Assessing Eutrophication Vulnerability as an Indicator of Cyanobacteria Presence in Kejimkujik National Park

by

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ABSTRACT

Kejimkujik National Park (KNP) and National Historic Site (Nova Scotia, Canada) is home to numerous lakes that support a multitude of recreational activities and aquatic habitats. The park is comprised of camp sites, canoeing routes, and hiking trails, making it a popular tourist attraction during the summer months. Aquatic systems in the park not only support these recreational activities, but also support diverse organisms that are important to the park's ecosystem. This thesis was the first study to assess the cyanobacterial populations within KNP. The objectives of this study were to identify lakes within the park that are susceptible to cyanobacteria blooms. Several factors could influence the proliferation of cyanobacteria in KNP, including nutrient abundance, light availability, water temperature, mixing regimes, and flushing rates. This study involves a phosphorus loading model, which uses publicly available spatial and water quality data, to characterize the lakes in KNP based on their eutrophication vulnerability, using the factors known to contribute to harmful cyanobacterial blooms. The results of the model were compared to information collected from a park-wide cyanobacteria sampling program and toxin survey conducted in 2023 to assess the applicability of this model for identifying lakes vulnerable to blooms. Next generation MiSeq illumina sequencing results indicate that cyanobacteria were present in small abundances in 10 of the 16 study lakes within the park, but little is known about whether these photosynthetic bacteria populations could proliferate and form blooms that could be harmful to humans and other animals. Three of the study lakes contained potentially toxin-producing cyanobacteria of the order Nostocales. Anatoxins and microcystins were detected in small quantities in several locations in the park. Water quality parameters collected from 2008 to 2021 indicate that cyanobacteria were primarily present in oligotrophic lakes. Cyanobacteria were additionally only shown to exist within certain thresholds of certain water quality parameters. Cyanobacteria were only found in lakes with a total phosphorus concentration between 4 to 10 µg/L, and a total organic carbon concentration between 2 to 5 mg/L. More research needs to be conducted to determine exactly what is driving the cyanobacteria proliferation in KNP.

Keywords: *Cyanobacteria, Eutrophication, Kejimkujik National Park, Phosphorus Loading Model, Oligotrophic lakes*

LIST OF ABBREVIATIONS

CCME	Canadian Council of Ministers of the Environment
CyanoHABS	Cyanobacteria Harmful Algal Blooms
DOC	Dissolved Organic Carbon
GHG	Greenhouse Gas
HRM	Halifax Regional Municipality
IRM	Integrated Microbiome Research
KNP	Kejimkujik National Park
N	Nitrogen
OM	Organic Matter
OWS	Onsite Wastewater Systems
P	Phosphorus
RA	Relative Abundance
SPATT	Solid Phase Adsorption Toxin Tracking
TOC	Total Organic Carbon
TP	Total Phosphorus
WHO	World Health Organization

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CHAPTER 1: INTRODUCTION

1.1 Motivation

Cyanobacteria have a long and notable history, dating back more than 2.5 billion years to the first mass extinction when the presence of cyanobacteria led to the emergence of aerobic metabolism on Earth (Aiyer, 2022). Cyanobacteria remain a vital ecosystem component, as these organisms fix nitrogen and supply large amounts of oxygen globally (Garcia-Pichel & Belnap, 2021). Although they provide important ecosystem functions, the proliferation of cyanobacteria in specific environments can potentially be harmful (Paerl et al., 2001). There has been an increase in the abundance and number of cyanobacteria blooms reported more recently (MacKeigan et al., 2023; Johnston et al., 2021).

1.2 Background, Context, and Definitions

1.2.1 Overview of Cyanobacteria

Cyanobacteria reside in a diverse group of prokaryotic organisms that have the ability to flourish in many different ecosystems (Vidal et al., 2021). Cyanobacteria have most notably been found in freshwater environments but have also been observed in marine environments around the world (Carmichael, 2008). There are many different cyanobacteria taxa found worldwide, ranging in size from about 0.2 µm to 40 µm (Vidal et al., 2021). Throughout cyanobacteria's history, it has been identified as a type of plant or plant-like organism and has been referred to as "Cyanophyta", "blue-green algae", "Cyanophyceae", and "Schizophyceae" (Vidal et al., 2021). Cyanobacteria are single-celled bacteria despite the common misconception of them being algae (Vidal et al., 2021). The primary distinction between cyanobacteria and algae is that cyanobacteria are prokaryotic, and algae are eukaryotic (Garcia-Pichel & Belnap, 2021). Cyanobacteria are seemingly the only prokaryotic organisms to possess chlorophyll-a, which give them their green pigment and allows them to perform oxygenic photosynthesis (Vidal et al., 2021).

1.2.2 Toxic Cyanobacteria

Freshwater cyanobacteria blooms have become a widespread global issue, with an increase in the number of blooms linked directly to climate change (Paerl & Paul, 2012). Cyanobacteria harmful algal blooms (cyanoHABs) are, for the most part, caused by pelagic cyanobacteria (cyanobacteria occurring in the open ocean), which can proliferate in freshwater bodies (O'Neil et al., 2011). Planktonic (surface-dwelling) and benthic (bottom-dwelling) species of cyanobacteria have the potential to severely impact ecosystem functions and produce high concentrations of toxins within the water bodies they intrude (Bláha et al., 2009). Some cyanobacteria are capable of producing cyanotoxins, which are toxic compounds produced by certain strains of cyanobacteria (Drugă et al., 2019). Ingestion of cyanotoxins has been associated with liver, skin, and digestive diseases, neurological impairment, and, in some cases, death (Chorus & Bartram, 1999). The World Health Organization (WHO) has developed tolerable daily intakes for many cyanotoxins to give a comprehensible guide of the amount of each cyanotoxin that can be ingested over a lifetime without considerable health risks (2021). The primary concern of cyanobacteria blooms is the presence and type of cyanotoxins found within these blooms and how these will harm aquatic environments and the consumers of these freshwater sources (WHO, 2021).

1.2.3 Cyanobacteria Blooms

A wide array of variables lead to the likelihood of cyanobacteria blooms occurring in freshwater bodies (Paerl et al., 2001). Relevant variables include but are not limited to nitrogen and phosphorus (P) concentrations, organic matter concentrations, water turbidity, climate change, and water column stratification and stability (Paerl et al., 2001). Detrimental effects occurring in freshwater bodies as a result of the presence of cyanoHABs include the loss of biodiversity, suppression of primary producers, and reduction in food web resilience (Carmichael & Boyer, 2016). Dense cyanobacteria blooms can also produce surface scums (Drugă et al., 2019). Surface scums alter the biological and chemical structure of freshwater bodies by blocking sunlight from reaching benthic plants and phytoplankton, and depleting oxygen in the water body (creating anoxic and hypoxic zones) (Drugă et al., 2019). Dense surface scums have also been associated with the release of large amounts of cyanotoxins (Drugă et al., 2019).

1.2.4 Eutrophication and CyanoHABs

Eutrophication is a primary factor linked to the potential for cyanobacteria blooms in freshwater bodies (Paerl et al., 2001), and it threatens the global supply of reliable drinking water (Sharabian et al., 2018). Eutrophication is distinguished as an excess growth in plant biomass due to enhanced primary productivity (Marvel, 2016). Studies have shown that eutrophication and climate change are linked due to several meteorological factors brought upon by climate change (Sharabian et al., 2018). Rising temperatures have created favourable conditions for cyanobacteria growth due to changing physical characteristics of aquatic environments, so cyanobacteria are more competitive (Raven & Geider, 1988). The critical nutrient that increases the risk of eutrophication is P, a limiting nutrient in freshwater bodies (Johnston et al., 2021; Trimbee & Prepas, 1987). Anthropogenic eutrophication is associated with agricultural, industrial, and urban development, which leads to higher levels of primary production in bodies of water, accelerating eutrophication and, therefore, leading to water quality impairment (Paerl et al., 2001).

