp.
		Melosira sp.

SITE	DIVISION	TAXON
		Navicula sp.
		Synedra acus
		Synedra ulna
	Chrysophytes	*Dinobryon divergens*
		Kephyrium sp.
	Chlorophytes	*Crucigenia sp.*
		Kirchneriella sp.
		Gloeocystis sp.
		Oocystis sp.
		Monoraphidium sp.
		unknown *ankistrodesmoideae sp.*
	Cryptophytes	*Plagioselmis sp.*
	Cyanobacteria	*Dolichospermum sp.*
	Charophyte	*Closterium sp.*
GSL 15-4	**Diatoms**	*Asterionella formosa*
		Cocconeis sp.
		Cyclotella sp.
		Cyclotella meneghiniana
		Pinnularia sp.
		Lindavia sp.
		Fragilaria sp.
		Navicula sp.
		Rhizolsolenia sp.
		Synedra acus
		Synedra ulna
	Chrysophytes	*Dinobryon divergens*
		Dinobryon sertularia
		Kephyrium sp.
	Chlorophytes	*Kirchneriella sp.*
		Oocystis sp.
		Scenedesmus sp.
		Monoraphidium sp.
	Cryptophytes	*Plagioselmis sp.*
	Cyanobacteria	*Dolichospermum sp.*
GSL ND5	**Diatoms**	*Asterionella formosa*
		Aulacodiscus sp.
		Cocconeis sp.
		Cyclotella sp.
		Cyclotella meneghiniana

SITE	DIVISION	TAXON
		Fragilaria sp.
		Synedra acus
		Tabellaria sp.
		Rhizolsolenia sp.
	Dinoflagellates	*Ceratium hirundella*
		Ceratium sp.
		Peridinium sp.
	Chrysophytes	*Desmatractum spryii*
		Dinobryon divergens
		Dinobryon sertularia
		Dinobryon stipitatum
		Kephyrion ovum
	Chlorophytes	*Scenedesmus sp.*
		Oocystis parva
		Oocystis submarina
		Oocystis sp.
		Crucigenia tetrapedia
	Cryptophytes	*Plagioselmis minuta*
	Cyanobacteria	*Dolichospermum sp.*
GSL DE3	**Diatoms**	*Asterionella formosa*
		Cocconeis sp.
		Cyclotella bodanica
		Cyclotella meneghiniana
		Diatoma sp.
		Synedra acus
		Tabellaria sp.
	Dinoflagellates	*Peridinium sp.*
	Chrysophytes	*Dinobryon sp.*
		Dinobryon divergens
	Chlorophytes	*Chlamydomonas sp.*
		Crucigenia tetrapedia
		Gleocapsa sp.
		Kirchneriella sp.
		Monoraphidium sp.
		Scenedesmus sp.
	Cryptophytes	*Cryptomonas sp.*
		Plagioselmis sp.
	Cyanobacteria	*Dolichospermum sp.*
		Aphanizomenon sp.
		Microcystis sp.

SITE	DIVISION	TAXON
		Small blue green
		Small blue green filament

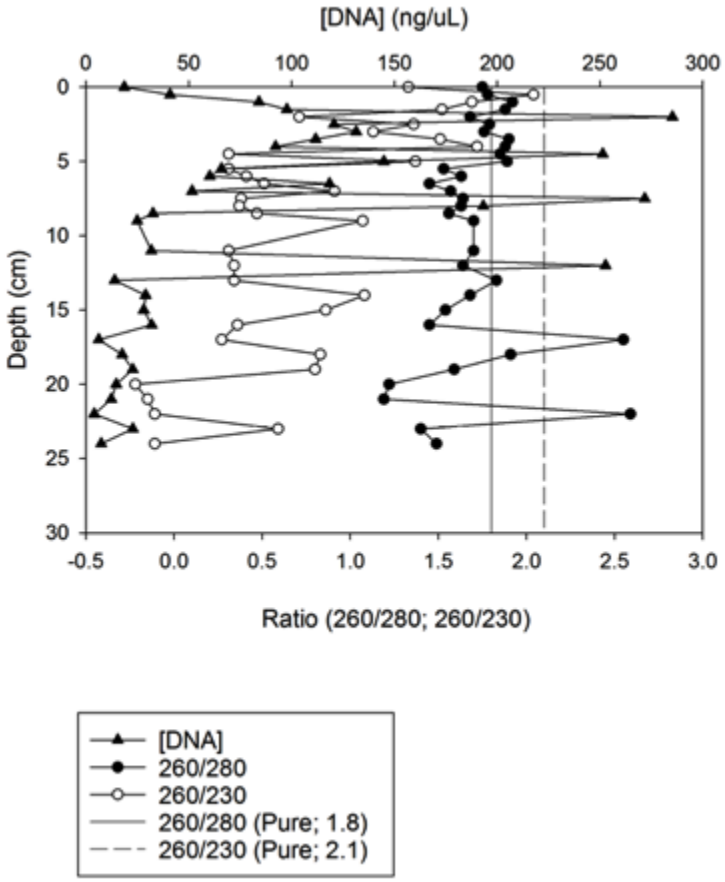

Figure B.1 DNA concentration ([DNA]) and quality (260/280; 260/230 ratio) of the 2013 Great Slave Lake core (GSL 8). Reference lines indicate 260/280 and 260/230 ratios consider "pure" for DNA.

