# TROPHIC STATE AND FACTORS RELATING TO PHYTOPLANKTON

# COMMUNITY COMPOSITION AND DISTRIBUTION IN LAKE

# DIEFENBAKER, SASKATCHEWAN, CANADA

Thesis Submitted to the College of

**Graduate Studies and Research** 

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Biology

University of Saskatchewan

Saskatoon

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### ABSTRACT

Planktonic algae are useful as indicators of water quality because their composition and distribution reflects environmental condition in lakes. Therefore, understanding their dynamics can aid certain water quality management goals. Lake Diefenbaker is a large mesotrophic reservoir in the Canadian Prairies. Approximately 98 % of its inflow is from the South Saskatchewan River. The composition and ecology of the phytoplankton community has not been reported comprehensively since the 1980s. This is a potential problem for a reservoir with multiple end users. Therefore, I collected epilimnetic whole water samples along its length from June to October in 2011 and in 2012. I examined the phytoplankton community and related their distribution to environmental factors. A total of 72 phytoplankton genera were observed with the chlorophytes having the highest number of genera (33). The increased nutrient load and non-algal turbidity associated with high inflow from the South Saskatchewan River may be related to the dominance of the cryptophytes and bacillariophytes (together constituting ~89 % of the total phytoplankton biomass). The cryptophytes were abundant during periods of high flow rates and thermal stratification whereas the bacillariophytes were abundant during cool, isothermal conditions.

Lake Diefenbaker is characterized by numerous embayments. Some of these embayments are exposed to human activities including development (housing, golf courses, marinas) and livestock operations (e.g., cattle watering). These localized activities could increase the frequency or size of algal blooms that will adversely affect the water quality. Therefore, I compared the phytoplankton community composition from eight exposed embayments, four unexposed embayments and six main channel sites.

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Phytoplankton community compositions were not significantly different in exposed, unexposed embayments and main channel sites (P > 0.05). High flows may have overridden localized influence from embayments. Hence, similar environmental conditions were present in the embayments and main channel.

Blooms of cyanobacteria are of concern because of the potential of some genera to produce cyanotoxins. I examined cyanobacteria in Lake Diefenbaker. Cyanobacterial biomass was low in Lake Diefenbaker (< 5 %). However, I observed some potential toxin and bloom-forming genera that may threaten the water quality under different environmental conditions in the future.

#### ACKNOWLEDGEMENTS

I would like to thank my supervisor Jeff Hudson for his patience, assistance, support and guidance throughout my degree. I am also very grateful to my committee members: John-Mark Davies, Mark Wickstrom, and John Sheard for their contribution and time toward my degree.

I am also very grateful to members of Hudson's lab: Jeff Sereda, Jessica Johansson, Kerry Head, Kristine Hunter, Chance Prestie, Rebecca North and David Vandergucht. I thank Hedy Kling, Paul Hamilton and Francis Pick for their assistance during my long hours of counting and identifying phytoplankton.

I am also grateful to my mother Mrs. Christiana Abirhire and my siblings for their prayers and support. I would like to acknowledge and thank the Delta State Government and the University of Benin, Nigeria for granting me study leave to undertake this degree.

I thank the Department of Biology, University of Saskatchewan, Global Institute for Water Security and Saskatchewan Watershed Agency for scholarship support during my degree.

Finally, I dedicate this thesis to the late Rev. A.A. Abirhire.

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#### **CHAPTER 1- General Introduction**

#### **1.1 Phytoplankton**

Planktonic algae are photosynthetic, oxygen-releasing micro-organisms, which include prokaryotic and eukaryotic forms that float freely in the water column (Salmaso et al., 2015; Bellinger and Sigee, 2010). The term "phytoplankton" has been often used to describe microalgae found in aquatic ecosystems. Reynolds (2006) used the term "limnoplankton" to describe phytoplankton found in lakes and reservoirs. Phytoplankton found in lakes and reservoirs are either meroplankton (planktonic algae that spend part of their life cycle in the sediments and are eventually recruited into the water column) or holoplankton (planktonic algae that are always suspended in the water column) (Kalff, 2002).

Approximately 4000 to 5000 planktonic algal species are believed to be present in freshwater lakes and reservoirs (Reynolds, 2006). Common planktonic algal divisions found in lakes and reservoirs include the eukaryotic Bacillariophyta (diatoms), Chlorophyta (green algae), Chrysophyta (golden algae), Cryptophyta (cryptomonads), Euglenophyta (euglenoids) and Pyrrhophyta (dinoflagellates) and the prokaryotic Cyanophyta (blue-green algae). A taxonomic understanding of phytoplankton in lakes and reservoirs can be used to achieve certain water quality management goals.

Light microscopy is the oldest and the most commonly used method for identifying phytoplankton. Light microscopy together with taxonomic keys allows for phytoplankton identification to species level (Bellinger and Sigee, 2010; Brook et al 2002; Wehr and Sheath, 2003). In addition, light microscopy provides the means to calculate the biovolume or biomass of the different planktonic algal groups. This is achieved by measuring and fitting each taxon to a geometric shape and a mathematical equation (Hillebrand et al., 1999).

#### **1.2 Ecological importance of phytoplankton**

Planktonic algae are the most important component at the base of the food web contributing to primary production in lakes and reservoirs (Lv et al., 2014; Winder and Sommer, 2012). They are also useful as indicators of water quality (Padisák et al., 2006) because their spatial and temporal patterns reflect both short- and long-term environmental conditions in lakes and reservoirs (Salmaso, 2010). For instance, paleolimnological studies use diatoms to track water quality changes attributed to climate change (Rúhland et al., 2008) and nutrient enrichment (Hall and Smol, 2010). Other studies have used the presence of cyanobacteria to indicate nutrient enrichment from anthropogenic activities (Rejmánková et al., 2011). Blooms of cyanobacteria are of concern because certain species are known to synthesize secondary metabolites that produce taste and odour in drinking water and "cyanotoxins" that pose serious health risks to humans and livestock (Landsberg, 2002; Izaguirre and Taylor, 2004). Therefore, understanding phytoplankton ecology requires monitoring environmental factors that drive their growth and distribution.

#### **1.3 Factors affecting phytoplankton distribution and abundance**

Several factors such as nutrients, light, temperature, pH, flushing rate, disease (e.g., bacteria, fungi and virus) and grazing pressure affect the growth, distribution, and composition of phytoplankton in lakes and reservoirs (Torremorell et al., 2009). Phosphorus (P) and nitrogen (N) are the main nutrients limiting the growth of phytoplankton (Reynolds, 2006). For instance, a low N:P ratio may favor the dominance of N-fixing cyanobacteria whereas a high N:P ratio may favor the dominance of non Nfixing cyanobacteria (Schindler et al., 2008; Paerl et al., 2014). Iron (Fe) is required by phytoplankton for chlorophyll production, but cyanobacteria use Fe specifically for N fixation and N assimilation (Wilhelm 1995; Molot et al., 2010). Silica is required for the growth of diatoms (Lampert and Sommer, 1997).

Hydrologic variables such as inflow rates influence water residence time, nutrient loading and turbidity, which in turn affect the distribution and growth of phytoplankton (Borges et al., 2008). For instance, inflow may carry suspended sediments that increases the turbidity and affects the light available for phytoplankton growth and photosynthesis in reservoirs (Straskraba, 1999). This in turn will affect the phytoplankton community composition by shifting it in favour of planktonic algae that can tolerate low light conditions (Obertegger et al., 2007). For example, the cryptophytes possess unique photosynthetic and accessory pigments, and mixotrophic habit that enable them to grow and reproduce under low light conditions (Tardio et al., 2003). High flows may wash out phytoplankton with loss exceeding growth rate (Roelke et al., 2010; Reynolds, 1990). In addition, inflow rates influence the water residence time and nutrient load in reservoirs, which in turn affects the phytoplankton community composition (Schindler, 2006). For examples, lakes and reservoirs with low flushing rates and high nutrient concentrations are mostly dominated by cyanobacteria (Huszar and Reynolds, 1997).

The growth rates of phytoplankton are affected by water temperature (Paerl et al., 2011). Most planktonic algae achieve maximum growth at a water temperature of about 20°C (Knappe et al 2004). For example, maximum growth for the cryptophytes occurs at 23.5  $^{0}$ C (Morgan and Kalff, 1979). The cyanobacteria can tolerate and out-compete other

phytoplankton groups such as cryptophytes, dinoflagellates and chlorophytes as water temperature increases above 25 °C (Paerl et al., 2011). Conversely, diatoms can tolerate low water temperature and are abundant in temperate lakes and reservoirs in fall and spring when water temperatures are cooler (Winder and Sommer 2012). Temperature also affects the intensity and duration of stratification with implications for phytoplankton community composition (Peeters et al., 2007). Planktonic algae that can regulate their position, including buoyant cyanobacteria and phytoflagellates (e.g., cryptophytes, dinoflagellate, chrysophytes) are favoured over non-buoyant planktonic algae (e.g., diatoms) during periods of thermal stratification (Jöhnk et al., 2008). Because of their dense siliceous cell wall, diatoms depend on mixing events to remain suspended in the water column and often thrive during periods of complete mixing (Winder and Sommer 2012).

Grazing by zooplankton is another factor that affects the abundance and distribution of phytoplankton. For instance, zooplankton excrete nutrients that phytoplankton can use for their growth (Grigorszky et al., 1999; Mazumder, 1994). Intense grazing can result in a clear water phase in lakes and reservoirs after spring runoff (Kalff, 2002). Furthermore, zooplankton grazing can be selective on the phytoplankton community. For instance, planktonic algae like the cryptophytes are consumed by zooplankton because they are easily ingested and contain a high proportion of certain essential fatty acids (Barone and Naselli-Flores, 2003), whereas cyanobacteria are less preferred food because they aggregate into colonies or filaments (DeBernardi and Giusanni, 1990), their biomass is of low nutritional value and many species produce toxins (Landsberg, 2002).

In general, nutrient enrichment and climate change are recognized as the major factors that may shift phytoplankton community composition in favour of cyanobacteria resulting in poor water quality (Brookes and Carey, 2011).

#### **1.4 Eutrophication and algal blooms**

Eutrophication is the release of nutrients into water bodies and it is a primary water quality problem because it encourages algal blooms (Smith and Schindler, 2009). The outcome of these blooms includes reduced dissolved oxygen concentrations and fish kills from anoxia (Smith et al., 2008; Schindler et al., 2008). In addition, some planktonic algae produce taste and odor and toxins (e.g., certain species of cyanobacteria species (Landsberg, 2002; Izaguirre and Taylor, 2004). The ecological and economic impacts of these algal blooms are significant because of deteriorating habitat, loss in biodiversity at all trophic level, losses in tourism and high water treatment cost (de Figueiredo et al., 2006; Dodds et al., 2009).

Human activities, including urban development and agricultural practices, have exacerbated the eutrophication of lakes and reservoirs by releasing nutrients into water bodies (Carney, 2009; Jeppesen et al., 2007; Lehman, 2014; Michalak et al., 2013). For example, Katsiapi et al. (2012) found a relationship between phytoplankton community composition and land use activities in 11 lakes and 7 reservoirs in Greece. According to the authors, cyanobacteria were mostly associated with artificial (e.g., non agricultural vegetated areas) and agricultural land use type, chrysophytes were associated with forested areas and chlorophytes were found in industrial and commercial land use type. Paul et al. (2012) also found a strong relationship between cyanobacteria and pastoral land and a strong relationship between chlorophytes and native forest and urban land use

in 11 lakes in New Zealand. Therefore, a good understanding of different nutrient sources and their influence on the phytoplankton community composition and distribution is important for proper management of reservoirs.

#### 1.5 Climate change and algal blooms

There is a significant increase in the temperature of lakes around the world, which is attributed to climate warming (Schindler and Smol, 2006). Climate change influences chemical and physical conditions of lakes, which in turn affects phytoplankton community composition (Kalff, 2002; Schindler and Smol, 2006). For example, climate warming may influence the intensity and duration of precipitation and drought (Paerl and Huisman, 2009). Intense precipitation will increase surface runoff from the watershed and increase nutrient loading in receiving lakes and reservoirs (Paerl and Huisman, 2009; Jeppesen et al., 2011). According to Paerl and Huisman (2009) runoff events after intense precipitation will washout and suppress the growth of cyanobacteria (Grover et al. 2011) But, if drought conditon (reduced water discharge and increased water residence time) follows high nutrient load, cyanobacterial blooms would be encouraged (Paerl and Huisman, 2008).

An increase in temperature will intensity and extends stratification in lakes and reservoirs (Peeters et al., 2007; Adrian et al., 2009). Extended thermal stratification may lead to the development of anoxic conditions in the hypolimnion and encourage internal loading of nutrients (phosphorus) from the sediments that may promote cyanobacterial blooms and affect water quality (Nurnberg 2009). Hence, empirical models for water quality should incorporate weather related variables such as flow rates and water temperature.

#### **1.6 Rationale and objectives**

Lake Diefenbaker is a large reservoir in the Canadian Prairies that receives about 98 % of its water from the South Saskatchewan River. Extensive studies on the phytoplankton community composition are limited. Royer (1972) reported that the diatoms dominated the phytoplankton from June to August in Lake Diefenbaker from 1967 to 1969. The cyanobacteria were the dominant group from July to October in 1984 and in June in 1985 (Saskatchewan Environment and Public Safety and Environment Canada [SEPS and EC], 1988). Siliceous algae (mainly diatoms and some chrysophytes) and cryptophytes dominated the phytoplankton in the Qu'Appelle arm of the reservoir from May to August from 1995 to 2003 (McGowan et al., 2005). Unfortunately, the dynamics of the phytoplankton community have not been extensively related to environmental factors.

Lake Diefenbaker is characterized by numerous embayments exposed to human activities such as development (housing, golf courses, marinas) and livestock operations (e.g cattle watering/wading). These localized activities are potential sources of nutrients to the reservoir. The influence of these localized human activities on the phytoplankton community in Lake Diefenbaker has not been investigated. Residents living along Lake Diefenbaker have complained about the occurrence of episodic algal blooms (Soggie, 2011), especially in the arms of the reservoir (Hecker et al., 2012). Therefore, the specific objectives of this study were

1. To characterize the trophic status of the reservoir

- 2. To examine the spatial and the temporal distribution of the major phytoplankton groups during the open water period of 2011 and 2012 and relate their distribution to environment factors.
- 3. To compare phytoplankton community composition and biomass of exposed and unexposed embayments and main channel sites in the reservoir.

