THE DYNAMICS OF

BIOLOGICAL NITROGEN FIXATION

IN PRAIRIE LAKES

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By

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ABSTRACT

Nutrient-rich lakes are highly susceptible to cyanobacterial blooms. Nutrient pollution can lead to worsening blooms and the loss of important ecosystem services. A key approach to managing aquatic health has been nutrient control. Although current technologies can remove a large proportion of nutrients from wastewater prior to discharge, agricultural nutrients are difficult to control, as are in-lake sources of nutrients, such as biological nitrogen fixation. The consumption of dissolved inorganic nutrients can be rapid and potentially lead to the depletion of nitrogen stores in highly productive lakes. Reductions in nitrogen loads can be compensated for through nitrogen fixation by select cyanobacteria (diazotrophic taxa) to a variable extent. The goal of this work is to support a better understanding of potential management options for eutrophication and for blooms by better understanding the uniquity of cyanobacterial blooms and the associated roles of nitrogen fixation in time and space. This study is the first to use $^{15}N_2$-calibrated acetylene reduction bottle assays to measure gross rates of nitrogen fixation in lakes located in southern Saskatchewan, Canada. Nutrient-rich lakes with differing nutrient sources were sampled over the course of one summer. Results indicated that upstream lakes receiving nutrients pulses from land-surface runoff and internal cycling experienced fluctuations in total biomass, predominantly cyanobacteria, and a mid-bloom shift in taxa dominance from diazotrophic to non-diazotrophic taxa. Meanwhile, downstream wastewater-impacted lakes receiving more constant nutrient supplies (in addition to nutrient pulses) were able to maintain more consistent levels of biomass, which were dominated by diazotrophic cyanobacteria. Nitrogen fixation rates were greatest in lakes with fluctuations in biomass, while the lakes with more consistent biomass dominated by diazotrophs actually exhibited relatively low rates of nitrogen fixation. A more intensive study of one upstream lake found that cyanobacteria rapidly increased with elevated water temperatures and increasing PAR. At the onset of the bloom, key nitrogen fixation events co-occurred with transient stratification in limnetic zones. Interestingly, some of the highest seasonal rates of nitrogen fixation occurred in surface scum formations, which were consistently dominated by diazotrophs, suggesting that fixation is important to scums. Although existing work in the region found that nitrogen fixation provides a relatively small proportion nitrogen compared to external and internal cycling, this thesis work shows that diazotrophic cyanobacteria may provide short-term relief from nitrogen depletion. Nitrogen fixation can help support high densities of cyanobacteria, and potentially toxin-producing taxa.

FOR SUBMISSION

This manuscript-based thesis contains two manuscripts to be prepared for journal submission:

1. Boyer, L.M., S.N. Higgins, and H.M. Baulch (2019). "Cyanobacterial composition and nitrogen fixation rates in prairie lakes with varied nutrient loading regimes" Journal **Vol**(Issue): pg – pg.

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2. Boyer, L.M., S.N. Higgins, and H.M. Baulch (2019). "Cyanobacterial bloom evolution: A high-resolution study of nitrogen fixation and phytoplankton composition within a shallow, polymictic prairie lake (Buffalo Pound Lake, Saskatchewan, Canada)" *Journal* Vol(Issue): pg – pg.

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For Gil Faizon and George St. Geegland

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δ^{15}N-POM	^{15}N enrichment of particulate organic matter
˚C	degrees Celsius
$\Delta\ I_P$	difference between the final and initial ^{15}N atom% of particulate nitrogen
Δt	incubation time
^{14}N	stable isotope of nitrogen (atomic mass number of 14)
^{15}N	stable isotope of nitrogen (atomic mass number of 15)
^{15}N-N_2	^{15}N-labelled nitrogen gas
^{15}N-NH_4Cl	^{15}N-labelled ammonium chloride
$^{15}N_2$	nitrogen gas from ^{15}N
A_{N_2}	atom percent of nitrogen gas
A_{PN}^{final}	atom percent of particulate nitrogen following the incubation period
$A_{PN}^{t=0}$	atom percent of particulate nitrogen at the start of the experiment
Aph.	*Aphanizomenon*
Ar	argon
ARA	acetylene reduction assay
avg	average
BP	Buffalo Pound Lake
C_2H_2	acetylene
C_2H_4	ethylene
CG	gas in headspace
CW	gas in water
D.	*Dolichospermum*
DIN	dissolved inorganic nitrogen
DO	dissolved oxygen
DOY	day of year
g	gram
GF/Fs	glass microfiber filter
h^{-1}	per hour
I_0	natural ^{15}N atom%

I_r	^{15}N atom percent of the tracer
KT	Katepwa Lake
kg	kilogram
L	litre
L^{-1}	per litre
m	meter
m^{-2}	per square meter
m^{-3}	per cubic meter
max	maximum
mg	milligram
min	minimum
mL	millilitre
mL^{-1}	per millilitre
N	nitrogen
n	number of samples/observations
$N\text{-}NH_4^+$	nitrogen from ammonium
N_2	nitrogen gas
NH_3	ammonia
NH_4^+	ammonium
NO_2^-	nitrite
NO_3^-	nitrate
P	phosphorus
PAR	photosynthetically active radiation
$[PN]_0$	concentration particulate nitrogen at the start of the experiment
$[PN]_f$	concentration particulate nitrogen following the incubation period
ppm	parts per million
PQ	Pasqua Lake
s^{-1}	per second
S_a	ambient ammonium concentration
S_t	added ammonium concentration
SD	standard deviation

SE	standard error of the mean
SRP	soluble reactive phosphorus
TDP	total dissolved phosphorus
TP	total phosphorus
VG	volume of headspace
VW	volume of water
WC	Wascana Lake
YSI	Yellow Springs Instrument
$\delta^{15}N$	measure of the ratio of the two stable isotopes of nitrogen, ^{15}N:^{14}N
µg	microgram
µM	micromoles per litre
µmol	micromoles

Chapter 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Ongoing degradation of freshwater and marine ecosystems is a global reality (Millenium Ecosystem Assessment 2005). Nutrient pollution to excess can lead to ecosystem degradation by supporting enhanced productivity of primary producers. This has resulted in notable increases in the frequency and severity of cyanobacterial blooms (Anderson et al. 2002). Anthropogenic sources of nutrients—such as point source wastewater effluent and non-point land surface runoff from agricultural and urban zones—can alter natural nutrient cycles and nutrient budgets, particularly those of nitrogen and phosphorus (Galloway et al. 2004; Fowler et al. 2013). Climate change has and will continue to further aggravate these vulnerable aquatic systems through warmer water temperatures, longer growing periods and altering rainfall/snowfall patterns which facilitate growth (Carey et al. 2012). The presence of uncharacteristically severe cyanobacterial blooms can devastate (or markedly affect) food-web dynamics via effects of hypolimnetic anoxia impacting fish, and trophic cascade, and also via direct effects of toxins (Watson et al. 2015). Controlling anthropogenic nutrient inputs is key in mitigating cyanobacterial blooms. However, managing blooms has proved to be challenging in many ecosystems. And while water quality concerns are a global reality, the solution for managing degradation is not universal (Paerl et al. 2016).

In systems where nitrogen is the limiting nutrient for lake productivity, atmospheric nitrogen may be introduced into a system through biological nitrogen fixation (Paerl et al. 2011). Nitrogen fixation may support species in times of nitrogen deficiencies by bringing in new nitrogen. This nitrogen can be recycled within the ecosystem, or removed via ecosystem processes. The role of nitrogen fixation on lake productivity is dependent on the rate at which nitrogen is fixed and the importance of other nitrogen sources. Nitrogen fixation rates are influenced by a number of factors, including light intensity, water temperature, nutrient concentrations, species presence, among others (Howarth et al. 1988b; a). A key meta-analysis conducted across a small number of lakes suggests that nitrogen fixation can be a significant proportion of the nitrogen budget of some lakes (Howarth et al. 1988a) which may be the case in some prairie lakes where fixation was shown to supply up to 77% of the nitrogen supply (Patoine et al. 2006).

There has been a global increase in research concerning the importance of nitrogen inputs from biological fixation, especially those conducted within marine environments (Sherwood et al. 2014; Messer et al. 2016; Knapp et al. 2018). However, few studies have assessed spatial and temporal variability of nitrogen fixation in freshwater systems (Schindler 1998) and existing knowledge is constrained by methodological issues (Mohr et al. 2010; Dabundo et al. 2014). Given the increase in frequency of severe cyanobacterial blooms (Paerl and Scott 2010; Small et al. 2014), it is important to better understand how nitrogen fixation plays into the issue of bloom formation, and may impact options for bloom control. While not all blooms include diazotrophic (nitrogen-fixing) taxa, many do—and these species may help support nitrogen needs of non-diazotrophic taxa.

1.1.1 Research objectives and thesis outline

The purpose of this thesis is to assess the importance of biological nitrogen fixation to the nitrogen cycle within freshwater systems during the bloom season to help address the question: 'Is nitrogen control necessary?' To do this, I examined the spatial and temporal variability of nitrogen fixation rates during the hottest months of the year when environmental conditions support enhanced lake productivity within the Qu'Appelle River Watershed in southern Saskatchewan, Canada. I assessed the rates of nitrogen fixation, and their temporal (diel and seasonal) and spatial variability, how rates change with evolution of a bloom, and change with taxa and heterocyte biovolume.

This thesis has been organized into 4 chapters including two connected manuscripts. The first manuscript presented (Chapter 2 – Cyanobacterial composition and nitrogen fixation rates in prairie lakes with varied nutrient loading regimes) assesses the seasonal variability in nitrogen fixation rates and phytoplankton communities in four lakes within a single watershed with differing external nutrient input sources. Here, I measured rates of nitrogen fixation by performing bottle assays during the 2017 bloom season while analyzing samples for dissolved nutrient and chlorophyll *a* concentrations, and examining standard water chemistry and phytoplankton composition. Assessments were conducted in lakes distributed across the Qu'Appelle River Watershed—Buffalo Pound, Wascana, Pasqua and Katepwa. Lakes Buffalo Pound and Wascana were considered 'upstream lakes' receiving pulsed nutrient supplies (non-point sources) from urban and/or agricultural runoff and internal loading. Pasqua and Katepwa Lakes were considered 'downstream lakes' receiving pulsed nutrient supplies from runoff and

internal loading as well as pressed nutrient supplies associated with wastewater effluent (point source). In this chapter, the following research questions were examined:

1) Do upstream and downstream lakes exhibit different seasonal trends in both rates of nitrogen fixation and phytoplankton community characteristics; and, if so, to what extent?

2) Do upstream and downstream lakes exhibit similar relationships between rates of nitrogen fixation and both dissolved nutrient concentrations and phytoplankton community characteristics; and, if not, what are the major differences?

The second manuscript (Chapter 3 - Cyanobacterial bloom evolution: A high resolution study of nitrogen fixation and phytoplankton composition within a shallow, polymictic prairie lake (Buffalo Pound Lake, Saskatchewan, Canada) takes a close look at Buffalo Pound Lake and examines spatial and temporal variability of phytoplankton composition and rates of biological nitrogen fixation in response to seasonal changes. As in the previous chapter, I measured rates nitrogen fixation in Buffalo Pound Lake using bottle assays during the 2017 bloom season and accompanied these experiments with a phytoplankton inventory, standard water chemistry measurements, dissolved nutrient and chlorophyll *a* analyses. These data were paired with synchronous high-frequency sensor data to describe seasonal biological, physical and chemical changes through fine-scale monitoring and identify short-term fluctuations characteristic of polymictic lakes. In this chapter, the following research questions were examined:

- Cyanobacterial bloom evolution
  1) What were the physiochemical and water chemistry conditions prior to the major bloom phase, during the bloom and following bloom collapse? And,
     a. Does the bloom become nitrogen-limited, and if yes, when?
  2) Does the phytoplankton composition change through the bloom period? If yes,
     a. Are cyanobacteria the dominant phytoplankton group across the season? And,
     b. Does the cyanobacterial community shift from dominant nitrogen-fixing (diazotrophic) to dominant non-diazotrophic taxa, and if yes, when and by which species?
  3) Do rates of nitrogen fixation vary over the course of the bloom season? If yes,

a. Are nitrogen fixation rates correlated to changes in heterocyte biovolume, dissolved nutrients (ammonium, nitrate, dissolve inorganic nitrogen (DIN), soluble reactive phosphorus (SRP) and total dissolved phosphorus), total phosphorus (TP) or nutrient ratios (DIN:SRP and DIN:TP)?

- Spatial variability
  4) Is nitrogen uptake from ammonium and nitrogen fixation in surface water and surface scums occurring at the same rate? If not, how do they differ?
  5) Do nitrogen fixation rates differ between limnetic and littoral zones? If yes, how?
  6) Is there a measurable amount of nitrogen fixation in surface scums? If yes,
     a. Is there a difference in nitrogen fixation rates and taxa composition between surface scums and surface water adjacent to scums, and if yes, what are they?

- Temporal variability
  7) Is nitrogen fixation negligible at night, and if no, then why not?
  8) Is the phytoplankton community present during the 2017 sampling period consistent with what is seen across the long-term record? If no,
     a. Were cyanobacteria also dominant through the long-term data?
     b. Were similar dominant species observed?

1.2 Literature review

This literature review will explore the linkages between nutrient pollution and enhanced productivity leading to pronounced cyanobacterial blooms (including surface scum development), and examine the potential implications with regards to biological nitrogen fixation rates. First, this literature review briefly discusses changes to the reactive nitrogen cycle resulting from anthropogenic additions of bioavailable, or fixed, nitrogen. Second, it outlines the issue of cultural eutrophication and associated management strategies with a focus on the ongoing disagreements surrounding nutrient control, before illustrating the uniquity of cyanobacterial blooms and the potential for surface scum development. Third, it provides an overview of nitrogen fixation as performed by diazotrophic cyanobacteria, then it identifies the various factors influencing process rates and the opportunities for spatial and temporal variability linked to these factors. Fourth, it discusses current methods for measuring nitrogen fixation rates and methodological issues surrounding each. One of the tools used to measure rates of nitrogen

fixation is the natural abundance (^{15}N stable isotope) method which determines net rates as nitrogen fixed minus nitrogen lost (i.e. denitrification losses). And, bottle assays which are used to measure gross nitrogen fixation rates using either an acetylene block or $^{15}N_2$-tracer. Finally, I will briefly introduce the prairie landscape and illustrate the complexity of polymictic prairie lakes with a focus on the Qu'Appelle River Watershed in southern Saskatchewan, Canada.

1.2.1 The nitrogen cycle & nitrogen budget

Nitrogen is essential for life. Aquatic primary producers, such as cyanobacteria, are able to take up dissolved nitrogen as a nutrient for growth (Daufresne and Loreau 2001). In organic form, nitrogen within these organisms may be taken up by primary consumers; or, upon death of the producer, decomposers may recycle the nitrogen by converting it into inorganic nitrogen through mineralization (Daufresne and Loreau 2001). And although the Earth's atmosphere is comprised of a seemingly-endless supply of nitrogen (Ferber et al. 2004), biologically-available nitrogen (both inorganic and organic) in a water column can be depleted over time resulting in a nitrogen-limited environment (Scott and McCarthy 2010). This is because atmospheric nitrogen (or nitrogen gas) is, in general, inaccessible to most organisms for use as a nutrient for growth (Vitousek et al. 1997). Prior to industrial development, atmospheric nitrogen could only be introduced to the reactive nitrogen cycle through lightning fixation or biological fixation (Schlesinger 2009). Nitrogen may be removed from the nitrogen cycle through sedimentation or permanently removed by denitrification (Finlay et al. 2013). In brief, sedimentation is the settling out of materials—either organic or inorganic (Findlay et al. 1994); and denitrification is an anaerobic biological process which converts inorganic nitrogen into gaseous nitrogen (Scott et al. 2008b).

Human interference in nitrogen bio-availability has significantly altered both the nitrogen cycle (Vitousek et al. 1997) and global nitrogen budget (Fowler et al. 2013). Specifically, reactive nitrogen availability has increased due to a variety of human activities, including industrial fixation and fossil fuel combustion (Fowler et al. 2013). Humans have more than doubled the annual input of reactive nitrogen to the landscape (Schlesinger 2009) and have concurrently increased the concentration of other important nutrients, such as phosphorus. This nutrient enrichment has led to degradation of many ecosystems (Dixit et al. 2000).

1.2.2 Eutrophication & management of aquatic systems

The term *eutrophication* has been defined by Small et al. as "a change in an aquatic system from a pre-industrial state to a highly degraded state, characterized by high algal productivity, nuisance algal blooms, low oxygen, and altered biodiversity, as a result of nutrient fertilization" (p. 108, 2014). A lake may undergo cultural eutrophication as a result of nutrient pollution from point sources and non-point sources, such as wastewater effluent or agricultural runoff, respectively (Falk et al. 2013). Even lakes with high background nutrients that are naturally productive can experience worsening occurrences of cyanobacterial blooms with increased nutrient loads (Doyle et al. 2010). Transport of nutrients from freshwaters to downstream marine ecosystems also has had massive impacts on coastal and estuarine ecosystems, and can lead to toxic cyanobacterial blooms on an even greater scale (Smith et al. 1998).

It is evident that cultural eutrophication is an issue that must be managed. For decades, researchers have been working to understand what triggers are prompting eutrophication (Schindler 1974; Howarth et al. 1988a; Paerl and Huisman 2008; Molot et al. 2014) and what solutions are needed to prevent and manage these situations (Schindler et al. 2008; Conley et al. 2009; Paerl et al. 2011). There is growing concern that climate change will worsen issues of harmful cyanobacterial blooms in response to elevated atmospheric carbon dioxide (Verspagen et al. 2014), warmer temperatures and longer growing periods (Paerl and Huisman 2008), hence a renewed focus on reducing nutrient pollution. While the need for prudent use of agricultural nitrogen is well recognized (Rütting et al. 2018), and management of nitrogen is widely seen as important in areas with high connectivity to the coast (Conley et al. 2009), the benefits of controlling nitrogen from wastewaters discharging to freshwaters remains a point of scientific debate (Schindler 1974; Schindler et al. 2016; Stroom and Kardinaal 2016).

There is agreement among limnologists that phosphorus must be controlled (e.g. Schindler 1974; Scott and McCarthy 2010); however, there are those that view nitrogen control of wastewater as costly and unnecessary. A revolutionary study in the boreal region of Canada has previously identified phosphorus as the limiting nutrient in phytoplankton growth as determined through an intensive, multi-decadal whole-lake experiment eutrophying an oligotrophic lake and is supported by the well-known split-lake fertilization experiment (Schindler 1974; Schindler D. W. 1977). This work has emphasized the ability of select

cyanobacteria (diazotrophs, or nitrogen-fixers) to supplement nitrogen requirements when ambient levels are insufficient (Howarth et al. 1988a). By fixing nitrogen gas to satisfy their own needs, diazotrophs make 'new' nitrogen available which can be recycled by others, thus maintaining biomass levels. In such cases, removal of nitrogen would be ineffective in controlling eutrophication as nitrogen removed would be reintroduced through nitrogen fixation until phosphorus inevitably becomes depleted. Conversely, others believe that contributions of nitrogen through this process are insufficient or inefficient, and, ultimately, nitrogen control—in addition to phosphorus control—is needed to prevent and manage eutrophication (Conley et al. 2009; Scott and McCarthy 2010).

While diazotrophs have historically been able to maintain phytoplankton biomass in the boreal region of Canada (Schindler 1974), subsequent studies in naturally eutrophic systems have been unable to confirm such a phenomenon (Conley et al. 2009; Scott and McCarthy 2010; Hayes et al. 2019). These studies have demonstrated that, often, nitrogen fixation by diazotrophs is less able to supply sufficient nitrogen to maintain phytoplankton biomass at pre-nitrogen-limitation levels where productivity is high, particularly in phosphorus-rich regions. Whether or not diazotrophs can or cannot maintain biomass levels across all trophic gradients, we have learned that excess nitrogen can encourage growth of toxin-producing taxa, such as *Microcystis*, and contribute to the production of the toxin itself (Gobler et al. 2016). Similarly, nitrogen limitation (low N:P) can lead to or maintain dominance by toxin-producing diazotrophs (Orihel et al. 2012). In either scenario, there is an opportunity for toxin-producing diazotrophic or non-diazotrophic taxa to thrive which highlights the complexity and potential implications of nutrient control strategies.

1.2.2.1 Cyanobacteria blooms

Cyanobacterial blooms can be described as a dense accumulation of cyanobacterial cells near the surface of a water body (Svrcek and Smith 2004). However, each bloom is unique. As a fingerprint can vary in a seemingly infinite number of ridge and furrow shape and size combinations, a bloom varies by taxa composition, cell sizes and counts, and factors such as strains of individual species. A review by Svrcek and Smith (2004) discusses the variations in bloom characteristics, including overall biomass, the range in species composition or whether a bloom is dominated wholly by a single species, the proportion of diazotrophic to non-diazotrophic taxa, the species composition and abundance or biovolume of heterocytes, the

proportion of toxin-producing species or strains and the type of toxins present and overall toxicity. Favourable conditions conducive to bloom formation are typically low/no wind, warm water temperatures, neutral to alkaline pH levels and high essential nutrient concentrations (Svrcek and Smith 2004). A bloom evolves following external or internal changes (such as weather conditions or nutrient depletion/enrichment, respectively). The combination of these external and internal changes with site properties (including pre-existing phytoplankton composition) produces the unique characteristics of the bloom.

Surface scums

Some cyanobacterial blooms remain distributed within the water column – with cyanobacteria aggregated in surface layers, but not at the surface. However, oftentimes cyanobacterial blooms have the capacity to evolve into surface scums (Ishikawa et al. 2002; Persaud et al. 2014). These scums are major aesthetic concerns but may also impact biogeochemistry. Scum formation can be induced by thermal stability of the water column and greater water residence times leading to indirect surface water temperatures effects which can improve microbial buoyancy and promote photosynthetic efficiency (Kromkamp et al. 1988). Low precipitation, runoff and wind speed coupled with high ambient temperatures support thermal stability and greater water residence times (Persaud et al. 2014), and these conditions are associated with bloom risk, and the risk of scum formation.

Using photosynthesis, cyanobacteria convert various forms of carbon into carbohydrates for growth—although it is suggested that cyanobacteria have a strong preference for carbon dioxide over both aqueous carbonate and bicarbonate (Paerl and Ustach 1982). Carbohydrates may also be stored for later use, doubling as ballasts to support buoyancy regulation. Gas vesicles combined with these ballasts allow the cyanobacterium to migrate vertically by manipulating cell density. When photosynthesis halts, the ballasts may be respired which decreases cell density, allowing the cyanobacteria to float to the surface (Howard 2001). Horizontal distribution of surface water can transport buoyant communities across a lake to join other transported communities to create a larger population (Ishikawa et al. 1999). This repositioning of the colonies within a lake shifts the biomass downwind (Ishikawa et al. 1999), creating a hot spot of primary productivity and potential nitrogen fixation. As primary productivity increases, the rate of photosynthesis increases, as do carbon requirements (Paerl and Ustach 1982). With an increase in inorganic carbon demand, the inorganic carbon supply within

the water column can become depleted over time. Able cyanobacteria can migrate to the surface to access carbon from the atmosphere.

Surficial aggregate or clustered cyanobacterial colonies are highly susceptible to horizontal distribution by wind (Ishikawa et al. 2002). Under ideal growth conditions—stable water column, warm temperatures, low grazing—buoyant colonies may evolve into surface scum, or potentially hyperscums (Soranno 1997). These scums are characterized by a thick layer of cyanobacteria, shading the water column below, and reducing the ability of lower communities to photosynthesize. As previously discussed, enhanced productivity consumes and depletes nitrogen stores. Once the nitrogen demand exceeds supply, diazotrophs will have the competitive advantage (Dolman et al. 2012).

1.2.3 Nitrogen fixation & controlling factors

The ability of select cyanobacteria to fix atmospheric nitrogen has been recognized for decades, though due to the sensitive nature of this process, the role of diazotrophs varies across and within trophic gradients. Fixing nitrogen gas has proved to use more energy than, for example, assimilating forms of bioavailable nitrogen, such as ammonium (Stam et al. 1987). Nutrients that would otherwise be used for growth are redirected to produce the enzyme required and, for heterocytous taxa, to convert a typical vegetative cell into a specialized cell that serves as the site of nitrogen fixation (Kumar et al. 2010). Both non-heterocytous and heterocytous diazotrophs produce the enzyme, *nitrogenase*, to break the nitrogen bond (or 'fix') a nitrogen gas molecule. This enzyme is inhibited by oxygen, therefore nitrogen fixation occurs in anaerobic conditions (Howarth et al. 1988a; Molot et al. 2014). Fixation occurs in the vegetative cells of a non-heterocytous taxa at night in conjunction with respiration when oxygen concentrations are low (Howarth et al. 1988a; Wasmund et al. 2001; Reddy et al. 1993). Or, in heterocytous taxa, fixation occurs within an oxygen-impermeable cell called a heterocyte (previously referred to as a heterocyst) during the day in conjunction with photosynthesis (Findlay et al. 1994; Kumar et al. 2010). The presence and quantity of heterocytous cyanobacteria can be used as an indicator of nitrogen fixation activity, though the relationship between heterocyte abundance and rates of nitrogen fixation are strongest when N:P loading is low (Findlay et al. 1994). Given the expense of performing this process, diazotrophs will preferentially select dissolved sources of nitrogen, such as ammonium, before resorting to nitrogen fixation (Howarth et al. 1988a; Galloway et al. 2004; Kumar et al. 2010).

Under nitrogen-fixing conditions, characterized by a low concentration of dissolved nitrogen and low N:P, rates of nitrogen fixation are influenced by additional elemental and physical factors (Figure 1.1). In particular, adequate amounts of trace metals, molybdenum and iron, are required to produce the nitrogenase enzyme and, if limited, can restrict or lead to a reduction in nitrogen fixation rates (Molot 2017). Under nitrogen-fixing conditions (low N, low N:P), where trace-metal limitation is not an issue, rates of nitrogen fixation are influenced by physical factors. Namely, turbulence and water-column mixing in deep lakes decrease nitrogen fixation, while a greater light intensity and warmer water-column temperatures increase fixation (Howarth et al. 1988a). Evidence has shown that in shallow lakes, given favorable conditions, nitrogen fixation may occur in both stratified and mixed water-columns (Howarth et al. 1988a).

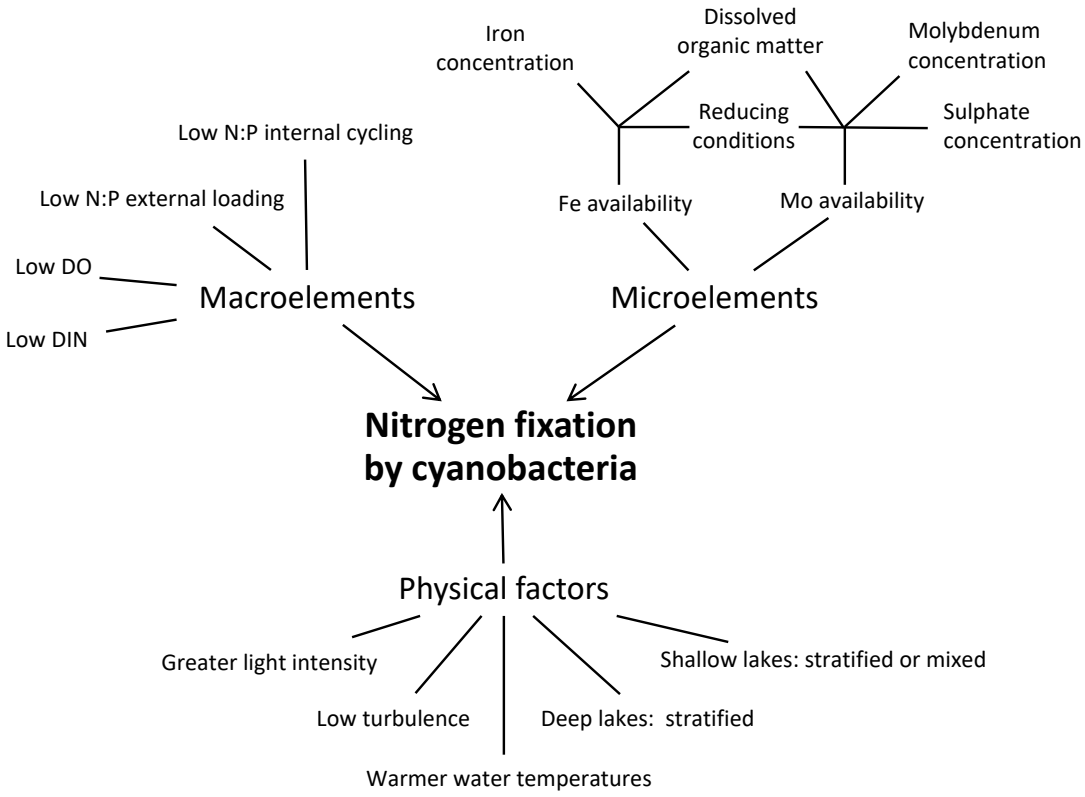

Figure 1.1. Diagram adapted from Figure 3 in Howarth et al. (1988a) illustrating the direct and indirect factors that influence rates of nitrogen fixation in aquatic systems.

1.2.3.1 Spatial & temporal variability

The rate at which nitrogen is fixed in an aquatic system varies both spatially and temporally. McClain et al. explains that increased fixation rates central to one particular location or at a point in time are indicators of hot spots and hot moments, respectively (2003). For nitrogen fixation, these responses are a result of favourable ambient temperature, light intensity and nutrient concentrations (Doyle et al. 2010). In shallow lakes that exhibit frequent, irregular mixing with intermittent thermal stratification events (namely, polymictic lakes), conditions can be altered by wind-induced mixing. Mixing may lead to surface-water cooling and resuspension of particles reducing light penetration through the water column and replenishing nutrient pools. While nitrogen fixation may occur at the sediment-water interface, the addition of nitrogen by fixation can be less than that of nitrogen loss by denitrification at the sediment level, and this can result in a net loss of nitrogen (McCarthy et al. 2016). Nitrogen fixation is typically greater in surface water regions within the photic zone (Mugidde et al. 2003), although nitrogen fixation may be light saturated under irradiant conditions as low as 50 μmol s^{-1} m^{-2} (Higgins et al. 2017).

In 'transition zones'—where a tributary mixes with resident lake water—the introduction of new water is often accompanied by essential materials needed to enhance lake productivity (McClain et al. 2003; Doyle et al. 2010). With rapid growth of cyanobacterial communities, the depletion of nitrogen in stratified water-columns can induce nitrogen fixation 'hot spots' (Wasmund et al. 2001; Doyle et al. 2010; Paerl et al. 2011).Without further addition of this essential nutrient, cyanobacteria capable of fixation will then resort to fixing atmospheric nitrogen near the water-atmosphere interface resulting in spatial variability (Howarth et al. 1988a). Although measurements of nitrogen fixation are typically performed at a single site in a lake, past work detailing spatial variation suggests that rates of nitrogen fixation can vary from ~1 – 8-fold across a lake, and 3 – 8-fold within a single profile (Mugidde et al. 2003).

Polymictic lakes can be a challenge to sample representatively as they typically exhibit irregular and unequal mixing and can have significant spatial variation in nutrient availability, light penetration and surface water temperature (Taranu et al. 2012). Continuous vertical mixing of the water column followed by a stratification event can incorporate nutrients (e.g. trace metals, nitrogen and phosphorus) released from the sediment into the photic zone (Wilhelm and Adrian 2008) and promote the growth of cyanobacteria tolerant to fluctuations in light penetration and turbulence (Wilhelm and Adrian 2008; Taranu et al. 2012). Stratification events

can induce ‘hot moments’ of cyanobacterial growth; this temporal variability replenishes nutrients and other important conditions, positively influencing lake productivity (Wilhelm and Adrian 2008; Doyle et al. 2010). Again, the depletion of nitrogen can trigger fixation in the presence of diazotrophic cyanobacteria (Doyle et al. 2010; Scott and Grantz 2013).

Rates of nitrogen fixation in a water column exhibit both long-term and short-term temporal variability. Specifically, there are predictable seasonal cycles with maximum rates of nitrogen fixation in summer and early fall (Horne and Goldman 1972; Mugidde et al. 2003; Doyle et al. 2010); and many regions experience significant inter-annual variation as well as diel variation in nitrogen fixation (Doyle et al. 2010). Inter-annual variation can relate to changes in hydraulic retention. Longer water residency times (i.e. low-flushing rates) can support slow growing species of cyanobacteria, including diazotrophic taxa, due to lower levels of ‘cell washout’. Given the photosynthetic nature of these organisms, variations in light penetration from year-to-year can affect rates of nitrogen fixation as well (Doyle et al. 2010).

Interestingly, as noted, nitrogen fixation can occur in the day, or at night. During daylight hours, heterocytous cyanobacteria are able to fix nitrogen concurrently with photosynthesis (Horne and Goldman 1972; Bentzon-Tilia et al. 2014). Non-heterocytous diazotrophic cyanobacteria are restricted to fixing nitrogen after sundown in the absence of photosynthesis (Wasmund et al. 2001). When measuring nitrogen fixation, we assume heterocytous cyanobacteria are solely fixing nitrogen during the day, and non-heterocytous diazotrophs are solely fixing nitrogen after sundown. In some regions, rates of nitrogen fixation are higher during daylight hours (Doyle et al. 2010) while others have documented an increase in fixation after sundown as performed by non-heterocytous cyanobacteria (Wasmund et al. 2001). Non-heterocytous cyanobacteria revert to fixing nitrogen at night when photosynthesis halts and the ambient oxygen level is reduced (Wasmund et al. 2001). This indicates that diel cycling of fixation is largely dependent on taxa present, as well as the environmental conditions that influence nitrogen fixation.

1.2.4 Measuring rates of nitrogen fixation

Measuring rates of nitrogen fixation has challenges. These challenges are associated with methodological and spatial and temporal variability. If not managed or accounted for, data may be flawed or falsely interpreted (Soranno et al. 1999; Klawonn et al. 2015). Nitrogen fixation in aquatic systems can be measured using a nitrogen mass balance approach (Patoine et al. 2006;

Hayes et al. 2019), acetylene block (Scott et al. 2008a; Beversdorf et al. 2013) or $^{15}N_2$-tracer (Ohlendieck et al. 2000; Kumar et al. 2017). Each method has its own set of methodological difficulties (Montoya et al. 1996; Mohr et al. 2010; Dabundo et al. 2014). In addition to methodological issues, spatial and temporal variability can confound results by creating biases or uncertainty in the data (Doyle et al. 2010).

1.2.4.1 Mass balance approach

One form of mass balance approach assesses net rates of nitrogen fixation by examining changes to the isotopic nitrogen signature of the particulate organic matter (δ^{15}N-POM) over time in a given system (Patoine et al. 2006). This approach—as performed by Patoine et al. (2006)—operates under a number of assumptions, including:

1) δ^{15}N-POM and the δ^{15}N of the dissolved nitrogen pool are at equilibrium,
2) changes in δ^{15}N-POM are a balance between nitrogen fixation (decreases δ^{15}N-POM) and other nitrogen transformations such as, denitrification (increases δ^{15}N-POM), and
3) other nitrogen inputs do not affect δ^{15}N.

That is, the observed changes to δ^{15}N-POM are a reflection of fractionation by primary producers—given the option, primary producers select ^{14}N, as it is isotopically 'light' compared to ^{15}N. Such an approach can be a valuable tool for demonstrating the importance of biological nitrogen fixation as a source of nitrogen in a defined ecosystem on a long-term scale (Leavitt et al. 2006; Patoine et al. 2006; Pinto and Litchman 2010; Hayes et al. 2019). Evaluation of (total) nitrogen to (total) phosphorus ratios are also deemed useful (Hellstrom 1996; Pinto and Litchman 2010), as these ratios have been previously identified as indicators of nitrogen fixation (Schindler et al. 2008). Additional taxonomic data is often collected to identify dominant cyanobacteria species and to assess the capacity of these species to fix nitrogen (Patoine et al. 2006; Pinto and Litchman 2010). Although valuable for long-term, large-scale studies, in order to understand the drivers of nitrogen fixation and examine the magnitude of spatial and temporal variability, more intensive methods are needed.

1.2.4.2 Bottle assays

The acetylene reduction (ARA) and $^{15}N_2$-tracer bottle assays are both commonly used methods for examining gross rates of nitrogen fixation. These methods may be used individually

or in parallel. Both methods require gaseous additives—acetylene for ARA and ^{15}N-N_2 for $^{15}N_2$-tracer assay—and incubation periods lasting 2 to 36 hours (Zehr and Montoya 2007). Incubations may be completed in-situ (Bombar et al. 2014; Berrendero et al. 2016) or in a laboratory setting (Zehr et al. 2007; McCarthy et al. 2013). Overall, the $^{15}N_2$-tracer is a more direct approach; although results are easily contaminated and the costs associated with the $^{15}N_2$ tracer are high—i.e. ^{15}N-N_2 tracer costs and analytical costs. On the other hand, ARA is relatively simple in procedure; however, the conversion factor used to interpret moles of ethylene produced to moles of nitrogen gas reduced is theoretically variable.

The ARA measures nitrogen fixation by introducing acetylene into a water sample. In the presence of active nitrogen fixation, the acetylene will be reduced by the diazotrophs at the same rate as nitrogen gas would be reduced, with the inclusion of a conversion factor (Liengen 1999). Because this approach analyzes concentrations of reduced acetylene rather than fixed nitrogen, the value of the conversion factor is critical (Zehr and Montoya 2007). An original factor of 3 was applied to early nitrogen fixation calculations (Mugidde et al. 2003; Gondwe et al. 2008). This factor is based on a theoretical ratio of 3:1—for every 3 moles of ethylene produced, 1 mole of N_2 will be fixed when stoichiometrically balancing electrons. More recently, a second conversion factor has been suggested; this factor takes into account the hydrogenase activity of the nitrogenase reaction in which two protons and two additional electrons are required to balance the equation, the outcome of which has been the introduction of a new conversion factor of 4 (Zehr and Montoya 2007). Some researchers have chosen to use this 4:1 theoretical ratio, as opposed to the 3:1 (Beversdorf et al. 2013; Mischler et al. 2014). There is still a clear disagreement in the literature about which theoretical conversion factor is more appropriate.

A study performed by Mulholland & Bernhardt (2005) experimentally concluded that molar ratios can range from 3 to 22—although typical ratios are between 3 and 4. Higher molar ratios were detected in samples having higher levels of growth during the incubation period. This is likely attributed to dissolved ^{15}N-nitrogen release following $^{15}N_2$ fixation (Mulholland and Bernhardt 2005). Rather than utilizing a theoretical factor, the ARA can be calibrated using the $^{15}N_2$-tracer assay, if performed in parallel (Ferber et al. 2004; Jäntti 2007; Zehr and Montoya 2007). By performing both bottle assay methods in parallel on a semi-regular basis, a regression analysis of ethylene produced using the ARA to nitrogen fixed using the ^{15}N-tracer assay can be used to determine a more suitable conversion factor (Ferber et al. 2004; Zehr and Montoya

2007). This factor can then be used to convert all sample measurements from rates of ethylene produced to nitrogen fixed.