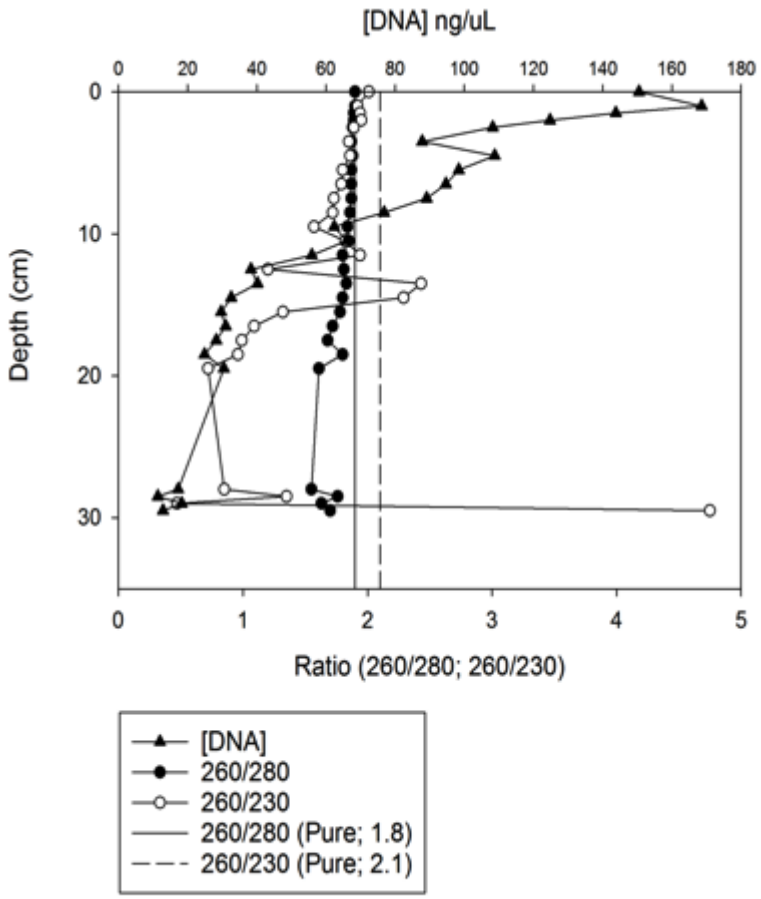

Figure B.2 DNA concentration ([DNA]) and quality (260/280; 260/230 ratio) of the 2015 Madeline Lake core. Reference lines indicate 260/280 and 260/230 ratios consider "pure" for DNA.

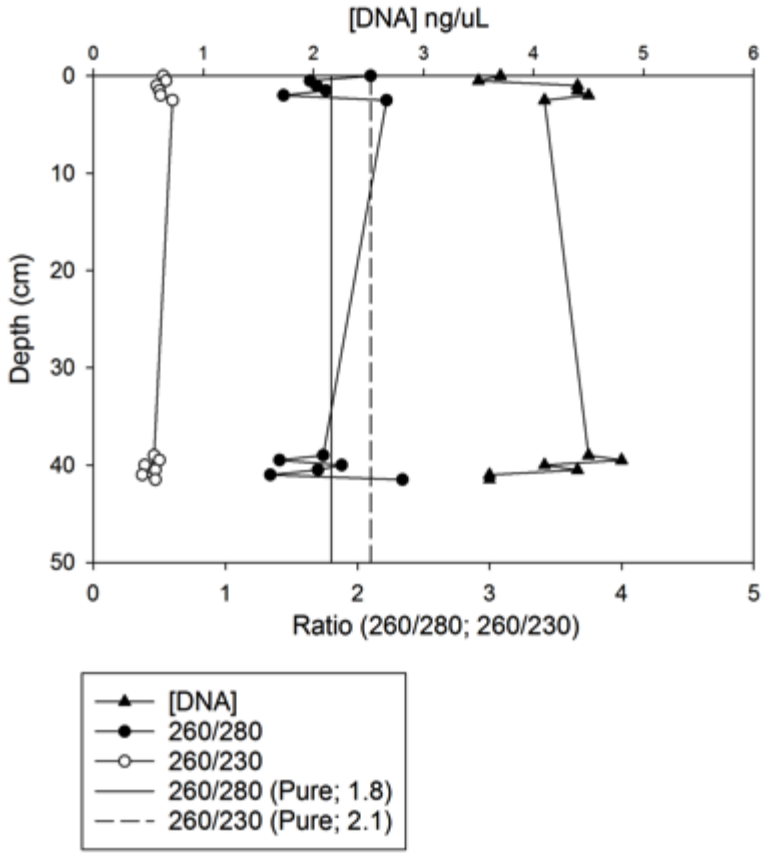

Figure B.3 DNA concentration ([DNA]) and quality (260/280; 260/230 ratio) of the 2015 Pontoon Lake core. Reference lines indicate 260/280 and 260/230 ratios consider "pure" for DNA.