# CHAPTER 2- Environmental factors influencing phytoplankton communities in Lake Diefenbaker, Saskatchewan, Canada.<sup>1</sup>

#### 2.1 Introduction

Planktonic algae are useful indicators of water quality (Padisák et al., 2006) because their spatial and temporal patterns reflect both short- and long-term environmental changes in lakes and reservoirs (Salmaso, 2010). For instance, the bacillariophytes have been used in paleolimnological studies to track water quality changes attributed to climate change (Rühland et al., 2008) and nutrient enrichment (Hall and Smol, 2010). Other studies have used the presence of cyanobacteria to indicate nutrient enrichment from anthropogenic activities (Rejmánková et al., 2011). Moreover, cyanobacterial blooms in freshwater lakes and reservoirs are of concern because certain species have been reported to produce compounds (e.g., geosmin and cyanotoxins) that affect water quality (Izaguirre and Taylor, 2004; Landsberg, 2002).

Several environmental factors, including nutrients, light, temperature, and grazing pressure affect the growth, distribution, and composition of phytoplankton communities in lakes and reservoirs (Torremorell et al., 2009). Reservoirs often have pronounced spatial gradients in these environmental factors, resulting in reservoirs being characterized into three general zones: riverine, transitional, and lacustrine (Kimmel et al., 1990). Changes in phytoplankton abundance and composition along nutrient gradients (especially phosphorus [P] and nitrogen [N]) have been reported (Reynolds, 2006). For

<sup>&</sup>lt;sup>1</sup> This chapter has been accepted for publication in J. Great Lakes Res. Abirhire, O., North, R., Hunter, K., Vandergucht, D., Sereda, J. and Hudson, J. There may be overlap with other chapters.

instance, P limitation or N limitation, particularly in the lacustrine zone, may favour non N-fixing or N-fixing cyanobacterial growth (Paerl et al., 2011; Schindler et al., 2008). Other secondary nutrients are also important for the growth and distribution of phytoplankton. For example, silica is essential for the growth of bacillariophytes (Lampert and Sommer, 1997).

In addition to nutrients, hydrological variables such as inflow rates and water residence time play an important role in regulating the growth, biomass, and composition of phytoplankton (Borges et al., 2008). River inflow often carries suspended sediments into reservoirs that increase turbidity and in turn affect the light available for phytoplankton growth and photosynthesis (Straskraba, 1999; Yip et al., 2014; Zohary et al., 2010). This may shift the phytoplankton composition in favour of planktonic algae that can tolerate low light conditions (Obertegger et al., 2007). For instance, cryptophytes have been reported to grow and reproduce under low light conditions due to their unique photosynthetic and accessory pigments (Tardio et al., 2003). In addition, cryptophytes possess flagella that enable them to regulate their position in the water column for optimum light conditions (Reynolds, 2006). High inflow may wash out phytoplankton (Roelke et al., 2010) and reduce their reproductive capacity (Reynolds, 1990). Furthermore, inflow also affects water residence time in reservoirs and nutrient loading (Schindler, 2006). Several studies have reported that lakes and reservoirs with low flushing rates are often dominated by cyanobacteria (Huszar and Reynolds, 1997).

Scientists have detected a significant increase in the temperature of lakes around the world, which they attribute to climate warming (Schindler and Smol, 2006).



Figure 2.1. The location of main channel sampling sites (squares) on Lake Diefenbaker (SK). Sites are labeled from upstream (Highway 4 Bridge) to downstream (arms) from June to October 2011 and 2012. M3 and M5 are upstream main channel sites, C1-M, C2-M and C3-M are associated cattle main channel sites, U1-M, U2-M, and U3-M are associated urban main channel sites, and F4-M is the associated fish-farm main channel site.

Climate warming will affect the intensity and duration of thermal stratification in lakes and reservoirs (Adrian et al., 2009) with implications for phytoplankton community composition (Peeters et al., 2007; Winder and Schindler, 2004). Buoyant cyanobacteria and phytoflagellates (e.g., cryptophytes) are favoured over non-buoyant planktonic algae (e.g., bacillariophytes) during periods of thermal stratification because they are able to regulate their position in the water column for optimum nutrient and light conditions (Jöhnk et al., 2008). Conversely, bacillariophytes often thrive during periods of complete mixing of the water column, because their dense siliceous cell wall causes them to sink during thermal stratification (Winder and Sommer, 2012).

Water temperature affects the growth rates of most planktonic algae (Paerl et al., 2011). Maximum growth rates are achieved by most phytoplankton at a water temperature of about 20°C (Knappe et al., 2004). For instance, the cryptophytes have been shown to achieve maximum growth at 23.5 <sup>o</sup>C (Morgan and Kalff, 1979). But as water temperatures increase above 25 °C, the cyanobacteria achieve maximum growth rates and may out-compete other phytoplankton groups such as cryptophytes, dinoflagellates and chlorophytes (Paerl et al., 2011). Conversely, bacillariophytes prefer colder water and are abundant in temperate lakes and reservoirs in fall and spring when water temperatures are cooler (Winder and Sommer, 2012).

Lake Diefenbaker is a large river-connected reservoir in the Canadian Prairies (Fig. 2.1). It receives about 98 % of its water from the South Saskatchewan River and serves as an important source of water for domestic consumption, irrigation, recreation, aquaculture, and power generation in southern Saskatchewan (Saskatchewan Water Security Agency, 2012). Despite its importance, extensive studies on the phytoplankton

community composition in Lake Diefenbaker are limited. The bacillariophytes dominated the phytoplankton from June to August in Lake Diefenbaker from 1967 to 1969 (Royer, 1972). Cyanobacteria dominated during the open water season from July to October in 1984, and in June in 1985 (Saskatchewan Environment and Public Safety and Environment Canada [SEPS and EC], 1988). McGowan et al. (2005) reported that siliceous algae (mainly bacillariophytes and few chrysophytes) and cryptophytes dominated the phytoplankton in the Qu'Appelle arm (Fig. 2.1) of the reservoir from May to August from 1995 to 2003. Unfortunately, a comprehensive analysis relating the water column phytoplankton community composition to environmental factors has been absent in Lake Diefenbaker.

Moreover, residents living along Lake Diefenbaker have complained about the occurrence of episodic algal blooms. Blooms have been reported, especially in the arms (Hecker et al., 2012; Fig. 2.1). Therefore, the specific objectives of this study were to 1) characterize the trophic status of the reservoir; 2) examine the spatial and the temporal distribution of the major phytoplankton groups; and 3) relate the phytoplankton distribution to environmental factors during the two years of study (2011 and 2012). Such information will improve and add to the knowledge of the extant phytoplankton population and distribution. It will also provide information on the cyanobacterial abundance which is essential for the management of Lake Diefenbaker.

## **2.2 Materials and Methods**

#### 2.2.1 Sampling sites

Lake Diefenbaker (51° 1'53"N, 106° 50'9"W) (Fig. 2.1) was created by the construction of dams in the Gardiner and Qu'Appelle arms in 1967 (Saskatchewan Water

Security Agency, 2012). The reservoir has a length of approximately 182 km and a width of 2-3 km. The volume and surface area of reservoir are approximately 9 km<sup>3</sup> and 394 km<sup>2</sup>, respectively (Sadeghian et al., submitted manuscript). The mean depth of the reservoir is 22 m and the maximum depth is 59 m near the Gardiner Dam.

During the open-water seasons, water samples were collected within the epilimnion from a 2 m depth (this represents the mixing zone that is relatively homogenous) at 9 sites located down the length of the reservoir (Fig. 2.1). Each site was sampled once every month from June to October. We avoided sampling in the month of May because of the presence of ice-cover. All water samples were collected with a Van Dorn sampler (6.4 L), poured into 20 litre poly-bags and kept in the dark in coolers. Water samples were returned to the laboratory at the University of Saskatchewan and stored at ambient condition (light and temperature) until processed for water chemistry the following day. Water samples that were collected for phytoplankton analysis were fixed immediately in 1% Lugol's solution.

#### **2.2.2 Physical variables**

Water temperature (WT), pH, conductivity (SpCon) and dissolved oxygen concentrations (DO) were measured using a YSI 6600 v2 multi-parameter sonde. The mixing depth ( $Z_{mix}$ ) was defined as the depth from the water surface to a point where the temperature change was greater than 0.5°C/m. A biospherical radiometer (Biospherical Instruments Inc. BIC 2104 submersible radiometer) was used to measure photosynthetically active radiation (PAR). The vertical extinction coefficient ( $k_d$ ) was derived from the linear regression of the natural logarithm of PAR with depth (Kirk, 2003). The euphotic depth ( $Z_{eu}$ ) was estimated as the depth from the water surface to the depth where the light intensity is 1 % of the intensity above the water surface. All of these variables were related to samples collected from a depth of 2 m. In addition, Secchi depth (SD) was used to estimate water transparency. Rates of inflow into the reservoir were obtained from Hudson and Vandergucht (2015).

#### 2.2.3 Water chemical variables

Water chemistry samples were collected at the 2 m depth at each date and analyzed. The method of Parsons et al. (1984) was used to determine total phosphorus (TP), total dissolved phosphorus (TDP) and dissolved reactive phosphorus (DRP). Total nitrogen (TN), total dissolved nitrogen (TDN) and nitrate  $(NO_3)$  were measured using second derivative spectroscopy (Bachmann and Canfield, 1996). The phenol-hypochlorite method (Stainton et al., 1977) was used to determine ammonium  $(NH_4^+)$  concentrations colorimetrically. Samples for TDP, DRP, TDN,  $NO_3^-$ , and  $NH_4^+$  were filtered through 0.2 µm polycarbonate filters using syringe filtration. Samples for dissolved organic carbon (DOC) were measured using an organic carbon analyzer (Shimadzu TOC – 5050A) as described in Sereda et al. (2012). The particulate nitrogen (PN) was measured using an ANCA-GSL sample preparation unit coupled to a Tracer 20 mass spectrometer as reported in Vandergucht et al. (2013). Particulate phosphorus (PP) was calculated by difference (TP - TDP = PP). Then, the particulate nitrogen to phosphorus molar ratio (PN:PP) was derived. Chlorophyll a (chl a) samples were collected on 47 mm GF/F filters with vacuum filtration (10 psi) under low light conditions. Pigments were extracted and analyzed according to Bergmann and Peters (1980) and the absorbance read at 665nm as described in Vandergucht et al. (2013).

#### 2.2.4 Trophic state indices and phytoplankton identification and counting

Trophic status of Lake Diefenbaker was estimated using Carlson's (1977) trophic state indices for chlorophyll *a* (TSI<sub>chl *a*</sub>), Secchi depth (TSI<sub>SD</sub>) and total phosphorus (TSI<sub>TP</sub>) (TSI values < 40 = oligotrophy; 40 – 50 = mesotrophy; 50 – 70 = eutrophy; and 70 – 80 = hypereutrophy). Preserved phytoplankton samples collected at 2m depth were settled in a settling chamber. Settled phytoplankton were identified and counted on an Olympus inverted (IX51) microscope using the technique of Utermöhl (1958). Each taxon was identified to genus level with the use of several keys (Bellinger and Sigee, 2010; Brook et al., 2002; Wehr and Sheath, 2003). Fields of view in transects (each transect represents a diameter of the counting chamber) were counted until a minimum of 400 cells were enumerated for each sample. I used image-Pro Analyser 7.0 computer software to estimate the size of the phytoplankton and used a computerized phytoplankton counting program "Algamica (Version 4.0)" developed by Gosselain and Hamilton (2000) to calculate final biomass of each taxon.

#### 2.2.5 Data analyses

I only analyzed phytoplankton groups that contributed > 10 % to the total phytoplankton biomass, which were the bacillariophytes and cryptophytes. Hence, I investigated the relationship between environmental factors and the biomass of bacillariophytes and cryptophytes using multiple linear regression (MLR) analyses. I reduced the number of predictor variables in our MLR by selecting variables that were correlated to the biomass of the bacillariophytes or the biomass of the cryptophytes at p < 0.1. In addition, when two or more covariates were significant, the strongest covariate was selected. The environmental predictors considered in our model for the

bacillariophytes were hydrologic (inflow), physical (WT,  $Z_{mix}$ ) and chemical (pH, TP, NH<sub>4</sub><sup>+</sup>, DOC and PN:PP). The environmental predictors considered in our model for the cryptophytes hydrologic (inflow), physical (WT,  $k_d$ ) and chemical (pH, TN, TP, NO<sub>3</sub>, DOC and PN:PP). Rows with missing values were removed from the data set. Dependent variables (bacillariophyte biomass and cryptophyte biomass) were transformed to meet assumptions of parametric statistics. Statistical significance was set at an alpha level of 0.05. I used second order Akaike's Information Criterion (AICc) from the package MuMIn (Barton, 2011) to select our best MLR models. Here we used *cor2pcor* to calculate partial correlates (Opgen-Rhein and Strimmer, 2007) and vif to estimate the variance inflation factor (VIF), a method to check for collinearity between predictor variables used in the model (Heiberger and Holland, 2004). VIF values greater than 5 are evidence of collinearity. However, large VIF values can be tolerated if all of the model coefficients were significantly different from zero (Heiberger and Holland, 2004). Spearman's rank correlation was used to examine other bivariate relationships between environmental variables when parametric statistics were not appropriate. All statistics were performed in R version 2.15.2 (R Development Core Team, 2012).

#### 2.3 Results

#### 2.3.1 Physical variables

Mean monthly WT was consistently above 15°C from upstream sections to downstream sections in both years during the open-water season (Table 2.1). Mean WT increased from June to July and then decreased in September and October (Fig. 2.2A). The maximum WT occurred in July and August while the minimum WT occurred in October in both years.

Table 2.1. Environmental variables measured: mean and standard error from sites located from the upstream sections to the downstream sections of LD during the 2011 and 2012 field season. Water temperature (WT), dissolved oxygen (DO), mixing depth ( $Z_{mix}$ ), euphotic depth ( $Z_{eu}$ ), extinction coefficient ( $k_d$ ), Secchi disk depth (SD), total phosphorus (TP), total dissolved phosphorus (TDP), dissolved reactive phosphorus (DRP), total nitrogen (TN), total dissolved nitrogen (TDN), nitrate (NO<sub>3</sub>), ammonium (NH<sub>4</sub>), dissolved organic carbon (DOC) and particulate nitrogen to particulate phosphorus molar ratio (PN:PP).