The $^{15}N_2$-tracer assay measures nitrogen fixation by enriching water samples with a stable nitrogen isotope (e.g. Stewart 1967; Montoya et al. 1996; Mulholland et al. 2006; Klawonn et al. 2015). This method is used to quantify the amount of ^{15}N incorporated into the biomass of diazotrophic taxa during the incubation period by determining the amount of ^{15}N found in the organic content and attributing that to the amount of nitrogen fixed in the sample. Subsequent calculations are used to estimate the overall rate of fixation in the sample (Montoya et al. 1996). It is important to highlight that this method can identify the amount of nitrogen fixed and incorporated into biomass; however, analysis of these samples will not detect nitrogen fixed and subsequently converted to either ammonium or dissolved organic nitrogen during incubation (Mulholland et al. 2006). Additional measurements and calculations are needed to determine the quantity of dissolved nitrogen that had previously been fixed during the incubation period. This can be achieved by measuring the excess ^{15}N and concentration of dissolved nitrogen following the incubation period (Mulholland and Bernhardt 2005).

Rates of fixation determined by the $^{15}N_2$-tracer assay can be confounded by contamination of either the samples or the stock gas itself (Dabundo et al. 2014; Klawonn et al. 2015). As nitrogen is naturally abundant in the atmosphere in gaseous form, samples are prone to contamination at any stage of the experimentation period through contact with air, glassware and other laboratory supplies (Dabundo et al. 2014). Alternately, factory produced ^{15}N-N_2 can be contaminated with other ^{15}N-species during the manufacturing process (Dabundo et al. 2014). Contamination of the gas with ^{15}N-ammonium, for example, can result in an overestimation of nitrogen fixation. In such an instance where alternate nitrogen species are added to a sample, the cyanobacteria present will first uptake the alternate species before they resort to fixing nitrogen; interpretation of the data will indicate a greater amount of nitrogen was fixed because the results include ^{15}N that was previously fixed. An analysis of the ^{15}N-N_2 is strongly recommended to minimize the level of error.

Another confounding variable for $^{15}N_2$ tracer-based methods is the approach used to add the ^{15}N-N_2 to a sample (Klawonn et al. 2015). Injecting a ^{15}N-N_2 bubble, for instance, into water samples has been shown to produce an underestimation of nitrogen fixation rates; the resulting underestimation occurs because the injected gas needs adequate time to dissolve in the sample

water (Mohr et al. 2010). Moreover, when comparing the $^{15}N_2$-tracer method to the ARA, the solubility of acetylene is greater than that of N_2 (Schöllhorn and Burris 1967). Preparation of a $^{15}N_2$-enriched stock solution of de-ionized water—for freshwater samples—more than 24 hours before use will circumvent underestimation by allowing the gas to dissolve prior to injection into the sample (Klawonn et al. 2015).

In addition to the methods outlined above, estimates of nitrogen fixation rates may be produced using heterocyte counts by assuming a constant rate of nitrogen fixation per heterocyte at a given light intensity. The accuracy of these estimates is better under low N:P conditions (Findlay et al. 1994). This is because although heterocytes may be present they would have been produced during a period of nitrogen-limitation. It does not imply that nitrogen-limitation is still an active issue requiring diazotrophs to produce and use nitrogenase to fix new nitrogen. Whether or not heterocytes are present, bioavailable forms of nitrogen, such as ammonium, are still preferentially used (Howarth et al. 1988a; Galloway et al. 2004; Kumar et al. 2010).

1.2.5 Canadian Prairies & polymictic prairie lakes

The Canadian Prairies are situated in the northern-most region of the Great Plains of North America, spanning southern Alberta, Saskatchewan and Manitoba. The current climate of this ecozone ranges from semi-arid to humid continental. This region is also referred to as the Prairie Pothole Region due to its vast number of wetlands. Shallow lakes and the abundance of wetlands are legacies of the glacial-interglacial period, the results of which have also produced phosphorus-rich landscapes (Klassen 1989 as cited in Leavitt et al. 2006; Patoine et al. 2006; Hayes et al. 2019). Prairie lakes are ice-covered in the winter and are often too shallow to undergo stable summer stratification, resulting in irregular mixing patterns and transient stratification events. Lakes with these characteristics are classified as continuous cold polymictic lakes (Lewis, William M. 1983, referred to as polymictic lakes in this thesis).

1.2.5.1 Nutrient pollution and consequences to prairie lakes

Prairie lakes are naturally productive meaning eutrophic lakes are not an uncommon occurrence. As with all freshwater and marine systems, eutrophic lakes, too, are susceptible to eutrophication (Hall et al. 1999; Dixit et al. 2000; Doyle et al. 2010; Finlay et al. 2010b). As prairie landscapes are both flat and naturally nutrient-rich, they are well-suited for agricultural production. Large amounts of fertilizers are amended to the soil to increase crop yields. Excess

nutrient pulses from fertilizers and soil erosion are able to enter waterways through land surface runoff during rainfall events and annual snowmelt (Liu et al. 2013). In addition to these non-point sources of nutrients, urbanization since European settlement in the 1930's has led to the production of wastewater and associated effluent (Dixit et al. 2000). Wastewater effluent can contribute continuous or pressed nutrient loads. And while wastewater is treated, water is not returned to its original condition, meaning excess nutrients are still entering aquatic systems (Falk et al. 2013). The implications of nutrient pollution to naturally productive lakes include worsening of cyanobacterial blooms and shifts to atypical phytoplankton communities (Dixit et al. 2000). Eutrophication of such systems can result in increases in biotoxin-production with enhanced growth (Smith et al. 1998) and an increase risk of anoxia with enhanced decomposition—which can lead to fish kills (Anderson et al. 2002). Work to remediate these systems through nutrient control is challenging as recovery can be delayed by internal loading of accumulated nutrients over time (Welch and Cooke 2005; Orihel et al. 2017).

1.2.6 Study area and lakes

This study was conducted in the Qu'Appelle River Watershed situated in the prairie region in the southern portion of the province of Saskatchewan, Canada. The average ice-free period in this cold region is short, extending from mid-April to mid-October (Finlay et al. 2010a) with annual growing periods ranging from 200 to 245 days (Hall et al. 1999). Average daily maximum temperatures are greatest during July and August (mean 25.0 and 24.5˚C, respectively; Environment and Climate Change Canada 2020).

The watershed drains an approximately 52,000 km^2 catchment and consists of nine lakes interconnected by the Qu'Appelle River or its tributaries forming a lake chain ranging from meso- to hypereutrophic (Patoine et al. 2006). From west to east, the most upstream reservoir in the watershed is Lake Diefenbaker. Downstream of the Lake Diefenbaker reservoir, the Qu'Appelle River links Buffalo Pound, Pasqua, Echo, Mission, Katepwa, Crooked and Round Lakes. Wascana and Last Mountain Lakes drain into the Qu'Appelle River between Buffalo Pound and Pasqua Lakes. Buffalo Pound Lake receives mesotrophic waters from the Lake Diefenbaker reservoir and, as with all Qu'Appelle lakes, Buffalo Pound Lake receives some agricultural runoff from the local catchment (Patoine and Leavitt 2008). Wascana Lake receives nutrient enrichment from urban runoff from the City of Regina (Finlay et al. 2010b). Downstream of Wascana Lake, effluent from the city's wastewater treatment plant is released

into Wascana Creek which introduces additional enrichment to this Qu'Appelle River tributary (Leavitt et al. 2006). As Pasqua, Echo, Mission, Katepwa, Crooked and Round Lakes are downstream of the Wascana Creek outlet, these lakes are subject to inputs from the upstream wastewater treatment plant, urban and agricultural runoff (Leavitt et al. 2006). This study focused on four of the Qu'Appelle lakes: two upstream of the city's wastewater treatment plant (Wascana and Buffalo Pound) and two downstream (Pasqua and Katepwa).

Chapter 2: CYANOBACTERIAL COMPOSITION AND NITROGEN FIXATION RATES IN PRAIRIE LAKES WITH VARIED NUTRIENT LOADING REGIMES

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2.0 Abstract

The importance of biological nitrogen fixation in aquatic nutrient cycling has confounded watershed management strategies. Although phosphorus control has been widely accepted as necessary to help mitigate eutrophication, given the potential for nitrogen-fixing (diazotrophic) cyanobacteria to support phytoplankton demands for nitrogen, reductions in nitrogen loading remain a topic of debate. Here, we measured rates of nitrogen fixation ($^{15}N_2$-calibrated acetylene reduction assays), water chemistry, and taxonomy in eutrophic, prairie lakes with differing nutrient sources in the Qu'Appelle River Watershed in Saskatchewan, Canada. This study examines four lakes receiving diffuse landscape nitrogen, two of which are located downstream of a point-source sewage input site. Upstream lakes unimpacted by sewage inputs, showed a key importance of nitrogen fixation in supporting blooms and more dynamic phytoplankton communities. Rates of nitrogen fixation up to 8.7 μg N L^{-1} h^{-1} were observed, though these fixation events were short-lived. Succession of diazotrophic taxa in early summer to non-diazotrophic taxa in late summer (predominantly *Dolichospermum flos-aquae* to *Planktothrix agardhii*, respectively) suggests potential for early nitrogen fixation to support end-of-season growth through recycling processes. Downstream lakes with high nitrogen concentrations were consistently dominated by diazotrophic taxa (*Aphanizomenon flos-aquae*) across the season but showed low rates of nitrogen fixation and heterocyte biovolumes. Peak rates of nitrogen fixation were, at minimum, five times lower in downstream lakes than upstream lakes. Within the context of mass balances for these lakes, nitrogen fixation inputs appear to be very small proportions of the nitrogen inputs, but, ecologically, nitrogen fixation appears to be important in sustaining blooms in upstream lakes.

2.1 Introduction

Anthropogenic nutrient inputs are the primary driver of eutrophication and have led to degraded water quality globally (Millenium Ecosystem Assessment 2005). Excess nutrients increase primary production causing ecosystem degradation (Dixit et al. 2000) by promoting growth of often toxic and bloom-forming cyanobacterial taxa, and impacting oxygen and food web dynamics (Watson et al. 2015).The addition of nutrients through anthropogenic sources has led to increased frequency and severity of harmful cyanobacterial blooms in many freshwater lakes—for example, Lake Erie, Ontario, Canada (Harke et al. 2016a); Lake Taihu, China (Paerl et al. 2014); Lake Victoria, East Africa (Mugidde et al. 2003) and lakes in the Qu'Appelle River Watershed, Saskatchewan, Canada (Quinlan et al. 2002)

Several studies in the 1970's identified phosphorus as the limiting nutrient for primary productivity (e.g., Dillon and Rigler 1974; Schindler D. W. 1977) which contributed to the implementation of phosphorus-centric management strategies for wastewater. A key example of which was the Great Lakes Water Quality Agreement of 1972 between Canada and the United States (revised in 1978) which sought reductions of phosphorus from point sources to protect the health of the Laurentian Great Lakes (as cited in Dove and Chapra 2015). Such phosphorus-centric strategies have been successful in decreasing eutrophication in these Great Lakes, along with many others from North America and Europe—e.g. Lakes Mälaren, Hjälmaren, Vättern and Vänern in Sweden (Willén 2001) and Moses Lake in Washington, USA (Welch 2009). There is much evidence globally to support the hypothesis that phosphorus is often the ultimate limiting nutrient (Schindler et al. 2016). The belief that only phosphorus control is needed is based on the assumption that, where nitrogen becomes the limiting nutrient over time, biological nitrogen fixation will incorporate enough nitrogen to the lake's nitrogen budget to maintain biomass, and that growth will continue until phosphorus is inevitably depleted (Howarth et al. 1988b). However, a number of studies have shown that nitrogen limitation or nitrogen and phosphorus co-limitation, can be proximate factors regulating growth of primary producers, such as cyanobacteria (Mischler et al. 2014), hence elevated nitrogen loads, while not an immediate driver of blooms, could make blooms worse. Key to this debate is the understanding of how important nitrogen fixation is to lake nitrogen budgets, and the role of nitrogen fixers (diazotrophs) in supporting cyanobacterial blooms.

In aquatic systems, biological nitrogen fixation is the process by which diazotrophic cyanobacteria are able to convert gaseous nitrogen into a usable form (Carey et al. 2012; Beversdorf et al. 2013). Diazotrophs use a specialized, oxygen-inhibited enzyme, *nitrogenase,* to split the N_2 molecule (Howarth et al. 1988a; Molot et al. 2014). The triple bond joining the two nitrogen atoms is severed, and these taxa are able to produce two ammonia molecules for every one molecule of nitrogen gas (Liengen 1999; Galloway et al. 2004). While non-heterocytous diazotrophs require low oxygen conditions to fix nitrogen (Howarth et al. 1988a), heterocytous taxa can generate an oxygen-impermeable heterocyte cell to protect the enzyme, allowing the fixation process to proceed in well-oxygenated environments (Wasmund et al. 2001).

While capacity for nitrogen fixation is an important mechanism for a subset of cyanobacteria, it is important to note that it is energetically taxing for the organism to synthesize the required enzyme and produce a heterocyte cell, and as such, nitrogen fixation by diazotrophic cyanobacteria tends to occur under nitrogen-limited conditions (Howarth et al. 1988a; Galloway et al. 2004). Typically diazotrophs dominate under nitrogen-depleted conditions exhibiting low N:P loading, while non-diazotrophic taxa tend to outcompete diazotrophs in systems with high N:P loading (Taranu et al. 2012). And although the presence and quantity of heterocytous cyanobacteria can act as an indicator for nitrogen fixation, the relationship between heterocyte abundance and rates of nitrogen fixation is strongest when nitrogen to phosphorus input ratios (N:P) are low (Findlay et al. 1994). When nutrient input levels are less than 16:1 (molar), rates of fixation significantly increase (Howarth et al. 1988a).

Nutrient loading in lakes is either a product of internal cycling or external loading. External inputs from wastewater effluent, in particular, are relatively stable, providing continuous (point source or pressed) nutrient supplies, while external inputs from agricultural or urban runoff are sporadic, providing irregular non-point source pulses of nutrients from the land surface following heavy rainfall events (Lürling et al. 2018). Further, internal loading in eutrophic lakes can lead to significant non-point source pulses of nutrients from sediments (D'Silva 2017) potentially greater than that of external loading sources (Wu et al. 2017). Whether nutrients are products of internal cycling or external loading, pulses of nutrients can lead to temporary and spatially-variable enhancements in biological productivity (Lürling et al. 2018) and alter N:P favouring either diazotrophic or non-diazotrophic taxa.

Although the strongest influence over nitrogen fixation is internal N:P cycling and external N:P loading, additional elemental factors and physical conditions influence rates. Since nitrogenase is oxygen-inhibited, the production of oxygen-impermeable heterocytes allows for nitrogen fixation during photic hours by heterocytous taxa while non-heterocytous, diazotrophic taxa restrict nitrogen fixation activities to the night when photosynthesis has stopped (Howarth et al. 1988a). In temperate lakes, greater light intensity and warmer water-column temperatures characteristic of the summer season are essential to heterocytous diazotrophs. These physical conditions can be altered by the level of turbulence and water-column mixing, a frequent occurrence in polymictic lakes (Howarth et al. 1988a).

Our primary objective was to evaluate the role of nitrogen fixation in supporting algal blooms within lakes influenced by varied nutrient sources. Using $^{15}N_2$-calibrated acetylene reduction assays, we a) assessed how short-term nitrogen input through nitrogen fixation changed seasonally, and with bloom development, and b) compared rates of nitrogen fixation between upstream lakes in a lake chain which received pulsed, non-point nutrient supplies (upstream runoff inputs from agricultural sources) and downstream lakes receiving both pulsed non-point source inputs, and pressed nutrient supplies associated with a wastewater effluent. Phytoplankton identification and enumeration was completed to a) identify seasonal trends in cyanobacterial and heterocyte composition and b) compare major differences in composition between upstream lakes and downstream lakes. Finally, we used this phytoplankton composition information and measured inorganic nutrient concentrations to identify possible predictors of nitrogen fixation in upstream and downstream lakes (including cyanobacterial biomass, cyanobacteria as a percent of total biomass, diazotrophic taxa as a percent of cyanobacterial biomass, heterocyte biovolume, ammonium, nitrate, soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen to SRP ratios). We hypothesized that nitrogen fixation in downstream, wastewater-impacted lakes, would be negligible due to high nitrogen inputs and high in-situ concentrations of dissolved inorganic nitrogen. Further, we predicted upstream lakes and downstream lakes would exhibit different seasonal trends in both rates of nitrogen fixation and phytoplankton community characteristics, although upstream lakes and downstream lakes would exhibit similar relationships between rates of nitrogen fixation and both dissolved nutrient concentrations and phytoplankton community characteristics.

2.2 Methods

2.2.1 Study area and sampling

The study lakes (Buffalo Pound, Wascana, Pasqua and Katepwa) are located within the Qu'Appelle River Watershed. The watershed as a whole includes nine interconnected lakes and reservoirs in Saskatchewan, Canada, which drain roughly 52,000 km^2 of mainly agricultural land (Patoine et al. 2006) in the local catchment. Much of the water flowing through the system comes from the upstream Lake Diefenbaker, a reservoir with a catchment area of ~135,500 km^2 (Giesy et al. 2009). The Qu'Appelle lakes are typically polymictic, with the exception of Katepwa, which is occasionally dimictic (McGowan et al. 2005), and current trophic statuses range from mesotrophic to hypereutrophic (Smith et al. 1998; Quinlan et al. 2002). Peak phytoplankton biomass typically occurs between August 3 (day of year, DOY, 215/216 leap) and August 18 (DOY 230/231 leap, McGowan et al. 2005). Major changes to the nutrient regime have stemmed from agricultural development and intense urbanization (Dixit et al. 2000); however, sediment cores by Dixit et al. (2000) revealed that prior to the 20th century, previous to urban development, species of diatoms and cyanobacteria—including diazotrophic taxa—indicative of eutrophic systems were present; this suggests the watershed was nutrient-rich prior to human interference.

Four Qu'Appelle lakes were selected for investigation, and sampled from July 10 – September 21 (DOY 191 – 264), 2017, including: Buffalo Pound, Wascana, Pasqua and Katepwa (see Table 2.1, Figure 2.1). Biweekly samples were collected from long-term monitoring sites at each lake from a depth of 0.3 m. Additional, discrete samples were collected from Buffalo Pound Lake from a depth of 0.1 m from a different monitoring site south of the biweekly site. Samples were collected using acid-washed HDPE bottles and couriered overnight under dark, ambient temperature conditions. Discrete water samples were partitioned for experimental use, phytoplankton inventory and water chemistry analysis. Water was analyzed for chlorophyll *a*, ammonium, nitrate and soluble reactive phosphorus (SRP) content at the University of Saskatchewan's biogeochemistry lab. Concurrent Yellow Springs Instrument (YSI) water quality sensor measurements were captured at each sampling interval (YSI multi-probe meter, YSI, Ohio, USA). Overall, each lake was sampled between four and fourteen times (N=29)—Buffalo Pound (n=4 + 10), Wascana (n=6), Pasqua (n=5) and Katepwa (n=4).

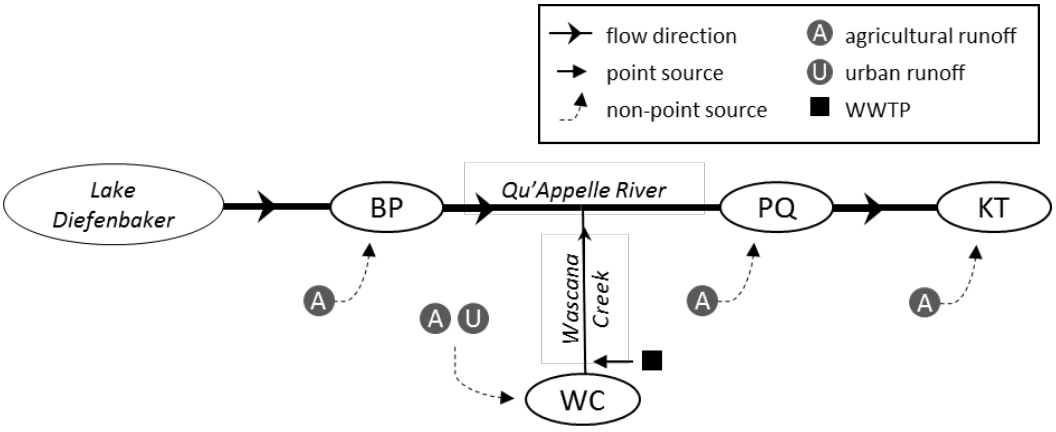

Figure 2.1 Simplified site map showing flow direction of study area and external sources of nutrient input for lakes Buffalo Pound (BP), Wascana (WC), Pasqua (PQ) and Katepwa (KT).

Table 2.1. Descriptive and measured characteristics of Buffalo Pound, Wascana, Pasqua, and Katepwa Lakes, in the Qu'Appelle watershed in Saskatchewan, Canada.

	Buffalo Pound	Wascana	Pasqua	Katepwa
Lat, long coordinates (location sampled)	50.5975 N, 105.4081 W[1] 50.5861 N, 105.3847 W[2]	50.4364 N, 104.6125 W[1]	50.7814 N, 103.9525 W[1]	50.6961 N, 103.6356 W[1]
Mean/max depth (m)	3.0 / 5.5	1.7 / 5.5[3]	6.0 / 15.5	14.3 / 23.2
Residence time (year)	0.7	0.02	0.7	1.3
Surface area (km^2)	29.5	0.5	20.2	16.2
Estimated annual TN Load (tonnes y^{-1}) [4]	190[5]	No data	1900	---[6]

[1] Latitude indicates location of sampling at 0.3 m depth by the University of Regina
[2] Latitude indicates location of sampling at 0.1 m depth by the University of Saskatchewan
[3] Leavitt et al. unpublished data
[4] Data reflect the average load over a 3 y study period from 2013 to 2016 (Water Security Agency 2018).
– indicates data were not reported
[5] Nutrient loading to Buffalo Pound in this period is thought to be atypical, due to lower than average flows from Lake Diefenbaker, and a greater flow proportion occurring from the local watershed, and backflow from the Moose Jaw River
[6] Data for Katepwa Lake is not reported, although estimates for the lake chain, including Pasqua, Echo, Mission and Katepwa are 1950 tonnes y^{-1}

2.2.2 Water Chemistry

Chlorophyll *a* was collected on Whatman® GF/F. Following Wintermans and DeMots (1954), the chlorophyll extraction method was used to calculate chlorophyll *a* concentrations using a UV-visible spectrophotometer (Shimadzu UV-1601PC) to measure absorbance at wavelengths of 665, 750 and 649 nm. For dissolved nutrient analysis, a discrete chemical analyzer (SmartChem 170©, Westco Scientific) was used to measure $NH_3 + NH_4^+$ (referred to as ammonium), $NO_2^- + NO_3^-$ (nitrate) and soluble reactive phosphorus (SRP). Methods used and method performance detection limits as follows: ammonium, AMM-001-A 5.0 µg L^{-1}; nitrate, NO3-001 4.4 µg L^{-1}; and SRP, PHO-001-A 1.8 µg L^{-1}. Each sample was filtered using pre-rinsed 0.45 µm nylon syringe filters (A.M.D. Manufacturing, Mississauga, Ontario). Soluble reactive phosphorus was analyzed within 24 hours of sample collection. Ammonium samples were preserved with sulphuric acid (pH<2), and nitrate and ammonium samples were frozen for no more than 28 days, and later thawed for analysis.

2.2.3 Phytoplankton community composition & biomass

Microscopic analysis was used to characterize the phytoplankton community in each sample, including total biomass, cyanobacterial biomass from different taxa and heterocytes by taxa. These data were used to determine the proportion of cyanobacterial biomass to total biomass in each sample, compare the biomass of diazotrophic to non-diazotrophic taxa, and observe changes to heterocyte biovolume in differing lakes over time. Samples were preserved for taxonomy by adding 1 mL of Lugol's iodine to 100 mL of raw water in a 100 mL graduated cylinder. After 24 hours of settling, the upper 80 mL was siphoned for disposal with minimal disturbance of settled material. The remaining 20 mL was transferred into vials and amended with additional Lugol's iodine if deemed necessary to ensure adequate preservation of densely-populated samples. Taxonomic identification and counts were conducted by Plankton R Us, Winnipeg, Manitoba (following Findlay and Kling 1998). Here, a known volume of the sample is selected, settled and enumerated using a simple counting chamber fitted to an inverted microscope. A minimum of 400 – 600 whole cells are enumerated— filaments are counted individually, and colonies are partially counted and remaining cells estimated. Cell wet-weight biomass is estimated by approximating cell volume and assuming a specific gravity of 1 for

cellular biomass. For heterocyte analyses, heterocyte biomass was converted to heterocyte biovolume by assuming heterocyte density was equal to water density (1 g mL^{-1}).

Heterocyte abundance is often compared to rates of nitrogen fixation (Findlay et al. 1994; Higgins et al. 2017) or used to estimate nitrogen fixation rates (Hayes et al. 2019), as rates of nitrogen fixation per heterocyte have been deemed constant at a given level of light saturation (Findlay et al. 1994). While the rate of nitrogen fixation per heterocyte may be constant at a given level of light saturation, fluctuations in sample-water turbidity resulting from enhanced productivity—as well as the probable presence of other suspended particles—led to self-shading during bottle assays. And as such, although each light-incubated sample received the same treatment, light intensities vary by water sample. Further, heterocyte biovolume rather than abundance (or density) was used in analysis as the size of individual heterocytes varied within and between species (see Appendix D, Figure D2.1). Assessing rates of nitrogen fixation per heterocyte suggests that, no matter the size of individual heterocytes, each will fix same amount of nitrogen, which may not be the case (Higgins et al. 2017).

2.2.4 Nitrogen fixation

Experiments were typically performed ~1 day after collection due to the transit time between sampling location and laboratory. All experiments were conducted using a water bath and artificial light source to simulate in-situ conditions. Nitrogen fixation rates were measured using $^{15}N_2$-calibrated acetylene (C_2H_2) reduction assays (ARA). This method uses a C_2H_2 amendment to measure gross nitrogenase activity in water samples (Flett et al. 1976). The addition of C_2H_2 prevents nitrogen fixers from using available N_2 by acting as an enzyme-blocker. Rather than fixing N_2, the nitrogen fixers will reduce the introduced C_2H_2 to ethylene (C_2H_4) (Mohr et al. 2010). Additional $^{15}N_2$-tracer bottle assays were performed regularly to determine a mole-to-mole conversion factor to translate rates of C_2H_4 production into N_2 fixed (Montoya et al. 1996). The $^{15}N_2$-tracer bottle assays use a stable isotope tracer (^{15}N) to measure net N_2 uptake by diazotrophs by analyzing the $\delta^{15}N$ of organic content. The calibration curve produced by performing these two methods in parallel was used to convert all other ARA measurements to rates of nitrogen fixation.

Treatments and control bottle assays were incubated in triplicate for 4 to 5 hours in a temperature-controlled water bath adjusted to simulate in-situ conditions (± 2°C). ARA samples were either incubated in the dark (aluminum foil protected) or light conditions using an artificial

sunlight source (PAR of ~125 μmol s^{-1} m^{-2}). Dark incubations were used to quantify fixation occurring in the absence of sunlight (and photosynthesis) under conditions which may facilitate non-heterocytous nitrogen fixation. Tracer assays ($^{15}N_2$) were performed under artificial sunlight only.

2.2.4.1 Acetylene reduction assays

ARA incubations were performed (following Findlay et al. 1994) using 160 mL glass serum bottles. Bottles were filled with water, sealed with a butyl stopper with no headspace, and inoculated with 20 cc C_2H_2 (99.6% purity, atomic absorption grade, Praxair) and placed in the incubation chamber. Sample blanks containing deionized water were inoculated to identify C_2H_4-contamination (see Appendix A). After incubation, a headspace of 20 mL was produced in each replicate bottle by replacing sample water with room air. Gaseous and aqueous phases were brought to equilibrium by shaking vigorously for 2 minutes and allowing sample bottles to sit for several minutes before subsampling the headspace. C_2H_4 concentrations were analyzed at the International Institute for Sustainable Development-Experimental Lakes Area field station using a Varian micro gas chromatograph (CP 4900) fitted with a 1 m COx^H column. To improve analytical precision and assist with error detection, neon was used as a conservative tracer to detect changes in GC performance (S. Higgins IISD-ELA, September 26, 2018, Personal communication).

Following a modification of the acetylene reduction method described in Hendzel et al. (1994), the rate of C_2H_4 production was calculated using the known values: the concentration of C_2H_4 in the headspace (*CG*) and in solution (*CW*—calculated by correcting the C_2H_4 in the headspace using the Henry's Law constant (Sander 1999), volume of the headspace (*VG*), water in the serum bottle (*VW*), and incubation time (Δt).

$$C_2H_4\ production\ rate = \left[\frac{(CG\ x\ VG) + (CW\ x\ VW)}{VW}\right]\left(\frac{1}{\Delta t}\right) \quad (2.1)$$

The rate of C_2H_4 production was converted to N_2 fixed using the factor determined through associated $^{15}N_2$ tracer assays.

2.2.4.2 $^{15}N_2$-tracer bottle assays

The $^{15}N_2$-tracer bottle assays were performed in parallel to ARAs at least once per week across the sampling season and incubated under the artificial light source to determine the C_2H_4:N_2 conversion factor. Following Klawonn et al. (2015), an enriched stock solution of 1% ^{15}N-N_2 in degassed, deionized water was prepared in advance for use in this method (see Appendix B). At the start of each $^{15}N_2$-tracer bottle assay experiment, an initial set of raw water samples were filtered onto pre-combusted Whatman® glass microfibre filters (GF/F, 4 hours at 450°C) to quantify the $\delta^{15}N$ of particulate organic matter (^{15}N-POM) in atom percent (atom%) in sample water at natural abundance. Water samples were spiked with the enriched solution in quadruplicate—6 mL of 1% stock solution for 60 mL of sample, and placed in the incubation chamber. Following incubation, all enriched samples were filtered as above. Natural abundance and ^{15}N-N_2-enriched filter samples were dried and stored in a desiccator, and filtrate preserved with hydrochloric acid (pH<4) and frozen (see Appendix D, Table D2.1). Filters were formed into pellets using ThermoScientific tin capsules. Seston on filters was analyzed for particulate nitrogen content and $\delta^{15}N$ using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to either a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) or an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK) at the University of California Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/). Four internal laboratory standards were used, calibrated to international reference materials. Those four standard materials were (bovine liver G-13, Glutamic acid G-20, Alanine G-21 and Nylon 6 G-6). Standard deviations for replicate analyses of the internal reference materials were within 0.15 per mil.

Following the summarized equation from Montoya et al. (1996, equations 6 & 7c), the net rate of nitrogen fixation was calculated using the following known values: the ^{15}N atom% of the N_2 available for fixation (A_{N_2}), ^{15}N atom% and concentration of particulate nitrogen at the start of the experiment ($A_{PN}^{t=0}$and $[PN]_0$) and following incubation (A_{PN}^{final} and $[PN]_f$), and incubation time (Δt). Initial and final *PN* are averaged to approximate the mean concentration of *PN* (mass/volume) during the incubation.

$$N_2\ fixation\ rate\ = \left(\frac{A_{PN}^{final} - A_{PN}^{t=0}}{A_{N_2} - A_{PN}^{t=0}}\right) x \left(\frac{1}{\Delta t}\right) x \left(\frac{[PN]_f + [PN]_0}{2}\right) \quad (2.2)$$

2.2.5 Data analyses

The normality status of datasets was assessed visually via frequency histograms and confirmed using the Shapiro-Wilk normality test (p-value<0.05, Ghasemi and Zahediasl 2012). A linear least squares regression was used to determine the relationship between N_2 fixed ($^{15}N_2$-tracer assay) to C_2H_4 production (ARA) and describe the level of unexplained variance. A two-tailed t-test was used to determine if this relationship was statically similar to the theoretical molar ratio (C_2H_4 produced:N_2 fixed) of 3:1. Kendall's tau was used to detect correlations between nitrogen fixation and select variables, including dissolved nutrient concentrations, cyanobacterial biomass and heterocyte biovolume. Measurements of dissolved nutrient concentrations below method detection were included in analysis as measured. These correlations were completed for all lakes combined, lakes grouped by upstream (pulsed nutrient supplies) or downstream of Regina's Wastewater Treatment Plant effluent (pulsed and pressed nutrient supplies), and individual lakes to detect if certain relationships were stronger in some lakes than others. All calculations, statistical analyses and graphics development (ggplot2 package, Wickham 2016) were performed in R (version 3.6.0).

2.3 Results

2.3.1 Seasonal changes in water chemistry across lakes

Lake transparency varied up to 1 m (secchi depth) across the season within each lake with Katepwa Lake exhibiting the greatest depth of transparency (mean 1.6 m) and Wascana Lake exhibiting the least (mean 0.36 m, Figure 2.2). Surface water temperatures reached between 22 and 25˚C at their peaks in study lakes. Increases in pH were observed mid-season with a similar mean pH in Buffalo Pound (8.8), Wascana (9.0), Pasqua (8.9) and Katepwa (8.8) Lakes. Nutrient concentrations were seasonally variable between lakes. Dissolved inorganic nitrogen (ammonium and nitrate) concentrations showed greater variability than soluble reactive phosphorus across the season in each lake (Figure 2.3). Variability in ammonium concentrations differ most in upstream lakes (Wascana and Buffalo Pound), with peaks reaching 435 and 750 µg N L^{-1}, respectively, compared to downstream lakes (Pasqua and Katepwa). Soluble reactive phosphorus was distinctly different between lakes with greatest concentrations ranging from approximately 300 and 550 µg P L^{-1} in Wascana Lake, 50 and 150 µg N L^{-1} in Pasqua and Katepwa Lakes and below 26 µg N L^{-1} in Buffalo Pound Lake.

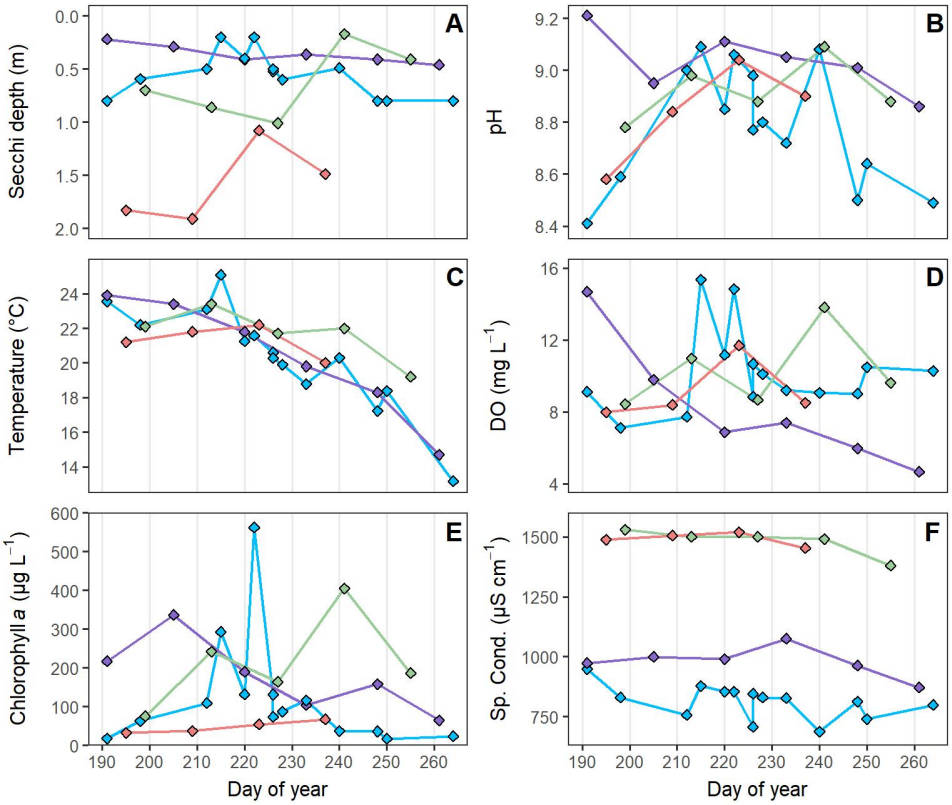

Figure 2.2 Surface water quality during the 2017 summer sampling season.in Buffalo Pound Lake (blue), Wascana Lake (purple), Pasqua Lake (green) and Katepwa Lake (red) by A) Secchi disk depth, B) pH, C) water temperature, D) dissolved oxygen, E) chlorophyll *a*, and F) specific conductivity.

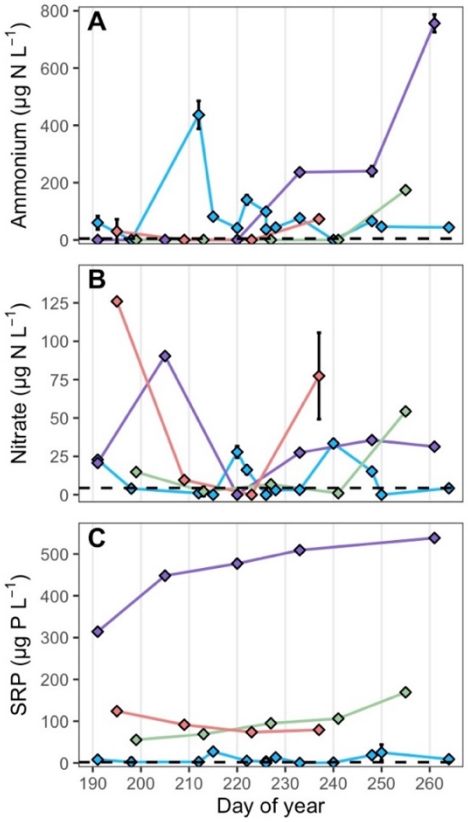

Figure 2.3 Dissolved nutrient concentrations during the 2017 summer sampling season in Buffalo Pound Lake (blue), Wascana Lake (purple), Pasqua Lake (green) and Katepwa Lake (red); A) ammonium, B) nitrate and C) SRP. Method detection limit identified indicated as dashed horizontal line at 5.0 µg L^{-1}, 4.4 µg L^{-1} and 1.8 µg L^{-1}, respectively. Points are the mean between duplicate runs, and error bars show the difference between duplicates.

2.3.2 Taxa composition & heterocyte biovolume across lakes

The proportion of heterocytous diazotrophs contributing to total phytoplankton biomass increased with increasing phytoplankton biomass within the four lakes. For instance, where heterocytous diazotrophs contributed 75% or more of total biomass, mean phytoplankton biomass was 51.6 mg L^{-1} (median 49.4 mg L^{-1}, n=14). Although heterocytous diazotrophs contributed less than 75% of total biomass, mean phytoplankton biomass was only 6.9 mg L^{-1} (median 3.9 mg L^{-1}, n=15) (Figure 2.4A, 2.4C, 2.4E). Elevated heterocyte biovolume often coincided with relatively rapid accumulations of biomass, specifically diazotrophic taxa, but not in every case (Figure 2.4B), though elevated levels of biomass did not necessarily denote a similar elevation in heterocyte biovolume. The proportion of cyanobacteria contributing to total biomass varied over the course of the sampling season and across lakes, with greater variability

appearing in upstream lakes (Figure 2.4D). A similar trend was observed for biomass of diazotrophic taxa. Lakes downstream of the wastewater effluent (i.e., Pasqua and Katepwa which are downstream of the confluence of the Qu'Appelle River and Wascana Creek) remained dominated by diazotrophic cyanobacteria throughout the season (99.8 ± 0.3% of cyanobacterial biomass, mean ± SD), while upstream lakes (Wascana and Buffalo Pound) showed a decline in the proportion of cyanobacteria as nitrogen fixers through the summer (57 ± 35% of cyanobacterial biomass, Figure 2.4F).