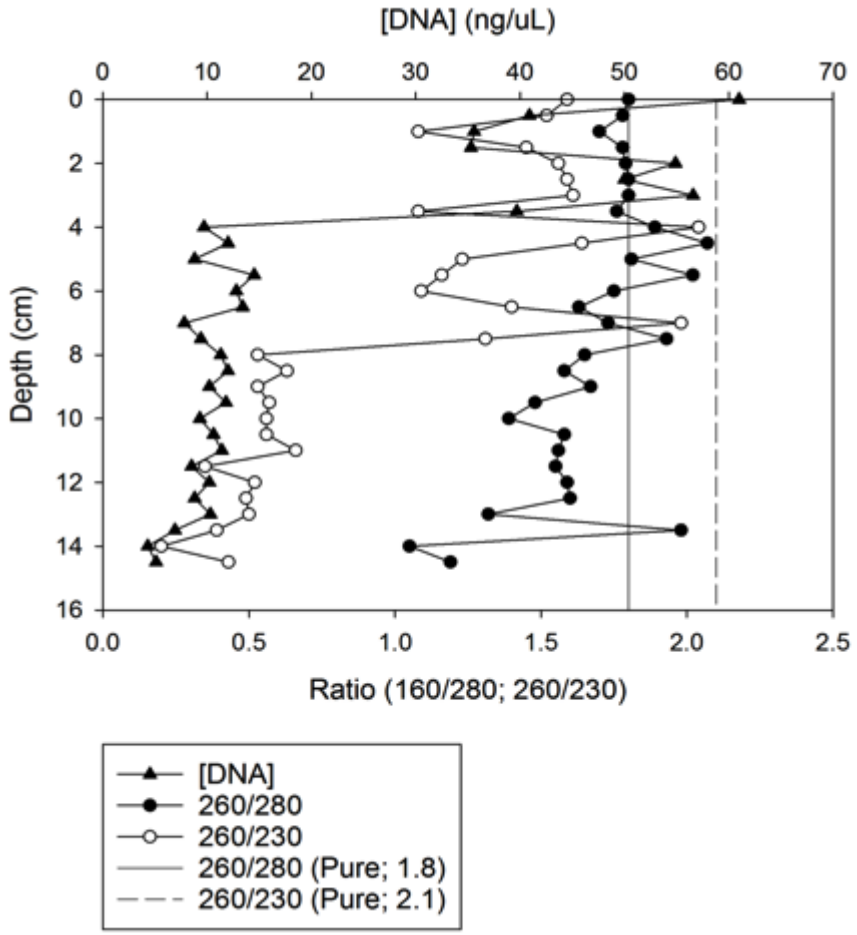

Figure B.4 DNA concentration ([DNA]) and quality (260/280; 260/230 ratio) of the 2014 Meretta Lake core. Reference lines indicate 260/280 and 260/230 ratios consider "pure" for DNA.

