PALEOLIMNOLOGICAL RECONSTRUCTION OF LONG-TERM TRENDS IN PHOTOTROPHIC COMMUNITIES IN PRAIRIE RESERVOIRS

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University of Saskatchewan
Saskatoon

by

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ABSTRACT

Anecdotal evidence suggested that Lake Diefenbaker, a large river-valley reservoir in Southern Saskatchewan, Canada, has been experiencing increased pelagic algal bloom frequency and intensity in recent years. This has raised concerns regarding possible deteriorating water quality, including taste and odour issues, and the potential production of harmful cyanobacterial toxins. Due to limited historical environmental monitoring data, paleolimnological investigations of Lake Diefenbaker sediments were conducted to assess spatial and temporal environmental trends. Lines of investigation included trends in organic input and changes within the phototrophic (algal) community, with a primary focus on cyanobacteria and associated toxins. Sediment cores were collected from Lake Diefenbaker (the test case site) and two smaller Prairie lakes, Buffalo Pound Lake, Saskatchewan (eutrophic lake with recurring cyanobacterial blooms) and Ross Lake, Manitoba (historically known to have inputs of raw sewage), both serving as positive reference sites for method development purposes (extraction of environment DNA and fecal sterols, respectively). These sediment cores were sectioned at 1-cm increments and analyses were conducted for the presence of fecal sterols, sedimentary algal pigments, algal biotoxins and preserved environmental DNA. Inference of historical changes in fecal pollution, algal and cyanobacterial community composition and toxin synthesis genes were completed using a variety of analytical methodologies (e.g. high performance liquid chromatography, high-resolution gas chromatography mass spectroscopy, and Q-exactive Orbitrap liquid chromatography mass spectroscopy) and next-generation sequencing techniques.

In Ross Lake, trends in raw and primary treated sewage was reconstructed through the analyses of coprostanol and cholesterol. Concentrations of coprostanol increased from <1 $\mu g\ g^{-1}$ in older sediments, to 252.3 $\mu g\ g^{-1}$ organic carbon at the peak (approximately in the early 1930s). Furthermore, ratios of coprostanol to cholesterol >1, peaking at 3.6 were consistent with anecdotal information that municipal sewage was discharged into Ross Lake during the early years of urbanization, prior to changes in treatment of sewage and discharge practices that began in 1951. These techniques were then applied to Lake Diefenbaker sediment cores to investigate the presence of sewage input.

For Buffalo Pound Lake, high-throughput next-generation sequencing techniques were used to reconstruct and identify the presence of potentially harmful cyanobacteria, including

Microcystis, *Dolichospermum*, and *Planktothrix*. Furthermore, the abundance of the microcystin synethetase *A* gene confirmed the presence of potentially toxic cyanobacteria and that the genetic potential to produce microcystins has been present since reservoir formation. These findings demonstrate a novel means to infer long-term dynamics of the cyanobacterial community in inland waters and highlights the power of paleo-16S-high-throughput sequencing to rapidly identify problematic organisms with high resolution. These techniques were also applied on Lake Diefenbaker sediments.

In Lake Diefenbaker, sedimentary pigment analyses combined with other lines of evidence (e.g. diatom remains and physicochemical parameters) suggested spatial and temporal trends in reservoir ecology. Distinct ecological regions of Lake Diefenbaker were identified, likely due to differences in the morphology and hydrology which exist along the longitudinal axis of this, and other similar river-valley reservoirs. Sediments from up-reservoir locations suggested relatively consistent primary production, nutrient loading and trophic status throughout the temporal coverage of the collected sediment cores. In general, primary productivity increases with distance down-reservoir, until available nutrients are depleted in the mid-reservoir region of Lake Diefenbaker. In addition, sediments from the furthest down-reservoir locations suggest increasing primary production over the last two decades, particularly in the Qu'Appelle arm. Reconstruction of the cyanobacterial community using next-generation sequencing revealed the presence of potentially problematic cyanobacteria (e.g. *Dolichospermum*, *Microcystis* and *Planktothrix*) in the Qu'Appelle and Gardiner arms, taxa capable of producing toxins and taste-and-odour compounds. The continued presence of these nuisance cyanobacterial taxa and the increasing relative abundance of the *mcyA* gene in Lake Diefenbaker suggested that the genetic potential to produce microcystins have been present since formation of the reservoir, and that this potential has varied over time. Altogether, the combination of traditional analytical methodologies and next-generation sequencing techniques represents a novel approach to identify relationships between environmental conditions and cyanobacterial communities in freshwater ecosystems. Further, the results detailed in this thesis identify the practicality of utilizing paleolimnological approaches to reconstruct historical environmental trends in spatially-complex aquatic systems where long-term monitoring data are lacking or absent.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AP	Apatite phosphorus
ATP	Adenosine triphosphate
ATX-a	Anatoxin-a
BLAST(N)	Basic local alignment search tool (nucleotide)
BPL	Buffalo Pound Lake
Chl	Chlorophyll
CIA	Co-inertia analysis
Ct	Threshold cycle
dcSTX	Decarbamoyl saxitoxin
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
EC	Environment Canada
eDNA	Environmental DNA
GC	Gas chromatography
GIWS	Global Institute for Water Security
GLM	Generalized linear model
GTX-2	Gonyautoxin-2
GTX-3	Gonyautoxin-3
HAB	Harmful algal bloom
LDGD	Lake Diefenbaker Gardiner arm
LDQA	Lake Diefenbaker Qu'Appelle arm
MC-LR	Microcystin-LR
MC-RR	Microcystin-RR
mcyA	Microcystin synthetase A gene
MIB	2-methylisoborneol
NAIP	Non-apatite inorganic phosphorus
NCBI	National Center for Biotechnology Information
NeoSTX	Neosaxitoxin

Nod	Nodularin
NRC	National Research Council
OC	Organic carbon
OP	Organic phosphorus
OTU	Operational taxonomic units
Pb-210	Lead-210
PCR	Polymerase chain reaction
PP1	Protein phosphatase 1
PP2A	Protein phosphatase 2a
PPi	pyrophosphate
PRSi	Reactive particulate silica
PSP	Paralytic shellfish poisoning
Ra-226	Radium-226
RDP	Ribosomal Database Project
rRNA	Ribosomal ribonucleic acid
SEPS	Saskatchewan Environment and Public Safety
SIM	Selective ion monitoring
SSI	Sterol source index
STX	Saxitoxin
SWSA	Saskatchewan Water Security Agency
TOC	Total organic carbon
UPGMA	Unweighted pair group method with arithmetic mean
US-EPA	United States - Environment Protection Agency
(U)HPLC	(Ultra) High performance liquid chromatography
UV	Ultraviolet
WHO	World Health Organization

GENERAL NOTE TO READERS

The thesis is written in manuscript style format with certain information, such as materials and methods, appearing in more than one chapter. Chapter 1 is a general introduction, and chapters 2 to 5 are written in the style of publishable manuscripts. Chapter 6 elaborates on the key findings, strengths and limitations of paleo-methods and conclusions observed in this thesis. Chapter 2 of this thesis was published in Chemosphere in May of 2014. Chapter 3 was published in the Journal of Great Lakes Research in September of 2015. Chapter 4 is being prepared for publication, although the journal has not yet been chosen. Finally, Chapter 5 is currently being prepared for submission to the International Society for Microbial Ecology (ISME) Journal.

To avoid redundancies in citation lists, all citations have been provided in Chapter 7. The contents of Chapters 2 and 3 were reprinted here with the permission of Elsevier publishers after minor adaptations to adhere to the thesis guidelines required by the College of Graduate and Postdoctoral Studies, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

CHAPTER 1

GENERAL INTRODUCTION

Chapter 1
General Introduction

1.0. Abstract

Water quality of lakes and reservoirs in some areas of North America and Europe continue to be deteriorating (Cooke et al., 2005; Loftin et al., 2016) and thus have received increased attention since the 1980s (Gulati et al., 2007). Furthermore if nutrient loadings of nitrogen and phosphorus continue to increase on a global scale, this could potentially result in a substantial amount of people being exposed to a high risk of water pollution by 2050 (IFPRI 2015). In particular, cultural eutrophication is causing undesirable changes in aquatic communities (Pretty et al., 2003) due to changes in trophic relationships and nutrient stoichiometry, elevated external nutrient loads, and increased transport of nutrients from benthic to pelagic regions (Schindler 2006). Water quality reduction is especially problematic for potable supplies where costly purification processes must be used to reduce offensive tastes, odours and algal toxins, while minimizing production of hazardous disinfection by-products. Furthermore, harmful algal blooms can also alter water chemistry parameters by altering pH and dissolved oxygen and carbon dioxide content (Verspagen et al., 2014), which can have serious consequences for animal habitat, as evidenced by fish kills due to anoxic conditions (Hallegraeff 1993; Anderson et al., 2002). As many aquatic ecosystems lack sufficient long-term monitoring data (Smol 2010), this study describes the use of paleolimnological techniques in reconstructing the history of community trends in the phototrophic community using a variety of analytical and metagenomic techniques. These techniques can describe the environmental conditions at the time of sediment deposition and can be used to investigate trends subsequent to reservoir formation. Reconstruction of the cyanobacteria community can provide insight on the presence of potentially harmful cyanobacteria and whether its abundance has been increasing during the history of the lake. As such, early identification of harmful cyanobacteria is critical in maintaining good water quality and can help water management agencies implement possible remedies to avoid future problems.

1.1. Background and approach

With a length of approximately 181.6 km, Lake Diefenbaker is the largest multi-purpose reservoir in the Canadian Prairies. Area residents along Lake Diefenbaker have raised concerns regarding an apparent increase in pelagic algal bloom intensity and frequency in the down-reservoir regions in recent years (Figure 1.1). The presence of pelagic algal blooms, including the emergence of problematic cyanobacteria, can result in taste and odour issues, and the presence of toxins can cause adverse health effects in humans and wildlife ingesting or in direct contact with these waters. It is important to, not only identify the presence of potentially harmful cyanobacteria, but also to identify the variables that can influence the growth and abundance of certain cyanobacteria genera over others. Therefore, if the environmental conditions are favorable for cyanobacteria growth and potentially harmful taxa are increasing in abundance then this can contribute to the overall deterioration in water quality of this important reservoir. Unfortunately, long-term monitoring data are limited for this system. Therefore, this paleolimnological study was conducted to reconstruct historical trends in algal and cyanobacterial abundance and community composition through the use of various analytical techniques focused on detecting the remains of biomolecules. Coupling established (phytopigments, sterols) and emerging (next-generation sequencing) technologies together has provided insight into the complex relationships among different environmental paleo-variables and the cyanobacterial community (Chapter 5). Future endeavors utilizing these paleolimnological techniques may help reservoir managers by providing historical information in lieu of detailed long-term monitoring data.

Figure 1.1. Surface algal bloom sampled in September 2011, Lake Diefenbaker, SK, Canada.

1.2. Paleolimnology

Many aquatic ecosystems lack long-term monitoring data (Smol 2010), with environmental studies normally initiated only after an effect has been noticed (Kohler 2010). This often makes it difficult for water management agencies to establish baseline conditions, long-term trends and the effects of multiple stressors on water quality (Schindler 1997). Paleolimnology focuses on the historical reconstruction of aquatic environmental conditions in freshwater ecosystems through the study of materials preserved within the layers of depositional sediments. Paleolimnological techniques have been useful in determining the efficiency of remedial actions on contaminated water bodies, many years after the fact (Smol 1992) and can be used when long-term monitoring data are lacking or absent (Smol 2010). Using paleolimnological techniques, the biological productivity of a water body through time can be examined. This involves the analysis of sediment cores and the identification of physical, chemical, and biological indicators preserved in the profiles (Smol 1992). This study used a suite of these techniques to establish historical environmental trends in different algal primary producers and possible sources of nutrients within Lake Diefenbaker. The various analyses used in this study are described and discussed below.

1.3. Fecal sterols

The presence of fecal wastes in natural waters can contribute to an increase in nitrogen, phosphorus, potassium and recalcitrant and labile organic matter (Nordin 2010) within the water column. In excess amounts, these nutrients can accelerate eutrophication within freshwater lakes. Fecal pollution can be identified and monitored through the analyses of fecal sterols, which are cholesterol derivatives. These sterols are relatively stable in sedimentary environments and are characteristically distributed (e.g. sterol ratios) in different organisms (Huang and Meinschein 1976). Therefore, identification of the presence and relative abundance of fecal biomarkers can shed light regarding the origins of the organic materials entrained in lake sediments. For example, coprostanol, a non-ionic, non-polar, organic molecule, which associates with sediments (Writer et al., 1995), has historically been used as a biomarker to identify fecal contamination originating from higher organisms (e.g. cattle and humans). The synthesis and secretion of this compound is a characteristic of only these higher organism classes.

Many different stanol/sterol ratios have been formulated for determining sources of fecal contamination within aquatic systems. A common stanol/sterol ratio used to investigate fecal contamination is the ratio of the concentration of 5β-coprostanol to that of cholesterol (Grimalt et al., 1990; Latif et al., 2009). In addition, a sterol source index (SSI) corresponding to terrestrially derived sterols has been derived (terrestrially derived sterols to cholesterol ratio; Mudge and Norris 1997) and can be used to provide an estimation of the loading of terrestrially derived sterols into an aquatic system.

1.4. Algal pigments

The trophic status of a lake can be estimated by several factors, one of which is through the analysis of chlorophyll *a* in surface water, the water column or water near the sediment surface. As an example, Alberta Environment and Parks (AEP) has set a guideline for chlorophyll *a* concentrations within the water column, for estimating trophic status (Table 1.1). According to these criteria, Lake Diefenbaker is currently in a state of moderate productivity with chlorophyll *a* concentrations between 2.5 and 8 μg/L in water samples collected in June-October 2008, with increased concentrations occurring later in the summer (Hecker et al., 2012).

The relative concentrations of algal pigments in the water column, such as carotenoids and chlorophylls, are typically used to infer trends in total algal abundance and total phototrophic activity for all photosynthesizing organisms (Reuss et al., 2005). The presence and ratios of various other pigment biomarkers can be used to determine the algal community composition and relative abundances and dominant algal taxa present within aquatic environments. For example, correlations between chlorophyll α and chlorophyll *b* or chlorophyll *c* may be indicative of different classes of algae (Kowalewska 2005). A strong correlation of chlorophyll *a* with chlorophyll *c* is a marker of the presence of diatoms, a strong correlation of chlorophyll *a* with chlorophyll *b* is a marker of green algae, and a weak correlation between chlorophylls *b* and *c* indicates elevated abundance of blue–green algae (Kowalewska 2005). However, many phototrophic organisms produce a wide variety of pigments; thus, due to their taxonomic specificity, various species can be differentiated and identified (Chapter 3) with some limits to this specificity (e.g. cannot distinguish among genera or species).

Pigments produced by photosynthesizing aquatic algal species can undergo sedimentation and become entrained and preserved within aquatic sediments under appropriate conditions

(Sanger 1988). These preserved pigments can be used to reconstruct historical trends in lake primary production, algal succession, invertebrate herbivory and ultraviolet radiation regimes (Wolfe et al., 2006). Previous investigations have shown that the abundance of preserved sedimentary plant pigments may be indicative of lake productivity (Sanger and Gorham 1970). As such, the primary role of pigments in early paleolimnological studies was as a biochemical marker for the former presence of populations of phototropic organisms or for the estimation of historic changes in lake primary production (Leavitt and Hodgson 2001).

Table 1.1. *Chlorophyll a* concentrations as a measure of trophic state **(**Alberta Environment and Parks 2006; modified from Smith et al., 1999).

Chlorophyll a concentration	**Trophic status**
<2.5 μg/L	Oligotrophic (low productivity)
2.5 – 8 μg/L	Mesotrophic (moderate productivity)
8 – 25 μg/L	Eutrophic (high productivity)
>25 μg/L	Hypereutrophic (very high productivity)

1.5. Cyanotoxins

Cyanobacteria are regularly observed in temperate lakes in the Northern Hemisphere generally during the summer and fall months. However, in the tropics and sub-tropics, blooms can last longer. Some cyanobacteria can produce toxins (cyanotoxins), that are hepatotoxic or neurotoxic (e.g. microcystins and saxitoxins) (Krishnamurthy et al., 1986; Shephard et al., 1998). There are many factors that can contribute to the production of toxins during blooms such as: salinity, nutrient concentrations and ratios (e.g. P and N loadings), irradiance and water clarity, temperature, stratification and residence times, among other factors (Berg and Sutula 2015). Ingestion of these toxins have resulted in death of livestock (Dillenberg and Dehnel 1960), wildlife (Krishnamurthy et al., 1986) and humans (Behm 2003). In addition, the proliferation of these harmful cyanobacteria can also result in taste and odour problems for consumers of the water and can also result in dermatological problems (e.g. skin rashes) (Vranješ and Jovanović

2011; US-EPA 2015), gastroenteritis and liver and kidney damage (US-EPA 2015). Some common cyanotoxins investigated in this study are described below.

1.5.1. Microcystins

The increasing frequency of cyanobacterial blooms around the world has become a concern, especially in waters used for human consumption. Microcystins are one of the most common cyanobacterial toxins found in freshwater systems. Over 230 different congeners of microcystins have been identified as of 2016 (T. Davis, National Oceanic and Atmospheric Administration – Great Lakes Environmental Research Laboratory, 2016, personal communication). The different variants of microcystins are identified by variations in their amino acid 'sequences'. Although structural variations can arise in all seven amino acids, the most frequently encountered microcystin variants are characterized according to methylation pattern, and the two variable L-amino acids located at positions 2 and 4 of its structure (McElhiney and Lawton 2005). According to Health Canada, microcystin-LR (MC-LR) is one of the more common microcystins found in water supplies around the world (Health Canada 2002, http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/cyanobacter-eng.php). The World Health Organization (WHO) has set a 1 μg/L exposure guideline for microcystin, based on MC-LR, in drinking water (WHO 1998). In addition, Health Canada has recently adopted a drinking water maximum acceptable concentration of 1.5 μg total microcystins/L. The LD_{50} for MC-LR in rats has been determined to be 5 mg/kg (oral) (Fawell et al., 1994; WHO 1999) and 43 μg/kg (Gupta et al., 2003).

Microcystin-LR, the primary microcystin congener that will be focused on in this study, and other microcystin variants are hepatotoxins (Figure 1.2). Its mechanism of toxicity is the inhibition of protein phosphatase type 1 and type 2a (PP1 and PP2A) activities in the cytoplasm of liver cells (MacKintosh et al., 1990). This inhibition results in an increase in phosphorylation of proteins in liver cells, which can result in rapid cell death, as well as the activation of transcriptional factors involved with tumor promoting activity.

1.5.2. Saxitoxins

Saxitoxins (STXs) (Figure 1.2) are potent inhibitory neurotoxins that induce flaccid paralysis, a lethal condition known as Paralytic Shellfish Poisoning (PSP) (Faber 2012). STXs block the voltage-gated sodium channels of nerve cells thereby preventing depolarization of the membranes and inhibiting impulse generation in the nerves. This results in relaxed state paralysis (Kao 1972; Kao 1983; ICPS 1984; Chorus and Bartram 1999; Faber 2012). The WHO has been unable to gather sufficient data to derive a guideline value for cyanobacterial toxins other than microcystin-LR. However, for areas harvesting shellfish, a guideline of 80 μg STX equivalents per 100-g shellfish has been implemented and is currently used in North America and much of the rest of the world (ICPS 1984; and Sivonen and Jones 1999).

There are currently 57 analogs of STX known (Figure 1.2), which are divided into several classes such as non-sulfated (e.g. saxitoxin, neosaxitoxin), mono-sulfated (e.g. gonyautoxin 2/3), di-sulfated, decarbamoylated (e.g. decarbamoyl-saxitoxin) and hydrophobic analogs (e.g. *p*-hydroxybenozoate analogues of gonyautoxin 2 and 3) (Wiese et al., 2010; Negri et al., 2003). These saxitoxin analogs share a common chemical structure (Figure 1.2) and are identifiable through the binding of different moieties to R-groups. Relative toxicities of saxitoxin and its analogs have been investigated using a standardized mouse bioassay (Table 1.2) (Usleber et al., 1997). To put these into perspective, the LD_{50} of STX in mice has been calculated to be 2.4 μg/kg (intravenous), 10 μg/kg (intraperitoneal) and 263 μg/kg (oral) (Johnson et al., 2009). For comparison, the oral LD_{50} of STX for humans is as low as 5.7 μg/kg and the toxin can also enter the body via open wounds, by which route it has been suggested that 50 μg per person would be a lethal dose (Rapala et al., 2005).

Table 1.2. Relative toxicity of paralytic shellfish toxins in relation to saxitoxin (from Usleber et al., 1997).

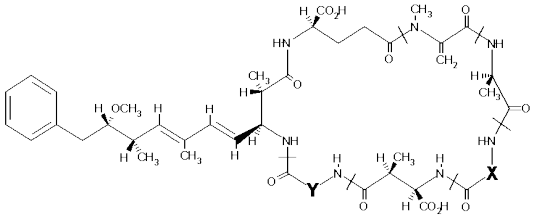
Toxin	**Relative toxicity**
Saxitoxin	1
Neosaxitoxin	0.5 - 1.1
Gonyautoxin 2/3	0.8 / 0.33 - 0.9 / 0.9
Decarbamoyl-saxitoxin	0.43

Saxitoxin and its analogs originate from a variety of cyanobacteria including *Phormidium* (Borges et al., 2015) and *Dolichospermum circinalis* (Wiese et al., 2010), formerly *Anabaena circinalis* (Wacklin et al., 2009), which was documented as the dominant cyanobacterial species present in Lake Diefenbaker in 2011 (Hecker et al., 2012).

1.5.3. Anatoxin-a

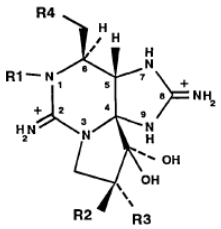
Anatoxin-a (ATX-a) (Figure 1.2), also known as Very Fast Death Factor, is another type of neurotoxin produced by cyanobacteria including *Dolichospermum* (*Anabaena*), *Oscillatoria*, and *Aphanizomenon* (Sivonen and Jones 1999). Ingestion of ATX-a results in acute neurotoxicity (Puschner et al., 2008). In addition, intraperitoneal injection of ATX-a in rats results in convulsions, paralysis and ultimately death within 2-7 minutes (Fitzgeorge et al., 1994; NCEA 2006). ATX-a acts as an analogue of acetylcholine through interaction with the nicotinic acetylcholine receptor. When bound to the receptor, it does not undergo degradation through the activity of cholinesterase; this results in permanent stimulation of muscle cells leading to paralysis. The LD_{50} of ATX-a in mice was determined to be >5 mg/kg (oral) (Stevens and Krieger 1991; Fitzgeorge et al., 1994;), 210 to 375 µg/kg (intraperitoneal) (Stevens and Krieger 1991; Fitzgeorge et al., 1994), 2mg/kg (intranasal instillation) (Fitzgeorge et al., 1994) and < 100 µg/kg (Astrachan et al., 1980). Detection and accurate quantification of ATX-a in water sources can be challenging due to the rapid degradation of ATX-a in the water column upon exposure to environmental UV light (e.g. sunlight). The half-life for photochemical breakdown is 1-2 hours (Sivonen and Jones 1999) Furthermore, degradation of these metabolites can be accelerated by alkaline conditions (Stevens and Krieger 1991; Matsunaga et al., 1989).

A)



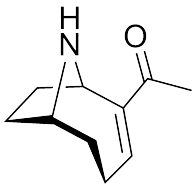
Toxin	**X-group**	**Y-group**
Microcystin-LR (MC-LR)	Leucine	Arginine
Microcystin-RR (MC-RR)	Arginine	Arginine

B)



Toxin	**R1**	**R2**	**R3**	**R4**
Saxitoxin (STX)	H	H	H	$OCONH_2$
Neosaxitoxin (neoSTX)	OH	H	H	$OCONH_2$
Gonyautoxin-2 (GTX-2)	H	H	OSO_3^-	$OCONH_2$
Gonyautoxin-3 (GTX-3)	H	OSO_3^-	H	$OCONH_2$
Decarbamoyl-saxitoxin (dc-STX)	H	H	H	OH

C)



Toxin
Anatoxin-a

Figure 1.2. General chemical structures of cyanotoxins: (A) microcystins, (B) saxitoxins and (C) anatoxin-a.

1.6. DNA preserved in lake sediments

DNA from algae, including cyanobacteria, can be preserved for long periods of time, up to thousands of years, in lake sediments (Domaizon et al., 2013; Boere et al., 2011; Möller and Jansson 1997). DNA analyses and gene sequencing of DNA from different microorganisms preserved in the sediment can provide taxonomic identification or information for ecological inference, thereby producing an archive of limnological history (Savichtcheva et al., 2011). Fossil DNA has been successfully employed to study the succession of species as a result of environmental changes (Coolen and Gibson 2009) in terrestrial ecosystems (Willerslev et al., 2007), marine sediments (Boere et al., 2009; Coolen et al., 2007; Coolen et al., 2009; Coolen and Overmann 2007; Manske et al., 2008) and lacustrine sediments (Coolen et al., 2004; Coolen et al., 2008; Coolen and Overmann 1998; D'Andrea et al., 2006; Epp et al., 2009).

DNA from prokaryotic and eukaryotic cells can be protected against nuclease degradation by its adsorption on soil colloids, sand particles (Pietramellara et al., 2009), minerals and organic matrices (Savichtcheva et al., 2011). Environmental DNA (eDNA) is fairly persistent in the aquatic environment and can be deposited and preserved in lake sediment (Domaizon et al., 2013). However, degradation of DNA to shorter fragments is possible, within the first several thousands of years after deposition, despite the existence of excellent preservation conditions. Therefore, only short base-pair fragments (<500 bp) have been suggested to be available for molecular methods and analyses (Coolen and Gibson 2009). Analysis of this genetic material can be used to infer recent or past site occupancy by aquatic organisms (Turner et al., 2015), information useful for water management practices in monitoring for invasive organisms and the presence for nuisance cyanobacteria (discussed in this thesis).

Modern molecular techniques, including polymerase chain reaction (PCR) have made it possible to obtain information on microbial community composition directly, without cultivation of target organisms. Real-time PCR has many advantages in monitoring phytoplankton activity in aquatic sediment. These include: high sensitivity, high specificity, potentially high throughput, and applicability to preserved environmental samples (Antonella and Luca 2012). Thus, an environmental sample can be catalogued for taxa present by direct nucleic acid isolation, amplification via PCR and gene sequencing analyses. The most widely used marker gene for taxonomic analysis is the small subunit rRNA gene (16S rDNA), and the recent application of

molecular techniques in a variety of habitats has produced a large set of sequences for this gene (Zwart et al., 2002). The 16S rRNA gene is a sequence of DNA that encodes the RNA component of the smaller ribosome subunit in all bacteria. Ribosomes are essential in all living organisms as they are responsible for the synthesis of proteins that are needed in some organelles of bacteria and eukaryotes. Sequences of 16S rRNA from many organisms have revealed that some portions of the molecule (e.g. hypervariable regions) undergo rapid genetic changes, thereby distinguishing between different species within the same genus (Yang et al., 2016). Conserved regions of this gene sequence allow for higher-level taxonomy and allow for primer development to target a broad group of microorganisms (e.g. Cyanobacteria). Therefore, sequencing of the 16S rRNA region can provide taxonomic information regarding cyanobacteria community composition, as is detailed herein. These techniques can also be applied to other genes such as the 23S rRNA gene, which encodes the larger 50S ribosomal subunit in bacteria (further discussed in Chapter 4). The 23S gene can also be found in cyanobacteria and plastids; therefore, some eukaryotic algae can be identified using this primer. However, 23S rRNA genes typically have higher sequence variations including insertions and deletions than the 16S rRNA gene (Pei et al., 2009).

Recently developed techniques for the large scale sequencing of DNA are known as "next-generation sequencing". The Illumina technology that was used in this thesis is based on the "sequencing-by-synthesis" principle. This method is more rapid than the Sanger method. It works through the implementation of sequencing primers that hybridize to the adapter sequences (i.e. barcodes) of a single stranded oligonucleotide (sequence of interest). With each cycle, fluorescently-tagged nucleotides compete for addition to the growing nucleotide chain during DNA extension. After the addition of each nucleotide the clusters are excited by a light source and a characteristic fluorescent signal is captured on camera. Each fluorescently-tagged nucleotide emits at a different wavelength or colour, thus allowing sequencing during DNA synthesis of the complementary strand. After each cycle, the fluorescent dye is cleaved from the nucleotide and washed away. This technique was applied to analysis of eDNA in Lake Diefenbaker sediment core increments to catalogue cyanobacterial species composition, as well as to identify the presence of toxin synthesis genes (e.g. *mcy* genes).

The use of high-throughput next-generation sequencing techniques for targeted sequencing has enabled researchers to investigate phylogenies among different organisms. Prior

to sequence alignment (e.g. taxonomically classifying the sequencing data), sequences are normally clustered into "Operational Taxonomic Units" (OTUs) dictated by percent sequence similarity (typically assessed at 97% similarity) (Nguyen et al., 2016) implying that from each OTU a single sequence is selected as a representative. Therefore, depending on the sequence similarity threshold, some bias can occur (e.g. two very similar species or strains may be reported as the same taxa) (Nguyen et al., 2016). A higher sequence similarity threshold will result in a lower taxonomic rank, whereas a lower sequence similarity threshold will result in a higher taxonomic rank. These sequences are then aligned against a reference database, such as SILVA, Greengenes or the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) to give these OTUs a taxonomic identity or rank. This is very useful in identifying taxa and performing biological community assessments using sequencing data, as will be demonstrated in Chapters 4 and 5, where these techniques were applied to reveal the cyanobacteria community composition and identify potentially nuissance genera.

To better understand the cyanobacterial community structure and investigate whether potentially harmful cyanobacteria have emerged in Lake Diefenbaker, preserved DNA from collected sediment cores was analyzed in this study. These analyses can also provide insight into the presence and relative abundances of cyanobacterial toxin-producing genes. Although some studies have shown that some cyanotoxins (e.g. microcystins) can be preserved in lake sediment (Zastepa et al., 2016), they can also degrade under aerobic conditions and even under anoxic conditions in the presence of nitrates (Holst et al., 2003). Furthermore, some cyanotoxins are photosensitive and can degrade before they reach the sediment (eg. ATX-a). Since it was possible that some of these toxins were not detectable in Lake Diefenbaker sediments, sedimentary DNA linked to toxin production was investigated. The presence of these toxin-producing genes can provide a measure of the potential to produce toxins. The development of these paleo-metagenomic techniques represents a new and novel tool for paleolimnological investigations.

1.7. Site description and sampling locations

1.7.1. Lake Diefenbaker

This study focused primarily on the river-valley reservoir, Lake Diefenbaker (Figure 1.3) in Southern Saskatchewan, but also made use of other lake systems as positive reference sites for method development purposes: Ross Lake, Manitoba (Figure 1.3), historically known to have inputs of raw sewage (discussed in Chapter 2), and Buffalo Pound Lake, Saskatchewan, which has frequent cyanobacterial blooms (Figure 1.3) (Chapter 4).

Narrow-river valley reservoirs tend to be spatially-variable, demonstrating different hydrological regimes within the reservoir. For example, up-reservoir locations are typically more turbid and experience faster flow-rates before a transitional zone where flow-rates decrease, the basin deepens, and turbidity decreases. At the terminal end in down-reservoir regions, the hydrology becomes more lake-like (e.g. deep basin, increased light penetration and slow flow-rates) (see review by Thornton et al., 1990). Lake Diefenbaker is an example of a narrow-river valley reservoir; therefore, it has distinct hydrological zones that can influence nutrient gradients and regimes, and which can have further impact on the phototrophic communities. Collection of sediment cores, along the longitudinal axis of this lake, captured these different hydrological zones which influence the algal community composition.

Lake Diefenbaker is the largest multi-purpose reservoir in the Canadian Prairies, running approximately 181.6 km. It was formed by the damming of the South Saskatchewan River and officially opened in 1967. The reservoir is controlled by two main dams, the Qu'Appelle River Dam and the Gardiner Dam, which control water release to the Qu'Appelle River and the South Saskatchewan River, respectively. Presently, Lake Diefenbaker provides water for the irrigation of nearby arid land, water for livestock, wildlife and municipal drinking water (~45% of Saskatchewan residents), and it is used for hydro electric operations. Furthermore, it is used for trout aquaculture and recreational activities.

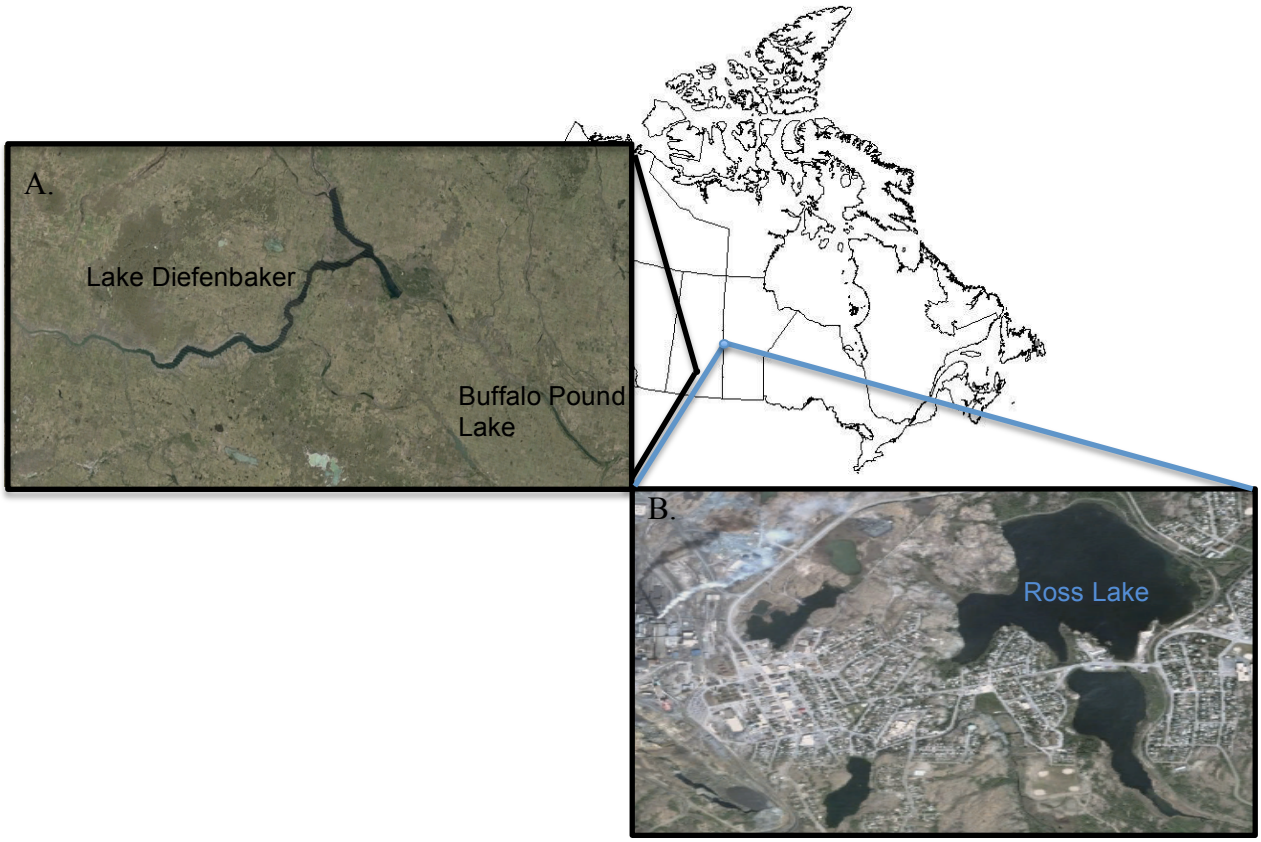


Figure 1.3. Map of Canada showing the study areas: Lake Diefenbaker and Buffalo Pound Lake (A), SK, Canada, and Ross Lake (B), MB, Canada.

Lake Diefenbaker was constructed to provide a wide range of uses including electricity production, flood control, irrigation, industrial and recreational uses and to provide drinking water for animals and people. The Saskatchewan Water Security Agency (SWSA) states that Lake Diefenbaker supplies water for 10 potash mines, four major irrigation projects, various industries, wildlife, the Wild West Steelhead Fish Farm® (Canada's largest freshwater fish farm), and provides domestic water for approximately 45 per cent of Saskatchewan residents (SWSA 2012).

The occurrence of periodic algal blooms (Figure 1.1) in the down-reservoir arms of the lake has raised public concerns regarding the water quality of Lake Diefenbaker. A study conducted in 2011, concluded that increased phosphorus loads in recent years have been directly related to increased cyanobacterial productivity and that the predominant cyanobacterial species

identified in Lake Diefenbaker was *Anabaena circinalis* (Hecker et al., 2012). To investigate whether cyanobacterial abundance has been increasing since the creation of the reservoir and whether sewage pollution may be contributing to increased algal abundance, pigment and sterol profiles were analyzed. In addition, toxins were analyzed to determine if concentrations pose risks of adverse effects for humans and the environment.

1.7.2. Ross Lake

Ross Lake (Figure 1.3) is a small, relatively shallow lake comprised of north (550,000 m^2) and south (150,000 m^2) basins having maximum depths of about 7.0 m and 2.3 m, respectively (Beck 1995; Evans 2000). Ross Lake lies within the City of Flin Flon (Manitoba, Canada), a mining community originally formed by the Hudson Bay Mining and Smelting Company (now Hudbay Minerals Inc.) in 1927. Flin Flon was incorporated as a municipality in 1933, and as a city in 1970. The construction (late 1920s) and operation of Hudbay resulted in a rapid influx of people with the local population increasing from a few hundred to several thousand residents in the 1930's. During the first two decades of urban development, raw and minimally-treated sewage were discharged into the north basin of Ross Lake (Evans 2000). This practice likely ended in 1951 with the construction of a sewage treatment facility at the southern end of Ross Lake, which discharges effluent into the mouth of Ross Creek. Effects of both industrial and municipal activities on the ecology of Ross Lake were unmonitored for the first four decades of operation. Biophysical data were not collected from Ross Lake until 1973 (e.g. Rowley 1975; Van Loon and Beamish 1977; Sergy and Fallis 1978), when it was revealed that Ross Lake was a highly degraded environment. This study site was used as a means to develop methodologies for the extraction and analyses of fecal and phytosterols prior to their application on sediment cores collected from Lake Diefenbaker.

1.7.3. Buffalo Pound Lake

Buffalo Pound Lake (Figure 1.3) is a shallow natural water body, created 10 000 years ago from the glacial spillways that formed the Qu'Appelle Valley (SERM 2001). The outflow of the original shallow lake was dammed in 1952 (Hall et al., 1999), resulting in the conversion of this water body to a reservoir. Buffalo Pound Lake is approximately 29 km long, with a width of 1 km (Buffalo Pound Water Treatment Plant 2015), and a volume of approximately 91,987 cubic

decametres (SWSA 2016). The majority of the water supply is from the Qu'Appelle arm of Lake Diefenbaker, a reservoir located approximately 60 km to the north-west. Buffalo Pound Lake has a maximum depth of 5.6 m and mean depth of 3.0 m (Hammer 1971) and is regulated to supply water to 25% of Saskatchewan's population, including the cities of Regina and Moose Jaw and several small communities (SWSA 2016; McGowan et al., 2005). This lake is characterized by regular blooms of planktonic cyanobacteria during the summer, and deepwater anoxia is common during winter and late summer (McGowan et al., 2005) and has been characterized with the onset of cyanotoxins in the past (Sharpe 2016; Roegner et al., 2013; H. Peng, unpublished data, University of Saskatchewan, 2015). Because this reservoir is often suffers from regular blooms of planktonic cyanobacteria (McGowan et al., 2005), this lake was used as a means to develop biomolecular methodologies for the extraction of environmental DNA and the targeted sequencing approach for the reconstruction of the cyanobacteria community prior to its application on sediment cores collected from Lake Diefenbaker.

1.8. Overview of thesis

Prior to the initiation of this research, anecdotal observations from local residents suggested a recent increase in frequency and intensity of algal blooms occurring in Lake Diefenbaker. This raised concerns regarding the potential impacts that these blooms could have on water quality of the lake. Increased bloom formation or increased production of algae in general could result in the production of biotoxins causing adverse health effects in humans and wildlife. The lack of long-term monitoring data has raised concerns regarding the past and recent trends of primary producers in the lake. If there is a continuing increase in algal and cyanobacterial abundance, this can result in taste and odour issues and cyanotoxin production leading to deteriorating quality of water. Therefore, paleolimnology techniques were applied to reconstruct trends in the phototrophic community. Using paleolimnological techniques involving organic biomarkers (e.g. fecal sterols) and sedimentary phytopigments (e.g. chlorophylls and carotenoids), profile analyses were used to infer historical trends and to help identify potential sources and sites of organic matter and nutrient loadings to Lake Diefenbaker. It was unknown whether toxin-producing cyanobacteria were increasing in Lake Diefenbaker. Preserved eDNA was subjected to gene sequencing to more definitively identify cyanobacterial community

structure and to assess the presence and trends in abundance of toxin-producing genes that may have resulted from past algal bloom activity.

1.9. Study objectives and hypothesis

The overall goal of this study was to reconstruct and interpret water quality trends, since reservoir formation, particularly with respect to algal and cyanobacteria community composition, toxin production and fecal contamination. As such, the aim of this investigation was to answer the following questions:

1) Have sterol concentrations or ratios in Lake Diefenbaker changed over time since the formation of the reservoir? A sediment core collected from Ross Lake, Manitoba was used as a positive reference site for method development purposes, and therefore comparisons between these two sites were not conducted.
    - H_{01} = There are no significant differences among fecal sterol concentrations through incremental sections of sediment core samples collected from Ross Lake, MB, Canada, indicating no temporal changes to anthropogenic contaminant contributions through sewage discharge to Ross Lake.
    - H_{03} = There are no significant differences among fecal sterol concentrations through incremental sections of sediment core samples collected from Lake Diefenbaker, SK, Canada, indicating no temporal changes to anthropogenic contributions of fecal material to Lake Diefenbaker.
2) Has the composition or biomass of the algal community changed over time since the formation of Lake Diefenbaker?
    - H_0 = There are no significant differences in algal and cyanobacterial phytopigment concentrations, or their rates of deposition through various increments in sediment core samples, thereby indicating no change to historical and modern-day algal assemblages or algal production.
3) Have cyanotoxins been produced in Lake Diefenbaker and has their production changed over time since the formation of the reservoir? If cyanotoxins cannot be detected in the sediment profile, are toxin-producing genes present and has there been a change to their abundance in Lake Diefenbaker over time?

- H_{01} = Preserved algal biotoxins show no significant differences through various increments in sediment core samples, thereby indicating no change in historical and modern-day algal biotoxin production.
- H_{02} = There is no increase/change in the abundance of cyanobacterial genes responsible for the production of biotoxins throughout the length of sediment cores collected from the Qu'Appelle and Gardiner arms of Lake Diefenbaker.