	M3	M5	U1-M	C1-M	C2-M	U2-M	F4-M	С3-М	M9	U3-M
2011										
WT (°C)	15.2	16.0	19.1	17.7	17.5	17.6	17.4	19.0	19.0	17.5
	+3.6	+3.0	+2 3	+1 9	+1.4	+1.4	+1.1	+1.0	+1.2	+1.5
DO (mg/L)	9.1	8.6	$\frac{12.5}{8.5}$	8.9	8.8	9.1	9.0	8.8	9.2	8.9
	+0.9	+0.8	+0.4	+0.3	+0.2	+0.3	+0.5	+0.1	+0.1	+0.1
$Z_{mix}(m)$	14.5	19.6	21.8	23.5	25.0	31.0	23.8	28.1	±0.1 25.7	32.9
Z <sub>eu</sub> (m)	±0.2	±0.2	±3.6	±5.4	±2.8	±3.7	±4.0	±4.0	$\pm 1.0$	±5.6
	3.2	3.2	4.6	4.7	4.7	5.0	6.9	9.3	6.5	7.5
$k_{d}(m^{-1})$	±0.9	±0.8	±1.3	±1.0	±1.0	±1.6	±1.2	±0.2	±0.4	±0.7
	2.2	1.7	1.6	1.2	1.1	1.2	0.7	0.5	0.7	0.6
	±0.9	±0.5	±0.8	±0.3	±0.3	±0.4	±0.2	±0.01	±0.04	±0.1
SD(m)	0.6	0.9	2.2	2.5	2.0	2.4	5.2	3.5	2.9	3.5
	±0.1	±0.3	±0.4	±0.5	±0.5	±0.6	±1.1	±1.0	±0.4	±0.5
рН	8.4	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.5	8.4
	+0.1	+0.03	+0.04	+0.1	+0.04	+0.03	+0.1	+0.1	+0.04	+0.02
TP (µmol/L)	1.09	1.23	0.90	0.85	0.74	0.66	0.65	0.57	0.58	0.56
	$\pm 0.3$	±0.5	$\pm 0.3$	±0.2	±0.2	±0.2	$\pm 0.2$	±0.2	$\pm 0.1$	±0.2

	M3	M5	U1-M	C1-M	C2-M	U2-M	F4-M	С3-М	M9	U3-M
DRP(µmol/L)	0.13	0.17	0.34	0.31	0.16	0.11	0.10	0.10	0.10	0.10
TN (µmol/L)	$\pm 0.07$ 29.5 $\pm 3.0$	±0.04 30.3 ±2.7	±0.2 44.4	±0.2 45.3	±0.05 45.5	±0.04 47.1	$\pm 0.01$ 48.1 $\pm 2.1$	±0.05 47.5 ±1.7	±0.01 51.3	±0.02 51.6 ±1.3
TDN(µmol/L)	±3.9	$\pm 2.7$	±0.4	±0.7	±3.3	±3.5	$\pm 2.1$	±1.7	±0.9	$\pm 1.3$
	21.4	25.5	38.8	41.4	41.7	42.6	44.2	45.0	45.6	46.9
	+4.1	$\pm 2.8$	+7.1	+6.3	+4.9	+4.8	+1.9	+2.3	+1.7	+1.0
$NO_3$ (µmol/L)	8.2 ±4.4	9.6 ±4.1	24.3 ±6.8	26.1 ±5.4	27.7 ±5.1	28.5 ±4.7	29.6 ±1.9	=2.3 31.3 ±2.3	26.9 ±0.6	$31.0 \pm 0.5$
$NH_4^+$ ( µmol/L)	0.8	2.6	1.7	1.6	0.6	0.3	0.7	0.3	1.5	1.0
	±0.3	±1.0	±1.0	±0.9	±0.2	±0.1	±0.4	±0.1	±0.3	±0.6
DOC (mg/L)	3.1	3.2	3.1	3.2	3.1	3.1	3.6	3.4	3.2	3.3
	±0.1	±0.1	±0.1	±0.2	±0.2	±0.1	±0.2	±0.1	±0.1	±0.1
PN:PP (molar ratio)	12.8	12.2	11.7	14.1	13.7	17.7	13.3	16.6	18.8	20.2
	±2.4	±2.6	±1.5	±3.3	±1.2	±2.1	±1.2	±2.2	±2.2	±4.3
2012										
WT (°C)	16.0	16.9	17.8	17.6	17.5	18.2	19.7	16.3	16.5	16.1
	+3.8	+3.4	+1.7	+1.5	+1.8	+1.7	+1.6	+2 8	+2.4	+2 9
DO (mg/L)	9.2	9.0	8.6	9.0	8.9	9.0	8.8	9.6	9.4	9.6
	±0.8	±0.7	±0.2	±0.3	±0.3	±0.3	±0.4	±0.6	±0.3	±0.4

	M3	M5	U1-M	C1-M	C2-M	<b>U2-M</b>	F4-M	С3-М	M9	U3-M
$Z_{mix}(m)$	14.2	17.8	24.0	22.3	24.9	22.4	31.0	27.8	21.1	37.8
	±0.3	$\pm 1.2$	±3.1	$\pm 4.2$	$\pm 5.1$	±6.1	$\pm 5.0$	$\pm 8.0$	$\pm 4.0$	$\pm 5.5$
$k_d(m^{-1})$	1.1	1.0	0.6	0.6	0.6	0.5	0.4	0.5	0.4	0.4
	$\pm 0.1$	$\pm 0.1$	$\pm 0.04$	$\pm 0.05$	$\pm 0.03$	$\pm 0.01$	$\pm 0.01$	$\pm 0.04$	$\pm 0.01$	$\pm 0.01$
SD(m)	0.2	0.9	2.2	2.6	2.4	3.3	4.0	5.0	4.2	4.6
	±0.03	±0.3	±0.6	±0.4	±0.6	$\pm 0.5$	±0.7	±0.2	±0.2	±0.1
рН	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
	$\pm 0.04$	$\pm 0.1$	$\pm 0.1$	$\pm 0.05$	±0.1	$\pm 0.02$	$\pm 0.1$	$\pm 004$	$\pm 0.05$	±0.1
TP (µmol/L)	0.87	0.68	0.56	0.47	0.39	0.33	0.39	0.37	0.36	0.39
	$\pm 0.2$	$\pm 0.1$	$\pm 0.1$	$\pm 0.1$	$\pm 0.1$	$\pm 0.02$	$\pm 0.1$	$\pm 0.05$	$\pm 0.02$	±0.03
TDP (µmol/L)	0.17	0.23	0.24	0.15	0.12	0.12	0.16	0.15	0.14	0.16
	$\pm 0.04$	$\pm 0.1$	$\pm 0.04$	±0.03	$\pm 0.02$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	±0.01	$\pm 0.01$
DRP (µmol/L)	0.11	0.18	0.15	0.1	0.1	0.1	0.05	0.1	0.04	0.1
	$\pm 0.1$	$\pm 0.1$	±0.03	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.02$	±0.02	±0.01	±0.02
TN (µmol/L)	29.8	26.1	31.2	30.8	30.5	31.0	35.4	34.2	34.9	34.6
	±4.9	±3.4	$\pm 3.8$	$\pm 2.0$	$\pm 2.4$	±1.4	±2.5	$\pm 1.0$	±1.3	±0.7
TDN (µmol/L)	21.7	21.3	27.9	26.2	27.0	27.7	31.0	30.8	28.6	31.1
·• ·	±4.3	±4.2	±4.6	±1.7	±2.3	±1.3	±1.5	±0.6	±1.7	±1.1
$NO_3$ (µmol/L)	13.0	10.8	16.2	15.6	14.3	15.1	18.9	18.5	16.4	17.5
• · ·	±4.6	±5.2	$\pm 4.8$	±1.6	±1.2	$\pm 1.8$	$\pm 0.8$	±0.7	±0.7	±0.5

	M3	M5	U1-M	C1-M	C2-M	U2-M	F4-M	С3-М	M9	U3-M
DOC (mg/L)	5.7	6.1	5.3	3.1	5.5	3.3	5.9	4.3	4.8	7.2
(8, _ )	±2.6	±2.4	±1.6	±0.3	±1.7	±0.3	±1.8	±0.8	±0.7	±2.4
PN:PP (molar ratio)	14.0	12.1	13.9	16.6	15.7	15.9	18.7	17.1	21.4	19.5
	$\pm 1.9$	$\pm 1.2$	$\pm 2.5$	$\pm 2.1$	$\pm 1.2$	$\pm 0.8$	$\pm 2.8$	$\pm 2.9$	±3.7	±6.6



Figure 2.2. Monthly average and standard errors of chemical and physical variables measured in Lake Diefenbaker from June to October in 2011(squares) and 2012 (triangles); WT= water temperature (°C) (A),  $Z_{mix}$ = mixing depth (m) (B),  $Z_{eu}$ = euphotic depth (m) (C),  $k_d$ = extinction coefficient (m<sup>-1</sup>) (D), inflow from the SSR (m<sup>3</sup>/s) (Peak in June) (E), TP= total phosphorus concentration (µmol/L) (F), TDP= total dissolved phosphorus concentration (µmol/L) (G),TN= total nitrogen concentration (µmol/L) (H), NH<sub>4</sub><sup>+</sup>= ammonium concentration (µmol/L) (I), and DOC= dissolved organic carbon concentration (mg/L) (J).

Mean epilimnetic DO concentrations were consistently observed above 7.0 mg/L throughout the reservoir in both years (Table 2.1). Conductivity was similar throughout the reservoir. Mean  $Z_{mix}$  was similar from June to August and deepened from September to October in both years (Fig. 2.2B). Mean  $Z_{mix}$  increased from upstream to downstream sections of the reservoir in both years (Table 2.1). Mixing and thermal stratification events were not uniform across sites in both years. However, the water column was stratified in the reservoir from July to September in both years (Hudson and Vandergucht, 2015). Mean  $Z_{eu}$  increased from June to October in both years (Fig. 2.2C). Mean  $Z_{eu}$  and mean SD were lowest at both M3 and M5 (upstream sites) and then increased further downstream. Mean  $k_d$  decreased from June to October in both years (Fig. 2.2D) and was greatest at M3 and M5 and then decreased downstream (Table 2.1). Peak flows into Lake Diefenbaker from the South Saskatchewan River occurred in June of both years (Fig. 2.2E).

#### 2.3.2 Water chemistry variables

Mean epilimnetic pH was consistently observed above 8.0 throughout the reservoir in both years (Table 2.1). In general, mean concentrations of TP, TDP, TN, and  $NH_4^+$ concentrations were greater in 2011 compared to 2012. Mean concentrations of TP, TDP, TN, and  $NH_4^+$  decreased from June to August and were similar from September to October in both years (Fig. 2.2F - I). Mean TP concentrations decreased from upstream sections to downstream sections, whereas mean TN, TDN, and  $NO_3^-$  concentrations followed a reverse pattern in both years (Table 2.1). Mean TDP, DRP, and  $NH_4^+$  concentrations varied from upstream sections to downstream sections of the reservoir in both years (Table 2.1). Generally, DOC concentrations were greater in 2012 compared to 2011. Mean DOC concentrations was similar from June to October, and throughout the reservoir in 2011, whereas concentrations increased from June to August and decreased from August to October (Fig. 2.2 J) and varied throughout reservoir in 2012 (Table 2.1). Mean PN:PP ratios varied from the upstream sections to the downstream sections of the reservoir in both years, with the largest ratios occurring at site U3-M (downstream in the Gardiner arm) in both years (Table 2.1).

Chlorophyll *a* concentrations varied along the length of the reservoir (Fig. 2.3). We observed a seasonal bimodal distribution of chl *a* concentration in both years with a peak from June to July, followed by a secondary peak from September to October. The lowest chl *a* concentrations were observed in August in both years. The greatest concentration of chl *a* occurred at the midstream sites (C1-M, C2-M, U2-M and F4-M) of the reservoir in both years except at site M3 (upstream sites close to Highway 4) in October. Chlorophyll a concentration was positively correlated with total phytoplankton biomass ( $r_s = 0.30$ , p = 0.004)

#### 2.3.3 Trophic state indices and phytoplankton composition and distribution

The mean  $TSI_{chl a}$ , mean  $TSI_{SD}$  and mean  $TSI_{TP}$  decreased down the length of the reservoir with the greatest values observed at the most upstream site (M3) in both years (Fig. 2.4). At midstream and downstream sites (i.e., U1-M, C1-M, C2-M, U2-M, F4-M, C3-M and U3-M)  $TSI_{chl a}$ ,  $TSI_{SD}$  and  $TSI_{TP}$  were similar to each other.

In both years, we identified 72 phytoplankton genera comprising 33 chlorophytes, 17 bacillariophytes, 12 cyanophytes, 3 euglenophytes, 3 pyrrophytes, 2 chrysophytes, and 2 cryptophytes (Table 2.2). The bacillariophytes and the cryptophytes dominated the phytoplankton seasonally and spatially in both years (Fig. 2.5A - D). The bacillariophytes contributed about 39 % and 46 % of total phytoplankton biomass in 2011 and 2012, respectively, while the cryptophytes contributed 43 % and 38 % of total phytoplankton biomass in 2011 and 2012, respectively. The cyanophytes and chlorophytes were always represented during the
sampling periods but contributed very little to the total biomass (< 5 % in both 2011 and 2012; Fig. 2.5). The other phytoplankton groups were not consistently observed during the 5 months of sampling.

The bacillariophytes consisted of small centric (*Cyclotella, Stephanodiscus*) to large centric (*Aulacoseira*) and pennate forms (*Asterionella, Fragilaria, Synedra, Tabellaria*). The genus *Aulacoseira* contributed about 80 % to the total bacillariophyte biomass (in both 2011 and 2012). The cryptophyte biomass consisted of members from the genera *Cryptomonas* and *Rhodomonas*. The cryptophytes dominated in June and July with peak contributions in June 2011 and in July 2012. Conversely, the bacillariophytes dominated in September and October with peak contributions in October in both years. The combined relative biomass of the cryptophytes and bacillariophytes were lowest in August of both 2011 and 2012 (Fig. 2.5A and B). The relative biomass of the bacillariophytes and cryptophytes varied along the length of the reservoir in both years. However, the greatest and the lowest contribution of the cryptophytes and the bacillariophytes, respectively, to the total phytoplankton biomass occurred at site M5 in both years (Figs 2.5C and D).