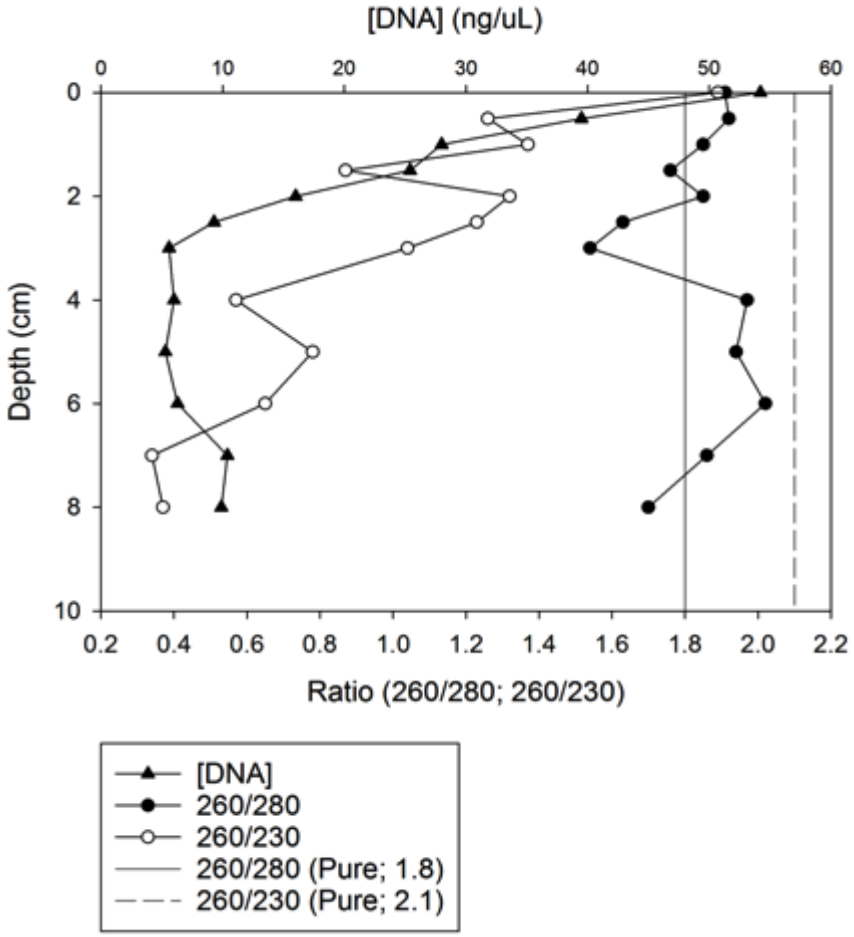

Figure B.5 DNA concentration ([DNA]) and quality (260/280; 260/230 ratio) of the 2014 Crazy Lake core. Reference lines indicate 260/280 and 260/230 ratios consider "pure" for DNA.

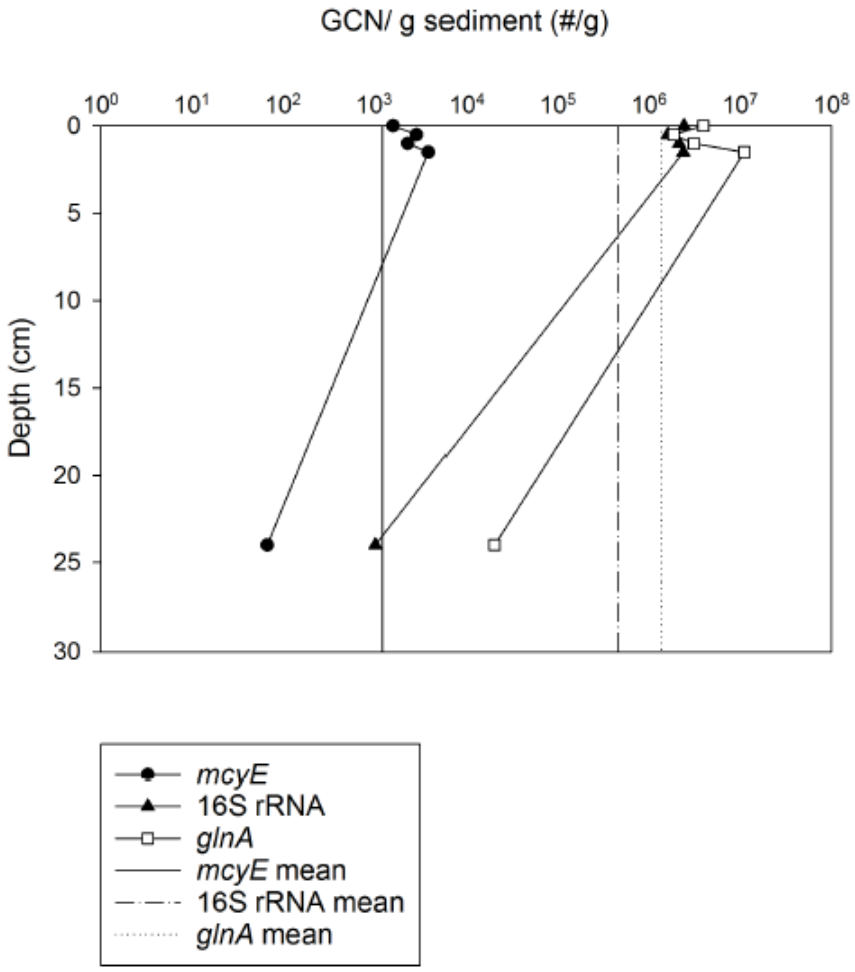

Figure B.6 Depth profile for the Yellowknife Bay, GSL (DE3) core (2014). Note logarithmic scale.