4) Have potentially harmful cyanobacterial genera emerged and has the cyanobacterial community composition changed over time in Buffalo Pound Lake, Saskatchewan and Lake Diefenbaker? A sediment core collected from Buffalo Pound Lake was used as a positive reference site for method development purposes. This lake is known to occasionally experience blooms containing toxin-producing algae (Kehoe et al., 2015).
    - H_{01} = There is no change in the abundance of individual cyanobacterial genera (e.g. composition of the cyanobacterial community) throughout the length of a sediment core collected from Buffalo Pound Lake, as inferred from percent relative abundance of operational taxonomic units (OTUs).
    - H_{02} = There is no change in the abundance of individual cyanobacterial genera (e.g. composition of the cyanobacterial community) throughout the length of sediment cores collected from the Qu'Appelle and Gardiner arms of Lake Diefenbaker, as inferred from percent relative abundance of operational taxonomic units (OTUs).

CHAPTER 2

Reconstructing long-term trends in municipal sewage discharge into a small lake in northern Manitoba, Canada

This chapter is based on the article published as Tse, T. J., Codling, G., Jones, P. D., Thoms, K., Liber, K., Giesy, J. P., Wheater, H., and Doig, L. E. 2014. Reconstructing long-term trends in municipal sewage discharge into a small lake in north Manitoba, Canada. Chemosphere. 103: 299–305.

NOTE TO THE READERS – CHAPTER 2

The proper citation for Chapter 2 is Tse, T. J., Codling, G., Jones, P. D., Thoms, K., Giesy, J. P., Wheater, H., and Doig, L. E. 2014. Reconstructing long-terms in municipal sewage discharge into a small lake in northern Manitoba, Canada. Chemosphere. 103: 299–305.

The author contributions to Chapter 2 were as follows:

- Timothy Tse (University of Saskatchewan) collected and processed all sediment samples (with assistance from Lorne Doig), performed all analytical methodologies, analyzed sample data, and drafted the manuscript.
- Ken Thoms (University of Saskatchewan) provided analytical support and helped develop the analytical methodology to run samples.
- Gary Codling (University of Saskatchewan), Paul Jones (University of Saskatchewan) and Lorne Doig (University of Saskatchewan) provided guidance in extraction method development and reviewed and revised the manuscript.
- John Giesy (University of Saskatchewan) and Karsten Liber (University of Saskatchewan) provided laboratory facilities to extract and process samples and reviewed and revised the manuscript.
- Howard Wheater (University of Saskatchewan) provided funding to Paul Jones and John Giesy to conduct the research and reviewed and revised the manuscript.

Chapter 2
Reconstructing long-term trends in municipal sewage discharge into a small lake in northern Manitoba, Canada

2.0. Preface

This chapter discusses the methodology used to successfully extract sterols and stanols for a sediment core collected from Ross Lake, Manitoba (positive reference site). Analyses of these compounds investigated the historical trend in fecal pollution within Ross Lake, Manitoba. The sterol source index was also investigated to identify contributions from terrestrial-based plants. Successful extraction and analytical methodologies, detailed herein, were then applied to sediment cores collected from Lake Diefenbaker, Saskatchewan (principle study site) for a similar analysis (discussed in Chapter 5).

2.1. Abstract

Ross Lake lies within the City of Flin Flon (Manitoba, Canada), a mining community originally formed by the Hudson Bay Mining and Smelting Company (now Hudbay Minerals Inc.) in 1927. At the time of this investigation, a continuous effluent stream from Hudbay Minerals (approximately 80 years) and a discontinuous and unknown amount of raw and minimally treated municipal sewage (>20 years, likely ending in 1951) was discharged into the north basin of the lake. Maximum concentrations of fecal sterols, such as coprostanol and terrestrial phytosterols, such as: β-sitosterol, campesterol, stigmastanol were measured in vertical sections of sediment cores, collected from Ross Lake, in the 15-16-cm section, which likely corresponds to 1930's. Concentrations of coprostanol increased from <1 μg g^{-1} in older sediments, to 252.3 μg g^{-1} organic carbon at the peak (approximately in the early 1930s). Observed changes in concentrations of sterols, in combination with radiometric dating and changes to sediment physicochemical characteristics, support the conclusion that sediments of a depth of less than 17.5-cm were deposited during the post-industrial era from approximately 1930 onwards. Ratios of coprostanol to cholesterol >1, peaking at 3.6 were consistent with anecdotal information that municipal sewage was discharged into Ross Lake during the early years of urbanization, prior to changes in treatment of sewage and discharge practices that began in 1951. Finally, historical concentrations of terrestrial phytosterols followed trends similar to

those of coprostanol and cholesterol and may possibly be the result of an increase in the flux of terrestrial organic matter into Ross Lake as the result of regional deforestation due to logging and fire.

2.2. Introduction

Sediments can act as sinks for organic and inorganic chemicals. Concentrations of these chemicals in vertical sediment profiles can provide understanding of historical natural processes and anthropogenic activities within a catchment. Thus, the sedimentary record can help to reconstruct the history of environmental changes associated with industrial, agricultural and urban development.

Sterols are a subgroup of steroids found naturally in animals, plants and fungi. These compounds can find their way into surface waters and sediments. Because of their long-term stability under a wide range of environmental and anaerobic conditions, sterols are ideal biomarkers for use in monitoring the conditions of lakes and their catchments (Huang and Meinschein 1976; Yde et al., 1982; Dureth et al., 1986; Norfariza et al., 2010).

For animals, the majority of sterols measured in the environment are from fecal matter. The fecal sterols present and the ratios of particular sterols are characteristic of different taxa and modes of feeding. For example, the sterol profile present in avian fecal matter is different from that in mammalian fecal matter. A simple ratio of coprostanol/cholesterol can be used to identify sources of fecal matter, such as livestock, versus municipal sewage (Grimalt and Albaiges 1990).

Coprostanol is frequently used as an indicator of municipal sewage (Humrawali et al., 2010) but can also be used to identify organic matter originating from other higher mammalian taxa (e.g. livestock) (Yde et al., 1982; Bayona and Albaiges 2006). However, anaerobic processes *in situ* can also produce coprostanol in sediments through anaerobic hydrogenation or from the reduction of cholesterol by microbial activity (Fattore et al., 1996). Thus, greater concentrations of coprostanol alone do not indicate the presence of contamination originating from municipal effluents or agricultural sources. Instead, a more robust assessment can be conducted by investigating ratios of sterols (Table 2.2). When investigating pollution related to municipal activities, the coprostanol/cholesterol ratio is commonly used as an indicator of municipal sewage. A ratio ≥ 0.2 is indicative of fecal matter from either sewage or biogenic (animal) contamination (Grimalt and Albaiges 1990). However, a ratio <1 is often considered to

be indicative of biogenic sources and a ratio >1 is indicative of municipal sewage source (Leeming et al., 1996; Patton and Reeves 1999; Humrawali et al., 2010). This technique was used to assess whether sewage/fecal matter contamination was present and to identify potential sources of contamination.

More than 40 different phytosterols have been identified (Tikkanen 2005). There are distinct sterols associated with either terrestrial or aquatic plants, and with either higher (vascular) or lower (e.g. mosses, liverworts and lichens) plants (Saliot et al., 2002). Previous studies have attributed the origin of the majority of C_{29}-sterols, such as stigmastanol and β-sitosterol, in freshwater lacustrine sediments to terrestrial-plants (Nishimura 1978; Ishiwatari et al., 2005**;** Hanisch et al., 2009; Matsumoto et al., 1982). Thus, temporal changes in patterns of vegetation can be identified through changes in the relative proportions of phytosterols within sediments of lakes.

The City of Flin Flon, which surrounds Ross Lake, was established in 1927 by the Hudson Bay Mining and Smelting Corporation, now Hudbay Minerals (Hudbay). Limited information exists regarding wastewater treatment and discharge for both Flin Flon and Hudbay during the early years of industrialization and urbanization. The goal of this experiment was to reconstruct the historical trends of sewage contamination in Ross Lake through changes in relative concentrations of sterols in the vertical profile of depositional sediments in the lake. This, in turn, provided complementary information for a companion study investigating temporal changes in the ecology of Ross Lake (Doig et al., 2013), where the temporal dating of sediment sections using radiometric techniques (e.g. the unsupported activity of Pb210) was less than definitive. In general, sterol concentrations and ratios preserved in sediment cores were used to attribute historical industrial activities and municipal wastewater management practices to specific periods of time. It was hypothesized that concentrations of various sterols and, in particular, the coprostanol/cholesterol ratio would be greater in sediments deposited after the onset of urbanization and lesser in those sediments deposited after a major change in waste water management, which occurred in 1951.

2.3. Methods

2.3.1. Study site

Ross Lake (54°46´N, 101°52´W) (Figure 2.1) is a small, relatively shallow lake comprised of north (550,000 m^2) and south (150,000 m^2) basins having maximum depths of about 7.0 m and 2.3 m, respectively (Beck 1995; Evans 2000). Geographically, the surrounding area is characterized by thin soils, outcrops of granite, and Quaternary and Holocene deposits including till, glaciolacustrine sediments and peatlands (Henderson and McMartin 1995). The main vascular terrestrial plants in the area include jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), white spruce (*Picea glauca*), trembling aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*) (Hogan and Wotton 1984).

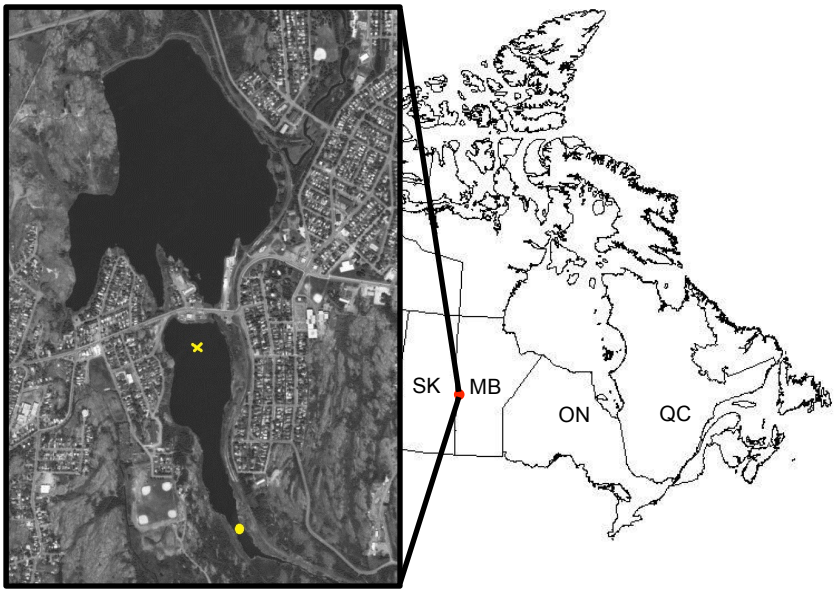


Figure 2.1. Map of Ross Lake, Manitoba, Canada. The X indicates the site location where the sediment core was collected. The O indicates the location of the sewage treatment plant discharge for the City of Flin Flon.

Flin Flon was incorporated as a municipality in 1933, and as a city in 1970. The construction (late 1920s) and operation of Hudbay resulted in a rapid influx of people with the local population increasing from a few hundred to several thousand residents in the 1930's. During the first two decades of urban development, raw and minimally-treated sewage were

discharged into the north basin of Ross Lake (Evans 2000). This practice likely ended in 1951 with the construction of a sewage treatment facility at the southern end of Ross Lake, which discharges effluent into the mouth of Ross Creek and flows south into Schist Lake. Effects of both industrial and municipal activities on the ecology of Ross Lake were unmonitored for the first four decades of operation. Biophysical data were not collected from Ross Lake until 1973 (e.g. Rowley 1975; Van Loon and Beamish 1977; Sergy and Fallis 1978), when it was revealed that Ross Lake was a highly degraded environment.

2.3.2. Sample collection and handling

A sediment core was collected from a single site in the deepest portion of the south basin of Ross Lake by use of a Wildco® Hand Core Sediment Sampler (Wildlife Supply Company, Yulee, FL, USA) fitted with an acrylic core tube (pre-cleaned, 5-cm diameter) on August 29, 2009**.** This sampling location was chosen to avoid difficultues associated with potentially sampling through thick layers of sludge (in the north basin), and avoid potentially confounding effects of disturbance to sediment stratigraphy caused by ice, scour, wave action or propeller wash (Doig et al., 2015). Upon collection of sediment, the core tube, including overlying water, was sealed and transported in a cooler with freezer packs to the Toxicology Centre, University of Saskatchewan, Saskatoon and stored at 4 °C in the dark. Prior to sectioning of the core, water overlying the core was siphoned off and a wooden plunger inserted into the base of the coring tube. The plunger was raised at 1-cm increments, extruding sediment out of the top of the core (under a stream of nitrogen gas). Each 1-cm section was collected using a plastic spatula and placed into a 20-mL glass scintillation vial. Samples were immediately mixed using a plastic rod and the vial was capped under a nitrogen atmosphere. The vials were then covered with Parafilm® and aluminum foil and stored at 4 °C, in the dark, prior to extraction and analysis.

2.3.3. Standards

Sterol standards (Table 2.1) were characteristic of a range of common plant and animal taxa. The internal standard was 5α-cholestan-3β-ol. The recovery standard 17-β-estradiol-2-4-16-16-d_4 (CAS number 66789-03-5) was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). Acetone (HPLC Grade) and methanol (Certified ACS), were both obtained from Fisher Scientific (Ottawa, Ontario, Canada).

Table 2.1. Ion masses of sterol compounds identified. Standards of all compounds were obtained from Sigma Aldrich, St. Louis, MO, USA.

Compound	**Ion (m/z)**	**Retention time (min)**	**[1]Limit of quantification (ng/ml)**	**[2]Limit of quantification for extracts (ng/g dry weight)**
Coprostanol-TMS	370.3600	7.42	3.50	0.61 to 1.80
Cholesterol-TMS	368.3443	7.88	9.27	0.23 to 0.68
Campesterol-TMS	382.3600	8.48	2.10	0.14 to 0.41
β-sitosterol-TMS	396.3756	9.02	8.47	0.55 to 1.65
Stigmastanol-TMS	383.3678	9.12	5.59	0.37 to 1.09
5α-cholestan-3β-ol*	370.3600	7.95	-	
17β-estradiol-d_4-TMS**	420.2818	5.86	-	

* Internal Standard

** Recovery Standard

[1]Limit of instrumental quantification in relation to standard curves

[2]Limit of quantification for extracts (e.g. how much sample is required to reach limit of instrumental quantification). Obtained through comparison of individual sterols in samples in relation to the limit of instrumental quantification.

2.3.4. Extraction and cleanup

An aliquot of each 1-cm sediment section (~5 g ± 0.5 g wet weight) was lyophilized for 72 hr in the dark at -80°C. Sediments were then sonicated in a solvent mixture (acetone:methanol 85:15 v/v) at room temperature for 3 min, followed by centrifugation at 2000 x *g* for 10 min before the supernatant was collected. The procedure was repeated twice. Each sample was concentrated under nitrogen gas and the chemicals of interest were separated from potentially interfering chemicals using a multilayer silica column consisting of 2 g prebaked silica and a small layer of anhydrous sodium sulphate in a 9-mm ID column. The column was conditioned with 30 mL each of acetone and methanol before use. The sample extracts were then passed through the column and concentrated by rotary evaporation to 1 ml. Removal of sulfur was accomplished by US-EPA Method 3660B (U.S. Environmental Protection Agency, 1996) in which pre-cleaned, copper fines are added to the extract.

Constituents of extracts were saponified by adding of 5% methanolic KOH (w/w), then sonicating for 3 hr at room temperature and taken to dryness under a gentle stream of nitrogen gas. To remove KOH, water and chloroform were introduced at a 1:5 ratio (v/v) and the chloroform fraction was collected. Extracts were then filtered using a Whatman 0.2 µm GD/X disposable Nylon syringe filter (Whatman 25 mm diameter; Maidstone, Kent, United Kingdom) and taken to dryness under N_2 gas and derivatized by use of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 60°C for 1 hr. BSTFA was removed under N_2-gas and the samples were redissolved in 100 µL of hexane containing 1 µg ml^{-1} of 5α-cholestan-3β-ol internal standard. The samples were then stored at -80 °C until analysis.

2.3.5. GC-MS instrumental analyses

Gas chromatographic analyses were performed by use of a Carlo-Erba 8060 GC (Thermo Instruments, USA) equipped with a 30-m DB-5 MS (250 µm x 0.25 µm film thickness) fused silica capillary column. The GC was operated at 225 °C and 1 µl of sample was manually injected. The initial oven temperature was 225 °C. After 1 min the oven temperature was increased to 325 °C at a rate of 20 °C per min and held for 8 min. The carrier gas was helium at a flow rate of 1 ml min^{-1}. A VG70SE series Mass Spectrometer (VG analytical Inc. Ltd, Manchester, UK) was used with a source temperature of 200 °C and a resolution of 5000. The ion masses used are provided (Table 2.1). Individual sterols were identified and quantified by use

of Masslynx® version 4.1 software.

As some bacteria can produce 5α-Cholestan-3β-ol from cholesterol, the data were normalized using blanks and standards that were spiked with 1 μg ml^{-1} of this sterol, which were analyzed with extracts every five samples. Concentrations of all the sterols were consistently above detection limits (Table 2.1). The sterol concentrations were converted to μg g^{-1} organic carbon by incorporating their respective dry weights and multiplying it by their organic carbon content (obtained from Doig et al., 2015)

The primary questions answered were i) was there an initial spike in sterol concentrations that could be used to indicate the sediment section corresponding to the onset of urbanization (i.e. approximately 1930) and ii) was this later followed by a significant decrease in sterol concentrations, indicating sediments deposited post-construction of the wastewater treatment plant (i.e. post-1951)? Data were assessed for normality using the Shapiro-Wilk test for normality and necessary comparisons were made using the non-parametric Mann-Whitney U test (SigmaPlot® 11.0) to identify statistical differences in fecal trends among the sediment increments.

2.3.6. Radiometric dating

Radiometric dating was performed through the analysis of Pb-210, Ra-226 and Cs-137 and is further discussed in Doig et al., (2015).

2.4. Results and discussion

All sterols analyzed followed a similar trend throughout the sediment core (Figures 2.2 and 2.3; each point represents a single analysis), with maximum concentrations occurring in the 15-16-cm section. Based on radiometric dating data and changes in physico-chemical characteristics of the sediments (Doig et al., 2015), the increases in sterol concentrations were consistent with both industrial development and urbanization that occurred in the vicinity of Ross Lake just prior to 1930. Simultaneous peaks in concentration for those sterols investigated suggested that their sources may be linked, i.e. they may all have derived from the same source or processes occurring during the same period.

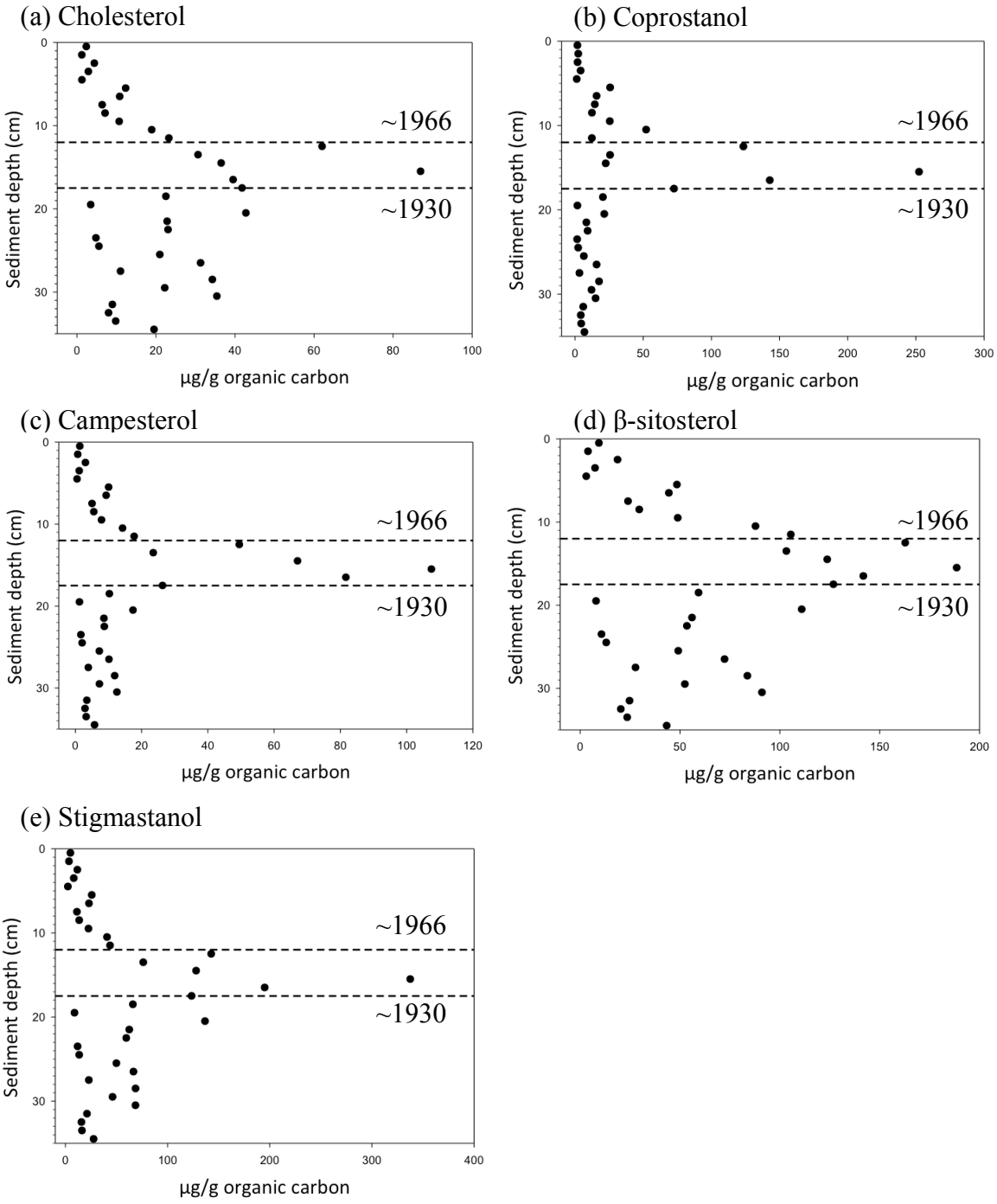


Figure 2.2. Sterol profiles: cholesterol (a), coprostanol (b), campesterol (c), β-sitosterol (d), stigmastanol (e), concentrations in a 35-cm sediment core collected from the south basin of Ross Lake in 2009.

2.4.1. Fecal sterols

Concentrations of coprostanol and cholesterol increased in the central portion of the sediment core, beginning at approximately 1930 (Figure 2.2) with maximum concentrations (normalized to organic carbon) of 252.3 μg coprostanol g^{-1} OC and 87.0 μg cholesterol g^{-1} OC occurring in the 15-16-cm section. Given that 10 μg g^{-1} dry weight of coprostanol in freshwater sediment has been reported as a strong sewage signature (Mudge and Bebianno, 1996), the early post-industrial sediments of the south basin of Ross Lake were clearly contaminated with human waste, because concentrations of coprostanol reached a maximum of 22.0 μg g^{-1} dry weight. The coprostanol/cholesterol ratio (3.61; Figure 2.3), which corresponds to peak concentrations of sterols also strongly suggests substantial loading of municipal sewage into Ross Lake during this time period. The 17-18 cm section likely represents the approximate boundary between pre- and post-industrial activities in the Ross Lake basin (approximately 1930; Doig et al., 2015). Practices of discharge of municipal effluent presumably changed in 1951 when a new wastewater treatment facility was constructed at the outflow of Ross Lake. Elevated concentrations of coprostanol and cholesterol were also observed, throughout the pre-industrial sediments within the collected core (34-35-cm deep up core to 19-20 cm). The corresponding coprostanol/cholesterol ratio consistently exceeded 0.2, which indicates that the south basin of Ross Lake received greater inputs of sterols from non-municipal, biogenic sources during the pre-industrial period (Fattore et al., 1996).

Concentrations of coprostanol and cholesterol in more recent sediments (12-13-cm and above) significantly declined compared to the mid-core (p-value < 0.005 and < 0.001 for coprostanol and cholesterol, respectively (Mann Whitney U Test). In the upper layers of the sediment core (4-5-cm and above), coprostanol and cholesterol concentrations were significantly less than those concentrations in pre-industrial sediments (p-value = 0.008 for both coprostanol and cholesterol). This suggests that changes to wastewater management (most notably in 1951) substantially reduced sewage input into Ross Lake and that inputs of biogenic matter, presently, are reduced compared to pre-industrial times. During sewage treatment, coprostanol can be converted to epi-coprostanol through microbial action (McCalley et al., 1981), and thus would result in a lower coprostanol signature. Although total concentrations of coprostanol and cholesterol in the more recent post-industrial sediments (12-13-cm section and up) are low compared to preindustrial concentrations, the coprostanol/cholesterol ratio (>1) indicates that at

least some portion of the organic matter in Ross Lake sediments in the south basin continues to originate from municipal sewage sources.

Table 2.2. Various sterol and stanol ratios cited in peer-reviewed literature

Sterol and stanol ratios	Value and indication	Reference
(5β-stanol + epi-5β-stanol) : (5β-stanol + epi-5β-stanol + 5α-stanol)	> 0.7 = fecal pollution <0.3 = non-contaminated sediments	Grimalt et al., 1990
Coprostanol : 5β-stigmastanol	1.5 to 5.5 = humans and pigs 0.25 = ruminants	Baeten et al., 2012
(3β, 5β)-stanol : (3α, 5β)-stanol	< 1 = Evidence for composting of fecal matter	Baeten et al., 2012; McCalley et al., 2981
Coprostanol : cholesterol	≥ 0.2 indicates sewage pollution < 1 indicates biogenic sources > 1 indicates sewage source	Leeming et al., 1996; Patton and Reeves 1999; Humrawali et al., 2009
Individual phytosterol : cholesterol	Sterol Source Index indicates proportion of sterols contributed from land plants	Ali et al., 2009

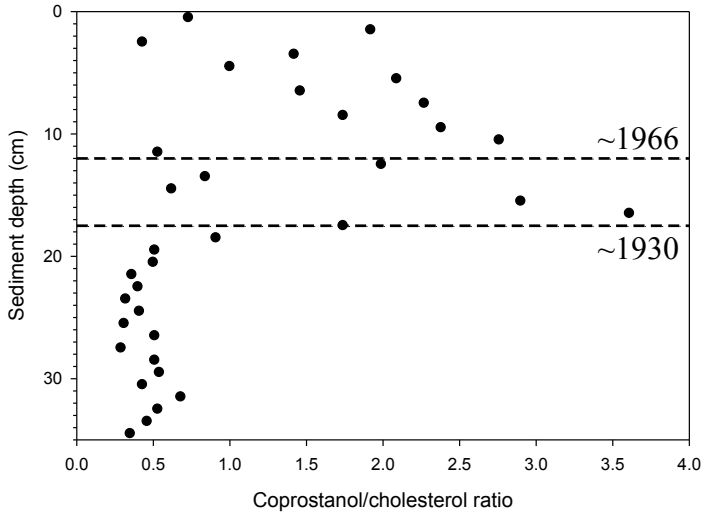


Figure 2.3. Ratio of coprostanol/cholesterol for a 35-cm sediment core collected from the south basin of Ross Lake in 2009.

2.4.2. Phytosterols

Historical concentrations of the phytosterols investigated exhibited trends similar to those of coprostanol and cholesterol (Figure 2.3). Phytosterols are steroid compounds similar to cholesterol that occur in plants; thus, the presence of various phytosterols provides insight into other potential sources of organic matter. Since it is difficult to identify the source of organic matter from a single sterol, a suite of sterol ratios was instead used to assess the potential sources of organic matter in Ross Lake sediments. The sterol source index (SSI) (SSI = terrestrially derived sterol/cholesterol; Figure 2.4) was used (Grimalt and Albaiges 1990; Masni and Mudge 2005) to assess terrestrial organic input into the aquatic environment of Ross Lake. The phytosterols investigated, which included campesterol, β-sitosterol and stigmastanol have traditionally been used to investigate terrestrial vascular plant input into an aquatic environment. However, these sterols have also been reported to be present in some algae and phytoplankton species (Volkman 1986; Mudge and Norris 1997). Increasing SSIs, in this study, may correlate to increased terrestrial organic material in Ross Lake from vascular plants. However, the presence of phytoplankton might have contributed to these increasing contributions.

Overall, the greater SSIs (Figure 2.4) for the three terrestrial sterols investigated in the post-industrial era suggested a concurrent increase in the relative contribution of terrestrial plant

material into Ross Lake during this period, but more than one interpretation is possible. Increasing stigmastanol concentrations might indicate increased herbivore fecal input into the aquatic system; as stigmastanol is the biohydrogenation product of sitosterol and stigmasterol within ruminants (Fahrenfeld 2008; Vane et al., 2010; Rogge et al., 2005). Based on photographs taken in the past, horses were routinely used in the early days of Flin Flon and might account for the increase in stigmastanol concentrations in the post-industrial sediments. Nevertheless, stigmastanol can also by produced by freshwater algae when aquatic systems are stressed by reducing conditions and the resulting low dissolved oxygen concentrations (Fahrenfeld 2008). The spike in the SSI for stigmastanol during the early 1930's might indicate that the character of Ross Lake fundamentally changed to a reducing environment, possibly the result of discharged mining and municipal sewage effluents.

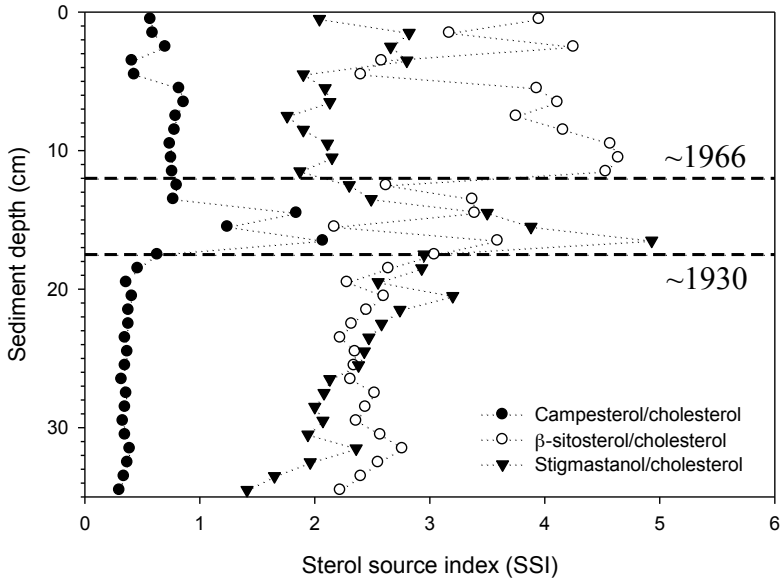


Figure 2.4. The Sterol Source Index (SSI), the ratio of phytosterol/cholesterol, for a 35-cm sediment core collected from the south basin of Ross Lake in 2009.

As a percentage of the total phytosterols, β-sitosterol was the dominant phytosterol throughout the majority of the Ross Lake core profile (Figure 2.4). The peak concentrations, in this sediment core, for campesterol, β-sitosterol and stigmastanol were 107.5, 188.6 and 337.6 μg g^{-1} OC, respectively (Figure 2.4). Greater concentrations of β-sitosterol and campesterol in the

early years of the post-industrial era (Figure 2.4) were possibly due to increased input of plant material from the Ross Lake basin. Sterol mixtures of several pine barks (pines are common in this region) have been shown to consist predominantly of β-sitosterol with about one-tenth as much campesterol (Rowe 1965). The sudden peak in phytosterol concentrations in the 15-16-cm section is possibly due to basin deforestation during the construction of Flin Flon. Trees were cut for fuel and lumber when the communities of Flin Flon and Creighton were established. Several years after the mine opened, a forest fire also consumed much of the nearby forest (StarPhoenix newspaper, Saskatoon, SK, December 10, 2012). These activities and events likely increased the input of terrestrial organic matter into Ross Lake, possibly through the decomposition of tree matter and eventual transport in runoff into the lake.

2.5. Conclusions

Concentrations of coprostanol and cholesterol and a coprostanol/cholesterol ratio consistently above 0.2 indicated that the south basin of Ross Lake historically received naturally high inputs of sterols from non-municipal, biogenic sources. Concentrations of coprostanol and cholesterol and their ratio increased dramatically in the south basin of Ross Lake, suggesting substantial municipal sewage loading with the onset of industrial activities and urbanization. Coprostanol and cholesterol concentrations were lesser in the more recent sediments and reflect changes to municipal effluent discharge practices (post-1951), which resulted in reduced sewage input into Ross Lake. Nevertheless, recent sediments continue to have coprostanol/cholesterol ratios indicative of municipal sewage contamination as an organic matter source.

Historical concentrations of phytosterols investigated followed trends similar to those of coprostanol and cholesterol and are possibly the result of the response of algae to altered environmental conditions and/or an increase in the flux of terrestrial organic matter into Ross Lake as the result of regional deforestation due to logging and fire.

Analyses of absolute and relative concentrations of sterols provided a means to identify those sediments formed under different municipal wastewater management practices. This provides greater confidence that inferences made based on biological remains isolated from such sediments can be correctly attributed to specific periods of time and hence specific historical municipal and industrial activities. The methodologies detailed in this chapter were applied to sediment cores collected from Lake Diefenbaker and are further discussed in Chapter 5.

CHAPTER 3

Long-term spatial trends in sedimentary algal pigments in a narrow river-valley reservoir, Lake Diefenbaker, Canada

This chapter is based on the article published as Tse, T. J., Doig, L. E., Leavitt, P. R., Quiñones-Rivera, Z. J., Codling, G., Lucas, B. T., Liber, K., Giesy, J. P., Wheater, H., Jones, P. D. 2015. Long-term spatial trends in sedimentary algal pigments in narrow-river-valley reservoir, Lake Diefenbaker, Canada. Journal of Great Lakes Research. 41: 56–66.

NOTE TO THE READERS – CHAPTER 3

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The author contributions to Chapter 3 were as follows:

- Timothy Tse (University of Saskatchewan) collected, processed, analyzed sample data, and drafted the manuscript.
- Peter Leavitt (University of Regina) and Zoraida Quiñones-Rivera (University of Regina) performed High-Performance Liquid Chromatography analyses on extracted samples and reviewed and revised the manuscript.
- Brett Lucas (University of Saskatchewan) helped collect sediment cores from the study site, Lake Diefenbaker, Saskatchewan, Canada.
- Gary Codling (University of Saskatchewan), Paul Jones (University of Saskatchewan) and Lorne Doig (University of Saskatchewan) provided guidance in method extraction development and reviewed and revised the manuscript.
- John Giesy (University of Saskatchewan) and Karsten Liber (University of Saskatchewan) provided laboratory facilities to extract and process samples and reviewed and revised the manuscript.
- Howard Wheater (University of Saskatchewan) provided funding required to conduct the research and reviewed and revised the manuscript.

CHAPTER 3

Long-term spatial trends in sedimentary algal pigments in a narrow river-valley reservoir, Lake Diefenbaker, Canada

3.0. Preface

This chapter discusses the methodology used in Chapter 2, to successfully co-extract algal pigments and fecal sterols (discussed in Chapter 5) from sediment cores collected from Lake Diefenbaker, Saskatchewan (case study test site). Analyses of these compounds investigated the historical trends in algal and cyanobacterial community composition in Lake Diefenbaker. Reconstruction of the phototrophic community using these pigment biomarkers revealed the presence of potentially harmful cyanobacteria in the down-reservoir arms of Lake Diefenbaker. Furthermore, spatial changes in the abundance of phototrophic taxa throughout the reservoir were attributed to different hydrological gradients typically experienced in these types of river-valley reservoirs.

3.1. Abstract

Narrow river-valley reservoirs are typically spatially heterogeneous. Little is known about how (a) spatial water quality and algal community composition change longitudinally along a narrow river-valley reservoir and (b) how algal composition and production change as these reservoirs age. To address these unknowns, multiple sediment cores were collected from mid-channel locations along the longitudinal axis of Lake Diefenbaker, Saskatchewan, Canada, a long, narrow river-valley reservoir on the Canadian prairies. Profiles of concentrations of various pigments in sediment cores were measured to infer spatial and temporal trends in algal biomass and community composition. Diverse mixtures of pigments derived from cyanobacteria, diatoms, chlorophytes, and other phyla were observed. Spatial patterns of sedimentation of pigments (nmol m^{-2} yr^{-1}) in surficial sediments suggested increases in algal biomass with distance down-reservoir, with maximum inferred biomass occurring in mid-reservoir. This is consistent with general knowledge of patterns of primary production in narrow, river-valley reservoirs. However, myxoxanthophyll, a biomarker of filamentous or colonial cyanobacteria and picocyanobacteria, detected only at sites furthest down-reservoir, did not follow this general trend. Temporally, an increase in algal biomass occurred at down-reservoir locations after 1990,

followed by a substantial increase after 2000 at the majority of sites. Depth profiles of concentrations of pigments exhibited no clear trends to support the prevailing paradigm that predicts an initial upsurge in trophic status upon formation of reservoirs. This pattern may result from limited penetration of light in the early years after reservoir formation due to turbidity. This study reinforces the need for paleolimnological analyses among hydrologic zones of large reservoirs.

3.2. Introduction

Water quality of lakes and reservoirs in some areas of North America and Europe is believed to be deteriorating (Cooke et al., 2005). In particular, cultural eutrophication is causing undesirable changes in aquatic communities (Pretty et al., 2003) due to changes in trophic relationships, elevated external nutrient loads, nutrient stoichiometry, and transport of nutrients from benthic to pelagic regions (Schindler 2006). Water quality loss is especially problematic for potable supplies where costly purification processes must be used to reduce offensive tastes, odours and algal toxins, while minimizing production of hazardous disinfection by-products. Unfortunately, long-term monitoring data are often lacking for potable water supplies within the Canadian Prairies. Such information is essential to assess long-term trends in environmental quality and inform resource management decisions. Monitoring can be performed by local water authorities, but parameters measured are often limited in scope and spatial extent. Long-term monitoring efforts tend to be initiated only after serious problems arise (Kohler 2010). Therefore, various paleolimnological tools, especially those involving analysis of chemical characteristics and biological remains within sediments, are gaining popularity as a means of reconstructing past changes in the environmental quality of inland waters. To date, such studies are abundant for natural lakes but are less commonly used for manmade reservoirs. This trend, however, appears to be changing as it has been recognized that stratigraphies of undisturbed sediments can be found in deeper regions of reservoirs (Sholbolt et al., 2001; Van Metre and Mahler 2004) and that these sediments represent potential sources of long-term environmental information that can be used to inform ongoing management decisions.

Paleolimnological investigations typically rely on a small number of sampling stations, often a single representative site, to assess the overall historical environmental changes of an inland body of water. For small or morphologically simple lakes this approach is often

appropriate. However, for longer morphologically complex reservoirs formed by damming of narrow river valleys, various longitudinal gradients are likely to occur and need to be considered in the designing, implementing and interpreting of retrospective studies (Kennedy et al., 1982). These gradients result in zones, each having different physical, chemical and biological properties (Thornton et al., 1981). As detailed in Thornton et al. (1981), a riverine zone occurs in the narrow, shallow, up-reservoir region. This zone is well-mixed and aerobic, but primary production is often light-limited as a consequence of abundant fine suspended particles and allochthonous organic matter. The riverine zone is followed downstream by a transitional zone, where sedimentation is substantially greater and light penetrates to greater depths. Finally, there is the down-reservoir lacustrine zone in which lake-like conditions predominate; it is characterized by increased water depth, greater light penetration, slower currents and constant sedimentation rates. Organic matter in the lacustrine zone is predominately autochthonous (Thornton et al., 1990).

Observational studies suggest that inundation of dry-valley reservoirs leads to predictable changes in aquatic productivity and composition of the phytoplankton community, a phenomenon termed a trophic upsurge (reviewed in Thornton et al., 1990). Immediately following flooding, production increases in phytoplankton, invertebrates and fish, over a period of 5−20 years due to the leaching of soluble nutrients from flooded soils, erosion of newly formed shorelines, and decomposition of inundated vegetation (Kennedy and Walker 1990; Ostrofsky and Duthie 1978). Although secondary production is thought to decline thereafter, little is known of the long-term trends in nutrient fluxes, production of phytoplankton (Donar et al., 1996), or changes in the benthic algal community composition (Thornton et al., 1990). While these patterns have been observed in more lacustrine regions of reservoirs, much less is known about how algal biomass and community composition vary through time at more riverine or transitional locations.

Algal pigments in sediments can be used as biochemical markers for mapping historical populations of phototrophic algae and prokaryotes (Leavitt and Hodgson 2001). These fossil pigments are relatively sensitive to external environmental factors. During sedimentation of phytoplankton, most pigments are largely degraded by irradiance, oxygen, microbes and grazers (Cuddington and Leavitt 1999), although once buried in anoxic sediments, pigments and their derivatives can be preserved for thousands of years (Kowalewska 2001; Sanger 1988; Watts and

Maxwell 1977). Lake sediments often preserve a range of carotenoids, chlorophylls, photoprotectant compounds and other lipid-soluble pigments produced by phototrophic organisms in both the lake and, potentially, its surrounding catchment (Table 3.1). The main sources of pigments in lakes include planktonic and benthic algal communities, phototrophic bacterial populations and macrophytes (Leavitt and Hodgson 2001). In Lake Diefenbaker, relatively steep banks, high seasonal turbidity and frequent drawdowns have resulted in minimal macrophyte growth (T. Tse, University of Saskatchewan, 2011, University of Saskatchewan, personal communication). Similarly, while in principle sediments can include pigments in detrital material from the terrestrial environment, the high rate of oxidation during transport usually minimizes the importance of allochthonous pigments (Leavitt and Hodgson 2001).

A paleolimnological study was conducted to assess whether 1) temporal changes in inferred algal biomass ("production" herein), especially that of cyanobacteria, has increased over time in Lake Diefenbaker; 2) there were spatial differences within the reservoir that can be linked to reservoir zonation; and 3) a period of trophic upsurge was observed during initial flooding of this narrow river-valley reservoir. Sediment cores were collected along the longitudinal-axis of this large river-valley reservoir in the grasslands of central Canada. Fossil pigments preserved within the vertical sediment profile were analyzed to reconstruct changes within the phytoplankton community. Our study builds on previous paleolimnological research at a single site within the lake (near the mouth of the Qu'Appelle arm) that demonstrated historical changes in inferred phytoplankton biomass based on fossil pigments (Hall et al., 1999). However, given that this type of reservoir is spatially complex along the longitudinal axis, and that relatively few paleoecological studies have addressed spatial variation in fossil records (e.g. Edlund et al., 2009), the present study was initiated to provide one of the first tests of how algal biomass varies through time among different reservoir zones.