We selected inflow, WT, Z<sub>mix</sub>, k<sub>d</sub>, pH, TN,TP, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, DOC, and PN:PP as explanatory variables in our models to reduce collinearity. We reported only our most parsimonious models because the AICc difference with the second best models were greater than 2 (Burnham and Anderson, 2004). The most parsimonious model (Table 2.3) confirmed that inflow and water temperature (WT) explained 41 % of the variability of the biomass of the cryptophytes. The biomass of cryptophytes showed an increasing relationship with inflow and WT.

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Figure 2.3. Spatial (from upstream sections to downstream sections) and temporal (June to October) distribution of chlorophyll *a* concentrations in Lake Diefenbaker for 2011 and 2012. Highway 4 is set to zero Km



Figure 2.4. Mean and standard errors of trophic state indices for chlorophyll *a* (TSI chl *a*), Secchi depth (TSI SD) and total phosphorus (TSI TP) from upstream sections to downstream sections in 2011 and 2012 in Lake Diefenbaker. The broken lines represents the threshold for trophic states according to Carlson (1977): O= oligotrophy < 40 ; M= mesotrophy = 40 - 50 ; E= eutrophy = 50 - 70.

Phyla	Genera	Phyla	Genera
Chloropytes	Actinastrum		Cyclotella
1.0	Ankistrodesmus		Cymatopleura
	Ankyra		Cymbella
	Botryococcus		Diatoma
	Carteria		Fragilaria
	Chlamydomonas		Gyrosigma
	Chlorella		Melosira
	Closteriopsis		Navicula
	Closterium		Nitzschia
	Coelastrum		Pinnularia
	Cosmarium		Rhizosolenia
	Crucigenia		Stephanodiscus
	Desmodesmus		Synedra
	Dictyosphaerium		Tabellaria
	Dispora	Cyanophytes	Anabaena*
	Elakatothrix		Aphanizomenon*
	Eudorina		Aphanocapsa
	Gleocystis		Aphanothece
	Koliela		Chroococcus
	Micractinium		Coelosphaerium*
	Monoraphidium		Gomphosphaeria*
	Oocystis		Microcystis*
	Pandorina		Planktolyngbya
	Paradoxia		Planktothrix*
	Pediastrum		Pseudanabaena*
	Scenedesmus		Woronichinia*
	Schroederia	Euglenophytes	Euglena
	Sphaerocystis		Trachelomonas
	Staurastrum		Lepocinclis
	Stichococcus	Chrysophytes	Dinobryon
	Tetraedron		Mallomonas
	Tetrastrum	Pyrrophytes	Ceratium
	Volvox		Peridinium
Bacillariophytes	Asterionella		Gymnodinium
	Aulacoseria	Cryptopytes	Cryptomonas
	Cocconeis		Rhodomonas

Table 2.2. List of phytoplankton genera identified in Lake Diefenbaker from June to October in 2011 and 2012.

\* Potential bloom forming and toxin producing cyanobacterial genera (Cronberg and Annadotter, 2006; Beaulieu et al., 2014)



Figure 2.5. Percentage of phytoplankton groups by total biomass from June to October 2011 (A) and 2012 (B) and from upstream sections to downstream sections in 2011 (C) and 2012 (D) in Lake Diefenbaker.

Table 2.3. The most parsimonious models that described the relationship between the major phytoplankton groups and environmental variables with second order Akaike's Information Criterion (AICc) and multiple linear regression models (MLR).  $Z_{mix}$ = mixing depth, PN:PP = particulate nitrogen to particulate phosphorus molar ratio, WT= water temperature, VIF= variance inflation factor.

	Explanatory variable	Estimate	Partial correlation	P value	VIF	AICc
Cryptophytes $R^{2}_{adj} = 0.41, n=$ 50, $P < 0.001$	Intercept	0.9181				
	Inflow $(m^3/s)$	0.0006	0.50	0.00022	1.05	65.4
	WT (°C)	0.0473	0.45	0.00124	1.05	
Bacillariophytes $R^{2}_{adj}=0.38$ , n= 50, P < 0.001	Intercept	1.5224				
	$Z_{mix}(m)$	0.0348	0.62	0.00001	1.04	65.6
	PN:PP (molar)	-0.0239	-0.35	0.0149	1.04	

The partial correlation coefficient between the biomass of cryptophytes and inflow (when WT was partialled out) was 0.5 whereas the partial correlation coefficient between the biomass of cryptophytes and WT (when inflow was partialled out) was 0.45. In a separate bivarate relationship, I found cryptophyte biomass to be positively correlated with dissolved organic carbon concentrations ( $r_s = 0.33 \ p = 0.018$ ).

The most parsimonous model (Table 2.3) explained 38 % of the variability of the biomass of the bacillariophytes. Mixing depth was postively related to the biomass of bacillariophytes (partial correlation of 0.62 when PN:PP was partialled out), whereas PN:PP was negatively related to the biomass of the bacillariophytes (partial correlation coeficient of -0.35 when PN:PP was partialled out).

#### **2.4 Discussion**

#### 2.4.1 Trophic status of Lake Diefenbaker

The assessment of the trophic status of Lake Diefenbaker using Carlson's trophic indices, revealed a decreasing general trend in all indices ( $TSI_{chl a}$ ,  $TSI_{SD}$  and  $TSI_{TP}$ ) along the length of the reservoir, (Fig. 2.4) as observed in other river-connected reservoirs (Kimmel et al., 1990). For instance, Bolgrien et al. (2009) noticed a similar trend in the trophic status from the riverine to the lacustrine zones in three large reservoirs on the Missouri River (Lake Oahe, Lake Sakakawea, and Fort-Peck Lake). Haggard et al. (1999) reported that the upstream riverine zone was the most productive in Beaver Lake.

Trophic state indices were different at upstream sites in Lake Diefenbaker. Specifically, the  $TSI_{SD}$  and the  $TSI_{TP}$  were greater and indicated a eutrophic condition, especially at M3 (Fig. 2.4), compared to the  $TSI_{chl a}$  in both years. This is related to the

large nutrient loads and the associated turbidity carried into Lake Diefenbaker from the SSR during the high flow events in 2011 and 2012. This non-algal turbidity associated with the high flows reduced water transparency (Hudson and Vandergucht, 2015), resulting in light limitation of phytoplankton communities (Dubourg et al., submitted manuscript). Most of the nutrients associated with such high flows in reservoirs are typically characterized by loads of non-bioavailable P (Kimmel et al., 1990). At midstream and downstream sites all three trophic state indices were similar due to the gradual settling of allochthonous organic matter from the water column and the attendant loss of P and turbidity from the reservoir (Hudson and Vandergucht, 2015; Kimmel et al., 1990). The similarity of all three trophic indices at midstream and downstream sites suggests P limitation according to Carlson and Havens (2005). Dubourg et al., (submitted manuscript) also reported that P was the major limiting nutrient of phytoplankton in Lake Diefenbaker in 2013. Because high flow events resulted in the overestimation of the TSI estimates of SD and TP, especially at upstream sites, I proceeded to only use chl a to estimate the trophic status of Lake Diefenbaker (Carlson and Havens, 2005).

The TSI as determined from chl *a* is the most definitive trophic status index, because chl *a* concentration is the only direct measure for algal biomass that is free from interference, e.g., from turbidity (Bolgrien et al., 2009; Carlson, 1977; Carlson and Simpson, 1996). The TSI<sub>chl *a*</sub> placed Lake Diefenbaker as a mesotrophic system (i.e., moderately productive, see Fig. 2.4). I also observed a diverse phytoplankton community (72 phytoplankton genera comprised of seven planktonic algal divisions, Table 2.2), that is consistent with the general phytoplankton diversity of temperate mesotrophic lakes (Watson et al., 1997). For instance, Leitão et al. (2003) observed 79 phytoplankton genera in the deep temperate mesotrophic Vouglas reservoir in France, and Negro et al. (2000) observed 72 phytoplankton genera in the deep temperate mesotrophic Valparaíso reservoir in Spain.

## 2.4.2 Major phytoplankton groups in relation to environmental variables

The cryptophytes and bacillariophytes were the dominant groups in terms of biomass (together they contributed ~ 89 % of total phytoplankton biomass) during our study and in previous Lake Diefenbaker studies. For instance, McGowan et al. (2005) reported that siliceous algae (mainly bacillariophytes and some chrysophytes) and cryptophytes were the dominant phytoplankton in the Qu'Appelle arm of Lake Diefenbaker from 1995 to 2003. The bacillariophytes and cryptophytes dominated from August 2008 to November 2011 within 20 km of the aquaculture facility in Lake Diefenbaker (M. Otu, Department of Fisheries and Oceans Canada, personal communication) (site F4-M; Fig 2.1). The dominance of the bacillariophytes and cryptophytes has also been reported in lakes and reservoirs of similar trophic status elsewhere. For instance, Tolotti et al. (2010) reported that the bacillariophytes and cryptomonads were the dominant groups in deep temperate mesotrophic Lake Santa Croce reservoir in Italy. Simek et al. (2008) reported that the cryptophytes dominated in spring to early summer and the bacillariophytes dominated in summer to fall in deep temperate meso-eutrophic Rimov Reservoir in the Czech Republic.

Cryptophytes have been reported to have high nutrient uptake and growth rates (Dokulil, 1988; Tolotti et al., 2010). Therefore, their positive relationship with inflow may be related to their ability to take up flow-associated available nutrients for rapid growth. As such, they have the ability to compensate for washout during high flow event

as observed in Lake Santa Croce, Italy (Tolotti et al., 2010). As phagotrophs, cryptophytes have the ability to engulf bacterial cells during low light conditions to compensate for low photosynthetic rates (Bellinger and Sigee, 2010), and to use organic matter as a source of carbon (osmotrophy) (Gillott, 1990). Interestingly, the greatest contribution of the cryptophytes to the total phytoplankton biomass occurred at M5 (Fig. 2.5C and D). This site was characterized by high turbidity due to large deposits of allochtonous organic matter during the high flow events (Hudson and Vandergutcht, 2015). Therefore, it is possible that the observed positive relationship between inflow and cryptophytes may be related to the increase in bacterial abundance linked to allochthonous organic matter or the allochthonous organic matter associated with the high flow events, both of which the cryptophytes can consume (Tranvik et al., 1989). For instance, Simek et al. (2008) reported that the summer abundance of cryptophytes in the mesotrophic Rimov reservoir may be related to the abundance of certain bacterioplankton and extracellular phytoplankton production. There are some indicators that osmotrophy was occurring in Lake Diefenbaker, due to the positive relationship I found between cryptophytes and DOC ( $r_s = 0.33 p = 0.018$ ).

Water temperature affects the growth of phytoplankton and is associated with thermal stratification (Adrian et al., 2009; Paerl et al., 2011). Cryptophytes have been shown to achieve maximum growth at 23.5 °C (Morgan and Kalff, 1979), and unlike the bacillariophytes, are favoured during periods of thermal stratification due to their ability to maintain an elevated position in the water column with their flagella (Reynolds, 2006). The greatest contribution of the cryptophytes to the total phytoplankton biomass coincided with the onset of thermal stratification, when the Lake Diefenbaker water temperature was warmest (July, ~ 22°C). Thus, the relationship with water temperature suggests the importance of warm conditions and thermal stratification on the abundance of cryptophytes in Lake Diefenbaker (Morgan and Kalff, 1979; Reynolds, 2006). Weyhenmeyer et al. (2004) also reported that the biomass of the cryptophytes remained high during summer stratification in Lake Mälaren, in Sweden.

Mixing depth and PN:PP explained 38 % of the variability in the biomass of the bacillariophytes. However, mixing depth explained a greater proportion of bacillariophytes biomass compared to the PN:PP ratio (partial correlation of 0.62 versus --0.35, respectively; Table 3). This suggests that the biomass of the bacillariophytes was more related to  $Z_{mix}$  than nutrients. The genus Aulacoseira contributed to the majority of bacillariophyte biomass (80 %). Diatoms, specifically the genus Aulacoseira, have been shown to be dependent on mixing to remain suspended in the water column (Reynolds, 2012). In addition, the greatest contribution of the bacillariophytes to the total phytoplankton biomass occurred during periods when the water temperature was lowest and isothermal. Bacillariophytes can grow rapidly and out-compete other phytoplankton groups under low water temperatures (Rothenberger et al., 2009). Thus, the relatioship between the biomass of bacillariophytes and the mixing depth suggests the importance of cool, isothermal conditions for bacillariophyte abundance in Lake Diefenbaker. Gillett and Steinman (2011) reported that bacillariophytes were abundant in Muskegon Lake (a mesotrophic lake in the USA) when the lake was also  $cool (~13^{\circ}C)$  and isothermal. However, Aulocoseira contributed the least (50 %) to the bacillariophytes biomass at upstream sites (especially at M5 where we observed the lowest proportion of the bacillariophytes to the total phytoplankton biomass) compared to midstream and

downstream sites (89 %) in both years. Because of the typical shallow, nutrient-rich, turbid and turbulent conditions at upstream sites, small centric diatoms (*Cyclotella* and *Stephanodiscus*), pennate diatoms (*Asterionella*, *Diatoma*, *Fragilaria*, *Navicula*, *Nitzschia* and *Synedra*), *Cymatopleura* and *Melosira* are better adapted to these conditions and constituted the remaining half of the bacillariophytes biomass (Lucas et al., 2015; Padisák et al, 2006; Padisák et al, 2009; Reynolds et al., 2002).

The negative relationship between the biomass of the bacillariophytes and the PN:PP is not fully understood. However, I speculated that the negative relationship between the biomass of the bacillariophytes and the PN:PP may be related to the more rapid loss of P from the water column, following the sedimentation of particles containing P, including bacillariophytes during periods of thermal stratification (Kufel, 2001). Moreover, nutrients do not seem to play a major role in the dominance of the bacillariophytes; silica was not found to be at a concentration in Lake Diefenbaker that would limit their growth (Gilpin et al., 2004; Maavara et al., 2015) and P was weakly deficient in Lake Diefenbaker (Dubourg et al., submitted manuscript).