1.3 Knowledge Gaps

The presence of cyanobacteria and cyanotoxin species can change depending on location (Carvalho et al., 2011). There have been many studies on the presence of cyanobacteria and cyanotoxins on a global scale but fewer studies on smaller spatial scales (Carvalho et al., 2011).

The presence of cyanobacteria was studied in four lakes in the Halifax Regional Municipality (HRM), providing preliminary data on the occurrence of cyanobacteria specific to the HRM (Betts, 2018). There have been a few other studies relating specifically to cyanobacteria blooms and toxic cyanobacteria in Nova Scotia (Johnston et al., 2021; Anderson et al., 2017), which serve as a basis for developing management plans and techniques to counteract these blooms. However, Nova Scotia has thousands of lakes which vary widely in terms of physical and chemical characteristics (Johnston et al., 2021). There is a lack of understanding of which lakes in the province are more susceptible to cyanoHABs (Johnston et al., 2021), making it difficult to narrow down the lakes needing management and monitoring.

1.4 Introduction to Study

This study will aim to characterize the cyanobacteria and cyanotoxin presence in the freshwater environments of Kejimkujik National Park (KNP) in Nova Scotia, Canada. A P loading model will be used to predict the eutrophic vulnerability of the study lakes in KNP. Additionally, water quality parameters collected by Parks Canada from 2008 to 2021, and cyanobacteria and cyanotoxin data collected during the summer of 2023 will be assessed. KNP is a widely used park during the summer months (Park Canada, 2023). There are many summer activities involving lakes within the park, and some campers use water directly from the lakes and rivers as potable water sources (Parks Canada, 2023). The presence of cyanobacteria has never been studied within the park, so this research serves as a preliminary study to possibly implement lake monitoring programs within KNP. The objectives of this study will aim to a) identify the lakes in KNP that are most vulnerable to eutrophication, b) identify the types of cyanobacteria and cyanotoxins present in the freshwater environments of KNP, and c) determine the factors that are correlated with the presence of cyanobacteria and cyanotoxins in the freshwater environments of KNP.

This study will look at data collected from 16 lakes in KNP that were sampled during the summer of 2023 and analyzed for bacterial community composition using DNA sequencing methods, and cyanotoxins. Chemical parameters were also assessed in this study and were collected by Parks Canada and are publicly available through the Canadian Federal Government's open data portal. The other data being studied is currently only available to select personnel working on research projects related to cyanobacteria in Nova Scotia.

This study will attempt to answer the research question: How can we characterize the occurrence, species, and factors that influence the presence of cyanobacteria and cyanotoxins in the freshwater environments of Kejimkujik National Park in Nova Scotia? While answering this research question, we expect to find a relationship between lakes of higher eutrophic vulnerability and lakes with higher cyanobacteria and/or cyanotoxin concentrations.

1.5 Summary of approach

The research question was answered by identifying the lakes in the park with the highest concentrations of cyanobacteria and their subsequent trophic status. Predicted P concentrations were identified in the P loading model, which were then compared to the measured P concentration to identify the feasibility of using this model as an accurate predictor of total phosphorus (TP). Following, the water quality parameters were assessed to identify trends and relationships in the data over time, and then these variables were compared to the RA of cyanobacteria in each of the lakes. This assessment allowed for the determination of the occurrence, species, and factors which influenced the presence of cyanobacteria and cyanotoxins in the freshwater environments of KNP.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This literature review used various sources to provide an overview of the current understanding of cyanobacteria blooms and management practices, specifically focusing on literature based on studies in Northeastern North America. Several references from the primary literature source, *Trophic triage: a tiered eutrophication vulnerability screening tool for lakes in sparsely monitored regions* (Johnston et al., 2021), were investigated and integrated into this review.

2.2 Cyanobacteria and Climate Change

2.2.1 Rising Temperatures

Many studies have shown that climate change has created favourable conditions for the growth of cyanobacteria (Paerl & Huisman, 2009; O'Neil et al., 2011; Raven & Geider, 1988). Cyanobacteria are exceptional organisms in their ability to adapt to the changing environments created by the anthropogenic warming of the Earth (Paerl & Huisman, 2009). Cyanobacteria compete most successfully with their primary competitors at higher temperatures (Jöhnk et al., 2008; Paerl & Huisman, 2009). Cyanobacteria growth increases in warmer temperatures, while most competing eukaryotic organisms experience a decline in growth rate (Jöhnk et al., 2008). Temperatures above 25°C have been shown to represent cyanobacteria's ideal thermal environment (Robarts & Zohary, 1987). Cyanobacteria proliferations may also contribute to warming local water temperatures through their light absorption propensity, which can further harm aquatic organisms living within these dense cyanobacteria blooms (Paerl & Huisman, 2009). Remote sensing was used to study surface blooms in Lake IJsselmeer, Netherlands, and it was documented that the water temperature was 3°C higher than surrounding surface waters (Ibelings et al., 2003).

Rising temperatures increase the geographical range of suitable environments for photosynthetic bacterial populations to thrive in (Paerl & Huisman, 2009). This is evident for the toxin-producing species, *Cylindrospermopsis,* which was once only found in tropical and subtropical environments and is now being found throughout Europe and the United States (Briand et al., 2003; Saker et al., 2003).

2.2.2 Stratification

Lake stratification occurs when the water in the water column of a lake becomes separated into distinct layers with different densities due to seasonal warming and cooling cycles (Fafard, 2018). Many bloom-forming cyanobacteria have been shown to flourish in stratified conditions (Paerl & Huisman, 2009). The periods of time in which lakes remain stratified have increased due to global warming (Paerl & Huisman, 2009). The warming of surface waters, possibly as a result of cyanobacterial growth, escalates vertical stratification (Paerl & Huisman, 2009).

In thermally stratified lakes, cyanobacteria are often found in the metalimnion (middle layer of the lake) during the summer months (Davis et al., 2003). Some bloom-forming cyanobacteria can form gas vesicles, making them more buoyant (Walsby, 1988). Given the applicable characteristics (rising temperatures and low wind mixing), the water column becomes still, and any buoyant cyanobacteria floats upwards, creating dense surface blooms in the epilimnion (upper layer of a lake) (Paerl & Huisman, 2009). The vast accumulation of cyanobacteria cells in the epilimnion has the potential to lead to exceptionally high toxic cyanobacteria concentrations (Paerl & Huisman, 2009). Cyanobacterial surface blooms have competitive dominance over other phytoplankton as they can maximize their photosynthetic needs in the surface layer (Huisman et al., 2004). Surface blooms of cyanobacteria typically suppress their non-buoyant eukaryotic competitors found at lower levels of the water column, as blooms create less light availability in the hypolimnion (lower layer of a lake) (Huisman et al., 2004; Jöhnk et al., 2008).

A study on the cyanobacterial genus *Anabaena* in a lake in Germany has shown that increased thermal stratification has benefited species within this genus (Wagner & Adrian, 2009). Due to the ability of *Anabaena* to regulate its buoyancy, it can access nutrients in the hypolimnion and migrate to the epilimnion for optimal light conditions (Wagner & Adrian, 2009).

Stratification also has the potential to lead to oxygen depletion in the hypolimnion (LeBlanc et al., 2008). Oxygen depletion in the hypolimnion can increase internal loading of nutrients like P and Fe (iron) (LeBlanc et al., 2008). The release of P and Fe have been shown to promote cyanobacterial growth (Reinl et al., 2021; Orihel et al., 2017).

2.2.3 Carbon dioxide

The catalyst for global warming stems from greenhouse gas emissions (GHG) (IPCC, 2021). The Earth's climate has already warmed more than 1℃ since the 19th century due to high GHG emissions, primarily from anthropogenic activities that emit carbon dioxide (CO_2) (IPCC, 2021). Carbon dioxide supports photosynthetic growth as it is one of the main contributors to photosynthesis ($CO_2 + H_2O \rightarrow C_6H_{12}O_6 + O_2$) (Thompson et al., 2017). Cyanobacteria and other phytoplankton species rely on CO_2 for their photosynthetic needs (Paerl & Huisman, 2009)

The expected relationship between cyanobacteria and increasing CO_2 is not well studied, but some models have predicted the prevalence of cyanobacteria in intensified carbon environments (O'Neil et al., 2011). Many studies have assessed the potential competitive dominance that cyanobacteria will have over eukaryotic species in these environments (Shapiro & Wright, 1990; Paerl et al., 2009). Paerl & Huisman (2009) have hypothesized that due to the proximity of surface-dwelling cyanobacteria to atmospheric CO_2, there will be a competitive advantage for cyanobacteria over other types of phytoplankton.