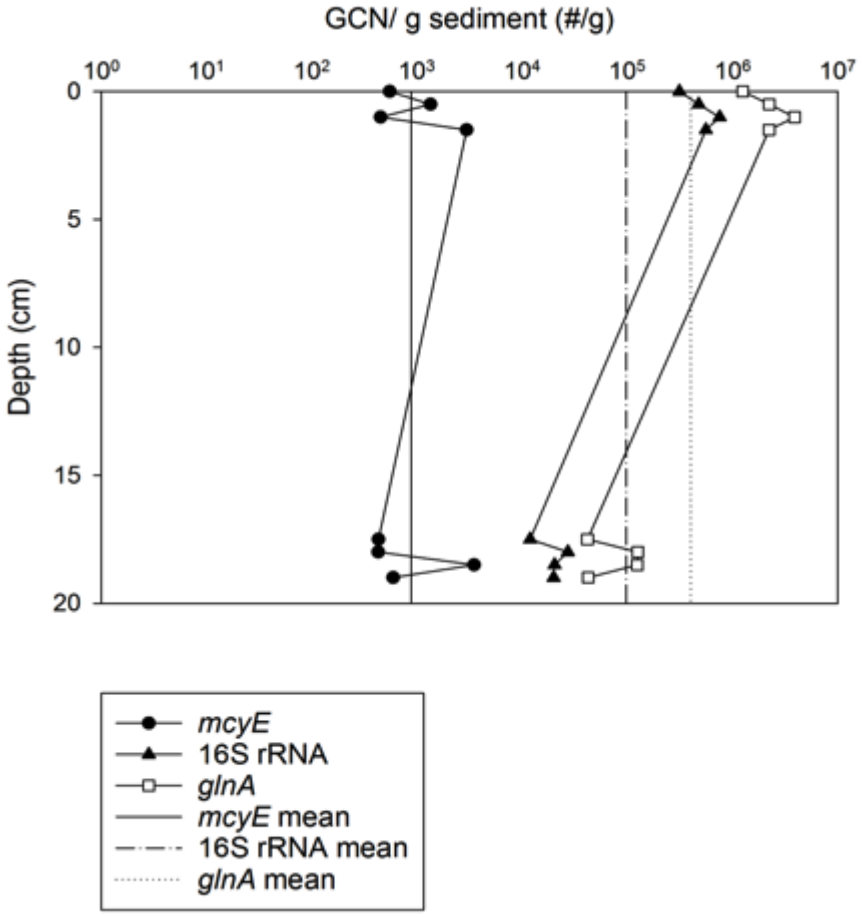

Figure B.7 Depth profile for the Yellowknife Bay, GSL (GSL 15-4) core (2015). Note logarithmic scale.

Table B.2 Pearson Product-Moment correlations (*r*) of molecular data (per gram of wet sediment) in the 2014 Crazy Lake core (d.f.= 4).

	mcyE	**16S RNA**	**glnA**	**DNA**
mcyE	1.00			
16S rRNA	0.793	1.00		
glnA	0.703	0.960*	1.00	
DNA	0.687	0.939*	0.950*	1.00

* p< 0.01

Table B.3 Spearman rank correlations (r_s) of molecular data (per wet gram of sediment) in the 2015 Madeline Lake core. Pearson Product-Moment correlation (*r*) indicated in bold for data that was normally distributed (d.f.= 16)

	mcyE	**16S CYA**	**glnA**	**DNA**
mcyE	1.00			
16S rRNA	0.220	1.00		
glnA	0.0258	0.901 ***	1.00	
DNA	**0.0691**	0.725**	0.702 **	1.00

** p<0.001
***p<0.0001

Table B.4 Pearson Product-Moment correlations (*r*) of molecular data (per wet gram of sediment) in the 2015 Pontoon Lake core (d.f.= 6)

	mcyE	**16S RNA**	**glnA**	**DNA**
mcyE	1.00			
16S rRNA	-0.0332	1.00		
glnA	-0.517	-0.555	1.00	
DNA	-0.166	0.108	0.347	1.0