3.3. Methods

3.3.1 Study site

Lake Diefenbaker (50°43' N, 107°30' W) (Figure 3.1), Saskatchewan, Canada, is the largest multi-purpose reservoir in the Canadian Prairies, with an approximate length of 181.6 km, a maximum depth of 59 m, an area of 394 km^2, and a volume of 9.03 km^3. The lake was formed in 1967 after the completion of the Gardiner and Qu'Appelle dams on the South Saskatchewan

River and Qu'Appelle River valley, respectively (Hall et al., 1999). The water that flows into Lake Diefenbaker has a residency time of approximately 1.3 years (Bogard et al., 2012) before exiting through the Gardiner arm (Site 7, 99% of the flow) and Qu'Appelle arm (Site 8, 1% of the flow) (Saskatchewan Water Security Agency 2012). The Saskatchewan Water Security Agency (SWSA) manages Lake Diefenbaker for power production, flood control, and water supply for mining, crop irrigation, various industries, wildlife, aquaculture, and as a supply of drinking water for approximately 45% of Saskatchewan's residents (SWSA 2012). Long-term water elevation trends have been summarized by Pomeroy and Shook (2012) with the relevant data presented graphically in Lucas et al. (2015a). There have been substantial yearly variations in both maximum and minimum annual water elevations since reservoir formation, with a progressive decrease in minimum water elevation over time (Pomeroy and Shook 2012).

Table 3.1. Pigments recovered from aquatic sediments and their taxonomic affinities (Leavitt and Hodgson 2001).

Pigment	**Affinity**	**Retention time (min)**	**Stability Index**
Alloxanthin	Cryptophyta	8.7	1
Lutein	Chlorophyta, Euglenophyta, Plantae	9.7	1
Zeaxanthin	Cyanobacteria	9.7	1
Fucoxanthin	Diatoms, Chrysophyta, Dinophyta	4.9	2
Diatoxanthin	Diatoms, Dinophyta, Chrysophyta	9.3	2
Chlorophyll *a*	Total algae abundance, Plantae	15	3
β-carotene	Total algae abundance, Plantae	20	1
Chlorophyll *b*	Plantae, Chlorophyta, Euglenophyta	13	2
Echinenone	Cyanobacteria	15.5	1
Myxoxanthophyll	Most cyanobacteria (including filamentous and colonial)	8	2
Canthaxanthin	All cyanobacteria	11.8	1
Phaeophytin *a*	Chlorophyll a derivative (general)	22.1	1
Phaeophytin *b*	Chlorophyll b derivative (general)	18.9	2

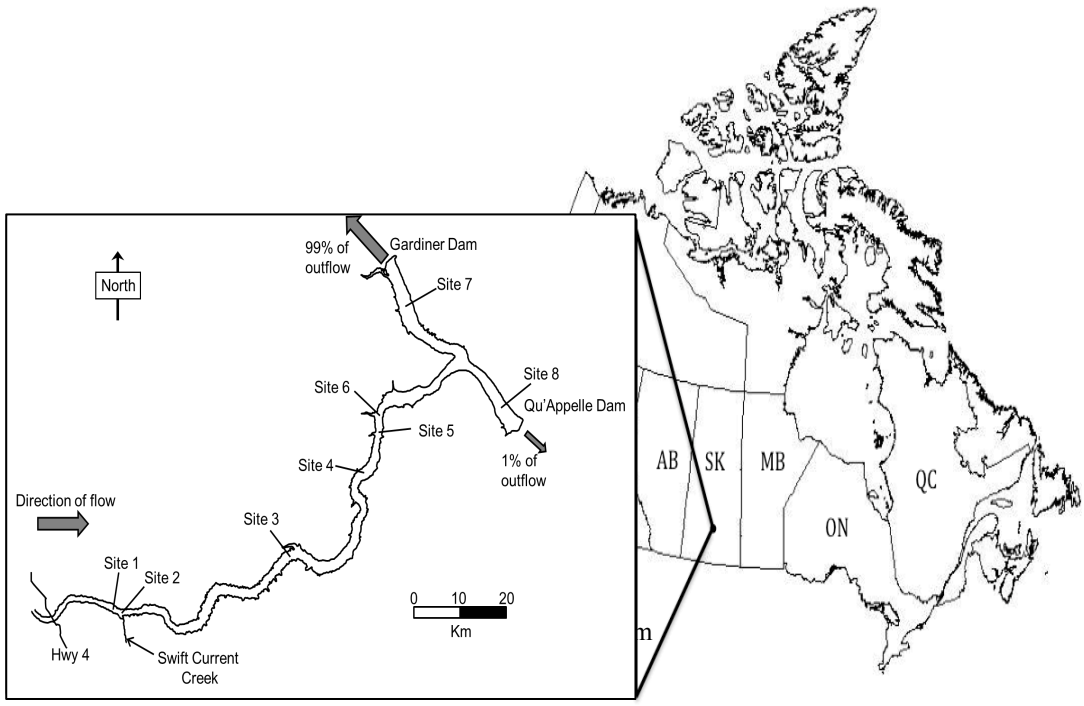

Figure 3.1. Map of Lake Diefenbaker, Saskatchewan, Canada, showing where cores of sediment were collected from the main channel in 2011 (Sites 1, 2, 4–7) and 2012 (Sites 3 and 8).

Consistent and detailed monitoring of water chemistry and limnology of Lake Diefenbaker over the past two decades has been confined to the Qu'Appelle arm (e.g. Vogt et al., 2015). Nevertheless, recent, short-term studies have been conducted of the broader reservoir. Briefly, Lake Diefenbaker is relatively fresh, slightly basic (mean conductivity 434 µS cm^{-1}, mean pH 8.2 to 8.4; Hecker et al., 2012), and mesotrophic (Abirhire et al., 2015), unlike most lakes in the region, which are eutrophic (Vogt et al., 2011). In addition, analysis of phytoplankton phenology shows that spring blooms of diatoms and cryptophytes give way to chlorophytes and cyanobacteria during late July and August (McGowan et al., 2005), with occasional blooms of colonial cyanobacteria in late July and August (Hecker et al., 2012; Patoine et al., 2006; Vogt et al., 2011). Assemblages of zooplankton are composed of ubiquitous copepods (*Diacyclops thomasii*, *Leptodiaptomus sililoides*), *Daphnia* spp. (*D. galaeta-mendotae*, *D. pulex*, *D. retrocurva),* predaceous *Leptodoura kindtii* and a diverse community of small-bodied crustaceans (Dröscher et al., 2009; Patoine et al., 2006). However, despite these studies,

little is known of the spatial pattern of temporal changes in algal production and water quality within this large reservoir.

In recent years, anecdotal observations by area residents suggested increasing spatiotemporal changes in algal production, as well as associated decline in water quality linked to cyanobacteria abundance. As well, sampling during 2008 demonstrated that the predominant cyanobacteria was *Anabaena circinalis* (Hecker et al., 2012), a taxon with strains known to produce hepato- (e.g. microcystins) and neuro-toxins (e.g. anatoxin-a, saxitoxin and its analogs) (Sivonen and Jones 1999). Therefore, to address concerns regarding a potential decline in water quality, the lake was sampled to investigate spatial patterns of water quality in Lake Diefenbaker. If harmful algal blooms are becoming more frequent, it is important to control them (if possible) as they can affect the livestock and wildlife that use the reservoir directly, increase costs associated with water treatment for human consumption, and potentially amplify water quality problems downstream (e.g. Buffalo Pound Lake and the Qu'Appelle River system).

In addition to the South Saskatchewan River inflow, a variety of potential point sources contribute nutrients and organic matter to Lake Diefenbaker. These sources include the largest freshwater fish farm in western Canada (Wildwest Steelhead Fish Farm®) currently licensed to produce 1,450 tonnes of fish annually and to release a maximum of 25 tonnes of phosphorus into the reservoir (Government of Saskatchewan 2007), numerous cattle operations with access to shoreline locations, breeding colonies of water birds, and municipal inputs (e.g. treated wastewater discharge from the town of Elbow and the city of Swift Current). Instantaneous algal growth in the lacustrine portion of Lake Diefenbaker is regulated mainly by the availability of phosphorus (Dubourg et al., 2015; Hecker et al., 2012; Vogt et al., 2015). Therefore, while Lake Diefenbaker is presently managed as a source of good quality water, increased nutrient availability could compromise or increase the costs of future water uses through increased frequency and extent of harmful algal blooms and their associated issues.

Sediment cores were collected from six locations along the reservoir between May and October of 2011 (Sites 1, 2, 4, 5, 6 and 7) and two locations between July and August of 2012 (Sites 3 and 8) (Figure 3.1) by use of a Glew Maxi Gravity Corer (John Glew, Kingston, Canada) fitted with an acrylic core tube (pre-cleaned, 7.6-cm inner diameter). Upon collection of the sediment core, the sediment and overlying water were sealed and transported in a cooler with freezer packs to the Toxicology Centre in Saskatoon (SK, Canada) and stored at 4 °C in the dark.

The overlying water remained clear throughout the transportation, suggesting minimal resuspension of sediment particles. Prior to sectioning of the core, the surface water was siphoned off and an extruder was inserted into the core base. Cores were then extruded and vertically sectioned at 1-cm increments under a constant stream of nitrogen (N_2) gas. Each 1-cm section was collected using a plastic spatula and placed in 20-mL glass scintillation vial (Fisher Scientific, Ottawa, Ontario). Samples were immediately mixed using a plastic rod and the vial capped under a N_2 atmosphere. The capped vials were then sealed with Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL) and aluminum foil and stored at -20 °C, in the dark, prior to extraction and analysis.

3.3.2. Sediment core dating

The disappearance of diatom fossil remains from the sediment profile (Lucas et al., 2015b) was used to determine the depth in sediments corresponding to the date of reservoir formation (1967). This analysis demonstrated that the complete sediment profile was captured at two of the eight sites sampled (Sites 5 and 7). Individual depth intervals could not be assigned precisely-known ages, so sediment depths were used as an approximation of fossil chronology.

Sediment depositional rates were calculated for up-reservoir regions by comparing historical and recent cross-sectional and bathymetric data (Sadeghian et al., 2015). Up-reservoir regions experienced much larger yearly deposition rates compared to down-reservoir regions (Sites 3 to 8). Depositional rates were determined to range between 0.05 m y^{-1} and 0.03 m y^{-1} for two stations straddling Sites 1 and 2, with an average deposition rate of 0.04 m y^{-1} assumed at Sites 1 and 2 (Lucas et al., 2015a). Therefore, the vertical profiles of the cores from Sites 1 and 2 represent approximately 8 and 13 years, respectively.

Although the beginning of the diatom stratigraphies were not clearly captured in the cores from Sites 4, 6, and 8, the similar distinctive patterns in diatom community stratigraphies among these and Sites 5 and 7 (entire core profile represented) suggested that the majority of the sediment profile was collected at these sites (detailed in Lucas et al., 2015a). Therefore, the core profiles for Sites 4 and 8 are assumed to approximate 44 and 45 years, respectively. A substantial change in fecal sterol concentrations (e.g. coprostanol:cholesterol ratios) (T. Tse unpublished data, University of Saskatchewan, 2013) coincided with the end of diatom remains in the Site 7 core, supporting the interpretation of a historical river valley-reservoir boundary. A substantial

deviation of the organic carbon:nitrogen ratio in the deepest sediment 1-cm increment in cores 4, 5, 7 and 8 (Lucas et al., 2015a) further suggested a change in parent materials, providing additional confidence that the historical reservoir bottom was reached or approached in these cores. A comparison of magnetic susceptibility profiles between two cores collected at Site 6 for either diatom or chironomid analysis showed that not all of the sediment profile was captured in the diatom core (also analyzed for pigments herein), with the bottom of the core representing approximately 1978. To facilitate a general comparison of temporal trends, core chronologies have been assigned based on the assumption of relatively constant deposition over time at Sites 4 to 8, but we acknowledge that there is uncertainty in this assumption.

It was not possible to assign ages to the sediment profile of Site 3. Nevertheless, Site 3 data have been included in Figure 3.3 for comparative purposes by assuming a chronology similar to that of Site 4, which is closest in proximity. Deposition rates for Site 3 are likely underestimated, as the sediment profile likely represents a shorter time period than Site 4 (i.e. Site 3 likely has a greater rate of deposition).

3.3.3. Pigment extraction and preparation

An aliquot of each 1-cm sediment core section (~5 g ± 0.5 g wet weight) was lyophilized for 72 h, in the dark. All samples were then sonicated in a solvent mixture of acetone:methanol (85:15 v/v) in an ice bath for 3 min, followed by centrifugation at 2000 x *g* for 10 min after which the supernatant was collected. This extraction was performed twice and the two supernatants were pooled. Each sample was concentrated under N_2 gas and then cleaned up using a silica column. The silica column consisted of 2 g pre-baked silica and a small layer of prebaked anhydrous sodium sulfate (baked at 450 °C for 24 h) in a 9-mm ID column preconditioned with 30 mL each of acetone and methanol. This silica column was used to remove polar compounds for a separate experiment. It is unlikely that this method compromised the integrity of the pigments extracted, as the pigment trends analyzed follow similar trends to organic carbon content and $\delta^{15}N$ values (Lucas et al., 2015a). Samples were passed through the column with the same acetone and methanol mix as eluent. Samples were then blown to dryness using N_2 gas and resuspended to a total volume between 0.5 ml to 2 ml in an ion-pairing reagent consisting of 0.75 g tetrabutyl ammonium acetate and 7.7 g ammonium acetate in 100 mL HPLC-grade water following the methods of Leavitt and Hodgson (2001). This solution also contained the internal

standard Sudan II (Sigma Aldrich, St. Louis, MO).

3.3.4. Pigment isolation and quantification

High performance liquid chromatography (HPLC) analyses were conducted according to standard procedures of Leavitt and Hodgson (2001). Pigments and their derivatives were separated and quantified by use of an Agilent (Hewlett Packard model 1100) HPLC system fitted with a model 1100 photodiode array detector and an HP fluorescence detector. The system was equipped with a Waters μBondapak C-18 pre-column (10 μm particles) and a Rainin C-18 column (10 cm, 5 μm particles). Analytical separation was achieved by isocratic elution with mobile phase A (10% ion-pairing reagent in methanol) for 1.5 min at 1.5 ml min^{-1} followed by a linear ramp to 100% solvent mixture B (27% acetone in methanol) over 7 min with an isocratic hold for an additional 12.5 min. The column was re-equilibrated with an isocratic delivery for 3 min, a linear return to 100% solution A over 3 min, and a further isocratic hold for 12.5 min (Leavitt and Hodgson 2001). Total run time was approximately 30 min.

Pigments isolated from sediments were compared to those from uni-algal cultures and authentic standards obtained from US Environmental Protection Agency and other suppliers (Leavitt and Hodgson 2001). Pigment identity was based mainly on spectral characteristics and chromatographic mobility of pigments from all sources (Table 3.1). Pigment analysis was restricted to taxonomically-diagnostic carotenoids characteristic of the following algal groups: diatoms, chrysophytes and some dinoflagellates (fucoxanthin); mainly diatoms (diatoxanthin); cryptophytes (alloxanthin); chlorophytes (phaeophytin b); chlorophytes and cyanobacteria (lutein-zeaxanthin); cyanobacteria (myxoxanthophyll); Nostocales cyanobacteria (canthaxanthin); all cyanobacteria (echinenone); and the major a, b, and c-phorbins (chlorophyll (Chl) and Chl derivatives). Finally, estimates of post-depositional pigment degradation were derived from analysis of ratios of the labile precursor, chlorophyll *a* (Chl *a*), to the chemically-stable degradation product, phaeophytin *a* (Phaeo *a*), as described by Leavitt and Hodgson (2001). Sedimentary pigment concentrations were expressed as nmol pigment g^{-1} organic carbon (OC) sediment dry weight.

Clay particulates in the water column can reduce primary productivity due to decreased light. Therefore, measured Chl *a* concentrations in the sediment profile of each site investigated were compared to co-occurring clay content (%) (data from Lucas et al., 2015a) using Pearson

Product Moment correlation.

3.4. Results

3.4.1. Spatial variability in lake sediments

Fossil pigments (Table 3.1) representative of Cryptophyta, Chlorophyta, diatoms, and cyanobacteria were observed in all cores of sediment from Lake Diefenbaker. In general, sedimentation rates of pigments (Chl *a* and total pigments) in surficial sediments (2009 until 2011/2012) increased along the longitudinal gradient (Figure 3.2). Sites in the up-reservoir regions (Sites 1 and 2) exhibited low pigment depositional rates in the superficial sediments (1,342 and 1,045 nmol m^{-2} $year^{-1}$ for Chl *a*, respectively; 5,608 and 4,433 nmol m^{-2} $year^{-1}$ for total pigments, respectively), while maximal depositional rates of Chl *a* and total pigments were observed at mid-reservoir locations (12,303 and 10,333 nmol m^{-2} $year^{-1}$ at Sites 5 and 6, respectively, for Chl *a*; 15,936 and 16,910 nmol m^{-2} $year^{-1}$, respectively, for total pigments). In addition, comparisons among depositional rates of cyanobacterial pigments, such as canthaxanthin, echinenone and myxoxanthophyll with distance down-reservoir suggested increased cyanobacterial presence in the lacustrine portion of the lake (Sites 7 and 8; Figure 3.2).

Deposition rates for specific algal groups, expressed as a percent of total pigment deposition rate for surficial sediments, were compared along a spatial gradient (Figure 3.3). Non-specific pigments (Chl *a* and β-carotene) and products of degradation of pigments (phaeophytins) are not shown. Pigments that were not mutually exclusive (diatoxanthin and fucoxanthin; the various cyanobacterial pigments) have been combined. Therefore, alloxanthin (Cryptophyta), diatoxanthin/fucoxanthin (Chrysophyta and Bacillariophyta [diatoms]), Chl *b* (Plantae, Chlorophyta, Euglenophyta), and cyanobacterial pigments were plotted to observe patterns in algal community composition with distance down-reservoir from Site 1. Pigments representative of Cryptophyta, Bacillariophyta (diatoms), and Chrysophyta increased with distance down-reservoir, with maximum dominance down-reservoir of Site 3. Although the maximum inferred cyanobacterial biomass occurred at Site 7 (Figure 3.2), the overall relative proportion of cyanobacterial pigments showed little trend with distance down-reservoir.

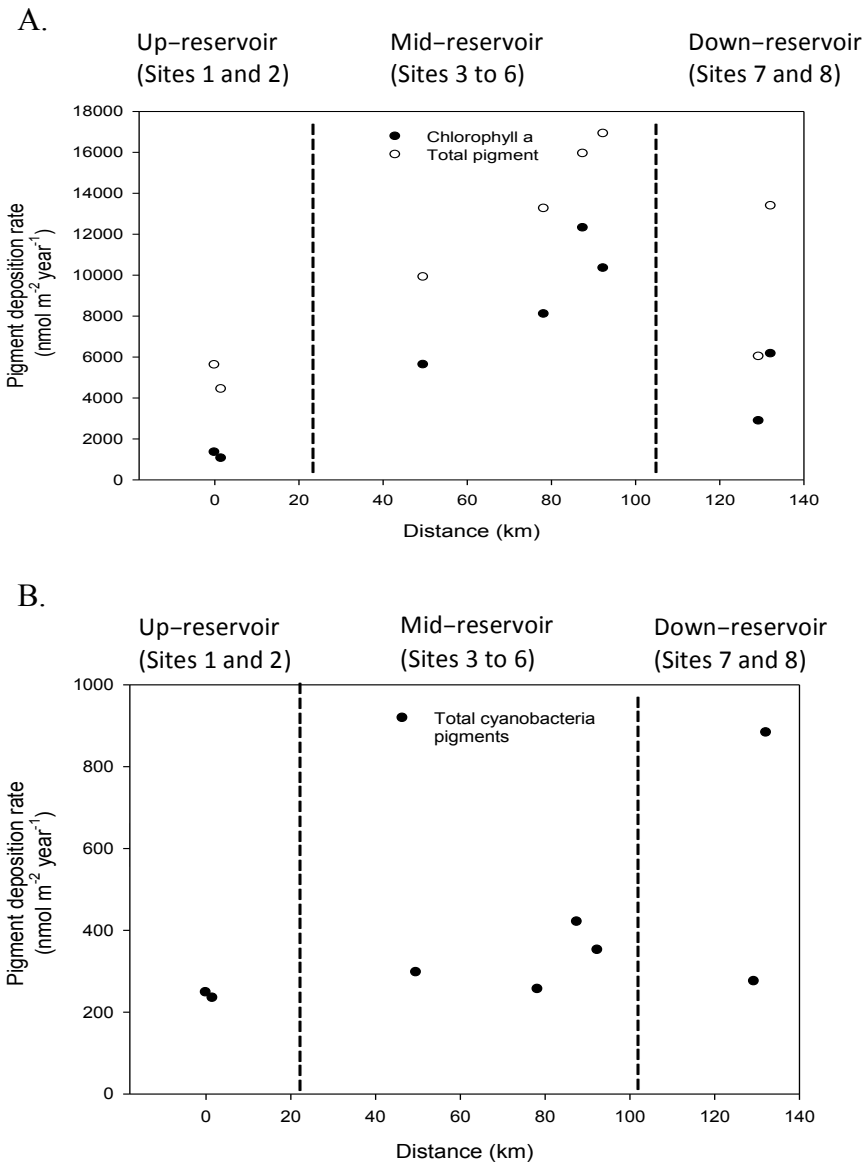

Figure 3.2. Pigment deposition rates in surficial sediments along Lake Diefenbaker, Saskatchewan, Canada. Spatial comparison of up-, mid-, and down-reservoir (separated by vertical dotted lines) locations of recent (2009 to 2011, Sites 1, 2, 4–7; 2009 to 2012, Sites 3 and 8) (A) Chl *a* and total pigment deposition rates (nmol m^{-2} $year^{-1}$) and (B) pigment deposition rates of total cyanobacterial pigments, both with distance down-reservoir from Site 1. Site 7, in the Gardiner arm, is the furthest distance from Site 1.

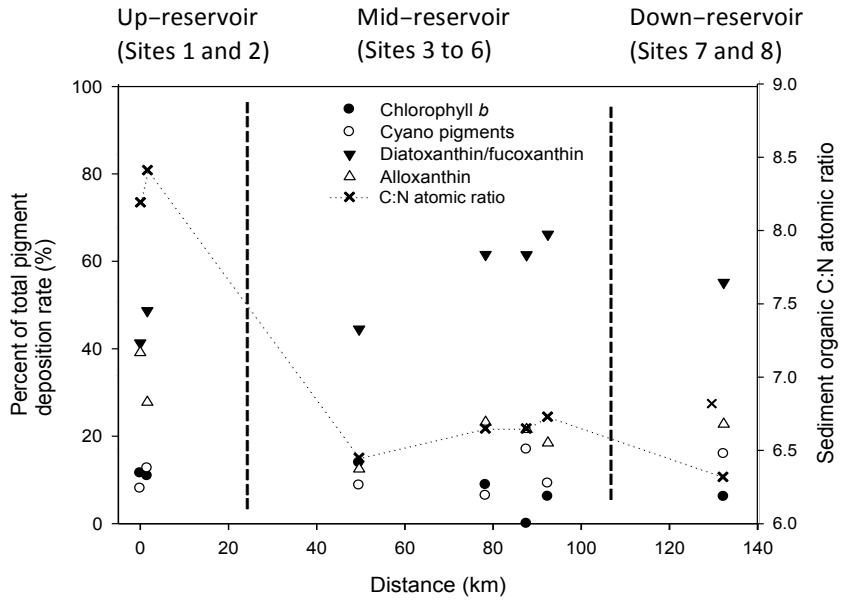

Figure 3.3. Algae community composition along Lake Diefenbaker, Saskatchewan, Canada. Algae community composition (presented as relative proportions of the total pigment deposited in recent sediments, 2009–2011/12) represented by diatoxanthin/fucoxanthin (combined), alloxanthin, chlorophyll *b* and total cyanobacterial pigments, over a spatial gradient. The associated organic carbon: total nitrogen ratio (C:N, data from Lucas et al., 2015a) along the main flow path (Sites 1 to 7) has been plotted (dashed line) for comparison.

3.4.2. Temporal trends in algal pigments and preservation

Concentrations of preserved chlorophyll and carotenoid pigments in lake sediments are related to inferred abundance of phytoplankton, as well as the integral of loss processes during sinking and incorporation into the sediments (Cuddington and Leavitt 1999; Leavitt and Hodgson 2001; Swain 1985). Phaeophytin *a*, the degradation product of Chl *a*, is more stable than Chl *a* and hence comparisons between Chl *a* and Phaeo *a* (Figures 4 and 5) can provide insight regarding pigment preservation conditions (Leavitt and Hodgson 2001; Reuss et al., 2010) or diagenetic activity (Das et al., 2005; Vinebrooke et al., 2002; Wolfe et al., 2006). The Chl *a*:Phaeo *a* ratio in the deeper portions of all cores suggested relatively constant preservation of pigments over time. However, there was an increase in Chl *a*:Phaeo *a* ratio in the upper portions of the mid-reservoir cores (Sites 4 to 6) which was generally more pronounced, in the vertical sediment core profiles, with distance down-reservoir.

Up- reservoir locations (Sites 1 and 2, Figure 3.4A and B) did not exhibit any clear temporal trends for any of the concentrations of pigments identified, although the period of time represented by these cores is much less (8 to 13 years) than Sites 4 to 8 (up to 45 years). The synchronous oscillations in pigment concentrations at Sites 1 and 2 may be a product of seasonal and inter-annual variability in pigment deposition; however, more research is needed to confirm this hypothesis. Increasing pigment trends over time begin to emerge at Site 3 (e.g. Chl *a* and some carotenoids; Figure 3.4C), with obvious increases in all pigments observed at Site 4 and onwards (Figure 3.4D, 3.5A, and 3.5B). Phytoplankton biomass showed a clear increase ca. 1990 at Sites 7 and 8 (Figures 3.5C and 3.5D). These trends subsequently remained relatively constant at Site 8, with Site 7 experiencing a substantial increase in pigment concentrations in recent years. Finally, myxoxanthophyll (Figure 3.5C and D, dashed outline) was undetectable at all sites except the most down-reservoir sites, 7 and 8, with pigment concentrations increasing over time at site 7.

3.5. Discussion

The geomorphological and hydrological characteristics of long, narrow-valley reservoirs can result in biological, chemical and physical gradients along the longitudinal axis (Thornton et al., 1990). These gradients can affect the location and intensity of blooms of algae (Kennedy et al., 1982). Analysis of concentrations of pigments in sediments and their conversion to depositional rates (nmol m^{-2} $year^{-1}$) revealed variability in inferred algal production within Lake Diefenbaker that was consistent with these spatial gradients. In addition, the general paradigm is that there is a predictable series of trophic events that follow formation of a reservoir, such as the *trophic upsurge* paradigm. In this theory, productivity is initially greater due to inundation of dry land and increased accessible nutrients. This is followed by a trophic depression as nutrient concentrations decline (Thornton et al., 1990). However, the absence of greater concentrations and accumulation rates of pigments in the oldest (deepest) sediments, compared to younger sediments within the sediment core profiles, gives little support for the existence of an initial trophic upsurge in this reservoir. These spatial and temporal observations are discussed further below.

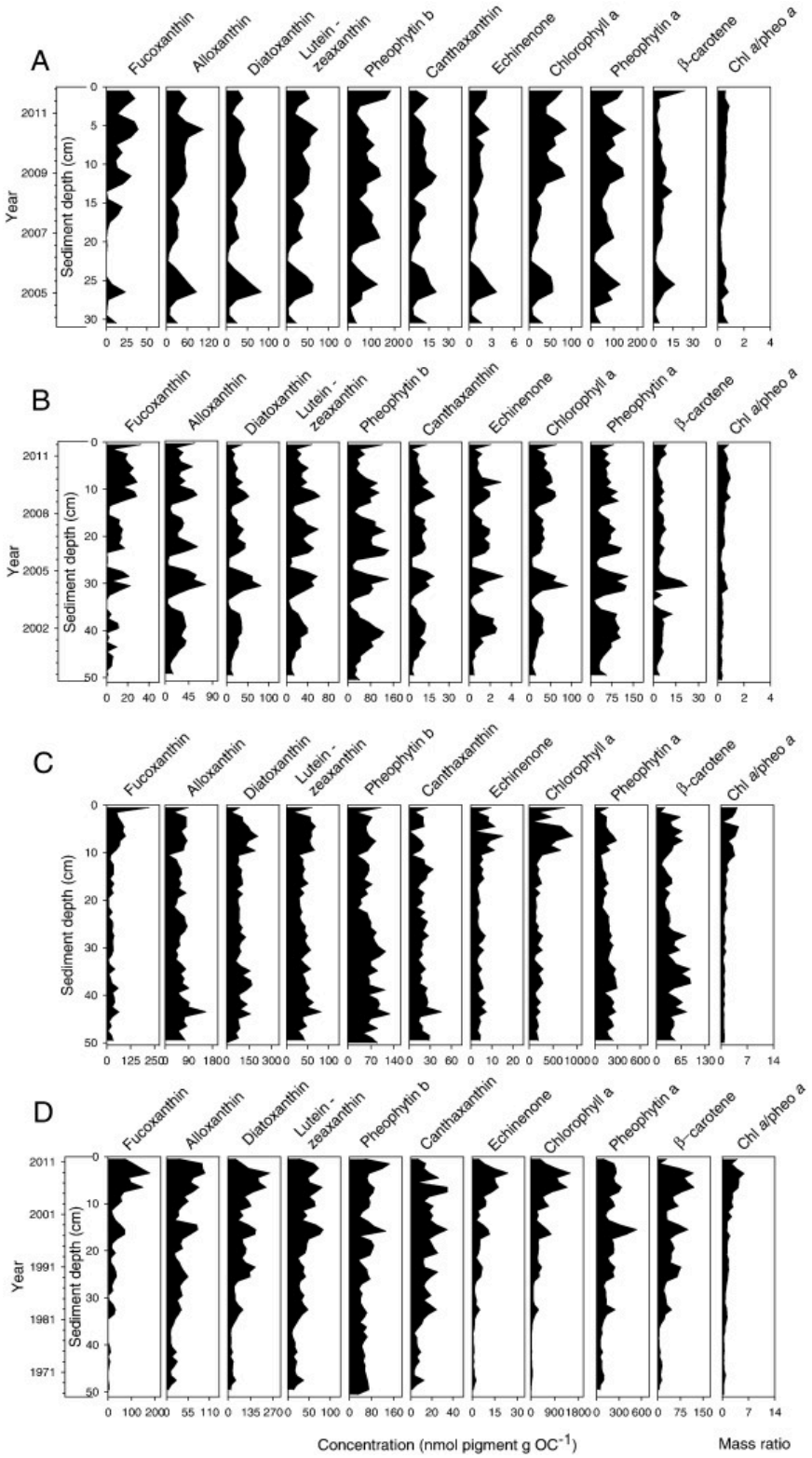

Figure 3.4. Temporal profiles of concentrations of sedimentary pigments for two up-reservoir (Sites 1 [A] and 2 [B]) and two mid-reservoir (Sites 3 [C] and 4 [D]) locations in Lake Diefenbaker, Saskatchewan, Canada. Note the different scales on the x-axis.

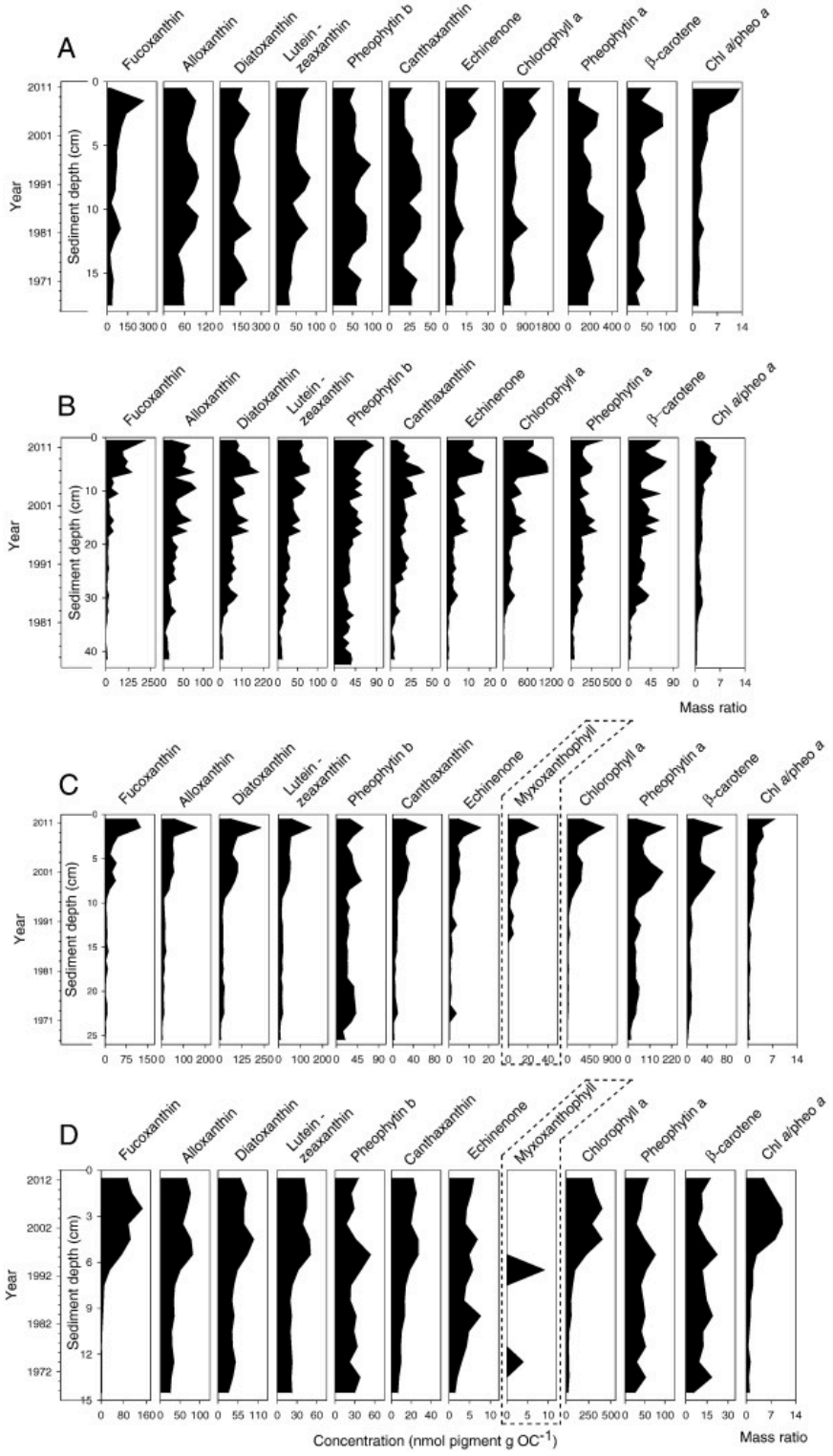

Figure 3.5. Temporal profiles of concentrations of sedimentary pigments for two mid-reservoir (Sites 5 [A] and 6 [B]) and two down-reservoir (Sites 7 [C] and 8 [D]) locations in Lake Diefenbaker, Saskatchewan, Canada. Note the different scales on the x-axis.

3.5.1. Spatial patterns of algal pigments

Reservoirs possess both longitudinal and vertical gradients, which can affect availability of nutrients, abundance of phytoplankton, productivity and standing crop, organic matter supply and trophic status within a reservoir (Kimmel and Groeger 1984). Regarding longitudinal gradients, advective processes in up-reservoir locations can result in greater concentrations of suspended solids and thus more turbidity (Kimmel and Groeger 1984). Light can therefore limit algal abundance in up-reservoir locations. Because flow rates decrease with distance down-reservoir through the transitional zone, suspended solids settle and water clarity increases. As a result of abundant light and nutrients, primary productivity is greatest in these transitional, mid-reservoir areas (Kennedy et al., 1982; Kimmel and Groeger 1984). In contrast, although clarity of water can be greater, nutrients may be depleted in the water column in more lacustrine regions down-reservoir (Kennedy et al., 1982; Kimmel and Groeger 1984).

Spatial trends in pigment depositional rates (Figure 3.2) in the uppermost portions of the sediment cores collected from Lake Diefenbaker, representing recently deposited materials (2009 to 2011/12), are consistent with the general paradigm that reservoir production is maximal in mid-reservoir transitional zones (e.g. between Sites 3 and 6). Modern algal biomass as inferred from accumulation rates of total pigment and Chl *a* in the surficial sediments (between 0−9 cm, depending on core chronology) increased with distance down-reservoir, with greatest concentrations occurring at Sites 5 and 6 and least production occurring furthest up-reservoir (Sites 1 and 2) and down-reservoir (Sites 7 and 8). Although, phytoplankton community composition has been found to be similar throughout this region, phytoplankton biomass in the water column tends to be greatest near the fish farm (Abirhire et al., 2015), which is proximate to our sediment core sampling Sites 4 through 6. It is possible that fish farm operations have influenced primary productivity in this region and hence sedimentary pigment concentrations; however, a detailed assessment found no evidence of spatial trends in phytoplankton as a result of fish farm operations (2009 and 2010 sampling years).

Analysis of bulk sediment deposition rates (J. Johansson, University of Saskatchewan, 2014, personal communication; Saskatchewan Environment and Public Safety and Environment Canada [SEPS and EC], 1988) for the longitudinal axis of Lake Diefenbaker showed that Sites 1 and 2 are located within a steep spatial gradient of sedimentation rates (i.e. within the transitional zone). Based on SEPS and ECCC (1988) and Sadeghian et al. (2015), the majority of materials

carried into the reservoir are deposited prior to Site 3. Trends in total organic carbon, nitrogen, organic carbon to nitrogen ratio (shown in Figure 3.3 for comparison to pigment trends), $\delta^{13}C$ and $\delta^{15}N$ values measured in surficial sediments in the same cores as analyzed in the present study also suggested that sediment parent materials shift in character from allochthonous materials up-reservoir (Sites 1 and 2) to autochthonous matter down-reservoir (Lucas et al., 2015a). Parent materials have similarly shifted in character from allochthonous to autochthonous over time at mid- and down-reservoir sites, with the Qu'Appelle arm showing the least change in sediment physico-chemistry over time. Therefore, it is likely that the lesser concentrations of total pigments at Sites 1 and 2 were due not only to lower productivity in this up-reservoir area, but were also a function of dilution with pigment-poor allochthonous organic matter in the bulk sediment and possibly advective currents carrying autochthonous phytoplankton remains further down-reservoir. With reduced deposition of allochthonous materials with distance down-reservoir, pigment concentrations per unit OC will increase, as autochthonous materials are less diluted with pigment-poor organic matter. This can create the appearance of increasing primary production with distance down-reservoir, regardless of actual production. However, the effect of dilution should be much reduced down-reservoir of Site 3 and pigment concentrations or deposition rate monotonic with distance. The consistency between our observed trends and the trends of algal biomass in the water column (Abirhire et al., 2015) suggest that greater accumulation rates of both Chl *a* and total pigments at mid-reservoir locations are likely due, at least in part, to a primary production maximum in this region. These trends were also supported by increasing Secchi disk depth and light availability in the mid-reservoir compared to the up-reservoir (Dubourg et al., 2015) due to reduced inorganic turbidity. Gross primary productivity was less in more turbid areas (e.g. up-reservoir, nearer to the main inflow) and increased in the more lacustrine areas down-reservoir where light penetration was deeper (Dubourg et al., 2015). Nevertheless, reduced dilution of sediment pigment content with allochthonous materials cannot be ruled out as a factor in the observed spatial trends.

Of the sites investigated, Sites 5 and 6 were observed to have the greatest concentrations of total pigments (up to 2141 nmol pigment g^{-1} OC; Figure 3.5A and 3.5B) and greatest pigment deposition rates (up to 16910 nmol m^{-2} $year^{-1}$) in Lake Diefenbaker, which suggests greater biomass of algae in this region (Figure 3.3). With regard to specific taxa, the greater concentrations and deposition rates of Cryptophyta (inferred from alloxanthin) and

Bacillariophyta/Chrysophyta (inferred from diatoxanthin and fucoxanthin) at Sites 5 and 6 are supported by Abirhire et al. (2015), who found that the largest biomass in the water column (2011 and 2012 data) for Cryptophyta and Bacillariophyta occurred at similar mid-reservoir locations.

Although the greatest algal biomass (measured as Chl *a*) has been observed in proximity to transitional locations (Kennedy et al., 1982), our results demonstrate that maximum biomass of certain taxa, such as colonial and filamentous cyanobacteria (Figures 3.5C and 3.5D) can occur in other regions of a river valley reservoir. For example, increased algal biomass was observed for chemically stable (e.g. alloxanthin, diatoxanthin and echinenone) and relatively labile pigments (fucoxanthin and Chl *a*) (Hall et al., 1999) in the Qu'Appelle arm within approximately the last 22 years. This is discussed further below.

3.5.2. Temporal trends in algal preservation and concentration

The trophic upsurge hypothesis was not supported by analysis of fossil pigment trends in the sediment cores, as there were no initial increases in pigment accumulation rate or concentration followed by a depression, which would be the expected pattern (Thornton et al., 1990). It is possible that a small trophic-upsurge may have occurred near the mouth of the Qu'Appelle arm (e.g. based on data from Hall et al., 1999), but it appears to have been minor compared with the much greater pigment concentrations observed in the more recent layers of sediment. Overall, it might be that high turbidity in the early years after reservoir formation limited the availability of light, and hence restricted primary productivity. Light remains a common limiting factor for primary production in Lake Diefenbaker (Dubourg, et al., 2015).

Pigments have been used successfully to infer changes in trophic state, algal abundance, and changes in lake production (Engstrom et al., 1985; Waters et al., 2005). However, sedimentary pigments are subjected to natural degradation processes within lake sediments, leading to differential preservation, which can result in changes to pigment concentrations throughout a sediment core (Riedinger-Whitmore et al., 2005). Such changes can confound temporal ecological interpretations. Fortunately, several lines of evidence suggest that overall preservation was adequate for reliable interpretation of pigment trends at all sites investigated in Lake Diefenbaker. First, highly labile pigments such as fucoxanthin (siliceous algae) and ubiquitous Chl *a* were present at concentrations well above detection limits in all cores. Second,

spatial patterns of Chl *a*:Phaeo *a* in the deeper portions of the sediment profile suggested that there was little relationship between preservation of pigments and core position in the lake. Third, comparison of concentrations of Chl *a* and Phaeo *a* can provide an assessment of sediment diagenesis (Vinebrooke et al., 2002). Relatively greater concentrations of Phaeo *a* concentrations and lesser Chl *a* concentrations (lesser Chl *a*:Phaeo *a* ratio) are expected if diagenetic processes have altered the pigment distribution during sinking or in sediments (Das et al., 2005). Chl *a:*Phaeo *a* ratios (Figures 4 and 5) do not suggest strong diagenetic processes occurring within the collected sediment cores, especially at upstream Sites 1 and 2 where sedimentation was greatest. Thus, increased deposition, at these up-reservoir locations, could results in higher preservation. Furthermore deposition during the winter could lead to better preservation due to slower bacterial processes in the sediments. Therefore, the observed increase in this ratio up-core might be attributed to an increase in recent phytoplankton abundance and resulting incomplete degradation of labile pigments (e.g. Chl *a* and fucoxanthin) (Reuss et al., 2010). Taken together these patterns suggest that comparison of pigment concentrations among sites can provide useful insights regarding the spatial and temporal variation of past algal populations.