All lakes were dominated by cyanobacteria (i.e. biomass of this group was higher than any other groups), with the exception of Wascana Lake (DOY 234) where dinoflagellates briefly dominated over cyanobacteria (see Appendix D, Figure D2.2). Upstream Buffalo Pound Lake and Wascana Lake exhibited similar proportions of cyanobacterial biomass, with ranges from 36.9 – 99.4% and 29.5 – 98.0%, respectively (Figure 2.4D). In the downstream Qu'Appelle lakes, Pasqua maintained cyanobacterial biomass dominance across the season with proportions ranging from 95.7 – 99.4% (Figure 2.4D). Further downstream, Katepwa began the sampling season (DOY 195) with 69% cyanobacteria and 26.3% diatoms, thereafter cyanobacteria exceeded 83% until sampling ceased at August end (see Appendix D, Figure D2.2).

Peaks in cyanobacterial biomass in Buffalo Pound Lake were observed on DOY 215, 222 and 228 of 110 mg L^{-1}, 159 mg L^{-1} and 34.5 mg L^{-1} (Figure 2.4C), otherwise, biomass did not exceed 9 mg L^{-1}. Cyanobacterial biomass in Wascana Lake reached 89.4 mg L^{-1} on DOY 191 and 35.8 mg L^{-1} on DOY 220; otherwise cyanobacterial biomass was consistently below 8.8 mg L^{-1}. In Pasqua Lake, cyanobacteria reached 59.1 mg L^{-1} on DOY 213, following a relatively low level at the start of the sample season, and maintained a consistent level of cyanobacterial biomass across the season (Figure 2.4C). Katepwa maintained a lower cyanobacterial biomass ranging from 2.4 mg L^{-1} at the start of the season to 11.5 mg L^{-1} at the end of August. It is unknown whether Katepwa would have followed in a similar trend had sampling continued beyond DOY 237.

Peaks in heterocyte biovolume in Buffalo Pound and Wascana Lakes corresponded to peaks in cyanobacterial biomass, with the exception of DOY 228 in Buffalo Pound Lake where the increase in heterocyte biovolume was not proportionate to the increase in cyanobacterial biomass (Figure 2.4B, 2.4C). In keeping with trends identified in Buffalo Pound and Wascana Lakes, following an increase in cyanobacterial biomass, heterocyte biovolume in Pasqua Lake

increased from ~0.16 µL L^{-1} on DOY 199 to ~0.71 µL L^{-1} on DOY 213 (Figure 2.4B). However, although cyanobacterial biomass was maintained between 58 and 60 mg L^{-1} for the remainder of the season, heterocyte biovolume consistently decreased following DOY 213 in Pasqua Lake. Heterocyte biovolume in Katewpa Lake remained relatively low (<1.8 µL L^{-1}) across the sampling season (Figure 2.4B).

Upstream Buffalo Pound Lake and Wascana Lake were dominated by diazotrophic cyanobacteria at the start of the sampling season, and, following some fluctuations, were dominated by non-diazotrophic cyanobacteria come mid-September. Notably, cyanobacterial composition in Pasqua and Katepwa Lakes was highly dominated by diazotrophic taxa (>99%, Figure 2.4F) across the entire sampling season and a minimum of ~0.05 and ~0.02 µL L^{-1} of heterocyte biovolume, respectively.

Phytoplankton counts showed similar cyanobacterial taxa community composition across all lakes. Overall, a total of eleven genera and fifteen species were detected (Figure 2.5). Six (6) taxa were observed in all lakes including: *Aphanizomenon flos-aquae* (*Aph. flos-aquae*), *Dolichospermum crassa* (*D. crassa*), *Dolichospermum flos-aquae* (*D. flos-aquae*), *Microcystis aeuginosa*, *Planktothrix agardhii* (*P. agardhii*) and *Woronichinia compacta*. The richness of cyanobacterial taxa in Buffalo Pound Lake (6 species detected in ≥50% of Buffalo Pound Lake samples) and Wascana Lake (5; as previous) was greater than that of Pasqua Lake (3; as previous) and Katepwa (4; as previous). As mentioned, Buffalo Pound and Wascana Lakes were dominated by heterocytous, diazotrophic species at the start of the season, then non-diazotrophic species became dominant for the remainder of the sampling season. Cyanobacterial biomass in Wascana Lake showed a strong variability in heterocytous taxa where diazotrophic taxa were dominant. While in Buffalo Pound Lake, *D. flos-aquae* consistently dominated heterocytous taxa. Heterocytous taxa dominance was parallel to heterocyte dominance (Figure 2.5; Appendix D, Figure D2.3, respectively). When heterocytous taxa diminished in these upstream lakes, *P. agardhii* was typically the dominant taxa. Downstream of the City of Regina's Wastewater Treatment plant, Pasqua and Katepwa Lakes were consistently dominated by *Aph. flos-aquae*. Although heterocyte production was relatively low—compared to upstream lakes—heterocytes from *Aph. flos-aquae* were greater than *D. flos-aquae* in all samples in downstream lakes. However, a similar biovolume of *D. flos-aquae* heterocytes to *Aph. flos-aquae* heterocytes were detected on DOY 199 and 227 in Pasqua Lake and DOY 223 in Katepwa Lake.

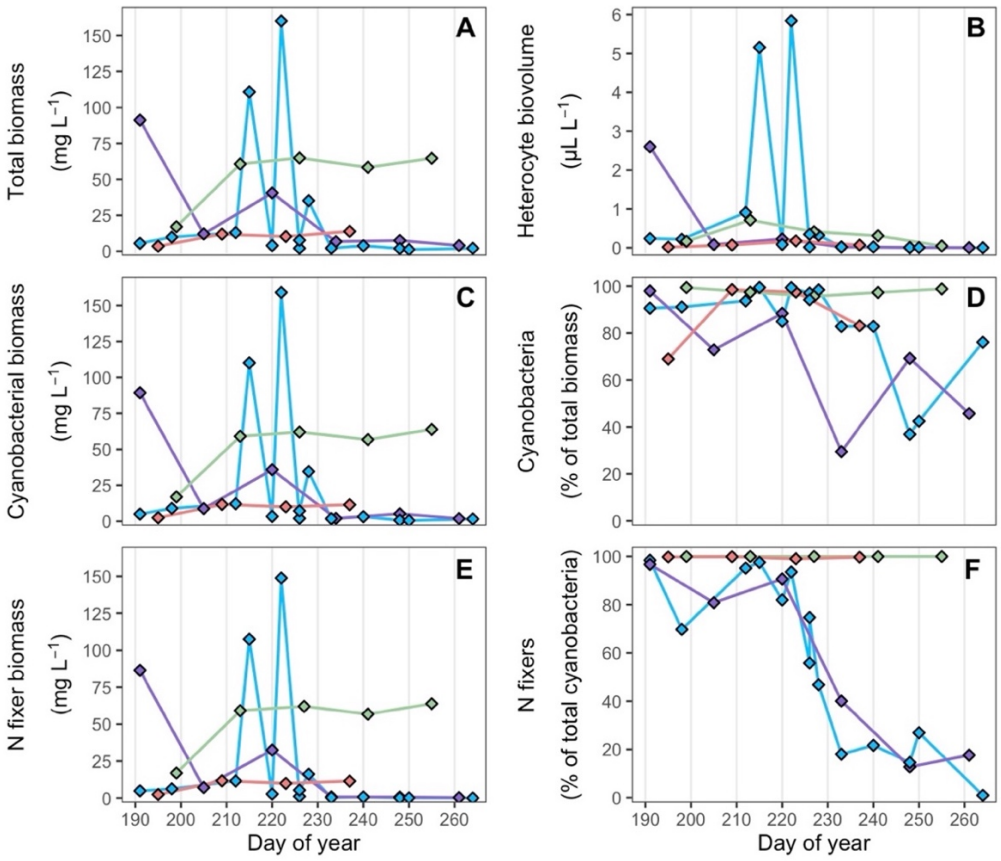

Figure 2.4 Seasonal variation in phytoplankton composition in surface waters during the 2017 sampling season in lakes Buffalo Pound (blue), Wascana (purple), Pasqua (green) and Katepwa (red) by A) total phytoplankton biomass, B) heterocyte biovolume, C) cyanobacterial biomass, D) cyanobacteria as a percent of total biomass, E) biomass of diazotrophs (N fixer), and F) biomass of N fixers as a percent of total biomass.

2.3.3 Nitrogen fixation across lakes

Parallel ex-situ ARA and $^{15}N_2$-tracer assays revealed a molar ratio of 2.87 C_2H_4:N_2. This ratio was then used to convert all C_2H_4 production rates to nitrogen fixed (Figure 2.6). This conversion factor was not statistically different (two-tailed t-test, p=0.9) from the theoretical ratio of 3:1 (based on the ratio of electrons required to reduce C_2H_2 and N_2, respectively). The relationship here between C_2H_4-produced and N_2-fixed ($^{15}N_2$-tracer method) is sensitive to outliers and should be interpreted with caution. Further, measured the rate of nitrogen fixation per unit heterocyte biovolume appears to decline with increased biomass (Figure 2.7). This is likely also true in-situ where changing levels in productivity, shifting bloom locations within the water column, and water column mixing are highly irregular in polymictic lakes such as these.

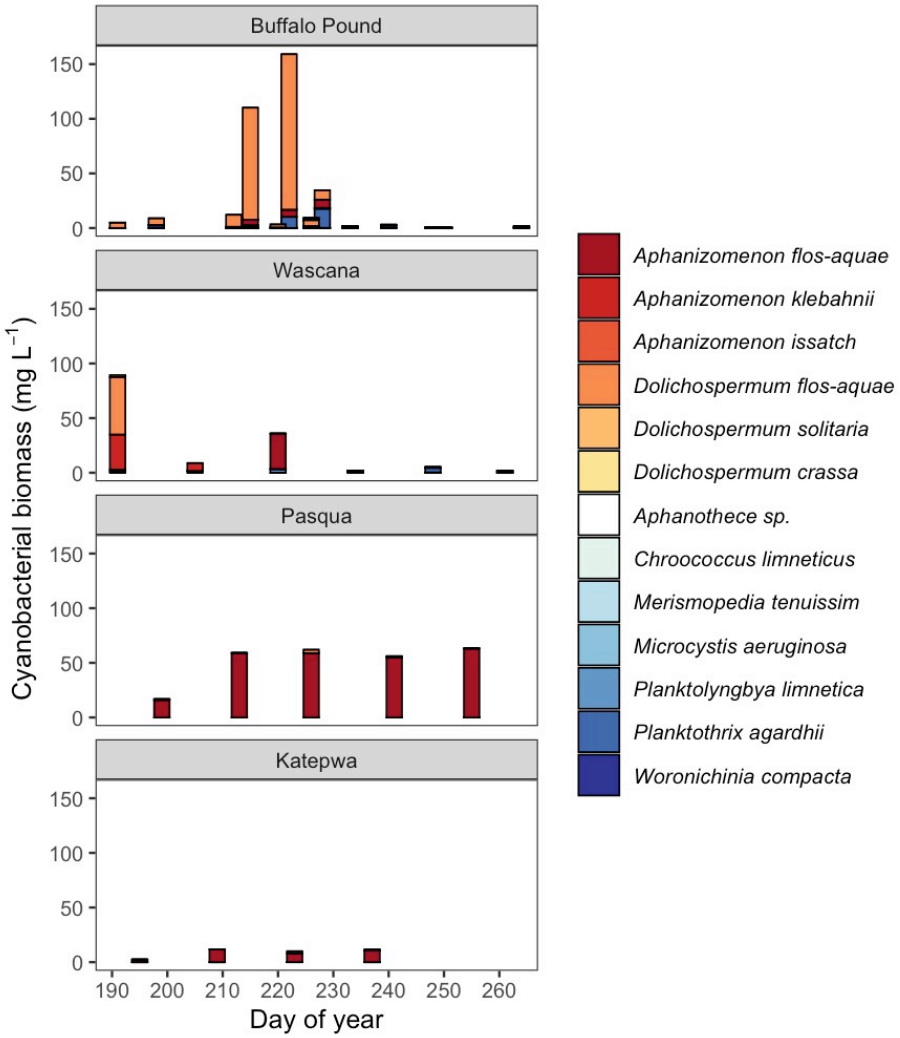

Figure 2.5 Cyanobacterial biomass by species for each lake observed during the 2017 sampling season in the Qu'Appelle River Watershed, Saskatchewan, Canada. The heterocytous *Aphanizomenon* and *Dolichospermum* genera have been widely researched as diazotrophic cyanobacteria (e.g. Findlay et al. 1994; De Nobel et al. 1997; Bradburn et al. 2012). The remaining species are non-heterocytous species with no such indications of nitrogen-fixing capabilities.

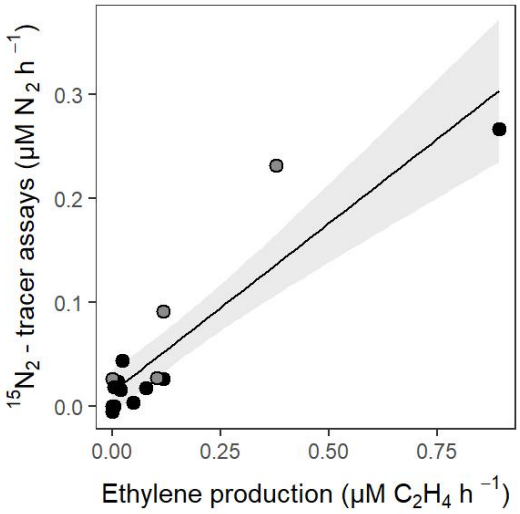

Figure 2.6 Relationship between nitrogen fixed ($^{15}N_2$-tracer assay method) and ethylene produced (ARA method) in experiments conducted during the summer of 2017 in the Qu'Appelle River Watershed ($y = 0.324x + 0.0135$, R^2=0.84, p=0.000002, n=15). Points indicate sample depth—black = 0.3 m and grey = 0.1 m, and shaded area indicates the 95% confidence interval. This regression is sensitive to the outliers (+) and should be interpreted with caution.

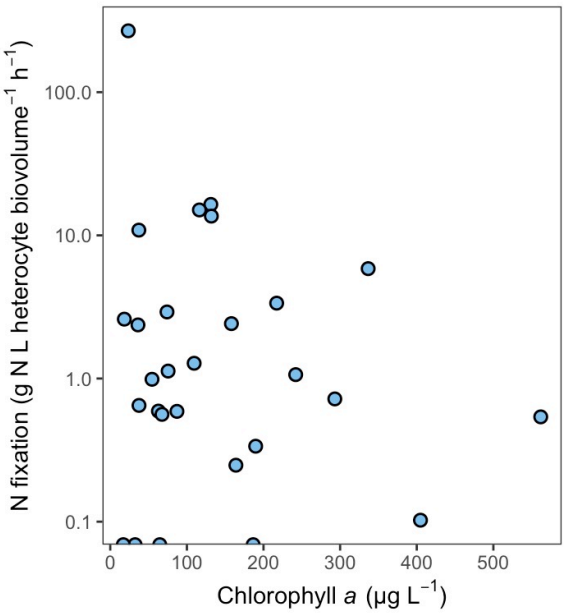

Figure 2.7 Variation in nitrogen fixation per unit heterocyte biovolume as a function of water column chlorophyll *a*. Note the log scale on the y-axis.

Bottle assays showed that nitrogen fixation occurred in all lakes to some extent (Figure 2.8, 2.9). Rates in Buffalo Pound Lake ranged from 0 µg N L^{-1} h^{-1} (DOY 250) to 3.7 µg N L^{-1} h^{-1} (DOY 215). Two key fixation events were observed mid-season on DOY 215 and DOY 222 (see Chapter 3 for an in-depth analysis of these events). Lesser peaks occurring in Pasqua and Katepwa Lakes were observed on DOY 213 and DOY 223, respectively; nitrogen fixation reached 0.76 µg N L^{-1} h^{-1} in Pasqua Lake and only 0.18 µg N L^{-1} h^{-1} in Katepwa Lake. Nitrogen fixation in Wascana Lake initially measured 8.7 µg N L^{-1} h^{-1} (DOY 191) where the water temperature was also at its peak (24˚ C) and rates exponentially declining thereafter (Figure 2.8A). The seasonally high level of cyanobacterial biomass (Figure 2.5) at the start of the sampling period indicates that the bloom was well-underway and a potential underestimation in the average rate of nitrogen fixation in Wascana Lake (Figure 2.8B).

Sampling of Wascana and Buffalo Pound Lakes had commenced on DOY 191. Nitrogen fixation was ~14-fold greater in Wascana Lake than Buffalo Pound Lake on this day; however, rates of fixation per unit of chlorophyll *a* were relatively similar (Figure 2.9A)—40.1 and 33.5 µg N mg Chl a^{-1} h^{-1}, respectively—as was nitrogen fixation per unit mass of cyanobacteria (Figure 2.9B)—0.10 and 0.12 µg N mg cyanobacterial $biomass^{-1}$ h^{-1}, respectively. Only Buffalo Pound lake exceeded 3.2 µg N mg Chl a^{-1} h^{-1} following DOY 191. Here, rates ranged from 5 – 15 µg N mg Chl a^{-1} h^{-1} from DOY 212 to DOY 240, and again on DOY 264.

2.3.4 Predictors of nitrogen fixation across lakes

Rates of nitrogen fixation were analyzed for correlations between dissolved nutrients, DIN:SRP ratios (Figure 2.10) and phytoplankton composition (Figure 2.11). In downstream, wastewater-impacted lakes (n=9), correlations were identified between rates of nitrogen fixation and SRP (negative, tau, $p<0.05$) and heterocyte biovolume (positive, $p<0.05$). Marginal correlations were identified between rates of fixation and both ammonium (negative, $p=0.07$) and nitrate (negative, $p=0.06$) in downstream lakes. While no significant or marginal correlations between nitrogen fixation and dissolved nutrients were observed in upstream lakes, positive correlations were significant between fixation rates and cyanobacterial biomass, heterocyte biovolume, percent cyanobacteria in total biomass and percent diazotrophs in cyanobacterial biomass ($p<0.05$).

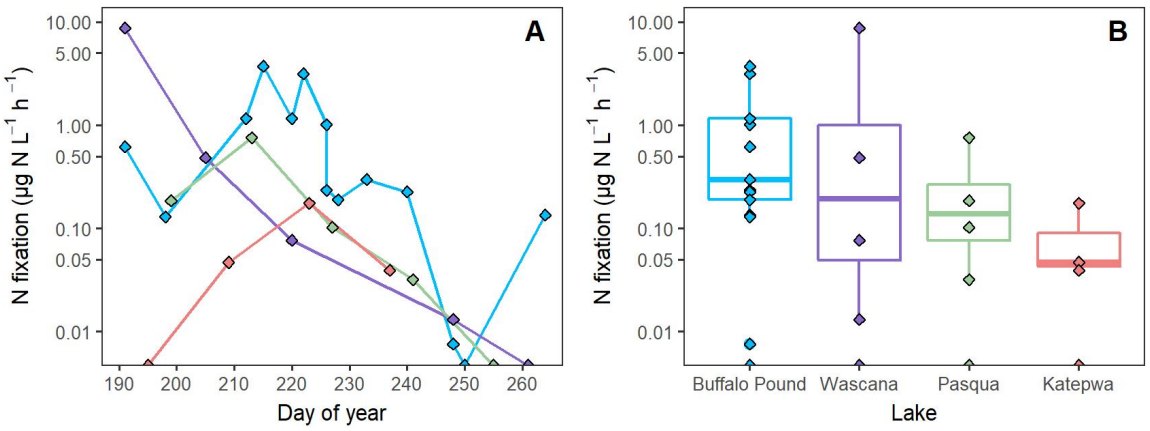

Figure 2.8 Nitrogen fixation rates calculated using $^{15}N_2$-calibrated acetylene reduction assays surface water during the 2017 summer sampling season in lakes Buffalo Pound (blue), Wascana (purple), Pasqua (green) and Katepwa (red). A) Measured rate changes across the season B) Distribution of rates by lake. Note the log scale on the y-axis. Within boxplots the outer boundaries indicate the 1st and 2nd quartiles (or Q1 and Q3, respectively), and the center line is the median. The two whiskers show the range from maximum to minimum excluding outliers, and each dot shows individual measurements. Dots beyond the range of whiskers are considered outliers (1.5 times the interquartile range (Q3 – Q1) below Q1 or above Q3.

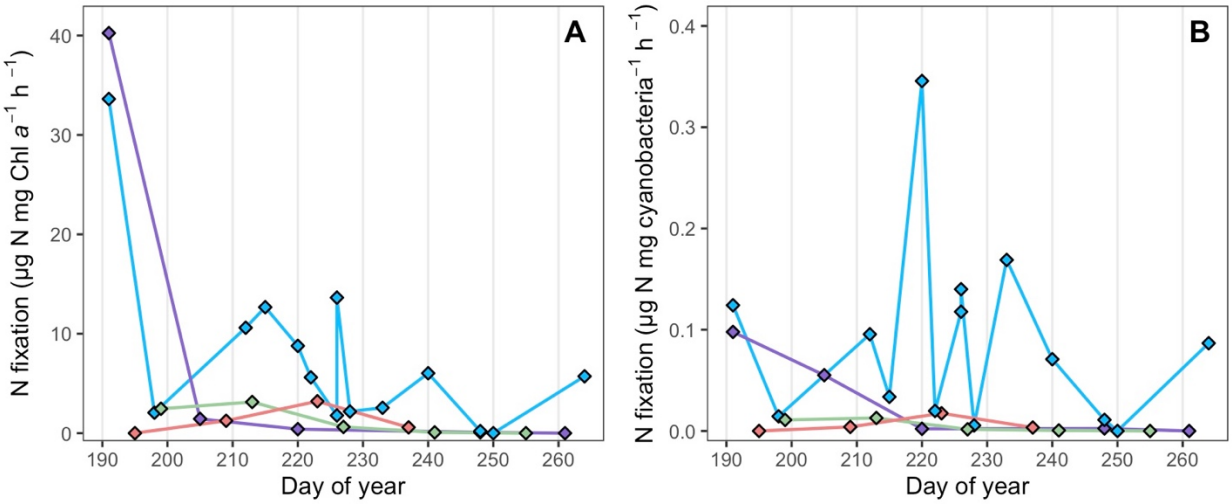

Figure 2.9 Change in nitrogen fixed over time A) per unit of chlorophyll *a* and B) per unit of cyanobacterial biomass in lakes sampled during the 2017 summer sampling season— Buffalo Pound (blue), Wascana (purple), Pasqua (green) and Katepwa (red).

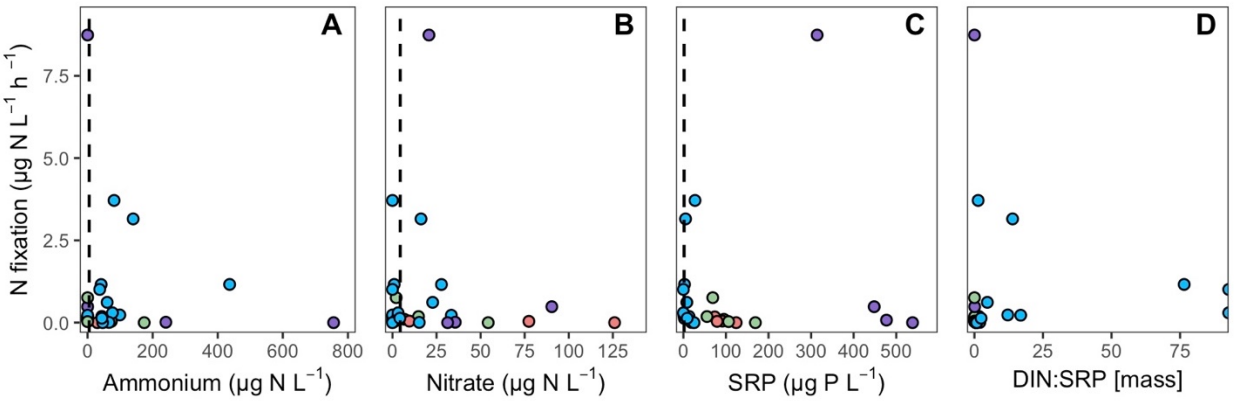

Figure 2.10 Rates of nitrogen fixation associated with A) ammonium, B) nitrate and C) soluble reactive phosphorus (SRP) concentrations and D) ratios of dissolved inorganic nitrogen to SRP by mass in lakes Buffalo Pound (blue), Wascana (purple), Pasqua (green) and Katepwa (red) in surface water during the 2017 summer sampling season. The vertical dotted lines represent the method detection limit (5.0 µg N L^{-1}, 4.4 µg N L^{-1} and 1.8 µg P L^{-1}, respectively).

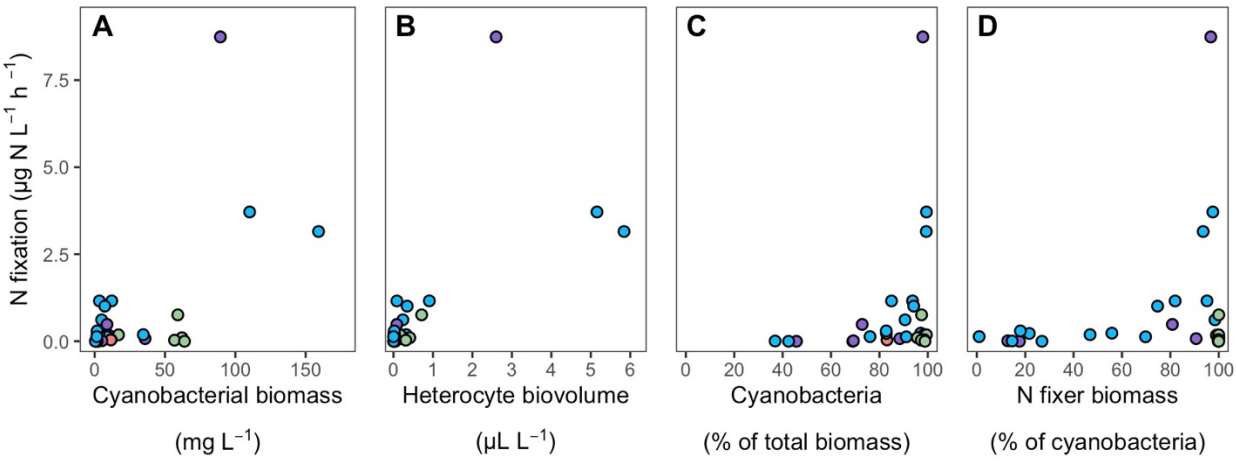

Figure 2.11 Rates of nitrogen fixation associated with A) cyanobacterial biomass, B) heterocyte biovolume, C) percent of total biomass from cyanobacteria, and D) percent of cyanobacterial biomass from diazotrophic taxa in lakes Buffalo Pound (blue), Wascana (purple), Pasqua (green) and Katepwa (red) in surface water during the 2017 summer sampling season.

2.4 Discussion

2.4.1 Nitrogen fixation rates

Nitrogen fixation has long been recognized as an important means by which some taxa can alleviate nitrogen limitation in aquatic systems (Howarth et al. 1988b). The research presented here represents the first measurements of gross biological nitrogen fixation for the Qu'Appelle region using ex-situ bottle assays, complementing previous assessments using an isotopic natural abundance method (NAM, Patoine et al. 2006; Hayes et al. 2019) and microscopic heterocyte enumeration (Hayes et al. 2019). A key question is whether nitrogen fixation is sufficient to meet cyanobacterial nitrogen demand—a question which underpins much of the debate regarding the need for nitrogen control. Nitrogen fixation has been shown to maintain biomass levels in a eutrophic boreal lake (Higgins et al. 2017). Although net rates of nitrogen fixation in the Qu'Appelle River Watershed have been thought to supply an insufficient amount of fixed nitrogen to these highly productive, phosphorus-rich lakes in the long-term (Hayes et al. 2019), in the short term, nitrogen fixation may be reasonably well-matched to demand. For example, gross measurements of nitrogen fixation measured using bottle assays ranged from 0 – 8.7 $\mu g\ L^{-1}\ hr^{-1}$ (0.8 ± 1.8 $\mu g\ L^{-1}\ hr^{-1}$, mean ± SD) during the 2017 sampling period. If we compare our overall rates of nitrogen fixation for the region to phytoplankton nitrogen demand, as calculated by Hayes et al (0.1 – 9.2 $\mu g\ L^{-1}\ hr^{-1}$, 2019) in the same study, the gap between demand and supply from fixed nitrogen is lessened, and suggests that that nitrogen fixation in the region is more important to sustaining high productivity conditions than originally believed.

Comparing rates between our study lakes, and other lakes globally, we observe high short-term rates of nitrogen fixation in some Qu'Appelle lakes, but note that the period of high rates of nitrogen fixation is much shorter in these Saskatchewan lakes than in many other bloom-affected lakes and reservoirs (Table 2.2). This contributes to a lower importance of nitrogen fixation in annual budgets, but does not negate the importance of nitrogen fixation in blooms. For example, peak rates of volumetric nitrogen fixation in Buffalo Pound and Wascana Lakes exceeded peak rates from Waco Reservoir, Texas, USA (roughly 1.8 $\mu g\ N\ L^{-1}\ h^{-1}$; Scott et al. 2008a) by approximately two and five-fold, respectively. These sites however have major differences in annual temperature regimes, and daylength due to differing latitudes which will impact nitrogen fixation. Many diazotrophic taxa require temperatures exceeding 11°C and a

minimum photoperiod of 10 light-hours per day for growth (at a pH of 8.2, Yamamoto 2009), however, nitrogen fixation rates are generally optimized at temperatures exceeding 20°C (Yamamoto and Nakahara 2009). In the Texas reservoir water temperatures exceed 20°C from roughly early May to November end (Scott et al. 2008, Figure 4A), and daylength in Texas is consistently above 10 light-hours per day during this period (maximum roughly 14.25 hours). In the Qu'Appelle Watershed region, water temperatures may exceed 20°C only in July and August (Baulch unpublished data, Buffalo Pound Lake sensor data) although daylength during these months reaches roughly 16.5 hours in June before decreasing to 13.5 hours come August-end. The time window at which temperature appears optimal for nitrogen fixation is more narrow here than for temperate systems at lower latitudes, though the window is accompanied by potentially up to ~2 additional daytime hours per day. Even so, although peak measured rates were greater in our study lakes, the nature of this cold region climate reduces the length of the optimal growth window for these diazotrophic taxa. This leads to lower annual inputs of nitrogen from fixation. Annual inputs of nitrogen via nitrogen fixation at the Waco Reservoir, a lake with a similar surface area to Buffalo Pound (28.5 and 29.5 km^2, respectively) were 50,675 kg N yr^{-1} during 2003 and 15,736 kg N yr^{-1} during 2004. These amounts of nitrogen loading are 5.8-fold and 1.8-fold greater than was estimated for Buffalo Pound Lake during the 2017 sample season (Figure 2.3A).

2.4.2 Drivers of peak fixation rates

Measured peak nitrogen fixation events occurred sequentially from upstream Wascana Lake (DOY 191) to downstream Pasqua Lake (DOY 213) then to Katepwa Lake (DOY 223). Buffalo Pound Lake showed two measured peaks on DOY 215 and DOY 222. As the speciation of heterocytes dominating in Wascana Lake at the peak of nitrogen fixation differed from taxa dominating in Pasqua Lake (see Appendix D, Figure D2.3), we believe the physical transfer of upstream blooms to downstream is limited. This apparent shift in peak rates from upstream to downstream may have been an indirect product of differing lake depths. Water temperatures in the shallow, upstream lakes tended to warm earlier in the season (Figure 2.2C) likely leading to staggered akinete germination (Pick 2016). Peak surface water temperature and low lake transparency of Wascana Lake on DOY 191 (Figure 2.2) which coincided with the greatest detectable rates of nitrogen fixation observed here (8.7 µg N L^{-1} hr^{-1}) suggests that seasonal peak nitrogen fixation may have preceded the sampling season.

Table 2.2. Maximum reported rates of nitrogen fixation rates in shallow, temperate lakes as reported in existing literature.

Date Location	Waterbody[1]	Mean depth (m)	Sampling depth	Max. N_2 fixation converted to $\mu g\ N\ L^{-1}\ hr^{-1}$	Method (conversion ratio)[2]	Reference
1994 – 2014 Saskatchewan, Canada	Buffalo Pound Lake[eu, p] Last Mountain Lake[m,p] Wascana Lake [he, p] Pasqua Lake [eu, p] Katepwa Lake [eu, p(di)] Crooked Lake [eu, p]	3.0 7.9 1.5 6.0 14.3 7.9	depth - integrated	3.0 1.5 3.0 <0.5 2.5 1.0	Natural abundance method (NAM)	Hayes et al. 2019[3]
2017 Saskatchewan, Canada	Buffalo Pound Lake[eu, p] Wascana Lake [he, p] Pasqua Lake [eu, p] Katepwa Lake [eu, p(di)]	3.0 1.5 6.0 14.3	0.1, 0.3 m grab 0.3 m grab 0.3 m grab 0.3 m grab	3.7 8.7 0.76 0.18	Acetylene reduction (AR, $^{15}N_2$-calibrated)	This study
1999 Vermont, USA	Shelburne Pond[he, p]	3.4	upper 1 m - integrated	0.13	AR ($^{15}N_2$-calibrated)	Ferber et al. 2004
2003, 2004 Texas, USA	Waco Reservoir[eu, p]	6.1[4]	0.3 m - grab	1.8	AR (3:1)	Scott et al. 2008;[3] Doyle et al. 2010[3]
2006 Texas, USA	8 reservoirs (85 locations total)	16.2 – 40.3 (range of reservoir max. depths)	0.3 m - grab	11.7	AR (3:1)	Forbes et al. 2008

[1] Waterbody superscripts indicate trophic status (o = oligotrophic, m = mesotrophic, eu = eutrophic, he = hypereutrophic or hypertropic) and lake type (p = polymictic, di = dimictic, mo = monomictic, p(di) = polymictic, occasionally dimictic, rl = river-lake mixing zone, well-mixed).

[2] Conversion ratio, where indicated, reflects the assumed ratio to calculate rates of nitrogen fixation from acetylene reduction assays (where $^{15}N_2$ calibration was not used).

[3] Rate was estimated visually from graph.

[4] Waco Reservoir mean depth from Conry 2010.

Table 2.2, cont'd. Maximum reported rates of nitrogen fixation rates in shallow, temperate lakes as reported in existing literature.

Date Location	Waterbody[1]	Mean depth (m)	Sampling depth	Max. N_2 fixation converted to $\mu g\ N\ L^{-1}\ hr^{-1}$	Method (conversion ratio)[2]	Reference
2008 Arkansas, USA	3 flood control impoundments[eu/m, mo]	mean euphotic depth euphotic zone	composite	15.2	AR (3:1)	Scott and Grantz 2013
2009 Arkansas, USA	3 flood control impoundments[eu/m, mo]	mean euphotic depth euphotic zone	composite	5.6	AR (3:1)	Scott and Grantz 2013
2009, 2010 Nevada, USA California, USA	Walker Lake[m,mo] Clear Lake[eu, p]	22 10	0.3 m – grab 0.3 m - grab	0.024 ± 0.02[3] 0.626 ± 0.03[3]	AR (4:1)	Romero et al. 2013
2011 Ontario, Canada	Lake 227[eu, d]	4.4	epilimnion—integrated	2.6	AR ($^{15}N_2$-calibrated)	Higgins et al. 2017
2012 Colorado, USA	17 vernal ponds[he, p]	3 (max depth)	depth-integrated	0.06 – 0.25, 12.5	AR (4:1)	Mischler et al. 2014[4]
2015, 2016 Ohio, USA	Sandusky Bay[he,rl]	2.6	1.0 m - grab	30.3	$^{15}N_2$-tracer assays	Salk et al. 2018

[1] Waterbody superscripts indicate trophic status (o = oligotrophic, m = mesotrophic, eu = eutrophic, he = hypereutrophic or hypertropic) and lake type (p = polymictic, di = dimictic, mo = monomictic, p(di) = polymictic, occasionally dimictic, rl = river-lake mixing zone, well-mixed).

[2] Conversion ratio, where indicated, reflects the assumed ratio to calculate rates of nitrogen fixation from acetylene reduction assays (where $^{15}N_2$ calibration was not used).

[3] mean rate ± standard error

[4] Rate was estimated visually from graph.

Past work on net nitrogen fixation within the study watershed identified spatial trends of increasing fixation rates moving eastward from the headwaters of Lake Diefenbaker to Crooked Lake (Patoine et al. 2006; Hayes et al. 2019). Considering this, we would expect rates of nitrogen fixation to be greater in Katepwa Lake than Buffalo Pound Lake. However, based on our biweekly experiments, gross volumetric nitrogen fixation rates in upstream lakes (Wascana and Buffalo Pound) were nearly 4-fold greater than that of downstream lakes (Pasqua and Katepwa). Numerous studies in the region have identified lake position as an influence over internal biogeochemical processes in wastewater-impacted systems due to lake interconnectivity. These lakes share similar morphology and exhibit a nutrient gradient when viewed as a whole (e.g. Dixit et al. 2000; McGowan et al. 2005; Patoine et al. 2006). Aside from their commonalities, individual lake characteristics, such as area and depth, are believed to influence phytoplankton taxonomy and biomass (McGowan et al. 2005). Our work demonstrates major differences in phytoplankton composition and nitrogen fixation rates between upstream (Buffalo Pound and Wascana) and downstream wastewater-impacted lakes (Pasqua and Katepwa). Beyond the influence of landscape position and wastewater influence, variability between these lakes likely reflects a variety of factors including non-point source and internal nutrient loads and timing, lake position and lake characteristics such as depth.

As expected, rates of nitrogen fixation in individual lakes were positively correlated with associated heterocyte biovolume in both the upstream, and downstream lakes. Relationships between nutrient concentrations and nitrogen fixation were however less consistent. Within downstream lakes, nitrogen fixation was negatively related to nitrate concentrations, as may be expected due to potential inhibitory effects of nitrate on nitrogen fixation, or competitive disadvantages for nitrogen fixers when nitrate is available (Ramos and Guerrero 1983; Agawin et al. 2007). Interestingly, nitrogen fixation was negatively correlated to SRP concentrations, which may reflect high biomass periods leading to drawing down of phosphorus, rather than a chemical change directly affecting rates of nitrogen fixation. Within the upstream lakes, nitrogen fixation was related to overall cyanobacterial biomass, and appears to be an important nitrogen source (see Appendix D, Tables D2.2 and D2.3), contributing more than 3 – 7 tonnes to each lake in summer. Importantly, in the context of estimated annual loads, it has been suggested that biologically-fixed nitrogen constitutes only a small proportion of total nitrogen inputs in the Qu'Appelle River Watershed (Patoine et al. 2006). However, evidence presented in this study

indicates an importance of inputs from nitrogen fixation to support the summer bloom season. Within the downstream lakes, phytoplankton groups or biomass were not related to nitrogen fixation, although there was relatively little variation in total phytoplankton biomass or cyanobacterial biomass within these lakes. Seasonal peaks in overall heterocyte biovolume and nitrogen fixation rates corresponded to peak surface water temperature in all lakes (exception: Buffalo Pound Lake on DOY 222, which was 4˚C below DOY 215).

2.4.3 Phytoplankton Composition and Drivers

The magnitude and temporal sustainment of the cyanobacterial bloom appeared most extensive in Pasqua Lake which was directly downstream of urban effluent. As dissolved inorganic nitrogen concentrations were lowest in Pasqua Lake compared to other lakes, we expect effluent nitrogen is rapidly consumed to sustain the high phytoplankton biomass. Diazotrophs also dominated the phytoplankton community structure of Pasqua Lake throughout the study period. However, despite the dominance by diazotrophs, heterocyte biovolumes were much lower in Pasqua Lake than those upstream of urban nutrient sources.