Studies have also shown that there may be a difference in how cyanobacteria genera will react to increasing CO_2 environments (O'Neil et al., 2011). A study conducted by Van De Waal and co-authors (2011) used a competition experiment to assess the difference between toxic and non-toxic strains of the freshwater cyanobacterium *Microcystis aeruginosa* under contrasting CO_2 conditions. The results showed that the toxic strain was dominant in low CO_2 conditions, and the non-toxic strain was dominant in high CO_2 conditions (Van De Waal et al., 2011). Additional studies need to be conducted to affirm the results included in this review regarding the implication of CO_2 on cyanobacteria.

2.2.4 Salinity

Many ramifications that occur as a result of climate change have been shown to increase salinities in various parts of the world (Paerl & Huisman, 2009). Rising sea levels, increased use of freshwater for agricultural purposes, road salt application, and summer droughts are only some of the primary contributors to the increased salinization of freshwater bodies (Paerl and Huisman, 2009). Studies have shown there are various genera of euryhaline (salt-tolerating) cyanobacteria found in estuarine environments (O'Neil et al., 2011). Research on blooms of freshwater cyanobacteria in brackish waters have been studied in several locations globally

(Paerl & Huisman, 2009). These studies indicate that salt-tolerant cyanobacteria may have a competitive advantage over other freshwater phytoplankton species (Paerl & Huisman, 2009).

The tolerance of several cyanobacteria species to salinity may have consequences on freshwater environments (O'Neil et al., 2011). *Microcystis*, a genus of freshwater cyanobacteria that commonly produces neurotoxins and hepatotoxins (WHO, 2021), have been shown to increase toxin production in elevated salinity (O'Neil et al., 2011). *Microcystis* have a higher salt tolerance than many phytoplankton species (Tonk et al., 2007), giving *Microcystis* a competitive advantage in freshwater environments with high salinity (Robson & Hamilton, 2003). Salinity is an important variable to consider when studying cyanobacteria proliferation, and many studies still need to be done to understand the full impacts of rising salinity levels on bloom-forming cyanobacteria.

2.2.5 Potable Water Sources

Water scarcity is a serious issue in many parts of the world (Tian et al., 2017). As a result of severe drought, increasing GHG emissions, and water contamination, we are seeing a global decrease in available water resources (Tian et al., 2017). Ecosystem degradation and external nutrient loads lead to eutrophication and, ultimately, the contamination of water bodies (Sharabian et al., 2018). Cyanobacteria blooms contribute to water contamination and the ongoing water scarcity issue that the world faces (Sharabian et al., 2018). To combat water scarcity and the other impacts of climate change, GHG emissions need to decline so the remediation of freshwater bodies can be initiated.

2.3 Phosphorus and Cyanobacteria

Increased P concentrations have been shown to impact the proliferation of cyanobacterial blooms immensely (MacKeigan et al., 2023; Trimbee & Prepas, 1987; Johnston et al., 2021). Lakes in Nova Scotia can generally be assigned a trophic status depending on their total P concentration value (Johnston et al., 2021). The classification system, developed by the Canadian Council of Ministers of the Environment (CCME), has been widely adopted throughout North America (Johnston et al., 2021; James et al., 2015).

Studies have shown that cyanobacteria require high nutrient inputs, primarily consisting of P, to create dense blooms (MacKeigan et al., 2023; Trimbee & Prepas, 1987). While P is a large determiner of the potential for cyanobacterial blooms, much of the research on the relationship between P and cyanobacterial blooms has typically been conducted in similar environments (usually lowland, alkaline, eutrophic lakes) (Carvalho et al., 2011; Westwood & Ganf, 2004). As a result of several homogenous studies, it is difficult to determine if P inputs are among the primary causes of cyanobacteria-bloom proliferation within contrasting environments (Carvalho et al., 2011). Further studies examining cyanobacterial abundance at a comprehensive quantitative level across different eutrophic gradients will allow for the creation of a more accurate predictor of bloom-forming cyanobacteria models to be developed (Carvalho et al., 2011).

There are many other parameters that have been examined and shown to impact the presence of bloom-forming cyanobacteria (Carvalho et al., 2011; James et al., 2015). When studying cyanobacterial blooms on a national scale, Carvalho and co-authors (2011) revealed that water colour and alkalinity may play a more significant role than nutrient concentrations. This study was conducted on lakes that were divergent to lakes typically studied for blooms as they exhibited low water colour and relatively neutral pH (Carvalho et al., 2011). However, the results showed that increased P concentrations, along with higher water retention times, still caused an escalation in cyanobacterial abundance (Carvalho et al., 2011).

Kerekes and co-authors (1989) found a strong relationship between total P and zooplankton abundance in lakes in Nova Scotia. This study could be relevant as other studies on zooplankton have suggested that grazing zooplankton eliminates cyanobacteria's primary competitors (MacKeigan et al., 2023; Haney, 2010). While high P concentrations remain a primary attributor to cyanobacterial blooms, the implications of P concentrations on freshwater bodies require advanced studies to preclude bloom-forming cyanobacteria occurrence (Orihel et al., 2017; Carvalho et al., 2011; Johnston et al., 2021; Trimbee & Prepas, 1987).

2.3.1 Phosphorus Sources in Nova Scotia

Nova Scotia is likely seeing increased eutrophication within freshwater bodies as a result of rising urbanization (Doucet et al., 2023; Schwartz & Underwood, 1986). Several local sources discharge nutrients into lakes around Nova Scotia, including septic systems, agricultural activities, and land runoff (Van Heyst et al., 2022). Southwestern Nova Scotia has several lakes classified as hyper-eutrophic and oligotrophic, indicating that anthropogenic runoff is the primary cause of elevated trophic states in the lakes found in these areas (Van Heyst et al., 2022).

Models and screening tools have been developed to predict the total P concentration within lakes in the province (Johnston et al., 2021; Canfield & Bachmann, 1981; Marvel, 2016).

Onsite wastewater systems (OWS) are a possible source of groundwater contamination in numerous bodies of water (Schellenger & Hellweger, 2019). Human waste and synthetic detergents are the primary sources of P found in domestic wastewater (McCray et al., 2005). Roughly 34% of Nova Scotians rely on OWS (Statistics Canada, 2015). Modern-day OWS typically consists of a septic tank that dispenses into a tile drainage disposal field, which is grounded by soil (Van Heyst et al., 2022). Sorption and precipitation are the primary forms of P removal from groundwater, and the effectiveness of these processes is dependent on the soil and hydrology parameters (McCray et al., 2005; Robertson et al., 1998). A study conducted by Schellenger & Hellweger (2019) indicated that P from OWS is a significant contributor to the eutrophication of downstream lakes.

The mink fur farming industry was established in Nova Scotia in the 1930s and peaked in 2012 when the demand for mink pelts increased (Van Heyst et al., 2022). Due to the sudden peak in the industry, a notable amount of nutrient-rich runoff was excreted from the farms, followed by an increase in algal blooms in nearby lakes (Campbell et al., 2022). Monitoring efforts were established to assess the impact of mink fur farming on water quality (Campbell et al., 2022). Campbell and co-authors (2022) found that mink fur farms contribute significant amounts of nutrients to freshwater lakes, creating enhanced anthropogenic eutrophication. The Nova Scotia Fur Industry Act was established to create regulations around allowable concentrations of different contaminants in groundwater and surface water, which resulted in reduced P loads from mink farms (Van Heyst et al., 2022). Even with these regulations in place, substantial nutrient runoff from mink farms can still be anticipated, and it is recommended that mink farms operate as zero discharge systems within their watersheds to reduce the potential of significant nutrient runoff (Van Heyst et al., 2022).