Comparison of up-, mid-, and down-reservoir sites suggested few consistent historical trends in algal biomass along the entire longitudinal axis of Lake Diefenbaker. For example, pigment concentrations in cores from Sites 1-3 showed no interpretable trends in inferred algal biomass during the period of time encompassed by these cores (8, 13 and an unknown number of years for Sites 1, 2 and 3, respectively). In general, the greater variation in pigment concentrations at sites 1 and 2 relative to other sites suggests that there may have been hiatus or changes in preservation status in sediments at these sites (Figure 3.4A and 4B), possibly due to variations in chemical stability among pigments (Bianchi et al., 2002), as well as, pre-depositional (i.e. photo-oxidation, grazing, microbial decay) and post-depositional (i.e. bioturbation oxygen concentrations, microbial decay) processes (Leavitt and Hodgson, 2001). Given the short time period represented by these core stratigraphies, some seasonal variability in taphonomic processes may also have been captured. Conversely, clear increasing trends of concentrations of pigments were observed at Sites 4 through 8 with inferred algal biomass being greater in the upper half of the cores profiles.

Clay content has generally decreased in the sediment profile over time at Sites 4 to 7 in Lake Diefenbaker (Lucas et al., 2015a), suggesting diminishing clay in the water column. If light

is a limiting factor, this could serve to increase primary production over time in these down-reservoir areas. Increasing the concentration of inorganic suspended solids (mg L^{-1}) while holding TP concentrations constant has been found to significantly decrease chlorophyll *a* concentrations in the water column in reservoirs (Hoyer and Jones 1983). However, increased chlorophyll per unit biomass has been observed to be a common response to decreased light intensity (Nicholls and Dillon 1978), such as that imposed by suspended inorganic materials (i.e. clay). Therefore, increasing water clarity in the presence of other limiting factors (i.e. P limitation) could decrease Chl *a* concentration per g OC. Nevertheless, clay content in the sediment profile was not statistically correlated with Chl a concentration ($p > 0.05$; $n = 9$ to 16), suggesting that temporal changes to algal biomass at Sites 4 to 7 were not linked to changes in water clarity due to suspended inorganic clay content.

As a result of river-valley reservoir hydrology, up-reservoir areas in Lake Diefenbaker (e.g. Sites 1 and 2) were more turbid than mid- and down-reservoir regions (Dubourg et al., 2015). Therefore, light limitation up-reservoir has likely persisted over time and limited changes to primary production due to any other environmental factors. Although some of the inflow at Site 2 is from Swift Current Creek (population of 17,535; Statistics Canada, 2011), which contains municipal effluent, trends in concentrations of pigments do not suggest that Swift Current Creek has had a measurable effect on local algal production in the lake. This finding could be due to: i) the minor nutrient contribution of this creek (J. Lawrence University of Saskatchewan, 2014, personal communication) compared to the total flow of the reservoir (<1%; Water Security Agency, 2012); ii) light being the limiting factor at this site; or iii) changes to algal biomass being recorded in sediments further down-reservoir.

Profiles of concentrations of fossil pigment for the Qu'Appelle arm varied little before ca. 1990, but the concentrations of various pigments increased substantially after 1990. Of the sites studied in Lake Diefenbaker in Lucas et al., (2015a), bottom sediment at this site has changed the least in its physicochemical properties over time (i.e. allochthonous versus autochthonous content). Therefore, observed temporal trends in pigment concentrations can be attributed to changes in algal biomass with greater confidence relative to other sites. In addition, the very strong correlations between both Chl *a* and total pigment concentrations with concentrations of other biological proxies such as more biologically available phosphorus (P) (non-apatite inorganic P + organic P, data from Lucas et al., 2015a) that are typically of non-detrital origin

(Hiriart-Baer et al., 2011) and reactive particulate silica (PRSi) (Maavara et al., 2014), support an interpretation of increasing primary production in the Qu'Appelle arm after 1990 (Figure 3.6).

The presence of myxoxanthophyll at Site 8 shows some consistency with findings reported by Hall et al. (1999) where an observed increase in myxoxanthophyll was noted in the mouth of the Qu'Appelle arm. This trend was not observed in the Qu'Appelle arm in this study; however, Hall et al. (1999) collected their sediment core closer to the junction of the Gardiner and Qu'Appelle arms. Phytoplankton biomass in the present study was inferred to have increased in the early 1990s at Sites 7 and 8 (consistent with Hall et al., 1999), rapidly reaching relatively stable values in the upper half of each core (particularly at Site 8). Although speculative, the observed elevated but stable algal biomass at Site 8, as well as the presence of potentially harmful colonial cyanobacteria (as myxoxanthophyll) is consistent with the trends observed during two decades of summer water quality monitoring (McGowan et al., 2005; Patoine et al., 2006; Vogt et al., 2011). Algal production in the Qu'Appelle arm of Lake Diefenbaker has varied coherently with other lakes in southern Saskatchewan, but has not exhibited a marked increase since the mid-1990s (Vogt et al., 2011). These increased trends in cyanobacterial pigments may be due to years of little mixing and periods of increased water temperature and thermal stratification; however, periods of drought have been relatively brief compared to the sustained trends observed at Site 7.

Finally, greater concentrations of algal pigments in more recent sediments at Site 7 were consistent with anecdotal observations of increased surface bloom occurrence reported by local residents in recent years. Increasing concentrations of myxoxanthophyll in the Gardiner arm (this study) and near the mouth the Qu'Appelle arm (Hall et al., 1990; Vogt et al., 2015), suggest a greater presence of potentially harmful cyanobacteria (Leeben et al., 2008; Riedinger-Whitmore et al., 2005; Swain, 1985). Myxoxanthophyll and some of its variants have been associated with toxic cyanobacteria (Schlüter et al., 2004) and have been strongly correlated with microcystin content in aquaculture impoundments (Zimba 2008).

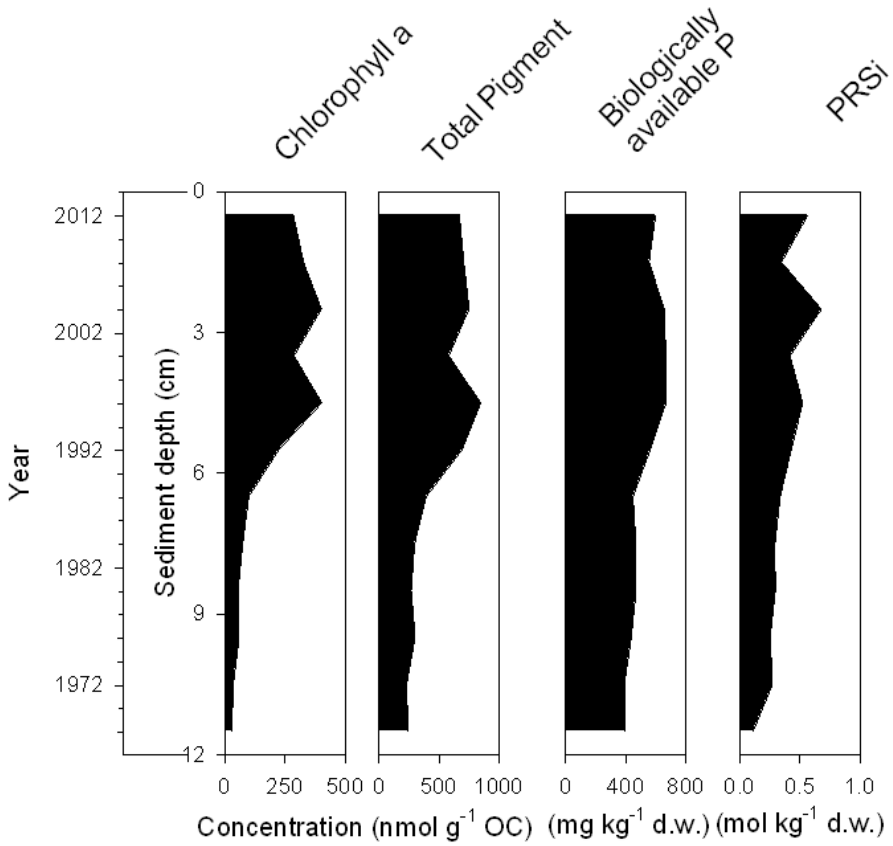

Figure 3.6. Sediment temporal trends for the Qu'Appelle arm (Site 8), Lake Diefenbaker, Saskatchewan, Canada, for chlorophyll *a* (nmol g^{-1} OC), the more biologically-available P fraction (non-apatite inorganic P + organic P, mg kg^{-1} d.w.), and reactive particulate silica (PRSi, mol kg^{-1} OC d.w.).

As demonstrated in this study, hydrological zonation in a narrow river-valley reservoir can influence algal production and community composition, with certain taxa preferring the environmental conditions of particular regions (e.g. colonial and filamentous cyanobacteria). For reservoirs having limited historical monitoring data, paleolimnological analysis of sedimentary pigments can provide management with insights into spatiotemporal trends of primary production and taxonomic distribution as a reservoir ages. This type of investigation can potentially identify localized or reservoir-wide issues of emerging concern, such as increasing toxin-producing cyanobacteria in the Gardiner arm of Lake Diefenbaker, and help management design focused monitoring efforts to validate and directly monitor variables of concern to current or future intended water uses.

3.6. Conclusions

Trends in our sedimentary pigment data were consistent with other studies that have assessed algal abundance and community composition in the water column of Lake Diefenbaker (Abirhire et al., 2015), with maximum total algal biomass occurring at mid-reservoir locations (i.e. in the transitional zone) and community dominance of Bacillariophyta/Chrysophyta and Cryptophyta. The trend in maximum total algal biomass, as inferred from sedimentary pigment deposition rates was consistent with the spatial pattern anticipated in narrow, river-valley reservoirs. Therefore, spatial trends in algal biomass and production in the water column are reflected in the materials entrained in reservoir sediments.

The prevailing reservoir paradigm predicts an initial trophic upsurge upon reservoir formation (Thornton et al., 1990). However, profiles of concentrations of pigments observed in this study showed no clear trends to support an initial elevation of phytoplankton biomass compared to later years. Given that portions of Lake Diefenbaker are often light limited (Dubourg et al., 2015), the absence of such a trend in the pigment record could be due to light limitation during the early years after reservoir formation. If an initial trophic upsurge did occur in down-reservoir regions, algal production was much less compared to more recent years.

The results of this study suggest that some cyanobacteria taxa did not follow the general paradigm regarding the distribution of alga biomass in this type of reservoir (i.e. greatest concentrations in the transitional zone). Myxoxanthophyll, a biomarker of filamentous and colonial cyanobacteria, was detected only in the sites furthest down-reservoir, the Gardiner (Site 7) and Qu'Appelle (Site 8) arms. The trend of increasing concentrations of myxoxanthophyll in the Gardiner arm suggests increasing productivity of potentially harmful cyanobacteria species. If the observed trend in cyanobacteria production continues, the water quality of this important water source could degrade in the future.

CHAPTER 4

Reconstructing the cyanobacterial community in a small eutrophic Prairie lake using 16S and 23S metagenomic sequencing

NOTE TO THE READERS – CHAPTER 4

Chapter 4 is currently being prepared for publication; however, the journal has not yet been selected. This manuscript will focus mainly on the findings from the 16S rRNA gene sequencing. The principle topic will be using high-throughput next-generation sequencing as a paleolimnological approach to reconstruct past trends in cyanobacteria community composition in the eutrophic Buffalo Pound Lake, Saskatchewan.

The author contributions to Chapter 4 were as follows:

- Timothy Tse (University of Saskatchewan) collected, processed, performed biomolecular and next-generation sequencing on samples, analyzed sample data, performed statistical analyses, created visual aids (e.g. figures) and drafted this chapter/ manuscript.
- Song Tang (University of Saskatchewan) helped with downstream bioinformatic analyses, including statistical operations and creating visual aids (e.g. figures).
- John Giesy (University of Saskatchewan) and Markus Hecker (University of Saskatchewan) provided the laboratory facilities and instruments to extract and sequence the samples.
- Paul Jones (University of Saskatchewan) and Lorne Doig (University of Saskatchewan) reviewed, revised and provided essential feedback in the drafting of the manuscript.

CHAPTER 4

Reconstructing the cyanobacterial community in a small eutrophic Prairie lake using 16S and 23S metagenomic sequencing

4.0 Preface

Many inland waters lack sufficient long-term monitoring to assess trends in the occurrences and intensities of harmful algal blooms. Furthermore, algal pigment analyses can only provide broad taxanomic identification, and cannot give high resolution insight into the presence of potentially harmful cyanobacterial genera. Fortunately, metagenomic sequencing of environmental DNA (eDNA) in cores of sediment is an emerging technique (Kyle et al., 2015; Domaizon et al., 2013; Hou et al., 2014) to reconstruct the ecological timeline of inland waters, and is rapidly gaining momentum in the fields of limnology and paleolimnology. As it was unknown whether preserved eDNA could be successfully extracted from sediment cores collected from mesotrophic Lake Diefenbaker, a eutrophic Prairie lake (Buffalo Pound Lake, Saskatchewan) was chosen for eDNA method development purposes. This chapter discusses the methodology used to successfully extract and sequence eDNA from the sediment of Buffalo Pound Lake, and to compare among different primers targeting conserved variable regions 16S and 23S to infer temporal trends in cyanobacterial diversity. In addition, algal toxins are not necessarily well preserved in sediment. Therefore the presence and abundance of the *mcyA* gene was also investigated to assess trends in potential for toxin production. The methodology described in this chapter was then applied to two sediment cores collected from the Qu'Appelle and Gardiner arms of Lake Diefenbaker, Saskatchewan (principle study site) to identify and reconstruct trends in the cyanobacterial community and to identify potentially nuisance genera (Chapter 5).

4.1. Abstract

High-throughput next-generation sequencing techniques can provide limnologists with a means to rapidly identify potentially problematic organisms, such as those found in harmful algal blooms. Early identification of changes in the composition of the cyanobacteria community can help inform water management, especially if toxic cyanobacteria are a recurring problem. Analysis of environmental DNA preserved in cores of sediment is emerging as a means to

reconstruct historical trends in cyanobacterial assemblages with greater resolution compared to traditional methodologies (e.g. light microscopy). In this study, targeted high-throughput sequencing was applied to a sediment core to reconstruct trends in cyanobacteria diversity and to identify the presence of potentially toxic genera in the eutrophic Buffalo Pound Lake, Saskatchewan. Sequencing of the 16S and 23S rRNA genes revealed different cyanobacterial assemblages. Diversity indices (α- and β) illustrated significant cyanobacteria community-level changes over time, including a significant increase in the potentially harmful genus *Planktothrix* in more recent sediments. The abundance of the microcystin synethetase *A* gene confirmed the presence of potentially toxic cyanobacteria and that the biomolecular machinery to produce microcystins has been present since reservoir formation. These findings demonstrate a novel means to infer long-term dynamics of the cyanobacterial community in inland waters and highlights the power of paleo-16S-high-throughput sequencing to rapidly identify problematic organisms with high resolution.

4.2. Introduction

Cultural eutrophication has led to incidences of cyanobacterial harmful algal blooms (HABs) in many freshwater systems (Shaw et al., 2003; Hudnell and Dortch 2008), including Prairie lakes (Barica 1993). Cyanobacteria, or blue-green algae, are a group of prokaryotic, photosynthetic bacteria that are frequently associated with water quality degradation. Cyanobacteria can live in a diverse range of environments including salty, brackish or fresh waters, cold and hot springs and environments where no other microalgae can exist (Mur et al., 1999). Their ability to colonize environments of extreme salinity and temperature (Chorus and Bartram 1999) is well documented, as is their potential to produce a variety of lethal toxins. These organisms become increasingly dominant as concentrations of total phosphorus and total nitrogen increase during eutrophication of lakes, rivers and estuaries (Havens 2008). This can become problematic in extremely shallow lakes (<2 m), where dominance of cyanobacteria can persist if the ratio of photic depth to mixed depth never falls to levels that prevent net growth of low-light adapted taxa (i.e. cyanobacteria), but remains low enough to exclude other plankton (Havens 2008).

Some cyanobacteria are capable of producing neuro- (e.g. saxitoxins and anatoxins) and hepato-toxins (e.g. microcystins), cytotoxins (e.g. cylindrospermopsins) and dermatoxins

(lyngbyatoxins) (Kurobe et al., 2013). Most of these toxins also have the ability to biomagnify within the food web, resulting in exposure and adverse effects to higher trophic level organisms (Cox et al., 2003). In addition to toxin production and associated risks to wildlife and humans, increased abundance of cyanobacteria activity within a reservoir can increase the production of taste-and-odour compounds such as trans-1,10-dimethyl-trans-decalol (geosmin) and 2-methylisoborneol (MIB), which are responsible for musty, earthy tastes and odours in drinking water (Journey et al., 2013). Many of these compounds are problematic for water management agencies, as conventional water-treatment procedures are not normally robust enough to remove these compounds (Suffet et al., 1996). Therefore, increasing abundance of cyanobacteria in water supplies can have multiple implications for the water treatment processes required prior to use.

Early identification of emerging trends in the abundance of potentially harmful cyanobacteria is critical in managing water supplies. In areas where long-term monitoring data are absent or lacking, reconstruction of the historical cyanobacterial community can provide insight into past or recent trends in harmful cyanobacteria taxa and their relative abundances (Chapter 5), as well as identify the presence or absence of invasive species. Further, when coupled with paleo-variable measurements, these techniques can provide a powerful tool in investigating and identifying relationships between environmental conditions and observed trends in cyanobacterial community composition (Chapter 5).

Reconstructive techniques for algae and cyanobacteria typically involve the analysis of sediment cores for pigment content (Chapter 3; Leavitt and Hodgson 2001) or taxonomic identification of physical remains (e.g. diatom remains) using microscopy. These techniques can be time consuming, limited to certain taxa (e.g. diatoms) or only provide broad taxonomic information (e.g. pigments). Because of the potential for taxonomic specificity across a diverse range of organisms, metagenomic sequencing is becoming more common as an alternative approach to assessing the environment, past or present.

To date, the 16S rRNA gene sequence (Nübel et al., 1996) has been the most common means to identify prokaryotic organisms, including cyanobacteria. However, a primer pair was recently developed to amplify the 23S rRNA region (Sherwood and Presting 2007), as cyanobacteria and chloroplasts of plants and algae are believed to share a common ancestor. The modern chloroplast is a remnant of an endosymbiosis between a eukaryotic cell and an ancestral oxygenic photosynthetic prokaryote (Florencio et al., 2006). This opens new possibilities to

identify cyanobacteria and eukaryotic algae using a single primer pair, thereby allowing comparisons of relative abundance across these taxa (Sherwood and Presting 2007).

In this study, the cyanobacterial community composition was reconstructed within a sediment core collected from a eutrophic Prairie lake, Buffalo Pound Lake, Saskatchewan, Canada. The original intent was to compare between eukaryotic algae and prokaryotic cyanobacteria using a single primer pair. However, because sequencing of the 23S rRNA region failed in this study to identify eukaryotic taxa in the preferred database (SILVA), the aim of this study component was modified to compare cyanobacterial community taxa richness and evenness and through time (α- diversity) using both the 16S and 23S gene sequences to assess methodological concordance. Finally, the presence of the *mcyA* gene, a gene component involved in microcystin production, was investigated and its relative abundance was measured, using fluorescence techniques, to assess whether the conditions (i.e. biomolecular machinery) to produce toxins have been historically present.

4.3. Methods

4.3.1. Study area and sediment core collection

Buffalo Pound Lake (50°38'840" N, 105°30'323" W) (Figure 4.1) is a shallow natural water body, created 10 000 years ago from the glacial spillways that formed the Qu'Appelle Valley (SERM 2001). The outflow of the original shallow lake was dammed in 1952 (Hall et al., 1999), resulting in the conversion of this water body to a reservoir. Buffalo Pound Lake is approximately 29 km long, with a width of 1 km (Buffalo Pound Water Treatment Plant 2015), and a volume of 91 987 cubic decametres (SWSA 2016). The majority of the water supply is from the Qu'Appelle arm of Lake Diefenbaker, a reservoir located approximately 60 km to the north-west. Buffalo Pound Lake has a maximum depth of 5.6 m and mean depth of 3.0 m (Hammer 1971) and is regulated to supply water to 25% of Saskatchewan's population, including the cities of Regina and Moose Jaw and several small communities (SWSA 2016; McGowan et al., 2005). This lake is characterized by regular blooms of planktonic cyanobacteria during the summer, and deepwater anoxia is common during winter and late summer (McGowan et al., 2005).

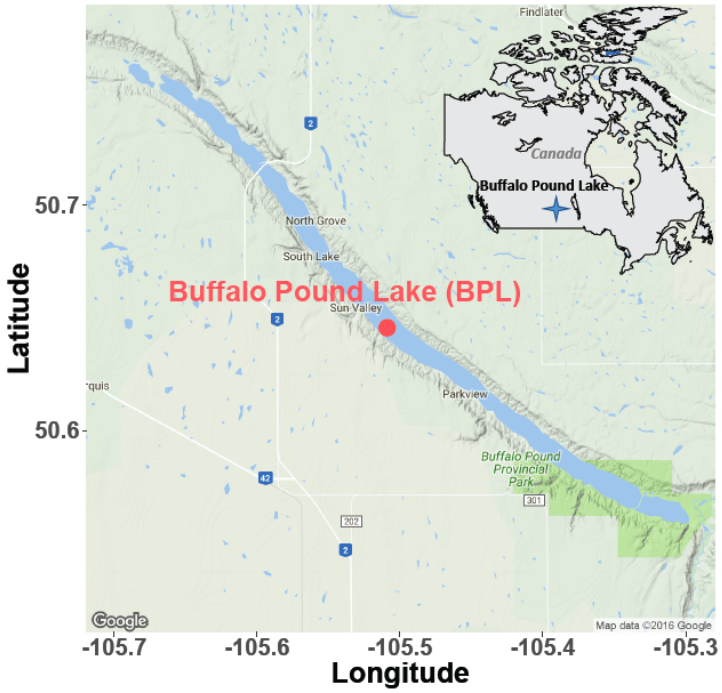

Figure 4.1. Sediment core location (red dot) in Buffalo Pound Lake, Saskatchewan, Canada collected in October, 2013. The location of Buffalo Pound Lake in Canada is indicated by a blue star.

Recent increases in surface bloom occurrence have resulted in public notification to avoid eating fish and avoid contact with the water in the summer of 2016 (Sharpe 2016; CBC News 2016). Previous analyses of water samples collected in the summer and fall of 2015 revealed the presence of microcysin-LR and –RR (H. Peng, unpublished data, University of Saskatchewan, 2015). Concentrations of these compounds (MC-LR and MC-RR) were found to be close to, or exceeding Health Canada's drinking water guidelines of 1.5 μg/L (Health Canada 2002; H. Peng, unpublished data, University of Saskatchewan, 2015). The concentration of total microcystins in the water column in the summer of 2015 ranged from 1.36 to 2.62 μg L^{-1} (H. Peng, unpublished data, University of Saskatchewan, 2015).

A 31-cm long sediment core was collected in October, 2013, from the deepest location identified in Buffalo Pound Lake (~4 m depth, Figure 4.1), using a Glew Maxi Gravity Corer (John Glew, Kingston, Canada). The core was transported to the laboratory at the Toxicology Centre, University of Saskatchewan, Canada, and immediately sectioned into 1-cm layers under nitrogen gas using plastic spatulas cleaned with 70% ethanol and nano-pure water between subsections. The outer ~0.5 to 1.0 cm rims of the subsections were discarded prior to removing the inner material of each section to avoid contamination that might result from contact with the acrylic tube. The samples were then stored in 50-mL sterile falcon tubes at -20 °C, in the dark.

4.3.2. Total Organic Carbon

Approximately 0.2 g of dried sediment was pulverized from each 1-cm section using a mortar and pestle and fumigated for 48 h with 12M HCl. Following acid fumigation, the samples were dried at room temperature for 48 hrs then placed in a drying oven at 105 °C overnight. Total organic carbon was measured on a LECO C-632 (Missouri, USA) fitted with antimony and chlorine scrubbers. Combustion temperature was set at 1100 °C.

4.3.3. Radiometric dating

Radiometric analysis was performed to quantify sediment deposition rate and facilitate sediment dating. Sediment sections 1 cm, 2 cm, 4 cm, 5 cm, 7 cm, 10 cm, 11 cm, 13 cm, 15 cm, 20 cm, 25 cm and 31 cm were analyzed for Pb-210 and Cs-137. Sediment sections 7 cm, 20 cm and 31 cm were analyzed for Ra-226. All radiometric analyses were performed by Flett Research Limited (Winnipeg, MB, Canada). Decay counts were collected using an 'Octet' alpha

spectrometer and HPGe gamma detector (Ortec, Oak Ridge, TN, USA). The method used to determine the decline of Pb-210 throughout the sediment profile is based on the method of Eakins and Morrison (1978). This method isolates Po-210, a decay product of Pb-210, through distillation, acid digestion, and plating onto silver disks, where it is quantified with an Ortec secular equilibrium with Pb-210 (i.e. the same activity). The constant rate of Pb-210 supply (CRS model) was then used to estimate sediment dates (Smol 2008). A quality check for Pb-210 analysis was performed by also analyzing for Cs-137, a product of nuclear weapon testing. Cs-137 is measured by counting the gamma emissions at 662 KeV. Radium-226 measurements were made to estimate background Pb-210.

4.3.4. DNA extraction and sequencing of 16S and 23S rRNA

DNA was extracted from a 1-g wet weight subsample of each sediment core increment using E.Z.N.A.® Soil DNA Kits (Omega Bio-tek, Georgia, USA) in accordance with the manufacturer's specifications. The quality and quantity of the isolated DNA were measured using a Nanodrop 1000 Spectrophotometer (Thermo-Fisher, USA). DNA samples were frozen at -80 °C until further analysis. The universal cyanobacterial primers CYA359F (5'-CGGACGGGTGAGTAACGCGTGA-3'), CYA781R(A) (5'-GACTACTGGGGTATCTAATCCCATT-3') and CYA781R(B)(5'-GACTACAGGGGTATCTTATCCCTTT-3') (Nübel et al., 1997) with attached Illumina bar-code overhangs were used to amplify the 379 bp fragments corresponding to the V3 and V4 regions of the bacterial small subunit ribosomal RNA (16S rRNA) (Boutte et al., 2006). The reverse primers CYA781R(A) and CYA781R(B) targeted mainly heterocystous cyanobacteria and Chroococcales-Oscillatoriales, respectively (Loza et al., 2013). Reaction solutions for PCR were made according to Illumina's 16S Metagenomic Sequencing Library Preparation (Part # 15044223 Rev. B) with 25 ng of environmental DNA used as template. The reverse primers CYA781(A) and (B) were used separately as they target filamentous and unicellular cyanobacteria, respectively, and their separate use allows the two different types of populations to be revealed (Coutte et al., 2006). However, there is cyanobacteria community overlap between the two reverse primers. PCR reactions included denaturing at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30s, extension at 72 °C for 30s and finally, an extension at 72 °C for 5 min.

The universal primer pairs amplifying the 23S rRNA plastid marker in eukaryotic algae and cyanobacteria were p23SrV_f1 (5'-GGACAGAAAGACCCTATGAA-3') and p23SrV_r1 (5' TCAGCCTGTTATCCCTAGAG-3') (Sherwood and Presting 2007). The PCR reactions were the same as above. PCR product size and integrity, for both the 16S and 23S amplification processes, were verified on a Bioanalyzer DNA 1000 chip using a 2100 Bioanalyzer Instrument (Agilent Technologies, California, USA). Next-generation sequencing was then performed on a MiSeq Desktop Sequencer (Illumina, California, USA) using a 2x300-bp paired-end protocol at the Toxicology Centre (University of Saskatchewan, Saskatoon, SK).

4.3.5. Sequencing data analysis

Trimming and selection of high-quality sequences were performed using the *Mothur* software package (v 1.18.1; Schloss et al., 2009). Only sequences ≥200 bp in length with no ambiguous bases and no homopolymer stretches of more than 7 bp were retained. Quality-checked sequences were aligned using the SILVA LSU reference alignment (Pruesse et al., 2007) for the 23S rRNA sequences and the SILVA SSU reference alignment for the 16S rRNA sequences. For all comparative analyses performed with sequences binned into Operational Taxonomic Units (OTUs), sequences were clustered (average neighbor algorithm) at 97% sequence identity. This definition was chosen as it has previously been associated with approximately species level differences in genomic DNA (Stackebrandt and Goebel 1994), it has been shown to limit the inflation of diversity estimates that have been associated with pyrosequencing (Hugenholtz et al., 2010), and it is greater than the known sequence divergence for most organisms that encode multiple 23S rRNA genes (Pei et al., 2009). The 99% OTU definition was maintained for calculations of coverage to give a more conservative estimate of the coverage of genetic diversity.

4.3.6. *mcyA* gene abundance

The previously reported primers of mcyA-Cd 1R (5'-AAAAGTGTTTTATTAGCGGCTCAT-3') and mcyA-Cd 1F (5'-AAAATTAAAAGCCGTATCAAA-3') were used to investigate the presence of the *mcyA* gene, a component of the biomolecular machinery involved in the production of microcystins (Beversdorf et al., 2015). Samples were removed every 5 cycles between cycle 5 and 40 to

determine the C_t, which was determined to be around cycle 25. The PCR cycle consisted of an initial denaturation at 95 °C for 3 minutes, followed by 25 cycles of denaturing at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s, and a final extension phase at 72 °C for 5 min. The PCR product was then applied to a freshly prepared 1% agarose gel and fluorescence, inferred abundance, was determined using a Bio-Rad VersaDoc MP 4000 Molecular Digital Imaging System (Hercules, CA, USA) running Quantity One software v.4.6.7 (Bio-Rad, Hercules, CA, USA). To confirm *mcyA* gene identity, the PCR product was sequenced at the University of Calgary Core DNA Services (University of Calgary, Alberta, Canada). The gene sequence was then compared against the NCBI BLAST database (nr/nt nucleotide collection).

4.3.7. Statistical analysis

All statistical analyses were completed using R software v 3.3.1 and differences were considered significant when $p<0.05$. Non-bacterial sequences were removed from all samples to obtain a strictly bacterial data set. Cyanobacteria sequences were separated from this data set to determine relative abundances of cyanobacterial genera within each sample. Numbers of shared bacterial or cyanobacteria OTUs among the 16S and 23S primers were determined using Venn diagrams in the "VennDiagram" package. A Monte-Carlo test with 9999 permutations was used for validating results of co-inertia analysis, a multivariate method that identifies trends or co-relationships in multiple data sets. α-diversities (number of observed OTUs and Shannon-Wiener index) for cyanobacteria were determined using "phyloseq" (McMurdie and Holmes 2013) and measures cyanobacterial community differences among sediment increments. For β-diversity (Bray-Curtis dissimilarity index), to avoid biases generated by different sequencing depths, all samples were normalized to the minimum number of total bacterial reads (10170) among all sequencing libraries. This analysis was used to investigate community composition differences across sediment sections. Reads of cyanobacteria communities were then separated from these normalized libraries.

4.4. Results

Preserved eDNA was successfully extracted from the complete temporal profile of the sediment core collected from Buffalo Pound Lake. Concentrations of eDNA ranged from 4.82 to 22.29 μg g^{-1} (wet weight), with greater eDNA concentrations occurring in the more recent sediments (Figure 4.2). Diverse communities of cyanobacteria were identified through the independent amplification of reverse 16S primers (A) and (B) and the 23S primer (C). The cyanobacteria community comprised up to ~30% of the overall bacterial community (Figure 4.3, indicated by the red bar), with highest relative abundance of cyanobacteria occurring within the more recent sediments. In total, 54 cyanobacterial genera were identified using all 3 primers (Figure 4.7). After normalizing the data by removing the bottom 0.5% of sequences, 26 cyanobacterial genera remained (Figure 4.4); however, sequences matching unclassified cyanobacteria genera were abundant in the sediments tested (Figure 4.4). The two predominant cyanobacteria genera identified through 16S reverse primers (A) and (B) were *Snowella* and *Synechococcus*, respectively. *Cyanobium* was the predominant genus identified through 23S amplification. However, other, potentially toxic, cyanobacteria were also identified using all 3 primers (e.g. *Dolichospermum*, *Planktothrix*, and *Microcystis*). The *mcyA* gene abundance was variable in the temporal profile, with peak concentrations occurring in approximately the late 1970s and the late 1990s until 2009 (Figure 4.2). Percent TOC was relatively consistent over time, ranging from 5.1% to 6.6% (Figure 4.2).

4.4.1. α-diversity

α-diversity estimates demonstrated significant changes in the composition of the cyanobacterial assemblage over time (years) for all three primers in the sediment core collected from Buffalo Pound Lake (Figure 4.5). In general, sediments from more recent years had greater richness of taxa, compared to sediments from earlier years, with similar but less marked increasing trends observed in the Shannon-Wiener index (evenness) for the 16S and 23S primers (Figure 4.5).

4.4.2. β-diversity

β-diversities were significantly different among years for the 16S reverse primer pairs and the 23S primer pair (Figure 4.6), suggesting a shift in composition within the cyanobacterial community over time. Also, some dissimilarities within the community structure were observed within the temporal profile between the 16S reverse primers (A) and (B) (Figure 4.6). In addition, although the sequencing of these three primers revealed distinct cyanobacteria assemblages (Figure 4.4), there was taxonomic overlap among all three primers (Figure 4.7).

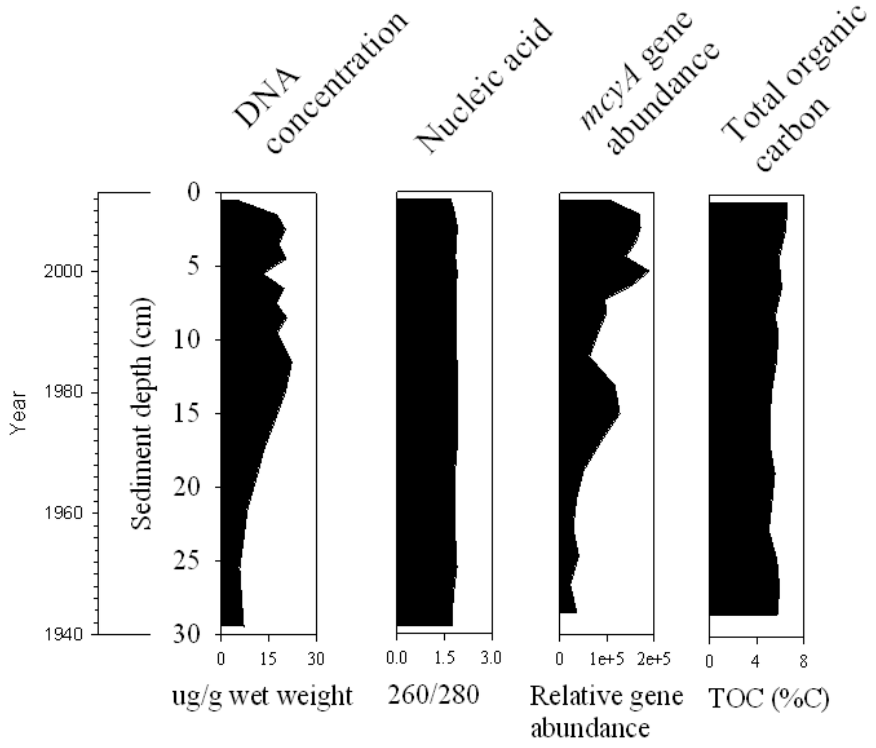

Figure 4.2. Concentrations of eDNA (μg g^{-1} wet weight), A260/280 ratio (an indicator of eDNA purity), *mcyA* gene abundance (fluorescence) and total organic carbon content (TOC) in a sediment core collected in October, 2013, from Buffalo Pound Lake, Saskatchewan, Canada.

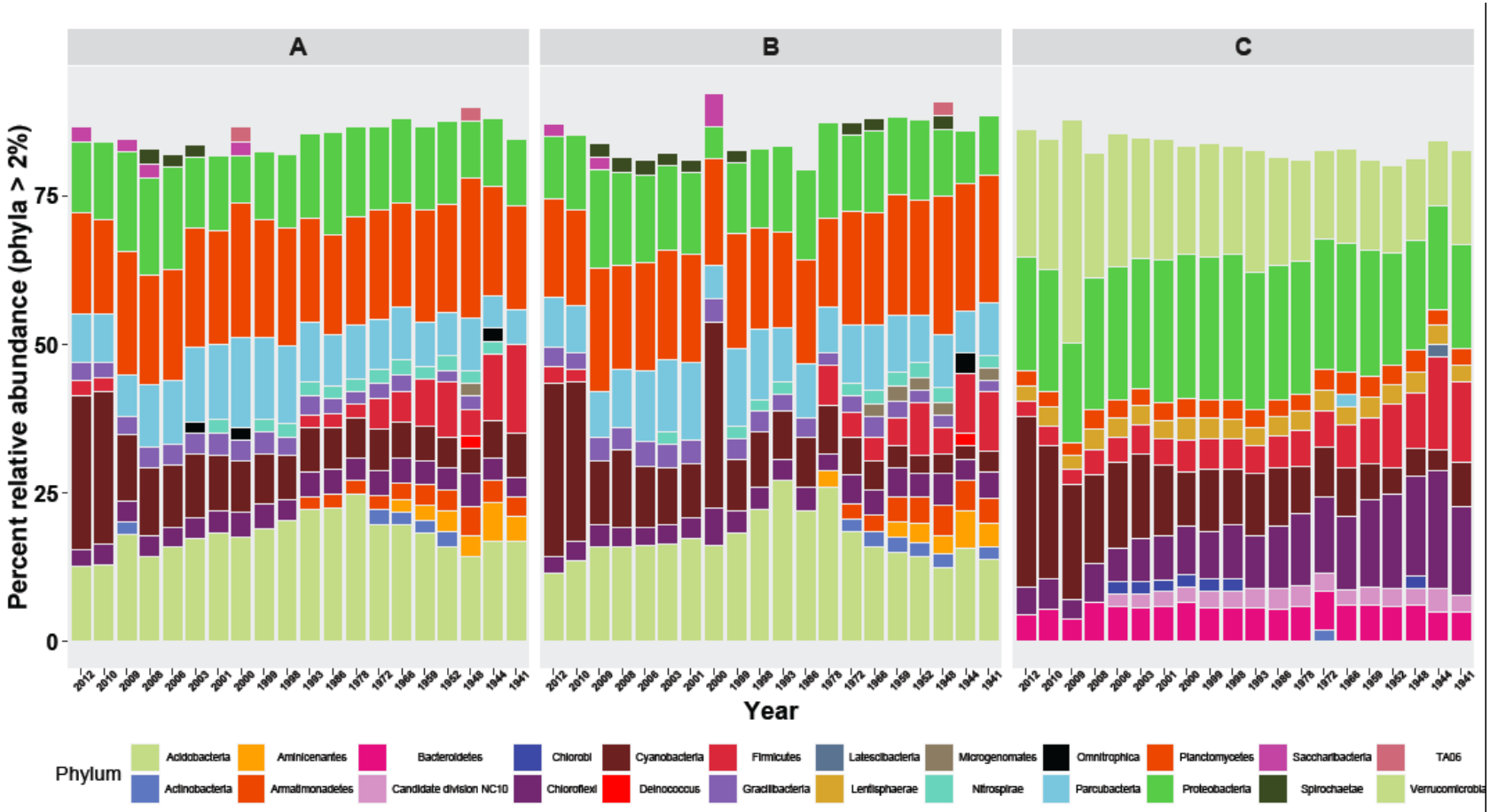

Figure 4.3. Relative abundances over time of bacterial community taxa (phylum level) observed in a sediment core collected from Buffalo Pound Lake, SK, Canada, determined using different cyanobacterial 16S rRNA primers (panels A and B) and 23S rRNA primer (panel C). Only taxa with relative abundance > 2% are shown.

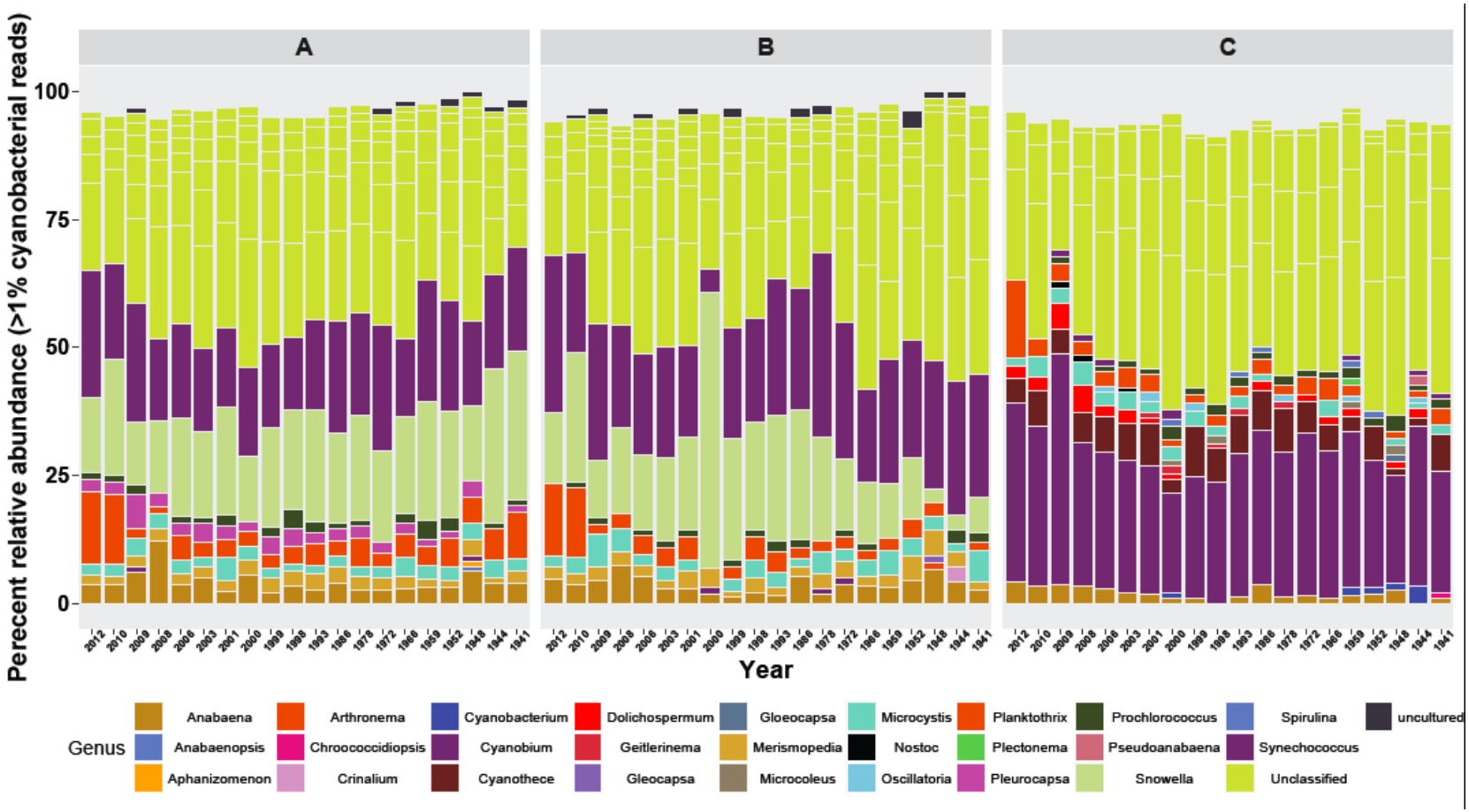

Figure 4.4. Relative abundances of gene sequences mapped to cyanobacteria genera over time observed in a sediment core collected from Buffalo Pound Lake, SK, Canada based on cyanobacterial 16S rRNA primers (panels A and B, respectively) and 23S rRNA primer (panel C). Only taxa with relative abundance > 0.5% are shown.