APPENDIX C: CHAPTER 3 SUPPLEMENTAL MATERIAL

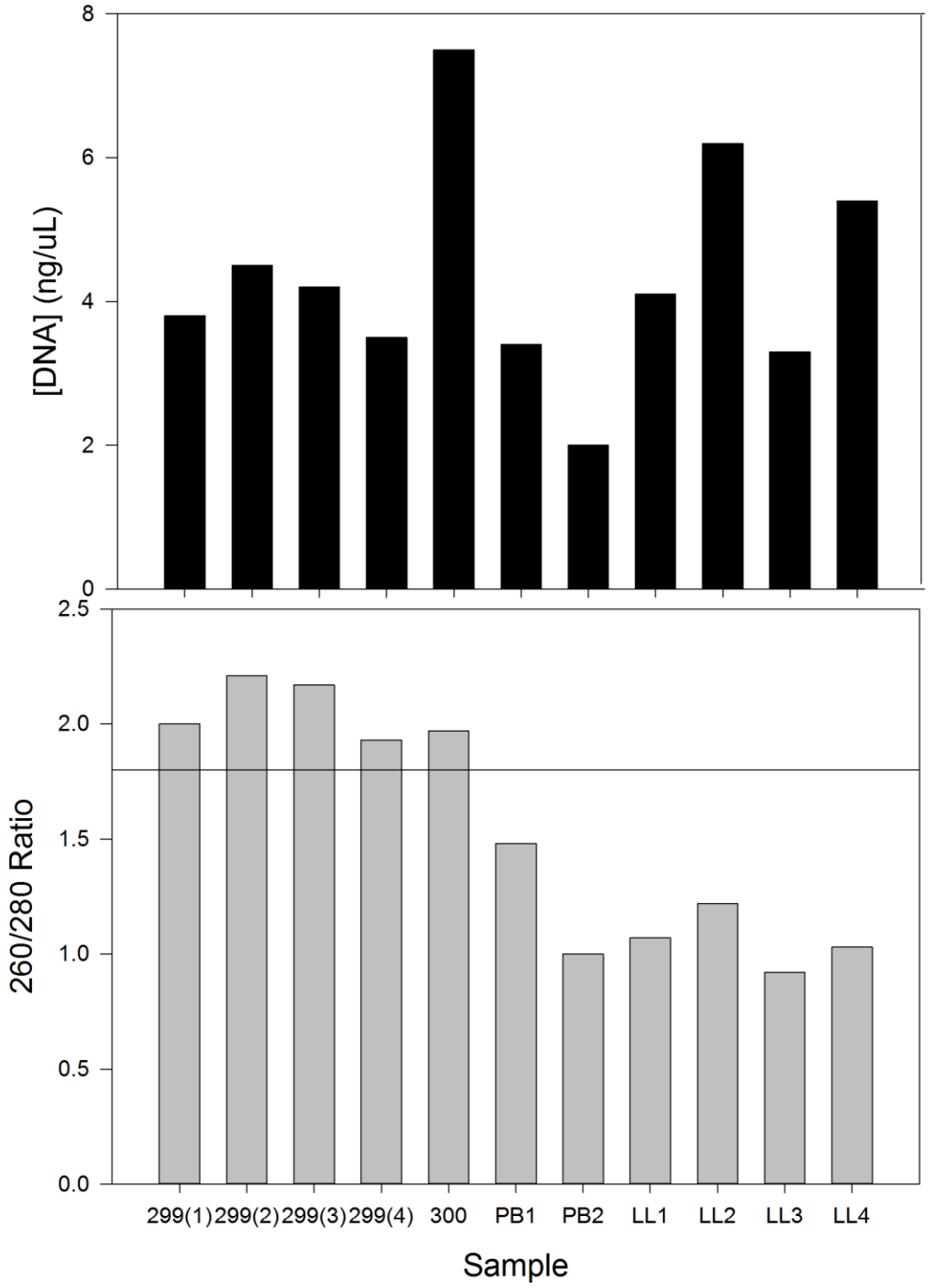

Figure C.1 DNA concentration ([DNA]) and quality (260/280) of single colony *Microcystis* sp. isolates. Horizontal line in lower panel indicates 260/280 ratio for "pure" DNA (~1.8)