While anthropogenic P sources are prominent within Nova Scotia, the natural P sources should also be considered. Natural sources of P in lakes can be attributed to atmospheric deposition, weathering geological material, soil erosion, and plant decomposition (USGS, 2018).

2.3.2 Internal Phosphorus Loading

The internal cycling of P has led to the impeded recovery of lakes (Orihel et al., 2017). Aquatic ecosystems with an extensive history of nutrient pollution have been shown to harbour nutrients within lake sediment, which is released to surface waters under certain conditions (Welch & Cooke, 1995). Internal P loading is enabled by anoxic conditions in the hypolimnion, which elevates the release of P from the sediment (Nürnberg, 2009). The release of internal P has been shown to stimulate algal blooms (Orihel et al., 2017). Internal loading is a complex process to predict in that its contribution of phosphorus to aquatic ecosystems varies widely between water bodies (Orihel et al., 2017). While Orihel and co-authors' (2017) study suggests that Nova Scotia has a relatively low potential for internal P loading, it should still be considered when assessing P sources within local lakes.

2.4 Past Studies on Kejimkujik National Park

Studies on the freshwater environments of KNP were documented in the 1970s and 80's (Kerekes & Freedman, 1989; Kerekes et al., 1989; Kerekes et al., 1986; Kerekes, 1975). Kerekes (1975) determined all 46 lakes in KNP to be oligotrophic. The lakes in KNP were also shown to be vulnerable to acidification (Kerekes et al., 1989). While lakes in Nova Scotia were likely naturally acidic as a result of the poorly buffered soils, the bedrock, and the organic acidity from the wetlands (Clair et al., 2007; Kerekes et al., 1986), the acidity in the lakes was further enhanced. In the 1980s, there was a significant increase in acidic deposition in Atlantic Canada (Jeffries et al., 1986). The pH of lakes in the province became so acidic that salmon runs in many rivers in southwestern Nova Scotia were eliminated or heavily impacted (Bowman et al., 2014). However, more recent studies on drinking water supplies in Nova Scotia indicate that lakes may be recovering from acidification (Anderson et al., 2017).

A study conducted by Kerekes (1975) calculated the total P concentrations within 17 lakes in KNP and found that most of the lakes had relatively low P concentrations (typical of what one would expect to see in oligotrophic lakes). Trends found by Kerekes's (1975) study show that lakes with low flushing rates are likely to have lower overall total P concentrations but slightly higher P concentrations in the hypolimnion (due to internal P loading). Past studies on water quality in KNP serve as a foundation for this study.

2.5 Management of Eutrophication and Associated Cyanobacterial Blooms

Different management strategies have been applied to lakes susceptible to bloom-forming cyanobacteria in hopes of understanding and decreasing the proliferation of these photosynthetic organisms (Johnston et al., 2021; Bennion et al., 2005; Londe et al., 2016). Different screening tools and models have been used to identify eutrophic susceptibility in hopes of monitoring bloom-forming cyanobacteria populations (Johnston et al., 2021; Bennion et al., 2005; Carvalho et al., 2011). A high-level, three-tiered screening tool was developed to identify the eutrophication vulnerability of lakes in Nova Scotia (Johnston et al., 2021). This tool was developed due to the recent increase in bloom-forming cyanobacteria in rural lakes (Johnston et al., 2021). Through the use of a GIS-based assessment and basic limnological data, this tool identifies lakes vulnerable to eutrophication. Bennion and co-authors (2005) developed a similar screening tool, where a GIS-based assessment was used to identify lakes more vulnerable to eutrophication so that management actions can be focused. However, this tool looks to characterize the eutrophic vulnerability of lakes at a broader scope (at a national instead of local scale) (Bennion et al., 2005).

Remote sensing has been used in Ibitinga's reservoir, Brazil, to determine how the changes in hydraulic residence time affect the spatial distribution of phytoplankton (Londe et al., 2016). Remote sensing has shown to be an adequate tool in assessing chlorophyll-a distribution over time and in distinguishing the relationship between hydraulic residence times and cyanobacterial blooms (Londe et al., 2016). However, findings suggest that more research needs to be conducted to assess this tool's feasibility (Londe et al., 2016).

A model was developed to assess the impact of past management practices on the water quality of a lake in Massachusetts, United States, which investigated the effects of switching from cesspools to septic systems and banning laundry detergent containing P (Schellenger & Hellweger, 2019). The model predicted that without the P detergent ban the P concentration would have been 31.4% higher, and without the ban on cesspools the P concentrations would have been 89.5% higher (Schellenger & Hellweger, 2019). This model further predicts that replacing all OWS with a sewer system and wastewater treatment facility would create an exponential decrease in external P loads (Schellenger & Hellweger, 2019).

2.6 Conclusion

Cyanobacterial blooms are a global issue that require extensive research and monitoring (Paerl & Huisman, 2009). Ongoing studies are essential to measure the full ramifications of climate change on the future of bloom-forming cyanobacterial populations. Research on decreasing P loads needs to be further examined. Studies specifically on P concentrations in rural, undeveloped areas should be assessed to address eutrophication in Nova Scotia. Eutrophication management tools must be developed, and screening tools need to be applied to combat eutrophication, and subsequently bloom-forming cyanobacteria.

CHAPTER 3: METHODS

3.0 Overview of Study

This study investigates the feasibility of using a P loading model to determine the potential P concentrations within lakes in KNP. This P model was developed in response to a recent increase in cyanobacteria blooms in rural lakes in Nova Scotia (Johnston et al., 2021). A comprehensive analysis of the water quality data was conducted to determine trends and relationships in the data collected by Parks Canada from 2008 to 2021. The cyanobacteria data collected by Dr. Rob Jamieson's summer research group in the summer of 2023 was assessed to characterize the species and abundance of cyanobacteria present in the park. Cyanotoxin data was also analyzed.

3.1 Study Area

3.1.1. Kejimkujik National Park

This study focuses on lakes in KNP, Nova Scotia, Canada. KNP is considered a National Historic Site due to its cultural landscape, which was home to the Mi'kmaq people for over 4000 years (Parks Canada, 2023). KNP is located in the southwestern portion of Nova Scotia (as shown in Figure 3.1), a location that is mainly unimpacted by residential and agricultural activities. However, KNP is heavily used during the summer months for recreational activities centred around the lakes within the park (Parks Canada, 2023). KNP's climatic patterns are typical of Nova Scotia's, with relatively warm summers and cold winters, and mean annual average temperatures of 6.5 ℃ (Webb & Marshall, 1999). There is substantial rainfall year-round, and the mean annual precipitation is about 1350 mm, with around 480 mm of precipitation occurring between May and September (Webb & Marshall, 1999). The southwestern portion of Nova Scotia primarily consists of metamorphic and weathered granitic bedrock (Johnston et al., 2021). KNP is within the Acadian Forest region, containing a blend of temperate tree species (Parks Canada, 2023).