Phytoplankton composition of shallow upstream lakes and deeper downstream lakes showed contrasting degrees of variability in phytoplankton groups, cyanobacterial taxa and biomass across the sampling season. The drivers of these different dynamics between upstream and downstream lakes likely relates to differences in nutrient regimes, along with effects of lake depth. All study lakes are expected to experience irregular nutrient pulses from runoff and internal loading contributing to alternating periods of rapid and reduced cyanobacterial growth (Lürling et al. 2018). Pulses are likely amplified in shallow upstream lakes as nutrient supplies from internal loading could more easily be transferred to the photic zone for assimilation during water column mixing events. Further, shallow lakes would experience sediment and water-warming earlier in the season compared to deeper downstream lakes, staggering bloom development across the watershed. Irregular nutrient pulses in downstream lakes are supplemented with pressed nutrient supplies from wastewater effluent, and as such, appear to support more constant levels of cyanobacterial biomass.

Upstream lakes showed a relatively high level of variability in phytoplankton group dominance as the sampling season progressed in comparison to downstream lakes. In phosphorus-rich lakes, such as the eutrophic Qu'Appelle lakes (Leavitt et al. 2006), chemical forms of available nitrogen—e.g. nitrate and ammonium— can influence phytoplankton

composition (Lomas and Glibert 1999; Heil et al. 2007), and urea (not measured here) may also be important (Finlay et al. 2010b). Elevated nitrate concentrations, for example, can be associated with increases in non-cyanobacterial taxa (Heil et al. 2007) which was seen in upstream Wascana Lake on DOY 205, 233, 248 and 261 (Figure 2.3; Appendix D, Figure D2.2) with dinoflagellate biomass surpassing cyanobacteria on DOY 233. Here, SRP concentrations remained high across the season (>300 μg L^{-1}) suggesting that the level of excess phosphorus permitted the shift in taxa based on nitrogen fluxes. In comparison to upstream Wascana Lake, Buffalo Pound Lake maintained lower levels of SRP (≤ 25 μg P L^{-1}). Here, there was no apparent taxa shift with nitrogen fluxes, perhaps reflective of phosphorus limitation. However, rapid growth of cyanobacteria in Buffalo Pound Lake (DOY 215) may have been associated with high ammonium (~435 μg N L^{-1}) prior to bloom development (discussed further in Chapter 3).

We observed a common successional pattern from diazotrophic, heterocytous dominance to non-diazotrophic dominance (cyanobacteria) in upstream lakes across the sampling season. Heterocytous cyanobacterial taxa tended to outcompete all other cyanobacterial taxa in upstream Buffalo Pound Lake prior to DOY ~228, likely due to their ability to efficiently fix nitrogen (De Nobel et al. 1997). Increases in heterocyte biovolume corresponded to rapid increases in biomass. Although *Aph. flos-aquae* were present, *D. flos-aquae* were the predominant taxa—known to be superior competitors for phosphorus under nitrogen-fixing conditions (De Nobel et al. 1997) and light saturation (De Nobel et al. 1998). Following DOY 228, cyanobacterial biomass was dominated by non-heterocytous taxa (predominantly *P. agardhii*). Species succession followed a similar trend in Wascana Lake, as heterocytous taxa tended to outcompete all other cyanobacterial taxa prior to DOY 234, and non-heterocytous taxa (predominately *P. agardhii*) thereafter. Similar successional phenomena from heterocytous taxa to non-heterocytous taxa have been observed in a number of shallow, eutrophic lakes—e.g. Ford Lake, USA (McDonald and Lehman 2013), Lake Wingra, USA (Miller et al. 2013) and Dianchi Lake, China (Wu et al. 2016) and attributed to changes in water temperature, thermal stratification, nitrate concentrations or SRP concentrations.

Downstream lakes showed little deviation from total cyanobacterial dominance. In contrast to upstream lakes, the consistent phytoplankton composition and biomass concentrations in downstream lakes is likely a result of pressed nutrient supplies from the upstream wastewater treatment plant combined with greater lake depths. Here, total biomass had stabilized for the

remainder of the sampling season in both Pasqua and Katepwa Lakes on DOY 213 and 209, respectively. Once high summer biomass was established, Pasqua Lake showed approximately five (5) times more total biomass per unit volume than Katepwa Lake, suggesting, as previously postulated, that upstream lakes help mitigate point-source nutrient inputs in downstream lakes (Dixit et al. 2000). The proportion of cyanobacterial biomass in Pasqua Lake exceeded 95% of total biomass across the season, which is characteristic of nutrient-rich lakes (Watson et al. 1997). Further downstream, in addition to ongoing cyanobacterial dominance, Katepwa Lake supported the growth of non-cyanobacterial taxa for brief periods (Figure 2.3; Appendix D, Figure D2.2)—possibly a result of elevated nitrate concentrations (Lomas and Glibert 1999; Heil et al. 2012).

Heterocytous taxa dominated the cyanobacterial community in downstream lakes for the entirety of the sampling season. These diazotrophic taxa (predominately *Aph. flos-aquae*) exceeded 99% of cyanobacterial biomass. The abundance of diazotrophic taxa compared to non-diazotrophic taxa suggests nitrogen-limitation in the systems; however, biovolume ratios of heterocytes to heterocytous taxa were consistently low (data not shown) which indicates that the heterocyte formation was somewhat suppressed. It is unclear why present non-diazotrophic species, such as *Microcystis aeruginosa*, were not more successful competitors. It is possible that productivity by these other species became light-limited due to the abundance of *Aph. flos-aquae*—a 'shade' species due to its broad light absorption spectrum and ability to thrive under low light conditions and tolerance to low nitrogen (De Nobel et al. 1998).

The dominant cyanobacterial taxa detected in these prairie lakes are known bloom-forming species with some potentially toxin-producing strains: heterocytous *D. flos-aquae* and non-heterocytous *P. agardhii* in upstream lakes and *Aph. flos-aquae* in downstream lakes (Carmichael 2001; Svrcek and Smith 2004; Donald et al. 2011; D'Agostino et al. 2016). While cyanotoxins were not a component of this study, concurrent analysis of surface water blooms in Buffalo Pound Lake detected low levels of microcystin-LR in whole samples from DOY 215 – 226, 2017 (Baulch unpublished data). Similarly, previous work in the region also identified relatively low levels of microcystin in whole-water samples during the same time period in Wascana Lake. Significant concentrations of microcystin were not observed until the first week of September (DOY 244 – 258) where diazotrophs had diminished, and microcystin production was primarily attributed to *P. agardhii* and *Microcystis spp.* (Donald et al. 2011).

2.5 Implications and Management

Eutrophication of the Qu'Appelle River Watershed's naturally meso-eutrophic lakes has followed agricultural development in the surrounding region. While agricultural impacts are widespread in the prairies, urbanization strongly affects lakes downstream of the City of Regina wastewater effluent (Hall et al. 1999; Dixit et al. 2000; Quinlan et al. 2002), along with impacts to urban lakes such as Wascana. Long-term assessments of biomass in response to land-use changes have demonstrated that nutrient pollution has caused a shift in food web dynamics in the Qu'Appelle watershed (Hall et al. 1999; Dixit et al. 2000; Quinlan et al. 2002).

This study supported our hypothesis that nitrogen fixation rates in wastewater-impacted lakes are negligible, likely as a result of high nitrogen loading and sometimes high in-situ concentrations of dissolved inorganic nitrogen. Ratios of DIN:SRP had limited value in understanding the potential for nitrogen fixation, likely because nitrogen and phosphorus were often in excess. We initially expected a higher biomass of diazotrophic taxa would be associated with increased rates of nitrogen fixation. This was not observed in the downstream, wastewater-impacted lakes, although this was shown in the upstream lakes. Indeed, the sustained dominance by diazotrophs in downstream (wastewater-impacted) lakes was unexpected. Our results suggest that diazotroph dominance is not related to nitrogen limitation, and instead that dominant taxa *Aph. flos-aquae* has other competitive advantages sustaining its dominance. This diazotroph dominance may suggest that reductions in nitrogen loads associated with the recent upgrade to the Regina wastewater treatment plant, or other actions to reduce watershed nitrogen inputs, may be balanced, at least in part, by increasing nitrogen inputs through biological nitrogen fixation. Although this suggests that continued bloom issues should be expected (a conclusion also supported by palaeolimnological evidence of blooms prior to agricultural development, Dixit et al. 2000), it is important to note that the investments in nitrogen removal were predicated based on acute toxicity effects of ammonia in Wascana Creek (Dylla 2019). As such the upgrade is still likely to lead to improved ecosystem health in this upstream ecosystem associated with decreased toxicity risks. Decreased phosphorus loading is also likely to have benefit, although downstream changes associated with this wastewater treatment plant upgrade are expected to be complex modulated by sediment nutrient stores which may support elevated internal loading (Schindler 2012; Orihel et al. 2017), changes in nutrient limitation, and long-term changes in nutrient stores within sediments.

Finally, as expected, upstream lakes tend to exhibit different seasonal trends in nitrogen fixation rates and phytoplankton dynamics compared to the aforementioned wastewater-impacted lakes. While downstream, wastewater-impacted lakes experience consistent biomass with a high degree of dominance by diazotrophic heterocytous cyanobacteria, upstream lakes experienced seasonally fluctuating biomass and cyanobacterial dominance from diazotrophic to non-diazotrophic taxa. Although downstream lakes receiving continuous nutrient loading regardless of catchment inputs showed more chronic effects, upstream lakes exhibit total biomass peaks exceeding those consistently-observed total biomass in downstream systems, particularly, in Pasqua Lake. Interestingly, the non-heterocytous taxa (*Planktothrix agardhii* and *Microcystis aeruginosa*) that dominated later in the summer in the upstream lakes did not dominate the downstream lakes. While toxin dynamics are difficult to predict in any ecosystem, we note that these shifts in taxa may mediate shifts in toxicity risk, and the potential for different toxins to be produced.

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2.7 Author Contributions

Lisa M. Boyer was the lead author on this thesis chapter, with advice and edits provided by Helen M. Baulch. HMB obtained research funding for the project and advised on research program design. Scott N. Higgins helped design research methods, analyze results, and provided feedback on text.

Chapter 3: CYANOBACTERIAL BLOOM EVOLUTION: A HIGH-RESOLUTION STUDY OF NITROGEN FIXATION AND PHYTOPLANKTON COMPOSITION WITHIN A SHALLOW, POLYMICTIC PRAIRIE LAKE (BUFFALO POUND LAKE, SASKATCHEWAN, CANADA)

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3.0 Abstract

We explored the trajectory of cyanobacterial bloom development by assessing phytoplankton community dynamics, nitrogen fixation rates and physical and chemical conditions in a shallow, polymictic and eutrophic lake. Using high-frequency sensor and discrete-sampling measurements, we characterized the phases of the bloom and determined the importance of nitrogen fixation to bloom development. The pre-bloom phase (defined as the period prior to rapid growth and biomass accrual) saw increases in cyanobacterial biomass, predominantly diazotrophic and heterocytous *Dolichospermum flos-aquae* (*D. flos-aquae*), associated with incident photosynthetically active radiation (PAR) and water temperatures reaching their annual maxima. Ammonium in surface water reached its seasonal peak leading up to the major bloom. With the onset of the bloom phase, high rates of nitrogen fixation were observed in limnetic surface water (up to 3.7 $\mu g\ L^{-1}\ h^{-1}$) during transient thermal stratification events which coincided with peak cyanobacterial biomass. Midway through the major bloom phase, as water temperatures and PAR decreased, we observed a shift from high biomass dominated by diazotrophs to lower biomass dominated by non-diazotrophic taxa, predominantly the lower-light adapted taxa *Planktothrix agardhii*. Nuisance surface scums are often associated with blooms in this lake and were observed across all bloom phases, though scums were most prevalent during the major bloom phase. Littoral surface scums displayed a very high potential for nitrogen fixation suggesting scums represent biogeochemical hotspots and hot moments. Although nitrogen fixation in the region hasn't previously been considered a viable source of nitrogen to maintain phytoplankton communities on a larger scale, managers and researchers should consider the impacts of nitrogen pulses from nitrogen fixation on succession and the potential effects on toxicity as blooms progress.

3.1 Introduction

Human interference in nutrient cycling has contributed to widespread water quality concerns from regional (e.g. southern Saskatchewan, Dixit et al. 2000; Taihu, China, Paerl et al. 2014) to global scales (Millenium Ecosystem Assessment 2005). Additions of nitrogen and phosphorus to marine and freshwater environments have compromised water quality by supporting higher levels of biological productivity (Galloway et al. 2004; Fowler et al. 2013). This has led to increased occurrences and severity of cyanobacterial blooms attributed to nutrient pollution—a phenomenon that has been well-documented for decades (Smayda 1989; Hallegraeff 1993; Anderson et al. 2002). The enhanced productivity can often lead to the formation of cyanobacterial blooms—"a marked visible discoloration of the water that is caused (predominantly) by cyanobacteria" (pg. 471, Huisman et al. 2018). While blooms bring significant aesthetic concerns, for example, associated with poor water clarity and scums, the development of cyanobacterial blooms also represents a substantive risk to water quality. Blooms carry health implications for humans and animals alike (Carmichael 2001). For example, anoxia resulting from increased decomposition rates can lead to fish kills (Anderson et al. 2002), while a suite of biotoxins which can be produced by cyanobacteria can have diverse health implications, including impacts on the nervous system, and on liver function (Carmichael 2001).

While increasing bloom risk in lakes has been documented for decades, mitigation efforts have had only mixed success (Paerl et al. 2016), and important questions remain regarding the best way to mitigate blooms. This is particularly important given the varied bloom-forming taxa, and great diversity in lake, watershed and climatic conditions. Whole-lake evidence supports phosphorus-only solutions to manage eutrophication and bloom risk, based initially on work in the Canadian shield (Schindler et al. 2008). However, the persistence of water quality issues in many lake ecosystems has led many to question whether phosphorus-centric management strategies are sufficient or whether lakes could benefit from phosphorus and nitrogen co-management strategies (Howarth and Marino 2006).

Central to the phosphorus-only management paradigm is the idea that diazotrophic cyanobacterial can compensate for reductions in nitrogen loads by fixing nitrogen in order to sustain blooms. However a contrary viewpoint is that nitrogen fixation is energy inefficient (Stam et al. 1987), hence nitrogen limitation may limit biomass accrual. Culture-based studies suggest the effects of nitrogen starvation and the consequent induction of nitrogen fixation on

growth rates in cyanobacteria are often small (Molot 2017). Yet nitrogen fixation remains a process that is measured only infrequently in lakes, hence the importance of nitrogen fixation to lake nitrogen mass balances on an annual basis, and perhaps more importantly, to sustaining blooms, remains poorly known across the diversity of lakes globally.

Some of the most commonly cited predictors of blooms relate to nitrogen dynamics. For instance, cyanobacteria tend to preferentially assimilate ammonium versus oxidized forms of nitrogen, such as nitrates (Flores and Herrero 2005; Andersen et al. 2020). It has been postulated that when sources of bioavailable nitrogen are insufficient (e.g., water column, sediments), the ability of diazotrophs to fix atmospheric nitrogen becomes critical to the progression of the bloom (Howarth et al. 1988a). Where inputs via nitrogen fixation are able supplement nitrogen loads to maintain phytoplankton biomass, growth may continue until phosphorus has been depleted (e.g. boreal Lake 227, Higgins et al. 2017).

The process of nitrogen fixation consumes more energy than uptake of bioavailable nitrogen (Howarth et al. 1988a). This is because the organism must first synthesize nitrogenase, an enzyme used to break the nitrogen gas triple bond—given the availability of trace metals (iron and molybdenum) which are major components of the enzyme (Howarth et al. 1988a; Romero et al. 2013). Further, nitrogen fixation must be performed in an anoxic environment as the nitrogenase enzyme is inhibited by oxygen. Certain cyanobacterial diazotrophs will restrict nitrogen fixation activities to periods of respiration when concentrations of ambient oxygen are low (Wasmund et al. 2001). Other taxa, such as *Dolichospermum* and *Aphanizomenon*) will fix nitrogen and photosynthesize concurrently using a specialized, oxygen-impermeable cell (called a heterocyte, known previously as a heterocyst). At the onset of nitrogen limitation, heterocytous taxa can convert vegetative cells into heterocytes, a process that takes about 20 hours at 30˚C to produce mature heterocytes (Kumar et al. 2010). Given the time and energy demands needed to fix nitrogen, the fundamental factor controlling nitrogen fixation in the water column is a lack of bioavailable nitrogen. Specifically, nitrogen limitation is believed to occur when the dissolved inorganic nitrogen concentration and the ratio of nitrogen to phosphorus from external loading and internal cycling is low—below the Redfield ratio of 16:1 (approximately 7.2:1 by mass), or below 20:1 (approximately 9.0:1 by mass) in freshwater lakes (Sterner et al. 2008), a notably widespread occurrence in prairie lakes (Salm et al. 2009).

The ability of diazotrophs to support growth by fixing atmospheric nitrogen is dependent on a number of physical factors. Nitrogen fixation can be optimized with high levels of light intensity, low water column turbulence, warmer water temperatures, and a stratified water column—although nitrogen fixation can occur in shallow lakes during water column mixing as well (Howarth et al. 1988a). Because the process of nitrogen fixation is sensitive to chemical and physical changes, the rate at which nitrogen is fixed varies temporally (from diurnal to seasonal to interannual variability) and spatially (Scott et al. 2009; Doyle et al. 2010; Hayes et al. 2019). Given the sensitive nature of nitrogen fixation rates, prior studies have identified spatial variations in nitrogen fixation rates when comparing 'in-shore' and 'off-shore' areas of larger lakes (e.g. Mugidde et al. 2003). Although large lakes have demonstrated greater 'in-shore' rates compared to 'off-shore', it is not clear whether rates in littoral and limnetic zones of smaller lakes share a similar variance. In polymictic lakes, irregular water column mixing alters the physical dynamics of the water column and nutrients therein (Taranu et al. 2010) which may lead to changes in biological activity, including nitrogen fixation (Doyle et al. 2010).

The development, or evolution, of cyanobacterial blooms can also lead to significant aesthetic concerns, such as the formation of foamy or thick mats of surface scum, which are also often associated with enhanced health risks (Carmichael 1995). The ecological function of these surface scums is largely unknown, though it has been hypothesized that surface scums are simply senescent (Reynolds and Walsby 1975; Reynolds 1987), although it is also recognized they may act as biogeochemical hotspots for biological activity (Paerl and Ustach 1982). Many species of cyanobacteria can regulate their density to adjust for optimal light conditions or nutrients by migrating. These individuals can migrate and accumulate at the water-atmosphere interface by adjusting their density using gas vesicles counteracted with carbon-based ballasts (Kromkamp et al. 1986, 1988). Under certain environmental, physical, and chemical conditions cyanobacteria can form a transient surface scum. Surface scums formed by buoyancy regulating taxa range in severity from some visible patches of aggregated communities to thick mats across the water's surface (Oyama et al. 2015). In addition to scums associated with buoyancy regulation, irreversible vertical migration has been documented. In colonies of *Microcystis aeruginosa,* extracellular accumulation of dissolved oxygen bubbles produced during photosynthesis can lead to positive buoyancy of the entire community and lead to upward migration. This form of migration leads to surface scums with a foamy appearance (Dervaux 2016).

The evolution of cyanobacterial blooms in nutrient-rich systems to surface scums has been linked to weather conditions very similar to those supporting nitrogen fixation. Although, to our knowledge, nitrogen fixation rates in surface scums have not been previously assessed. Conditions, including wind velocities below a certain threshold (Soranno 1997), often lead to reduced water-column mixing (Wanninkhof and Bliven 1991) and potentially transient thermal stratification events. Stratification allows buoyancy-regulating taxa to move freely within the water column. For fast-migrating taxa, such as *Microcystis* and *Dolichospermum* species, upward vertical migration is a strong competitive advantage against non-buoyant taxa (Carey et al. 2012). However, irregular mixing in polymictic lakes can prompt a redistribution of buoyancy-regulating taxa that have accumulated at the water's surface back into the water column. Notably, the occurrence of favourable environmental conditions does not guarantee the formation of surface scums, cyanobacterial bloom development (Soranno 1997) or nitrogen fixation (Howarth et al. 1988a).

This study examines changes to cyanobacterial blooms and surface scum development within a eutrophic prairie lake. Our aim is to work at time-scales more suited to cell turn-over times and bloom development in polymictic lakes using a combination of high-frequency sensors and frequent chemical, physical and process-based measurements. We consider the specific adaptations of cyanobacterial taxa which may have contributed to biomass increases or to bloom collapse (including diazotrophic *Dolichospermum flos-aquae* and non-diazotrophic *Planktothrix agardhii*). Our first objective was to examine the importance of nitrogen fixation to cyanobacterial bloom development by describing the trajectory of biological, chemical and physical changes associated with bloom development from pre-bloom to post-bloom phases. Our second objective was to assess the level of spatial variability within the bloom season by identifying the circumstances resulting in surface scum development and comparing rates of nitrogen fixation and phytoplankton composition, particularly cyanobacteria, in surface scums to that of surface water in adjacent littoral or limnetic zones. Overall, this study will illustrate a high-resolution picture of bloom formation from pre- to post-bloom. Our findings will help inform bloom management and adaptation strategies for similar systems elsewhere which is very important given key predictors (temperature and nutrients) of bloom formation, are increasing worldwide.

3.2 Methods

3.2.1 Study area and sampling

Buffalo Pound Lake is a polymictic prairie lake located within the Qu'Appelle River Watershed, Saskatchewan. This shallow lake receives mesotrophic waters from the Lake Diefenbaker reservoir and some agricultural runoff from the surrounding region (Patoine and Leavitt 2008). Buffalo Pound Lake (50°45' N – 50°33' N, 105°41' W – 105°17' W), which was originally dammed in the late 1930's to regulate water levels and downstream flow, now serves as a water source for 25% of the province of Saskatchewan via the Buffalo Pound Water Treatment Plant. At 29 km in length, Buffalo Pound has an average width and depth of only ~1 km and ~3 m, respectively—water storage capacity reaches 9 x 10^7 m^3 (Water Security Agency).

Buffalo Pound Lake is heavily monitored through frequent discrete sampling by the Buffalo Pound Water Treatment Plant (BPWTP), University of Saskatchewan and University of Regina, complemented by high-frequency water quality measurements during the open water season performed by a lake monitoring buoy (50°35'10.1" N, 105°23'5" W) deployed annually during the ice-free season. The buoy includes a weather station, YSI sensors measuring several parameters, including chlorophyll, phycocyanin, carbon dioxide, pH, turbidity, dissolved oxygen, and thermistors at five depths (see Appendix E, Table E3.1) which are regularly maintained. Phycocyanin (a cyanobacteria indicator) and chlorophyll are presented in relative fluorescence units (RFUs). The carbon dioxide sensor was online for a limited period from August 8 to September 17 (DOY 220 – DOY 260), 2017. Sensors measurements are made at 10-minute intervals from roughly May to October of each year. Optical sensors are fitted with wipers to minimize lens obstruction from accumulation of organic matter. Sensors are maintained at least every two weeks by the Saskatchewan Water Chemistry and Ecology laboratory based out of the University of Saskatchewan's Global Institute for Water Security. Measurements of instrumental drift, and drift associated with fouling were made at each maintenance interval.

Discrete samples were collected from the lake's limnetic zone using acid-washed, opaque HDPE bottles between July 10 (day of year, DOY, 190), 2017, and September 21 (DOY 264), 2017. This included biweekly sampling (4 samples from July 18 (DOY 198) – August 28 (DOY 240), 2017, at a depth of 0.3 m from a long-term monitoring site north of the high-frequency monitoring buoy (50°35'51" N, 105°24'29" W), and 10 samples obtained by the University of

Saskatchewan's Global Institute for Water Security between July 10 (DOY 198), 2017, and September 21 (DOY 264), 2017, at a depth of 0.1 m at a nearby site, adjacent to the lake monitoring buoy. Littoral surface samples were collected between August 10 (DOY 222), 2017, and September 7 (DOY 250), 2017, (50°34'56" N, W long 105°23'7.8" W) to pair with buoy-adjacent limnetic samples. Littoral diel sampling was completed on August 23 (DOY 235, solar noon and sundown), 2017, August 24 (DOY 236, sunrise and solar noon), 2017, and August 28 (DOY 240, sunrise, solar noon and sundown), 2017. Some littoral samples were collected with the presence of biological surface accumulation (scum). Scums were collected by scooping a wide-mouth HDPE bottle across the water's surface. Concurrent measurements of water temperature, dissolved oxygen, conductivity, pH (YSI multi-probe meter, YSI, Ohio, USA), basic current weather information (hand-held Kestrel 2500), and Secchi depth were made at each sampling event. Lake water samples were mixed well then partitioned for chlorophyll *a* and dissolved nutrient analysis, microscopic phytoplankton enumeration and experimental use. Archived water samples collected from the BPWTP intake location (situated near the monitoring buoy at a depth of 3 m, approximately 1 m from the sediment-water interface) were used to assess interannual changes to cyanobacterial dominance and speciation.

Phytoplankton enumeration was performed on archived samples collected between 1996 and 2016, inclusive, during previous bloom seasons. Each sample selected for analysis was collected between July 25 (DOY 207) and August 21 (DOY 233), inclusive, of the calendar year represented (years: 1996, 1998, 2000 – 2004, 2006 – 2016). These archived samples were compared with median phytoplankton biomass and heterocyte biovolume observed between DOY 207 and 233. One additional archived water sample from the BPWTP intake location collected on July 31(DOY 212), 2017, was used to represent the phytoplankton composition on DOY 212, 2017, due to a preservation issue with the original sample. Water samples partitioned for chlorophyll *a*, dissolved nutrient analysis and experimental use were unaffected.

3.2.2 Water Chemistry

Chlorophyll *a* and nutrient analyses were completed at the University of Saskatchewan's SaskWatChE laboratory in Saskatoon. Chlorophyll *a* samples were filtered onto Whatman® GF/Fs, dried in a desiccator and stored in a freezer until analyzed. As described in Wintermans and DeMots (1954), filters were place in ethanol for 24 hours, dissolving chlorophyll into solution. Absorbance of the solution was measured at wavelengths of 649, 665 750 nm using a

spectrophotometer (Shimadzu UV-1601PC). Ammonium, nitrate, soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP) and total phosphorus (TP) were preserved and analyzed with a discrete chemical analyzer (SmartChem 170©, Westco Scientific) using standard EPA methods (shown in Table 3.1). Nitrogen to phosphorus ratios (by atomic mass) were calculated as dissolved inorganic nitrogen (DIN, mass per unit volume of nitrogen from ammonium and nitrate) over SRP and TP (mass per unit volume of phosphorus from SRP and TP, respectively).

Table 3.1 Analysis of dissolved nutrients and total phosphorus: method identification, detection limits and sample preservation.

Analyte(s)	Method (MDL)	Sample preparation, preservation and storage
$NH_3 + NH_4^+$ (ammonium)	AMM-001-A (5.0 µg N L^{-1})	0.45 µm nylon syringe filter, H_2SO_4 (to pH<2), freeze (-40˚C), analysis within 28 days
$NO_2^- + NO_3^-$ (nitrate)	NO3-001 (4.4 µg N L^{-1})	0.45 µm nylon syringe filter, freeze (-40˚C), analysis within 28 days
Soluble reactive phosphorus (SRP)	PHO-001-A (1.8 µg P L^{-1})	0.45 µm nylon syringe filter, freeze (-40˚C), analysis within 24 hours
Total dissolved phosphorus (TDP)	PHO-001-B (1.4 µg P L^{-1})	0.45 µm nylon syringe filter, dark cupboard, digestion
Total phosphorus (TP)		Dark cupboard, digestion

3.2.3 Phytoplankton community composition & biomass

Phytoplankton inventories were conducted on all water and scum samples. Samples were preserved with Lugol's iodine solution and concentrated by five when total biomass was less than 200 mg L^{-1}. Briefly, preserved samples were subsampled and analyzed using an inverted microscope fitted with a simple counting chamber. All counts were completed by Plankton R Us, Winnipeg, Manitoba (following Findlay and Kling 1998; for more details see Section 2.2.3 of this thesis). Heterocyte abundance, or density, is commonly used to estimate rates of nitrogen fixation, although an argument has been made for the need for comparisons to be made between nitrogen fixation and heterocyte biovolume, rather than heterocyte density, as heterocytes can differ in size and such methods assume all heterocytes fix nitrogen at a consistent rate regardless of size (Higgins et al. 2017).

3.2.4 Surface scum observations

We tabulated occurrences of identifiable surface scums, light scums and blooms using hourly on-shore photographs of Buffalo Pound Lake from DOY 187 (July 6, 2017) to DOY 264 (September 21, 2017) between 08:00 to 16:00, inclusive. Each photo was examined with the unaided eye to identify discernible surface scums, patches or streaks of surface scum or subsurface blooms. Note that occurrences of surface scum, light scums or a bloom may not have been discernible by photograph where lighting was poor (normally due to cloud cover) and/or where scum was a very thin accumulation on the surface.

3.2.5 Experimental methods for measurement of nitrogen fixation and ammonium uptake

Water and scum samples designated for ex-situ experiments were transported under dark, ambient temperature conditions. Water samples collected by the University of Regina (DOY 198, 212, 236 and 240) were couriered overnight from Regina to Saskatoon, Saskatchewan. Sample processing began at 07:00 the following day. As these experiments started from 20 – 22 hours after collection, all other water and scum samples were stored in similar conditions until the following day for consistency. Laboratory experiments were conducted regularly to measure nitrogen fixation rates, and periodically to measure nitrogen uptake from ammonium, using a temperature-regulated water bath (± 2˚C of lake temperature at time of collection), and incubation time was between 4 and 5 hours. An artificial light source was used to simulate light conditions at the time of collection (consistent PAR of ~125 μmol m^{-2} s^{-1}). Diel experiments were completed on-site using a simple floating structure designed to suspend secured sample bottles near depth of collection without impeding natural light penetration.

3.2.5.1 Nitrogen fixation

Nitrogen fixation was measured using calibrated acetylene reduction assays (ARA). The ARA method measures gross nitrogen fixation using an enzyme-blocker to reappropriate nitrogenase activity. In the presence of the enzyme-blocker (here, acetylene, C_2H_2), diazotrophs will preferentially reduce C_2H_2 to ethylene (C_2H_4), rather than reduce (or 'fix') available nitrogen gas (N_2). The rate of nitrogen fixation is determined by, first, calculating the rate of C_2H_4 production and, then, converting this to N_2 fixed using a theoretical or experimentally-determined molar ratio (C_2H_4 produced: N_2 fixed). The molar ratio used here was determined by conducting frequent parallel $^{15}N_2$-tracer assay experiments (n=15) for comparison at least once

per week throughout the sampling period. This 'labelling' method uses an isotopic tracer to assess the change in the ^{15}N (atom%) of particulate nitrogen during incubation. The nitrogen fixation methods described here are discussed in greater detail in Section 2.2.4 of this thesis.

Nitrogen fixation rates of each water sample were measured using ARAs. Treatments were either incubated under light (artificial sunlight or natural for diel work) or dark (covered with foil) conditions. It is important to note that not all ARA samples were incubated in both light and dark conditions and that only paired light and dark samples were used in the analysis. Serum bottles (160 mL) were filled with sample water, sealed with a butyl stopper and secured with an aluminum cap. Stoppers were vented with syringe needles prior to fitting; this was done to remove any headspace present and allow for pressure equalization between the sample bottle and the atmosphere. Next, each bottle was inoculated with 20 mL of C_2H_2 (99.6% purity, atomic absorption grade, Praxair) which dissolved quickly into solution (within 30 seconds with shaking) then treatments were place in the incubation chamber. Following the incubation period, (1) a 20 mL headspace was introduced into each serum bottle, (2) serum bottles were shaken for 2 minutes to allow for equilibrium between gaseous and aqueous phase C_2H_4, and (3) the headspace subsampled. The headspace collected was analyzed for C_2H_4 concentrations using a gas chromatograph. Henry's Law was applied to calculate the rate at which C_2H_4 was produced during the incubation period in light and dark samples following Hendzel et al. (1994):

$$C_2H_4\ production\ rate = \left[\frac{(CG\ x\ VG) + (CW\ x\ VW)}{VW}\right] \times \left(\frac{1}{\Delta t}\right) \quad (3.1)$$

Where *CG* represents the concentration of C_2H_4 in the headspace and *CW* the concentration in solution—the concentration in solution was calculated by correcting the C_2H_4 in the headspace using the Henry's Law constant (Sander 1999)—*VG* represents the volume of the headspace, *VW* the volume of water in the serum bottle, and (Δt) as the incubation time. Sample blanks, consisting of deionized water inoculated with C_2H_2, accompanied each experiment to identify any C_2H_4 contamination. All treatment bottles and sample blanks were performed in triplicate.

The calculated rate of C_2H_4 production was converted to N_2 fixed by comparing ARA treatments incubated under light conditions with results from the $^{15}N_2$-tracer bottle assay

treatments incubated under light conditions. The $^{15}N_2$-tracer bottle assays were conducted frequently (n=15), but not at each sampling event. Here, 6 mL of a 1% ^{15}N-N_2 enriched solution was gently added to the bottom of glass-stopped bottles (60 mL BOD). Quickly, each bottle was filled with sample water and sealed without a headspace, and these treatments were incubated adjacent to ARAs. As soon as possible, additional 60 mL BOD bottles containing sample water (without enrichment) were filtered onto pre-combusted (450˚C for 4 hours) GF/Fs. Seston on filters and filtrate was analyzed to determine the natural abundance $\delta^{15}N$ of the sample water. Following the incubation period, enriched treatments were filtered using acid-washed filtering equipment. Seston and filtrate of the enriched treatments were analyzed to determine final δ ^{15}N, and natural abundance and enriched samples were used to calculate excess enrichment of particulate and dissolved nitrogen. Filters were covered and air dried, before being stored in a desiccator; and filtrate was preserved with hydrochloric acid (to pH<4) and stored at -40˚C until shipment for analysis.

Both seston on filters and filtrate were assessed for isotopic enrichment externally. Each filter was individually packed into tin capsules, then shipped to the UC Davis Stable Isotope Facility for particulate analysis using an elemental-analyzer-isotope ratio mass spectrometer (described in Section 2.2.4 $^{15}N_2$-tracer bottle assays of this thesis). Frozen filtrate was shipped to the University of Waterloo and analyzed for $\delta^{15}N$ using an elemental analyzer (4010 Elemental Analyzer, Costech Instruments, Italy) coupled with a continuous flow isotope ratio mass spectrometer (Delta Plus XL, Thermo-Finnigan, Germany).

3.2.5.2 Ammonium uptake

We conducted ammonium uptake experiments on select water samples to assess the rate at which N-NH_4^+ is assimilated compared to N-N_2. Ammonium concentrations at the surface of Buffalo Pound Lake are characteristically low during the bloom season due to high rates of primary productivity (Terry 2020). Samples were amended with ^{15}N-NH_4Cl with a known isotopic composition (98%), and as ammonia concentrations were expected to be near zero, we minimized stimulation of ammonium uptake by amending each sample to a final concentration of 0.05 μmol ^{15}N L^{-1}. Ammonium uptake experiments were performed in quadruplicate alongside $^{15}N_2$-tracer assays. Parallel to the $^{15}N_2$-tracer assays, ammonium uptake experiments were filtered onto pre-combusted GF/F's after incubation using a separate filtering apparatus to

reduce risk of contamination between methods. Seston on filters was analyzed for δ ^{15}N of particulate nitrogen with an elemental analyser-isotope ratio mass spectrometer at UC Davis Stable Isotope Facility.

Ammonium uptake was calculated following Kumar et al.(2008) using the known values: the final concentration of particulate nitrogen ([PN]), difference between the final and initial ^{15}N atom% of particulate nitrogen (ΔI_P), ambient ammonium concentration (S_a), added ammonium concentration (S_t), ^{15}N atom% of the tracer (I_r), natural ^{15}N atom% (I_0), and incubation time (Δt).

$$NH_4^+ \ uptake \ rate = [PN] \times \frac{\Delta I_P}{\Delta t \times \left(\frac{I_0 S_a + I_r S_t}{S_a + S_t} - I_0\right)} \quad (3.2)$$

Note in cases where ambient ammonium concentrations were below the method detection limit (5.0 µg N L^{-1}), ammonium uptake was calculated and reported by assuming the concentration of ambient nitrogen from ammonium was 5.0 µg N L^{-1}.

3.2.6 Statistical analyses

Normality status of datasets was assessed visually via frequency histograms and confirmed using the Shapiro-Wilk normality test (p-value<0.05, Ghasemi and Zahediasl 2012). Comparisons of (1) ethylene production between sample and blank treatments and (2) N_2 fixed between light and dark incubations were conducted using the exact form of the Wilcoxon signed-ranks test for nonparametric, paired data. Linear regression was used to determine the relationship between N_2 fixed ($^{15}N_2$-tracer assay) to ethylene production and describe the level of unexplained variance in the fit. Kendall's tau was used to detect correlations between nitrogen fixation and select variables, including nutrients (ammonium, nitrate, soluble reactive phosphorus, total dissolved phosphorus and total phosphorus), percentage of cyanobacteria in total biomass, percentage of cyanobacteria capable of fixing nitrogen, and heterocyst abundance. All calculations (including Schmidt stability; rLakeAnalyzer package, Winslow et al. 2014), statistical analyses and graphics development (ggplot2 package, Wickham 2016) were performed in R (version 3.6.0).

3.3 Results

3.3.1 Bloom evolution & water quality dynamics

High-frequency monitoring of Buffalo Pound Lake showed short-term and seasonal variability in conditions including (light intensity, water temperature, pH and dissolved oxygen, Figure 3.1). We describe the pre-cyanobacterial bloom phase here as the period prior to rapid growth and biomass accrual—identified as July 6 to 31 (DOY 187 – DOY 212), 2017. During this phase, although the proportion of biomass from cyanobacteria was consistently high, biomass remained relatively low (<10 mg L^{-1}). The major cyanobacterial bloom spanned from August 1 to 26 (DOY 213 – DOY 238), 2017, as indicated by sensor-based measurements of phycocyanin. These corresponded to microscopic count data showing peak cyanobacterial biomass in surface water samples exceeding 150 mg L^{-1} within that phase. Following this peak, both total and cyanobacterial biomass declined to levels below that of the pre-bloom phase. These results from the discrete sampling campaign showed similar patterns to the high-frequency monitoring buoy. For example, reduced photosynthetically active radiation (PAR) penetration through the water column along with elevated chlorophyll and phycocyanin showed similar timing of changes leading up to DOY 215. During this time, secchi depth decreased and chlorophyll *a* increased from 0.5 m and 100 µg L^{-1} on DOY 212, respectively, to 0.2 m and 300 µg L^{-1} on DOY 215. Although sensor-based measurements of pH, dissolved oxygen (DO) and specific conductivity were generally similar to discrete measurements over the course of the 2017 sampling season (Figure 3.1; Appendix E, Figure E3.1), high-frequency sensor measurements reveal diel changes not seen in discrete measurements. High-frequency monitoring and discrete sampling results will be described in concert where appropriate.