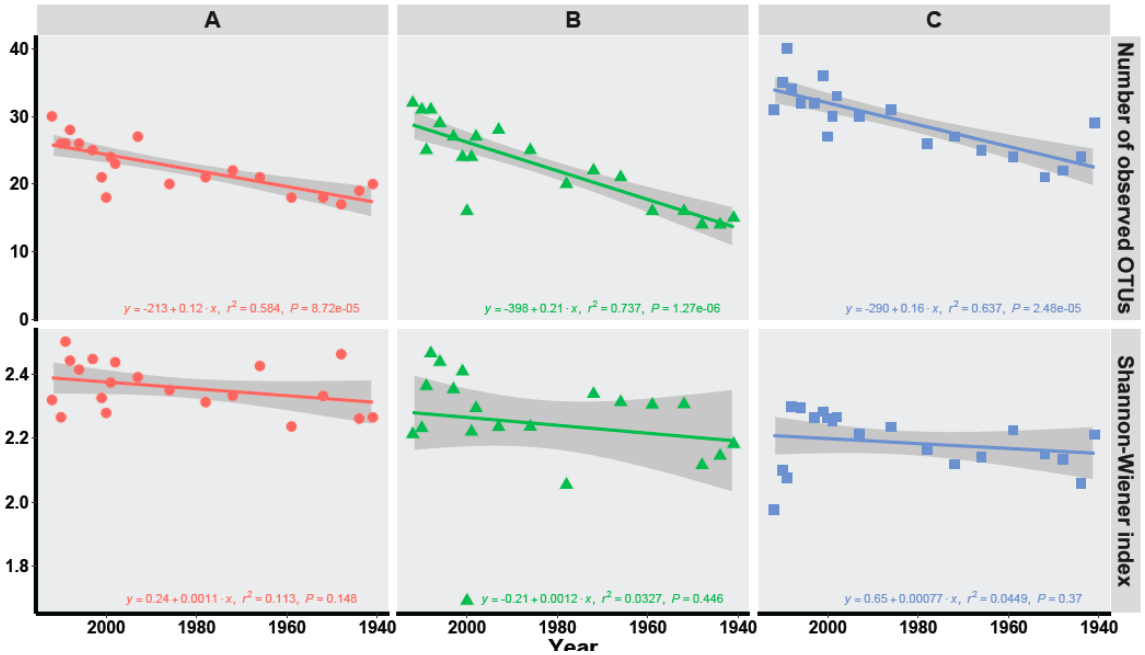

Figure 4.5. Shifts in α-diversity of cyanobacterial OTUs in Buffalo Pound Lake over time (years) based on cyanobacterial 16S rRNA primers (panels A and B, respectively) and 23S rRNA primer (panel C). Linear regressions between alpha diversities (number of observed OTUs and Shannon-Wiener index) and time (year) are given. Shaded areas are the 95% confidence intervals for each model. The equation and adjusted r^2 for the specific linear regressions are given in each panel.

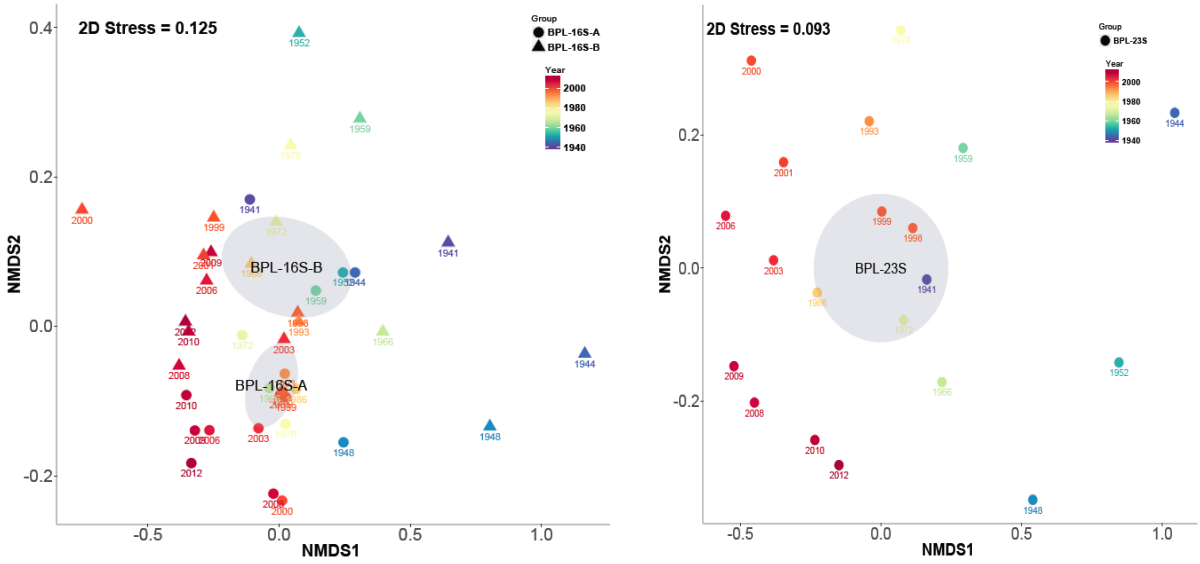

Figure 4.6. Shifts in Beta diversity of cyanobacterial OTUs over time (years). The non-metric multidimensional scaling (NMDS) visualizes the relative dissimilarities of 16S-cyanobacterial OTUs (left; **A**-Primer A and **B**-Primer B) and 23S rRNA OTUs (right) over time from a sediment core collected from Buffalo Pound Lake, Saskatchewan, Canada.

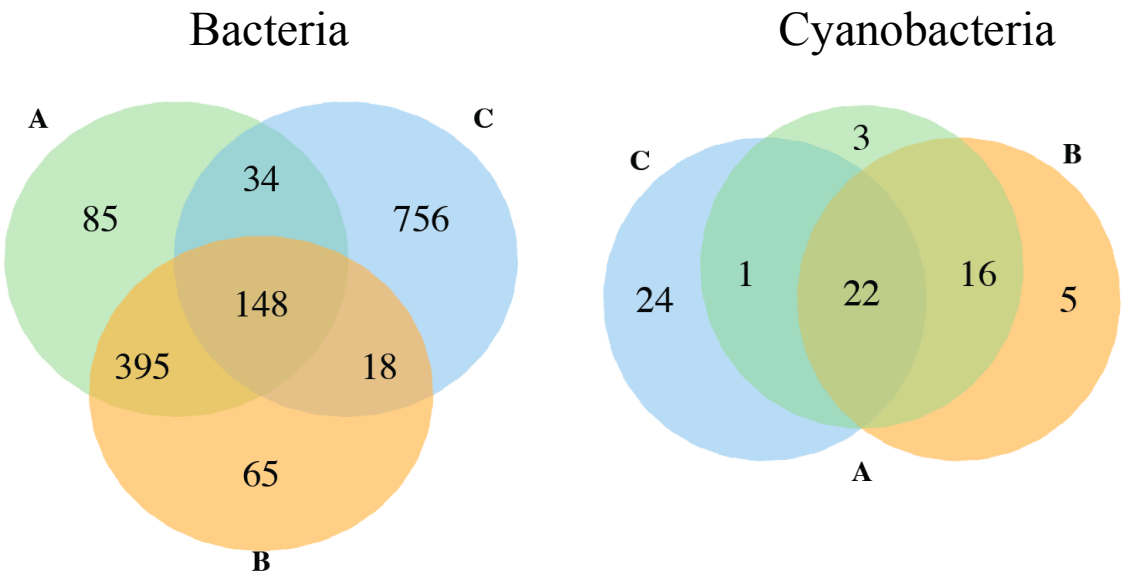

Figure 4.7. Venn diagrams showing the number of shared operational taxonomic units (OTUs) of bacteria (left) and cyanobacteria (right) identified in a sediment core collected from Buffalo Pound Lake, SK, Canada using different 16S cyanobacterial primers (A and B) and a 23S rRNA primer (C).

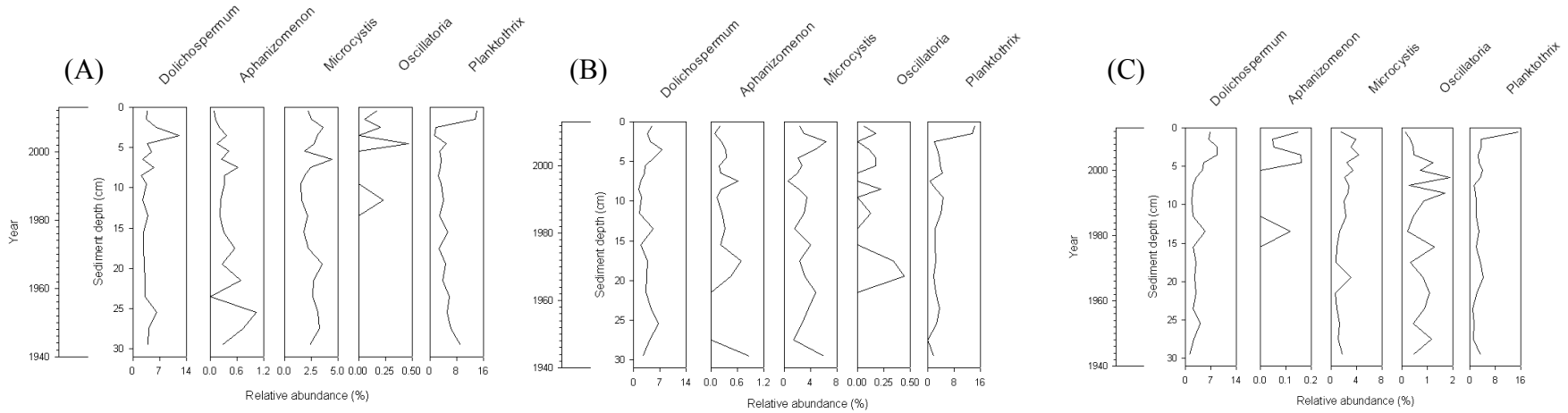

Figure 4.8. Relative abundances of gene sequences of potentially toxic cyanobacteria over time observed in a sediment core collected from Buffalo Pound Lake, SK, Canada based on cyanobacterial 16S rRNA primers (panels A and B, respectively) and 23S rRNA primer (panel C).

4.5. Discussion

4.5.1. Composition of the cyanobacterial community

Cyanobacteria constituted a major proportion of the overall bacterial OTUs (up to ~30%) identified in the sediment profile of Buffalo Pound Lake, with variable but higher relative abundance within more recent time intervals (Figure 4.3). Curiously, eukaryotic algae were not identified within the temporal profile of the sediment core through 23S rRNA sequencing using the SILVA LSU database (data not presented). This could be due to limitations in the 23S SILVA LSU database (Steven et al., 2012). For example, sequences aligned against the NCBI BLAST database produced results, however when applied to the SILVA LSU database, no positive hits were recorded. Therefore, this study exclusively investigated and compared temporal trends in the cyanobacterial community using both the 16S and 23S rRNA genes. Unlike cyanobacteria, eDNA from non-phototrophic bacteria can originate within either the water column or sediment profile. Therefore, general temporal trends in non-phototrophic bacteria were not inferred herein.

Three diverse assemblages of cyanobacteria were identified using the 16S and 23S primers. Although a combined total of 54 different genera of cyanobacteria were observed (Figure 4.7), many of these genera were screened out when the data were trimmed to exclude the bottom 0.5% of cyanobacterial genera sequences. In addition, the large portion of unclassifiable genera (up to ~50%) indicated the existence of potentially novel cyanobacteria. Cyanobacteria genera previously identified from Buffalo Pound Lake using light microscopy include *Anabaena/Dolichospermum*, *Aphanizomenon*, and *Oscillatoria* (Kehoe et al., 2015). In the present study, *Dolichospermum* and *Planktothrix* were the predominant potentially harmful genera (Figure 4.8), based on gene abundance. The occurrence of any one of these taxa is usually followed by the appearance of pronounced taste and odours issues (Slater and Blok 1983) resulting from the production of geosmin or MIB.

The cyanobacterial community has changed over time in Buffalo Pound Lake. The α-diversity (Figure 4.5) for both reverse 16S primers and the 23S primer indicates a significant increase in the richness of the cyanobacterial community since reservoir formation. The Shannon-Wiener index (α-diversity), which also considers evenness, suggests more subtle changes to the characteristics of the cyanobacterial community over time (Figure 4.6).

This study identified several potentially harmful taxa commonly recurring in the

historical cyanobacterial community of Buffalo Pound Lake, *Planktothrix*, *Microcystis*, and *Dolichospermum* (formerly *Anabaena*). This is unsurprising as the reservoir has recorded cases of microcystin-producing blooms, one that led to numerous deaths of cows and dogs in 1960 (Dillenberg and Dehnel 1960; Roegner et al., 2013) and, more recently that led to closure of beaches and swimming and drinking water advisory warnings (Sharpe 2016).

Dolichospermum species are common to freshwater lakes worldwide and can severely degrade water quality through production of cyanotoxins (Al-Tebrineh et al., 2012), including both hepato- and neuro- toxins (Sivonen and Jones 1999). The consistent presence and high relative abundance of *Dolichospermum, Planktothrix* and *Microcystis* over time in Buffalo Pound Lake (based on gene abundance) is of concern, particularly if their production increases (Figure 4.8). These genera are known to include toxin-producing species and were determined to be the predominant potentially harmful genera in the assemblage of identified cyanobacteria. Based on all three primers investigated, there appears to be a significant increase in *Planktothrix* in the more recent sediments. The trend for *Dolichospermum* and *Microcystis* are variable across all three primers, with no clear trends.

Some cyanobacterial taxa can produce geosmin and 2-methylisoborneol (MIB), which are responsible for taste and odour issues in drinking water (Journey et al., 2013). Genera of cyanobacteria that have strains known to produce geosmin and MIB identified in this study included: *Dolichospermum, Planktothrix, Oscillatoria, Aphanizomenon,* and *Symploca* (Izaguirre et al., 1982). These compounds frequently co-occur with cyanotoxins (Journey et al., 2013), although many species are unable to produce taste/odour compounds and cyanotoxins simultaneously (Chorus & Bartram 1999; Graham et al., 2010). Many of the genera reported here have previously been identified in Buffalo Pound Lake using light microscopy (Kehoe et al., 2015).

The assemblage of cyanobacteria identified varied depending on which conserved region was amplified (16S vs 23S), demonstrating some discord between both primer types. For example, the dominant genera identified through 16S sequencing included *Snowella* (primer A) or *Synechroococcus* (primer B), while 23S sequencing identified *Cyanobium* as the dominant genus. Sequencing of the universal 16S ribosomal RNA gene (16S rDNA) is frequently conducted to identify the presence of bacteria and cyanobacteria (Nübel et al., 1997). The 23S plastid primer was more recently developed to simultanously identify both eukaryotic algae and

cyanobacteria (Sherwood and Presting 2007; Steven et al., 2012). However, sequencing using the 23S rRNA gene is currently limited (Steven et al., 2012), because of the smaller databases of reference sequences for this large sub-unit in comparison with small sub-unit (SSU) databases (e.g. 16S databases) (Yilmaz et al., 2011). Currently, many of the recovered 23S rRNA genes can only be classified to the phylum level (Steven et al., 2012). This may explain the discrepancy between the cyanobacteria community observed from these two databases (LSU vs SSU**).**

4.5.2. *mcyA* gene abundance

The presence of potentially harmful cyanobacteria throughout the temporal profile of Buffalo Pound Lake raised concerns regarding trends in cyanotoxin production, as microcystins are periodically detected in the surface water of Buffalo Pound Lake (Sharpe 2016; Roegner et al., 2013). In cases were cyanotoxins are not readily preserved in the sediment record, toxin-producing genes within the sediment can provide historical insight into the potential for toxin production. The continued presence of the *mcyA* gene throughout the temporal profile of the sediment core suggests that genera able to produce microcystins have been present since formation of this reservoir. The increase in *mcyA* abundance leading up to the mid-1980s (Figure 4.2) is concerning, but appears to have then plateaued. *McyA* was used herein as a means to explore trends in microcystin production, but this method is transferable to other toxin genes, such as those associated with saxitoxin or anatoxin-a production. The abundance of the *mcyA* gene in the water column does not necessarily correlate with microcystin production (Beversdorf et al., 2015). Nevertheless, the *mcyA* gene can provide insight into whether potentially toxic cyanobacteria are or were present (Beversdorf et al., 2015) and the trends in biomolecular machinery to produce toxins.

4.5. Conclusion

Cyanobacteria taxa known to produce toxins and taste and odour compounds, were identified using high-throughput sequencing of the 16S and 23S rRNA regions. These taxa were similar to those found in previous investigations of this reservoir (Hall et al., 1999; Kehoe et al., 2015). This demonstrated the feasibility and usefulness of next-generation sequencing as applied to sedimentary eDNA for the reconstruction of the historical assemblages of cyanobacteria and long-term trends in freshwater systems. High-throughput NGS provided more detailed

taxonomic information than the pigment data reported in Hall et al. (1999) regarding relative abundances of potentially problematic cyanobacteria. Although the rationale for sequencing using the 23S rRNA gene is the ability to identify both eukaryotic algae and prokaryotic cyanobacteria using a single primer (Sherwood and Presting 2007), the LSU database can be limiting (Steven et al., 2012) and unable to provide high resolution taxonomic identification (Steven et al., 2012). However, potentially harmful cyanobacteria were identified using both the 16S and 23S rRNA gene regions. Furthermore, the abundances of the *mcyA* gene confirm the presence of potentially nuisance cyanobacteria and the presence of the toxin production machinery. The findings reported here confirm that potentially harmful cyanobacteria and the biomolecular mechanisms to produce toxins have been present since reservoir formation.

The use of next-generation sequencing can provide a rapid screening alternative or complementary addition to traditional limnological or paleolimnological techniques, and can provide a more taxonomically-sensitive approach. However, due to the limitations of the 23S LSU databases, if cyanobacteria are the principle focus of a study, the 16S SSU databases are better developed and currently provide more comprehensive results. As sequencing techniques become more affordable, the use of paleo-metagenomic-sequencing can be utilized to investigate long-term changes in the ecology and quality of aquatic ecosystems.

CHAPTER 5

Using paleo-16S-high-throughput sequencing to infer historical trends in cyanobacterial community composition in a freshwater lake

This chapter is based on the article: Tse, T. J., Song, T., Doig, L. E., Zhang, X., Xin Feng, C., Sun, W., Wiseman, S. B., Giesy, J. P., Hecker, M., and Jones, P. Paleo-16S-metagenomics as a means to infer historical trends in cyanobacterial community composition in a freshwater lake. The ISME Journal. Submitted February 2017.

NOTE TO THE READERS – CHAPTER 5

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The author contributions to Chapter 5 were as follows:

- Timothy Tse (University of Saskatchewan) collected, processed, performed biomolecular and next-generation sequencing on samples, analyzed sample data, performed statistical analyses and drafted the manuscript.
- Song Tang (University of Saskatchewan), Cindy Feng (University of Saskatchewan), Weimin Sun (Guangdong Institute of Eco-Environmental Science and Technology), and Xiaohui Zhang (Nanjing University) helped with downstream bioinformatic analyses, including statistical operations and creating visual aids (e.g. figures).
- Steve Wiseman (University of Saskatchewan) helped develop methodology to determine the threshold cycle for the quantification of the microcystin toxin-producing gene (*mcyA*).
- John Giesy (University of Saskatchewan) and Markus Hecker (University of Saskatchewan) provided the laboratory facilities and instruments to extract and sequence the samples and reviewed and revised the manuscript.
- Paul Jones (University of Saskatchewan) and Lorne Doig (University of Saskatchewan) reviewed, revised and provided essential feedback in the drafting of the manuscript.

CHAPTER 5

Using-paleo-16S-high-throughput sequencing to infer historical trends in cyanobacterial community composition in a freshwater lake

5.0. Preface

This chapter discusses the methodology used to extract and sequence environmental DNA collected from two sediment cores collected from Lake Diefenbaker, Saskatchewan (case study test site). Using biomolecular and high-throughput next-generation sequencing techniques and the 16S rRNA gene, historical trends in the cyanobacterial community were reconstructed. Potentially toxic cyanobacteria were identified and the presence of the toxin-producing *mcyA* gene suggests that the conditions to produce toxins have been present since reservoir formation. The data presented here also incorporated supplementary data discussed in Chapter 3 and obtained from Lucas et al. (2015a,b) to reveal complex relationships among different environmental variables that may influence the relative abundance of certain cyanobacteria.

5.1. Abstract

Many inland waters lack sufficient long-term monitoring to assess trends in the occurrences and intensities of harmful algal blooms. Analysis of environmental DNA in cores of sediments is emerging as a means to reconstruct past microbial assemblages and the effects of environmental conditions on those communities. In this study, paleo-16S-high-throughput sequencing was employed to explore multidecadal shifts in the cyanobacterial community of a Prairie reservoir, and to assess the covariance of sedimentary DNA with other, environmental paleo-proxy data, such as physicochemical variables, and biomolecular and subfossil remains. Two sediment cores were collected and DNA extracted for 16S-rRNA sequencing. α- and β-diversity indices illustrated significant community-level changes over time, including relative abundances of cyanobacteria, such as the potentially harmful genus *Dolichospermum*. Results of correlation-based network analysis further revealed that the changes in relative abundances of cyanobacterial genera significantly co-varied with other paleo-proxies. The increasing trend in *Dolichospermum* was positively correlated to the abundance of the microcystin synthetase *A* (*mcyA*) gene as well as other paleo-proxies, indicating a potentially deteriorating water quality. These findings demonstrate a novel means to infer long-term

dynamics of the cyanobacterial community in inland waters and highlights the power of paleo-16S-high-throughput sequencing. When used with other lines of evidence, this information can be used to construct trajectories of environmental quality and inform management decisions.

5.2. Introduction

Cultural eutrophication has become a worldwide environmental issue for freshwater lakes and reservoirs (Shaw et al., 2003) contributing to deteriorating conditions and increased production of harmful algal blooms (HABs) (Shaw et al., 2003) seen in many Prairie and potable lakes (Barica 1993). Consequent to HAB formation, production of cyanobacterial toxins can cause adverse effects to both wildlife (Briand et al., 2003) and humans (Funari and Testai 2008). Further, expensive treatment processes are often required for the removal of HAB compounds from drinking and agricultural waters.

One of the greatest challenges faced by water quality managers is that little information is available on a timescale sufficient to assess long-term, decadal, changes to environmental quality, how they relate to HABs, and what the primary and secondary drivers of such changes are (Smol 2010). This can make it difficult to evaluate long-term trends in abiotic and biotic stressors, which are critical in predicting future water scenarios (Schindler 1997). Systematic monitoring approaches are frequently initiated only in response to the emergence of serious problems, such as HABs (Kohler 2010), and therefore, are reactive rather than proactive. The analysis of multiple physiochemical, subfossil or biomolecular proxies within sediments can provide insight into historical events or trends in aquatic ecosystems, such as past and recent trends in primary producers (Chapter 3; Lucas et al., 2015a, b). By understanding the environmental trends in water quality and ecology in vulnerable systems managers can proactively respond to changing conditions and avoid undesirable ecological consequences. Traditional paleolimnological techniques, such as sedimentary pigment analysis, often rely on analysis of non-genetic biomolecules to infer past and recent trends in the ecology of aquatic systems. However, these techniques can be time-consuming and exhibit limited taxonomic specificity (Sivonen and Jones 1999). Analysis of environmental DNA (eDNA) is becoming a popular tool in limnology (Singh et al., 2014) because it can achieve a high level of taxonomic specificity without the need for labor-intensive techniques, such as traditional microscopy.

Environmental DNA is fairly persistent in the aquatic environment and can be deposited

and preserved for lengthy periods of time in lake sediment (Domaizon et al., 2013). Analysis of this genetic material can be used to infer recent or past site occupancy by aquatic organisms (Turner et al., 2015), information useful for water management practices. However, until recently the application of eDNA analysis as a molecular fingerprinting technique to reconstruct past microbial assemblages and to detect toxic cyanobacteria (Martinez et al., 2014) has been limited because of the time-, labor- and cost-intensive nature of traditional, low-throughput sequencing techniques. However, the rapid development of high-throughput next-generation sequencing (NGS) technologies, bioinformatics tools and the establishment of taxonomic reference databases have laid the foundations for novel, highly efficient approaches for eDNA analysis of complex microbial consortia (Shokralla et al., 2012; Sogin et al., 2006).

Recently, a variety of paleolimnological techniques were employed to reconstruct historical environmental trends along the longitudinal axis of Lake Diefenbaker, a large prairie reservoir in Saskatchewan, Canada (Chapter 3; Lucas et al., 2015a, b). The results of those investigations suggested temporal changes in the ecology of this reservoir. Of particular interest, the Gardiner and Qu'Appelle arms were the only regions to have measurable concentrations of myxoxanthophyll, a pigment marker for cyanobacteria; however, this biomarker lacks taxonomic specificity. Therefore, in the present study, paleo-16S-high-throughput sequencing was applied to sediment cores collected from these two locations. The objectives of this study were to: i) reconstruct the temporal trend of multidecadal shifts in the cyanobacterial community from the two different locations in the lake; ii) explore the relationships of these taxa with other relevant paleo-variables (pigments, sterols, stanols, *mcyA*, and composition of the diatom community); iii) identify the presence and relative abundance of potentially harmful cyanobacterial species; and iv) detect and quantify the microcystin synthetase A (*mcyA*) gene involved in microcystin production.

5.3. Methods

5.3.1 Study area and sediment core collection

Lake Diefenbaker (Figure 5.1) (50°43' N, 107°30' W) was formed in 1967, following the completion of the Gardiner and Qu'Appelle dams on the South Saskatchewan River and Qu'Appelle River valley, respectively (Hall et al., 1999). It is the largest multi-purpose reservoir on the Canadian Prairies, supplying potable water to approximately 45% of Saskatchewan's

residents (SWSA 2012). With an approximate length of 181.6 km, a maximum depth of 59 m, an area of 394 km^2 and a volume of 9.03 km^3 (Sadeghian et al., 2015), Lake Diefenbaker is the largest multi-purpose reservoir in the Canadian Prairies. It provides power production, flood control, and water supply for mining, crop irrigation, various industries, wildlife and aquaculture. The water entering Lake Diefenbaker has a residence time of roughly 1.3 years (Bogard et al., 2012) before exiting through the Gardiner (99% of the flow) or Qu'Appelle (1% of the flow) arms. Based on volume and average dam outflow, the Qu'Appelle arm has a residence time of ~26 years (Costa 2011). Trophic status varies longitudinally in the reservoir, but is generally mesotrophic (Hecker et al., 2012; Abirhire et al., 2015).

Sediment cores were collected from two hydromorphologically distinct regions, the Gardiner arm (LDGD, 52.9-m water depth) and the Qu'Appelle arm (LDQA, 19.1-m water depth) (Figure S1) in September, 2011 and August, 2012, respectively, using a Glew Maxi Gravity Corer (John Glew, Kingston, Canada) fitted with 7.5 cm diameter acrylic core liners. These locations were selected for eDNA analysis based on sedimentary pigment trends that suggested increasing cyanobacteria activity over time at these two locations (Chapter 3). In addition, long-term monitoring data are lacking for this reservoir, with monitoring of primary producers being limited to the Qu'Appelle arm and commencing almost three decades after reservoir formation. After collection, cores were sealed and transported on ice to the laboratory for sectioning into 1-cm layers under nitrogen gas. Edges of subsamples (approximately outer 1-cm rim) were discarded to preclude potential contamination resulting from contact with the inner surface of the acrylic tube. Samples were stored in 50-mL sterile glass vials at -80 °C, in the dark. Sample subsets were well-mixed using sterile techniques prior to further analysis.

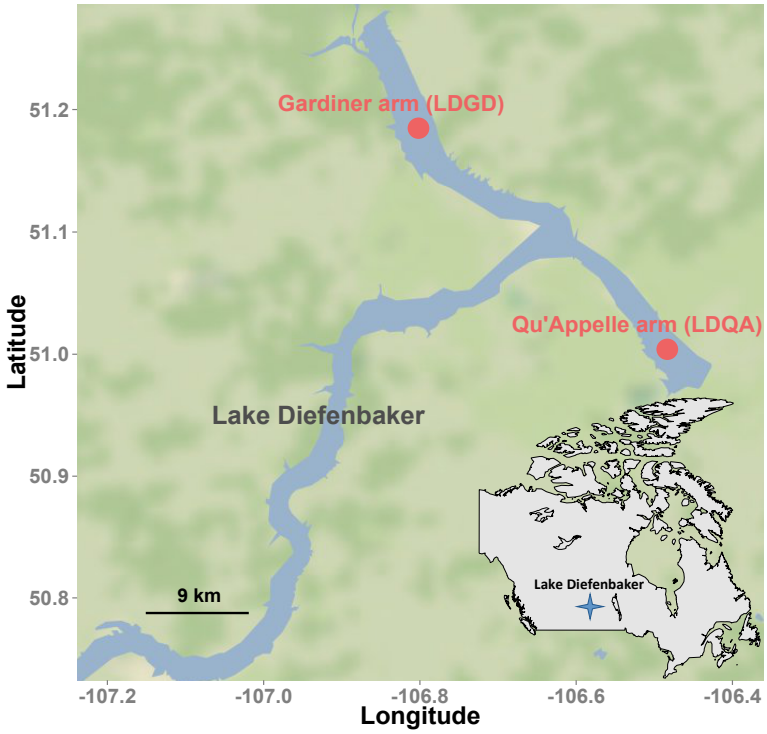


Figure 5.1. Sediment core locations in the Gardiner (LDGD) and Qu'Appelle arms (LDQA) of Lake Diefenbaker, Saskatchewan, Canada. The location of Lake Diefenbaker in Canada is indicated by a blue star.

5.3.2. Dating and analytical analyses of sediment cores

The complete or majority of the sediment profile, post-reservoir flooding, was captured in the sediment cores (Chapter 3; Lucas et al., 2015a, b; approximately 44 and 45 years for the Gardiner and Qu'Appelle arms, respectively). Data regarding other paleo-physicochemical and -biological variables from the same cores included concentrations of phosphorus (apatite phosphorus, AP; non-apatite inorganic phosphorus, NAIP; organic phosphorus, OP; and total phosphorus, TP), nitrogen (N) and carbon (C) ($\delta^{15}N$, percent total N, $\delta^{13}C$, percent organic C and molar C/N ratios), and metals (manganese, iron and reactive particulate silica, PRSi), fecal sterols, sedimentary pigments, and diatom community composition (Chapter 3; Lucas et al., 2015a, b; Maavara et al., 2015).

5.3.3. Extraction of DNA and sequencing of 16S rRNA

DNA was extracted from 1-g (wet weight) of sediment increments using E.Z.N.A.® Soil DNA Kits (Omega Bio-tek, Norcross, GA, USA) in accordance with the manufacturer's specifications. Purity and concentration of DNA were determined by measuring the A_{230}, A_{260}, and A_{280} using a Nanodrop 1000 Spectrophotometer (Thermo-Fisher, Waltham, MA, USA). DNA samples were frozen at -80°C until further analysis. Universal cyanobacterial primers CYA359F, CYA781R(A) and CYA781R(B) (Nübel et al., 1997) with attached Illumina bar-code overhangs were used to amplify V3 and V4 regions of bacterial small subunit ribosomal RNA (16S rRNA) (Boutte et al., 2006). The thermocycling program was as follows: 95 °C for 3-min, followed by 25 cycles of denaturation at 95 °C for 30s, annealing at 55 °C for 30s, and extension at 72 °C for 30s, and finally an extension at 72 °C for 5-min. PCR product size and integrity was verified on a Bioanalyzer DNA-1000 chip using a 2100 Bioanalyzer Instrument (Agilent Technologies, California, USA). Reverse primers CYA781R(A) and CYA781R(b), identified as primers (a) and (b) in this paper, were used separately to differentiate between two different cyanobacterial populations, targeting mainly filamentous and heterocystous cyanobacteria and unicellular cyanobacteria, such as *Chroococcales* and *Oscillatoriales*, respectively (Loza et al., 2013; Boutte et al., 2006). However, there is taxonomic overlap, or shared operational taxonomic units (OTUs) between the two reverse primers (i.e. they are not mutually exclusive). Reaction solutions for PCR and library preparation were made according to Illumina's 16S Metagenomic Sequencing Library Preparation (Part # 15044223 Rev. B) with 25-ng of eDNA used as template and sample libraries were pooled to a final concentration of 6 pM prior to paired-end multiplex sequencing performed on a MiSeq Desktop Sequencer (Illumina, California, USA). For unknown reasons, some sediment increments in the Gardiner arm core could not be amplified: 13-14 cm, 15-16 cm, 17-18 cm, 20-21 cm, 22-23 cm and 24-25 cm for primer (a) and 17-18 cm for primer (b). Raw sequences have been made available (National Center for Biotechnology Information (NCBI) Sequence Read Archive (Accession#: PRJNA350697).

5.3.4. Sequencing data analysis

Sequencing data were processed using a cloud-based microbiome-seq analysis workflow in BaseSpace (https://basespace.illumina.com), which automatically demultiplexed, quality filtered and joined paired-end read files. The 16S Metagenomics v1.0 application utilizes a high-

performance version of the Ribosomal Database Project (RDP) algorithm (Wang et al., 2007) and an Illumina-curated version of Greengenes database (May 2013) to taxonomically classify 16S rRNA amplicon reads at the 97% identity level. The output is a classification of reads at multiple taxonomic levels from kingdom to species.

5.3.5. Abundance of *mcyA* gene

The presence of potentially toxin-producing cyanobacteria raised concerns as to whether the genetic components for toxin production were also present. The *mcyA* primers used (*mcyA*-CD 1F and 1R) were designed to target the *Planktothrix*, *Microcystis* and *Dolichospermum* genera (Hisbergues et al. 2003), but have also been used to gain an overview of cyanobacterial genera with the potential to produce microcystins (Beversdorf et al., 2015). The thermocycling program consisted of initial denaturation at 95 °C for 3-min, followed by 25 cycles of 95 °C for 30s, 60 °C for 30s and 72 °C for 30s, and a final extension phase at 72 °C for 5-min. The threshold cycle (C_t) was determined using traditional RT-PCR methodology where samples were taken every 5 cycles. The C_t was determined to be cycle 25. An equal volume of amplicon was then loaded onto a freshly prepared 1% agarose gel containing SYBR Safe DNA gel stain (Thermo Fisher Scientific, Massachusetts, USA) and relative intensities of their fluorescence (proportional to semi-quantitative abundances of *mcyA*) were determined using a Bio-Rad VersaDoc MP 4000 Molecular Digital Imaging System (Hercules, CA, USA) running Quantity One software v.4.6.7. To confirm *mcyA* gene identity, the amplicon was sequenced (University of Calgary, Alberta, Canada), and the sequence was confirmed with 99% identity when compared against the NCBI BLASTN (nr/nt nucleotide collection) database.

5.3.6. Extraction of sterol and stanol compounds and cyanotoxins

Sterols and stanols were extracted following the procedure detailed in Chapter 2. Cyanotoxins were co-extracted with algal pigments as outlined in Chapter 3.

5.3.7. GC-MS instrumental analysis for sterols and stanols

The analytical methodology and standards used to identify and quantify various sterols and stanols are outlined in Chapter 2.

5.3.8. Cyanotoxins

Four variants of saxitoxin (saxitoxin; STX, Neosaxitoxin; NEOSTX, decarbamoyl saxitoxin; dcSTX, and gonyautoxin-2 and -3; GTX-2 and GTX-3) and anatoxin-a cyanotoxin standards (Table 6) were purchased from the Certified Reference Material Programme from the National Research Council (NRC) of Canada (Halifax, Canada). The hepatotoxin, nodularin (NOD) was also purchased from NRC. Microcystin-LR (MC-LR) and –RR (MC-RR) was purchased from Sigma Aldrich (St. Louis, MO, USA). Acetone (HPLC Grade), methanol (Certified ACS) and acetonitrile (Certified ACS), were obtained from Fisher Scientific (Ottawa, Ontario, Canada). Finally, formic acid (CAS# 64-18-6) was purchased from Sigma Aldrich (St. Louis, MO, USA).

5.3.9. Q-exactive orbitrap ultra-high-performance liquid chromatography mass spectrometry analysis

Extracts were analyzed using a Q Exactive™ mass spectrometer (Thermo Fisher Scientific, Toronto, ON) interfaced to a Dionex™ UltiMate™ 3000 ultra-high-performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific, Toronto, ON). Separation of chemicals was achieved with a TSKgel Amide-80 (3 μm; 4.6 mm × 150 mm; TOSOH; Grove City, OH, USA) with an injection volume of 5 μl. Ultrapure water ontaining 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) were used as mobile phases. Initially 90% B was decreased to 30% in 8 min, then decreased to 25% at 16 min, followed by an increase to initial conditions of 90% B and held for 3.5 min to allow for column re-equilibration. The flow rate was 0.50 mL/min. The column and sample chamber temperatures were maintained at 40 °C and 10 °C, respectively. Data were acquired using full scan mode and selected ion monitoring (SIM). Briefly, MS scans (100 to 1000 *m/z*) were recorded at resolution R = 70000 (at m/z 200) with a maximum of 3×10^6 ions collected within 200 ms, based on the predictive automated gain control. SIM scans were recorded at a resolution R = 35000 (at *m/z* 200) with a maximum of 1×10^6 ions collected within 80 ms, based on the predictive automated gain control, with the precursor isolation width set at 2.0 *m/z*. General mass spectrometry settings applied for positive ion mode were: spray voltage, 3.0 kV; capillary temperature, 400 ^{o}C; sheath gas, 46 L/h; auxiliary gas, 15 L/h; probe heater temperature, 350 ^{o}C. The ion masses used to identify each cyanotoxin are provided in Table 5.1.

Table 5.1. Ion masses of cyanobacterial toxins identified.

Compound	m/z	Retention time (min)	Calibration curve	Instrument Detection Limit (μg/L)
MC-LR	995.5470	6.59	>0.99	0.03
MC-RR	519.7894	8.06	>0.99	0.006
STX	300.1390	11.81	>0.99	0.02
NeoSTX	315.1300	11.91	>0.99	0.005
GTX	396.0900	10.85	>0.99	0.03
dc-STX	257.1340	11.84	>0.99	0.05
Nod	825.4428	6.79	>0.99	0.01
ATX-a	166.1220	6.64	>0.99	0.007

5.3.10. Numerical analysis

Statistical analyses were completed using packages *phyloseq*, *ggplot2*, *vegan*, *corrplot*, and *mvabund* in R software v3.3.1 (R Development Core Team, 2006) and differences were considered significant when $p<0.05$. The temporal patterns of key paleo-variables (e.g. biomolecules, *mcyA* gene abundance, physicochemical variables, diatom subfossil remains) from the two sites sampled were visualized using "tabplot" package.

Non-bacterial sequences were removed from all samples to obtain a strictly bacterial dataset. Cyanobacteria sequences were separated from this dataset to determine relative abundances of cyanobacterial genera within each sample, and this data was visualized using a Circos plot (Krzywinski et al., 2009). Numbers of shared bacterial or cyanobacteria OTUs between sampling sites and primers and global similarity (co-inertia) between bacterial or cyanobacteria assemblages of two primers were determined using Venn diagram and co-inertia analysis (CIA) in the "VennDiagram" and "ade4" packages, respectively. A Monte-Carlo test with 9999 permutations was used for validating results of CIA. α-diversities (number of observed OTUs and Shannon-Wiener index) for cyanobacteria or bacterial communities were determined using "phyloseq" (McMurdie and Holmes 2013) which measures cyanobacterial community differences within each sediment increment. For β-diversity (Bray-Curtis dissimilarity index), to avoid biases generated by different sequencing depths, all samples were normalized to the minimum number of total bacterial reads (10170) among all sequencing

libraries. This analysis was used to investigate community composition differences across sediment sections and sampling site. Reads of cyanobacteria community were then separated from these normalized libraries.

The Mantel test (9999 permutations) was used to investigate correlations between β-diversities of cyanobacterial genera (Bray-Curtis distance) and all measured paleo-variables (Euclidean distance) for each sampling site and primer using "vegan" package. The multivariate package "mvabund" was used to make inferences about the differences in relative abundance for each cyanobacteria genus in the cyanobacteria community due to differences in sample site, primer and paleo-environmental variables. This statistical package has been shown to be more informative and powerful than distance-based community analysis approaches (Wang et al., 2012). A generalized linear model (GLM) was fitted to the normalized abundance counts of each cyanobacteria genus separately across all samples by 'manyglm' function (negative binomial model, 9999 resamples to control for the family-wise error rate) using site, primer and paleo-environmental variables as predictor variables. The univariate hypothesis tested for each cyanobacteria genus within the multivariate model was also provided.

Spearman's ranked correlation was conducted to visualize correlations among the relative abundances of each cyanobacteria genus and with all available paleo-variables via "Corrplot" and "Hmisc" packages. Similarities of these correlation coefficients (ρ) across all samples were further determined by "dendextend" package using the average (UPGMA) clustering method. Network visualization and module detection of the co-occurrence relationships of all measured variables and cyanobacteria genera were conducted on the interactive platform of Gephi v 0.9.1 (WebAtlas, Paris, France) with Fruchterman-Reingold placement algorithm (Bastian and Heymann 2009). The topology of the resulting network was described by a set of measures, including average node connectivity, average path length, diameter, cumulative degree distribution, clustering coefficient and modularity. In the co-occurrence network, the nodes represent the measured variables and genera and the edges correspond to robust and significant correlations ($|\rho|>0.7$ and $p<0.01$) between nodes.