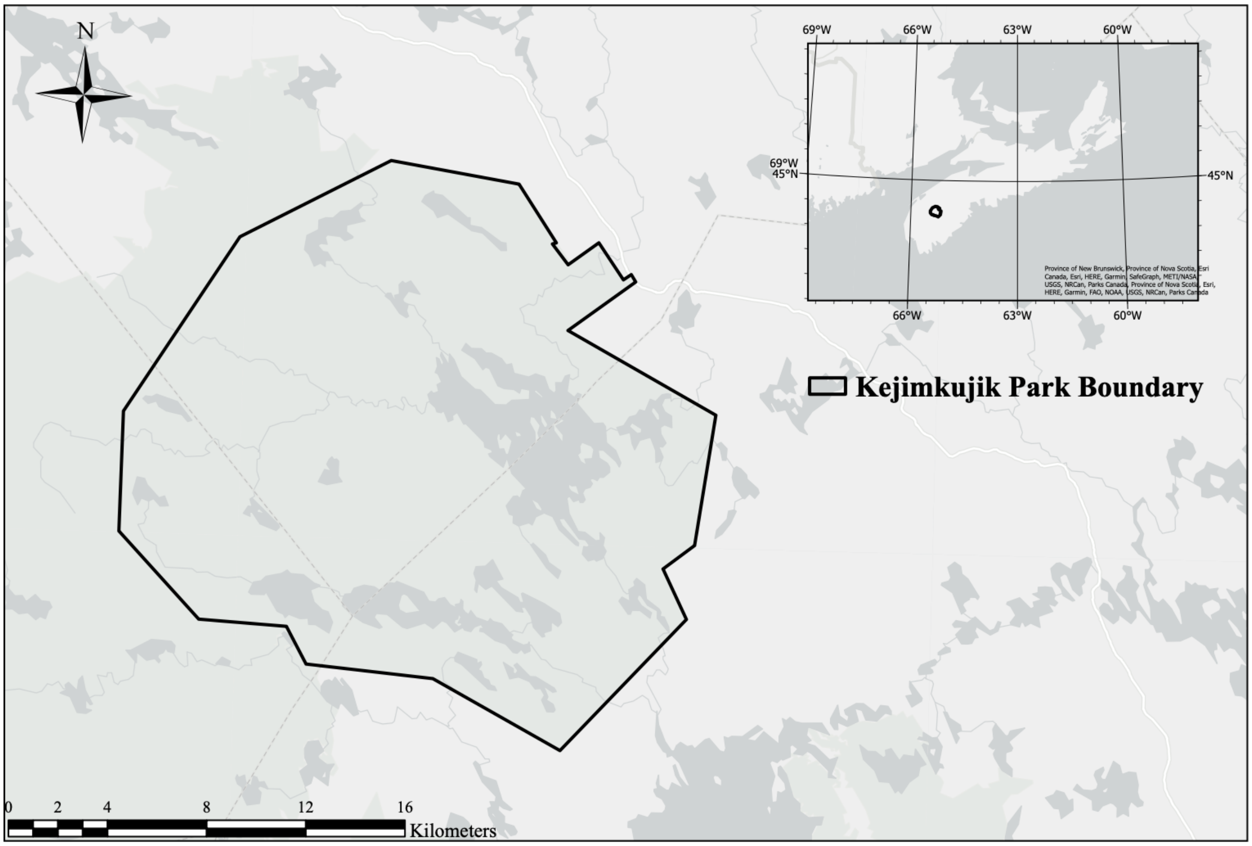


Figure 3.1 Study area for screening tool assessing eutrophication vulnerability in KNP, Nova Scotia, Canada. An inset of the map is provided for geographical context.

3.1.2 Lakes within Kejimkujik National Park

There were 16 lakes chosen for this study. The lakes were chosen based on their locations within the park, as they encompass most of the area (north to south and east to west) within the park boundary. Previous studies on water quality within the park, conducted in the 1980s, concluded that all 46 lakes in the park were oligotrophic (Kerekes & Freedman, 1989). The lakes are relatively shallow, with mean depths between 1 and 4.4 metres (Kerekes, 1975). The lakes have shown to be especially dilute, with salinities ranging from 11 to 24 mg/L (Kerekes, 1975). Kerekes (1975) revealed that many of the lakes in the park are dark-coloured due to the dissolved humic substances which may have been leached from organic soils located throughout the many wetlands in the park. Some of the lakes being studied do not have surface inflows, but all the lakes have permanent surface outflows (Kerekes et al., 1989). Many of the lakes have a coarse bottom substrate, dominated by rocky till (Kerekes et al., 1989). Below the bottom substrates are fine-grained sediments containing organic materials, silty minerals, and clay minerals (Stewart &

Freedman, 1982). The lakes chosen for this study (as shown in Figure 3.2) represent many of the limnological features of all the lakes within the park.

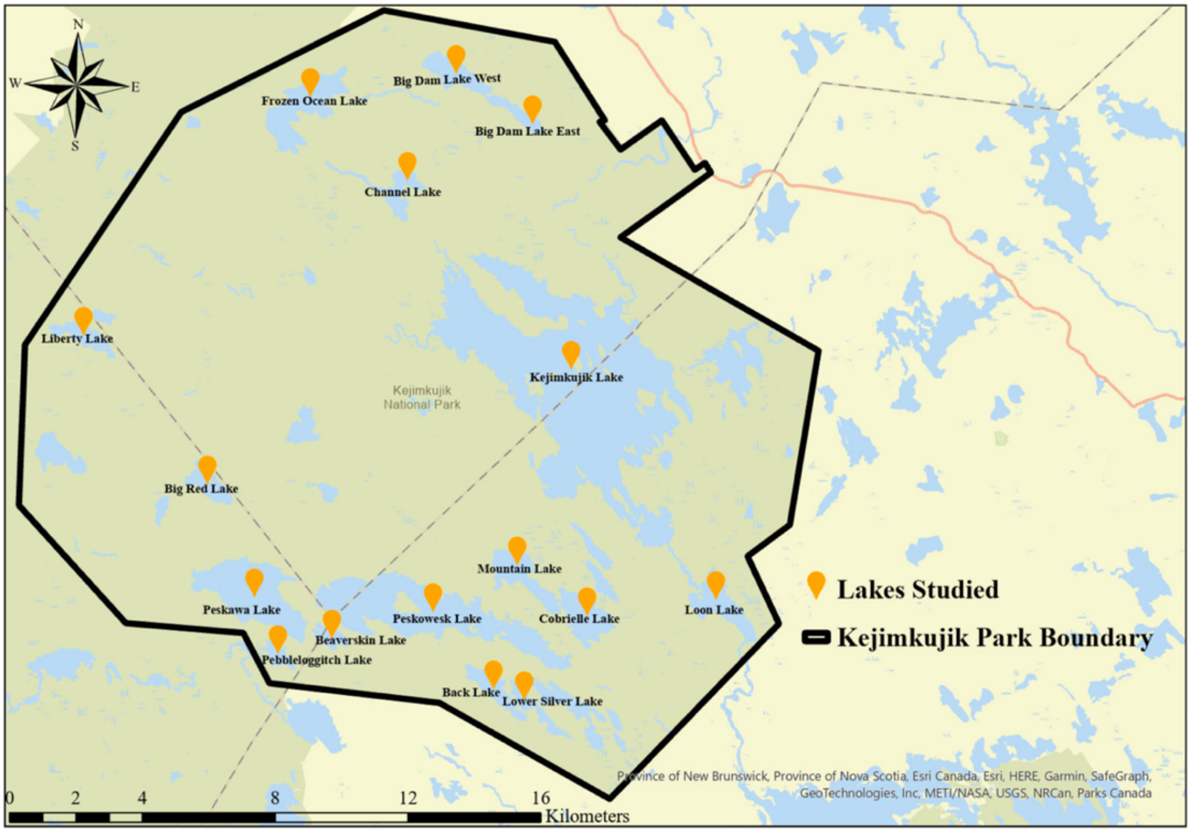


Figure 3.2 The lakes chosen for this study identified in the Kejimkujik Park Boundary map.

3.3 Phosphorus Loading Model

The P loading model estimates the TP (total phosphorus) concentration (μg/L) in each of the 16 lakes using a GIS-based assessment (Johnston et al., 2021). P is the primary nutrient shown to influence eutrophication in lakes in Nova Scotia, making it of paramount importance to this study (Johnston et al., 2021). The P loading model utilized publicly available spatial data to perform a GIS-based assessment. The spatial datasets chosen for this analysis (as shown in Appendix A, Table A.1) were chosen based on factors that typically influence the concentration of P in a water body (Johnston et al., 2021).