Incident PAR near the lake surface averaged a daily maximum of 1447 ± 117 µmol $s^{-1}m^{-2}$ (mean ± SD) in the weeks leading up to the bloom (DOY 187 – DOY 212). Incident PAR decreased to 1304 ± 222 µmol s^{-1} m^{-2} at the peak of the bloom season (DOY 213 – DOY 238) and decreased further to 1088 ± 249 µmol s^{-1} m^{-2} in the weeks following (August 26 to September 21, 2017, DOY 239 – DOY 264). Irradiance at 0.62 m during pre-bloom, major bloom and post-bloom phases averaged 45%, 14% and 35% of incident PAR, respectively, and at 0.78 m, light penetration averaged 27%, 5.8% and 19%, respectively (Figure 3.1A). Secchi disk measurements followed a similar trend, decreasing from 80 cm early in the season to 20 cm at DOY 215 and DOY 222 sampling, thereafter, stabilizing around 80 cm (see Appendix E,

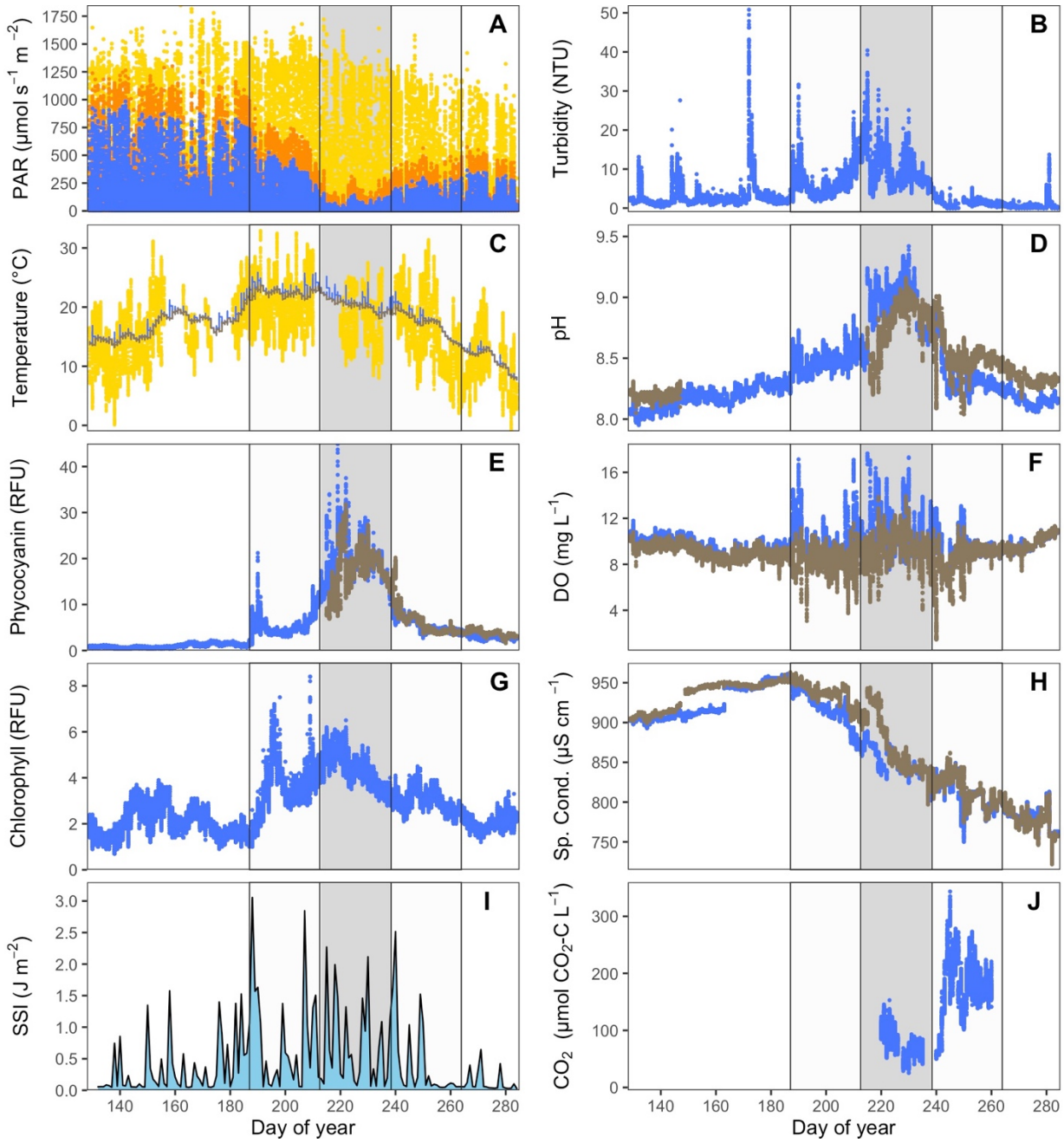

Figure 3.1 Time series high-frequency in-situ measurements from May 10 – October 11 (DOY 130 – 284), 2017—pre-bloom (DOY 187 – 212), major bloom (grey area, DOY 213 - 238) and post-bloom (DOY 239 – 264) phases in Buffalo Pound Lake A) Photosynthetically active radiation at ~1 m above (yellow), and 0.62 m (orange) and 0.78 m (blue) below the water surface. B) Turbidity (Formazin Nephelometric Turbidity Units) at 0.82 m. C) Temperature reading for air (yellow), and 0.45 m (blue) and 3.18 m (brown) below surface. D) pH at 0.82 m (blue) and 2.85 m (brown). E) Phycocyanin (relative fluorescence units) at 0.82 m (blue) and 2.85 m (brown). F) Dissolved oxygen concentration at 0.82 m (blue) and 2.85 m (brown). G) Chlorophyll (relative fluorescence units) at 0.82 m. H) Specific conductivity at 0.82 m (blue) and 2.85 m (brown). I) Daily Schmidt stability index (SSI). J) Carbon dioxide concentration at 0.82 m. Gaps in data represent periods of sensor failure.

Figure E3.1A). Lake temperatures increased roughly from daily averages of 14°C mid-May to 24°C mid-July (Figure 3.1C). The lake typically showed little variation in temperature between deep (sensor 3.18 m below surface) and shallow depths (sensor 0.45 m below surface) with maximum temperature differences averaging 1.1°C on any given day. Transient thermal stratification events were observed during each bloom phase—pre-bloom: DOY 188, 190, 207, 210, 211, major bloom: DOY 215, 218, 222, 230 and post-bloom: DOY 239, 240. These stratification events were identified as having a 1˚C change in temperature per metre of depth (Taranu et al. 2012) or a temperature difference of at least 2.73˚C between 3.18 m and 0.45 m. Further, although the peak measured temperature of discretely-sampled surface water occurred on DOY 215 during the major bloom phase (see Appendix E, Figure E3.1C) preceding a steady decline, the high-frequency temperature monitoring sensor at 0.45 m detected temperatures from 25 – 26˚C on DOY 188, 190, 207, 210, 211 (during the pre-bloom phase) and on DOY 215.

Sensor measurements of phycocyanin (0.8 m below surface) experienced an initial peak reaching 21.2 RFU on DOY 190 with an observed lag in peak chlorophyll on DOY 196 prior to major bloom development (Figure 3.1E, 3.1G). Phycocyanin across the major bloom phase averaged a daily maximum of 19.2 ± 4.9 RFU. Phycocyanin appears to follow the same trend in deep (2.8 m) compared to shallow water, with a reduction in deep water phycocyanin following DOY 228 (note deep phycocyanin sensor was offline prior to DOY 215). On the surface, discrete measurements show chlorophyll *a* concentrations increase to ~300 µg L^{-1} on DOY 215, and following a brief dip at the surface (100 µg L^{-1} on DOY 220), reaching ~570 µg L^{-1} on DOY 222 before then tapering off (see Appendix E, Figure E3.1E).

We identified two periods of elevated phycocyanin within the major bloom phase. The first (referred to as the primary bloom) occurred between DOY 215 and 222, inclusive, showing irregular peaks reaching a maximum of 44.9 RFU on DOY 219. While the major bloom phase showed light penetration (PAR) at 0.78 m averaging a daily maximum of 79 ± 37 µmol s^{-1} m^{-2} and a surface water temperature averaging 20.8 ± 1.2˚C, the primary bloom exhibited a lower average in daily maximum light penetration (45.3 µmol s^{-1} m^{-2}) and above average water temperatures (21.7˚C). The second period (referred to as the secondary bloom) occurred between DOY 226 and 233, inclusive, with consistently lower-level peaks between 25 and 30 RFU which coincide with below average PAR at 0.78 m (70.0 µmol s^{-1} m^{-2}) and water temperatures similar

to that of the major bloom phase average (20.4˚C). Carbon dioxide decreased during the secondary bloom and rapidly increased following collapse of the major bloom (Figure 3.1I).

Periodic spikes in turbidity were observed across the sampling season, often coinciding with phycocyanin peaks (Figure 3.1B, 3.1E, 3.1G). Turbidity unexplained by biological activity was likely caused by wind events leading to sediment resuspension (see Appendix E, Figures E3.2B and E3.2D). Further, seasonal elevations in turbidity reflected those of phycocyanin to a varying degree during the pre-bloom, major bloom and post-bloom phases.

Sensor-based measurements show pH levels gradually increased throughout the season, with values of ~8.0 at DOY 130, reaching a peak of approximately 9.4 on DOY 230 prior to collapse of the secondary bloom, and decreasing thereafter (Figure 3.1D; Appendix E, Figure E3.1B). Mean fluctuations in pH on a daily scale ranged from 0.22 pH units during the pre-bloom phase and 0.28 pH units during the major bloom phase, and 0.16 pH units post-bloom collapse. The greatest diel range in pH occurred during the primary bloom on DOY 215 (0.78 pH units) though daily pH ranges exceeding 0.40 pH units occurred during each phase of the bloom. Dissolved oxygen in discretely-sampled surface water fluctuated strongly, ranging from 6.1 to 17.6 mg L^{-1} with relatively high peaks occurring on DOY 215 and 222 (Figure 3.1F; Appendix E, Figure E3.1D). The high-frequency sensor showed a daily range of up to 10.0 mg L^{-1} (DOY 215), and that high DO concentrations in shallow waters (0.8 m sensor) tended to co-occur with low concentrations within the deeper waters (2.85 m) during periods of stratification (Figure 3.1F), with the first period of lower oxygen (4.7 mg L^{-1}) occurring on DOY 191. Sensor-based measurements of specific conductivity decreased from 950 µS cm^{-1} at the onset of the bloom to ~800 µS cm^{-1} in shallow surface water following bloom decline (Figure 3.1H). Although exhibiting an overall trend of decline in surface water, conductivity fluctuated from ~750 to ~900 µS cm^{-1} during the discrete sampling season (see Appendix E, Figure E3.1F). There were identifiable differences in surface water conductivity in samples collected from the University of Regina long-term monitoring site and the University of Saskatchewan buoy monitoring site, with higher levels of conductivity detected at the latter.

Ammonium exhibited a seasonal peak of 436 µg N L^{-1} in limnetic surface waters on DOY 212 immediately preceding the major bloom phase, and lesser peaks during the major bloom period on DOY 222, 226, 233 (Figure 3.2). Nitrate concentrations were relatively low compared to ammonium, with minimal peaks occurring on DOY 220 and 240 (maximum 33.4

µg N L^{-1}). Phosphorus from SRP was low compared to TP with low peaks occurring on DOY 215 and 228 (seasonal high of 27.3 µg P L^{-1} on DOY 215. Total phosphorus ranged from below detection (1.4 µg P L^{-1}) to 198 µg P L^{-1}. Key peaks in TP concentrations occurred on DOY 215 and DOY 222; while concentrations of TP below detection co-occurred with low (or undetectable) concentrations of ammonium). Finally, low peaks of phosphorus from TDP were observed on DOY 228, 233 and 250 (up to 29 µg P L^{-1}).

3.3.2 Bloom evolution & taxa composition & heterocyte biovolume

Biomass from cyanobacteria in limnetic surface water ranged from 0.5 – 160 mg L^{-1} across the season and diazotrophic taxa dominated all other taxonomic groups during both the pre-bloom and major bloom phases (Figure 3.3). The main constituents of cyanobacteria were heterocytous *Dolichospermum flos-aquae* (*D. flos-aquae*) and non-heterocytous *Planktothrix agardhii* (*P. agardhii*) though other species were observed to a lesser extent, including heterocytous *D. crassa* and *Aphanizomenon flos-aquae* (*Aph. Flos-aquae*) and non-heterocytous *Woronichinia compacta* and *Microcystis aeruginosa*. Heterocytes were produced by four heterocytous taxa: *D. flos-aquae*, *Aph. flos-aquae*, *D. crassa*, and *D. solitaria*; however, *D. flos-aquae* heterocyte biomass dominated all others in Buffalo Pound Lake (see Appendix E, Figure E3.4). Following cyanobacterial bloom collapse, biomass from cyanobacteria, as with total biomass, remained relatively low for the remainder of the sampling season with increases in chlorophytes and chrysophytes.

Phytoplankton counts indicated *D. flos-aquae* –a common heterocytous species–was the dominant taxa during the pre-bloom phase (DOY 187 – 212) with diazotrophs accounting for 98.4% of the cyanobacterial biomass on DOY 191 (Figure 3.3A). Cyanobacterial biomass and heterocyte biovolume were moderate at the onset of the sampling season (DOY 191 ~5 mg biomass L of $water^{-1}$ and 0.24 µL heterocyte biovolume L of $water^{-1}$, respectively, Figure 3.3A, 3.3B). And although cyanobacterial biomass increased through the pre-bloom phase, the extent at which diazotrophs dominated over other taxa experienced a brief decline to enhanced growth of *Woronichinia compacta* mid-phase (27% of cyanobacteria composition from *Woronichinia compacta*). Leading up to the major bloom phase, cyanobacterial biomass and heterocyte biovolume increased to 12.2 mg L^{-1} and 0.91 µL L^{-1}, respectively, on DOY 212.

Phytoplankton composition diverged between the primary and secondary bloom periods of the major bloom phase—separate periods defined as DOY 215 – 222 (primary) and DOY 226

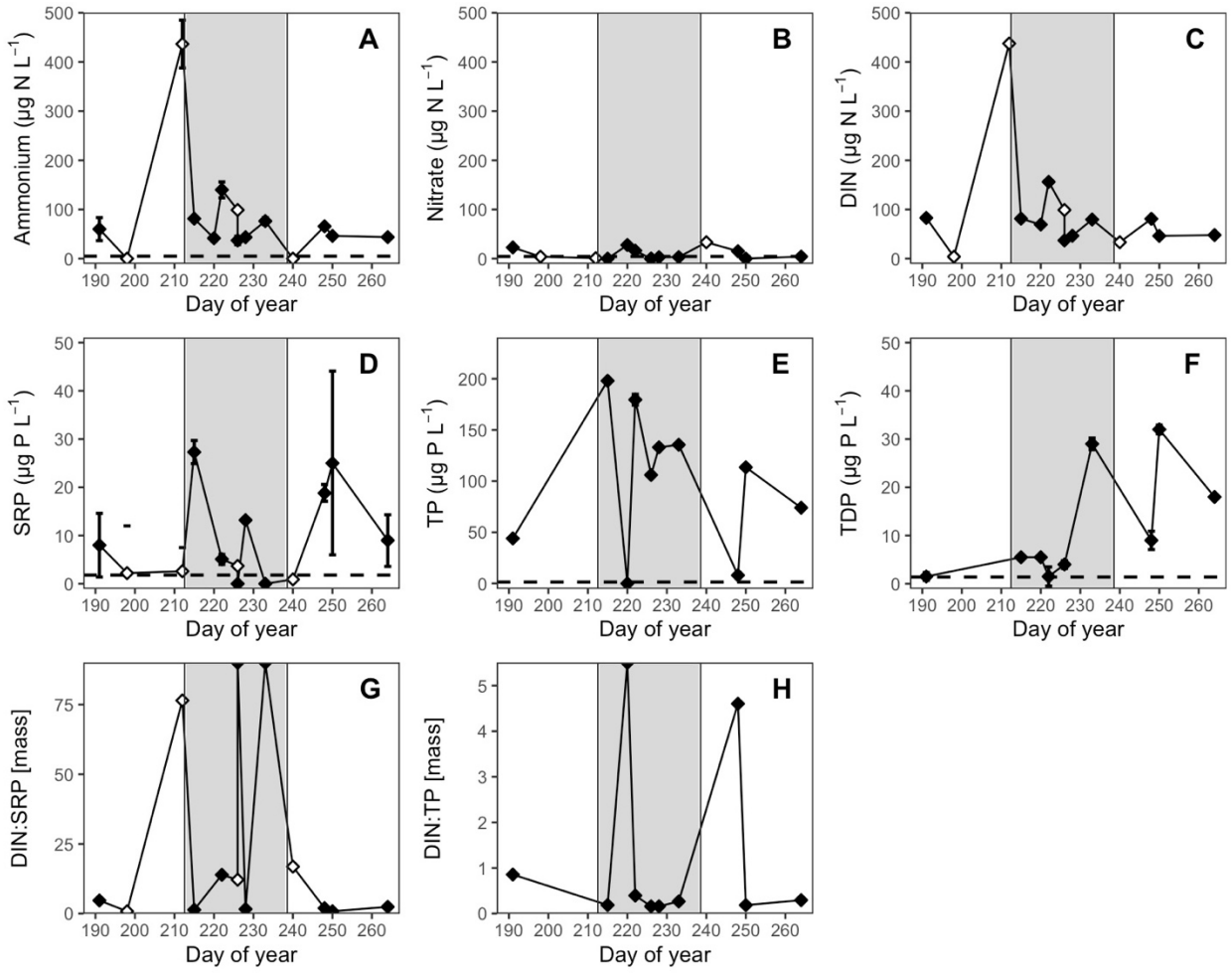

Figure 3.2 Time-series concentrations of nutrients during the pre-bloom (DOY 187 – 212), major bloom (grey area, DOY 213 - 238) and post-bloom (DOY 239 – 264) phases in Buffalo Pound Lake. A) Ammonium, B) nitrate, C) dissolved inorganic nitrogen, D) soluble reactive phosphorus, E) total phosphorus, F) total dissolved phosphorus, and ratios of G) DIN to SRP and H) DIN to TP by mass. Note the horizontal dotted lines are the MDLs for ammonium (5.0 µg N L^{-1}), nitrate (4.4 µg N L^{-1}), SRP (1.8 µg P L^{-1}) and TP/TDP (1.4 µg P L^{-1}). Points are the mean between duplicate runs, and error bars show the difference between duplicates in A, B, D, E, F. Black points indicate sampling was collected from a depth of 0.1 m adjacent to the high-frequency monitoring buoy, and white points were collected from the University of Regina's long-term monitoring location north of the buoy from a depth of 0.3 m. Note: TP and TDP were only measured in 0.1 m samples.

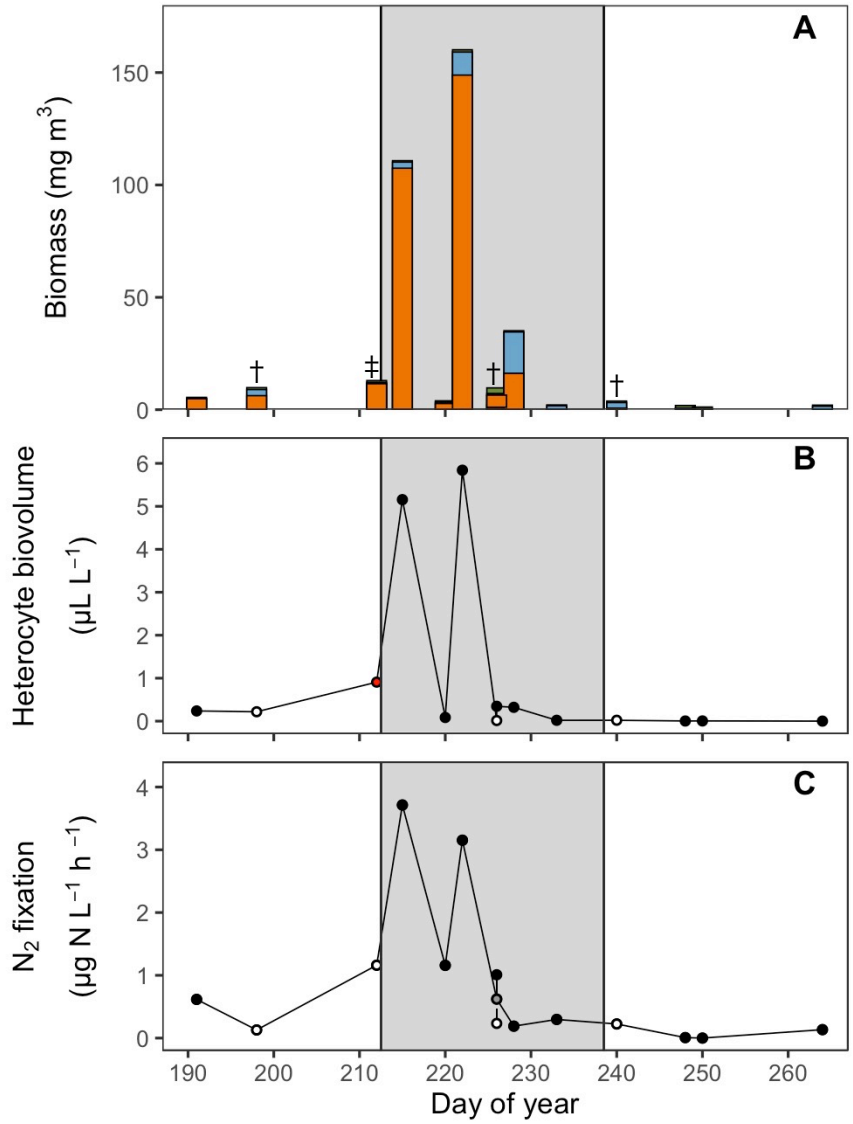

Figure 3.3 Time-series changes in phytoplankton biomass, heterocyte biovolume and nitrogen fixation with bloom development during the pre-bloom (DOY 187 – 212), major bloom (grey area, DOY 213 - 238) and post-bloom (DOY 239 – 264) phases in Buffalo Pound Lake. A) Phytoplankton biomass where diazotrophic cyanobacteria are shown in orange, non-diazotrophic cyanobacteria in blue and all other phytoplankton biomass in green. Samples were collected from a depth of 0.1 m adjacent to high-frequency monitoring buoy, 0.3 m north of the high-frequency monitoring buoy (†) and Buffalo Pound Water Treatment Plant intake near to the buoy (‡). B) Heterocyte biovolume and C) nitrogen fixation rates. Black points indicate sample was collected from 0.1 m depth (adjacent to the high-frequency monitoring buoy), white points from 0.3 m (north of the buoy), and the red points in B indicate phytoplankton counts collected by the Buffalo Pound Water Treatment Plant intake near the buoy (DOY 212). Note that samples were collected from both the 0.1 m and 0.3 m locations on DOY 226. The grey point between both measurements in C is the average rate of nitrogen fixation between the two locations.

– 233 (secondary) identified by exaggerated peaks and valleys in high-frequency measurements of phycocyanin and PAR, respectively, described in 3.3.1. As with the pre-bloom phase, phytoplankton composition of the primary bloom was dominated by *D. flos-aquae* contributing a minimum of 82% of the total cyanobacterial biomass. Within the primary bloom itself, surficial cyanobacterial biomass was highly variable ranging from approximately 2.0 – 160 mg L^{-1}, with greater biomass coinciding with thermal stratification. Cyanobacterial biomass and heterocyte biovolume increased from DOY 212 to 110.2 mg L^{-1} and 5.2 µL L^{-1}, respectively, on DOY 215, reflecting 9-fold and nearly 6-fold increases. Cyanobacterial biomass decreased to 3.4 mg L^{-1} on DOY 220 coinciding with water column mixing and increasing to the seasonal cyanobacterial biomass peak of 159.1 mg L^{-1} on DOY 222 with thermal stratification.

Following the collapse of the primary bloom, *D. flos-aquae* were unable to maintain dominance in limnetic surface waters and a shift to non-heterocytous *P. agardhii* was observed. During the secondary bloom, the percent of biomass from diazotrophs declined from 74.7% on DOY 226 to 17.3% on DOY 233 while the percent biomass from *P. agardhii* simultaneous increased from 24.2% on DOY 226 to 77.6% on DOY 233 (Figure 3.3A; Appendix E, Figure E3.3). Cyanobacterial biomass was lower during the secondary bloom with peak biomass reaching 34.6 mg L^{-1}.

The end of the major bloom phase was signified by a rapid decrease in phycocyanin. Post-bloom, *P. agardhii* was the dominant taxa prior to DOY 250. Biomass was relatively low on DOY 250 and no one taxa held a dominant position (all taxa less than 50% of total cyanobacterial biomass). Contributions from *P. agardhii* were further reduced to 17.1% on DOY 264 when *Woronichinia compacta* played a dominant role (70.1%). Biomass from taxa in surface water did not exceed 26% of the biomass in limnetic surface water.

Notably, the majority of shore water samples were dominated by *P. agardhii*—including those collected prior to the limnetic shift from diazotrophic to non-diazotrophic taxa during the primary bloom. As with the limnetic surface water on DOY 250, there was no dominant taxa in littoral water. However, surface scums were always dominated by *D. flos-aquae* at a minimum 64% of the total cyanobacterial biomass, including scum sampled on DOY 250. Further, cyanobacterial biomass increased as the level of dominance by *D. flos-aquae* increased from a cyanobacterial biomass and percent *D. flos-aquae* of 6.11 mg L^{-1} and 65% (DOY 235), respectively, to 8390 mg L^{-1} and 99.95% (DOY 222).

3.3.3 Bloom evolution & nitrogen fixation

The molar ratio of C_2H_4 produced to N_2 fixed was approximately 2.87:1 (discussed in Section 2.3.3 of this thesis) which falls near theoretical ratios (3:1 or 4:1, Montoya et al. 1996). Rates of nitrogen fixation at the limnetic surface of Buffalo Pound Lake ranged from 0 – 3.7 µg N L^{-1} h^{-1} between DOY 191 and DOY 264 (Figure 3.3C). A moderate level of fixation (0.62 µg N L^{-1} h^{-1}) had occurred at the start of the sampling season (DOY 191) but was reduced to rates approaching zero on DOY 198. Rates were moderate on DOY 212 (1.2 µg N L^{-1} h^{-1}), then on DOY 215, the rate of fixation reached its seasonal peak (3.7 µg N L^{-1} h^{-1}). Conversely, the concentration of ammonium in limnetic surface water reached its peak (436 µg L^{-1}) on DOY 212 and displayed a notable decrease in concentration to 81.4 µg L^{-1} on DOY 215.

Two important peak fixation events were captured during the major bloom phase—one on DOY 215 and the other on DOY 222 (3.7 µg L^{-1} h^{-1} and to 3.1 µg L^{-1} h^{-1}, respectively) occurring during the primary bloom. These peak events corresponded with key biogeochemical parameters, namely: low light penetration and high pH, surficial DO, chlorophyll *a* (Figure 3.1), TP concentrations (Figure 3.2), total and cyanobacterial biomass and heterocyte biovolume (Figure 3.3). On DOY 220, nitrogen fixation decreased to 1.2 µg N L^{-1} h^{-1}), separating the two peak fixation events on DOY 215 and 222. This relatively low level of nitrogen fixation corresponded with increased light penetration, decreased chlorophyll *a*, and TP concentrations below detection. Measured rates of nitrogen fixation did not exceed 1.0 µg N L^{-1} h^{-1} following DOY 222 reflecting a shift in taxa from diazotrophic to non-diazotrophic taxa.

3.3.4 Predictors of nitrogen fixation rates

A significant positive correlation (Kendall's tau 0.49, p= 0.01) between heterocyte biovolume and nitrogen fixation was identified (Figure 3.4I); however, heterocyte biovolume in surface water was mainly limited to extreme values (biovolumes either less than 0.32 µL L^{-1} or greater than 5.2 µL L^{-1} in 13 of 14 samples). There were not significant correlations between nitrogen fixation to nutrient concentrations, DIN:SRP or DIN:TP ratios. However, we did observe instances where nitrogen fixation rates were relatively high and ammonium concentrations were relatively low, and low rates of nitrogen fixation coinciding with higher ammonium concentrations. Similarly, we observed some higher rates of nitrogen fixation

associated with lower DIN:SRP and DIN:TP ratios and, conversely, some lower nitrogen fixation rates associated with higher DIN:SRP and DIN:TP ratios (Figure 3.4A – G).

3.3.5 Diel cycling of biogeochemistry & nitrogen fixation

High-frequency monitoring showed physical, chemical and biological fluctuations on a diel time-scale. Results reveal rapid and irregular changes in thermal stratification, primary production and associated physiochemical factors leading up to and following a key nitrogen fixation event of 3.1 μg N L^{-1} h^{-1} (DOY 222). On DOY 221, temperature differences between shallow surface water (0.45 m) and deep water (3.18 m) were 0.2°C at their maximum, and became homogenous overnight as temperatures declined to ~20.5°C. On DOY 222, shallow water increased to a maximum of 23.6°C, while deep water maintained overnight temperatures throughout the day, leading to a 3.2 °C temperature gradient. Again, as shallow water temperatures declined overnight, water column temperature became uniform as mixing occurred. Temperatures between depths differed by ~1°C on the date following the measured peak fixation event (DOY 223).

Across this 3-day period, phycocyanin and chlorophyll (RFUs) did not exhibit the observed daily increases and decreases seen in PAR and temperature measurements in shallow water (Figure 3.5). Rather, these indicators, in general, increased from DOY 221 through DOY 222, reached a plateau (phycocyanin up to 35 RFU and chlorophyll up to 6.5 RFU) and then began a decline on the evening of DOY 222 following the major fixation event. Meanwhile, pH, DO and CO_2 had similar cycles to PAR and temperature, (Figure 3.5). Fluctuations of pH and DO concentrations were synchronous with a 0.4 range in pH units and nearly 7 mg L^{-1} range in DO. Notably, measurements of pH and DO were highest on DOY 222. As expected, the concentration of carbon dioxide decreased throughout the day and increased over night following diel patterns of photosynthesis and respiration, although the magnitude of these changes varied by day. Concentrations of CO_2 were lowest on DOY 222.

The comparison between water samples incubated under the artificial light source (simulating in-situ conditions) and dark-incubated samples revealed a significant difference in nitrogen fixation rates under the differing light conditions (Wilcoxon signed rank test, $p<0.05$, Figure 3.6). The median rate of nitrogen fixation for 'dark' incubations was 0.12 μg N L^{-1} h^{-1}, while the median rate of fixation for 'light' incubations was 0.23 μg N L^{-1} h^{-1}. Although nitrogen fixation under dark conditions was significantly lower, some samples exhibited high rates of

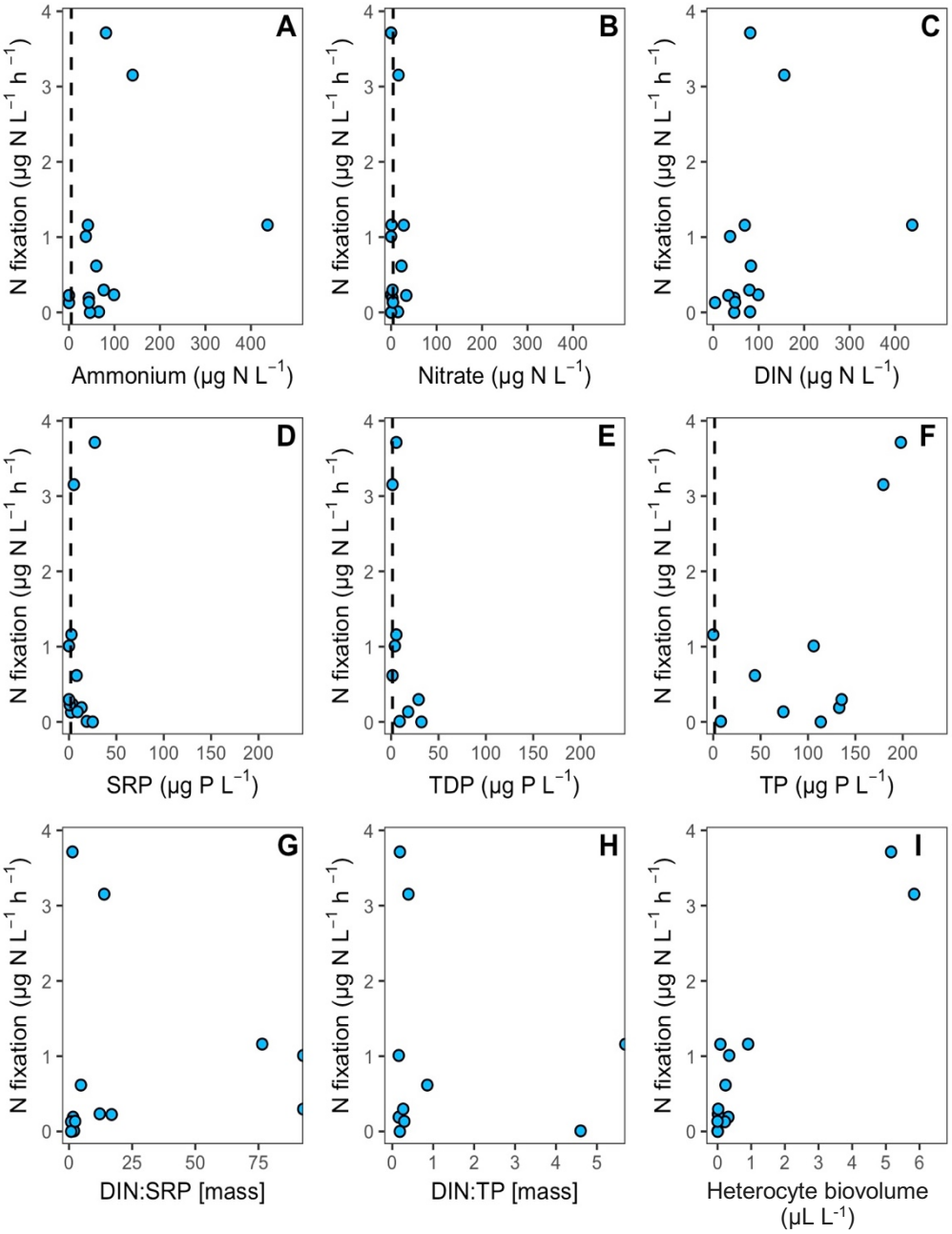

Figure 3.4 Association between rates of nitrogen fixation and A) ammonium, B) nitrate, C) dissolved inorganic nitrogen (ammonium + nitrate), D) soluble reactive phosphorus (SRP), E) total dissolved phosphorus (TDP), F) total phosphorus (TP), ratios of G) DIN to SRP and H) DIN to TP by mass, and I) heterocyte biovolume (µL L^{-1}) in discrete samples from the 2017 sampling season at Buffalo Pound Lake at limnetic depths of 0.1 or 0.3 m. Vertical dotted lines are method detection limits for each analyte—ammonium (5.0 µg N L^{-1}), nitrate (4.4 N µg N L^{-1}), SRP (1.8 P µg L^{-1}), and TP/TDP (1.4 µg P L^{-1}). Points in G, H are ratios of N-DIN to P-SRP and P-TP by mass.

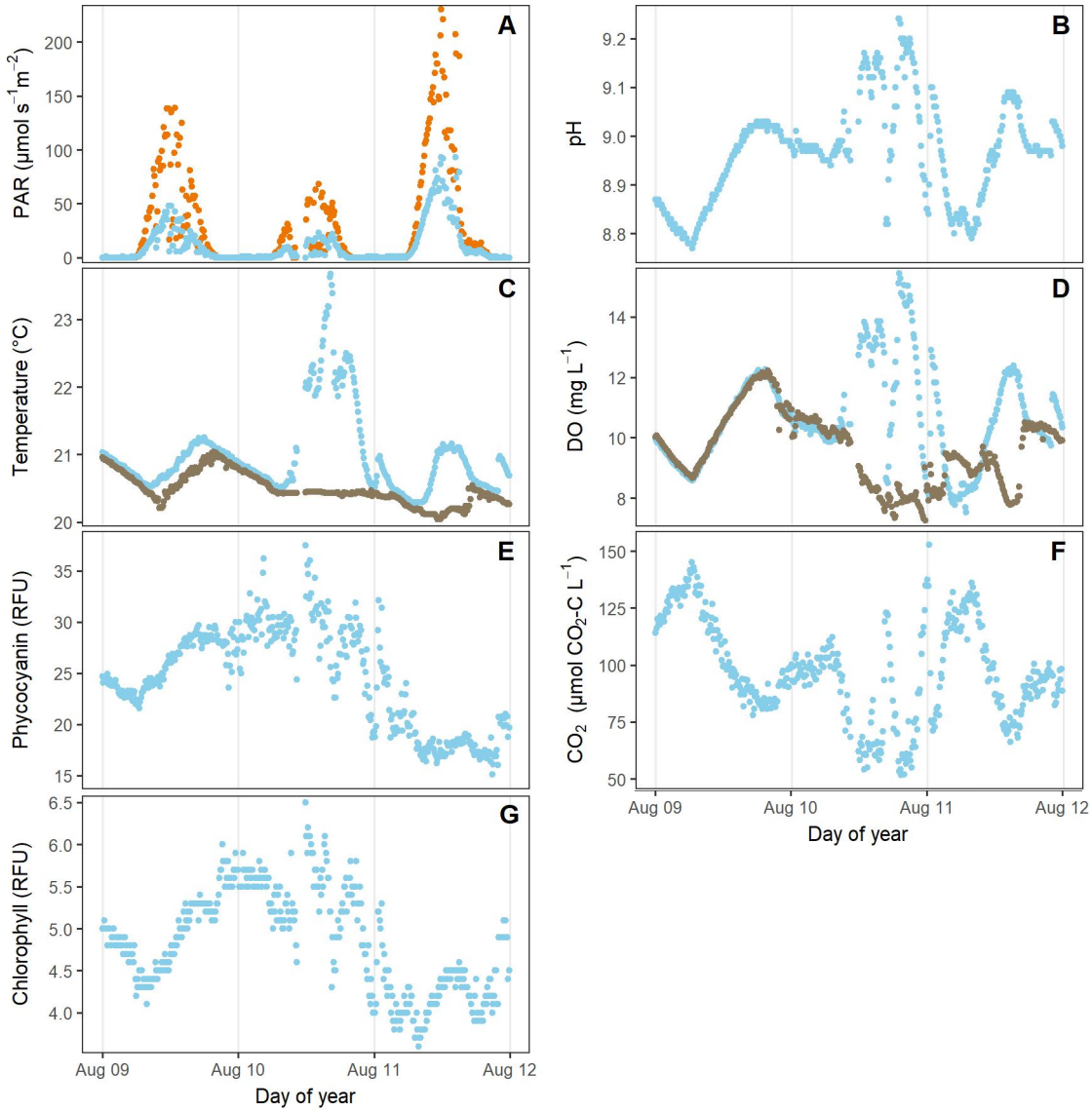

Figure 3.5 Time series high-frequency in-situ measurements for August 9 – 11 (DOY 221 – 223), 2017 during the major bloom phase. Gray vertical lines indicate 12:00 a.m. A) PAR at 0.62 m (orange) and 0.78 m (blue) below surface. B) Temperature at 0.45 m (blue) and 3.18 m (brown) below surface. C) Phycocyanin (relative fluorescence units) at 0.82 m. D) Chlorophyll (relative fluorescence units) at 0.82 m. E) pH at 0.82 m. F) Carbon dioxide concentration at 0.82 m. G) Dissolved oxygen concentration at 0.82 m (blue) and 2.85 m (brown).