Nevertheless, the lowest contribution of both the bacillariophytes and cryptophytes to the total phytoplankton biomass and the lowest chl *a* concentrations occurred in August (Fig 2.5). This corresponds to a period when water clarity increased in Lake Diefenbaker (Yip et al., 2014; Fig. 2.2 C and D). Although zooplankton abundance was not investigated during our study period, we speculated that the low relative biomass of bacillariophytes and the cryptophytes and the low chl *a* concentrations in August, may be related to an increase in zooplankton grazing (Vogt et al, 2014). Vogt et al. (2014) found a negative relationship between mean summer phytoplankton abundance (as chl *a* concentration) and total zooplankton abundance (explained 19 % of the variation) in the Qu'Appelle arm of Lake Diefenbaker in their decadal study.

Cyanobacteria contributed < 5 % of the total phytoplankton biomass over the period of study (Fig. 2.5). This may be attributed to washout from the high flow events (Roelke et al., 2010) and suppression of their growth from non-algal turbidity associated with the high flow (Paerl and Huisman, 2009; Reynolds, 1990). Godlewska et al. (2003) reported that high water flow eliminated the usual cyanobacterial blooms that occur in autumn in Dobczce reservoir in Poland. However, it is well documented that high nutrient loads from high flow events followed by drought conditions (reduced water discharge and increased water residence time) can promote cyanobacterial blooms in lakes and reservoirs (Paerl and Huisman, 2009). For instance, in a previous study on Lake Diefenbaker, cyanobacteria dominated the phytoplankton biomass (79 %) during a drought period with low flow from the SSR (SEPS and EC, 1988). Hecker et al. (2012) commented on a cyanobacterial bloom that occurred in the southern and western parts of the reservoir in fall 2007, which also corresponded with low flow conditions (Hudson and Vandergucht, 2015).

Cyanobacterial blooms have been reported to occur frequently during increased water residence time, extended and stable thermal stratification and internal loading of nutrients from sediments (Nürnberg, 2009; Paerl et al., 2011; Paerl and Huisman, 2009). Despite the low cyanobacterial biomass in Lake Diefenbaker, I observed some potential toxin and bloom forming genera (Table 2). Such genera of cyanobacteria may become an issue and threaten the water quality of Lake Diefenbaker, if early summer peak flow events are followed by the environmental conditions mentioned above and elsewhere (Nürnberg, 2009; Paerl et al., 2011; Paerl and Huisman, 2009).

#### **2.5 Conclusion**

The TSI<sub>chl a</sub> indicates that Lake Diefenbaker is a mesotrophic system, with a highly diverse phytoplankton community (72 phytoplankton genera). In both years of study, Lake Diefenbaker received high flows associated with high nutrient loads and nonalgal turbidity from the South Saskatchewan River which may be responsible for the high biomass of cryptophytes and bacillariophytes reported. The cryptophytes were abundant during high flow rates and when the reservoir's water temperature was warmest and stratified. The bacillariophytes were abundant during cool, isothermal conditions in the reservoir. There was no evidence to support an immediate threat to the water quality of Lake Diefenbaker because cyanobacterial biomass was low during this study. Therefore, I do not recommend any immediate management strategy for cyanobacteria in Lake Diefenbaker. However, I have provided useful information concerning the potential issues to the water quality of Lake Diefenbaker by some potential toxin and bloomforming cyanobacterial genera under certain conditions. Most of the conditions highlighted are related to climate change. Therefore, future studies conducted in drought years may help elucidate the effect of climate change on phytoplankton community composition in Lake Diefenbaker.

# **CHAPTER 3-** Localized human activities in relation to phytoplankton in Lake Diefenbaker, SK, Canada.<sup>2</sup>

# **3.1 Introduction**

Eutrophication is one of the primary water quality issues affecting aquatic ecosystems globally (Smith and Schindler, 2009). Algal blooms due to eutrophication can result in a decline in water quality and fish kills due to low dissolved oxygen concentrations (Smith et al., 2008; Schindler et al., 2008). Furthermore, the blooms of cyanobacteria are of great concern because of the potential for many cyanobacterial species to synthesize geosmin that causes taste and odor in drinking water and cyanotoxins that may pose serious health risk to humans and livestock (Landsberg, 2002; Izaguirre and Taylor, 2004). Moreover, algal blooms have huge ecological and economic impacts such as the deterioration of habitat, the loss of biodiversity at all trophic levels and the high cost of water treatment (de Figueiredo et al., 2006; Dodds et al., 2009).

Human activities such as urban development and agricultural practices in the surrounding watershed of lakes and reservoirs have exacerbated the release of nutrients into these systems (Carney, 2009). Several studies have reported the run off of nutrients such as ammonium, nitrate, organic nitrogen and dissolved phosphorus from urban (housing development, tourism and leisure) and agricultural practices (livestock waste, fertilizer application on cropland and turf grass maintenance) into lakes and reservoirs (Howarth et al., 1996; Mallin and Wheeler 2000; Kuo et al, 2008; Winter et al., 2003). These nutrients have profound effects on the phytoplankton community composition and

<sup>&</sup>lt;sup>2</sup> This chapter was written in manuscript style. Hence, there may be some overlap with other chapters

may encourage the growth and the occurrence of algal blooms (Jeppesen et al., 2007; Lehman, 2014; Michalak et al., 2013). For example, Katsiapi et al. (2012) found a strong correlation between phytoplankton species composition and some land use activities in 11 lakes and 7 reservoirs in Greece. The authors reported that cyanobacteria were associated with artificial (e.g., commercial mine, dump and non agricultural vegetated areas) and agricultural activities; chrysophytes were associated with forested areas; and chlorophytes were found most often associated with industrial, and commercial land use activities. Paul et al. (2012) also found a strong positive correlation between cyanobacteria and pastoral land and a strong positive correlation between chlorophytes and native forest and urban land use in 11 lakes in New Zealand.

Canadian prairie lakes and reservoirs lie in fertile agriculture soil with high phosphate, sulfate and organic matter content (Mitchell and Prepas, 1990). Consequently, most of the lakes and reservoirs in the Canadian prairie are mesotrophic to hypereutrophic and are frequently dominated by cyanobacteria. Lake Diefenbaker is a large mesotrophic reservoir in the Canadian prairie. It receives most of its water from the South Saskatchewan River and serves as an important source of water for domestic consumption, irrigation, recreation, aquaculture, and power generation to Saskatchewan, Canada. The effective drainage area of Lake Diefenbaker is 86,900 km<sup>2</sup> with about 91 % of this area located in southern Alberta. Most of the catchment area of the reservoir in Alberta and the state of Montana and is agricultural cropland (SIPA, 2008; Hall et al., 1999; Saskatchewan Water Security Agency, 2012). In addition, the reservoir is characterized by numerous embayments exposed to human activities such as urban (housing developments, golf courses, marinas) and cattle operations (manure inputs). These localized urban activities and cattle operations may be potential sources of nutrient to the reservoir. However, the relationship between these localized human activities (urban and cattle) and the phytoplankton community composition in Lake Diefenbaker have not been investigated. Therefore, I compared phytoplankton community composition in embayments exposed to human activities (i.e. urban and cattle), unexposed embayments (no apparent anthropogenic activity) and main channel sites (to account for gradient across the length of the reservoir). I also tested if the total phytoplankton biomass and number of phytoplankton genera collected from exposed embayments with urban activities and cattle operations were different from those in unexposed embayments and main channel sites in Lake Diefenbaker.

# **3.2 Materials and Methods**

## 3.2.1 Study area

Lake Diefenbaker is located in south Saskatchewan, Canada (51° 1'53"N, 106° 50'9"W). The reservoir has a length of approximately 225 km and a width of 2-3 km. The volume and surface area of reservoir are approximately 9 km<sup>3</sup> and 349 km<sup>2</sup>, respectively (Saskatchewan Water Security Agency, 2012). Initially, I had an experimental block design with three cattle embayments and three urban embayment sites, with their corresponding reference embayments and main channel sites (Table 3.1). However, as the summer of 2011 progressed, I noticed cattle at some of my unexposed/ reference embayments. Therefore, I merged sites with cattle and urban embayments classes together to form a single set of exposed embayments (comprised of urban activities

Table 3.1. The initial experimental design consisted of three potential cattle embayments (C1-I, C2-I and C3-I) with corresponding reference embayment (C1-C, C2-C and C3-C) and associated main channel sites (C1-M, C2-M and C3-M) and three potential urban embayments (U1-I, U2-I, and U3-I) with its corresponding reference (U1-C, U2-C, and U3-C) and main channel sites (U1-M, U2-M and U3-M).

TREATMENTS						
	Potential impact sites	Reference embayment sites	Reference main channel sites			
Cattle	C1-I	C1-C	C1-M			
	C2-I	C2-C	C2-M			
	C3-I	C3-C	С3-М			
Urban	U1-I	U1-C	U1-M			
	U2-I	U2-C	U2-M			
	U3-I	U3-C	U3-M			

Exposed embayments	Unexposed/ reference embayment	Main channel sites		
E1 (U)	R1	U1-M		
E2 (C)	R2	C1-M		
E3 (C)	R3	C2-M		
E4 (C)	R4	U2-M		
E5 (U)		C3-M		
E6 (C)		U3-M		
E7 (C)				
E8 (U)				

Table 3.2. The revised design consist of eight exposed embayments (E1 to E8), four unexposed/reference embayment (R1 to R4) and six main channel sites (U1-M, C1-M, C2-M, U2-M C3-M, and U3-M). C= cattle and U= urban.



Figure 3.1. Sampling sites: reference embayment (open triangles), exposed embayment (cattle operations (solid black circles) and urban activities (open circles)) and main channel sampling sites (open squares) in Lake Diefenbaker (SK), from upstream (Highway 4) to downstream (Gardiner and Qu'Appelle dams). Each site was sampled from June to October in 2011 and in 2012.

[settlements, golf courses and marinas] and cattle operations [with manure input from cattle watering]). Consequently, I had eight exposed embayments (three urban and five cattle) and four unexposed embayments with no perceived human activities. The six main channel sites were not modified (Table 3.2, Fig. 3.1). Each site was sampled once every month from June to October in 2011 and 2012. We avoided sampling in the month of May because of the presence of ice-cover. All water samples were collected at 2 m with a Van Dorn sampler, poured into 20 litre poly-bags and kept in the dark in coolers. Water samples were returned to the laboratory at the University of Saskatchewan and stored at ambient condition (light and temperature) until processed for water chemistry the following day. Water samples that were collected for phytoplankton analysis were fixed in Lugol's solution.

## **3.2.2 Chemical analysis**

Total phosphorus (TP), total dissolved phosphorus (TDP) and dissolved reactive phosphorus (DRP) were determined using the method of Parsons et al. (1984). Samples for TDP and DRP were filtered through 0.2  $\mu$ m polycarbonate filters using syringe filtration. Second derivative spectroscopy was used to measure total nitrogen (TN), total dissolved nitrogen (TDN) and nitrate (NO<sub>3</sub><sup>-</sup>) (Bachmann and Canfield, 1996). Samples for TDN and NO<sub>3</sub><sup>-</sup> were filtered through 0.2  $\mu$ m polycarbonate filters using gentle syringe filtration. The phenol-hypochlorite method by Stainton et al. (1977) was used to determine ammonium (NH<sub>4</sub><sup>+</sup>) concentrations colorimetrically after filtering samples through 0.2  $\mu$ m polycarbonate filters. Samples for dissolved organic carbon (DOC) were measured as described in Sereda et al. (2012) using an organic carbon analyzer (Shimadzu TOC – 5050A)

#### **3.2.3 Physical factors measured**

Water temperature (WT), pH, and dissolved oxygen concentrations (DO) were measured using a YSI 6600 v2 multi-parameter sonde. I used a biospherical radiometer (Biospherical Instruments Inc. BIC 2104 submersible radiometer) to measure photosynthetically active radiation (PAR). The vertical extinction coefficient ( $k_d$ ) was dervied from the linear regression of the natural logarithm of PAR and depth (Kirk, 2003). I estimated the euphotic depth ( $Z_{eu}$ ) as the depth from the water surface to the depth where the light intensity is 1 % of the water surface. All of these variables were related to samples collected from a depth of 2 m. Secchi disk (SD) depth was used to estimate water transparency.

# 3.2.4 Phytoplankton identification and counting and chlorophyll a measurement

Samples were identified and counted on an Olympus inverted (IX51) microscope using the technique of Utermöhl (1958). Each taxon was identified to genus level with reference to taxonomic keys (Bellinger and Sigee, 2010; Brook et al 2002; Wehr and Sheath, 2003). A minimum of 400 cells were enumerated per sample. I used image-Pro Analyser 7.0 computer software to estimate the size of the phytoplankton and used a computerized phytoplankton counting program "Algamica (Version 4.0)" developed by Gosselain and Hamilton (2000) to calculate final biomass for each taxon.

Chlorophyll *a* samples were collected on 47 mm GF/F filters (nominal pore size 0.7  $\mu$ m) with vacuum filtration (10 psi under low light). Pigments were extracted and analyzed according to Bergmann and Peters (1980) and the absorbance was read at 665 nm as described in Vandergucht et al. (2013).

## **3.2.5 Data analyses**

A linear mixed effects model (LME) was used to compare the total phytoplankton biomass found in exposed embayment versus unexposed embayments and main channel sites for each year. Distance down the length of the reservoir was the random term in the model (Pinheiro and Bates 2000). Total phytoplankton biomass was transformed (i.e., log<sub>10</sub>) to homogenize variance. In addition, I compared chemical and physical variables in exposed embayment versus reference embayments and main channel sites for each year using LME. All statistics were performed in R version 2.15.2 (R Development Core, Team, 2012).

# **3.3 Results**

# 3.3.1 Envriomental variables

Mean concentrations of TP, TDP, DRP, TN, TDN and NO<sub>3</sub><sup>-</sup> from all sites (exposed, unexposed and main channel) were almost twice as high in 2011 than in 2012 (P < 0.001). In contrast, mean DOC concentrations from all sites were higher in 2012 (n= 168, P= 0.001). Mean NH<sub>4</sub><sup>+</sup> concentrations from all sites were similar in both years (n= 180, P= 0.954) (Table 3.3). Overall, mean concentrations of TP, TDP, DRP, TN, TDN, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and DOC were similar in reference, exposed and main channel sites in 2011 (P > 0.05) and 2012 (P > 0.05), respectively (Table 3.3). Mean epilimnetic DO, pH, SD and WT were similar in reference, exposed and main channel sites in 2011 (P > 0.05) and 2012 (P > 0.05), respectively. Table 3.3. The mean and the range of environmental variables measured at reference, exposed and main channel sites in 2011 and 2012. TP= total phosphorus, TDP= total dissolved phosphorus, DRP= dissolved reactive phosphorus, TN= total nitrogen, TDN= total dissolved nitrogen,  $NO_3^-$  = nitrate,  $NH_4^+$  = ammonium, DOC= Dissolved organic carbon, DO= Dissolved oxygen, SD = Secchi disk depth and WT= Water temperature.