TP concentration was estimated with the following (Schnoor, 1996):

$$TP = \frac{\mathrm{L}}{q_s + v_s}$$

- TP = Total P concentration (μg/L)
- L = TP loading rate (g/m^2/yr)
- q_s = lake areal surface overflow rate (m/yr)
- v_s = apparent settling velocity of TP (m/yr)

The TP loading rate was calculated by analyzing land use, geology, and the number of residential properties within each of the lakes watersheds (Johnston et al., 2021). The authors of the P loading model conducted literature reviews and regional studies to estimate export coefficients of each land use P source (as shown in Appendix A, Table A.2) (Johnston et al., 2021).

The surface overflow rate was calculated as follows (Schnoor, 1996):

$$q_s = \frac{\text{Annual outflow rate}}{\text{Area of lake}}$$

The apparent settling velocity of TP was assumed to be 0.07 m/d (Schnoor, 1996). ArcGIS Pro 3.0.2 was used to assess each of the lakes, and Arc Hydro Tools 1.0 was used to delineate each of the lakes' watersheds.

The P loading model was coded within an RStudio script accessible using the R software package (script shown in Appendix B). The GIS layers that were previously screened (catchment shapefile, lake shapefile, land use raster, geology raster, and property shapefile) were input into this script. Based on the number of campers that came to the park over the summer of 2023 and the number of OWS, there was an estimated P load coefficient designated to each camper in the park, which was also included in the RStudio data analysis. The RStudio script outputs a variety of different numerical values for each of the lakes, which include: total precipitation (mm), P export coefficient (mg/m^2/ yr), sedimentary proportion, number of residences, septic load (g/yr), runoff load (g/yr), predicted TP concentration, and the predicted trophic status. Final eutrophication vulnerability assignments were dependent on the lakes' trophic status (see Appendix A, Table A.3). The results of the P loading model were compared to the actual measured TP concentrations of each lake to determine if the model was an accurate predictor of eutrophic vulnerability.

3.3.1 Assessing and Validating Phosphorus Model

The P loading model aims to reduce the amount of field sampling required to measure eutrophic vulnerability and to inform more specific eutrophication management practices in KNP (Johnston et al., 2021). Provincial and municipal governments can apply this model to increase the efficiency and prioritization of lake monitoring programs in the province (Johnston et al., 2021). Prior to this study, the presence of cyanobacteria in KNP had never been assessed, making this research of paramount importance to the safety of the water bodies within the park. The results of this study will inform whether lakes within KNP will require management and/or monitoring initiatives.

The P loading model was initially developed for lakes in the rural municipality of Cumberland County, Nova Scotia. There was a 2-year program conducted on 5 lakes in the county (which included lakes of varying trophic status', water quality conditions, flushing rates, and morphologies) to identify the key indicators and thresholds promoting eutrophication in rural lakes in Nova Scotia (Johnston et al., 2021). The indicators and thresholds determined during this program informed the P loading model parameters (Johnston et al., 2021). During the subsequent assessment of all 17 lakes in the county, it was demonstrated that the number of lakes requiring monitoring-based assessments was halved following the models assessment (Johnston et al., 2021). Additionally, the model was further validated on 29 lakes in the province, where it corroborated the trophic statuses of the lakes (Johnston et al., 2021). These studies inform the validity of this P loading model in focusing management and monitoring practices on lakes designated as moderate and/or high vulnerability.

3.4 Water Quality Parameters

The water quality parameters collected by Parks Canada from 2008 to 2021 were analyzed to determine any trends in the data that could be correlated to cyanobacteria presence. Parks Canada collected surface samples twice each summer (once in June and once in August) of each year, collecting the following parameters: Total phosphorus (TP), total organic carbon (TOC), pH, maximum depth of each lake, flushing rate, and nitrate-nitrogen. Trends in the data from 2008 to 2021 were graphed using bar and box plots in Microsoft Excel. The samples taken by Parks Canada were averaged (using the geometric mean) for each lake for each year and assessed, and the average (using the geometric mean) water quality parameters for each year accounting for all of the lakes collectively were also assessed. The flushing rate was calculated for each lake, using the outflow (m^3/yr) derived from the RStudio Script, divided by the volume (m^3) of the lake.

For one of the 16 study lakes, water quality samples were taken at Upper Silver Lake as opposed to Lower Silver Lake, which is where the cyanobacteria data was collected and GIS-based assessment was conducted.

3.5 Cyanobacteria and Cyanotoxin Data

The cyanobacteria data was collected in August of 2023 and assessed using 16S rRNA amplicon gene sequencing at Dalhousie University's Integrated Microbiome Research (IMR) centre. Pearson correlation tests were used to identify the relationship between the water quality parameters (TP, TOC, pH, flushing rate, maximum depth, and nitrate-nitrogen) and the Relative Abundance (RA) of Cyanobacteria. An additional sampling event was conducted on Peskowesk lake in October of 2023 after a reported cyanobacteria bloom.

Solid Phase Adsorption Toxin Tracking (SPATT) devices were employed at lake outlets over several weeks during the summer of 2023 to sample cyanotoxins. These samplers were analyzed for several toxin classes (e.g. anatoxins, microcystins) by the National Research Council's Biotoxin Metrology Group. This data was mapped to illustrate lakes with the highest concentrations of cyanotoxins.

3.6 Limitations

Due to the complexity of cyanobacteria presence/growth (Carvalho et al., 2011; Johnston et al., 2021), predicting the relevant processes that increase their proliferation can be difficult. While eutrophication has predominantly been shown to be a good predictor of bloom-forming cyanobacteria presence (Johnston et al., 2021), recent studies have shown that other parameters influenced by climate change might also play a vital role (Paerl & Huisman, 2009; O'Neil et al., 2011; Raven & Geider, 1988). The P loading model does not account for all the potential variables that could partake in bloom-forming cyanobacteria presence, which might make it difficult to equate certain variables to the presence of cyanobacteria in KNP.

The water quality data collected only accounts for the surface layers of each of the lakes. While this is valuable data, additional information on TP, pH, TOC, and nitrate-nitrogen, at different depths of each of the study lakes would provide more information on the nutrient profiles of each of the lakes. Additionally, this would increase the ability to compare the collected data with other studies that have assessed water quality at KNP.

The cyanobacteria data represents one sampling event during the summer of 2023. Given that this is the only data that has been collected on cyanobacteria in KNP, trends in the data over time cannot be determined.

CHAPTER 4: RESULTS

4.0 Results

The *GIS screening analysis* was used to characterize potential phosphorus loading and trophic status of lakes within the park. Further refinement of the tool is needed to calibrate the tool to KNP to make it an accurate predictor of trophic status for these lakes. Water quality parameters (TOC, pH, TP, flushing rates, maximum depth and nitrate-nitrogen) were also assessed to examine potential trends and correlations between these parameters and cyanobacteria proliferation. Additionally, the bacterial composition of the study lakes was evaluated to assess the presence and relative abundance of cyanobacteria within the study lakes.

4.1 Predicted and Measured Phosphorus Concentrations

Based on the results of GIS-based P loading analysis, eight of the lakes (Big Red Lake, Liberty Lake, Mountain Lake, Pebbleloggitch Lake, Peskawa Lake, Beaverskin Lake, Big Dam W Lake, and Big Dam E Lake) had predicted TP concentrations which were ≤ 4 μg/L (Table 4.1). Seven of the lakes (Back Lake, Channel Lake, Frozen Ocean Lake, Peskowesk Lake, Cobrielle Lake, Lower Silver Lake, and Kejimkujik Lake) had predicted TP concentrations (μg/L) which were between 5 μg/L to 10 μg/L (Table 4.1), and one of the lakes (Loon Lake) had a predicted TP concentration between 10 μg/L to 20 μg/L (Table 4.1).

The measured TP concentrations were different. There were no lakes with measured TP < 4 μg/L. Eight of the lakes had measured TP concentrations between 5 μg/L to 10 μg/L (Back Lake, Mountain Lake, Peskowesk Lake, Peskawa Lake, Cobrielle Lake, Lower Silver Lake, Big Dam W Lake, Beaverskin Lake), seven of the lakes had measured TPs between 10 μg/L to 20 μg/L (Channel Lake, Frozen Ocean Lake, Liberty Lake, Pebbleloggitch Lake, Loon Lake, Big Dam E Lake, and Kejimkujik Lake), and one of the lakes had a measured TP concentration between 20 μg/L to 35 μg/L (Big Red Lake) (Table 4.1).