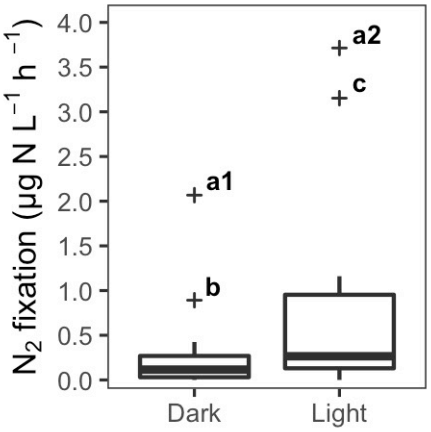

Figure 3.6 Comparison between rates of fixation observed in samples exposed to the artificial light source during incubation (*right*, n=17) and samples protected from exposure (*left*, n=17). The horizontal line through each box indicates the sample median. The rectangular areas below and above the median indicate ranges from the median to the first quartile and third quartile, respectively. Whiskers extend from each box to indicate the inter-quartile range x 1.5. Outliers (+) identified by i) the same letter in 'light' and 'dark' are rates of nitrogen fixation observed in the same surface water sample under differing light conditions and ii) different letters are from different surface water samples.

fixation. The highest rate of nitrogen fixation in the limnetic zone (measured ex-situ under artificial light source) was observed on DOY 215 (3.7 µg N L^{-1} h^{-1}). The rate of fixation in the paired dark-incubated limnetic sample on this day was also high (2.1 µg N L^{-1} h^{-1}). Conversely, nitrogen fixation under the ex-situ light source was second highest in the limnetic zone on DOY 222 (3.1 µg N L^{-1} h^{-1}); however, dark-incubated samples were limited to fixing 0.23 µg N L^{-1} h^{-1} (within the third quartile range of dark-incubated samples). The final dark-incubated sample outlier was measured on DOY 235 during the first day of in-situ diel experiments. Evening fixation (incubation period from 19:30 to 23:00) within the littoral region under natural lighting was 1.0 µg N L^{-1} h^{-1} in exposed water samples and 0.89 µg N L^{-1} h^{-1} in dark-incubated samples (Figure 3.6, Table 3.2). Rates of fixation here were relatively similar, which was expected, as the water column receives low to negligible light penetration at this time.

Diel experiments of littoral water performed in-situ were conducted at the end of the major bloom phase between DOY 235 – 240. On DOY 235, nitrogen fixation was unexpectedly higher in the evening than the hours of peak light penetration (Table 3.2, 11:30 & 19:30). In addition to higher nitrogen fixation rates (by 0.55 µg N L^{-1} h^{-1}), water temperature was 2.3˚C warmer (23˚C), chlorophyll *a*, heterocyte biovolume and cyanobacterial biomass were 1.35, 13

and 11 times greater, respectively, in 0.3 m samples collected at 19:30 than samples collected at 11:30. Temperature differences between deep and shallow high-frequency sensors were greater at 19:30 than 11:30 (1.6˚C compared to 0.8˚C, respectively; shown on Table E3.5C found in Appendix E). Meanwhile, accumulated surface scum within the littoral zone at 11:30 on DOY 235 fixed more nitrogen than either 0.3 m water sample—nitrogen fixation in scum was 4.8 µg N L^{-1} h^{-1} (Table 3.4) compared to nitrogen fixation in water samples at 11:30 and 19:30 at rates of 0.45 and 1.0 µg N L^{-1} h^{-1}, respectively (Table 3.2). On DOY 236, heterocyte biovolume was reduced by a factor of 6 and concentrations of cyanobacterial biomass were reduced from 6.95 mg L^{-1} on the evening of DOY 235 to 1.68 mg L^{-1} on the morning of DOY 236 as the lake shifted from a stratified to a mixed water column (Table 3.2; Appendix E, Figure E3.5). Though heterocyte biovolume, cyanobacterial biomass and chlorophyll *a* had decreased, rates of fixation were relatively moderate at 05:15 under both light and dark conditions (0.58 µg N L^{-1} h^{-1} and 0.36 µg N L^{-1} h^{-1}, respectively). Concentrations of measured inorganic nutrients (SRP, nitrate and ammonium) were consistently below detection (1.8 µg P L^{-1}, and 4.4 and 5.0 µg N L^{-1}, respectively) during our diel sampling campaign, with the exception of 05:15 on DOY 236 where ammonium was 130 µg L^{-1}. By noon on DOY 236, nitrogen fixation had increased somewhat to 0.69 µg N L^{-1} h^{-1} in light conditions but decreased to 0.15 µg N L^{-1} h^{-1} in dark conditions. Heterocyte biovolume and chlorophyll *a* concentrations were lowest during diel work completed on DOY 240, and rates of fixation were either very low or undetectable in samples collected at 6:00, 13:15 and 20:00.

3.3.6 Nitrogen fixation & ammonium uptake

On the five occasions when ammonium uptake was measured (surface water n=4, surface scum n=1, Table 3.3), rates of uptake ranged from 0.68 – 6.5 µg N L^{-1} h^{-1}. Nitrogen fixation in parallel $^{15}N_2$-calibrated ARAs found that nitrogen fixed ranged from near zero to 1.2 µg N L^{-1} h^{-1} in surface water samples, rates which matched up to one-third of the nitrogen obtained from ammonium. Surface scum samples notably darkened during the incubation period of the ARAs, suggesting bloom collapse within sample bottles, a potential artefact of incubation conditions. Nitrogen fixation ceased at 8.4 µg N L^{-1} h^{-1} using this method, though simultaneous $^{15}N_2$-tracer assays showed that nitrogen fixation reached 71.0 µg N L^{-1} h^{-1} in surface scum. The parallel ammonium uptake measurement of surface scum (0.71 µg N L^{-1} h^{-1}) indicates that nitrogen inputs were up to 100 times greater from fixation compared to ammonium uptake.

Table 3.2 Nitrogen fixation measured in-situ on DOY 235, 236 and 240 from 0.3 m littoral zone during the 2017 sampling season of Buffalo Pound Lake. Time and temperature indicate the time each sample was collected and the water temperature of the sample. Nitrogen fixation rates presented with dissolved nutrients and biological activity. Dissolved nutrients displayed as the mean concentration unless measured concentration is below instrument detection (ammonium 5.0 µg N L^{-1}, nitrate 4.4 µg N L^{-1}) and SRP 1.8 mg P L^{-1}). Note heterocyte biovolume is displayed in millilitres per litre to reflect low values.

Sampling date, times and in-situ water temperature	N fixation µg N L^{-1} h^{-1}		Ammonium µg N L^{-1}	Nitrate µg N L^{-1}	SRP µg P L^{-1}	Chl *a* µg L^{-1}	Heterocyte biovolume mL L^{-1}	Cyanobacteria biomass mg L^{-1}
	light	dark						
23-Aug-2017 DOY 235								
11:30 @ 20.7˚C	0.45	0.42	36	20.2	3.3	110	16.8	0.64
19:30 @ 23.0˚C	1.0	0.89	44	20.7	1.6 (<1.8)	149	217.1	6.95
24-Aug-2017 DOY 236								
05:15 @ 17.9˚C	0.58	0.36	129.5	29.3	3.2	89.2	28.73	1.68
12:00 @ 19.4˚C	0.69	0.15	49	37.3	3.2	76.2	18.55	1.80
28-Aug-2017 DOY 240								
6:00 @ 19.1˚C	0.03	0.07	59.5	9.8	3.1	35.9	13.9	3.37
13:15 @ 22.2˚	0.00	0.00	-	11.6	4	31.8	12.4	2.22
20:00 @ 19.8˚C	0.00	-	0 (<5.0)	7.6	3.6	41.6	3.5	2.34

Table 3.3 Comparison of nitrogen fixation rates to ammonium uptake completed with limnetic surface water during the major bloom phase (DOY 220 and 226) and post-bloom phase (DOY 240 and 248) and littoral surface scum during the post-bloom phase (DOY 250) during the 2017 sampling season at Buffalo Pound Lake. Ammonium and nitrate are displayed as mean concentrations with method detection if measured value below detection (ammonium 5.0 µg N L^{-1} and nitrate 4.4 µg N L^{-1}). Ammonium uptake in samples with ambient concentrations below detection was calculated using method detection limit. Nitrogen fixation determined using $^{15}N_2$-calibrated acetylene reduction assays unless otherwise noted.

Sampling date and location	N fixation µg N L^{-1} h^{-1}	NH_4^+ uptake µg N L^{-1} h^{-1}	Ammonium µg N L^{-1}	Nitrate µg N L^{-1}	DIN µg N L^{-1}
08-Aug-2017 DOY 220 Limnetic, surface	1.2	5.7	41.6	27.8	69.4
14-Aug-2017 DOY 226 Limnetic, surface	1.0	3.7	37.1	0.0 (<4.4)	37.1
28-Aug-2017 DOY 240 Limnetic, 0.3 m	0.22	0.68	0.0 (<5.0)	33.4	33.4
05-Sept-2017 DOY 248 Limnetic, surface	0.01	6.5	65.8	15.2	81.0
07-Sept-2017 DOY 250 Littoral, surface[1]	71.0[2]	0.71	0.0 (<5.0)	13.2	13.2

[1] Surface scum accumulation present.
[2] Rate measured using $^{15}N_2$-tracer assay presented as bloom collapsed during acetylene reduction assays. Nitrogen fixation ceased at a measured 8.4 µg N L^{-1} h^{-1}.

3.3.7 Spatial variability in nitrogen fixation

Rates of nitrogen fixation in limnetic zones were seldom similar to rates measured in littoral zones when concurrently measured (Table 3.4). Although chlorophyll *a* concentrations were always greater in littoral zones compared to limnetic zones, rates of fixation were, on occasion, greater in limnetic zones (i.e. DOY 226 and DOY 240). On DOY 240 (evening sampling event), no fixation was observed in the littoral zone, but limnetic fixation occurred at a rate of 0.47 µg N L^{-1} h^{-1}. At this time limnetic chlorophyll *a* was 83% that of the littoral zone, yet, heterocyte biovolume was an order of magnitude greater in the limnetic zone than littoral samples approximately 500 m away.

Surface scums formed during each phase of the bloom—pre-bloom (n=7), major bloom (n=12) and post-bloom (n=4, Figure 3.7). Phytoplankton enumeration indicates that surface scums were dominated by the diazotrophic *D. flos-aquae*, while surface water within the same region was typically dominated by *P. agardhii.* The highest rates of nitrogen fixation were

observed in surface scums. and nitrogen inputs were at least 1.7 µg N L^{-1} h^{-1} in surface scum communities.

Although limnetic subsurface measurements of the cyanobacterial bloom identified occurrences of high nitrogen fixation as previously discussed, nitrogen inputs were consistently greater in surface scum compared to parallel subsurface littoral and subsurface limnetic measurements. For example, surface scum collected on DOY 222 was found to fix nitrogen at a rate of 9.5 µg N L^{-1} h^{-1}. Compared to the concurrent rate of fixation occurring in the limnetic region, 3.2 µg N L^{-1} h^{-1}, surface scum fixed 3 times the amount of nitrogen as non-scum. Scum formations in littoral zones on DOY 235, 240 and 250 exhibited rates of 4.7 µg L^{-1} h^{-1}, 1.7 µg L^{-1} h^{-1} and 71.0 µg L^{-1} h^{-1}, respectively, where rates were either low in comparison (2% that of scum) or undetectable in littoral non-scum samples collected on the same date.

3.3.8 Interannual variability in the phytoplankton community during the bloom phase

To understand how typical the taxonomic composition was during our intensive sampling year, as compared to past years, we assessed the difference in dominant taxa during peak biomass using archived samples. Phytoplankton composition varied in taxa dominance between 1996 and 2016. During the 2017 intensive sampling year, the proportion of total biomass composed of cyanobacterial taxa did not fall below 75% in samples collected between DOY 207 – 233 (long-term phytoplankton data collect during this range, Figure 3.8). The long-term record presented here shows that cyanobacterial biomass fell below 75% during 10 of the 18 study years in single samples collected during the same period of time; further, cyanobacteria biomass was below 50% during 6 of those years. Although it is possible that some single samples presented here are seasonal anomalies, and therefore not representative of the cyanobacterial bloom period, when taken at face value, these data indicate that while cyanobacteria dominated all other taxa during the 2017 sampling year, 2017 may have had higher cyanobacterial dominance than typical years. Diazotrophs were not consistently outcompeting non-diazotrophic taxa across all years. In 2017, the proportion of cyanobacteria comprised of diazotrophs ranged from 37 – 99.5% between DOY 207 – 233. Similar community compositions were present in the long-term record with diazotrophs ranging from 7.6 – 100% (Figure 3.9).

Table 3.4 Spatial variation between limnetic and littoral zones and between samples with and without scums present in samples collected on DOY 222, 226, 233, 235, 240 and 250 during the 2017 sampling season at Buffalo Pound Lake. Nitrogen fixation rates presented with dissolved nutrients and biological activity. Dissolved nutrients displayed as the mean concentration unless measured concentration is below instrument detection (ammonium 5.0 µg N L^{-1}, nitrate 4.4 µg N L^{-1} and SRP 1.8 mg P L^{-1}). Nitrogen fixation determined using $^{15}N_2$-calibrated acetylene reduction assays unless otherwise noted.

Sampling date and location	N_2 fixation µg N L^{-1} h^{-1}		Ammonium µg N L^{-1}	Nitrate µg N L^{-1}	SRP µg P L^{-1}	Chl *a* µg L^{-1}	Heterocyte biovolume µL L^{-1}	Cyanobacteria biomass mg L^{-1}
	light	dark						
10-Aug-2017 DOY 222								
Littoral, surface[1]	9.5[2]	-	12	-	1.8	29,745.8	523.0	8390
Limnetic, surface	3.2	0.23	140	16.2	5.1	561.8	5.84	159.1
14-Aug-2017 DOY 226								
Littoral, surface	0.38	-	93	13	0 (<1.8)	99.8	0.0022	0.74
Limnetic, surface	0.97	-	37.1	0 (<4.4)	0 (<1.8)	74.0	0.346	7.21
21-Aug-2017 DOY 233								
Littoral, surface	0.56	-	56.5	25.8	0 (<1.8)	122.1	0.055	3.83
Limnetic, surface	0.30	-	76.4	3.2 (<4.4)	0 (<1.8)	116.3	0.020	1.76
23-Aug-2017 DOY 235								
Littoral, surface[1]	4.8	-	130.5	24	2.4	152.9	0.195	6.11
Littoral, 0.3 m	0.45	0.42	36	20.2	3.3	110.0	0.017	0.64
28-Aug-2017 DOY 240								
Littoral, surface[1]	1.7	-	115	17.6	2.8	100.32	0.206	7.06
Littoral, surface	0.0	-	50	12.2	1.4 (<1.8)	28.3	0.013	2.4
Limnetic, 0.3 m	0.22	0.0	0 (<5.0)	33.3	0.9 (<1.8)	37.2	0.02	3.17
28-Aug-2017 DOY 240[3]								
Littoral zone, surface	0.0	-	0 (<5.0)	7.6	3.6	41.6	0.004	2.34
Limnetic zone, surface	0.47	-	0 (<5.0)	27.4	2.3	34.5	0.03	2.83
07-Sept-2017 DOY 250								
Littoral surface[1]	71.0[4]	-	0 (<5.0)	13.2	-	1720	2.87	86.0
Littoral surface	1.4	-	263.5	5	-	129.5	0.03	1.71
Limnetic surface	0.0	0.13	46.3	0 (<4.4)	25	17.0	0.005	0.50

[1] Surface scum accumulation present.
[2] Rate measured using $^{15}N_2$-tracer assay presented as bloom collapsed during acetylene reduction assays. Nitrogen fixation ceased at a measured 0.18 µg N L^{-1} h^{-1}.
[3] Irregular early evening sampling time
[4] Rate measured using $^{15}N_2$-tracer assay presented as bloom collapsed during acetylene reduction assays. Nitrogen fixation ceased at a measured 8.4 µg N L^{-1} h^{-1}.

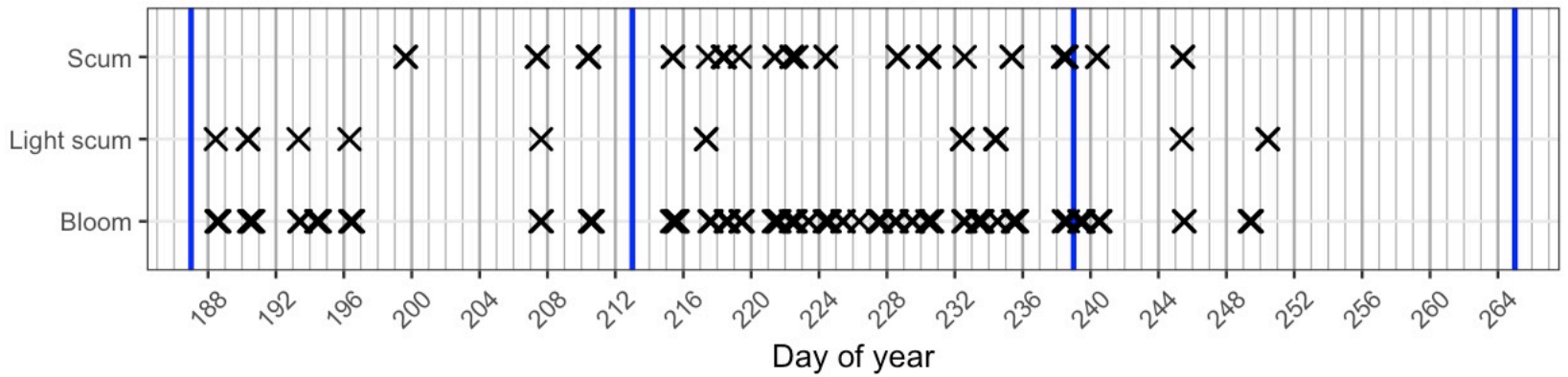

Figure 3.7 Detailed schematic of scum behavior tabulated from on-shore camera. Photos were captured hourly from 08:00 to 16:00, inclusive. Each black X indicates the appearance of a surface scum, a light scum or a clearly identifiable bloom—a maximum of one observation per photo. Overlapping Xs indicate that the same observation was made multiple times on that day. Vertical blue lines separate pre-bloom, major bloom and post-bloom phases (from left to right), and vertical gray lines are used to differentiate individual days.

A total of 14 cyanobacterial genera were detected in Buffalo Pound between 1996 and 2016 (Figure 3.9). Several species were detected in the long-term record that were not present in 2017, including both diazotrophic and non-diazotrophic species. Additional species were identified from the heterocytous *Aphanizomenon* (*Aph.*) and *Dolichospermum* (*D.*) genera, and *Microcystis* genera. *D. flos-aquae* was the only species to be detected across each of the sampling years. During 2017, *D. flos-aquae* showed the greatest biomass of any diazotroph. The long-term record showed this was true for most years—with the exception of *D. crassa* in 1996 and 2003 and both *D. crassa* and *Aph. flos-aquae* greater than *D. flos-aquae* in 2005. While *D. crassa* showed greater biomass than *D. flos-aquae* during the aforementioned years, heterocyte biovolume was greater in *D. flos-aquae* in 1996 (104 and 135 µL m^{-3}, respectively; Figure 3.10). As with biomass, heterocyte biovolume was greatest in *D. flos-aquae* across all years—with the exception of 2003 and 2005 where heterocytes from *D. crassa* were present at a biovolume 2-fold that of *D. flos-aquae*, although the overall heterocyte biovolume was relatively low here (0.11 µL L^{-1}). In terms of common non-diazotrophic species, *P. agardhii* was first observed in 1998, increased each year to a maximum of 7.4 mg L^{-1} in 2003, before becoming undetectable in the 2005 sample. Notably, the long-term record did now show *Microcystis* sp. as the dominant taxa in any of the sampling years, nor did it play a dominant role during the higher-frequency 2017 sampling year.

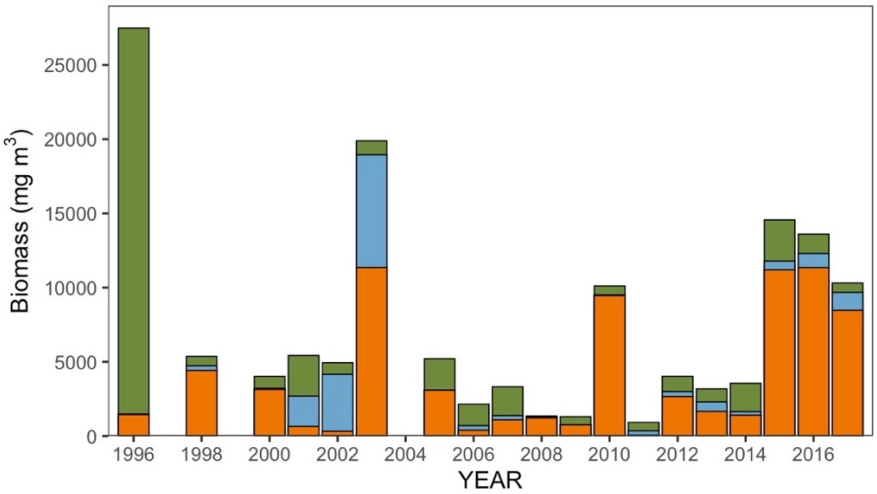

Figure 3.8 Variability of phytoplankton composition from discrete sampling of Buffalo Pound Lake from 1996 – 2017. Bar colour indicates differing taxa: diazotrophic cyanobacteria (orange), non-diazotrophic cyanobacteria (blue), and all other taxa (green). Data for 1997, 1999 and 2004 not reported.

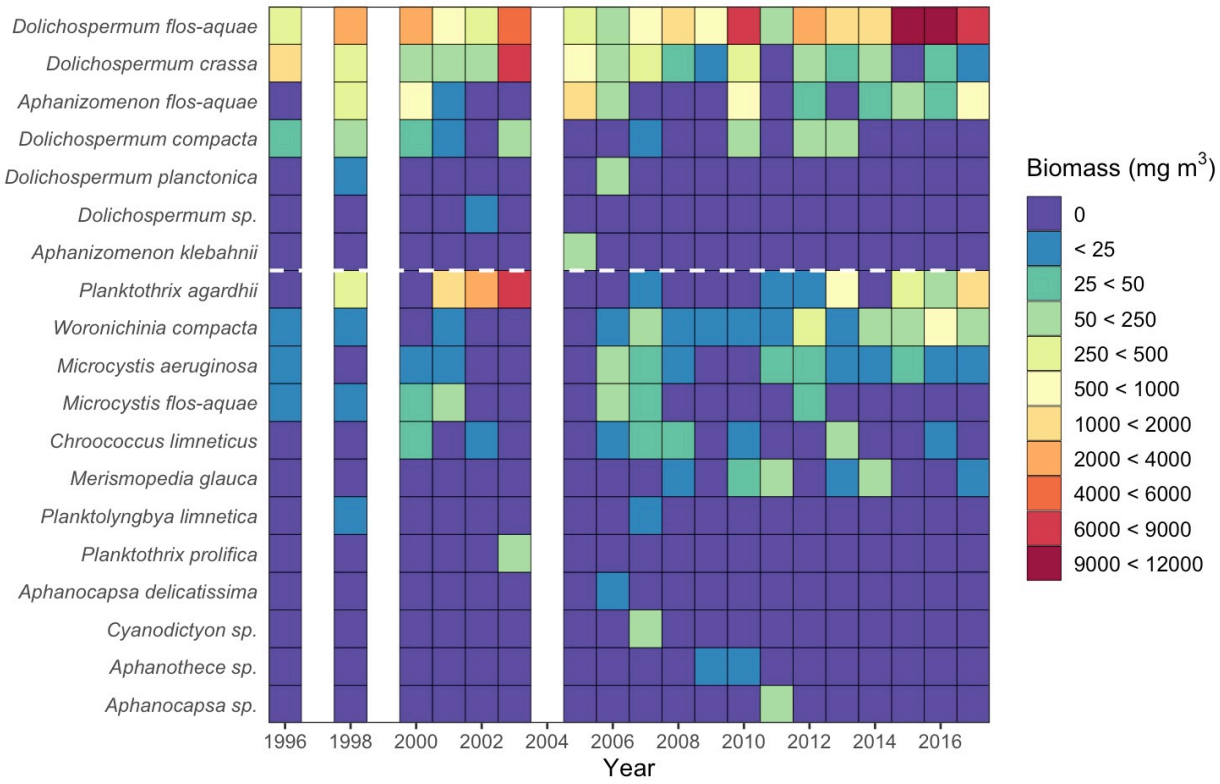

Figure 3.9 Long-term variation in cyanobacterial taxa composition and biomass from discrete sampling of Buffalo Pound Lake from 1996 – 2017. Diazotrophic cyanobacteria above white dashed line, non-diazotrophic cyanobacteria below—taxa are ordered from most to least abundant (top down) through the time series. Species biomass reflects a single point representing a given sampling year. Biomass has been categorized into unequal ranges identified by tile colour. Purple tiles indicate the species was not detected during the phytoplankton assessment. Data for 1997, 1999 and 2004 not reported.

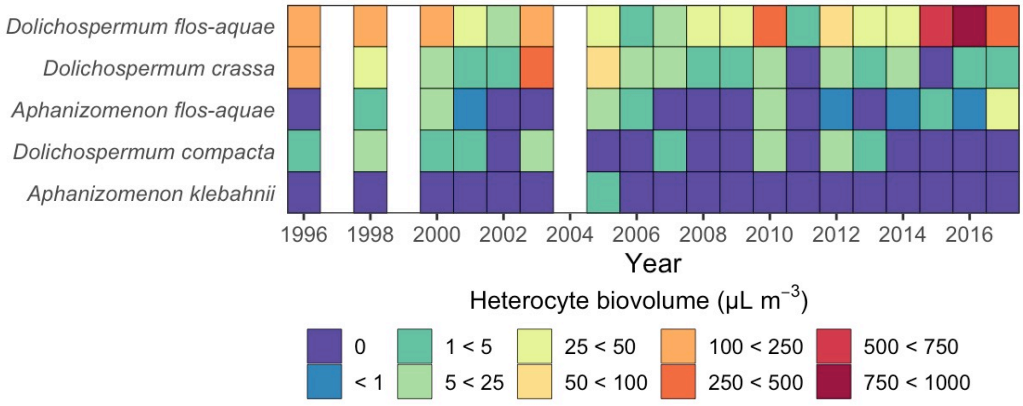

Figure 3.10 Long-term variation in heterocyte taxa composition and biovolume by species from discrete sampling of Buffalo Pound Lake from 1996 – 2017. Heterocyte biovolume reflects a single point representing a given sampling year which has been categorized into unequal ranges identified by tile colour. Purple tile indicates that heterocytes produced by the given species were not detected during the phytoplankton assessment. Data for 1997, 1999 and 2004 not reported.

3.4 Discussion

Using high-frequency sensors and frequent chemical, physical and process-based measurements, we followed the trajectory of the bloom, considering the specific adaptations of cyanobacterial taxa which may have contributed to biomass increases or to bloom collapse. Key attributes of the ecosystem are important to consider. First, although Buffalo Pound Lake is eutrophic, bioavailable nutrients were often low during the bloom phase, presumably due to high rates of uptake by primary producers. Second, this is a shallow, polymictic hardwater lake, and as such, thermal, chemical, and biotic changes were rapid. For example, pH and dissolved oxygen showed variations of up to 0.78 pH units and 10.0 mg L^{-1} in a single day, respectively, when the lake underwent transient stratification. Further, high variation in biomass was observed both in space, and in time. A description of the physicochemical changes from the pre-bloom, major bloom to post-bloom phases have been synthesized in Table 3.5.

3.4.1 Physical and chemical changes associated with bloom development

Increased phytoplankton biomass and the development of the cyanobacterial bloom in Buffalo Pound Lake coincided with high incident light and maximum water temperatures. This is characteristic of temperate water bodies (Paerl and Huisman 2008), and compounds concerns about how increased water temperatures and duration of the open-water season may impact bloom risk in this crucial regional drinking water supply. Growth rates were amplified with increasing water temperatures consistent with previously calculated mean rates ranging from 0.42 d^{-1} at 20˚C to 0.65 d^{-1} at 29.2˚C (Lürling et al. 2013). Increased biomass leading up to and through the major bloom phase restricted light penetration, reducing light availability due to self-shading, potentially favoring taxa with high irradiance requirements that can migrate toward the surface (Andersson et al. 2019), or possibly favouring taxa subsurface that can acclimate to lower light conditions (Bradburn et al. 2012).

Cyanobacteria have a number of adaptions that allow them to gain and maintain dominance within phytoplankton communities, particularly in nutrient-rich systems like Buffalo Pound. Adaptions for superior nutrient uptake mechanisms and buoyancy regulation were evident here. Specifically, rapid growth observed through the pre-bloom phase and much of the major bloom phase requires high rates of photosynthesis, and high rates of photosynthesis lead to CO_2 depletion. Indeed, through the bloom, pH became very high, and CO_2 may become limiting,

Table 3.5 Narrative description of physicochemical changes during the pre-bloom, major bloom, and post-bloom phases in Buffalo Pound Lake. Data synthesized here are presented in Figures 3.1 – 3.3 and 3.7, Table 3.4 and Appendix E, Figure E3.3.

Descriptor	Pre-bloom phase	Major bloom phase	Post-bloom phase
Date (DOY)	July 6 – 31 (187 – 212)	August 1 – 26 (213 – 238)	August 27 – September 21(239 – 264)
Maximum daily incident PAR ($\mu mol\ s^{-1}\ m^{-2}$)	Median, range 1413, 1271 – 1679	Median, range 1318, 752 – 1720	Median, range 1155, 416 – 1576
Maximum daily irradiance 0.62 m 0.78 m	Median, range 656, 337 - 991 383, 152 – 734	Median, range 175, 48 – 360 74, 17 – 156	Median, range 414, 107 – 564 224, 55 - 296
Water temperature (0.45 m)	DOY 190/191: max. 24 hr mean 23.9˚C DOY 190: seasonal maxima 24.9˚C	DOY 213: max. 24 hr mean 22.9˚C	DOY 240: max. 24 hr mean 20.7˚C temperature consistently declining
Thermal stratification	DOY 188, 190, 207, 210, 211	DOY 215, 218, 222, 230	DOY 239, 240
Phycocyanin (0.82 m)	DOY 190: transient peak 21.2 RFU at maximum	DOY 219: seasonal maxima 45 RFU Primary bloom: DOY 215 – 222 max. 24 hr mean 28.8 RFU Secondary bloom: DOY 226 – 233 max. 24 hr mean 22.7 RFU	DOY 239: max. 24 hr mean 10.4 RFU sharp decline
Chlorophyll (0.82 m)	DOY 193 – 198: chlorophyll peak lags initial phycocyanin peak	chlorophyll during bloom phase precedes increase in phycocyanin	gradual decline

Table 3.5, cont'd Narrative description of physicochemical changes during the pre-bloom, major bloom, and post-bloom phases in Buffalo Pound Lake. Data synthesized here are presented in Figures 3.1 – 3.3 and 3.7, Table 3.4 and Appendix E, Figure E3.3.

Descriptor	Pre-bloom phase	Major bloom phase	Post-bloom phase
Date (DOY)	July 6 – 31 (187 – 212)	August 1 – 26 (213 – 238)	August 27 – September 21(239 – 264)
Oxygen supersaturation (top sensor 0.82)	DOY 188 – 191 & DOY 207 – 211: transient high oxygen events above 15 mg L^{-1}	Primary bloom: frequent high oxygen events above 15 mg L^{-1} Secondary bloom: high oxygen events above 15 mg L^{-1} on DOY 228 – 230	Prior to DOY 251: transient high oxygen events above 11 mg L^{-1} DOY 251 – 264: high oxygen events absent. Oxygen below 10.5 mg L^{-1}
Oxygen undersaturation (bottom sensor, 2.85 m)	DOY 191/193 & DOY 208/211: transient low oxygen events below 5 mg L^{-1}	Primary bloom: low oxygen events below 5.0 mg L^{-1} on DOY 216/218 Secondary bloom: low oxygen events below 5.0 mg L^{-1} on DOY 235/239	Prior to DOY 251: frequent low oxygen events below 5 mg L^{-1} DOY 251 and on: low oxygen absent. Oxygen remains above 7 mg L^{-1})
pH (0.82 m)	transient peak pH 8.7, DOY 190	seasonal maxima pH 9.4, DOY 230	gradual decline
Inorganic nutrients (discrete)	Seasonal ammonium peak on DOY 212 with relatively high DIN:SRP ratio. Nitrate consistently lower than ammonium and both SRP and TDP consistently lower than TP.	Ammonium fluctuates with lesser peak of on DOY 222 followed by decline. Nitrate consistently lower than ammonium. TP fluctuates with peak during highest biomass, TP below detection on DOY 220 only. Low level TDP peak on DOY 233. Both SRP and TDP consistently low compared to TP.	Ammonium relatively low, nitrate typically lower than ammonium (exception: DOY 240). General decline in TP with some elevations in SRP and TDP.

Table 3.5, cont'd Narrative description of physicochemical changes during the pre-bloom, major bloom, and post-bloom phases in Buffalo Pound Lake. Data synthesized here are presented in Figures 3.1 – 3.3 and 3.7, Table 3.4 and Appendix E, Figure E3.3.

Descriptor	Pre-bloom phase	Major bloom phase	Post-bloom phase
Date (DOY)	July 6 – 31 (187 – 212)	August 1 – 26 (213 – 238)	August 27 – September 21(239 – 264)
Dominant taxa (limnetic, discrete)	*D. flos-aquae* (diazotrophic)	Primary: D. flos-aquae (diazotrophic) Secondary: *P. agardhii* (non-diazotrophic)	*P. agardhii* (non-diazotrophic)
Nitrogen fixation (limnetic, discrete)	moderate rates DOY 191 + 212, near-zero fixation DOY 198	peak nitrogen fixation on DOY 215, 222	low nitrogen fixation
Observations of scum during discrete sampling	none	DOY 222 predominantly *D. flos-aquae* DOY 235 predominantly *D. flos-aquae*	DOY 240 predominantly *D. flos-aquae* DOY 250 predominantly *D. flos-aquae*

favouring phytoplankton with efficient mechanisms for carbon uptake under low CO_2 conditions, namely cyanobacteria (Shapiro 1997; Ji et al. 2017). We note that the low CO_2 values reported (Figure 3.1, 3.5, E3.5) may indeed be overestimates. Measurements of pH, DIC and alkalinity from the water treatment plant for similar periods of time suggest CO2 may be lower than sensor-based measurements suggest. We also note that the error range of these sensors is rather high for the observed CO_2 concentrations (Table E3.1). Rapid growth also necessitates adequate nutrient availability. While cyanobacterial bloom-prone lakes tend to have high nutrient loading, they do not always have high dissolved nutrient concentrations. Low concentrations of SRP during the major bloom phase, combined with high cyanobacterial biomass suggests that available phosphorus was quickly recycled (Hudson et al. 2000). Rapid phosphorus recycling has been demonstrated within the water column across trophic gradients (Vandergucht et al. 2013).

Internal loading is an important source of nutrient replenishment in Buffalo Pound Lake (D'Silva 2017). It has been hypothesized that internal nutrient loading helps support blooms, particularly in polymictic lakes because of effects on sediment anoxia during transient stratification events (Taranu et al. 2010). Stratification events were apparent during all three phases of the bloom (pre-bloom, major bloom and post-bloom), however, internal loading in Buffalo Pound Lake remains relatively high under both anoxic and oxic conditions (D'Silva 2017). This phenomenon could provide a further advantage to taxa that can regulate buoyancy levels and access sediment nutrients.

Strong buoyancy regulation is likely a competitive advantage under the bloom conditions we describe. Counteracting gas vesicles and ballasts (typically carbon stores) provide many cyanobacterial species with the ability to migrate vertically during periods of low water-column turbulence to improve access to light or nutrients (Kromkamp et al. 1986, 1988). All of the abundant cyanobacterial taxa in Buffalo Pound Lake—including *Dolichospermum* sp. (Li et al. 2016), *Aphanizomenon flos-aquae* (Cirés and Ballot 2016), *Planktothrix agardhii* (Kurmayer et al. 2016), *Microcystis aeruginosa* (Harke et al. 2016b)—have shown migratory behaviour. Upward vertical migration was apparent during periods of thermal stratification in limnetic zones. Although summer stratification events are not necessarily associated with bloom conditions, some of the most extreme biomass levels (constituted predominantly by *D. flos-aquae*) were associated with periods of stratification during the primary bloom period. This suggests that, within a given year, timing of stratification events could be important to bloom

evolution. As cyanobacterial biomass before and after these transient stratification events was considerably lower during water-column mixing, we believe that the elevations in biomass at the surface of the stable water column can be attributed to a combination of community growth but also accumulating buoyancy-positive communities at or near the water's surface.

3.4.2 Nitrogen, nitrogen fixation and bloom dynamics

Diazotrophic cyanobacteria often dominate under low nitrogen conditions, and low nitrogen to phosphorus ratios (below the Redfield N:P ratio of 7.2 by mass or below 9:1 by mass in lakes) are often cited as an antecedent condition to nitrogen-fixing blooms (Howarth et al. 1988a; Vitousek et al. 2002). Here, ammonium concentrations were elevated leading up to the major bloom phase though surprisingly, nitrogen fixation rates were moderate (DOY 212) despite this ammonium availability. Shortly thereafter, cyanobacterial biomass increased markedly, ammonium concentrations dropped dramatically, and heterocytes and nitrogen fixation increased rapidly, suggesting that high ammonium may have helped hasten the bloom, but nitrogen fixation was required to sustain it. Further, higher rates of nitrogen fixation did coincide with lower ratios of DIN to both SRP and TP during transient stratification events, and, conversely, lower rates of nitrogen fixation occasionally coincided with higher DIN:SRP and DIN:TP. However, while these associations between nutrient ratios and nitrogen fixation were evident, significant correlations were not detected. Possible explanations for low nitrogen fixation rates during periods of low N:P could be trace metal limitation or environmental conditions unfavourable for nitrogen fixation, such as lower water temperatures or light intensities. Or, given the potential for rapid recycling of phosphorus and nitrogen, these ratios may not be an effective indicator of nitrogen limitation and may generally be limited in their usefulness. We can, however, infer that the system was nitrogen deficient based on continued dominance by diazotrophic *D. flos-aquae* with developed heterocytes until the secondary bloom. Notably the non-diazotrophic *Microcystis aeruginosa*, the sole *Microcystis* species observed in Buffalo Pound Lake during the 2017 sampling campaign, did not dominate, possibly due to its preference for high nitrogen and low phosphorus conditions (Harke et al. 2016b). As *Microcystis* has a history of being a superior competitor in many aquatic systems (Zohary and Breen 1989; Blomqvist et al. 1994; O'Neil et al. 2012) and was regularly observed in surface water here, the absence of *Microcystis* dominance suggests conditions, such as low nitrogen availability, may be suppressing this taxa of concern.

The trajectory of biological change observed here is common among many ecosystems (Paerl and Otten 2016; Higgins et al. 2017). Diazotrophs dominated surface water from pre-bloom to primary bloom collapse. Enhanced primary productivity of cyanobacteria associated with the bloom contributed to dissolved inorganic nitrogen depletion. Low nitrogen availability favours nitrogen-fixing taxa (Ferber et al. 2004), which helped sustain diazotroph dominance, until conditions shifted. We encountered two key nitrogen fixation events in limnetic surface water during transient thermal stratification and several more fixation events in littoral surface scums. The two genera of diazotrophs present in Buffalo Pound Lake, *Dolichospermum* and *Aphanizomenon*, may be common in prairie environments, because they are tolerant of systems with higher conductivity (Cirés and Ballot 2016; Li et al. 2016). The ability of *Dolichospermum* to thrive in nitrogen-fixing conditions under high light intensity, relative to *Aphanizomenon*, shown elsewhere (De Nobel et al. 1997, 1998; Bradburn et al. 2012), was also demonstrated by our study. High incident radiation and long day length favoured *Dolichospermum*, particularly during periods of thermal stratification where *Aphanizomenon* contributions to total biomass were minimal. The low concentration of soluble reactive phosphorus in the water column in conjunction with the high concentration of total phosphorus and high level of biomass from *D. flos-aquae* suggests the potential for rapid uptake of newly available phosphorus by that taxa.