		2011			2012	
Variables	Reference	Exposed	Main	Reference	Exposed	Main
TP	0.68	0.75	0.72	0.38	0.42	0.42
(µmol/L)	(0.28-1.80)	(0.30-1.81)	(0.34-2.05)	(0.20-0.73)	(0.19-0.90)	(0.27-0.83)
TDP	0.25	0.29	0.30	0.16	0.16	0.16
(µmol/L)	(0.14-0.63)	(0.14-0.73)	(0.14-0.99)	(0.08-0.32)	(0.10-0.27)	(0.09-0.34)
DRP	0.11	0.14	0.18	0.09	0.08	0.08
(µmol/L)	(0.02-0.62)	(0.02-0.59)	(0.02-1.15)	(0.02-0.30)	(0.02-0.19)	(0.02-0.23)

		2011			2012	
Variables	Reference	Exposed	Main	Reference	Exposed	Main
TN	47.00	47.98	46.90	32.53	32.51	32.03
(µmol/L)	(27.84-65.11)	(32.86-78.95)	(30.76-76.81)	(24.00-37.46)	(23.87-56.89)	(24.09-44.85)
TDN	41.91	43.31	42.74	28.79	28.29	28.44
(µmol/L)	(21.59-60.49)	(29.21-71.29)	(28.11-66.54)	(20.57-36.29)	(20.69-40.90	(20.47-44.79)
NO <sub>3</sub> <sup>-</sup>	27.37	28.16	28.15	16.06	16.15	16.18
(µmol/L)	(6.65-44.72)	(15.11-53.71)	(14.47-51.21)	(3.37-20.49)	(7.93-27.41)	(7.77-28.92)
$\mathrm{NH_4}^+$	0.76	0.97	0.91	0.71	0.75	0.85
	(0.07-3.48)	(0.03-6.30)	(0.06-5.69)	(0.10-2.28)	(0.01-6.44)	(0.08-7.02)

		2011		2012		
Variables	Reference	Exposed	Main	Reference	Exposed	Main
DOC (mg/L)	3.24	3.22	3.20	7.12	5.26	4.86
рН	(2.75-3.02) 8.43 (8.17.8.58)	(2.04-3.71) 8.39 (8.09.8.61)	(2.00-3.73) 8.40 (8.18.8.54)	(2.47-21.28) 8.43 (8.20, 8.61)	(2.38-31.33) 8.42 (8.09.8.63)	(2.32-13.22) 8.41 (8.10.8.59)
DO (mg/L)	9.06	(8.09-8.01) 8.97 (7.12.9.65)	(8.18-8.54) 8.86 (7.63.9.66)	9.29	9.09	9.09
SD (m)	(8.29-10.23)	2.31	2.65	2.72	2.49	3.26
WT(°C)	(0.50-4.50) 17.87	(0.35-5.10) 17.84	(0.40-5.30) 17.79	(0.60-4.50) 17.06	(0.50-4.75) 17.40	(0.45-5.50) 17.34
	(12.27-21.27)	(12.19-24.06)	(12.19-22.53)	(7.89-23.10)	(7.90-22.61)	(8.07-22.06)

#### 3.3.2 Phytoplankton composition in exposed, unexposed and main channel sites

A total of 76 phytoplankton genera were observed consisting of the seven major algal taxonomic groups (bacillariophytes, chlorophytes, chrysophytes, cryptophytes, cyanophytes, euglenophytes and pyrrophytes) from all sites in both years. Mean generic richness was similar in reference embayments, exposed embayments and main channel sites in 2011 (P > 0.05) and 2012 (P > 0.05), respectively (Fig. 3.2). I observed similar phytoplankton compositions in reference embayments, exposed embayments (urban and cattle) and main channel sites (Fig. 3.3). The diatoms and the cryptomonads accounted for about 87 - 91% of the total phytoplankton biomass across all sites. The other phytoplankton taxonomic groups (chlorophytes, chrysophytes, cyanophytes, euglenophytes and pyrrophytes) accounted for 9 - 13% of the total phytoplankton biomass across all sites.

#### 3.3.3 Phytoplankton biomass in exposed, reference and main channel sites

Total phytoplankton biomass was highly correlated to chlorophyll *a* concentrations in 2011 (Spearman's  $r_s = 0.62$ , P < 0.001, n= 90) and in 2012 (Spearman's  $r_s = 0.71$ , P < 0.001, n= 90). Mean chlorophyll *a* concentrations were similar in reference, exposed and main channel sites in 2011 (P > 0.05) and 2012 (P > 0.05), respectively. Total phytoplankton biomass was not different in reference embayments compared to exposed embayments and main channel sites in 2011 and in 2012. However, in comparison with reference/unexposed embayments in 2012, the difference between the mean total phytoplankton biomass approached significance in exposed embayments (P= 0.09, t=1.8290) and was not significant for main channel sites (P= 0.25, t= 1.1864) (Table 3.4).



Figure 3.2. Mean and standard error of generic richness in reference embayments, exposed embayments and main channel sites for 2011 and 2012



Figure 3.3. Percentage of major phytoplankton groups by total biomass in reference embayments, exposed embayments and Main channel sites in Lake Diefenbaker from June to October (results from 2011 and 2012 are combined).

Table 3.4. Comparing the total phytoplankton biomass from the exposed embayments against the reference/unexposed embayments and the main channel sites for 2011 and 2012 using linear mixed effect models. Distance of each site down the length of the reservoir was the random term in our models. Reference/unexposed embayments were set as the intercept in the models. E= Exposed embayment and M= Main channel sites.

	Estimate	Std. error	t-value	p-value
2011				
Fixed effect				
Total phytoplankton biomass				
Intercept	2.6062			
E	0.0303	0.0697	0.4348	0.6699
M	0.0071	0.0735	0.0971	0.9239
Random effect				
	Std. Dev.			
Distance	0.0000			
2012				
Fixed effect				
Total phytoplankton biomass				
Intercept	2.3719			
E	0.1511	0.0826	1.8290	0.0874
Μ	0.1033	0.0871	1.1864	0.2539
Random effect				
	Std. Dev.			
Distance	0.0000			

# 3.4 Discussion

In general, human activities in the large catchment area surrounding reservoirs may release large nutrient loads into these systems (Jørgensen et al., 2005; Kalff, 2002). These large nutrient loads may impact the water quality of reservoirs, with serious ecological and economic consequences such as algal blooms, taste and odour problems, deterioration of habitat and the loss of biodiversity at all trophic levels and high cost of water treatment (de Figueiredo et al., 2006; Dodds et al., 2009). Hence, management strategies of reservoirs are focused on mitigating such human impacts before it becomes problematic (Carney, 2009).

Despite the number of selected exposed embayments (eight) with human activities and the isolation of these embayments from the main channel of the reservoir, phytoplankton taxonomic compositions (mainly cryptophytes and bacillariophytes, see Fig. 3.3) were similar in reference embayments, exposed embayments and main channel sites. This is further supported by the lack of significant difference in chemical variables (TP, TDP, DRP, TN, TDN, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, DOC and pH) and physical variables (epilimnetic DO, SD and WT) measured in reference embayments, exposed embayments and main channel sites in 2011 and 2012. Therefore, differences in algal taxonomic composition in exposed, reference and main channel sites were not anticipated as similar environmental conditions prevailed in reference embayments, exposed embayments and main channel sites. Similar phytoplankton composition, dominated by the diatoms and the cryptophytes was observed from August 2008 to November 2011 within 20 km of the aquaculture facility in Lake Diefenbaker (M. Otu, Department of Fisheries and Oceans Canada, personal communication). Similarly, the taxonomic composition was consistent with those reported in Abirhire et al. (2015) across the main channel of Lake Diefenbaker, irrespective of embayments and main channel sites. Furthermore, Katsiapi et al. (2012) in their study of 11 lakes and 7 reservoirs in Greece, found no effect between land use and phytoplankton community in their analysis performed separately on reservoirs. They attributed the lack influence of land use activities on the phytoplankton community to the shorter water residence time of the reservoirs compared to that of lakes. For example, Marathonas reservoir (one of the reservoirs in their study) with a small catchment area of 118 km<sup>2</sup> (maximum depth of 54 m and mean depth of 15 m) had a water residence time of ~ 2.8 years (Katsiapi et al., 2011)

In this study, the total phytoplankton biomass and the generic richness in exposed embayments were not different from those in reference embayments and main channel sites in both years. The lack of difference may be attributed to the high flow events that carried nutrient-loads from South Saskatchewan River into Lake Diefenbaker, overriding any localized embayment's influence on the phytoplankton. Abirhire et al. (2015) found inflow to be a significant predictor of phytoplankton (e.g., cryptomonads) in Lake Diefenbaker along the main channel. However, the total phytoplanktion biomass in exposed embayments was nearly significantly different (P = 0.0874) from the total phytoplankton biomass in reference embayments in 2012 (Table 3.4). Although both years were characterized by high flow from the South Saskatchewan River into Lake Diefenbaker, the mean peak flow was higher in 2011 (2300 m<sup>3</sup>/s in June) than in 2012 (1505 m<sup>3</sup>/s in mid July) (Hudson and Vandergucht, 2015).

Therefore, it is possible that the phytoplankton biomass and composition in the embayments (exposed to human activities) may be different in years with low flow from the South Saskatchewan River to Lake Diefenbaker. For example, algal blooms have been sighted in Lake Diefenbaker especially in the Qu'Appelle arm (Hecker et al. 2012; Soggie, 2011). Although the Qu'Appelle arm is not comparable with the other embayments (e.g., in terms of size), according to Hudson and Vandergucht (2015), the Qu'Appelle arm should be a considered as a large embayment because most of the flow leaves Lake Diefenbaker through the Gardiner Dam and only a minor amount of water flows out from the Qu'Appelle Dam (Saskatchewan Water Security Agency, 2012). Furthermore, in a previous study on Lake Diefenbaker (1984/1985), cyanobacteria dominated the phytoplankton, contributing 79 % of the total phytoplankton biomass during a drought period with low flow from the South Saskatchewan River (e.g., flow declined to 40  $\text{m}^3$ /s in mid June in 1984) and longer water residence time (2.27 years in 1984) (SEPS and EC, 1988). In contrast, the cryptophytes and bacillariophytes dominated the phytoplankton in the present study with high flow from the South Saskatchewan River and short water residence time (0.72 years in 2011 and 0.99 years in 2012).

## **3.5 Conclusions**

Phytoplankton community composition was similar among exposed embayments, reference embayments and main channel sites. Furthermore, the total phytoplankton biomass and the generic richness in exposed embayments were not different from those in reference embayments and main channel sites. The lack of difference may be attributed to the high flow from the South Saskatchewan River into Lake Diefenbaker, overriding any localized embayments influence. Hence, similar environmental conditions were present in the embayments and main channel of Lake Diefenbaker, because during high flow events there is corresponding increase in water level in Lake Diefenbaker and a greater likelihood of water exchange between embayments and main channel

# **CHAPTER 4-** Variability of cyanobacteria in Lake Diefenbaker, SK, Canada: comparison of two years with high inflow.<sup>3</sup>

# 4.1 Introduction

The occurrence of cyanobacterial blooms is a major threat to the water quality of freshwater lakes and reservoirs all over the world (Paerl et al., 2011). Many of the bloom-forming cyanobacterial species have the potential to synthesize taste and odour compounds in drinking water. Others produce "cyanotoxins" that present health risks to humans and livestock (Landsberg, 2002; Izaguirre and Taylor, 2004). Furthermore, the outcome of cyanobacterial blooms may cause a decline in the aesthetic value of water bodies. Finally, blooms can result in reduced dissolved oxygen concentrations that may result in fish kills (Schindler et al., 2008). In temperate regions, blooms of cyanobacteria usually occur during summer and fall in mesotrophic and eutrophic lakes and reservoirs (de Hoyos et al., 2004).

Several environmental factors, including high nutrient concentration, high pH, high water temperature and stable thermal stratification affect the growth and distribution of cyanobacteria in lakes and reservoirs (Dokulil and Teubner, 2000). Nutrient enrichment is the primary factor responsible for the expansion of cyanobacterial blooms in lakes and reservoirs (Brookes and Carey, 2011). External nutrient loading from anthropogenic activities affects the nitrogen (N) to phosphorus (P) ratios, which in turn influences the cyanobacterial composition. For instance, at a low N:P ratio, N-fixing

<sup>&</sup>lt;sup>3</sup> This chapter was written in manuscript style. Hence, there may be some overlap with other chapters.

cyanobacteria are favored, whereas at a high N:P ratios, non N-fixing cyanobacteria are favoured (Schindler et al., 2008; Paerl et al., 2014).

Climate change is another factor influencing the prevalence of cyanobacteria in Lakes and reservoirs (Paerl and Huisman, 2009). Scientists have detected a significant increase in the temperature of lakes around the world (Schindler and Smol, 2006). Climate warming affects water temperature and the intensity and duration of thermal stratification, which may encourage cyanobacterial growth (Peeters et al., 2007). For instance, at water temperatures above 25°C, cyanobacteria achieve maximum growth rates and out-compete other phytoplankton groups such as diatoms, cryptophytes, dinoflagellates and chlorophytes (Paerl et al., 2011). Some cyanobacterial species possess gas vesicles that enable them to regulate their buoyancy for optimum nutrient and light conditions during thermal stratification of the water column (Paerl and Huisman, 2008). In addition, extended thermal stratification may lead to the development of anoxic conditions in the hypoliminion and encourage the internal release of P from the sediment that favours the growth of cyanobacteria (Nurnberg 2009). In non- acidic lakes, cyanobacteria may use the iron released during anoxic conditions for rapid growth (Molot et al., 2014). Climate warming may also influence the intensity and duration of precipitation and drought (Paerl and Huisman, 2009). For example, runoff from intense precipitation events will increase nutrient loading in receiving lakes and reservoirs (Borges et al., 2008; Paerl and Huisman, 2009). If drought conditions follow such events, cyanobacteria often develop to blooms due to increased loads of nutrients (Paerl and Fulton, 2006).