Table 4.1 Comparison of measured and predicted TP concentrations in the study lakes.

Lake Name	P export coefficient ($mg/m^2/yr$)	Runoff Load (g/yr)	Predicted TP Concentration (μg/L)	Measured TP Concentration (μg/L)
Back	38.7	1.60E+05	6	7
Beaverskin	33.5	3.75E+04	3	9
Big Dam E	17.8	3.90E+04	3	7

Big Dam W	4.6	2.00E+05	3	17
Big Red	15.4	9.60E+04	4	21
Channel	8.8	1.10E+06	8	16
Cobrielle	27.3	2.70E+05	6	5
Frozen Ocean	8.4	1.00E+06	6	16
Kejimkujik	15.3	1.20E+07	9	14
Liberty	25.4	9.40E+04	4	11
Loon	15.4	1.30E+07	17	10
Lower Silver	35.9	6.10E+04	7	6
Mountain	20.4	1.40E+05	3	5
Pebbleloggitch	25.7	3.80E+04	4	11
Peskawa	10.1	4.60E+05	3	7
Peskowesk	16.2	1.50E+06	5	7

The predicted and measured trophic status of each lake was based off the predicted and measured TP concentration of each of the lakes (Table 4.1 and Table 4.2). The TP concentrations were assigned a trophic status based off the CCME classification system (CCME, 2021). The predicted and measured vulnerability were assigned based on the predicted and measured trophic status, a metric adapted by Johnston et al., (2021). This approach predicted the correct trophic status for only three of the lakes (Table 4.2). Eleven out of 16 of the lakes had a predicted trophic status that was less than the measured trophic status (Table 4.2). Of the underpredictions, Big Red Lake was notably underpredicted to be ultra-oligotrophic, where measured TP concentrations indicated it was meso-eutrophic (Table 4.2).

Table 4.2 Predicted and measured trophic status and vulnerability of the study lakes. Trophic state based off of the predicted and measured TP values given in Table 4.1, and vulnerability based off the trophic status.

Lake	**Predicted Trophic Status**	**Predicted Vulnerability**	**Measured Trophic Status**	**Measured Vulnerability**
Back	Oligotrophic	Low	Oligotrophic	Low
Beaverskin	Ultra-oligotrophic	Low	Oligotrophic	Low
Big Dam E	Ultra-oligotrophic	Low	Oligotrophic	Low
Big Dam W	Ultra-oligotrophic	Low	Mesotrophic	Moderate
Big Red	Ultra-oligotrophic	Low	Meso-eutrophic	High
Channel	Oligotrophic	Low	Mesotrophic	Moderate

Cobrielle	Oligotrophic	Low	Oligotrophic	Low
Frozen Ocean	Oligotrophic	Low	Mesotrophic	Moderate
Kejimkujik	Oligotrophic	Low	Mesotrophic	Moderate
Liberty	Oligotrophic	Low	Mesotrophic	Moderate
Loon	Mesotrophic	Moderate	Mesotrophic	Moderate
Lower Silver	Oligotrophic	Low	Oligotrophic	Low
Mountain	Ultra-oligotrophic	Low	Oligotrophic	Low
Pebbleloggitch	Ultra-oligotrophic	Low	Mesotrophic	Moderate
Peskawa	Ultra-oligotrophic	Low	Oligotrophic	Low
Peskowesk	Oligotrophic	Low	Oligotrophic	Low

4.2 Water Quality Trends and Relationships

The water quality parameters, pH, TOC, and TP were represented collectively for all the study lakes to show the relationships and trends between the parameters over time (Figure 4.1). Notably, TP and TOC exhibited relatively similar trends (Figure 4.1), with an evident amount of fluctuation in TOC (+/- 2 mg/L) and TP (+/-2 μg/L) occurring from 2008 to 2021. However, it is worth noting that as TP increased in 2016, TOC decreased (Figure 4.1). pH remained relatively consistent over time (Figure 4.1).