5.4. Results

5.4.1. eDNA, paleo-variables and *mcyA* gene

eDNA was successfully extracted from the complete temporal profile of each sediment core, with greater eDNA concentrations occurring in the more recent sediments (Figure 5.2). Concentrations of DNA, ranged from 1580 to 13743 ng g^{-1} and 4641 to 16762 ng g^{-1} (wet mass) in the Gardiner and Qu'Appelle arms, respectively. Similar to total concentrations of eDNA, abundances of the *mcyA* gene were greater in more recently deposited sediments of the Gardiner and Qu'Appelle arms (Figures 1 and S3), although abundance plateaued in the late 1990's in the Qu'Appelle arm. Concentrations of various key paleo-variables (biologically available P, PRSi, and cyanobacterial pigments) illustrated trends similar to that of *mcyA* gene abundance within the vertical sediment profile of the Qu'Appelle arm, but not in the core from the Gardiner arm (Figures 5.3 and 5.4; coefficients of determination provided in Figure 5.5).

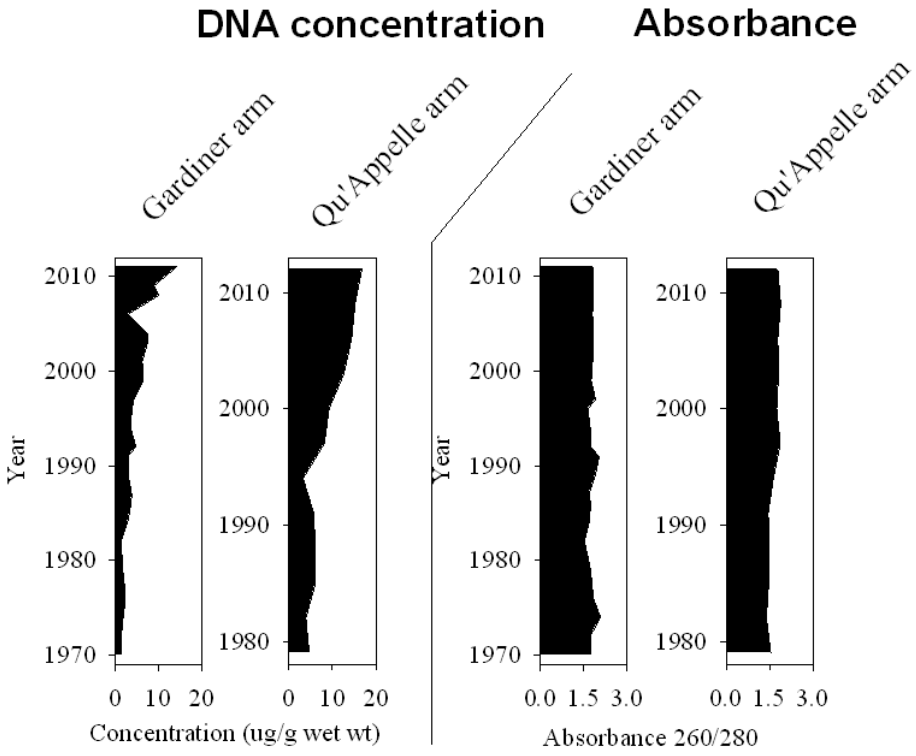


Figure 5.2. Concentrations of eDNA and A260/280 ratios of extracted eDNA (measuring eDNA purity) in sediment cores collected from the Gardiner and Qu'Appelle arms (LDGD and LDQA) of Lake Diefenbaker, Saskatchewan, Canada.

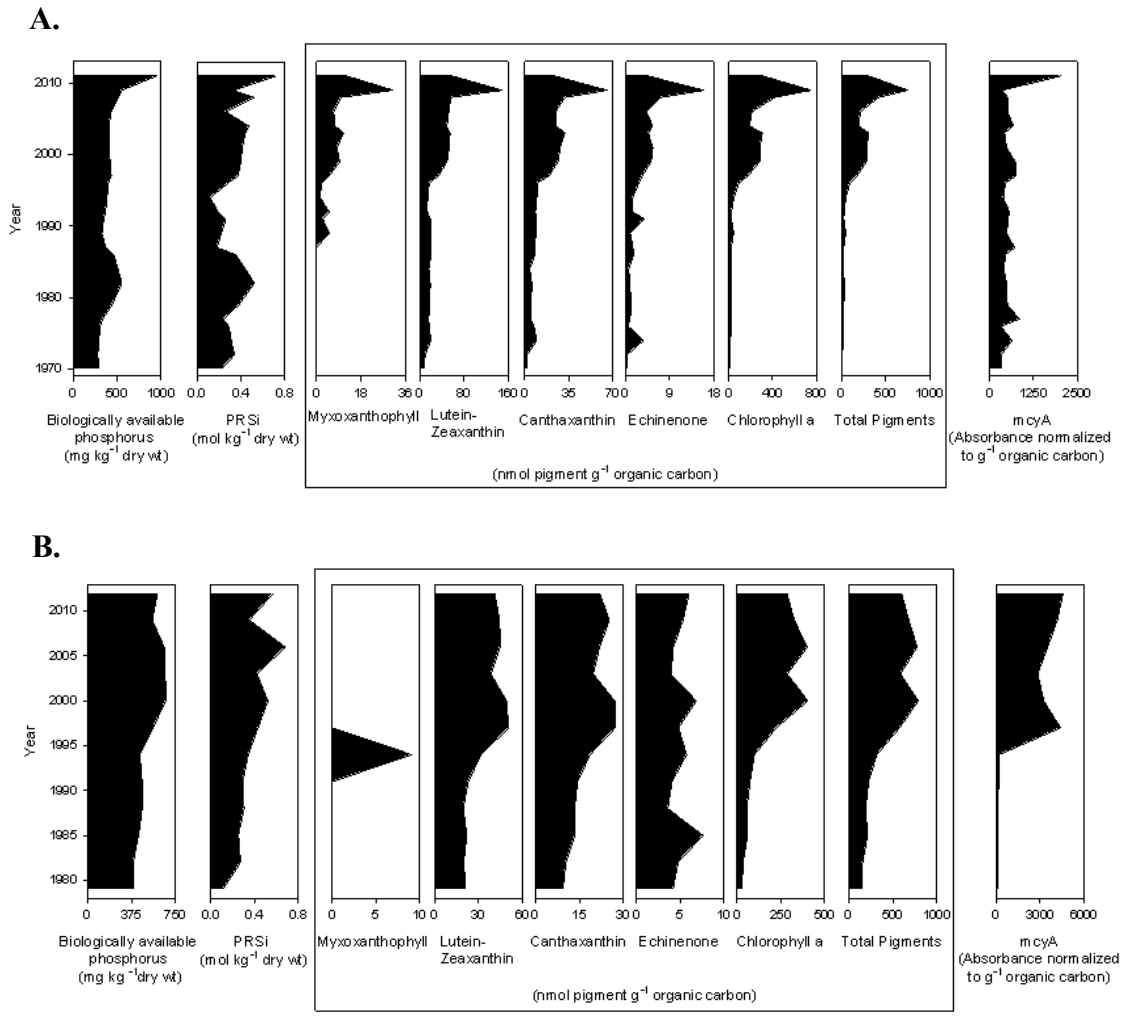
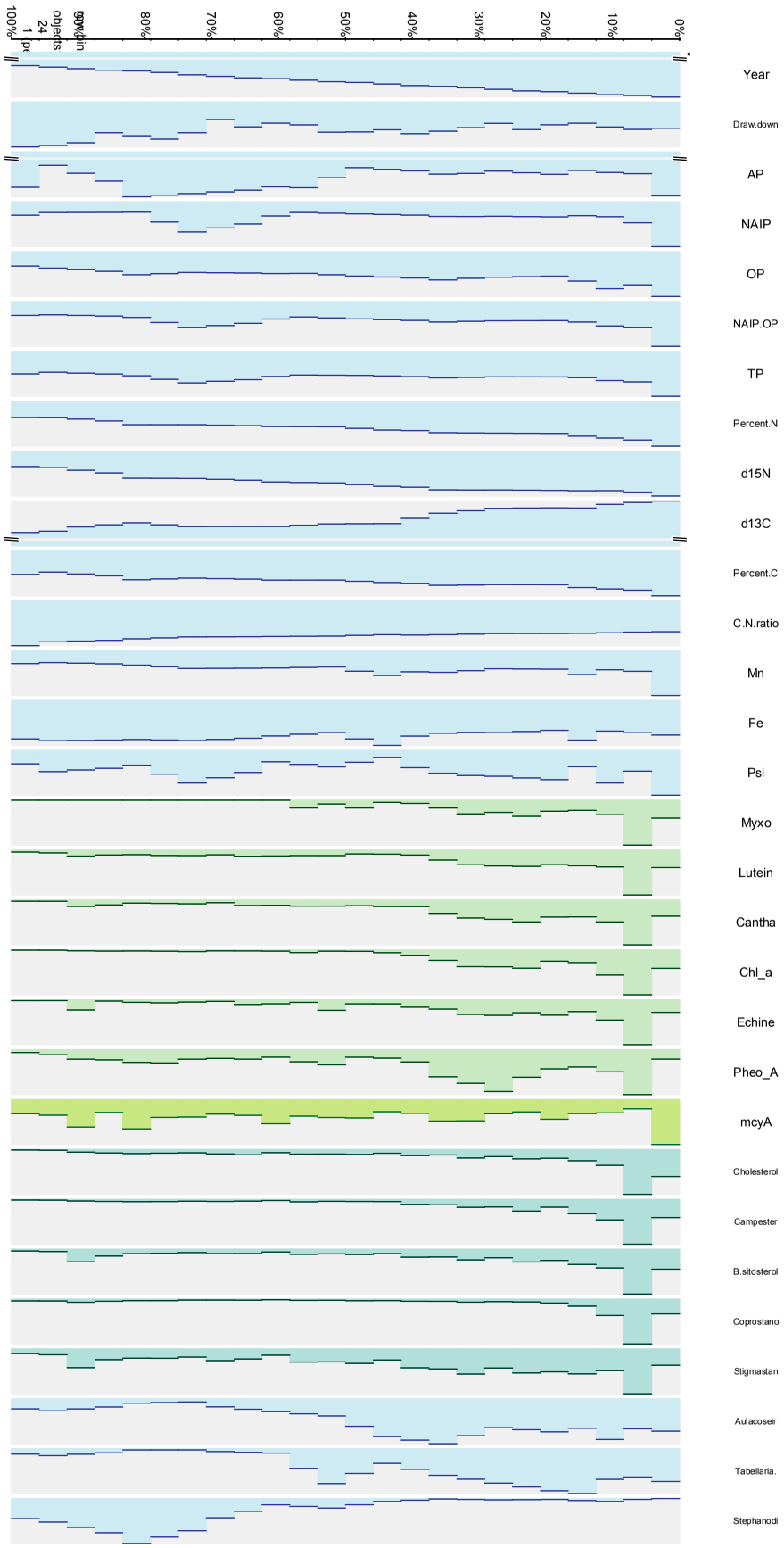


Figure 5.3. Temporal trends in concentrations of select paleo-variables, including physicochemical variables (biologically available P and particulate reactive silica), Cyanobacteria pigments (myxoxanthophyll, lutein-zeaxanthin, canthaxanthin, echineone and chlorophyll *a*) and *mcyA* abundance (as absorbance) in sediment cores collected from **A**) the Gardiner arm (2011) and **B)** the Qu'Appelle arm (2012), Lake Diefenbaker, Saskatchewan, Canada.

A.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Lutein
Cantha
Chl_a
Echine
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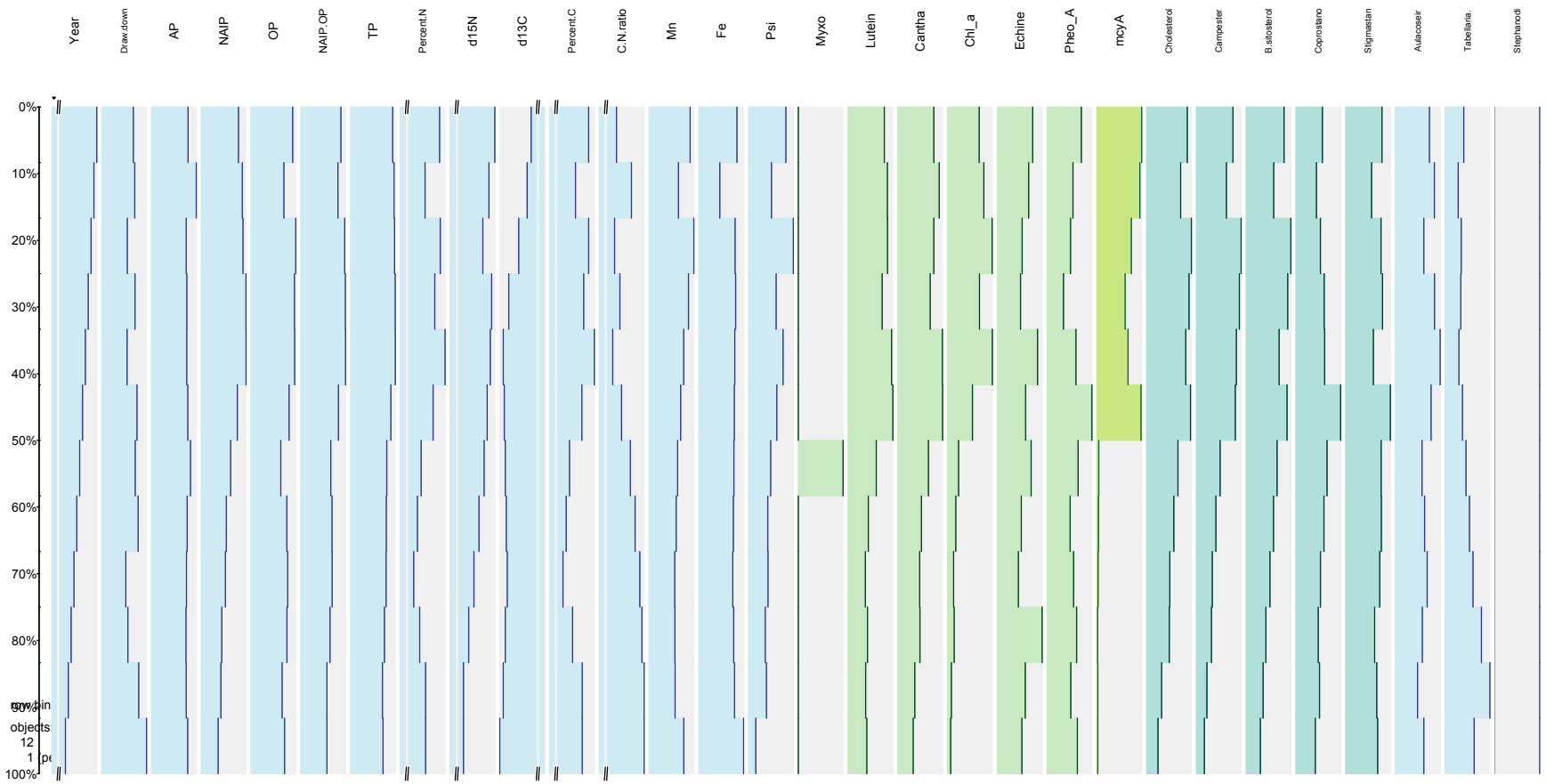
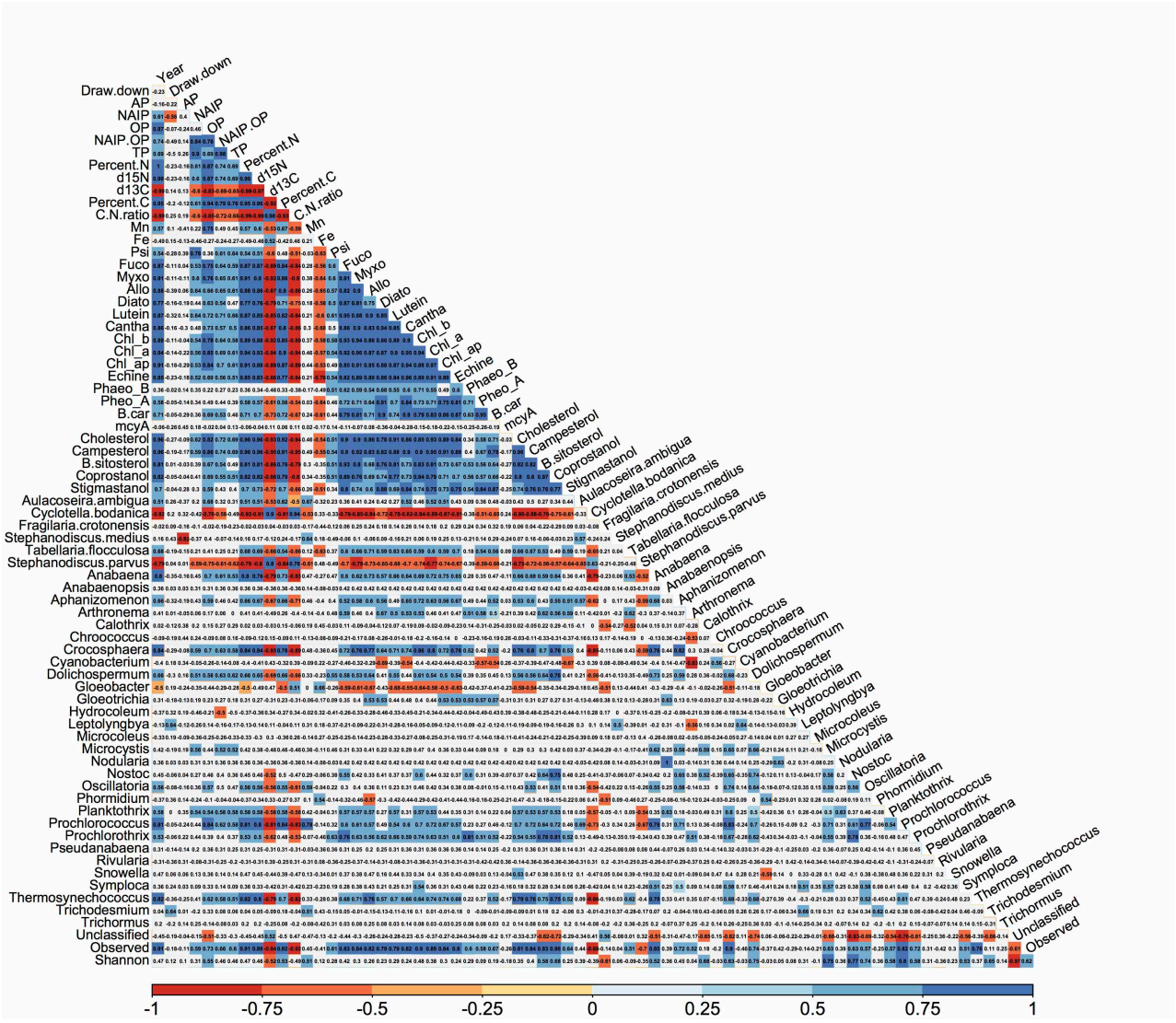


Figure 5.4. Table plots to visualize the temporal patterns between paleo-physicochemical characteristics and paleo-biological variables (concentrations of key sedimentary pigments, *mcyA* gene, sterols, stanols and key diatom species) at two sampling sites (**A**-LDGD and **B**-LDQA).

A)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Lutein
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Chl_a
Chl_ap
Echine
Phaeo_B
Pheo_A
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mcyA
Cholesterol
Campesterol
B.sitosterol
Coprostanol
Stigmastanol
Aulacoseira.ambigua
Cyclotella.bodanica
Fragilaria.crotonensis
Stephanodiscus.medius
Tabellaria.flocculosa
Stephanodiscus.parvus
Anabaena
Anabaenopsis
Aphanizomenon
Arthronema
Calothrix
Chroococcus
Crocosphaera
Cyanobacterium
Dolichospermum
Gloeobacter
Gloeotrichia
Hydrocoleum
Leptolyngbya
Microcoleus
Microcystis
Nodularia
Nostoc
Oscillatoria
Phormidium
Planktothrix
Prochlorococcus
Prochlorothrix
Pseudanabaena
Rivularia
Snowella
Symploca
Thermosynechococcus
Trichodesmium
Trichormus
Unclassified
Observed
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-0.75
-0.5
-0.25
0
0.25
0.5
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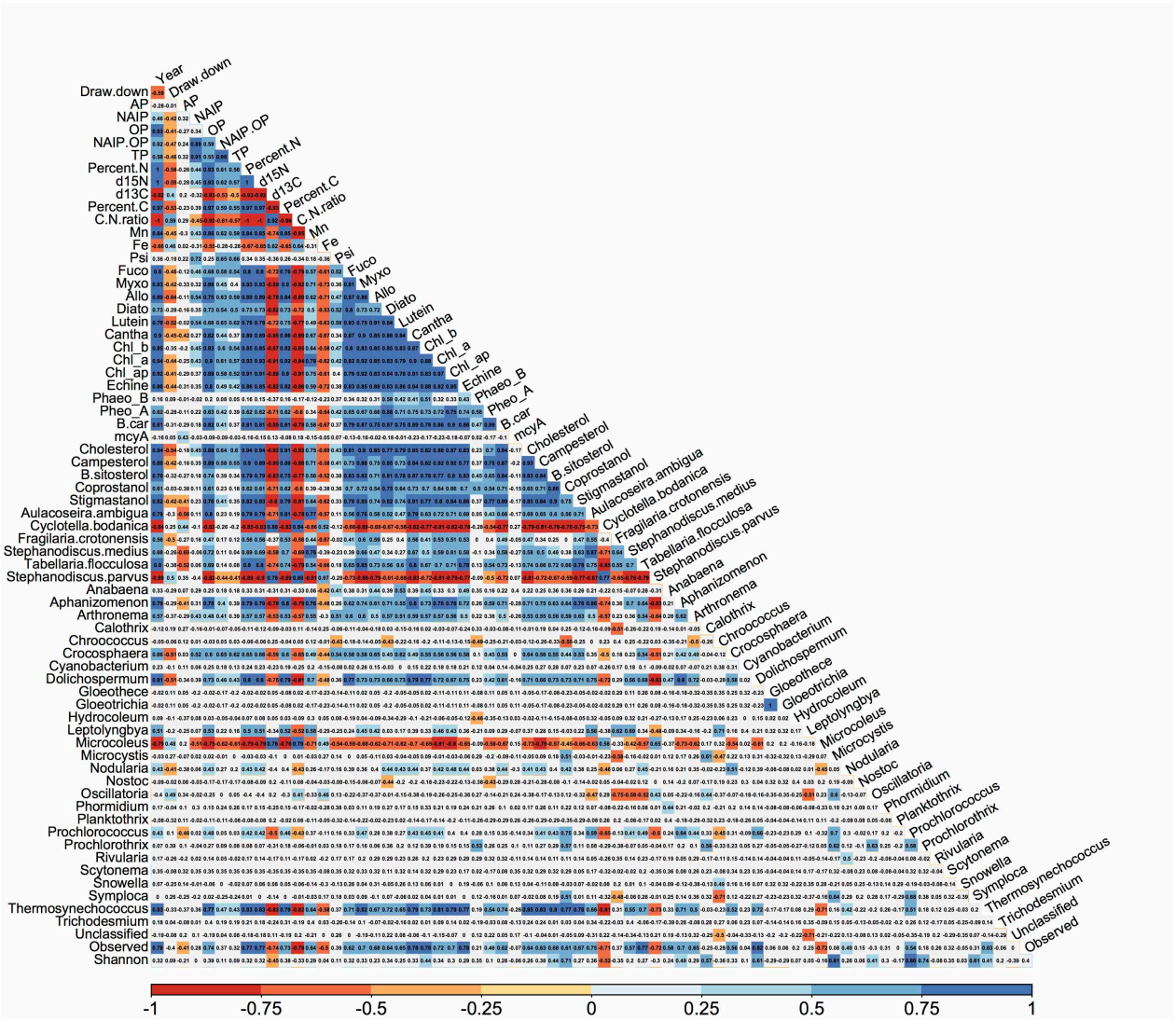
1

B)


Year
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Percent.N
d15N
d13C
Percent.C
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Psi
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Myxo
Allo
Diato
Lutein
Cantha
Chl_b
Chl_a
Chl_ap
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Phaeo_B
Phaeo_A
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mcyA
Cholesterol
Campesterol
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Cyanobacterium
Dolichospermum
Gloeothece
Gloeotrichia
Hydrocoleum
Leptolyngbya
Microcoleus
Microcystis
Nodularia
Nostoc
Oscillatoria
Phormidium
Planktothrix
Prochlorococcus
Prochlorothrix
Rivularia
Scytonema
Snowella
Symploca
Thermosynechococcus
Trichodesmium
Unclassified
Observed
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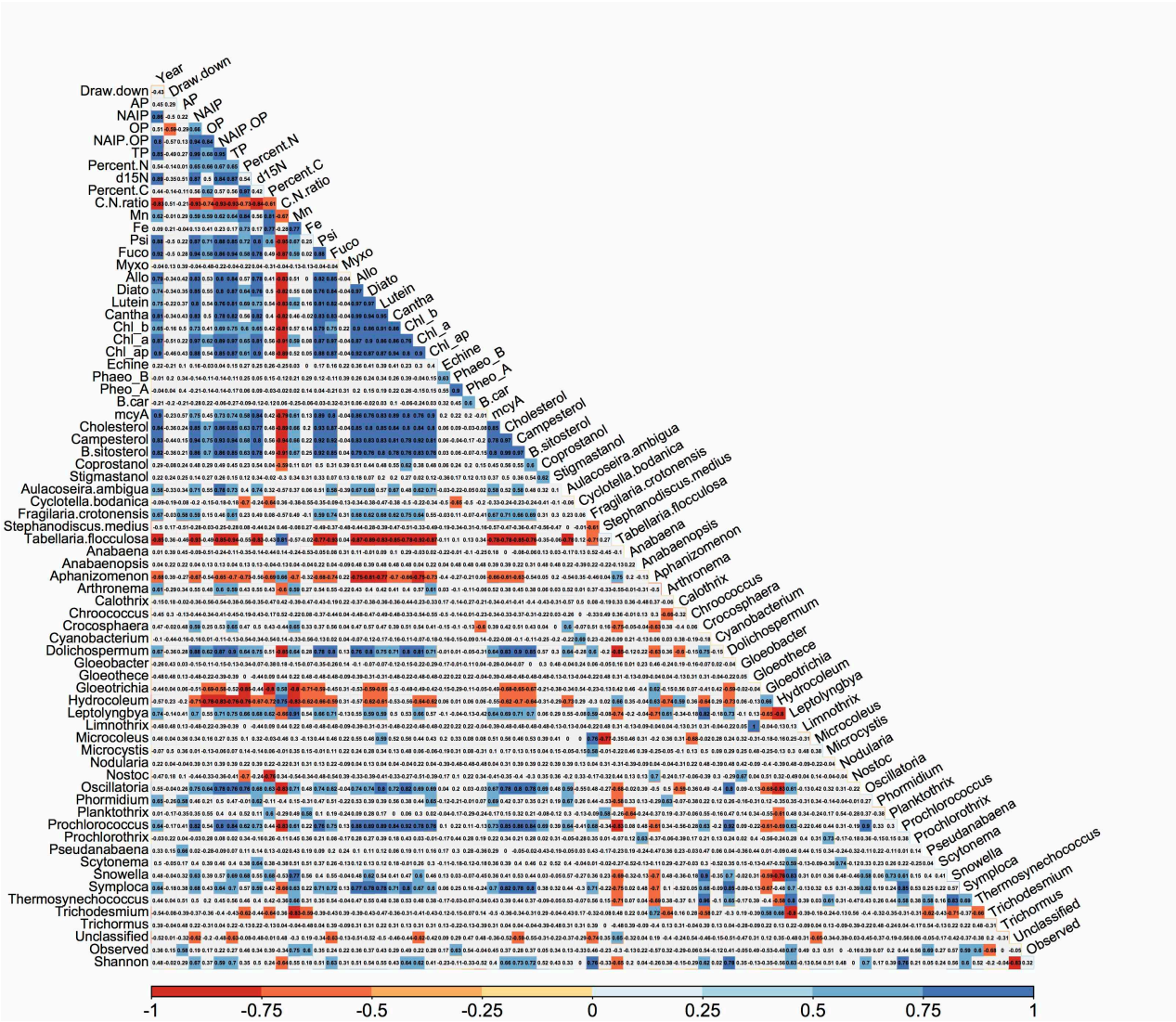
C)


Year
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Percent.N
d15N
Percent.C
C.N.ratio
Mn
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Myxo
Allo
Diato
Lutein
Cantha
Chl_b
Chl_a
Chl_ap
Echine
Phaeo_B
Phaeo_A
B.car
mcyA
Cholesterol
Campesterol
B.sitosterol
Coprostanol
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Aphanizomenon
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Chroococcus
Crocosphaera
Cyanobacterium
Dolichospermum
Gloeobacter
Gloeothece
Gloeotrichia
Hydrocoleum
Leptolyngbya
Limnothrix
Microcoleus
Microcystis
Nodularia
Nostoc
Oscillatoria
Phormidium
Planktothrix
Prochlorococcus
Prochlorothrix
Pseudanabaena
Scytonema
Snowella
Symploca
Thermosynechococcus
Trichodesmium
Trichormus
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D)

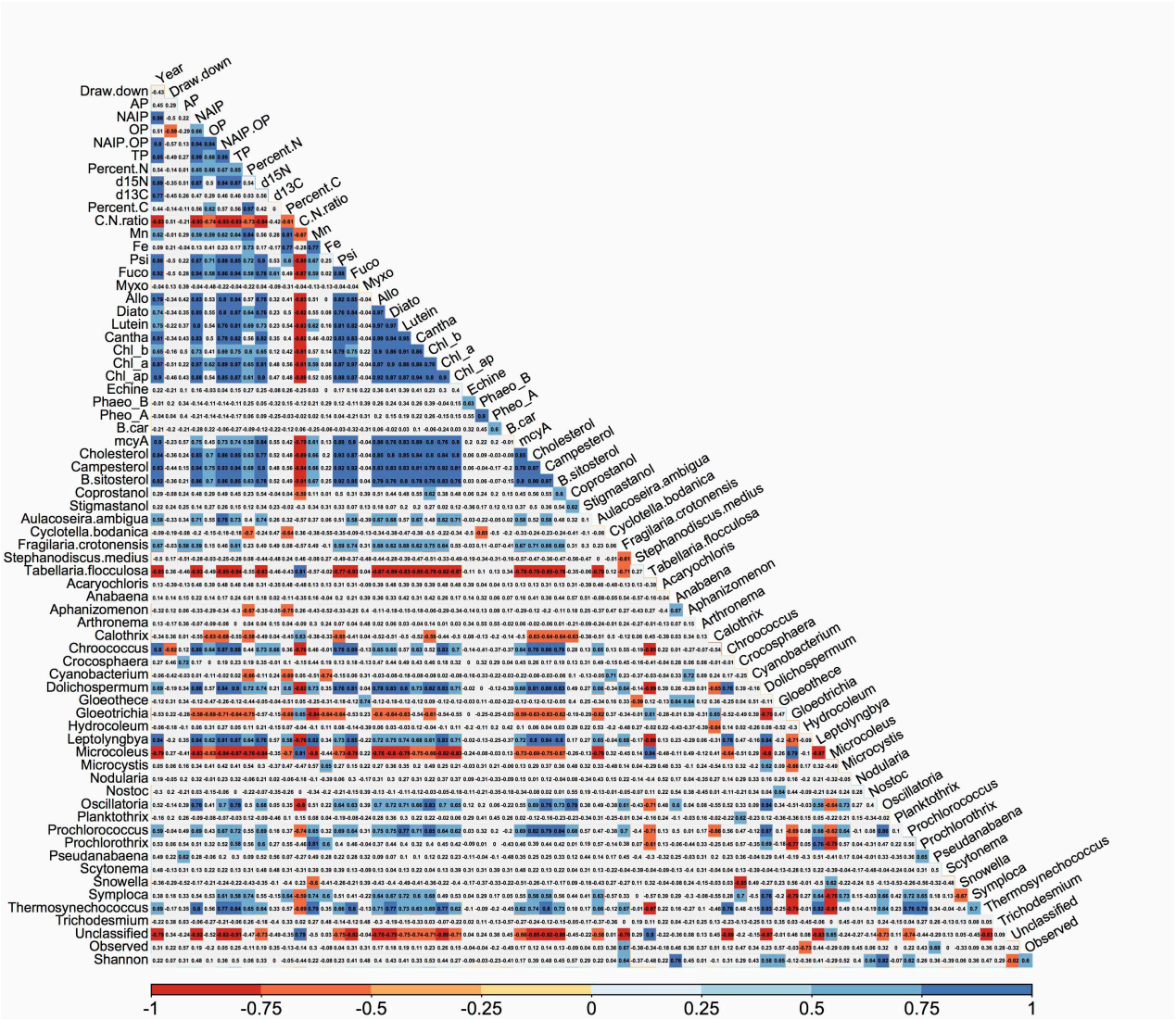

Figure 5.5. Spearman's Rank correlation coefficients (ρ) among paleo-physicochemical characteristics, paleo-biological variables (sedimentary pigments, *mcyA* gene, sterols, stanols and key diatom species), relative abundances of cyanobacterial genera in each cyanobacterial community, and alpha diversities of cyanobacterial community for the two sampling sites and each primer (panel **A**-LDGD-A, panel **B**-LDGD-B, panel **C**-LDQA-A, and panel **D**-LDQA-B). The intensity of colour for each square represents the strength of the correlation (the darker squares demonstrate a strong correlation ρ). Blue colours illustrate significantly positive correlations and red colours illustrate significantly negative correlation coefficients (p<0.05). Insignificant correlations are not coloured.

5.4.2. Composition of the cyanobacterial community

Diverse communities of cyanobacteria were identified, through the independent amplification of reverse primers (a) and (b) (Figure 5.8), for each site. A total of 10,739,269 and 658,714 reads were obtained for bacteria and cyanobacteria, respectively, in the 64 sequencing libraries. The cyanobacterial community represented <10% of the overall bacterial OTUs identified from the sequencing libraries (Figure S4A). Global similarities between the products of amplification with reverse primers (a) and (b) and sampling locations for bacteria and cyanobacteria were 20.1% (p=0.156, permutation test n=9999) and 13.8% (p=0.199, permutation test n=9999), respectively (Figure 5.7).

In total, 33 genera of cyanobacteria were detected by combining the datasets from amplification of both reverse primers (Figure 2). Sequences matching unclassified cyanobacterial 16S rRNA genes were abundant (at least 40%) in the samples tested. *Prochlorococcus* was the predominant cyanobacterial genus identified using the reverse primer (a) (average relative abundance: 7.3% and 4.3% for the Gardiner and Qu'Appelle arms, respectively). *Chroococcus* was the dominant genus using the reverse primer (b) (average relative abundance: 17.6% and 10.9% for the Gardiner and Qu'Appelle arms, respectively. *Dolichospermum* was the predominant potentially harmful genus of cyanobacteria identified (average relative abundance: 2.6% in both down-reservoir arms), although *Planktothrix* and *Microcystis* were also present. However, *Dolichospermum* was the only genus to exhibit an increasing trend within the temporal profile of both sediment cores (Figure 2).

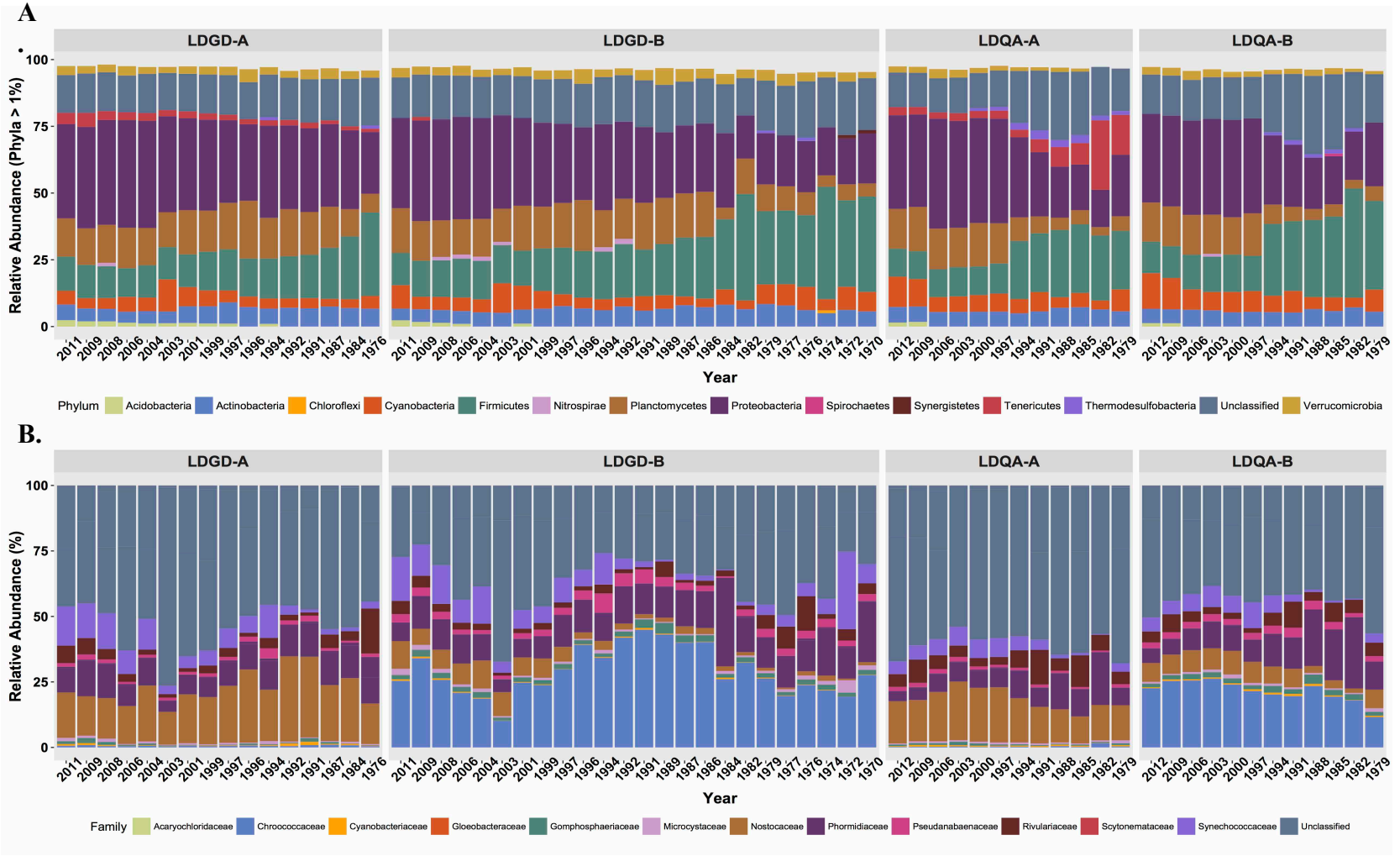

Figure 5.6. Panel **A.** Relative abundances over time of the bacterial community (phylum level) observed at two sampling sites (LDGD and LDQA) determined using different cyanobacterial 16S rRNA primers (A and B). Only taxa with relative abundance > 1% are shown. Panel **B.** Relative abundances (%) over time of cyanobacterial family in each cyanobacterial community observed at the two sampling sites for 16S rRNA primers A and B.

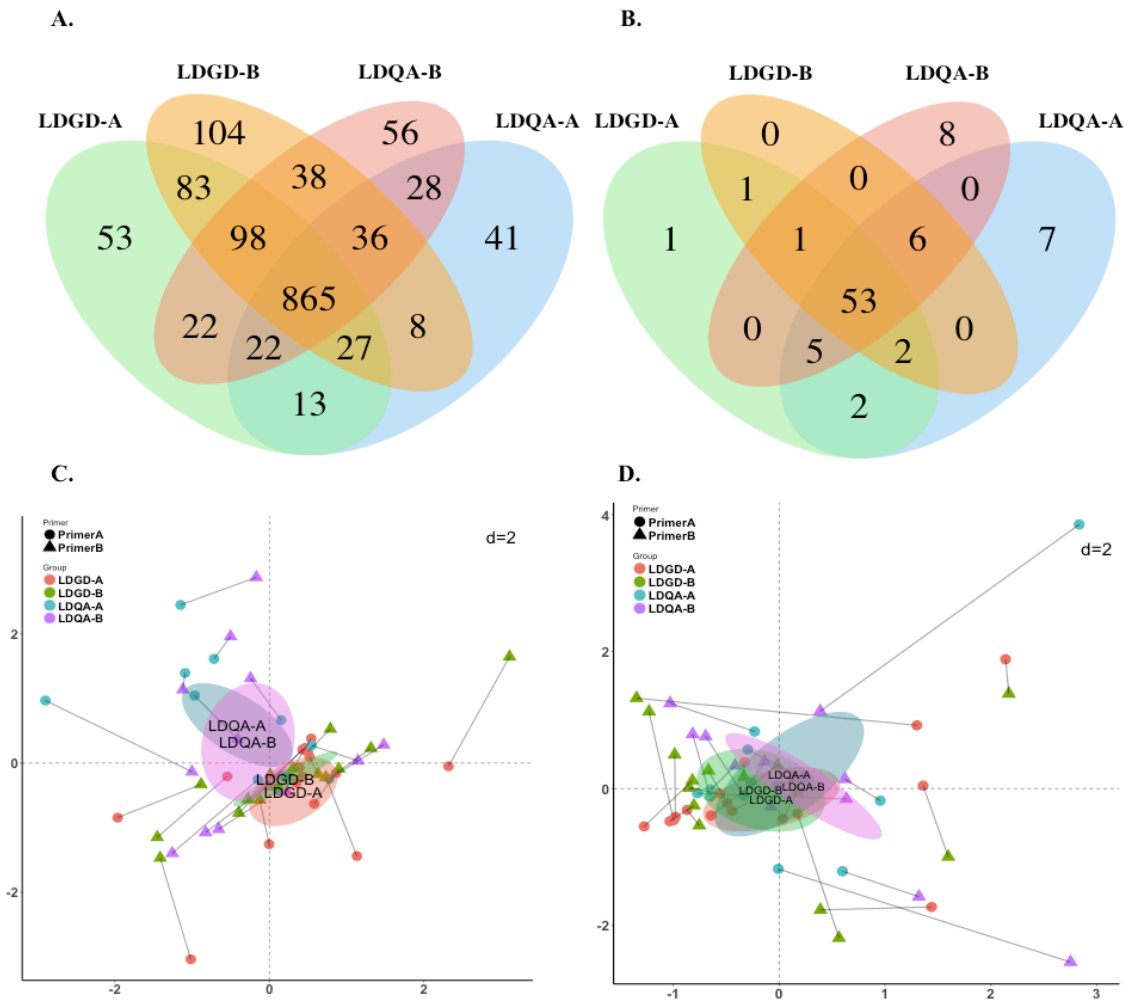

Figure 5.7 Venn diagrams showing the number of shared operational taxonomic units (OTUs) of bacteria (panel **A**) and cyanobacteria (panel **B**) between sampling sites and cyanobacterial 16S rRNA primers. Association between bacterial (panel **C**) or cyanobacterial (panel **D**) assemblages of primer A (circle) and primer B (triangle) in LDGD and LDQA sampling sites by use of co-inertia analysis (CIA). Each data point in panels C and D represents a 16S rRNA metagenomic sequencing library, and libraries from the same sample are linked together via a gray line. CIA is a multivariate method that identifies trends or co-relationships in multiple data sets. Global similarities (co-inertia) between primer A and primer B datasets for bacteria and cyanobacteria are 20.11% (p=0.156, permutation test n=9999) and 13.78% (p=0.199, permutation test n=9999), respectively.