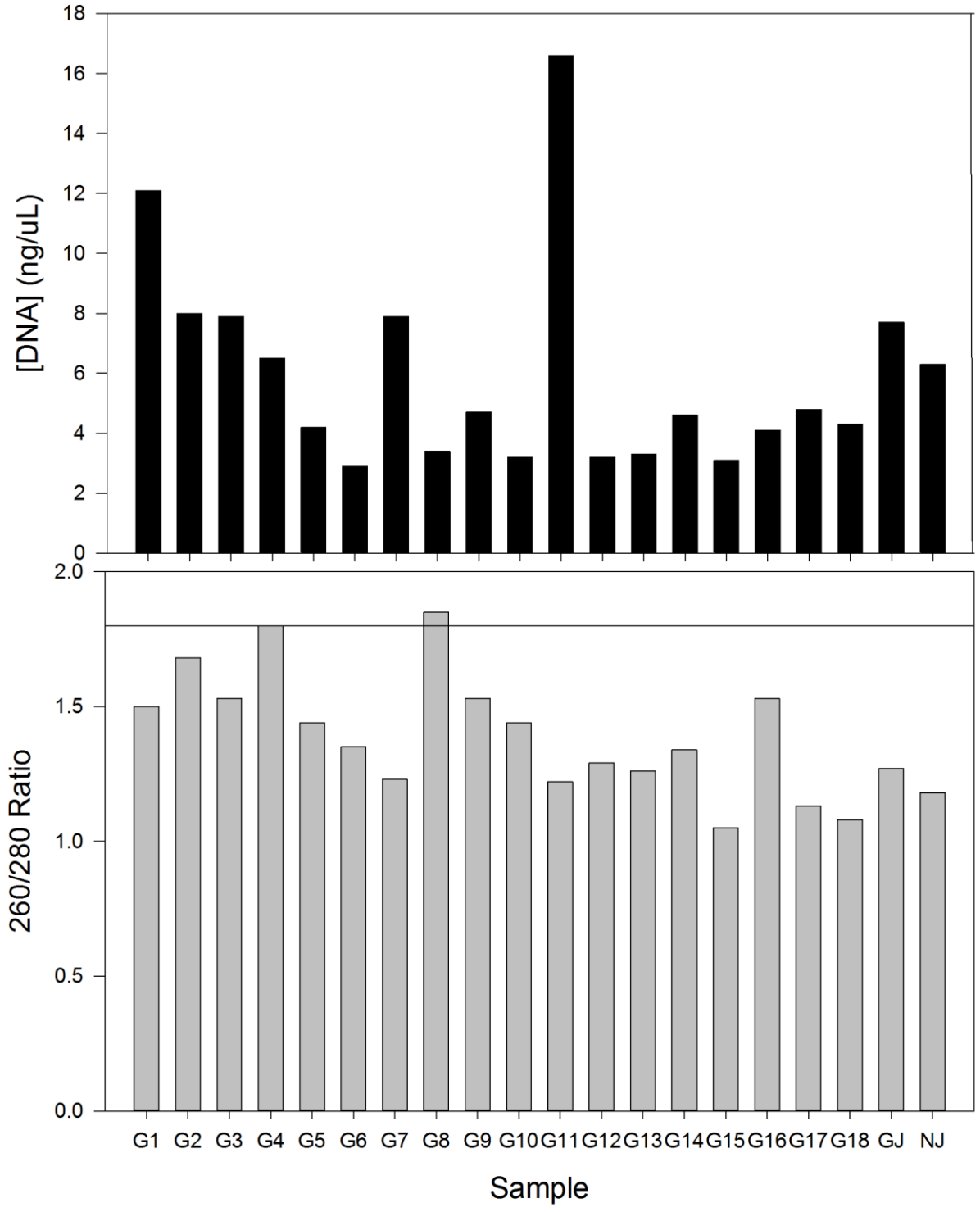

Figure C.2 DNA concentration ([DNA]) and quality (260/280) of single colonies of *Gloeotrichia echinulata and Nostoc* sp. isolates. Horizontal line in lower panel indicates 260/280 ratio for "pure" DNA (~1.8)

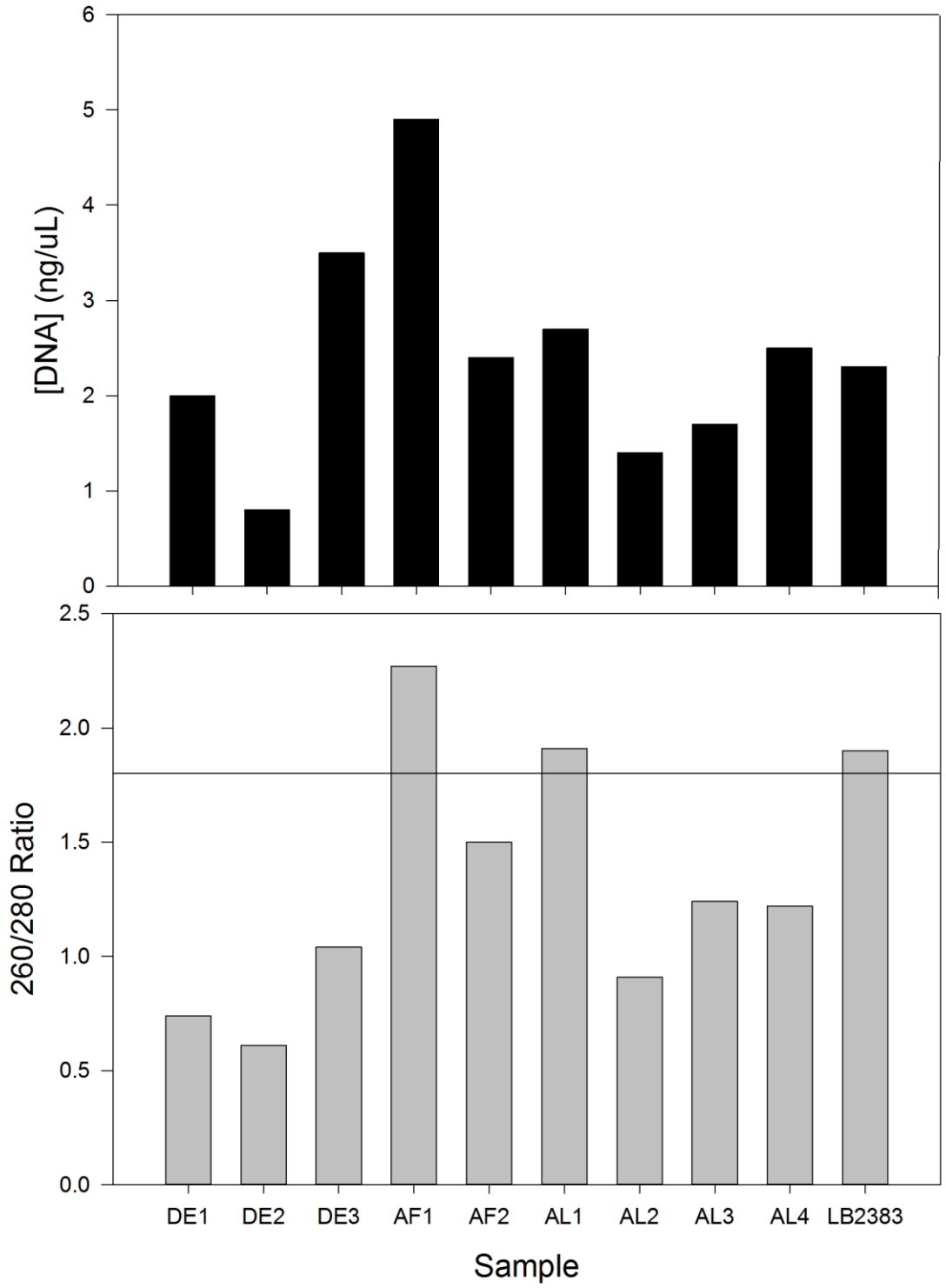

Figure C.3 DNA concentration ([DNA]) and quality (260/280) of single colonies of *Dolichospermum* sp. isolates. Horizontal line in lower panel indicates 260/280 ratio for "pure" DNA (~1.8)