In addition to efficient phosphorus uptake, *Dolichospermum* is able to optimize nitrogen fixation under higher light intensities; and this was evident by the speciation of heterocytes observed, showing an abundance of *D. flos-aquae* heterocytes, proportional to that of *D. flos-aquae* biomass. Although *Aphanizomenon* was not able to achieve a dominant position among the phytoplankton of this particular community, species of this genera are more adept at growth and nitrogen fixation under weak irradiance (De Nobel et al. 1998; Bradburn et al. 2012). It remains unclear why, of the three species of *Dolichospermum* observed during the 2017 bloom season, *D. flos-aquae*, *D. crassa* and *D. solitaria*, the superior competitor was consistently *D. flos-aquae* during the major nitrogen fixation events that occurred in both limnetic surface water and littoral surface scums.

Cooling water temperatures and decreases in incident PAR as the season progressed are suboptimal for *Dolichospermum* (Li et al. 2016) likely contributing to primary bloom collapse. Following the collapse of the primary bloom, phytoplankton composition shifted from diazotrophic to non-diazotrophic taxa. The cooler water and lower light conditions that ensued

following the primary bloom are favourable for *Planktothrix* growth (Kurmayer et al. 2016). In addition, we expect that nitrogen fixed by *D. flos-aquae* and other lesser heterocytous species during the primary bloom (and continued nitrogen fixation in surface scums following the primary bloom) was recycled, facilitating succession to non-diazotrophic taxa (Paerl and Otten 2016).

The hypothesis that the succession from *Dolichospermum* to *Planktothrix* was driven by declining light and water temperatures is further supported by the observation that even after *Planktothrix agardhii* reached dominance during the secondary bloom period (DOY 226 – 233), and during the post-bloom phase, *D. flos-aquae* continued to dominate surface scum formations. These scums were not senescent, and instead showed high rates of nitrogen fixation. We suggest continued dominance of *D. flos-aquae* in scum was probably due to warmer water temperatures along the shallow shoreline permitting enhanced productivity in the littoral zone leading to nitrogen deficits. In addition, *Dolichospermum* accrued at the surface where irradiance is stronger while *Planktothrix* dominated in the subsurface where irradiance was weaker (Kurmayer et al. 2016; Li et al. 2016).

Notably, *Woronichinia compacta* was the dominant taxa on the last day of the sampling season (DOY 264). And although this species of cyanobacteria was common across the study period and frequently the second or third highest contributor to biomass, current knowledge of *Woronichinia compacta* is very limited. A lack of information surrounding the ecology and phylogeny of this taxa is likely due to either its sensitivity, which has led to cultivation difficulties under laboratory conditions (Rajaniemi-Wacklin et al. 2006), or the limited availability of studies focused on *Woronichinia compacta*. As such, we are unable to interpret the change in abundance of this taxa over time.

3.4.2.1 Diel variability

Diel monitoring and measurements of nitrogen fixation showed a high degree of temporal variation in conditions, and rates of nitrogen fixation. Based on our knowledge of taxa in the region, we expected nitrogen fixation to be dominated by heterocytous diazotrophs (Patoine et al. 2006; Finlay et al. 2010b) with negligible nitrogen fixation rates under dark conditions given the absence of non-heterocytous diazotrophic strains (Wasmund et al. 2001). Nitrogen fixation was significantly higher under light conditions; however, we also identified two occurrences of atypically high rates of nitrogen fixation during dark incubations: the first on DOY 215 which

coincided with the highest rates of nitrogen fixation in limnetic water (light 3.7 μg L^{-1} h, dark 2.1 μg L^{-1} h^{-1}), and the second on the evening of DOY 235 measured in-situ (light 1.0 μg L^{-1} h, dark 0.89 μg L^{-1} h^{-1}). In these cases, the rate of nitrogen fixed under dark conditions could not be considered negligible. We suspect that carbon stored during hours of photosynthesis was used to fuel nitrogenase production, and therefore nitrogen fixation, under dark conditions (Postgate 1998, as cited in Gettel et al. 2013).

Overlaying the effects of diurnal light and temperature cycles with occurrences of frequent irregular mixing and transient stratification events in polymictic lakes will inevitably yield high temporal variation in rates of nitrogen fixation. For example, mixing alters chemical and physical parameters—such as turbidity and water temperature—which can regulate nitrogen fixation, and the turbulence of mixing reduces the ability of migrating taxa to regulate their buoyancy (Peterson et al. 1977). However, the variation we observed between afternoon and evening nitrogen fixation was unexpected. Despite lower incident PAR, evening rates of nitrogen fixation in surface water could surpass afternoon rates. This phenomenon, which was associated with greater cyanobacterial biomass and heterocyte biovolume, was possibly a response to elevated water temperatures (Carey et al. 2012). Likewise, nitrogen fixation in afternoon surface scums formed at lower water temperatures under higher incident PAR was greater than that of evening surface water at a greater temperature. This shows us that diurnal patterns of nitrogen fixation rates in a polymictic lake are subject to both temporal and spatial variation.

Spatial variability and surface scums

Nitrogen fixation rates in littoral zones were not always greater than that of limnetic zones. This is likely attributed to the lake's length, width and depth, making this prairie lake more susceptible to thorough wind-induced mixing (Verhagen 1994; Magee and Wu 2017). This characteristic could, hypothetically, alter biological, chemical and physical properties in littoral and limnetic zones during mixing events, and affect horizontal bloom distribution by shifting the existing bloom (Ishikawa et al. 1999; Doyle et al. 2010).

The severity of surface scums ranged from thin patchy accumulations to thicker mats. Photographic observations showed that surface scums were only formed during periods of little to no surficial turbulence, and established surface scums were disrupted by wind-induced disturbances. Surface scums formed in Buffalo Pound Lake did not have a foamy appearance at times of sampling, a characteristic of irreversible scums formed by a supersaturation of oxygen

(Dervaux et al. 2015). Rather, their appearance suggests that these scums were formed through buoyancy-regulated vertical migration.

While taxa from both diazotrophic and non-diazotrophic genera are capable of forming surface scums through self-regulating mechanisms (Wiśniewska et al. 2007; Persaud et al. 2014; Harke et al. 2016b) and a minimum of five species from both groups were observed in littoral surface scums, diazotrophs were consistently the major biomass contributors. The dominant taxa in surface scums was diazotrophic, heterocytous *D. flos-aquae*, even in littoral samples where surface water was typically dominated by *Planktothrix agardhii*.

We consistently observed substantially higher rates of nitrogen fixation in littoral surface scums than surface water. Where nitrogen fixation occurred in surface water, rates were consistently below that of nitrogen uptake from ammonium, consistent with previous work in the region showing that nitrogen fixation is less able to wholly support to biomass in highly productive systems (Patoine et al. 2005; Hayes et al. 2019). However, our single sample experiment on surface scum indicates that the extent of nitrogen fixation occurring in surface scum formations is over and above that of ammonium uptake, signifying the potential for nitrogen fixation to support these biological hot spots. High rates of nitrogen fixation within scum formations may indicate localized nitrogen deficiencies as evidenced by the ability of diazotrophic taxa which are tolerant of low nitrogen conditions to dominate (Li et al. 2016). Observed rates of nitrogen fixation also suggest these blooms were not senescent and the observed scums were therefore likely associated with buoyancy regulation. Because these surface scums could be maintained for up to 4 hours, they may contribute a substantive amount of new nitrogen via nitrogen fixation. Further, the repeated occurrences of surface scums in conjunction with low surficial turbulence, and not necessarily thermal stratification, during both the major and post-bloom phases indicate that high nitrogen fixation events were more frequent in littoral surface scums than limnetic surface water.

It is important to note that the measured rates of nitrogen fixation in surface scums sampled on August 10 (DOY 222), 2017, and September 7 (DOY 250), 2017, showed some irregularities. In addition to the community collapse within acetylene reduction bottle assays previously identified (Section 3.3.6, Table 3.4, 3.5), it is likely that underestimations in nitrogen fixation rates measured using the $^{15}N_2$ tracer assay also occurred. When comparing surface scum measurements to one another, DOY 222 scum exhibited extremely high concentrations of

chlorophyll *a*, cyanobacterial biomass and heterocyte biovolume (approximately 29,750 µg L^{-1}, 8,389 mg L^{-1} and 523 µL L^{-1}, respectively; Table 3.4), while DOY 250 exhibited much lower concentrations of those same parameters (chlorophyll *a* was 17 times lower, cyanobacterial biomass was 97 times lower and heterocyte biovolume was 182 times lower). However, the measured rate of nitrogen fixation on DOY 250 was nearly 7.5 times greater than that of DOY 222 (71.0 µg L^{-1} h^{-1} compared to 9.5 µg L^{-1} h^{-1} using $^{15}N_2$-tracer assays). We believe that the opacity of the more severe surface scum collected on DOY 222 restricted light penetration through assay bottles which led to a lower rate of nitrogen fixation. Assuming the reduced light intensity led to an underestimation in nitrogen fixation on DOY 222, we believe that the actual rate of nitrogen fixation in-situ was much higher.

3.4.3 Interannual variability

The long-term record of phytoplankton composition for Buffalo Pound Lake between 1996 and 2016 shows that cyanobacteria were the dominant phytoplankton group in 67% of the sampling years. Further, dominance by other phytoplankton was uncommon (11%), as anticipated in a reservoir with high nutrient loading (Watson et al. 1997). No specific taxa were dominant for the remaining years. Given the high level of internal nutrient loading from sediments (D'Silva 2017), we suspect that elevated biomass from other phytoplankton groups, such as chlorophytes, cryptophytes and dinoflagellates, were potentially a response to differing water residence times and flows.

The dominance of cyanobacteria by *D. flos-aquae*, as demonstrated in 2017, was consistent with the long-term record. This diazotrophic taxa was observed in all years (only species to appear in all years) and the dominant taxa for the majority of years. The presence of this diazotrophic taxa in conjunction with developed heterocytes suggests that nitrogen deficiencies are not an uncommon occurrence here. *Planktothrix* meanwhile was typically the dominant non-diazotrophic species during the 2017 sampling year; however, the long-term record suggests that this toxin-producing taxa tends to cycle in and out of the community in Buffalo Pound Lake.

3.5 Conclusion

In naturally eutrophic catchments, such as the Qu'Appelle River Watershed, water quality degradation or eutrophication is still a concern (Dixit et al. 2000). As noted, central to the

phosphorus-only eutrophication management paradigm is the idea that diazotrophic cyanobacteria can sometimes compensate for bioavailable nitrogen depletion via nitrogen fixation to sustain bloom development (Schindler et al. 2008). Existing literature has repeatedly postulated that nitrogen fixation is an energetically-taxing process for diazotrophs, and that these taxa will only resort to fixing nitrogen when bioavailable nitrogen is limiting growth (Stam et al. 1987; Howarth et al. 1988a; Galloway et al. 2004). Ammonium, specifically, is considered the preferred nitrogen species for uptake (Flores and Herrero 2005; Andersen et al. 2020) and, when present, nitrogen fixation rates are expected to remain low. The occurrence of nitrogen fixation in the presence of bioavailable nitrogen identified in this study suggests that the controls over nitrogen fixation and its associated costs in highly productive, nutrient-rich ecosystems needs to be revisited.

Further, in surface scum accumulations, nitrogen fixation rates may be 100 times that of ammonium uptake. Research studies that examine the role of nitrogen fixation in surface scums are largely absent in the literature. The demand for resources, specifically light and essential nutrients, required to sustain the biological activity of scums is evidently high, based on the occurrences of bloom collapse within bottle assays in this study. This quality of surface scums makes capturing process rates ex-situ very challenging. Regardless, this study has demonstrated that surface scums formed during and following the major bloom phase are potentially nitrogen fixation hotspots. Although scums were not consistently present, our findings from Buffalo Pound Lake show that scums could be formed in littoral zones of shallow lakes where surficial turbulence is low, regardless if the water column is stratified or not. We believe that the remarkably high rates of nitrogen fixation observed in these surface scums warrants further investigation into a previously overlooked source of nitrogen.

Given the high spatial and temporal variation in biological, chemical and physical water column attributes, estimations of robust lake-wide nitrogen inputs from nitrogen fixation are also challenging. However, our study demonstrated the usefulness of frequent gross measurements of nitrogen fixation to identify biological hot moments and hot spots. Moreover, our study demonstrates that transient stratification events promote nitrogen loading through biological fixation at the surface level in Buffalo Pound Lake. Net rates of nitrogen fixation in the region by diazotrophic taxa are, in general, unable to support phytoplankton growth to the point of phosphorus limitation in the long-term (Hayes et al. 2019); however, we found that gross

nitrogen fixation could be a critical source of nitrogen to phytoplankton in the short-term during said transient thermal stratification events. Importantly, nitrogen fixation also appears sufficient to allow secondary blooms of non-diazotrophic taxa, in this case, *Planktothrix*, a taxon with potentially high toxicity due to its capacity to produce anatoxin (a toxin observed in this ecosystem; Baulch et al. unpublished data).

The question of how to manage these blooms is a vexing one, particularly in an important regional drinking water supply. The reservoir is shallow, with high rates of internal loading, and has a large watershed, if one considers the area of the upstream reservoir, Lake Diefenbaker. As such, internal cycling and external loading are challenging to control. There are no point source inputs to the lake which means topical questions about the importance of nitrogen control have limited relevance here, given it is clear from a sustainability perspective both nitrogen and phosphorus merit careful use (Harrison et al. 2009). Nonetheless, we note that both diazotrophic and non-diazotrophic taxa observed here are potential toxin producers, including *Dolichospermum* sp. (Li et al. 2016), *Aphanizomenon flos-aquae* (Cirés and Ballot 2016), *Planktothrix agardhii* (Kurmayer et al. 2016), *Microcystis aeruginosa* (Harke et al. 2016b). The annual bloom is a substantive nuisance due to effects on recreational use of the lake and impacts on the water treatment utility, yet practical management solutions suitable to this ecosystem are lacking.

The findings of our study provide new information about the potential for nitrogen fixation to support bloom development and highlight key knowledge gaps surrounding spatial and temporal variability. As cyanobacterial dominance was noted relatively early in summer, we expect factors contributing to their dominance, such as adequate ferrous iron availability (Molot 2017), temperatures and light intensity (Carey et al. 2012), were achieved earlier in the year pointing towards the possible importance of lag effects on bloom development. Future studies should consider a longer time frame to capture the processes that occur earlier in the year leading up to dominance by cyanobacteria. Further, with warming water temperatures and extending growing periods associated with climate change and the early dominance by cyanobacteria, the seasonal initiation of nitrogen fixation could occur earlier in the year.

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3.7 Author Contributions

Scott Higgins provided detailed methodological advice, and input at multiple stages of research design and manuscript preparation. LMB was the lead author on this thesis chapter, with advice and edits provided by Helen M. Baulch. HMB obtained research funding for the project and advised on research program design and analyses.

Chapter 4: GENERAL CONCLUSION

4.1 Summary of findings and final considerations

Water quality management challenges remain in many lakes where significant nutrient inputs have proven difficult to control. These inputs include runoff sources (Baulch et al. 2019), internal loading from sediment (Orihel et al. 2017) and nutrient inputs from biological processes, specifically nitrogen fixation (Scott and Grantz 2013). In this thesis, I sought to explore the spatial and temporal variability of nitrogen fixation rates and phytoplankton composition—with a focus on diazotrophic cyanobacteria—to help address the decades-old question: 'Is nitrogen control necessary?' Given the high degree of variability in these shallow, eutrophic prairie lakes, the answer is complicated. However, these lakes appear to be primed to replace nitrogen through fixation, suggesting it is unlikely that nitrogen removal would lead to a sudden shift to a lower trophic status.

This study took place in the upper region of the Qu'Appelle River Watershed lake chain located in southern Saskatchewan, Canada, during the 2017 open-water period. The research presented here has been the first to measure gross rates of nitrogen fixation using $^{15}N_2$-calibrated acetylene reduction assays (ARA). Previous studies in this region have measured net rates of nitrogen fixation along the lake chain using an alternate method that evaluates changes to the natural abundance of the ^{15}N isotope in particulate matter overtime (Patoine et al. 2005, 2006; Patoine and Leavitt 2008; Hayes et al. 2019). These studies provide critical high-level insights into the overall importance of nitrogen fixation to the watershed's nitrogen budget by determining how much fixed nitrogen remains in the system following losses through denitrification. This existing research does not, however, consider the temporal variability of rates that accompany transient stratification events typical of polymictic lakes, spatial variability associated with irregular mixing patterns or short-term impacts of these nitrogen inputs to the phytoplankton community. Productivity and nitrogen fixation rates aren't expected to be limited by trace metals in study lakes during the growth period (see Appendix C).

This thesis was presented in two parts. The first examined changes in rates of nitrogen fixation and phytoplankton composition across lakes within a single watershed with differing nutrient loading sources (Chapter 2). The second provided a more detailed look at nitrogen

fixation rates and phytoplankton associated with cyanobacterial bloom evolution, spatial variability and interannual variability (Chapter 3).

The opportunity for nitrogen fixation in northern lakes is limited by below-optimal water temperatures (Robarts and Zohary 1987; Yamamoto 2009; Li et al. 2016) for the majority of each calendar year. Though brief, the growth period in shallow prairie lakes is, in general, highly productive. A comparison between the measured nitrogen fixation rates presented in Chapter 2 of this thesis and phytoplankton nitrogen demand calculated by Hayes et al. (2019) showing a similar range of nitrogen inputs and requirements, respectively, suggests that nitrogen fixation in the region may be more important to productivity than previously believed.

Further, Chapter 2 described differences in nitrogen fixation rates and phytoplankton composition in lakes receiving differing nutrient sources within the same watershed. In general, lakes receiving pulsed nutrient supplies from agricultural runoff (upstream and relatively shallow) showed a higher level of variability in nitrogen fixation rates and shifting phytoplankton dominance compared to downstream lakes. Lakes receiving both pulsed and pressed nutrient supplies from wastewater effluent (downstream) showed low rates of nitrogen fixation accompanied by continued dominance by diazotrophic taxa across the season. Essentially, a positive correlation between nitrogen fixation rates and cyanobacterial biomass identified in shallow, upstream lakes, not seen in deeper, downstream lakes, was attributed to pulsed nutrient input patterns in response to land-surface runoff and internal loading mechanisms. A more steady level of biomass across the season in downstream lakes is consistent with continually pressed nutrient supplies; however, the combination of diazotrophic (*Aphanizomenon flos-aquae*) dominance and low rates of nitrogen fixation was surprising. It is suspected that this could be a lag effect resulting from legacy akinete germination (Yamamoto and Nakahara 2009) and rapid growth producing a light-limited environment favourable to this particular “shade”-adapted taxa (De Nobel et al. 1998). This chapter concludes with implications of phytoplankton community dynamics with differing nutrient regimes, indicating potential for taxa shifts to facilitate shifts in toxicity in upstream lakes.

In Chapter 3 of this thesis, we investigated one upstream lake in detail. Results of this chapter identified a considerable amount of physicochemical variation in relation to nitrogen fixation rates and phytoplankton composition with seasonal bloom evolution and spatial and temporal variations within the major bloom phase. The bloom season was segmented into three

phases: pre-bloom, major bloom and post-bloom. Rapid growth of cyanobacteria seen at the onset of the major bloom phase proceeded pre-bloom peak light, water temperatures and ammonium. Here, the major cyanobacterial bloom itself was further segmented into two periods: the primary bloom and the secondary bloom. It was determined that early on during the major bloom, conditions were favourable for dominance by nitrogen-fixing taxa.

Key nitrogen fixation events in limnetic surface water occurred during the primary bloom period, coinciding with transient thermal stratification events and lower nitrogen to phosphorus ratios. In the littoral zone, surface scums formed where surficial water turbulence was low, regardless of whether or not the water column had stratified. Importantly, results of this study showed nitrogen fixation rates in these scum formations were some of the highest of the season. As incident PAR and water temperatures declined with the season, these conditions coincided with lower biomass and a successional shift in taxa dominance from diazotrophic (predominantly *Dolichospermum flos-aquae*) to non-diazotrophic (predominantly *Planktothrix agardhii*) cyanobacteria. However, while shifts in taxa dominance have been well documented (e.g. Paerl and Otten 2016), continued dominance of surface scum formations by *Dolichospermum flos-aquae* following surface water succession has not.

4.2 Limitations and future research opportunities

This research provides a novel contribution to the current state of knowledge concerning the possible importance of nitrogen fixation in surface scum-impacted lakes. Surface scums formed by aggregations of buoyant cyanobacteria are widely documented as nuisances and potentially harmful. Evidence presented here now indicates that nitrogen fixation is greater in scums compared to adjacent surface water. These results highlight a need for more in-depth analyses of surface scums as potential hotspots for both biological activity and nitrogen fixation. While the methods used did identify generally high rates of nitrogen fixation in surface scum, we would recommend certain adjustments to mitigate methodological concerns. First, bloom collapse occurred within two acetylene reduction bottle assays containing surface scums. This was possibly a result of carbon limitation, and as such, reducing incubation time in surface scum samples could allow for a more accurate estimation of high nitrogen fixation rates by terminating the experiment prior to carbon depletion. It would be prudent to prepare additional replicate bottles and collect headspace samples for ethylene analysis at different time intervals—for example, at 1, 2 and 4 hours—this would allow the researcher to ensure sample collection before

bloom collapse if such events occur in bioassays. Similarly, a number of water samples exhibited ethylene concentrations below detection, and as such, it may also be prudent to increase incubation time to calculate a more accurate hourly rate. Whether rates of nitrogen fixation will be high or low is unclear based on the appearance of the sample; and therefore, in order to provide the most accurate measurement collecting headspace samples at multiple timepoints would be beneficial.

Overall, this thesis demonstrated the high degree of spatial and temporal variability of nitrogen fixation rates and phytoplankton composition that occur within a single prairie watershed. The level of seasonal variation observed across lakes with differing nutrient loading regimes and spatially within a single lake highlights the complexity of water quality management in naturally nutrient-rich regions. We were able to capture strong fluctuations in nitrogen fixation rates and associated changes to phytoplankton communities and water surrounding transient stratification events and surface scum accumulation chemistry in one shallow upstream lake. And although we found these short-term pulses of nitrogen inputs to be important to bloom evolution and species succession later in the year in lakes lacking point-source nutrient inputs, it is unclear whether or not similar occurrences exist within wastewater impacted lakes. Assuming biological activity was constant between sampling points, the applied sampling strategy collecting samples once every two weeks suggests that nitrogen fixation rates were low and both biomass levels and phytoplankton composition were consistent in deeper downstream lakes. However, consistent dominance by diazotrophic taxa combined with low rates of nitrogen fixation raises further questions surrounding potential variation in nitrogen fixation rates and phytoplankton composition with transient stratification events and the potential for surface scum development in prairie lakes receiving wastewater effluent.

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APPENDIX

Appendix A: Acetylene Reduction Assay blanks

Each acetylene reduction bottle assay experiment included sample blanks in triplicate—deionized water inoculated with acetylene incubated alongside lake treatments. The calculated rates of ethylene production in sample blanks ranged from 0 – 0.017 μM L^{-1} (Figure A.1) with a median value of 0.006 μM L^{-1}. Rates of production in treatments ranged from 0 – 0.892 μM L^{-1}. The comparison of sample blanks to treatments showed that blanks were lower than treatments (Figure A.2, Wilcoxon signed rank test, $p<0.05$).

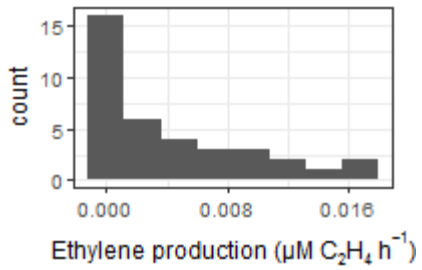


Figure A.1 Distribution of ethylene production in sample blanks.

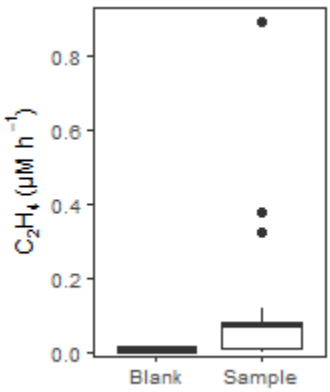


Figure A.2 Comparison between rates of ethylene production in sample blanks (left, n=37) and treatments (right, n=37). The horizontal line through each box indicates the sample median. The rectangular area below indicates the range from the median to the first quartile, and the area above indicates the range from the median to the third quartile. Whiskers extend from each box to indicate the inter-quartile range (IQR) x 1.5. Black circles identify outliers (above IQR x 1.5).

Appendix B: Additional information on $^{15}N_2$-tracer assay method

^{15}N-N_2-enriched solution

A ^{15}N-N_2 -enriched stock water solution was used to introduce dissolved gas to lake water samples. Previous studies have demonstrated that directly injecting ^{15}N-N_2 into a sample when conducting the $^{15}N_2$-tracer assay can lead to underestimation of N_2 fixation rates due to the relative insolubility of N_2 (Klawonn et al. 2015). We produced 1% ^{15}N-N_2-enriched stock water solutions more than 24 hours prior to use to limit underestimation of N_2 fixation rates resulting from undissolved N_2.

Deionized water was degassed at low pressure (600 mbar) for 45 minutes in a 1000 mL filtering flask prior to enrichment. The degassed water was enriched with ^{15}N-N_2 ($^{15}N_2$, 98% +, Cambridge Isotope Laboratories, lot#I-19197/AR0586172). Prior to the summer field season, single-use enriched solutions were prepared in 120 mL serum bottles by slowly pouring the degassed water into the bottle, inserting stopper and crimping bottle before injecting 1.2 mL of gas. Mid-season, multiple-use enriched solutions were prepared in 1 L Tedlar® bags. Degassed water was transferred into bags using 60 mL syringes with limited interaction with the atmosphere. ^{15}N-N_2 was injected through the septum at 1 mL per 100 mL of degassed water. Serum bottles and Tedlar® bags were vigorously agitated after ^{15}N-N_2 addition.

Measuring ^{15}N-N_2-enrichment

Isotopic enrichment of ^{15}N-N_2 stock solutions was measured by analyzing dissolved gas in solution using a membrane-inlet mass spectrometer. The MIMS instrument was equipped with a Pfeiffer HiCube® 80 Eco Turbo pumping station, PrismaPlus® Quadrupol-Analyzer with tungsten filament (QMA 200), and Gilson Minipuls®3 peristaltic pump (Bay Instruments, Easton, Maryland). We used a copper reduction tube heated to a temperature of 600°C (ThermoScientific Lindenberg/Blue M™ tube furnace) to decrease the oxygen to nitrogen ratio ($O_2/N_2 < 1\%$) to reduce the risk of over-estimation from the detection of NO in the sample (Eyre et al. 2002).

Mock samples were analyzed for ^{15}N-N_2 enrichment at the start of the field sampling period—tap water was enriched in lieu of lake water—tap water was allowed to sit on the counter to degas for 24 hours prior to enrichment. Sample water was continuously 'sipped' directly from the bottom of the 60 mL BOD bottle, and three data points were collected for each sample replicate once the signal stabilized. Mock samples amended with enriched solution prepared in serum bottles were analyzed (~2 weeks after solution was prepared), and found to have an atom% of 2.85 ± 0.20 (mean ± SD) in the dissolved N_2 pool; and similarly, mock samples amended with enriched solution from the Tedlar® bags had an atom% of 2.73 ± 0.12 atom% which were analyzed ~3 weeks after solution was prepared. Following the field sample period, the Tedlar® solutions were re-analyzed, and it was determined that enrichment of mock samples had dropped to 0.67 ± 0.07 atom%. Given the significant reduction in ^{15}N-enrichment in Tedlar® treatments, we performed a time-series experiment to evaluate how the level of enrichment changed over time in both Tedlar® and serum bottle treatments.

Method

To examine this decrease in enrichment, we performed a series of measurements over the course of ~5 weeks to compare changes in both Tedlar® and serum bottle solutions with newly prepared stock solutions. A total of twenty serum bottles and three Tedlar® bags (60% full) were enriched. The Tedlar® bags plus one or two serum bottles selected at random were analyzed on a given day. Each serum bottle and Tedlar® bag was used to produce tap water mock samples in duplicate, and measurements were repeated every 6 – 12 days.

Results & Discussion

There was a fluctuation in measured enrichment from 2.08 – 3.96 atom% in treatments enriched with serum bottle stock solution (Figure B.1). Enrichment ranged from 1.21 – 4.1 atom% in treatments enriched with Tedlar® stock solution. In general, Tedlar® treatments decreased over time, while serum bottle treatments appear to increase slightly. As each serum bottle is a closed system, the fluctuation in enrichment is likely a result of an issue with the mass spectrometer signals.

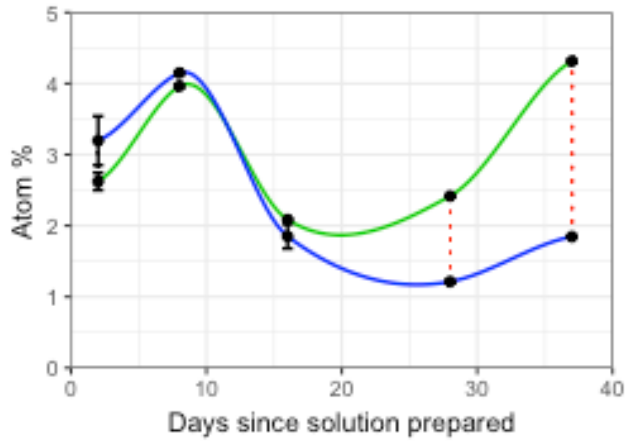


Figure B.1 Measured changes in enrichment over time in treatments amended with serum bottle (black points, blue line) and Tedlar® (black points, green line) stock solutions. Vertical red dashed lines highlight the differences in atom% between methods. Error bars are mean ± 2 SE.

Moving forward, there appeared to be a relationship between Tedlar® and serum bottle treatments in terms of enrichment. The differences between methods between samples enriched with the Tedlar® (A_{tedlar}) and serum bottle (A_{serum}) stock solution, and calculated as follows:

$$\text{difference between methods (\%)} = (A_{tedlar} - A_{serum})/((A_{tedlar} + A_{serum})/2)\ x\ 100 \quad \text{(B. 1)}$$

A significant relationship when comparing the difference between methods and the number of days since the solution had been prepared to use (or solution age/shelf life) was identified (p=0.0015, Figure B.2) in a downward trending slope. Enrichment of samples was greatest in Tedlar® treatments at the start of the series compared to serum bottle treatments. However, the

difference between methods steadily decreased from 18.5% to -81.0%, indicating that the difference in enrichment between the two vessels continuously increased over time following day 10 of the experiment.

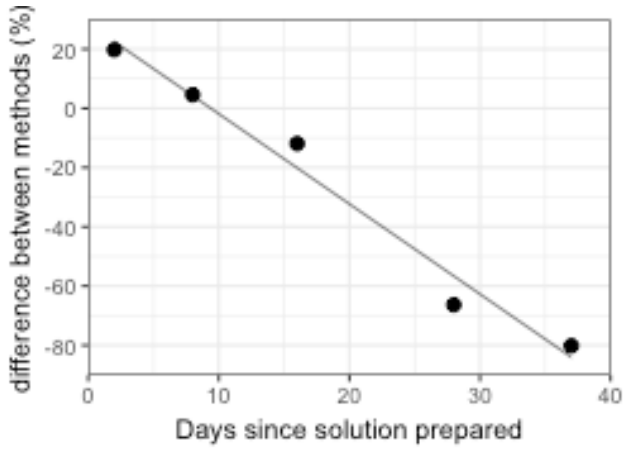


Figure B.2 Comparison of the difference between methods and days since stock solution was prepared (Equation B.2: $y = 28.56 - 3.04x$, n=5, R^2=0.98).

Based on the results of the time-series experiment, the dissolved N_2 pool of serum bottle treatments were assigned an enrichment level of 2.71 atom% for all nitrogen fixation calculations—assigned based on the average enrichment (^{15}N atom %) of the serum bottle treatments. For Tedlar® treatments, the dissolved N_2 pool was linearly adjusted to correct for changes in enrichment. To do this, 1) an adjusted 'difference between methods' value was determined using Equation B.2 (see Figure B.2) for each measurement date. Then, 2) the atom% of Tedlar® treatments (A_{tedlar}) was back-calculated using Equation B.1, where the enrichment of serum treatments was 2.71 atom% (A_{serum}) and the difference between methods was equal to the adjusted 'difference between methods'. Lastly, 3) the relationship between Tedlar® treatments and 'Days since solution prepared' was evaluated using linear regression (R^2=0.98, p=0.001). The following linear model was applied to $^{15}N_2$-tracer assay calculations:

$$A_{N_2} = 3.003 - 0.058\text{x}. \quad \text{(B. 3)}$$

where the enrichment of the dissolved N_2 pool (A_{N_2}) for each experiment was determined as a function of 'Days since solution prepared' (x = Date of experiment - Date solution was prepared). These additional calculations should result in a more accurate estimate of the amended $^{15}N_2$ pool. This was done to mitigate over or underestimates in nitrogen fixation rates measured using $^{15}N_2$-tracer bottle assays.

Appendix C: Trace-metal limitation experiments

Similar to macroelement limitation (i.e. nitrogen, phosphorus), cyanobacteria may be limited by metals required for enzyme and coenzyme development. Comparative $^{15}N_2$-tracer experiments were conducted to test if any trace metals are limiting nitrogen fixation in the Qu'Appelle River Watershed. These experiments were conducted following K. Salk's protocol for evaluating potential metal-limitation in L227—International Institute of Sustainable Development-Experimental Lakes Area—using a "super mix" of trace metals. We incubated 12-60 mL BOD bottles containing raw lake water for 24 hours (light 12 ON: 12 OFF). Four of these bottles were amended to a final concentration of 6.54 µmoles of added metal compounds per litre—"sulphate-free trace metal solution" prepared by Neufeld Lab, University of Waterloo. Solution originally from Imhoff and Trüper, 1977, as cited in Imhoff, 2014). After twenty-four hours of trace metal solution amendment, we performed the $^{15}N_2$-tracer assay using these samples. Eight bottles were enriched with the ^{15}N-N_2 enriched solution—including the trace metal amended samples—and, the remaining four bottles were filtered for natural abundance of ^{15}N-POM. Rates of nitrogen fixation were calculated for both the trace metal amended samples and the control samples (using equations 2 and 3) with the natural abundance samples representing $A_{PN}^{t=0}$, $[PN]_0$, and $A_{TDN}^{t=0}$ in both calculations. Trace metal amended treatments were compared with control treatments to determine whether or not rates of fixation were elevated in amended samples.

Trace metal additions were used in combination with $^{15}N_2$-tracer assays to determine whether nitrogen fixation was metal-limited on seven occasions (Table C.1). A comparison of treatment and control samples indicated that there was no difference between rates in samples subject to trace metal amendment and those without (Wilcoxon Signed Rank Test, p=0.30, V=7). This suggests that nitrogen fixation was not trace metal-limited at the time of the experiments. Ambient concentrations of metals varied across the season in each sample lake (Figure C.1).

Table C.1 Rates of nitrogen fixation in metal addition treatments compared to control treatments.

		N_2 fixation (μg N L^{-1} h^{-1})	
Lake	DOY	Control	Treatment
Buffalo Pound	220	1.83	1.88
Buffalo Pound	227	1.06	0.70
Buffalo Pound	248	0.64	0.49
Wascana	262	0.00	0.00
Pasqua	241	3.05	1.18
Pasqua	255	0.05	0.50
Katepwa	224	0.71	0.38

Figure C.1 Time-series changes of metals over time in surface water sampled during the 2017 growing period in the Qu'Appelle River Watershed. Metals included (top-down): boron, vanadium, cobalt, selenium, strontium, molybdenum, aluminum, chromium, copper, zinc and barium, uranium.