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Lake Diefenbaker is a large mestrophic reservoir in the Canadian Prairies. It receives about 98 % of its water from the South Saskatchewan River. It serves as an important source of water for domestic consumption, irrigation, recreation, aquaculture, and power generation for southern Saskatchewan (Saskatchewan Water Security Agency, 2012). Only a few limited studies have reported on the cyanobacterial distribution and abundance in Lake Diefenbaker. For example, cyanobacteria dominated in the reservoir during the summers of 1984/1985 (Saskatchewan Environment and Public Safety, 1988). Royer (1972) reported that blue- green algae (Aphanizomenon) developed to bloom intensity at the Qu'Appelle arm of the reservoir in July, 1967. Moreover, residents living along Lake Diefenbaker have complained about the occurrence of episodic algal blooms. For instance, Hecker et al. (2012) commented on a cyanobacterial bloom that occured in the southern and western parts of the reservoir in fall 2007. Therefore, the objective of this study was to characterize the cyanobacterial community composition and distribution. The relationship between cyanobacterial biomass and environmental variables was also examined in order to better understand potential mechanism during cyanobacterial blooms.

# 4.2 Materials and Methods

# 4.2.1 Sampling sites

Lake Diefenbaker (51° 1'53"N, 106° 50'9"W) was created from the construction of dams in the Gardiner and Qu'Appelle Arms in 1967 (Saskatchewan Water Security Agency, 2012) (Fig. 4.1). The reservoir has a length of approximately 182 km and a width of 2-3 km. The volume and surface area of reservoir are approximately 9 km<sup>3</sup> and



Figure 4.1. Sampling sites (squares) in Lake Diefenbaker (SK). Sites are labeled from upstream (Highway 4 Bridge) to downstream (Arms) from June to October 2011 and 2012. M3 and M5 are upstream main channel sites, M9 is in the Qu'Appelle arm, C1-M, C2-M and C3-M are associated cattle main channel sites, U1-M, U2-M, and U3-M are associated urban main channel sites, and F4-M is the associated fish-farm main channel site.

394 km<sup>2</sup>, respectively (Sadeghian et al., submitted manuscript). The mean depth of the reservoir is 22 m and the maximum depth is 59 m near the Gardiner Dam. During the open-water seasons of 2011 and 2012, water samples were collected at a 2 m depth from ten sites located down the length of the reservoir (Fig. 4.1). Each site was sampled onceevery month from June to October. I avoided sampling in the month of May because of the presence of ice-cover. All water samples were collected with a Van Dorn sampler. Lake water was poured into 20 litre poly-bags and kept in the dark in coolers. Water samples were returned to the laboratory at the University of Saskatchewan and stored at ambient conditions (light and temperature) until processed for water chemistry the following day. Water samples that were collected for phytoplankton analysis were fixed in Lugol's solution.

### 4.2.2 Physical variables

Water temperature (WT), pH and dissolved oxygen concentrations (DO) were measured using a YSI 6600 v2 multi-parameter sonde. These variables are reported at the 2 m depth to correspond with water chemistry and phytoplankton analyses. The mixing depth ( $Z_{mix}$ ) was defined as the depth from the surface down to a depth where the water temperature gradient was greater than 0.5°C m<sup>-1</sup>. A biospherical radiometer (Biospherical Instruments Inc. BIC 2104 submersible radiometer) was used to measure photosynthetic available radiation (PAR). I derived the vertical extinction coefficient ( $k_d$ ) from the linear regression of the natural logarithm of PAR and depth (Kirk, 2003). The euphotic depth ( $Z_{eu}$ ) was estimated from the water surface to the depth where the light intensity is 1 %. In addition, Secchi depth (SD) was used to estimate water transparency.



Figure 4.2. Inflow into Lake Diefenbaker from May 2011 to October 2012. Peak flow from the South Saskatchewan River occurred in mid June in 2011 and early July 2012. Date was written in day/month/year format. Adapted from Hudson and Vandergucht (2015)

Rates of inflow into the reservoir were obtained from Hudson and Vandergucht (2015) (Fig. 4.2.)

#### 4.2.3 Chemical analyses

The method of Parsons et al. (1984) was used to determine total phosphorus (TP), total dissolved phosphorus (TDP) and dissolved reactive phosphorus (DRP). Samples for TDP and DRP were filtered through 0.2  $\mu$ m polycarbonate filters using syringe filtration. Total nitrogen (TN), total dissolved nitrogen (TDN) and nitrate (NO<sub>3</sub><sup>-</sup>) were measured using second derivative UV spectroscopy (Bachmann and Canfield, 1996). Samples were filtered through 0.2  $\mu$ m polycarbonate filters and the phenol-hypochlorite method (Stainton et al. 1977) was used to determine ammonium (NH<sub>4</sub><sup>+</sup>) concentrations colorimetrically. Samples for dissolved organic carbon (DOC) were measured with an organic carbon analyzer (Shimadzu TOC – 5050A) as described in Sereda et al. (2012). The particulate nitrogen (PN) was measured using an ANCA-GSL sample preparation unit coupled to a Tracer 20 mass spectrometer as reported in Vandergucht et al. (2013). The particulate phosphorus (PP) was calculated by difference (TP – TDP = PP). Then, the particulate nitrogen to phosphorus molar ratios (PN:PP) were calculated and used as a measure of nutrient limitation (Healey and Hendzel, 1979)

## 4.2.4 Chlorophyll *a* measurement and phytoplankton identification and counting

Chlorophyll *a* (chl *a*) samples were collected on 47 mm GF/F filters with vacuum filtration (10 psi) under low light conditions. Pigments were extracted and analyzed according to Bergmann and Peters (1980) and the absorbance read at 665nm as described in Vandergucht et al. (2013). Samples were identified and counted on an Olympus inverted (IX51) microscope using the technique of Utermöhl (1958). Each taxon was

identified to genus level with reference to taxonomic keys (Bellinger and Sigee, 2010; Brook et al 2002; Wehr and Sheath, 2003). A minimum of 400 cells were enumerated for each sample. I used Image-Pro Analyser 7.0 computer software to estimate the size of the phytoplankton and used a computerized phytoplankton counting program "Algamica (Version 4.0)" developed by Gosselain and Hamilton (2000) to calculate the biomass of each taxon.

#### 4.2.5 Data analyses

I investigated the relationship between environmental factors and the biomass of cyanobacteria using multiple linear regression (MLR) models. Rows with missing values were removed from the data set. I used second order Akaike's Information Criterion (AICc) from the package MuMIn (Barton, 2011) to select the best model (Burnham and Anderson, 2004). Statistical significance was set at an alpha level of 0.05. I used the function *cor2pcor* to estimate partial correlation (Opgen-Rhein and Strimmer, 2007). The function *vif* was used to estimate variance inflation factor (VIF) to check for collinearity in the model (Heiberger and Holland 2004). A VIF value greater than 5 is evidence of collinearity. However, large VIF values can be tolerated if all of the model coefficients were significantly different (p < 0.05) from zero (Heiberger and Holland, 2004). All statistics were performed in R version 2.15.2 (R Development Core Team, 2012).

#### 4.3 Results

### **4.3.1 Environmental variables**

Mean WT increased from June to July and then decreased from August to October (Fig 2.2A). The maximum WT occurred in July and August while the minimum WT occurred in October in both years. Mean WT was above 15°C from upstream

sections to downstream sections in both years (Table 2.1). Mean epilimnetic DO concentrations was above 7.0 mg L<sup>-1</sup> in both years. Mean  $Z_{mix}$  was similar from June to August and deepened from September to October in both years (Fig. 2.2B). Mean Z<sub>mix</sub> was shallowest and deepest at sites M3 and U3-M, respectively, in both years (Table 2.1). Mixing episodes and thermal stratification were not uniform across sites; thermal stratification occurred from July to September and isothermal conditions occurred in both June and October in both years (Hudson and Vandergucht, 2015). Mean  $Z_{eu}$  increased from June to October in both years (Fig. 2.2C). Mean Zeu and SD were lowest at both M3 and M5 (upstream site of the reservoir) and increased downstream (Table 2.1). Conversely, mean extinction coefficients were greatest at M3 and M5 and decreased downstream (Table 2.1). Mean k<sub>d</sub> decreased from June to October in both years (Fig. 2.2 D). Mean pH in the reservoir was about 8.0 in both years (Table 2.1). Mean TP concentrations decreased from upstream to downstream sections of the reservoir in both years. Mean TN increased downstream and was greatest at site U3-M in both years (Table 2.1). The mean concentrations of TP, TDP, TN and TDN decreased from June to August and remained the same from September to October in both years (Fig. 2.2 F - D). Mean TDP, SRP,  $NO_3^-$  and  $NH_4^+$  concentrations varied from the upstream section to the downstream section of the reservoir in both years. Dissolved organic carbon was lower in 2011 (3.2 mg  $L^{-1}$ ) compared to 2012 (5.1 mg  $L^{-1}$ ). Mean DOC remained the same from June to October in 2011, whereas mean DOC increased from June to August and decreased from August to October in 2012 (Fig. 2.2 J). Mean PN:PP varied from upstream sections to downstream sections of the reservoir in both years. Mean PN:PP was greatest at site U3-M (downstream in the Gardiner arm) in 2011 (Table 2.1).

But in 2012, mean PN:PP was greatest at site M9 (downstream in the Qu'Appelle arm) (Table 2.1 ).

## 4.3.2 Cyanobacterial composition

A total of 12 cyanobacterial genera were observed. These include *Anabaena* (now called *Dolichospermum*), *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Coelosphaerium*, *Gomphosphaeria*, *Microcystis*, *Planktolyngbya*, *Planktothrix*, *Pseudanabaena* and *Woronichinia*. Cyanobacterial biomass was low and ranged from 0.11 to 104.94 mg/m<sup>3</sup> in 2011 and from 0.06 to 53.78 mg/m<sup>3</sup> in 2012. Minimum and maximum cyanobacterial biomass occurred in June (4.12 mg/m<sup>3</sup> in 2011 and 3.05 mg/m<sup>3</sup> in 2012) and in September (56.23 mg/m<sup>3</sup> in 2011 and 26.23 mg/m<sup>3</sup> in 2012), respectively (Fig 4.3A and B). In 2011, *Aphanizomenon* and *Planktothrix* were the dominant cyanobacteria in September (Fig. 4.3A and B). Cyanobacteria biomass varied from upstream sections to downstream sections (Fig. 4.3C and D). Maximum cyanobacterial biomass occurred at site M9 (Qu'Appelle arm of the reservoir) in 2011 (59.86 mg m<sup>-3</sup>) and in 2012 (27.60 mg m<sup>-3</sup>). In both years, *Aphanizomenon* and *Anabaena* were the dominant cyanobacterial at M9 (Fig. 4.3C and D).

## 4.3.3 Relationship between cyanobacterial biomass and environmental variables

The change in the second order Akaike Information Criterion ( $\Delta$ AICc ) was < 2 between the most parsimonious and the second best model (Table 4.1). The most parsimonious regression model (*n*=34) explained 49 % of the variability of the cyanobacteria (Table 4.1). The biomass of cyanobacteria showed an increasing relationship with the PN:PP, TP and Zeu and a decreasing relationship with dissolved



Figure 4.3. Cyanobacterial biomass and composition from June to October 2011 (A) 2012 (B) and from upstream sections to downstream sections in 2011 (C) and 2012 (D) in Lake Diefenbaker

Table 4.1. The relationship between cyanobacterial biomass (Log 10 + 1 transformed) and environmental variables using multiple linear regression models (MLR) and AICc. The top two models were selected based on  $\Delta$ AICc (change in second order Akaike Information Criterion). DOC =dissolved organic carbon, PN:PP = ratio of particulate nitrogen to particulate phosphorus, TP = total phosphorus, Z<sub>eu</sub> = euphotic depth, VIF= variance inflation factor.

Models	Explanatory variable	Estimate	t value	P value	Partial correlation	VIF	ΔAICc
	Intercept	-0.299					
First model	1/(DOC) <sup>2</sup> (mg/L)	6.602	4.238	0.0002	0.61	1.06	0.00
$R^2 = 0.49,$	PN:PP (molar ratio)	0.051	3.578	0.0012	0.55	1.65	
P < 0.001, n= 34	1/TP (µmol/L)	-18.472	-3.631	0.0011	-0.55	5.86	
	$Z_{eu}(m)$	0.123	2.391	0.0235	0.40	5.68	
	Intercept	1.013					
Second model	$1/(DOC)^2 (mg/L)$	5.668	3.369	0.0022	0.53	1.28	1.03
$R^2 = 0.50,$	N:P (molar ratio)	0.048	3.316	0.0025	0.53	1.71	
<i>P</i> < 0.001, n= 34	1/TP (µmol/L)	-20.847	-3.926	0.0005	-0.59	6.58	
	$Z_{eu}(m)$	0.122	2.410	0.0228	0.41	5.68	
	Log 10 (Inflow)( $m^3/s$ )	-0.403	-1.359	0.1849	-0.25	2.24	

organic carbon. Dissolved organic carbon had the highest partial correlation coefficient (0.61), followed by PN:PP (0.55) and TP (0.55) and Zeu (0.40). The most parsimonious model was superior to the second best model by 1.03 AIC units. Inclusion of inflow in the second best model increased the AICc and increased the predictive power of the model ( $R^2_{adj}$ = 0.50). However, inflow was not a significant predictor in the second best model making this model less satisfactory than the first (Table 4.1). However, in a separate bivarate relationship, I found inflow to be positively correlated with dissolved organic carbon concentrations ( $r_s$ = 0.32 p= 0.003)

### **4.4 Discussion**

### 4.4.1 Cyanobacterial composition

Cyanobacterial biomass was low in Lake Diefenbaker in the summers of 2011 and 2012. *Aphanizomenon* and *Anabaena* were the dominant genera in terms of cyanobacterial biomass (both constituted ~ 80 % of the total cyanobacterial biomass). Despite the low biomass, of greater concern, were the presence of some potential bloom forming (*Anabaena*, *Aphanizomenon, Gomphosphaeria, Microcystis, Planktothrix* and *Woronichinia* according to Beaulieu et al., 2014 and Komárek and Hauer, 2013) and toxin producing genera (*Anabaena*, *Aphanizomenon, Coelosphaerium, Gomphosphaeria, Microcystis, Planktothrix, Pseudanabaena* and *Woronichinia* according to Beaulieu et al., 2014 and Cronberg and Annadotter, 2006) that may become abundant and threaten the water quality of Lake Diefenbaker under suitable environmental conditions (i.e., increased water residence time, extended and stable thermal stratification and internal loading of nutrients from sediments) (Dokulil and Teubner,2000; Nürnberg, 2009; Paerl et al., 2011; Paerl and Huisman, 2009). For example, cyanobacteria dominated the phytoplankton (79 %) in Lake Diefenbaker in 1984 and 1985, during a period of drought with a water residence time of 2.75 years compared to water residence time of 0.72 years in 2011 and 0.99 years in 2012 (SEPS and EC, 1988).