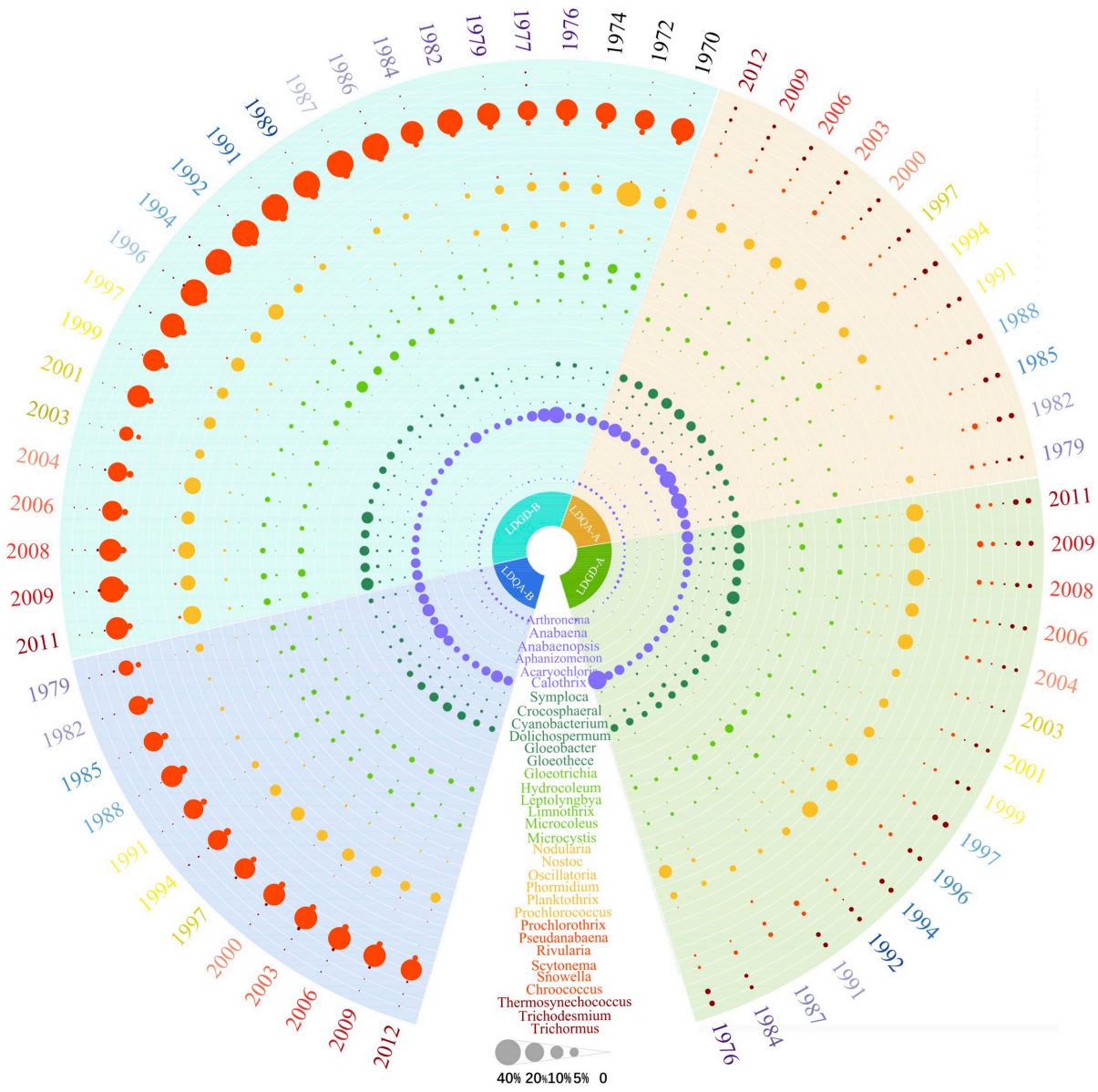

Figure 5.8. Shifts in taxonomic profiles of cyanobacteria in two arms of a Prairie reservoir over time (years). Circos plot shows the relative abundances of each Cyanobacteria genus at two sampling sites (Gardiner arm, LDGD and Qu'Appelle arm, LDQA) based on two different primer sets (A and B) of the Cyanobacterial 16S rRNA gene across the time scale (years are colour coded). The area of each circle is proportional to the relative abundance of each identified genus, in the Cyanobacterial communities each time interval. Dates represent the mid-point of each 1-cm sediment increment.

5.4.3. α-diversity

α-diversity estimates demonstrated significant changes in the diversity of the cyanobacterial assemblage over time (years) for both primers and at both sites sampled (Observed OTUs: ANOVA-year p=1.93e-08, -site p=3.32e-07, -primer p=0.48; Shannon-Wiener index: ANOVA-year p=0.021, -site p=0.782, -primer p=0.193) (Figure 5.9). In general, sediments from more recent years had greater richness of taxa, compared to sediments from earlier years, with similar but more variable increasing trends observed in the Shannon-Wiener index for both primers and both sampling locations (Figure 5.9).

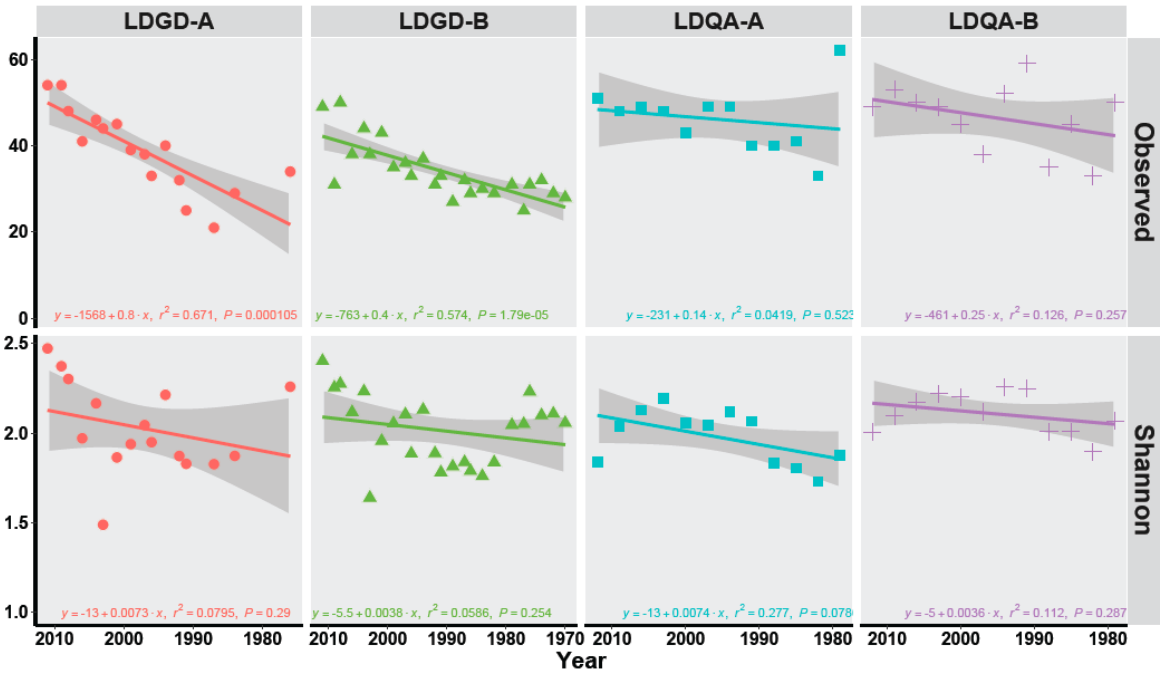

Figure 5.9. Shifts in Alpha diversity of cyanobacterial OTUs at the two sampling sites over time (years). Linear regressions between alpha diversities (number of observed OTUs and Shannon-Wiener index) and time (year) are given. Shaded areas are the 95% confidence intervals for each model. The equation and adjusted r^2 for the specific linear regressions are given in each panel.

5.4.4. β-diversity

β-diversities were significantly different among years (primer (a): Adonis test-year p= 0.0005; primer (b): Adonis test-year p= 0.0058) (Figure 5.10), suggesting a shift in composition within the cyanobacterial community over time. Also, dissimilarities within the community structure were observed within the temporal profile between the Gardiner and Qu'Appelle arms (primer (a): Adonis test-site p= 0.0071; primer (b): Adonis test-site p= 0.0054), as indicated by the non-overlapping centroids (Figure 5.10).

5.4.5. Paleo-variables and β-diversity of cyanobacterial community

In general, β-diversities of cyanobacterial assemblages were significantly correlated with year, $\delta^{15}N$, $\delta^{13}C$ and *Tabellaria flocculosa* for both primers and both sampling sites (Mantel test (Table 5.4). Significant positive correlations between cyanobacterial assemblages and NAIP, TP, C:N ratio, abundances of multiple sedimentary pigments (fucoxanthin, alloxanthin, canthaxanthin, lutein-zeaxanthin, Chlorophyll *a* and Chlorophyll *ap*), the *mcyA* gene, and sterols (cholesterol and campesterol), and *Fragilaria capucina* were observed in the Qu'Appelle arm sediment core for both primers.

5.4.6. GLM of paleo-physicochemical variables and cyanobacterial community

Across all sedimentary samples from the two sites and for both reverse primers, the relative abundances of individual genera of cyanobacteria differed significantly by sampling site, primer, year, and co-occurring paleo-physicochemical variables, such as, AP, percent N, $\delta^{15}N$, $\delta^{13}C$, C:N ratio, and PRSi, including annual reservoir draw-down (m), as determined by a GLM (Tables 5.2 and 5.3). Univariate analysis further indicated that the abundances of *Arthronema*, *Crocosphaera*, *Dolichospermum*, *Leptolyngbya*, *Microcoleus* and *Thermosynechococcus* were significantly affected by year (Table 5.3). Abundances of *Microcystis* and *Prochlorococcus* were significantly correlated with $\delta^{15}N$, and abundances of *Dolichospermum*, *Microcystis*, *Nostoc*, and *Prochlorococcus* significantly co-varied with C:N ratio.

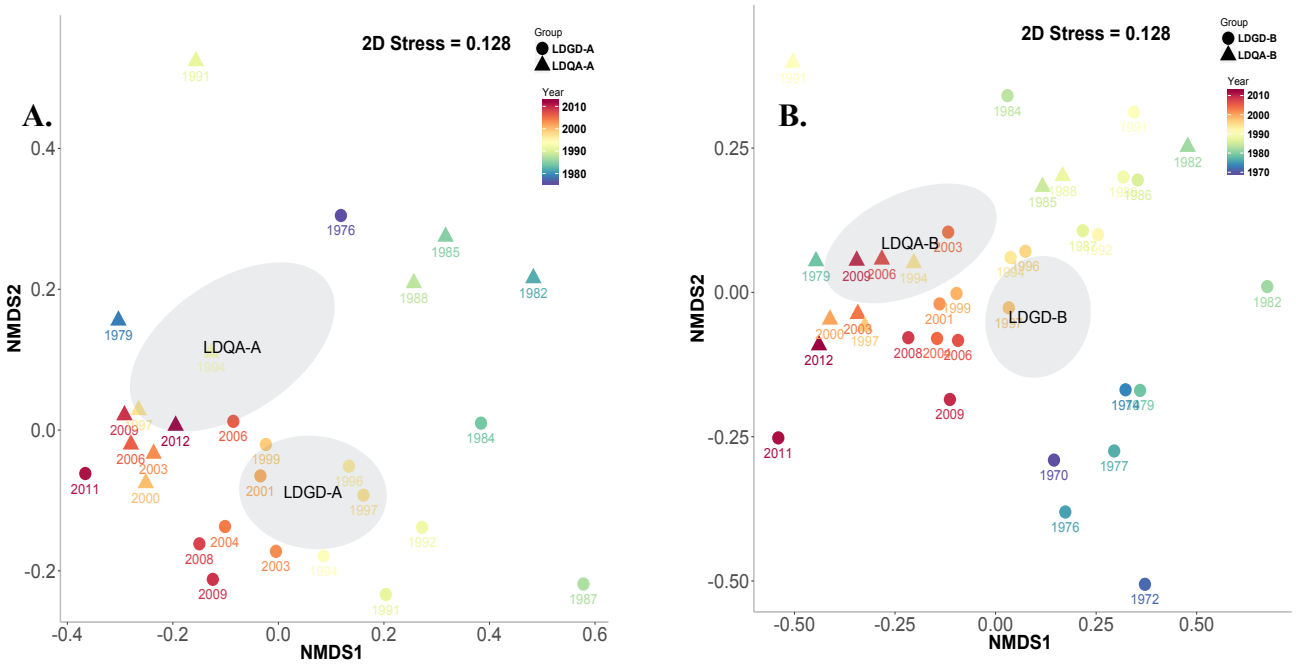

Figure 5.10. Shifts in Beta diversity of cyanobacterial OTUs over time (years). Non-metric multidimensional scaling (NMDS) visualizes the relative dissimilarities of cyanobacterial OTUs (**A**-Primer A and **B**-Primer B) over time and among sampling sites (circle-LDGD and triangle-LDQA). Distance matrices were generated using Bray-Curtis dissimilarities of normalized read counts and ordination was selected by minimizing stress on two dimensions. Each point corresponds to cyanobacterial reads from a normalized 16S rRNA gene sequencing library and years are colour coded in the corresponding legends. Shaded ovals represent the 95% confidence ellipse around the centroids of sampling sites. Permutational multivariate analysis of variance test (PERMANOVA, n=9999) further showed that cyanobacterial OTUs over time (years) and among sampling sites (centroids of LDGD and LDQA) are significantly different (Primer A: Adonis test-year p= 0.0005***, -site p= 0.071**; Primer B: Adonis test-year p= 0.0058**, -site p= 0.0054**).

Table 5.2. Generalized linear model (GLM) of sampling sites, primers and paleo-physicochemical variables for normalized abundance data of cyanobacterial genera in Lake Diefenbaker, Saskatchewan, Canada. Significance levels (adjusted $p<0.05$*, $p<0.01$**, and $p<0.001$***) for the negative binomial model (9999 permutations) of the overall model (multivariate test for data from two sampling sites and two primers) are indicated. Significance levels for the univariate tests of each cyanobacterial genus within the multivariate model are given in Table 5.3.

Parameters	**Multivariate Test**
	Overall model
Site	<2e-16***
Primer	<2e-16***
Year	<2e-16***
Draw down	0.001***
AP	0.047*
NAIP	0.062
OP	0.051
Percent N	<2e-16***
d15N	<2e-16***
d13C	0.004**
Percent C	0.354
C:N ratio	<2e-16***
Mn	0.039*
Fe	0.028*
PRSi	0.011*

Table 5.3. Generalized linear model (GLM) of sampling site, primer, year and paleo-physicochemical variables for abundance data of cyanobacterial genera in Lake Diefenbaker, Saskatchewan, Canada. Significance levels (adjust $p<0.05$*, $p<0.01$**, and $p<0.001$***) are given for the negative binomial model (9999 permutations) of the univariate tests of each cyanobacterial genus within the multivariate model.

Name	Site	Primer	Year	Draw down	AP	NAIP	OP	% N	d15N	d13C	% C	C:N ratio	Mn	Fe	Psi
Anabaena	0.458	0.995	0.965	0.998	1.000	0.966	0.992	0.058	1.000	1.000	0.999	0.992	1.000	1.000	1.000
Aphanizomenon	**0.029***	0.893	0.704	0.998	1.000	0.801	0.985	**0.023***	1.000	1.000	0.999	0.996	1.000	1.000	1.000
Arthronema	**0.015***	0.989	**0.005****	0.998	1.000	0.860	0.985	0.861	1.000	0.388	0.999	0.477	1.000	1.000	0.534
Calothrix	0.054	0.995	0.678	0.998	**0.015***	0.999	0.128	0.343	0.244	0.089	0.987	0.061	0.991	1.000	0.863
Chroococcus	0.980	**<2e-16*****	0.653	0.998	0.255	0.999	0.992	0.176	0.478	0.256	0.999	0.984	1.000	1.000	1.000
Crocosphaera	**0.027***	0.995	**0.002****	0.998	0.983	0.872	0.548	0.309	0.996	1.000	0.961	0.637	1.000	0.451	0.939
Cyanobacterium	0.123	0.989	0.937	0.992	0.842	0.998	0.971	0.444	0.828	0.996	0.956	0.996	0.991	0.964	0.990
Dolichospermum	0.833	0.580	**<2e-16*****	0.992	1.000	0.799	0.992	0.586	0.505	0.279	0.996	**0.026***	1.000	1.000	0.939
Gloeotrichia	0.089	0.995	0.857	0.998	1.000	0.985	0.945	0.213	1.000	1.000	0.999	0.996	1.000	1.000	1.000
Hydrocoleum	0.931	**<2e-16*****	0.965	0.998	1.000	0.998	0.992	0.954	1.000	1.000	0.999	0.900	0.822	0.944	1.000
Leptolyngbya	0.965	**0.036***	**0.001*****	**0.012***	1.000	0.976	0.987	0.586	1.000	**0.026***	0.999	0.984	**0.002****	1.000	0.352
Microcoleus	0.980	**<2e-16*****	**<2e-16*****	0.948	1.000	0.860	0.830	0.058	0.996	0.998	0.999	0.984	0.991	0.933	0.990
Microcystis	0.980	**0.004****	0.965	0.284	1.000	0.417	0.987	0.954	**0.001****	0.612	0.820	**0.026***	0.760	1.000	0.863
Nostoc	0.277	0.989	0.965	0.996	1.000	0.985	0.992	0.652	0.868	0.874	0.999	**0.026***	0.892	1.000	0.992
Oscillatoria	0.124	**0.011***	0.42	0.195	0.191	0.985	0.548	0.532	0.221	1.000	0.449	0.992	0.822	**0.025***	0.144
Phormidium	0.458	**<2e-16*****	0.194	0.990	0.971	0.999	0.992	0.097	0.121	1.000	0.849	0.061	1.000	0.531	1.000
Prochlorococcus	0.912	0.913	0.254	**0.002****	1.000	0.966	0.986	0.652	**0.005****	0.866	0.911	**<2e-16*****	0.648	0.573	0.939
Prochlorothrix	0.458	**<2e-16*****	0.99	0.991	0.953	0.995	0.548	0.885	0.450	0.256	0.999	0.992	1.000	0.999	0.996
Pseudanabaena	0.277	0.989	0.937	0.998	0.839	0.354	0.992	0.954	1.000	1.000	0.999	0.996	1.000	1.000	1.000
Rivularia	0.915	0.913	0.937	0.998	0.997	0.800	0.992	0.954	1.000	1.000	0.999	0.996	1.000	1.000	1.000
Snowella	0.931	**<2e-16*****	0.523	0.998	0.613	0.995	0.987	0.651	0.450	1.000	0.961	0.477	0.999	0.933	1.000
Symploca	0.980	0.989	0.965	0.990	1.000	0.995	0.977	0.954	0.055	1.000	0.961	0.477	0.991	0.999	0.939
Thermo-synechococcus	**<2e-16*****	0.995	**<2e-16*****	0.990	1.000	0.995	0.683	0.176	1.000	1.000	0.996	0.735	1.000	0.590	1.000
Trichodesmium	0.856	**<2e-16*****	0.965	0.998	1.000	0.995	0.979	0.992	0.999	1.000	0.999	0.061	1.000	0.995	1.000
Unclassified	0.058	0.462	0.704	0.954	0.971	0.803	0.927	0.954	0.848	0.715	0.999	0.562	0.957	0.703	**0.007****

Table 5.4. Mantel tests (9999 permutations) correlations between paleo-variables (sedimentary pigments, *mcyA* gene, sterols, stanols, and compositions of diatom community) (Euclidean distance) and beta diversity of cyanobacterial community (Bray-Curtis distance) at each sampling site. Data in bold indicate significant correlations at $p<0.05$*, $p<0.01$**, and $p<0.001$***.

Items	LDGD-A		LDGD-B		LDQA-A		LDQA-B	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Year	**0.345**	**0.042***	**0.231**	**0.006****	**0.476**	**0.003****	**0.603**	**0.001*****
Draw down	-0.045	0.591	0.112	0.150	-0.065	0.598	-0.035	0.537
AP	-0.026	0.464	0.116	0.076	0.093	0.291	0.136	0.236
NAIP	-0.004	0.353	0.097	0.167	**0.285**	**0.023***	**0.361**	**0.01****
OP	0.107	0.174	0.050	0.275	0.025	0.388	0.025	0.388
NAIP.OP	0.063	0.212	0.110	0.130	0.220	0.053	**0.276**	**0.028***
TP	0.044	0.246	0.110	0.130	**0.333**	**0.01****	**0.418**	**0.004****
Percent. N	0.261	0.090	0.180	0.063	0.106	0.217	0.107	0.216
d15N	0.280	0.084	**0.240**	**0.015***	**0.452**	**0.007****	**0.527**	**0.002****
d13C	**0.367**	**0*****	**0.181**	**0.023***	0.287	0.080	**0.392**	**0.032***
Percent. C	0.232	0.094	0.165	0.081	0.015	0.424	0.004	0.464
C.N. ratio	0.326	0.062	0.215	0.058	**0.284**	**0.023***	**0.329**	**0.017***
Mn	0.033	0.263	0.125	0.118	0.217	0.096	0.204	0.116
Fe	-0.022	0.481	0.032	0.295	0.071	0.334	0.140	0.248
Psi	0.077	0.225	0.069	0.216	0.044	0.371	0.092	0.286
Fuco	0.137	0.136	0.051	0.255	**0.250**	**0.04***	**0.317**	**0.017***
Myxo	0.128	0.111	0.028	0.328	-0.189	0.914	-0.183	0.835
Allo	0.122	0.118	0.022	0.346	**0.308**	**0.016***	**0.396**	**0.008****
Diato	0.201	0.065	0.052	0.253	0.181	0.120	0.221	0.084
Lutein	0.141	0.094	0.028	0.325	**0.314**	**0.012***	**0.386**	**0.006****
Cantha	0.226	0.066	0.094	0.150	**0.280**	**0.029***	**0.365**	**0.013***
Chl_b	0.120	0.158	0.060	0.238	-0.028	0.491	-0.009	0.459
Chl_a	0.208	0.069	0.036	0.288	**0.238**	**0.047***	**0.312**	**0.017***
Chl_ap	0.117	0.120	-0.018	0.486	**0.333**	**0.016***	**0.427**	**0.004****
Echine	0.056	0.226	-0.017	0.477	-0.072	0.621	-0.110	0.716
Phaeo_B	0.010	0.354	0.038	0.298	-0.316	0.996	-0.263	0.954
Pheo_A	0.158	0.134	0.060	0.251	-0.250	0.933	-0.204	0.837
B.car	0.123	0.153	0.004	0.410	-0.199	0.872	-0.190	0.858
mcyA	-0.011	0.386	0.046	0.270	**0.347**	**0.008****	**0.457**	**0*****
Cholesterol	0.059	0.232	-0.017	0.481	**0.310**	**0.032***	**0.388**	**0.014***
Campesterol	0.112	0.135	-0.032	0.554	**0.303**	**0.018***	**0.358**	**0.011***
B.sitosterol	0.046	0.238	-0.038	0.575	0.197	0.089	**0.248**	**0.044***
Coprostanol	0.010	0.316	-0.093	0.775	-0.197	0.855	-0.146	0.702
Stigmastanol	0.038	0.275	0.008	0.395	-0.179	0.815	-0.125	0.718
Aulacoseira ambigua	0.022	0.366	0.016	0.375	0.152	0.183	0.148	0.184
Asterionella formosa	0.179	0.102	0.145	0.079	0.067	0.277	0.148	0.149
Cyclotella bodanica	0.073	0.233	**0.158**	**0.033***	0.025	0.420	-0.019	0.494
Fragilaria crotonensis	0.206	0.120	**0.228**	**0.006****	0.065	0.318	0.066	0.316
Stephanodiscus medius	-0.151	0.854	-0.025	0.556	-0.037	0.453	0.042	0.367
Tabellaria flocculosa	0.193	0.133	**0.151**	**0.024***	**0.605**	**0.001*****	**0.626**	**0.001*****
Stephanodiscus niagarae	0.096	0.168	**0.187**	**0.025***	NA	NA	NA	NA
Stephanodiscus parvus	0.165	0.115	**0.205**	**0.039***	NA	NA	NA	NA
Fragilaria capucina	NA	NA	NA	NA	**0.242**	**0.047***	**0.234**	**0.047***
Cocconeis placentula	-0.168	0.904	-0.032	0.539	NA	NA	NA	NA
Cyclotella	0.003	0.347	0.045	0.288	NA	NA	NA	NA

5.4.7. Correlation of paleo-variables and individual genera of cyanobacteria

Spearman's ranked correlation coefficient was applied to examine possible relationships among α-diversities, individual paleo-variables, and relative abundances of individual cyanobacterial genera (Figures 5.5 and 5.11). Between the two sites and two reverse primers, *Dolichospermum*, *Prochlorococcus*, and *Thermosynechococcus* generally co-varied with the above variables in similar ways. These genera were significantly and negatively correlated with C:N ratio, but significantly and positively correlated with some paleo-variables (year, percent N; abundances of NAIP+OP, TP, Allo, Lutein, Cantha, Chl_a, Chl_b, Chl-ap, Echine, cholesterol, campesterol, and β-sitosterol; and percent relative abundances of *Aulacoseira ambigua* and *Tabellaria flocculosa*) as well as the Shannon-Wiener index. Abundance of the *mcyA* gene was significantly and positively correlated to the relative abundance of *Dolichospermum*, as well as other co-occurring genera, within the profile from the Qu'Appelle arm (Figure 5.11). *Microcoleus* showed an opposite trend (primer (b) data) compared to *Dolichospermum*, *Prochlorococcus*, and *Thermosynechococcus*, for both the Gardiner and Qu'Appelle arms.

5.4.8. Network analysis between paleo-variables and genera of cyanobacteria

Network analysis of co-occurrence was performed on all available paleo-variables to visualize correlations between individual parameters and with each variable and each of the cyanobacterial genera (Figure 5.12 and Table 5.5). Based on both reverse primers and the two sites investigated, this analysis revealed complex networks of both positively and negatively robust correlations, and 7 different major modules were identified in these interaction networks. Overall, the crucial nodes, determined by eigenvector centrality of the modules, of cyanobacteria genera were *Dolichospermum*, *Prochlorococcus*, and *Thermosynechococcus*, and they tended to co-occur with each other and with certain paleo-variables (NAIP, percent N, $\delta^{15}N$, percent C, percent N, C:N ratio, Cantha, Chl_a, Chl_b, Chl-ap, cholesterol, campesterol, and B.sitosterol) as well as diatom species including *Tabellaria flocculosa* in both Gardiner and Qu'Appelle arms.

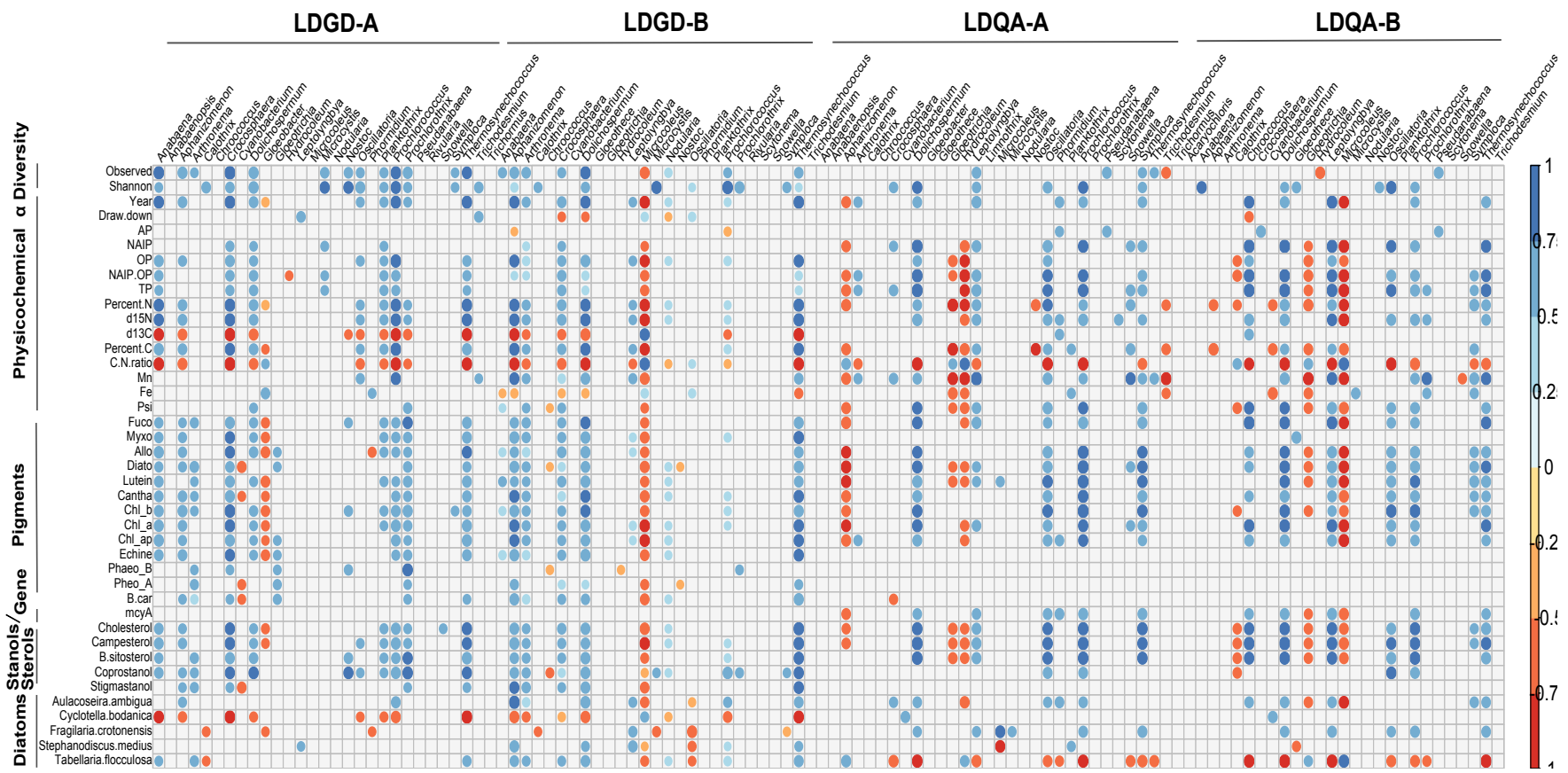

Figure 5.11. Matrix of Spearman's ranked correlation coefficients (ρ) between alpha diversities, individual paleo-physicochemical characteristics and paleo-biological variables (sedimentary pigments, *mcyA* gene, sterols, stanols and main diatom species) and relative abundances of individual cyanobacterial genera in each cyanobacterial community. The size and intensity of colour for each circle represents the strength of the correlation (the larger, darker circles demonstrate robust and significant correlation coefficients ρ). Blue colours illustrate positive correlations and red colours illustrate negative correlation coefficients. Only correlations that are statistically significant (p<0.05) are shown.

5.4.9. Network analysis of the co-occurrence patterns

It was hypothesized that the non-random co-occurrence patterns between genera of cyanobacteria and paleo-proxies could indicate the relative importance of some variables in shaping the diversity or composition of cyanobacterial community if they possessed significantly similar or opposite abundance trends among the different paleo-environments. Analysis of patterns of co-occurrence by use of correlation-based network analysis allows detection of pairwise relationships between taxa and between taxa and characteristics of the environment. This approach has been successfully applied to discern ecological linkages among microorganisms in marine water (Steele et al, 2011) and soil (Barberán et al, 2011), as well as to elucidate interactions between microorganisms in marine or lacustrine ecosystems and their environments (Ruan et al, 2006; Eiler et al, 2011; Kara et al, 2012). Graphs of co-variance (Figure 5.10) demonstrate associations or interactions between specific genera of cyanobacteria and a variety of paleo-variables for each primer used. For primer (a) and the Gardiner arm (Figure 5.12A), *Prochlorococcus*, the most abundant cyanobacteria identified, was associated with several physicochemical factors. For the same site/primer, *Dolichospermum* appears to be associated with biomolecules, such as coprostanol, while the C:N ratio was correlated with abundances of *Nostoc* and *Prochlorothrix*. Based on this analysis, the cyanobacteria community can be arranged into different groups. For example, Mod 1 shows linkages among *Prochlorococcus, Crocosphaera, Oscillatoria, Anabaena, Thermosynechococcus, Aphanizomenon* and *Microcystis*; whereas, *Dolichospermum, Nostoc* and *Prochlorothrix* exhibited linkages among each other. For primer (b) (Figure 5.12B), *Dolichospermum* appears to have a more dominant appearance, with strong correlations with other cyanobacteria and with diatoms. Linkages within the cyanobacteria community were observed, with diatom genera sharing correlations with cyanobacteria genera and paleo-variables. For the Qu'Appelle arm and primer (a) (Figure 5.12C), three dominant groups were observed, with diatoms *Fragilaria, Stephanodiscus,* and *Asterionella* comprising one group; *Prochlorococcus, Symploca, Dolichospermum* and *Oscillatoria* consisting of another group; and *Crocosphaera, Thermosynechococcus, Leptolyngbya, Snowella, Trichodesmium, Planktothrix, Gloeotrichia, Nostoc* and *Aphanizomenon* constituting the third major group. These groups all exhibited linkages with different measured variables. Similarly, linkages among various groups of cyanobacteria, diatoms, and physicochemical variables were observed for primer (b) in the

Qu'Appelle arm (Figure 5.12D). Again, *Dolichospermum* predominated within the cyanobacterial community and seems to be associated with several physicochemical parameters (Figures 5.12B, C and D).

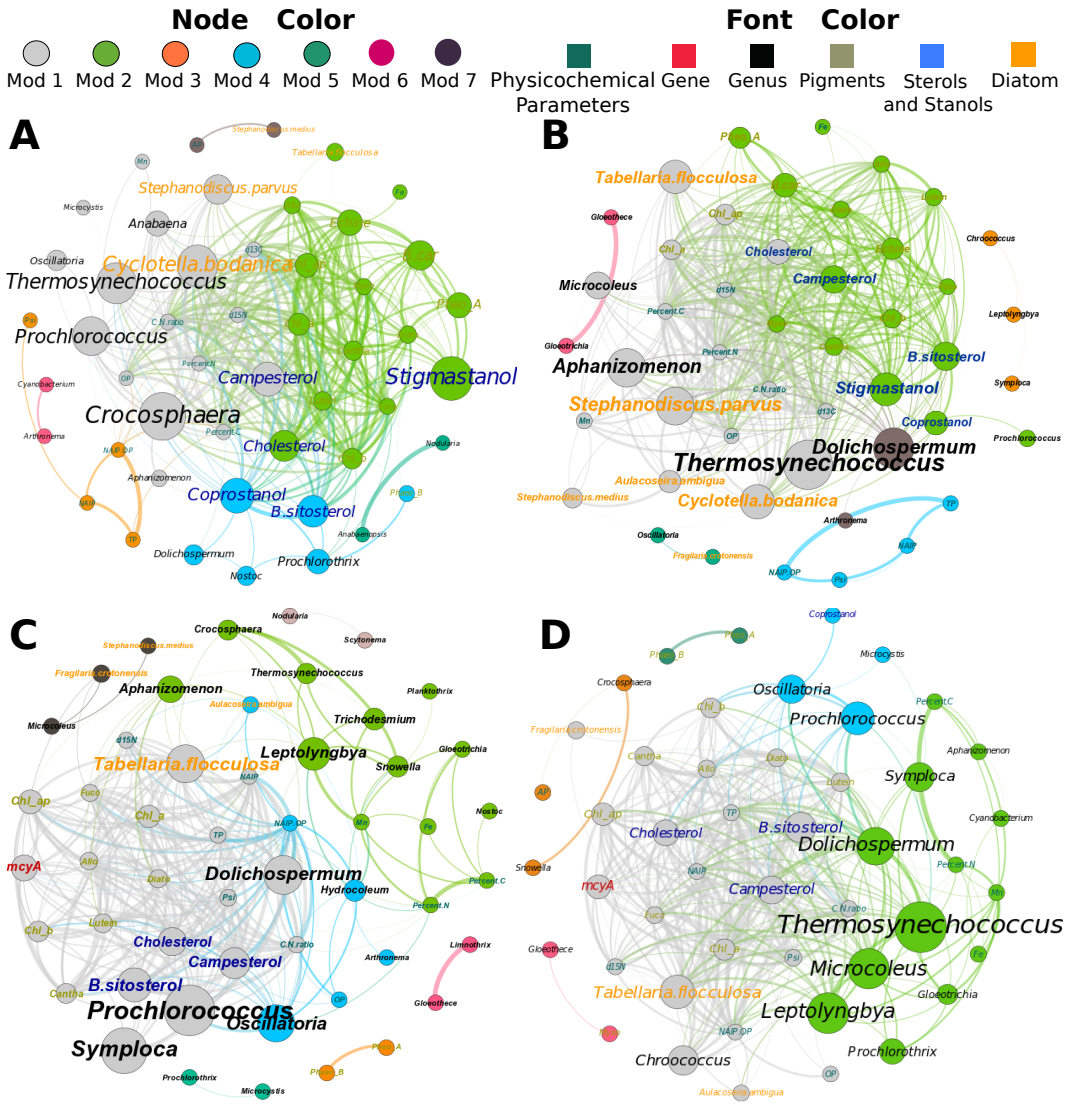

Figure 5.12. Network analysis of co-occurrence patterns among paleo-physicochemical characteristics, paleo-biological variables (sedimentary pigments, *mcyA* gene, sterols, stanols, and diatom communities) and cyanobacterial genus (**A**: LDGD-A, **B:** LDGD-B, **C:** LDQA-A, and **D:** LDQA-B). A connection stands for a robust and significant correlation (Spearman's correlation coefficients $|\rho|>0.7$ and $p<0.01$). The node size and font size of each node is proportional to the eigenvector centrality; the thickness of each edge is proportional to Spearman's $|\rho|$ ranging from |0.7| to |1|. The nodes were coloured by modularity class.

T**able 5.5.** Topological statistics for co-occurring networks among paleo-physicochemical characteristics, paleo-biological variables (sedimentary pigments, *mcyA* gene, sterols, stanols and core diatom communities) and cyanobacterial genus (Figure 5.11).

Name	**LDGD-A**	**LDGD-B**	**LDQA-A**	**LDQA-B**
Nodes	49	47	51	47
Edges	353	411	307	326
Average Degree (AD)	14.408	17.489	12.039	13.872
Network Diameter (ND)	5	3	4	3
Graph Density (GD)	0.3	0.38	0.241	0.302
Modularity (MD)	0.119	0.095	0.191	0.152
Clustering coefficient (CC)	0.315	0.335	0.281	0.303
Average path length (APL)	1.67	1.337	1.586	1.403

5.5. Discussion

Based on LandSat imagery from the last 29 years, surface algal blooms in Lake Diefenbaker primarily occur within the Qu'Appelle arm (Yip et al., 2015; Vogt et al., 2015). Recent blooms observed in this region by local residents led to concerns regarding water safety and ongoing trends (e.g. declining water quality). Paleolimnological studies of the Qu'Appelle arm have indicated that this region of the reservoir experienced a change in trophic status in the mid to late 1990's (Tse et al., 2015), that plateaued in the late 1990's. In this study, cyanobacterial genera with species known to produce toxins were identified within the cyanobacterial community of both arms of Lake Diefenbaker (e.g. *Dolichospermum* sp. and *Microcystis* sp.). Presence of these organisms suggests water quality issues in the reservoir, which is currently the primary source of potable water for much of Southern Saskatchewan (North et al., 2015). In particular, trends in the relative abundance of *Dolichospermum* are a cause of concern, as further discussed below.

5.5.1. Composition and biodiversity of the community of cyanobacteria

Using NGS, diverse communities of cyanobacteria were identified in each arm of the reservoir. The large portion of unclassifiable genera indicates the existence of potentially novel cyanobacteria. However, taxa that were identified (Figure 5.8), were similar to those taxa recently identified using traditional microscopy observations of water from Lake Diefenbaker (6 similar cyanobacterial genera; *Anabaena*, *Aphanizomenon*, *Chroococcus*, *Microcystis*, *Planktothrix* and *Pseudanabaena*) (Abirhire et al., 2015). Furthermore, the shift in α-diversity over time (Figure 3) indicates a significant change in composition of the cyanobacterial community during the life of the reservoir. Some similar cyanobacteria were common to both the Gardiner and Qu'Appelle arms (Figures 5.8 and 5.7). Nevertheless, recent communities of cyanobacteria in either arm were distinctly different from those of the early reservoir, based on changes in both the number and relative abundance of the identified taxa. The β-diversity (Figure 5.10), for both reverse primers, also illustrated a significant change in cyanobacterial community composition through time at both locations. This was not unexpected given that reservoirs typically undergo ontogenic changes post-formation, changes known to influence reservoir water quality, biology and trophic status (Thornton et al., 1990).

The cyanobacterial community also consisted of several potentially harmful taxa, *Planktothrix*, *Microcystis*, and *Dolichospermum* (formerly *Anabaena*; Waklin et al., 2009). *Dolichospermum circinalis* was previously identified as the dominant cyanobacterium in surface waters of Lake Diefenbaker in summer of 2008 (Hecker et al., 2012). However, results of the current study suggest that *Dolichospermum* has not been the dominant taxon during any period since filling of the reservoir. Therefore, there is some discontinuity between the dominant genera identified through eDNA analysis (*Chroococcus* and *Prochlorococcus*) and traditional cell counting techniques (*Dolichospermum*). *Dolichospermum* species are common to freshwater lakes worldwide and can severely degrade water quality through production of cyanotoxins (Al-Tebrineh et al., 2012), including both hepato- and neurotoxins (Sivonen and Jones 1999). The consistent presence and increasing trend in relative abundance of *Dolichospermum* over time in both the Gardiner and Qu'Appelle arms is of concern, particularly if these trends in the Gardiner arm continue, because it may signify an increasing presence of this potentially toxic cyanobacterium in the region.

Some cyanobacterial taxa can produce trans-1,10-dimethyl-trans-decalol (geosmin) and

2-methylisoborneol (MIB), which are responsible for taste and odour issues in drinking water (Journey et al., 2013). Genera of cyanobacteria known to produce geosmin and MIB identified in this study included: *Dolichospermum, Planktothrix, Oscillatoria, Aphanizomenon,* and *Symploca* (Izaguirre et al., 1982). These compounds frequently co-occur with cyanotoxins (Journey et al., 2013), although many species are unable to produce taste/odour compounds and cyanotoxins simultaneously (Chorus and Bartram 1999; Graham et al., 2010).