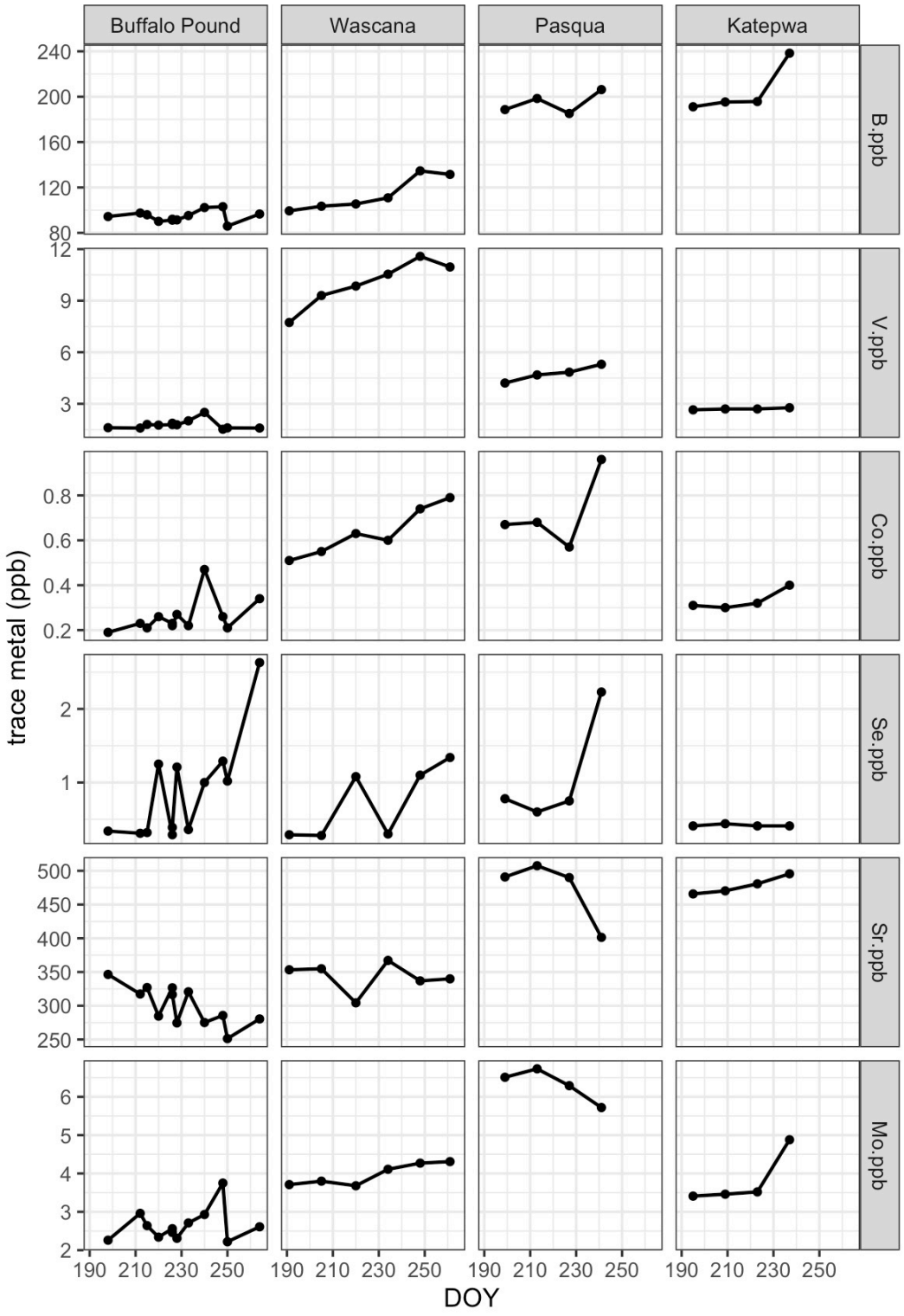
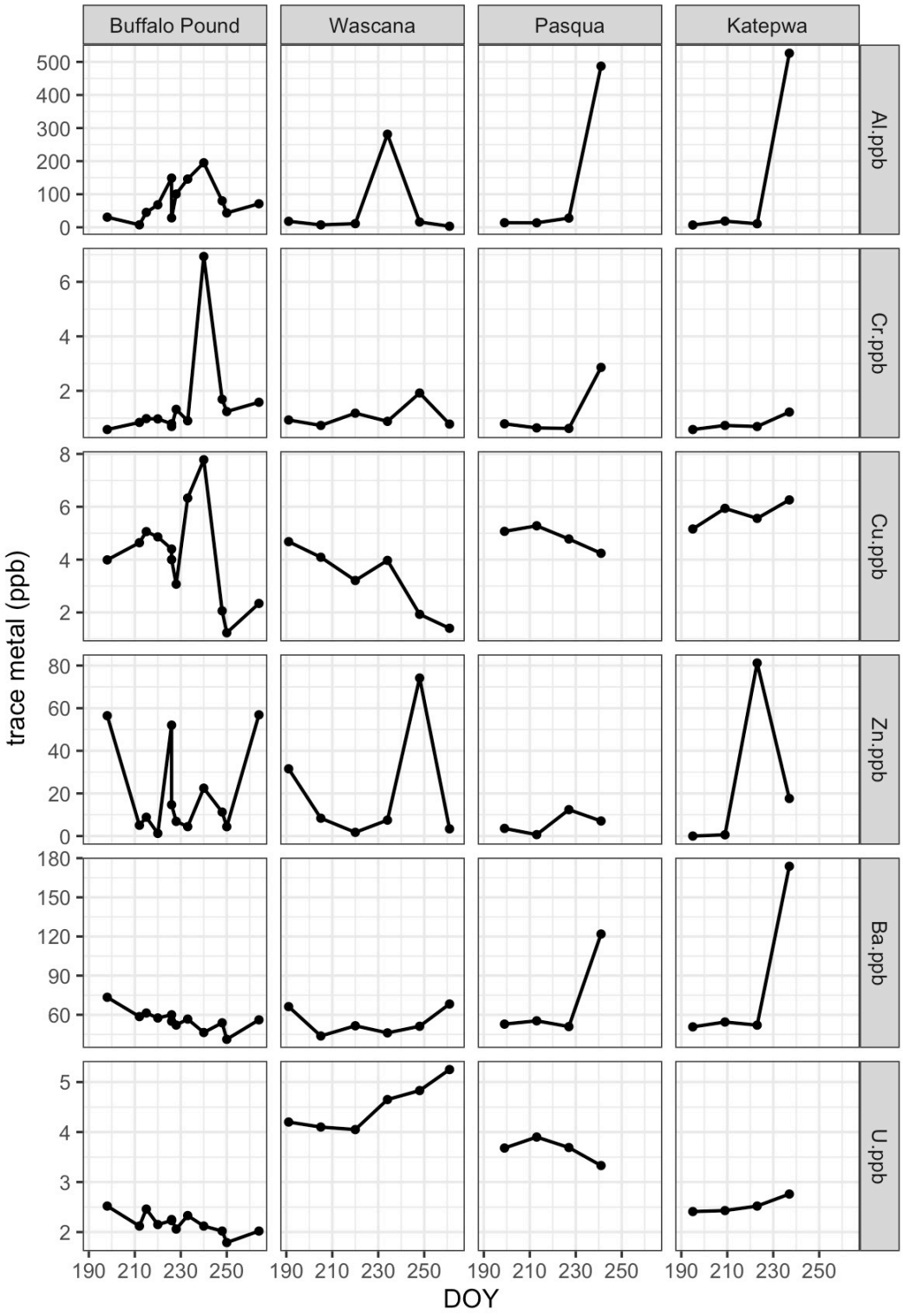


Figure C.1, cont'd. Time-series changes of metals over time in surface water sampled during the 2017 growing period in the Qu'Appelle River Watershed. Metals included (top-down): boron, vanadium, cobalt, selenium, strontium, molybdenum, aluminum, chromium, copper, zinc and barium, uranium.



Appendix D: Supplemental Information for Chapter 2

The supplemental information for Chapter 2 contains tables that provide additional information regarding the enrichment of filtrate from $^{15}N_2$-tracer bottle assays and basic descriptive statistics of nitrogen fixation (hourly, daily and seasonally volumetric and daily areal rates). This section also provides the mass and size distribution of inventoried heterocytes from each heterocytous taxa. Finally, it shows the time-series changes in phytoplankton groups over time by lake and time-series changes in heterocyte biovolume per unit volume by taxa.

Table D2.1 ^{15}N-enrichment in $^{15}N_2$-tracer bottle assay filtrate prior to (natural abundance) and following amendment ($^{15}N_2$) and incubation. Enrichment of filtrate was used to detect the potential release of nitrogen fixed during the incubation period.

Date (DOY)	Lake	Natural abundance	$^{15}N_2$-enriched
		$\delta^{15}N$ (AIR ± .3‰)	
17-Jul-17 (198)	Buffalo Pound	1.43	4.24
31-Jul-17 (212)	Buffalo Pound	8.67[1]	2.88
3-Aug-17 (215)	Buffalo Pound	1.34	19.54
8-Aug-17 (220)	Buffalo Pound	4.95[1]	6.21
14-Aug-17 (226)	Buffalo Pound	2.25	6.07
28-Aug-17 (240)	Buffalo Pound	5.29	5.09
5-Sep-17 (248)	Buffalo Pound	2.44	2.43
25-Aug-17 (237)	Katepwa	6.47	7.02
18-Jul-17 (199)	Pasqua	3.49	6.12
10-Jul-17 (191)	Wascana	2.95	4.39[1]
24-Jul-17 (205)	Wascana	3.08	4.30
21-Aug-17 (233)	Wascana	5.10	6.66
18-Sep-17 (261)	Wascana	9.49	8.76

[1] Sample has a higher percent nitrogen element content than the rest of the samples—based on sample weight.

Table D2.2 Basic descriptive statistics of nitrogen fixation rates assessed during the 2017 sampling season of this study:

D2.2A Measured hourly volumetric rates.

	Volumetric rates (measured hourly, reported in µg L^{-1} h^{-1})			
Lake	Average ± Std deviation	Median	Min	Max
Buffalo Pound	0.68 ± 0.9	0.27	0.0	3.71
Wascana	1.05 ± 2.2	0.06	0.0	8.74
Pasqua	0.24 ± 0.24	0.1	0.0	0.76
Katepwa	0.08 ± 0.05	0.07	0.0	0.17

Table D2.2B Estimated daily volumetric rates calculated based on measured hourly rates.

	Volumetric rates (Estimated daily[1], reported in µg L^{-1} d^{-1})			
Lake	Average ± Std deviation	Median	Min	Max
Buffalo Pound	10.0 ± 13.1	3.8	0.0	55.2
Wascana	16.6 ± 35.3	0.9	0.0	141.2
Pasqua	3.6 ± 3.7	4.5	0.0	11.4
Katepwa	1.1 ± 0.8	0.95	0.0	2.5

[1] Assumptions: i) rates are linear between points and ii) rates are consistent for 8 hours per day.

Table D2.2C Estimated daily areal rates calculated based on measured hourly rates at mean lake depth.

	Areal rates (estimated daily[2], reported in mg m^{-2} d^{-1})			
Lake	Average ± Std deviation	Median	Min	Max
Buffalo Pound	4.0 ± 3.73	2.1	0.0	12.8
Wascana	4.15 ± 8.26	0.35	0.0	31.1
Pasqua	3.6 ± 3.7	1.47	0.0	9.8
Katepwa	1.1 ± 0.8	1.64	0.0	2.7

[2] Assumptions: i) rates are linear between points, i) rates are consistent through the photic zone which is considered the distance from surface to secchi depth, iii) rates are constant during daylight hours, iv) daylight decreases linearly from July 10th (DOY 191, 16.15 hours) to September 21st (DOY 264, 12.27 hours).

Table D2.3 Estimated seasonal nitrogen fixation rates in 2017 including volumetric, areal rates and total nitrogen fixed for each study lake across the simultaneous sampling period from July 18 to August 25 (DOY 199 – 237) and total nitrogen fixed across individual sampling periods for each lake.

	Volumetric rates µg L^{-1}	Areal rates mg m^{-2}	Total nitrogen fixed Kg N	
Lake	DOY 199 – 237[3]	DOY 199 – 237[3]	DOY 199 – 237[3]	Full season[4]
Buffalo Pound	660.1	241.9	7135.4	8777.5
Wascana	320.3	92.1	46.1	147.4
Pasqua	202.3	170.7	3448.4	3475.1
Katepwa	49.1	68	1101.6	1111

[3] Comparable seasonal assumptions: i) rates are linear between points, ii) rates are constant during daylight hours, iii) daylight decreases linearly from July 10th (DOY 191, 16.15 hours) to September 21st (DOY 264, 12.27 hours).
[4] Date ranges of full season sampling: Buffalo Pound, July 6 – September 21 (DOY 187 – 264); Wascana, July 10 – September 18 (DOY 191 – 261); Pasqua, July 18 – September 12 (DOY 199 – 255); Katepwa, July 14 – August 25 (DOY 195 – 237).

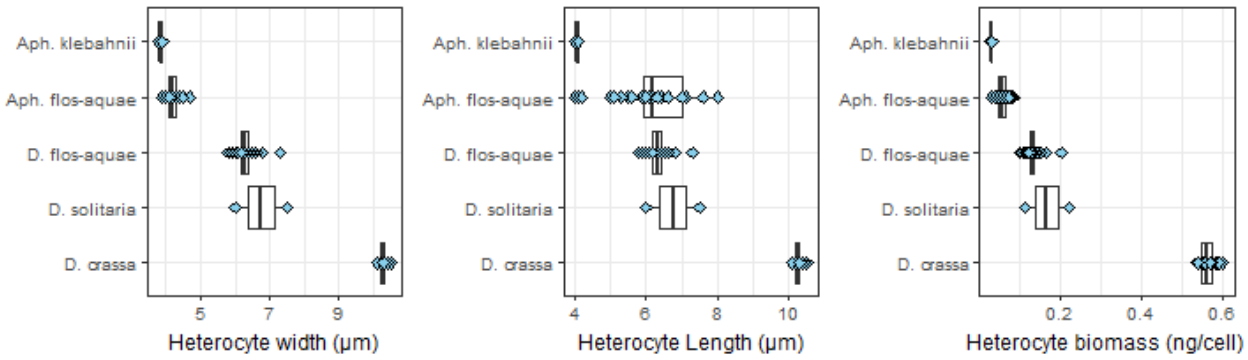


Figure D2.1 Distribution of heterocyte width, length and biomass by species. Blue points identify each sample point. The vertical line through the square identifies the median value; the boxes on the left and right of the line identifies the first and third quartiles, respectively. Of the 71 enumerated samples, the taxa *Aphanizomenon klebahnii* was observed in two (n=2), *Aphanizomenon flos-aquae* was observed in fifty-one (n=51), *Dolichospermum flos-aquae* was observed in seventy-one (n=71), *Dolichospermum solitaria* was observed in two (n=2) and *Dolichospermum crassa* was observed in thirty-two (n=32). All points are shown with blue points, and inside the boxes are the first and third quartiles with the median represented as the center line.

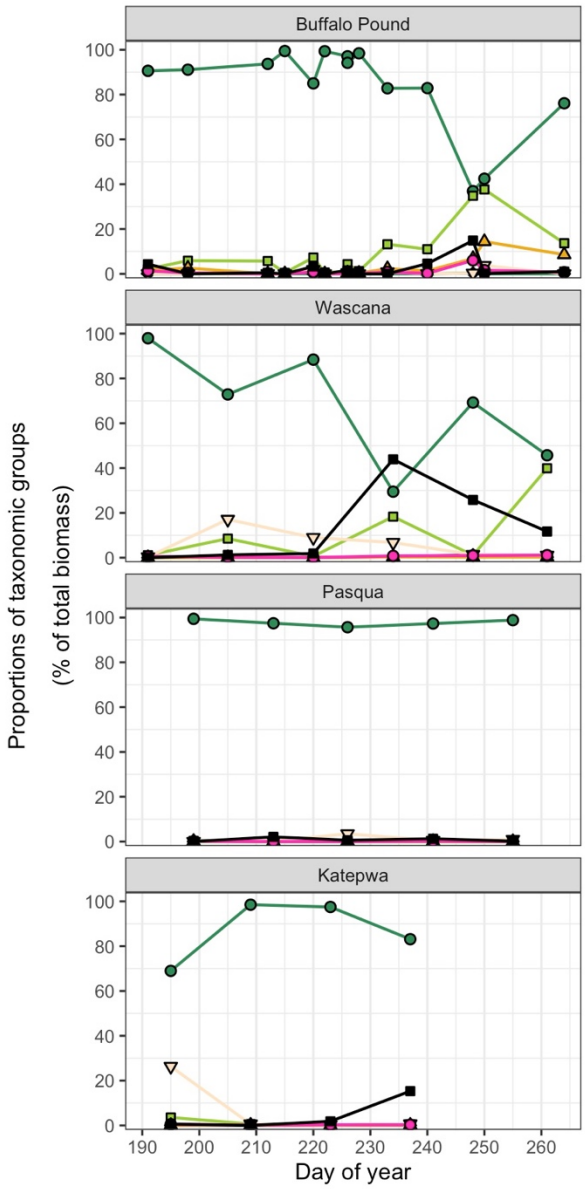


Figure D2.2 Changes in phytoplankton composition during the 2017 summer sampling season in Buffalo Pound Lake, Wascana Lake, Pasqua Lake and Katepwa Lake by taxonomic category as a percent of total biomass: cyanobacteria (green circles/lines), chlorophytes (light green squares/lines), chrysophytes (gold upward triangles/lines), cryptophytes (pink circles/lines), diatoms (beige downward triangles/lines) and dinoflagellates (black squares/lines).

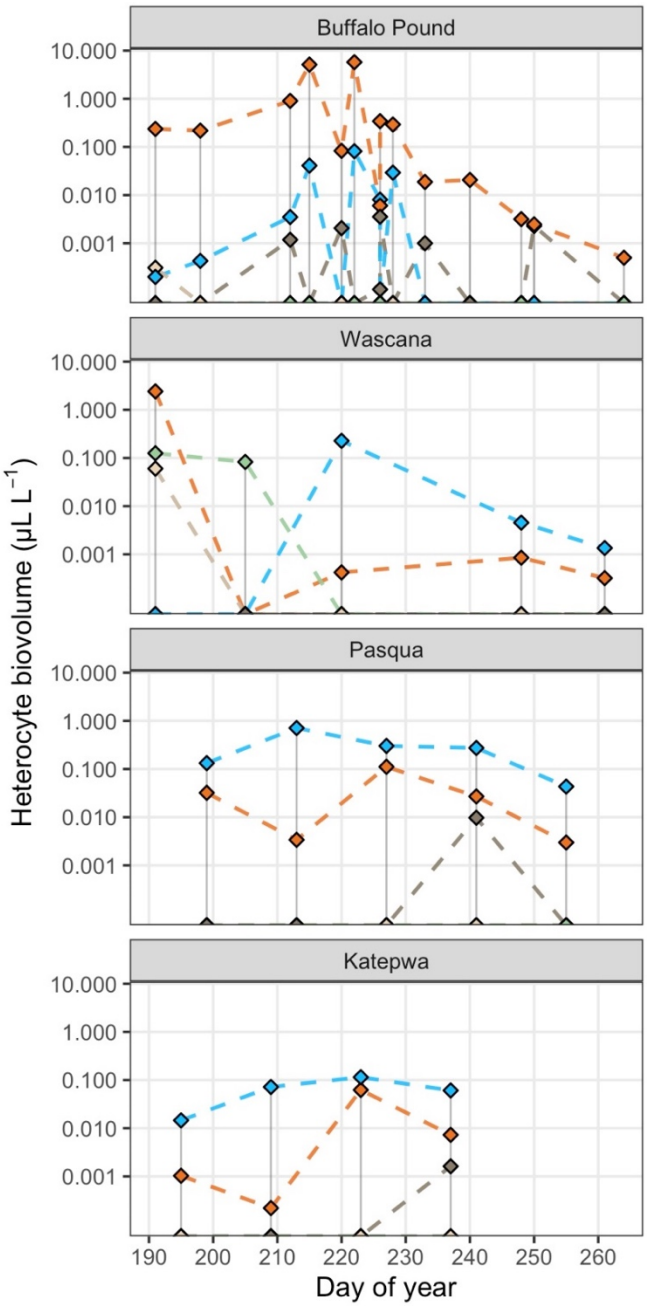


Figure D2.3 Variations in heterocyte biovolume and speciation across the 2017 summer sampling season in Buffalo Pound, Wascana, Pasqua and Katepwa Lakes: *Aphanizomenon flos-aquae* (blue), *Aphanizomenon klebahnii* (green), *Dolichospermum crassa* (brown), *Dolichospermum flos-aquae* (red) and *Dolichospermum solitaria* (beige). Points along the bottom of each plot indicate that the taxa was unobserved at the time of sampling.

Appendix E: Supplemental Information for Chapter 3

The supplemental information for Chapter 3 contains figures that provide additional information regarding the Buffalo Pound Lake monitoring buoy, including sensor models, operating range and sensor accuracy of the range for the full suite of weather station and water quality sensors and discrete measurements of parametres complimenting buoy data (e.g. secchi depth, water temperature and pH). This section also provides a time-series showing changes to surface turbidity with respect to wind speed and biological activity. This section also shows the composition of cyanobacterial biomass and heterocyte biovolume by taxa. And, it provides the monitoring buoy data that accompanies in-situ diel sampling. Finally, it compares the relationship between ethylene produced and nitrogen fixed in surface water samples compared to surface scum.

Table E3.1 Full characteristics of the Buffalo Pound Lake monitoring buoy: Weather station

Variable	Sensor	Height/depth (m)	Operating range	Sensor accuracy
Weather station		*Above water surface*		
Wind direction	Vaisala—WXT510/520	1.66	0 – 360°	± 3°
Wind speed	Vaisala—WXT510/520	1.66	0 – 60 m s^{-1}	± 3%
Air temperature	Vaisala—WXT510/520	1.66	-52 – 60 °C	± 3 °C
Relative humidity	Vaisala—WXT510/520	1.66	0 – 100 %RH	± 3%
Barometric pressure	Vaisala—WXT510/520	1.66	600 – 1100 hPa. Converted as 17.7 – 32.5 inHg	± 0.5% (0 – 30°C) ± 1% (-52 – 60°C)
PAR (air)	LiCor—L1-190	1.06	Not given directly	± 5%

Table E3.1 cont'd Full characteristics of the Buffalo Pound Lake monitoring buoy: Water quality

Variable	Sensor	Height/depth (m)	Operating range	Sensor accuracy
Water quality		*Below water surface*		
PAR	LiCor—L1-192	0.62, 0.78	Not given directly	± 5%
CO_2	Vaisala—GMP252	0.80	0 – 1,000 ppm	± 0.5%
Temperature	NexSens—T-Node FR	0.45, 0.77, 1.23, 2.18, 3.18	0 – 50 °C	± 0.53 °C
	YSI—Sonde 6600 YSI—Sonde 6820	0.82 2.85	-5 – 50 °C	± 0.15 °C
Specific conductivity	YSI—Sonde 6600 YSI—Sonde 6820	0.82 2.85	0 – 100 mS cm^{-1}	± 0.5%
pH	YSI—Sonde 6600 YSI—Sonde 6820	0.82 2.85	0 – 14 units	± 0.2
Turbidity	YSI—Sonde 6600	0.82	0 – 1000 NTU	± 2%
Chlorophyll	YSI—Sonde 6600	0.82	0 – 400 μg L^{-1}	0.5 μg L^{-1}
Chlorophyll RFU	YSI—Sonde 6600	0.82	0 – 100 RFU	N/A
Phycocyanin	YSI—Sonde 6600 YSI—Sonde 6820	0.82 2.85	0 – 280,000 cells mL^{-1}	± 160 cells mL^{-1}
Phycocyanin RFU	YSI—Sonde 6600 YSI—Sonde 6820	0.82 2.85	0 – 100 RFU	N/A
ODO saturation	YSI—Sonde 6600 YSI—Sonde 6820	0.82 2.85	0 – 500% air saturation	± 15%
ODO	YSI—Sonde 6600 YSI—Sonde 6820	0.82 2.85	0 – 50 mg L^{-1}	± 1.5 mg L^{-1}

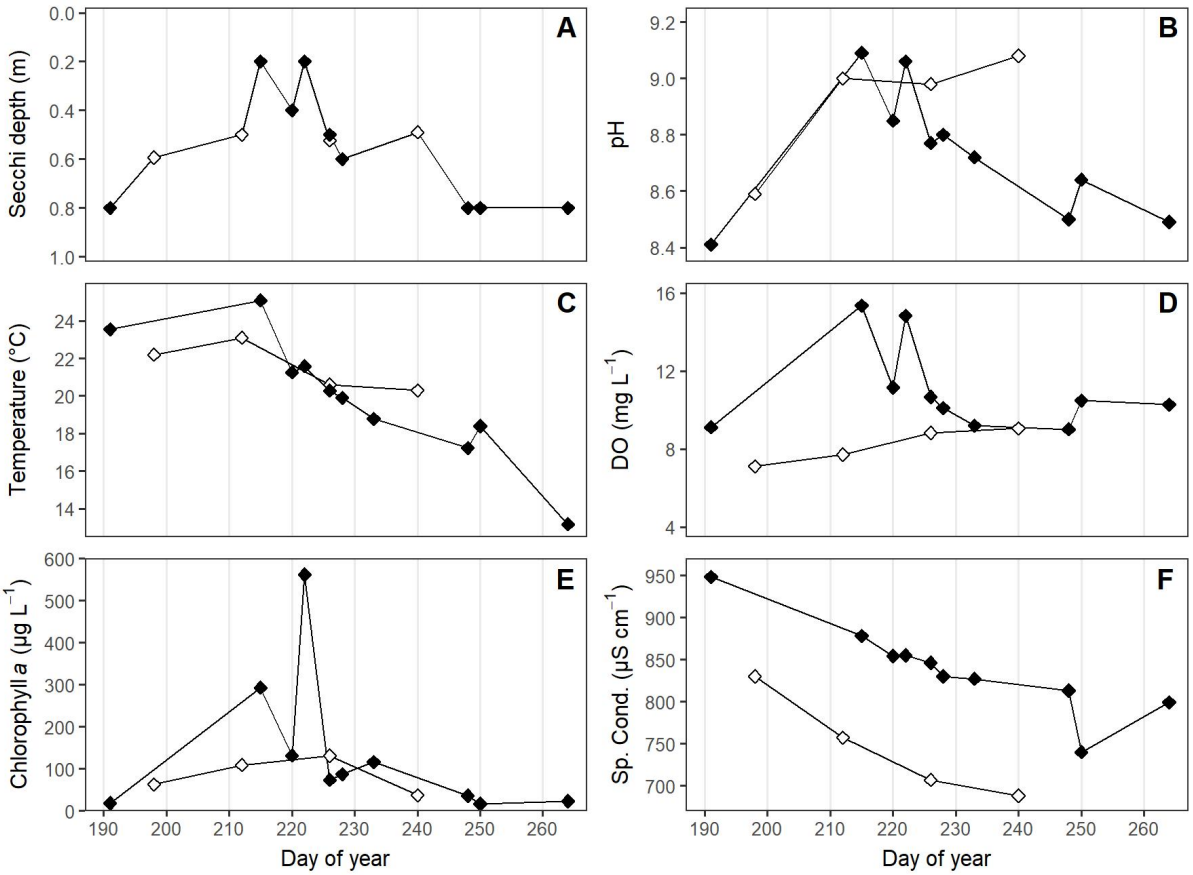


Figure E3.1 Time series measurements derived from discrete sampling from July 10 – September 21 (DOY 191 - 264), 2017. Black points indicate sample was collected from 0.1 m depth (adjacent to the high-frequency monitoring buoy) and white points from 0.3 m (north of the buoy). Values differ between sampling sites as a result of spatial variation within the lake. A) Secchi depth at time of collection (Note: y-axis is reversed to reflect the change in disk position), B) pH, C) temperature, D) dissolved oxygen (mg L^{-1}), E) chlorophyll *a* (µg L^{-1}), and F) specific conductivity (µS cm^{-1}).

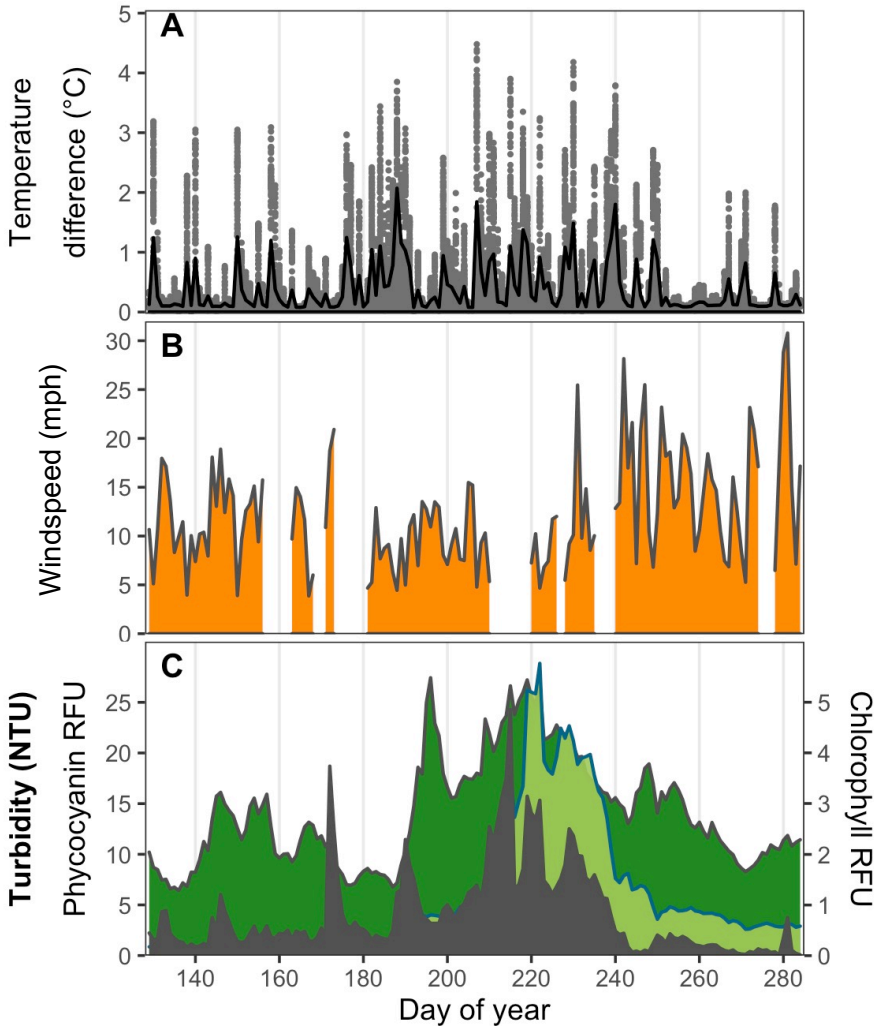


Figure E3.2 Time-series in-situ measurements of A) high-frequency (grey points) and mean daily (black line) temperature differences between 0.45 – 3.18 m depths; B) mean daily windspeeds—gaps indicate missing values; and C) overlay of mean daily turbidity (Formazin Nephelometric Turbidity Units, grey) with phycocyanin (relative fluorescence units, lime green) and chlorophyll (relative fluorescence units, dark green)—note primary y-axis (*inner left*) ranges from 0 – 25 RFUs of phycocyanin and the secondary y-axis (*right*) ranges from 0 – 5 RFUs of chlorophyll.

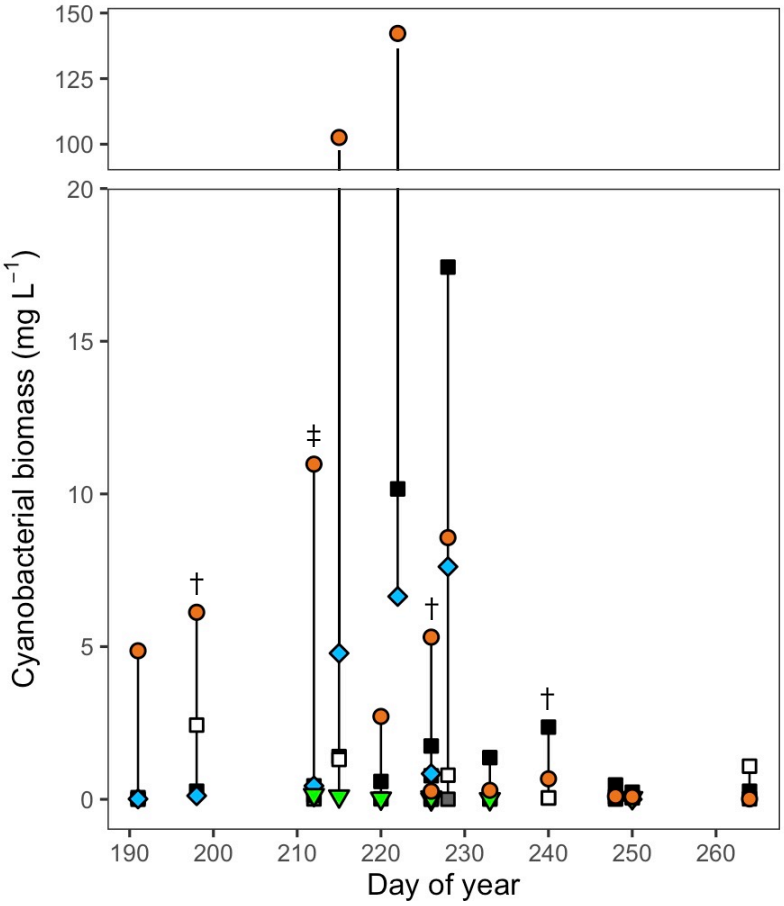


Figure E3.3 Time-series changes in cyanobacterial biomass by most observed taxa: heterocytous *Aphanizomenon flos-aquae* (blue diamonds), *Dolichospermum flos-aquae* (orange circles), and *Dolichospermum crassa* (green triangles), and non-heterocytous *Microcystis aeruginosa* (grey squares), *Planktothrix agardhii* (black squares), and *Woronichinia compacta* (white squares). Vertical lines represent a single sample containing multiple species. Samples were collected from a depth of 0.1 m adjacent to high-frequency monitoring buoy, 0.3 m north of the high-frequency monitoring buoy (†) and Buffalo Pound Water Treatment Plant intake near to the buoy (‡).

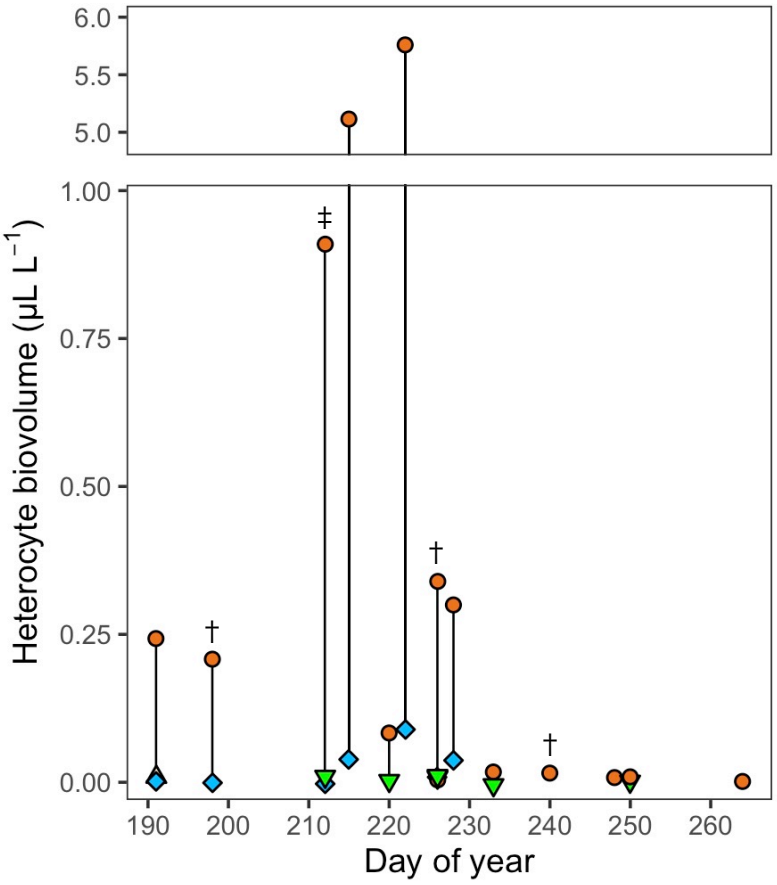


Figure E3.4 Time-series changes in heterocyte biovolume: *Aphanizomenon flos-aquae* (blue diamonds), *Dolichospermum flos-aquae* (orange circles), *Dolichospermum crassa* (green triangles) and *Dolichospermum solitaria* (single beige triangle on DOY 191). Vertical lines represent a single sample containing multiple species. Samples were collected from a depth of 0.1 m adjacent to high-frequency monitoring buoy, 0.3 m north of the high-frequency monitoring buoy (†) and Buffalo Pound Water Treatment Plant intake near to the buoy (‡).

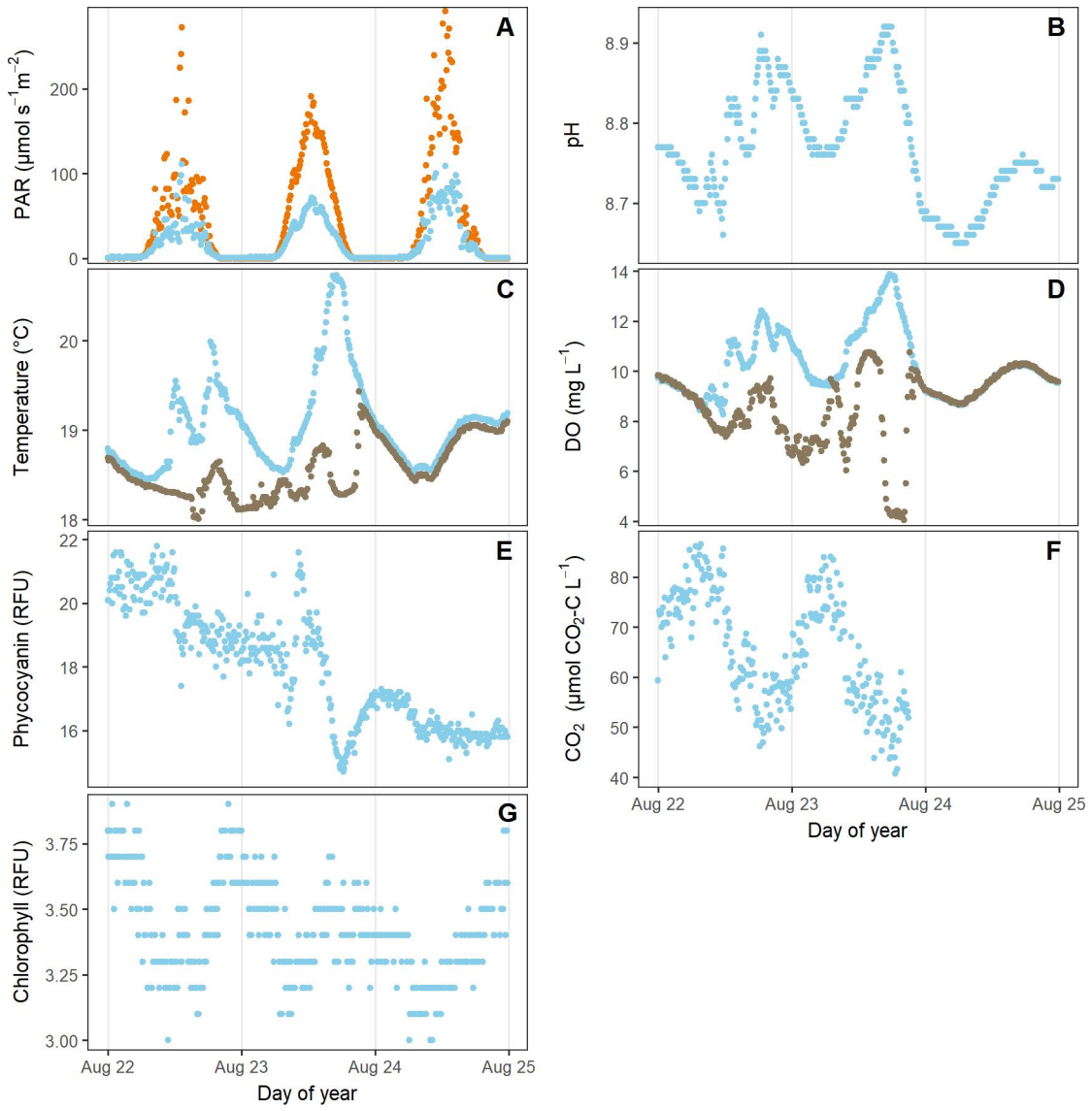


Figure E3.5 Detailed time series of high-frequency in-situ measurements for August 22 – 24 (DOY 234 – 236), 2017; during the major bloom phase. Gray vertical lines indicate 12:00 a.m. A) PAR at 0.62 m (orange) and 0.78 m (blue) below water surface. B) Temperature at 0.45 m (blue) and 3.18 m (brown) below water surface. C) Phycocyanin (relative fluorescence units) at 0.82 m. D) Chlorophyll (relative fluorescence units) at 0.82 m. E) pH at 0.82 m. G) Dissolved oxygen concentration at 0.82 m (blue) and 2.85 m (brown). F) Carbon dioxide concentration at 0.82 m. Note carbon dioxide sensor went offline on the evening of August 23 (DOY 235), 2017.

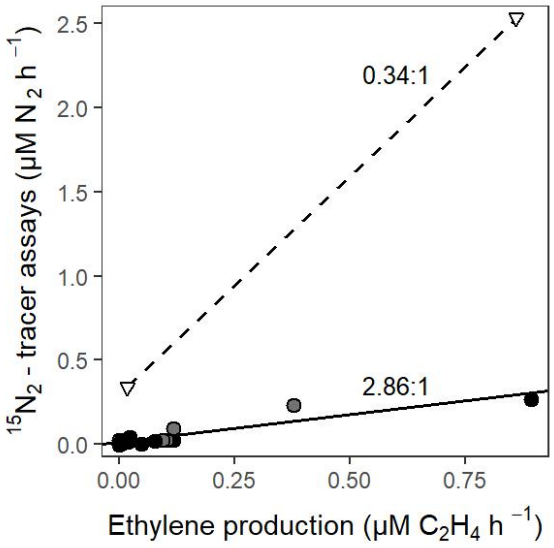


Figure E3.6 Relationship between ethylene produced and nitrogen fixed using acetylene reduction and ^{15}N-tracer assays, respectively, in surface water (black circles/solid line) and scum (hollow triangles/dotted line) sampled during the 2017 bloom season. Note simultaneous $^{15}N_2$-tracer and acetylene reduction assays were only performed twice using surface scum.