#### 4.4.2 Environmental factors related to cyanobacterial biomass

Dissolved organic carbon, PN:PP, TP and Zeu explained 49 % of the variability in biomass of the cyanobacteria. Dissolved organic carbon explained the greatest proportion of the biomass of cyanobacteria (partial correlation of -0.61). The relationship between the biomass of cyanobacteria and DOC is not fully understood in Lake Diefenbaker. This is partly because DOC influences a variety of factors (e.g., DOC binds to nutrients and affects light atteniation, pH and alkalinity) (Pace and Cole 2002; Leavitt et al., 2003; Waters et al., 2012), which in turn affects phytoplankton composition. Specifically, DOC has been reported to bind micronutrients such as iron (Fe), which is required by cyanobacteria for N fixation and N assimilation (Wilhelm 1995; Molot et al., 2010). Moreover, the relationship between the biomass of cyanobacteria and DOC is further confounded by the positive relationship between DOC and inflow ( $r_s = 0.32$ , p = 0.003). This is because inflow influences other factors, including water residence time, nutrient loading and water clarity. For example, high flows have shown to wash out phytoplankton with loss exceeding growth rate (Roelke et al., 2010; Reynolds, 1990) and suppress growth of phytoplankton due to high non-algal turbidity (Paerl and Huisman, 2009; Reynolds, 1990). Godlewska et al. (2003) observed that the usual cyanobacterial blooms that occur in autumn in Dobczce reservoir in Poland were eliminated during high water flows. Therefore, I conclude that any of these factors could be behind the mechanism influencing the relationship between the cyanobacterial biomass and DOC and further investigation is needed to understand this relationship.

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Cyanobacteria use their gas vesicles and/or mucilaginous sheaths to regulate their position in the water column to capture high surface irradiance required for their growth (Paerl, 1988; Paerl and Huisman, 2008; Reynolds et al., 2002). The greatest cyanobacterial biomass occurred in the fall (September) when water clarity was greatest and the reservoir was still stratified. Thus, their relationship with Z<sub>eu</sub> suggests the importance of increased water clarity for their growth in Lake Diefenbaker (Paerl and Huisman, 2008; Reynolds et al., 2002). For instance, Hecker et al. (2012) commented on cyanobacterial blooms in the southern and western parts of Lake Diefenkaer that occurred in the fall of 2007, which corresponded with the period of increased water clarity. Borges et al. (2008) attributed the dominance of cyanobacteria (especially *Anabaena*) to their ability to remain in the euphotic zone for optimum light and nutrient conditions in Segredo Reservoir, Brazil.

Cyanobacteria have been reported to use both dissolved and particulate organic P for growth (Davis et al., 2010). Empirical models predict cyanobacterial dominance in temperate lakes and reservoirs with increasing TP concentrations (100 to 1000  $\mu$ g/L) (Downing et al., 2001; Watson et al., 1997). For example, Beaulieu et al. (2014) found total phosphorus (2 to 513  $\mu$ g/L) as one of the positive predictors of cyanobacterial biomass (1 to 28,360 mg/m<sup>3</sup>) using data from 149 lakes across Canada in their model. This is consistent with the positive relationship between cyanobacterial biomass and TP in this study. However, TP concentrations ranged from 8 to 104  $\mu$ g/L and cyanobacterial biomass ranged from 0.06 to 104.94 mg/m<sup>3</sup> in the present study.

The particulate N:P ratios have been used to ascertain nutrient status of phytoplankton in lakes and reservoirs (Healey and Hendzel, 1979). Phosphorus is a major nutrient limiting cyanobacterial abundance in temperate lakes and reservoirs (Downing et al., 2001). The

relationship between cyanobacterial biomass and PN:PP suggests that P limited the growth of cyanobacteria in Lake Diefenbaker. This may be related to the loss of P from the water column because majority of the P in Lake Diefenbaker are in particulate form. Dubourg et al., (submitted manuscript) reported that P was the major limiting nutrient of phytoplankton in Lake Diefenbaker in 2013. An earlier study (1984/1985) also reported that P limited the growth of phytoplankton in Lake Diefenbaker (SEPS and EC, 1988).

The occurrence of the greatest cyanobacterial biomass at M9 (Fig. 4.1) may be related to the location and morphology of the site in the reservoir (Hudson and Vandergucht, 2015; Sadeghian et al., submitted manuscript). The Qu'Appelle arm is isolated from the major flow path of the reservoir; about 99 % of the flow leaves Lake Diefenbaker through the Gardiner dam and only 1 % leaves through the Qu'Appelle dam (Saskatchewan Water Security Agency, 2012). Hence, the Qu'Appelle arm has a longer water residence time (26 years compared to 1.5 years for the whole reservoir) (Costa, 2011). Huszar and Reynolds (1997) reported that reduced flushing rate or increased water residence time favours the dominance of cyanobacteria. In addition, the shallowness of the Qu'Appelle arm and the seiche events that occur in late summer at this location, may lead to the re-suspension of nutrients that may promote cyanobacterial biomass (Hudson and Vandergucht, 2015; Sadeghian et al., submitted manuscript).

Understanding the entire N budget in Lake Diefenbaker is still ongoing. Nevertheless, Patione et al. (2006) reported that the contribution of N-fixation to the total nitrogen budget in Lake Diefenbaker is negligible. This is consistent with the present study and may be related to the low biomass of N-fixing cyanobacteria genera (*Aphanizomenon* and *Anabaena* together only contributed < 2 % of the total phytoplankton biomass) and the attendant low heterocyst biomass (~ 1 mg/m<sup>3</sup>) observed in the summer of 2011 and 2012.

# 4.5 Conclusions

Cyanobacterial biomass was low. However, I observed the presence of some potential bloom forming and toxin producing cyanobacterial genera that may become abundant if environmental conditions become suitable for their growth. Cyanobacterial biomass decreased with increasing DOC and increased with increasing  $Z_{eu}$ , TP and PN:PP ratios. The greatest cyanobacterial biomass was observed in the fall (September), which corresponds to a period of increased water clarity. The greatest cyanobacterial biomass was observed in the Qu'Appelle arm (M9). The occurrence of the greatest biomass of the cyanobacteria at M9 may also be related to the isolation of this site from the major flow path of the reservoir (i.e., increased water residence time) and its shallowness with frequent seiche events that may re-suspend nutrients in the water column (Hudson and Vandergucht, 2015; Sadeghian et al., submitted manuscript). Future study will include meteorological patterns in relation to cyanobacterial biomass in Lake Diefenbaker.

### **CHAPTER 5-** General conclusions

### 5.1 Phytoplankton composition and the distribution of the major groups

Phytoplankton community composition and distribution reflect environmental condition in lakes and reservoirs (Padisák et al., 2006 and Salmaso, 2010). Comprehensive studies on the phytoplankton community and ecology in Lake Diefenbaker are limited. My work adds to the existing knowledge of the phytoplankton community composition in Lake Diefenbaker and provides current understanding of the factors influencing their distribution.

Based on the trophic state index calculated from chlorophyll *a* concentrations, Lake Diefenbaker is a mesotrophic system (Carlson and Simpson 1996). Similar to other temperate mesotrophic systems, I observed a total of 72 phytoplankton genera along the main channel of the reservoir with the chlorophytes (33) having the highest number of genera represented (Watson et al., 1997). The cryptophytes and bacillariophytes were the dominant groups in Lake Diefenbaker (together they contributed 89 % of the total phytoplankton biomass) as observed in other temperate mesotrophic reservoirs. The other groups (chlorophytes, chrysophytes, cyanophytes, euglenophytes and pyrrhophytes) contributed about 11 % of the total phytoplankton biomass (Fig. 2.5).

Mean chlorophyll *a* concentrations varied spatially and followed a seasonal biomodal distribution that reflects the seasonal distribution of the major phytoplankton groups in Lake Diefenbaker (Fig. 2.3). For example, the lowest chlorophyll *a* concentrations and lowest biomass of both the cryptophytes and the bacillariophytes occurred in August, which corresponds with the period of increased water clarity in Lake Diefenbaker (Fig. 2.2 C and D). It is well established that grazing by zooplankton can reduce phytoplankton abundance in Lakes and Reservoirs (Grigorszky et al., 1999; Mazumder, 1994). Vogt et al. (2014) reported that zooplankton grazing

explained 19 % of the mean summer chlorophyll *a* concentrations in the Qu'Appelle arm of Lake Diefenbaker. Although zooplankton abundance was not investigated in this study, I speculate that zooplankton grazing may be related to the low chlorophyll *a* concentrations and low biomass of the cryptophytes and the bacillariophytes in August. Therefore, a comprehensive study on the zooplankton population and further study on their abundance and distribution in relation to phytoplankton community composition and distribution in Lake Diefenbaker is required.

High flow (associated with nutrient rich loads and sediments) from the South Saskatchewan River is another factor that affects the phytoplankton distribution in Lake Diefenbaker. I found that cryptophytes biomass increased with increasing flow rates. Cryptophytes are able to use up flow-associated available nutrients for rapid growth to compensate for washout (Tolotti et al., 2010). They are also able to consume allochtonous organic matter and/or bacteria associated with allochtonous organic matter under low light conditions during high flow (Simek et al., 2008). However, our understanding of phagotrophic and osmotrophic habits of the cryptophytes in Lake Diefenbaker remains poor.

Water temperature is another factor that affects phytoplankton distribution in Lake Diefenbaker, as it affects the growth rate of phytoplankton and is associated with stratification (Adrian et al., 2009; Paerl et al., 2011). Peak biomass of the cryptophytes coincides with periods when the water temperature was warmest (Fig. 2.2 A) and with the onset of thermal stratification in Lake Diefenbaker. The bacillariophytes depend on mixing to remain suspended in the water column (Reynolds, 2012). I found that bacillariophytes biomass (*Aulacoseira* contributed 80 % to their biomass) increased with increasing mixing depth and their peak biomass occurred when water temperature was lowest in Lake Diefenbaker. The decline in the bacillariophytes biomass as the PN:PP ratios tends to increase is not yet understood. I speculate therefore that it may be related to the rapid loss P containing particles, including the bacillariophytes from the water column during stratification (Kufel, 2001).

#### 5.2 Phytoplankton in exposed, unexposed and main channel sites

Eutrophication increases the occurrence of algal blooms and it is one of the primary water quality issues globally (Smith and Schindler, 2009). This has been exacerbated by increased external loads of nutrients from urban activities, agricultural practices and shoreline erosion (Lehman, 2014; Michalak et al., 2013). Lake Diefenbaker is characterized by numerous embayments that contain anthropogenic activities (e.g., housing, golf courses, marinas and livestock operations [cattle watering]) that may impact the water quality. I found that the phytoplankton community composition was not significantly different in exposed embayments, reference embayments, and main channel sites in 2011 and 2012 (P > 0.05) (Fig 3.2; Fig 3.3; Table 3.3; Table 3.4). The flushing of these embayments during high flow events may have dampened localized influences on the embayments.

# 5.3 Cyanobacterial distribution

Residents living along Lake Diefenbaker have sighted and complained about the occurrence of episodic algal blooms especially at the arms (Soggie 2011; Hecker et al., 2012). Blooms of certain cyanobacteria impact water quality by causing taste and odour problems and some cyanobacteria genera produce "cyanotoxins" that may pose serious health risks to humans and livestock (Landsberg, 2002; Izaguirre and Taylor 2004). In general, cyanobacterial biomass was low, contributing < 5 % of the total phytoplankton biomass (*Aphanizomenon* and *Anabaena* were the dominant genera in terms of biomass) in Lake Diefenbaker. I observed peak

cyanobacterial biomass in the fall (September) and at M9 (Fig. 4.3). This suggests that cyanobacteria require high surface irradiance for their growth (Paerl, 1988). Furthermore, the increased water residence time in the Qu'Appelle arm may explain the observed peak in cyanobacterial biomass at M9 (Costa, 2011; Hudson and Vandergucht, 2015). My model revealed a positive relationship between cyanobacterial biomass and TP concentrations. However, the relationship between cyanobacteria biomass and DOC is not fully understood in Lake Diefenbaker.

#### **5.4 Summary**

The cryptophytes and bacillariophytes dominated the phytoplankton during both years with high flows from the South Saskatchewan River. For example, the cryptophytes were abundant during high flow rates and when the water temperature was warmest and the reservoir was stratified whereas the bacillariophytes were abundant during cool, isothermal conditions. It is possible that under different environmental conditions (low inflow, increased water residence time and extended and stable thermal stratification) that the phytoplankton composition may shift in favor of the cyanobacteria over the cryptophytes and the bacillariophytes (Nürnberg, 2009; Paerl et al., 2011; Paerl and Huisman, 2009). For example, in a previous study on Lake Diefenbaker, cyanobacteria dominated the phytoplankton biomass (79%) during a drought period with low flow from South Saskatchewan River and a corresponding increased water residence time (2.5 years) (SEPS and EC, 1988). Therefore, future research will benefit from monitoring drought years with low flow from the South Saskatchewan River and consequently long water residence time. I recommend the incorporation of meteorological variables in models to more fully understand the effect of weather patterns on phytoplankton composition and distribution (especially the cyanobacteria).

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Current studies on algal taxonomy have used molecular techniques to identify algal species and strains that produce toxins (Al-Tebrineh et al., 2012). I recommend the use of molecular techniques to improve the resolution of phytoplankton taxonomy, particularly the phytoplankton in blooms that may cause taste, odour and toxin issues. The hepatotoxic microcystins are the most common and widely studied toxins synthesized by toxin- producing cyanobacteria (Ho and Michalak, 2015). Future management of the water quality of Lake Diefenbaker will benefit from the analysis of microcystins concentrations.

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