Sequencing results of single colony isolates

LB2383_Oscillatoria_or_Phormidium_16S

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NJ_Nostoc_16S

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G3_Gloeotrichia_16S

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G9_Gloeotrichia_16S

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G2_Gloeotrichia_16S

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G16_Gloeotrichia_16S

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G17_Gloeotrchia_16S

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G15_Gloeotrichia_16S

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G5_Gloeotrichia_16S

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G18_Gloeotrichia_16S

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G12_Gloeotrichia_16S

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G6_Gloeotrichia_16S

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G14_Gloeotrichia_16S

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G8_Gloeotrichia_16S

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G11_Gloeotrichia_16S

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G1_Gloeotrichia_16S

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G4_Gloeotrichia_16S

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GGGAGGCAGCAGTGGGGAATTTTCCGCCATGGA

G7_Gloeotrichia_16S

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CTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC
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DEI1_Dolichospermum_or_Anabaena_16S

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DEI3_Dolichospermum_or_Anabaena_16S

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DEI2_Dolichospermum_or_Anabaena_16S

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AF1_cyano16S

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AF2_cyano_16S

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PB1_cyano_16S

>GGTGAGTAACGCGTGAGAATCTAACTTCAGGACGGGGACAACAGTTGGAAA
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PB2_cyano_16S

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GGAGGCAGCAGTGGGGAATTTTCCGCCATGGA

LL32_Microcystis_16S

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LL34_Microcystis_16S

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2991_Microcystis_16S

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LL33_Microcystis_16S

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LL31_Microcystis_16S

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300CP_Microcystis_16S

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2993_Microcystis_16S

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GGAGGCAGCAGTGGGGAATTTTCCGCCATGGA

2992_Microcystis_16S

>GGTGAGTAACGCGTGAGAATCTAACTTCAGGACGGGGACAACAGTTGGAAA
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TTGCGTCTGATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCGACGATCAGTAGC
TGGTCTGAGAGGATGAGCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTGGGGAATTTTCCGCCATGGA

2994_Microcystis_16S

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GGAGGCAGCAGTGGGGAATTTTCCGCCATGGA

APPENDIX D: ddPCR EFFICIENY WITH *mcyE*, *glnA* and 16S rRNA GENES

To verify the efficiency of droplet digital PCR with our genes of interest and the specific primers used, standard curves for each primer set were run on linearized plasmids of each gene. R^2 values are indicated on each graph

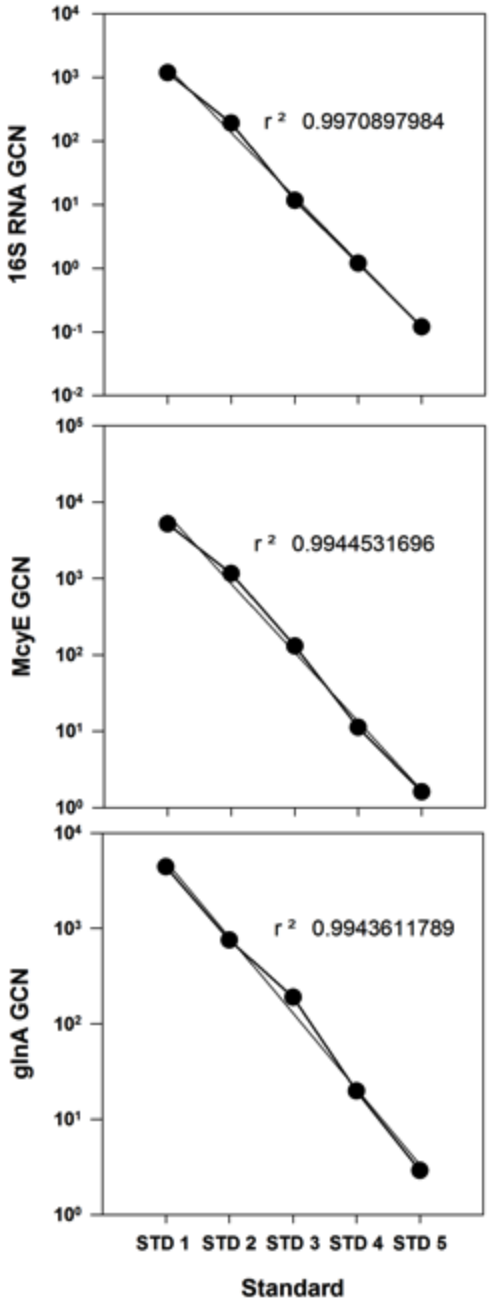

Figure D.1 Standard curves for decreasing concentrations of linearized plasmids of the *mcyE*, 16S rRNA and *glnA*.