Cyanobacteria are a minor component (<10%) of the overall bacterial OTUs identified in profiles of sediments from Lake Diefenbaker, similar to observations made in 2011-2012 (Abirhire et al., 2015). However, the relative proportion of cyanobacteria was less than observed in another nearby prairie reservoir, Buffalo Pound Lake, where the community of cyanobacteria represented upwards of ~30% of overall OTUs identified (Chapter 4). Unlike cyanobacteria, eDNA from non-phototrophic bacteria can originate in either the water column or sediment profile. Therefore, general temporal trends in non-phototrophic bacteria cannot be inferred. Nevertheless, our data suggest differences in relative abundances of phototrophic versus non-phototrophic bacteria between the mesotrophic (Lake Diefenbaker) and eutrophic (Buffalo Pound Lake) systems.

5.5.2. *mcyA* abundance

The presence of potentially harmful genera of cyanobacteria raised concerns regarding the potential production of toxic secondary metabolites, or cyanotoxins, in the Gardiner and Qu'Appelle arms of Lake Diefenbaker. A suite of hepato- and neuro-toxic secondary metabolites of cyanobacteria was previously investigated using the same samples; however, all compounds investigated were below the detection limit (<1 ppb) in the sediment profiles of each arm (T. Tse, unpublished data, University of Saskatchewan, 2015). This suggests that production of toxins is low or that preservation of toxins in sediment is poor, or that both conditions exist. In lieu of quantifying toxins, quantification of toxin-producing genes can provide insight into trends in the potential for production of toxins.

The community of cyanobacteria identified by NGS and microscopic observation included two potentially harmful genera, *Dolichospermum* and *Microcystis,* with the former illustrating an increasing trend in relative abundance and the latter exhibiting a continued presence in each arm (Figure 5.8). Bloom-forming species of *Microcystis* generally synthesize

toxic products (Carmichael 1995), however non-toxic strains do exist (WHO 1999). Although no microcystins were detected in sediment profiles (T. Tse unpublished data, University of Saskatchewan, 2015), presence of the *mcyA* gene throughout the core suggests that genera able to produce microcystins have been present since formation of the reservoir. Relative abundances of the *mcyA* gene illustrated positive correlations in the Qu'Appelle arm with various genera of cyanobacteria, including *Dolichospermum*. Further, observations in trends of cyanopigments, chlorophyll *a*, and total pigment production were similar to trends in abundances of the *mcyA* gene in both arms (Figure 5.3). Although non-toxic genera followed similar trends to *Dolichospermum*, the positive correlation with abundances of *mcyA* gene suggests that this genus was and continues to be the primary source of this gene in Lake Diefenbaker. However, the *mcyA* gene mapped to uncultured cyanobacterium (99% confirmed identity) when compared against the NCBI BLAST nucleotide nr/nt database.

5.5.3. Correlations of relative abundances of cyanobacteria with other environmental proxies

Multiple paleo-variables have been investigated in Lake Diefenbaker and inferred temporal trends indicate changes in the ecology of both the Gardiner and Qu'Appelle arms. Trends in eDNA data were compared to assess concordance among investigations (e.g. physicochemical, biochemical and subfossil remains). For example, temporal trends in sterols and stanols were similar to trends observed for various pigments produced by cyanobacteria and illustrated covariance with multiple cyanobacterial taxa (Figures 5.11, 5.12 and 5.5). In this study, 24-ethylcholesterol (β-sitosterol) and campesterol concentrations were positively correlated with abundances of various cyanobacteria, including *Dolichospermum*. β-sitosterol and campesterol can be synthesized by many microalgae and cyanobacteria (Matsumoto et al., 1982; Volkman 2003), with β-sitosterol used to characterize cyanobacteria (Volkman 2003). Some terrestrial plants and green algae can also produce these compounds, including cholesterol (Ikekawa et al., 1968; Grunwald 1975), but in Lake Diefenbaker, due to the draw-down regime and frequent turbidity of water, macrophytes are limited. Furthermore, the sterol source index demonstrated fairly constant contributions from terrestrial plants throughout the history of Lake Diefenbaker (see Appendix). In addition, the low coprostanol/cholesterol ratio (< 1) signifies that sewage/fecal pollution is not of concern within this reservoir. Interestingly, the

coprostanol/cholesterol ratio increased (>0.2) in the sediment increments below 25-cm. Analyses of diatom remains marked the bottom of the Gardiner arm at the 25-cm increment (Lucas et al., 2015a); therefore, the elevated coprostanol/cholesterol ratio in the lower portion of the sediment core likely reflects the pre-flooding origin of this material.

Correlations among relative abundances of genera of cyanobacteria and concentrations of various algal pigments in sediments (Figure 5.11) suggest an increase over time in both overall algal production and the production of cyanobacteria. This increase has favored certain taxa in the Gardiner and Qu'Appelle arms. For example, myxoxanthophyll, echinenone and zeaxanthin are typically biomarkers for cyanobacteria (Leavitt and Hodgson 2001). These cyanopigments were positively correlated with the relative abundance of various genera of cyanobacteria including *Dolichospermum* in the Gardiner arm. This suggests that these cyanobacteria exhibit not only a continued presence within this reservoir, but that the total production of *Dolichospermum* and other cyanobacteria has increased.

The GLM revealed positive correlations between measured paleo-variables and relative abundances of genera of cyanobacteria, as inferred from eDNA, particularly for the Qu'Appelle arm (Table 5.4). These results indicate concordance between various paleolimnological proxies related to the trophic status of the lake and paleometagenomic data. Although there may be some uncertainty in interpreting sedimentary phosphorus variables individually (discussed in Lucas et al., 2015a), the positive correlation of the relative abundances of *Dolichospermum*, the *mcyA* gene (in the Qu'Appelle arm), and multiple proxies of trophic state (Figure 5.3B) suggest concordance among proxies and provide additional confidence in the inferred trends. A continued increase in the relative abundance of *Dolichospermum* might contribute to future degradation in the water quality of Lake Diefenbaker. In addition, the pattern of concentrations of eDNA observed in the Qu'Appelle arm deviates from the monotonic pattern anticipated if eDNA distribution in the vertical sediment profile was due to degradation of DNA over time. That is concentrations of eDNA decreasing progressively as a function of depth in the core. Therefore, similar trends in other environmental proxies, including cyanobacterial pigments, biologically available P, PRSi and abundances of the *mcyA* gene in the Qu'Appelle arm suggests that potential for production of cyanobacterial toxins co-varies with trophic status within this region of the reservoir.

Although eDNA from potentially harmful cyanobacteria was found in both arms, each

arm exhibited somewhat different trends in the metagenomic data and environmental proxies that were measured. These differences are likely attributable to the distinct hydrology and geomorphology of each of these two arms. (Chapter 3; Lucas et al., 2015 a, b; Maavara et al., 2015).

5.6. Conclusions

The assemblage of cyanobacterial taxa identified using a paleo-16S-high-throughput sequencing approach was consistent with results of previous studies that have assessed absolute and relative abundances of phytoplankton in Lake Diefenbaker (Abirhire et al., 2015). This demonstrated the feasibility and usefulness of NGS as applied to sedimentary eDNA for the reconstruction of the historical assemblages of cyanobacteria and long-term trends in freshwater systems. Previously, observations of myxoxanthophyll within the two arms of the lake suggested the occurrence (Qu'Appelle arm) or an increase (Gardiner arm), respectively, in the abundance of potentially harmful cyanobacteria (Chapter 3). Paleo-16S rRNA sequencing results provided more detailed taxonomic information regarding relative abundances of taxa linked to myxoxanthophyll, including the harmful *Dolichospermum*, *Microcystis* and *Planktothrix.* In addition, the presence of *Anabaena, Planktothrix, Oscillatoria, Aphanizomenon, Lyngba, Symploca* and *Synechococcus* might indicate the potential for taste-and-odour issues to arise because of the ability of these taxa to produce geosmin and MIB metabolites. Furthermore, some non-cyanobacterial heterotrophs and bacteria are also known to produce geosmin and MID (Klausen et al., 2005), thus the bacterial community composition could also be investigated to identify potential contributors for these nuisance compounds. Although no cyanotoxins from cyanobacteria were detected in sediments, the presence of the *mcyA* gene suggests that the potential for toxin production has been present since formation of Lake Diefenbaker in 1967. Furthermore, abundances of the *mcyA* gene demonstrated trends similar to those of other proxies for primary producers. Findings reported here demonstrate how a combination of eDNA and paleo-proxies can be used to retrospectively assess the influence of environmental conditions on cyanobacterial communities in freshwater ecosystems. This approach might also provide data critical to resolving outstanding controversies about the evolution and global dispersal of toxic cyanobacterial species. Depending upon project goals, NGS technology can be used as a rapid screening alternative or complementary addition to traditional limonological or

paleolimnological techniques and can provide a more taxonomically-sensitive approach than analyses of phytopigments. This study reinforces the utility of the eDNA-based paleolimnological methods to study long-term changes in the ecology and quality of aquatic ecosystems.

CHAPTER 6

General Discussion

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6.1. General Discussion

Cultural eutrophication has become an environmental issue for many freshwater lakes, worldwide (Shaw et al., 2003). Nutrient enrichment can encourage excess primary productivity resulting in detrimental effects to the affected waterbody. Part of this thesis is one component of a larger collaborative research program to investigate and reconstruct historical trends in the environmental quality of an important freshwater reservoir, Lake Diefenbaker, Saskatchewan, Canada. This reservoir supplies water to various municipalities (~45 of Saskatchewan residents), as well as to industries and agricultural operations and is a site of substantial recreational activities. Lake Diefenbaker and the waters of the South Saskatchewan River, in their current state, represent a significant source of revenue for the province of Saskatchewan, with a valuation in the tens of millions of dollars associated with water use, hydroelectric power production and waste assimilation (Kulshreshtha and Gillies 1993; Smith and Kells 1993). Therefore, it is critically important to understand the trajectory of the water and environmental quality of this reservoir. Here, paleolimnological techniques were used to investigate these trends in Lake Diefenbaker to help inform water management.

In the absence of long-term monitoring data, the analysis of physicochemical (Lucas et al., 2015a), subfossil (Lucas et al., 2015b), and biomolecular variables (described in this thesis) within the sediment archive were assessed to provide a clearer understanding of the temporal trends in some environmental aspects of Lake Diefenbaker. For example, sterols and stanols were used to investigate the presence of fecal pollution, which can be a significant source of nutrients that may contribute to the growth of algae within an aquatic system, although this was not observed in Lake Diefenbaker, as sewage pollution was minimal (see appendix A). By modifying existing pigment methodology, a method was successfully implemented for the simultaneous extraction of algal pigments and sterols and stanols from sediment. This methodology was demonstrated in Chapters 2 and 3 and was initially tested on a sediment core collected from Ross Lake, Manitoba, to reconstruct fecal pollution trends. The trends observed illustrated the clear presence of fecal pollution during

the expansion of mining operations and the municipality, and subsequent decrease after the construction of the waste water treatment plant. Fecal pollution appears to have been minimal since the formation of Lake Diefenbaker, as indicated by a low coprostanol to cholesterol ratio (Appendix A) throughout the temporal profile of all sediment cores collected. However, the fecal sterol and stanol ratios in the Gardiner arm of Lake Diefenbaker proved useful in distinguishing between pre- and post- flooding sediment.

Sedimentary pigment analyses (Chapter 3), combined with other lines of evidence (e.g. diatom remains and physicochemical parameters; see Lucas et al., 2015a, b) revealed spatial and temporal variation in reservoir ecology. Distinct ecological regions of Lake Diefenbaker were identified, likely due to differences in the morphology and hydrology which exist along the longitudinal axis of this and other similar river-valley reservoirs (Thorton et al., 1990). Sediments from up-reservoir locations suggested relatively consistent primary production, nutrient loading and trophic status throughout the temporal coverage of the collected sediment cores (two cores representing approximately 8 and 13 years of deposition). However, sediments from down-reservoir locations suggest increasing primary production over the last two decades, particularly in the Qu'Appelle arm (Chapter 3). An increase in trophic status was observed in the Qu'Appelle arm in the early 1990s, but this trend plateaued in the late 1990s (Chapter 3; Lucas et al., 2015a).

Primary production appears to increase in Lake Diefenbaker with increasing distance down-reservoir based on strong correlations between distance down-reservoir and primary productivity proxies ($\delta^{13}C$, C:N ratio, TOC and rates of algal pigment deposition) (Chapter 3; Lucas et al., 2015a) in surficial sediments. This is likely explained by typical river-valley reservoir limnology whereby up-reservoir locations have increased turbidity and light attenuation compared to down reservoir locations (Kimmel et al., 1990; Kimmel and Groeger, 1984). Increases in primary productivity with distance down-reservoir, until available nutrients are depleted, has been demonstrated elsewhere (Kennedy et al., 1982; Kimmel and Groeger 1984). In addition, the current paradigm is that following reservoir impoundment there is an increase in nutrient availability resulting from the dissolution of soluble soil nutrients from inundated lands. This then leads to a trophic up-surge following reservoir formation (Thornton et al., 1990). However, the temporal profiles of sedimentary algal pigments from the sediment cores collected at down-reservoir locations in Lake Diefenbaker failed to demonstrate this phenomenon. If an

initial nutrient increase did occur in the down–reservoir regions, it could be that algal production was much less compared to more recent years, possibly due to high turbidity associated with eroding shoreline in the early years, resulting in light limitation and restricted primary productivity (Chapter 3).

Algal pigment analysis indicated the presence of a broad range of phototrophic taxa, and provided information regarding long-term environmental change along Lake Diefenbaker (Chapter 3). The algal community assemblages in up-reservoir locations were fairly consistent for the 8–13 years represented by the sediment cores. However, an increasing trend in pigment concentration was observed at mid- to down-reservoir locations over the past two decades. Although evidence of cyanobacteria taxa was observed in all investigated regions of the reservoir, certain taxa were found only in the Gardiner and Qu'Appelle arms. Myxoxanthophyll, a pigment biomarker for cyanobacteria (Leavitt and Hodgson 2001), was observed and quantified only within these two down-reservoir arms. This suggests that potentially harmful cyanobacterial genera may have occured at these locations. This raises concerns because myxoxanthophyll indicates the presence of potentially problematic cyanobacteria (Schlüter et al., 2004). Although pigment analyses is rapid and can give broad insight into the algal community composition within an aquatic system, these data cannot provide high resolution analyses (i.e. identification of organisms to lower taxonomical levels). Therefore, next-generation sequencing was conducted to provide high-resolution taxonomy of the cyanobacterial community in the sediment cores collected from the Gardiner and Qu'Appelle arms of Lake Diefenbaker (Chapters 4 and 5).

Environmental DNA can be used to identify past and recent presence of aquatic organisms, such as invasive or nuisance organisms (Turner et al., 2015). Metagenomic sequencing of the V3 region of the cyanobacterial 16S rRNA gene (~379 bp) was conducted on eDNA extracted from sediment cores to reconstruct the historical composition of the cyanobacterial community in the Gardiner and Qu'Appelle arms. Because it was unknown whether eDNA was preserved within the sediment profile of Lake Diefenbaker, or whether it was possible to extract and sequence such DNA, Buffalo Pound Lake was chosen as a positive reference site. This nearby reservoir is fed through the Qu'Appelle River–system from Lake Diefenbaker and is known to suffer from frequent cyanobacterial blooms.

Metagenomic reconstruction of the cyanobacterial community of Lake Diefenbaker revealed the presence of potentially problematic cyanobacteria (e.g. *Dolichospermum*, *Microcystis* and *Planktothrix*) capable of producing cyanobacterial toxins (Wiedner et al., 2001; Frank 2002), as well as taste-and-odour compounds (Zhang et al., 2009). Using microscopy, the dominant problematic cyanobacteria from surface water samples was previously identified as *Dolichospermum* (Hecker et al., 2012). *Dolichospermum* was not the dominant cyanobacterial genus identified in sediment cores collected from the Gardiner and Qu'Appelle arms of Lake Diefenbaker (Chapter 5), indicating discordance between surface water observations and next-generation sequencing. The dominant cyanobacteria genera identified through 16S-metagenomic sequencing were *Prochlorococcus* (primer A) and *Chroococcus* (primer B), which are common bloom-forming taxa, although not necessarily harmful, in many aquatic systems (Steffen et al., 2012). Nevertheless, there was a constant and increasing presence of *Dolichospermum* throughout the sediment temporal profile in both down-reservoir arms. This is concerning because some species of *Dolichospermum* are known to produce cyanotoxins (WHO 1998).

The sequencing results (i.e. genera identified) corroborated with some of the pigment analyses, discussed in Chapter 5, and with taxonomic identifications made using traditional microscopy techniques from water samples collected in this lake in 2011-2012 (Abirehire et al., 2015). For example, cyanobacterial pigment markers myxoxanthophyll, echinenone and zeaxanthin positively correlated with the relative abundance of various genera of cyanobacteria including *Dolichospermum.* In addition, many of the cyanobacteria taxa observed in Abirehire et al. (2015) were also identified in this study. Correlations between the cyanobacterial community and the environmental proxies presented herein (sterols/stanols, algal pigments, *mcyA* gene, 16S rRNA sequences data), and from Lucas et al. (2015a and b) illuminate the complex relationships between these paleo-variables and cyanobacterial assemblages. This multi-proxy paleolimnological approach can reveal relationships within the cyanobacteria community and between certain taxa and environmental variables, possibly identifying factors driving the relative abundance of certain cyanobacteria taxa.

The increasing presence of certain cyanobacteria (*Planktothrix*, *Microcystis* and *Dolichospermum*) suggests an increasing potential for the production of a variety of

cyanotoxins (Wiedner et al., 2001; Frank 2002). Accordingly, the presence of toxic secondary metabolites (cyanotoxins) and toxin-producing genes (e.g. *mcyA*), in the temporal profile of collected sediment cores, was investigated. Microcystins have been successfully extracted and analyzed from lake sediment before (Zastepa et al., 2016). However, all investigated cyanobacterial toxins investigated here (e.g. saxitoxin, decarbamoyl saxitoxin, anatoxin-a, gonyautoxin-2 and -3, and microcystins-LR and -RR) were below instrumental detection limits. Nevertheless, the continuous presence and increasing relative abundance of the *mcyA* gene in Lake Diefenbaker suggests that the genetic potential to produce microcystins have been present since formation of the reservoir and that this potential has varied over time. General primers for *stxA* (Al-Tebrineh et al., 2010) and *atx-a* (Rantala-Ylinen et al., 2011) were also investigated, but failed to produce an amplicon, suggesting the absence of these genes, and therefore the absence of these neurotoxins in this reservoir. The presence and abundance of the *mcyA* gene does not necessarily imply that microcystins were produced (Beversdorf et al., 2015), but that the mechanisms to produce them are present. Therefore, the absence of microcystin metabolites in the sediment profile may be attributable to the absence or low concentrations of these toxic metabolites, or due to degradative processes occurring during sedimentation, or to a combination of the two.

The results of correlation-based network analysis revealed that changes in relative abundances of cyanobacterial genera significantly co-varied with other measured paleo-variables or proxies. The main findings in the Gardiner and Qu'Appelle arms demonstrated an increasing trend in *Dolichospermum* that was positively correlated to an abundance of the *mcyA* gene as well as other paleo-proxies (e.g. pigments and physicochemical parameters), suggesting possible deteriorating conditions in water quality. Paleolimnological studies in the Qu'Appelle arm have observed that the trophic status experienced a change in the mid-1990s, but that it plateaued in the late-1990s (Chapter 3; Chapter 5; Hall et al., 1990; Lucas et al., 2015a, b; Maavara et al., 2015). Similar trends in multiple environmental proxies (e.g. cyanobacterial pigments, biologically available phosphorus, particulate reactive silica and abundances of *mcyA* gene) in the Qu'Appelle arm suggests that potential for production of cyanobacterial toxins co-varies with trophic status within this region of the reservoir (Chapter 5). If current trends in cyanobacteria (specifically *Dolichospermum*) continues in the Gardiner and Qu'Appelle arms, then the

future water quality of Lake Diefenbaker could fall into decline and risks associated with its direct use could increase.

6.2 General implications

This study has direct implications regarding monitoring efforts for Lake Diefenbaker and other potable waters. Distinct zones were identified in this reservoir. Therefore, the design of future monitoring efforts should take existing spatial gradients in hydrology, chemistry, and biology into consideration. For Lake Diefenbaker, trends towards increasing primary productivity over time (inferred by sedimentary algal pigments) are concerning, given the importance of this water source. This is particularly worrisome because of the presence of potential toxin-producing cyanobacteria (e.g. *Dolichospermum, Microcystis* and *Planktothrix*) and increasing trends in their relative abundance inferred through DNA sequencing techniques. Furthermore, presence of genera of *Oscillatoria, Aphanizomenon, Lyngba, Symploca* and *Synechococcus* suggests that taste-and-odour compounds (e.g. geosmin and MIB) may be synthesized and could increase proportionally to primary productivity. Although cyanotoxins were absent from sediment profiles throughout the lake, the continued presence of the *mcyA* gene suggests that the capability to produce toxins has been present since the formation of the lake and that the potential has increased during the past two decades.

If the current increasing trends in potentially harmful cyanobacteria taxa (e.g. *Dolichospermum*) and the *mcyA* gene abundance continue in the Gardiner arm or resume in the Qu'Appelle arm, it may only be a matter of time before deteriorating conditions become problematic. The presence of cyanotoxins and taste-and-odour compounds normally require expensive treatment options for drinking water generation to remove these nuisance substances and their harmful metabolites (Jütter and Watson 2007). Additionally, recurrent or severe algal blooms can result in areas or periods of reduced oxygen concentrations, with direct effects on aquatic animals, when algal blooms sink to the lake bottom, consuming oxygen during decay or respiration (CENR 2003). Low oxygen conditions in bottom waters can also stimulate the remobilization of phosphorus from the sediment via redox reactions (Mortimer 1941). The remobilized phosphorus can then encourage additional primary productivity and further formation of harmful algal blooms (Lucas et al., 2015a; Monbet et al., 2007; Coelho et al., 2004). Therefore, increased cyanobacterial HAB duration or frequency can have detrimental effects in

an aquatic system both directly (e.g. exposure to toxins) and indirectly (e.g. changes in dissolved oxygen concentrations and nutrient cycling). The complex relationship among cyanobacteria and other environmental proxies demonstrates the need to monitor those variables that can influence primary productivity and bloom formation so as to establish emerging trends and inform proactive water management decisions (e.g. identify areas where to implement water monitoring efforts).

Although fecal pollution was limited in this reservoir, the contrasting results from an impacted site (e.g. Ross Lake; Chapter 2) demonstrated the usefulness of sterol analysis to reconstruct the occurrence and trends in fecal pollution. These methodologies could be used to reconstruct trends and monitor for sewage discharge into Lake Diefenbaker from point sources (e.g. sewage lagoons in close proximity to the municipality of Elbow, Saskatchewan, beside Lake Diefenbaker). Furthermore, the dual extraction method detailed in this thesis allows for the simultaneous extraction for fecal sterols, and algal pigments, allowing the investigation of both cause (increased nutrient source materials) and effect (changes to primary productivity or the phototrophic community). In addition, investigation of a larger suite of sterols, stanols and bile acids could potentially be used to assess contributions of fecal matter from specific organisms (e.g. livestock vs waterfowl) (Tyagi et al., 2008).

Potentially harmful cyanobacteria, identified through myxoxanthophyll, occur within the Gardiner and Qu'Appelle arms of Lake Diefenbaker. However, algal pigments could not provide high-resolution data to assess the taxa that this pigment might represent. Next-generation sequencing techniques can augment pigment analysis by providing high-resolution data that can be used to investigate the bacterial community to gain insight into the recent and historical composition of the cyanobacterial community (Chapter 4 and 5), and investigate other bacterial microorganisms commonly associated with fecal materials from humans and other environmental sources (Tan et al., 2015).

This study focused on the identification of potentially harmful cyanobacteria using next-generation sequencing techniques. However, eDNA were not analyzed in any degree of detail to investigate other benign or problematic bacterial organisms (e.g. pathogenic bacteria). Unlike cyanobacteria, non-phototropic bacteria can live and reproduce within the sediment profile. It is difficult to reconstruct and interpret the trends of these organisms, as

increased gene abundance could simply be due to replication and not increased deposition of genetic material from the water column. In addition, the primers used herein were designed to mainly target cyanobacteria (Nübel et al., 1997) and were therefore not necessarily universal for bacteria. However, some bacteria can produce taste-and-odour compounds (Klausen et al., 2004) and pathogenic strains can be introduced through fecal material deposits (Tyagi et al., 2008). Therefore, it may be beneficial to investigate the bacterial community of Lake Diefenbaker further, but using a primer pair specific for bacteria (further discussed below). Nevertheless, this study illustrated proof of concept for the investigation of both cyanobacteria and non-phototrophic bacteria using sediment-preserved DNA.

Next-generation sequencing applications are becoming increasingly more affordable. Use of these techniques in this study provided rapid identification of gene sequences to lower taxonomic levels, giving increased resolution and insight into cyanobacteria community composition and dynamics compared to more traditional techniques. However there are biases and limitations to the use of NGS technologies on environmental samples. For example, the relative abundance of taxa identified using PCR-based methodologies can be influenced by: the secondary structure or nucleotide bases (e.g. GC content) of the amplified product; errors in sequencing; choice of primers targeting different rRNA hypervariable regions (Tan et al., 2015); and, downstream bioinformatic data processing (e.g. OTU clustering and alignment databases) (Kennedy et al., 2014; Nelson et al., 2014). In addition, other studies have observed that sequencing replicates were found to be more consistent than sample preparation replicates (Poretsky et al., 2014) and significant bias may be observed depending on which sequencing platform was used (Claesson et al., 2010). Some of these observations were discussed in Chapter 4 when comparing the 16S rRNA to 23S rRNA metagenomic sequencing to characterize the cyanobacterial community. Given the above, it is unlikely that a single universal approach can be applied to all sample types to achieve quantitative measurements of the sequenced community (Tan et al., 2015). Despite these limitations, the analyses presented herein demonstrate that NGS techniques, targeting the 16S rRNA gene specific for cyanobacteria, can provide a good approximation of the cyanobacteria community composition in the temporal profiles of sediment cores collected from freshwater lakes.

There can also be some ambiguity when sequencing the bacterial community composition from a sediment profile, as the detection and quantification of eDNA can be derived from both living organisms and naked or free DNA present within the sample. Therefore, comparisons between DNA copies and cell abundance can be difficult, due to confounding factors such as intracellular versus extracellular DNA. Nevertheless, biomolecular techniques (e.g. PCR-based detection and quantification) using eDNA have proven useful in identifying for the presence of potentially nuisance or problematic organisms. For example, the US Environmental Protection Agency has approved NGS methodologies to investigate water quality by measuring microbial indicator organisms and the quantification of fecal matter using *Enterococcus* (US-EPA 2009, 2013). These sequencing methodologies could potentially open new investigations into whether fecal coliforms align with fecal sterol data (e.g. coprostanol/cholesterol ratio in Ross Lake, MB).

With many lakes lacking long-term monitoring data, the use of paleolimnological techniques is becoming more widespread in an effort to reconstruct environmental trends in inland waters. These techniques can offer a substitute in the absence of long-term monitoring data (Smol 2010). The combined methodology demonstrated in this thesis, is the first to offer a powerful and novel approach to identify complex environmental relationships and dynamics that may influence the emergence or abundance of nuisance, toxin-producing cyanobacteria in many freshwater environments. The knowledge gained from this study focuses on the importance of using multiple lines of evidence (e.g. physicochemical, subfossil and biomolecular techniques) to reconstruct the trajectory of environmental quality and highlights the complex relationships between primary producers and their environment. These techniques can help generate the data necessary to inform water management agencies regarding environmental trends associated with algal and cyanobacteria bloom activity, and possibly identify where in spatially-complex systems to place intake sources for potable water, to minimize the need for costly purification processes.

6.3. Future Work

Paleolimnological investigations into Lake Diefenbaker as a whole (i.e. Lucas et al., 2015a, b and herein), did not suggest an immediate risk to water quality. However, the increasing presence of the *mcyA* gene and *Dolichospermum* is worrisome, due to the increasing potential to produce microcystins. In addition, *Dolichospermum*, *Oscillatoria, Aphanizomenon, Lyngba, Symploca* and *Synechococcus* were found in the down-reservoir arms. These taxa have frequently been associated with the production and presence of taste-and-odour compounds, such as geosmin and 2-methylisoborneol (Zhang et al., 2009), and although the relative abundance of some of these taxa may not necessarily be increasing, their constant presence may be of concern. Humans have extremely low taste and odour thresholds for geosmin and MIB (10 and 29 ng/L, respectively) (Cees et al., 1974; Persson 1979) and the removal of these compounds and cyanotoxins can be difficult, time-consuming and expensive (Jütter and Watson 2007). Consequently, it may be valuable to investigate the presence and trends of these compounds within Lake Diefenbaker and other systems of interest or importance.

Geosmin and 2-MIB can rapidly be extracted and identified through the use of solid phase microextraction techniques (Llyod et al., 1998; Klausen et al., 2004) followed by GC/MS methodologies (Hurlburt et al., 2009; Klausen et al., 2004). In addition, biomolecular approaches such as DNA sequencing and gene expression have identified genes involved in the production of these two compounds (Wang et al., 2011; Giglio et al., 2008; Wang et al., 2015). Biomolecular methodologies coupled with physical and chemical approaches, similar to the lines of investigation used in this study, can be used to identify relationships among influential environmental variables, such as gene expression, and production of these compounds in this, or other systems, such as Buffalo Pound Lake (Kehoe et al., 2015).

Given the above concerns regarding the potential production of cyanotoxins and taste-and-odour compounds, it is recommended that monitoring efforts be initiated to generate data that can be used to assess future trends in cyanobacteria and associated by-products in Lake Diefenbaker, especially in the down-reservoir arms. The web of complex relationships revealed through the comparisons among multiple paleolimnological variables, illustrates that many factors can potentially influence the phototrophic community and

primary productivity in this lake. Therefore, monitoring for significant changes in cyanobacterial community composition and these drivers should help inform future strategies to maintain water quality.

In addition to cyanobacteria, eukaryotic algae should also be investigated to confirm the observed increasing trends in pigment data. Unfortunately, the 23S rRNA alignment SILVA database (Quast et al., 2013) is currently limited (Steven et al., 2012), preventing comparisons across the entire phototrophic community; however, the 18S rRNA gene provides an alternative gene sequence that has successfully been used to identify eukaryotic microalgae and diatoms (Haddad et al., 2014; Bérard et al., 2005; Moro et al., 2009) and universal eukaryotic 18S rRNA primers are currently available (López-García et al., 2002). In addition, the SILVA 18S rRNA database provides comprehensive and quality checked sequences that are regularly updated (Quast et al., 2013).

Biomolecular methodologies such as DNA sequencing and gene expression are becoming more mainstream in the environmental sciences. These techniques have been widely successful in a monitoring capacity (Turner et al., 2015) and have been used to identify invasive fish (Turner et al., 2015), cyanobacteria (Nübel et al., 1997) and algae (Sherwood and Presting 2007; Haddad et al., 2014; Bérard et al., 2005; Moro et al., 2009), in toxin-producing gene analyses (Beversdorf et al., 2015), and identifying genes involved in the production of taste-and-odour compounds (Wang et al., 2011; Wang et al., 2015, Giglio et al., 2008) and nitrogen fixation in bacteria and cyanobacteria (Farnelid et al., 2011; Zehr and Turner 2001). With sequencing technologies continuing to develop and becoming more affordable, barriers are decreasing in genomic sequencing, permitting the study of new, rare and novel sequences in more complex samples (Tringe and Rubin 2005). These new technologies, coupled with analytical instrumentation and physicochemical measurements, can open new doors for a multitude of applications for environmental scientists to identify and understand complex mechanisms influencing ecosystem dynamics.

6.4. Conclusions

The data compiled in this thesis highlight the importance of understanding the hydrological, chemical and biological gradients typically associated with narrow river-valley reservoirs, such as Lake Diefenbaker. Using a multicore and multiproxy approach, the spatial and temporal characteristics of the phototrophic community of Lake Diefenbaker were examined. The evaluation of multiple environmental-proxies, illuminated the importance of using a multiple lines-of-evidence strategy to construct trajectories of environmental quality and inform water management decisions.

Algal pigment analyses revealed that primary productivity varies spatially in Lake Diefenbaker, with productivity increasing with distance down-reservoir until nutrients become limiting (Thorton et al., 1990). Furthermore, primary productivity in up-reservoir regions of Lake Diefenbaker was relatively consistent over the past decade, whereas primary productivity appears to have increased during the past two decades in the down-reservoir arms. Algal pigment analyses revealed that not all reservoirs follow the trophic upsurge paradigm, characterized by the emergence of algal blooms in newly impounded lakes. The presence of filamentous cyanobacteria, identified through the presence of myxoxanthophyll, prompted further examination into the cyanobacteria community using high-throughput NGS methodologies.

Characterization of the cyanobacterial community within the Gardiner and Qu'Appelle arms using NGS technology provided insight into the presence and temporal trends of potentially harmful cyanobacteria. Although cyanotoxins were not detected in reservoir sediment, the presence of the *mcyA* gene suggested that the biomolecular machinery to produce microcystins was present within Lake Diefenbaker and that the potential for toxin production increased in the mid-1990s in the Qu'Appelle arm. Trends in the Gardiner arm are less clear because of the increasing monotonic trend in the *mcyA* gene, which may suggest an increase in toxin-producing taxa, or a progressive degradation of the genetic material after deposition. Nevertheless, the presence of the *mcyA* gene and the identification of potential nuisance cyanobacteria in the Gardiner arm is still concerning. The paleolimnological techniques demonstrated in this thesis illuminate the importance of using a weight of evidence approach to investigate the complex web of interactions that can influence the growth and abundance of cyanobacteria within a freshwater lake. Because of

the significant economic importance of this particular reservoir, future monitoring of this lake should be spatially representative and be conducted frequently to best inform water management agencies about emerging threats to water quality. Furthermore, with decreasing operational costs, biomolecular approaches such as NGS can become a viable option in providing rapid high resolution data, for paleolimnological investigations and other environmental assessment applications.

CHAPTER 7

References

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References

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Appendix A – SUPPLMENTARY MATERIAL FOR CHAPTER 5

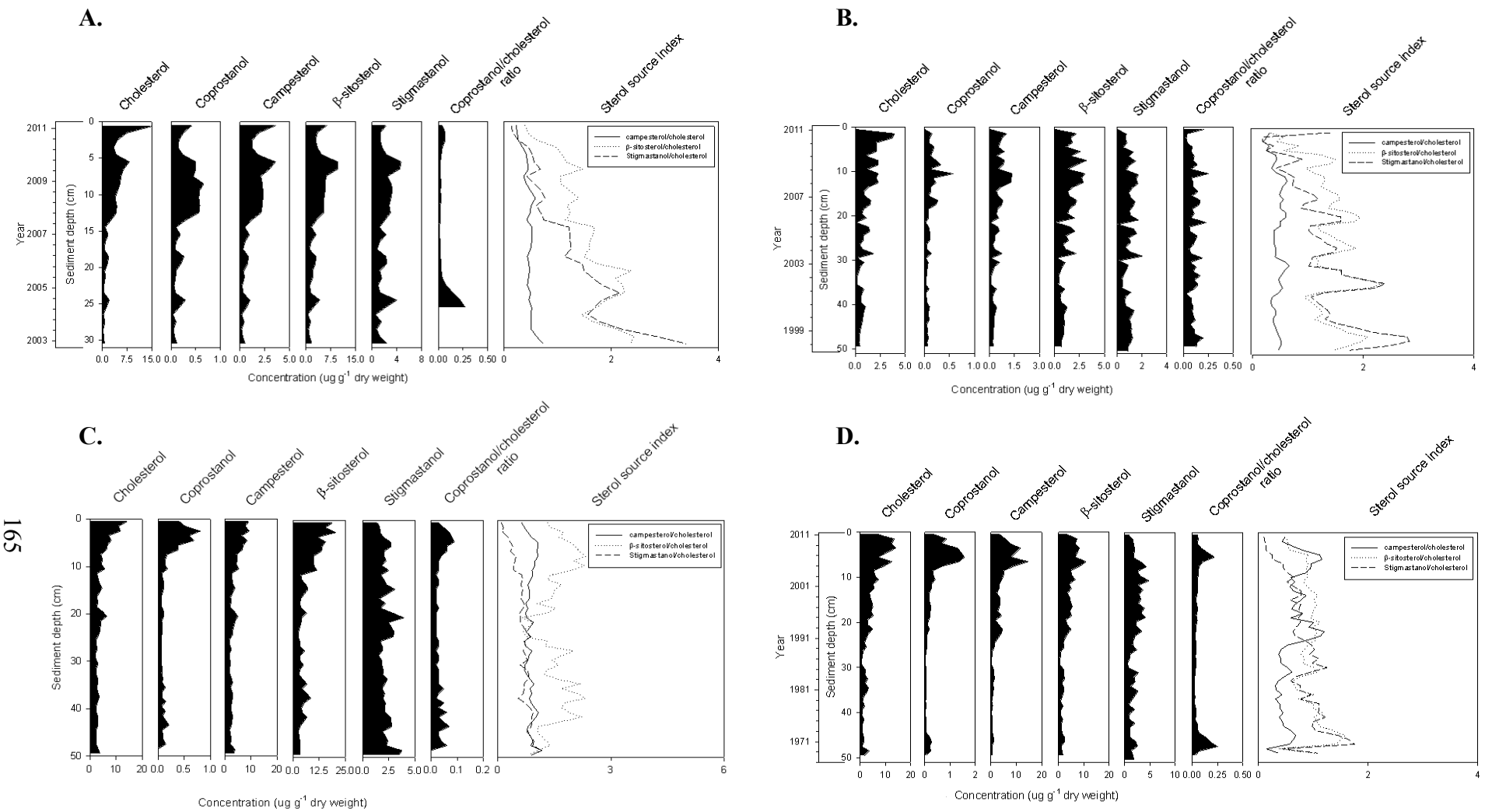

Figure A1. Temporal trends in cholesterol, coprostanol, campesterol, β-sitosterol, stigmastanol, coprostanol/cholesterol ratio and sterol source index for sediment cores collected from sites 1 (A), 2 (B), 3 (C), and 4 (D) from Lake Diefenbaker in the summer of 2011 (sites 1, 2 and 4) and the fall of 2012 (site 3).

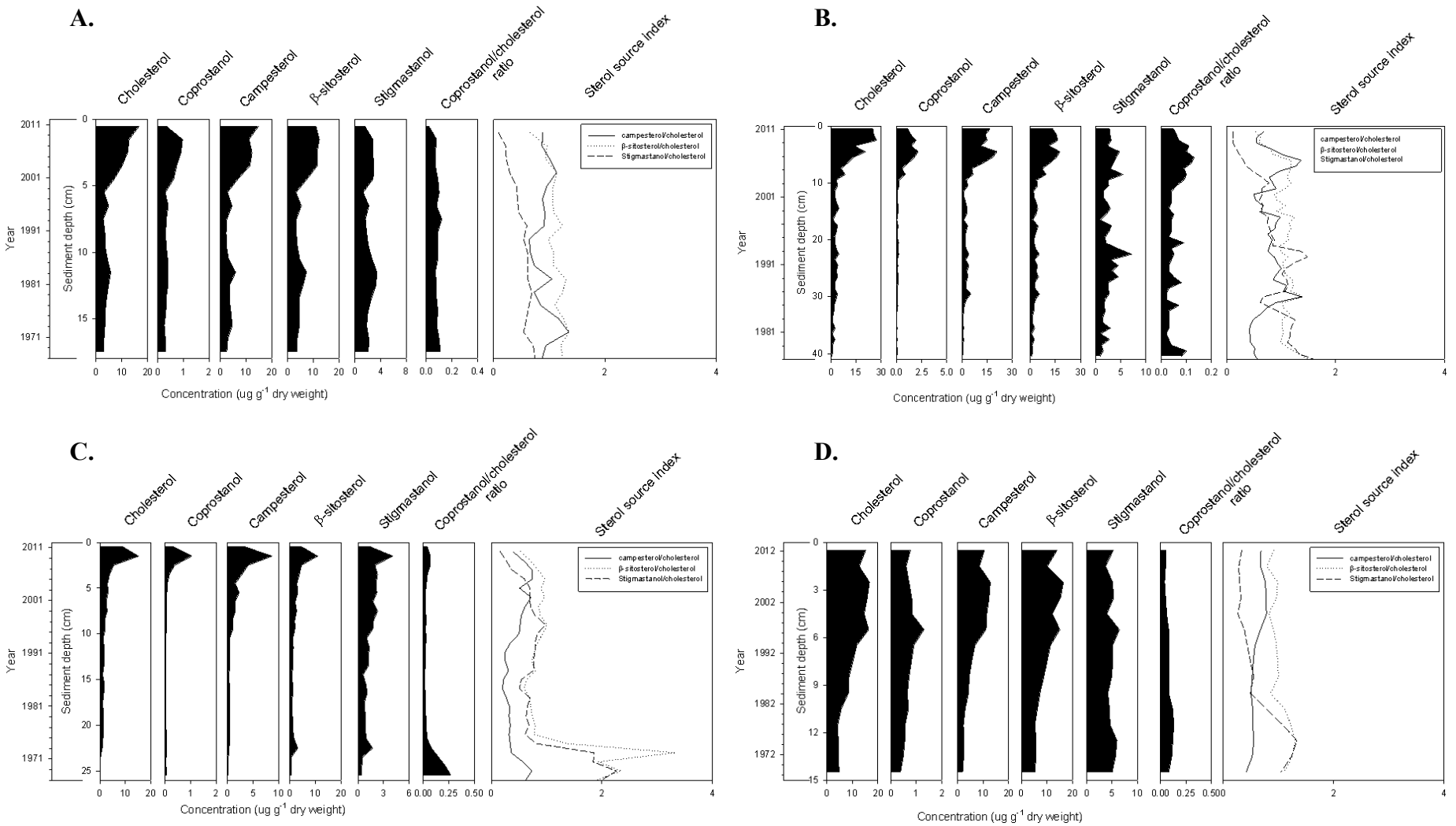

Figure A2. Temporal trends in cholesterol, coprostanol, campesterol, β-sitosterol, stigmastanol, coprostanol/cholesterol ratio and sterol source index for sediment cores collected from sites 5 (A), 6 (B), 7 (C), and 8 (D) from Lake Diefenbaker in the summer of 2011 (sites 5 to 7) and the fall of 2012 (site 8).

Table A1. Detection of cyanotoxins: MC-LR, -RR, STX, GTX-2, GTX-3, dcSTX, NeoSTX, ATX-a using Q-Exactive Orbitrap Mass Spectroscopy in the top 3-cm increments in eight sediment cores collected from Lake Diefenbaker in the summer of 2011 (sites 1, 2 and 4 to 7) and fall of 2012 (sites 3 and 8).

Site	Sediment Increment (cm)	Concentration (ng/µL)							
		MC-LR	MC-RR	STX	GTX-2	GTX-3	dcSTX	NeoSTX	ATX-a
1	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
2	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
3	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
4	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
5	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
6	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
7	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
8	001	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	002	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects
	003	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects	No Detects