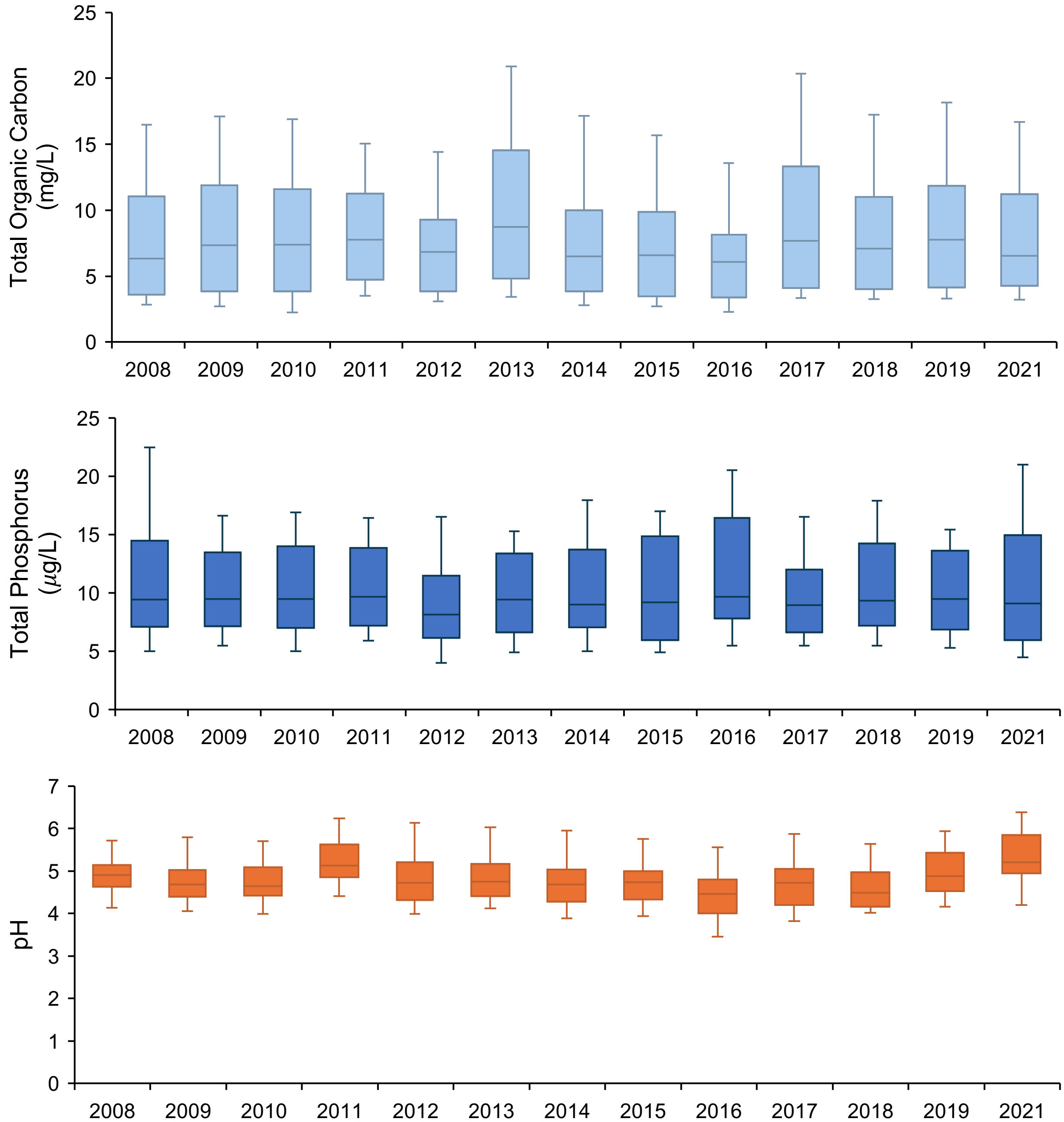


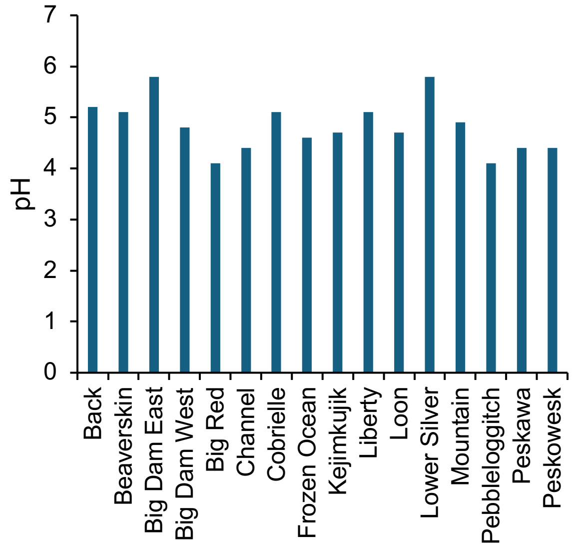
Figure 4.1 Water quality characteristics of the 16 study lakes, showing pH, TP (μg/L), and TOC (mg/L). Data from 2020 is missing from the figure due to absence of data collection that year. Data represents surface samples collected by Parks Canada once in June and once in August each year.

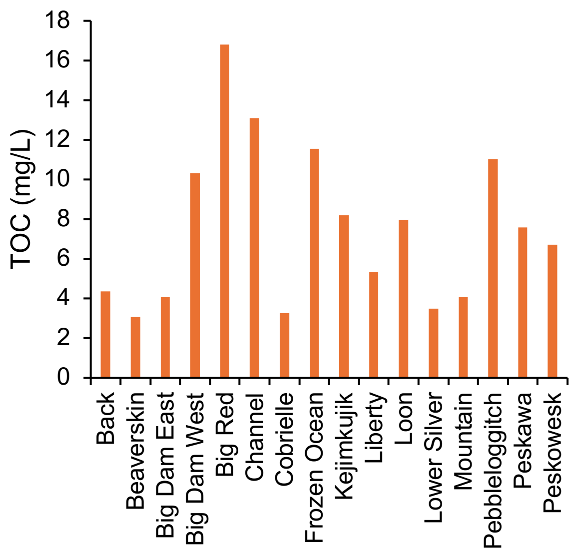
The pH of the lakes in the park ranged from 4.1 (Big Red Lake and Pebbleloggitch Lake) to 5.8 (Big Dam East Lake and Lower Silver Lake) (Table 4.3 and Figure 4.2). TOC ranged from 3.1 mg/L (Beaverskin Lake) to 16.8 mg/L (Big Red Lake) (Table 4.3 and Figure 4.2). The lowest

TP concentration in the park was 5.3 μg/L (Mountain Lake), and the highest TP concentration was 16.4 μg/L (Big Red Lake) (Table 4.3 and Figure 4.2).

Table 4.3 Water quality parameter data of 16 study lakes in KNP, Nova Scotia. Averaged values from data collected by Parks Canada in 2008 to 2021.

Lake	pH	TOC (mg/L)	TP (μg/L)
Back	5.2	4.4	9.5
Beaverskin	5.1	3.1	7.6
Big Dam East	5.8	4.1	6.7
Big Dam West	4.8	10.3	15.5
Big Red	4.1	16.8	16.4
Channel	4.4	13.1	14.5
Cobrielle	5.1	3.3	5.4
Frozen Ocean	4.6	11.5	14.3
Kejimkujik	4.7	8.2	11.7
Liberty	5.1	5.3	7.8
Loon	4.7	8.0	11.6
Lower Silver	5.8	3.5	7.0
Mountain	4.9	4.1	5.3
Pebbleloggitch	4.1	11.0	14.1
Peskawa	4.4	7.6	8.8
Peskowesk	4.4	6.7	6.7


pH
7
6
5
4
3
2
1
0
Back
Beaverskin
Big Dam East
Big Dam West
Big Red
Channel
Cobrielle
Frozen Ocean
Kejimkujik
Liberty
Loon
Lower Silver
Mountain
Pebbleloggitch
Peskawa
Peskowesk


TOC (mg/L)
18
16
14
12
10
8
6
4
2
0
Back
Beaverskin
Big Dam East
Big Dam West
Big Red
Channel
Cobrielle
Frozen Ocean
Kejimkujik
Liberty
Loon
Lower Silver
Mountain
Pebbleloggitch
Peskawa
Peskowesk

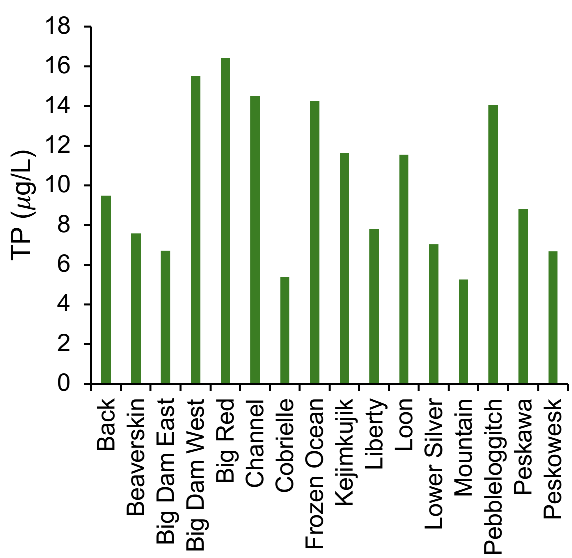

TP (µg/L)
18
16
14
12
10
8
6
4
2
0
Back
Beaverskin
Big Dam East
Big Dam West
Big Red
Channel
Cobrielle
Frozen Ocean
Kejimkujik
Liberty
Loon
Lower Silver
Mountain
Pebbleloggitch
Peskawa
Peskowesk

Figure 4. 2 Water quality parameters of the 16 lakes in KNP from 2008 to 2021. Averaged values from data collected by Parks Canada in 2008 to 2021.

The characteristics of the lakes in the park showed that lakes with larger surface areas typically had larger watershed areas (Table 4.4). Similarly, lakes with smaller surface areas had smaller watersheds (Table 4.4). The surface area (Ha) of the lakes in the park ranged from 35.3 Ha (Pebbleloggitch Lake) to 2575.4 Ha (Kejimkujik Lake) (Table 4.4). The watershed area (Ha) of the lakes in KNP ranged from 100 Ha (Beaverskin Lake) to 76,990 Ha (Loon Lake) (Table 4.4). The flushing rates of lakes in the park ranged from 1.3 times per year (Beaverskin Lake) to 447.2 times per year (Loon Lake) (Figure 4.4). Lakes with larger watershed areas were primarily shown to flush more than lakes with smaller watershed areas (Figure 4.4); however, this was not always shown (Kejimkujik Lake had a large watershed area but a low flushing rate due to its large volume) (Table 4.4). Nitrate-nitrogen for lakes in the park ranged from 6.4 μg/L (Frozen Ocean Lake) to 11.4 μg/L (Liberty Lake) (Table 4.4). The maximum depth (m) of the lakes in the park ranged from 1 m (Channel Lake) to 30 m (Kejimkujik Lake) (Table 4.4). The lakes were considered thermally stratified if the maximum depth was > 7 m (Table 4.4).

Table 4.4 Characteristics of the 16 study lakes in KNP, Nova Scotia.

Lake	**Surface Area (Ha)**	**Watershed Area (Ha)**	**Flushing Rate (yr)**	**Nitrate-Nitrogen (μg/L)**	**Maximum Depth (m)**	**Thermally Stratified**
Back	84.2	345	2.0	6.6	5	No
Beaverskin	45.4	100	1.3	7.1	2	No
Big Dam East	48.9	187	2.0	6.6	4	No
Big Dam West	113.3	3986	15.9	7.3	8	Yes
Big Red	80	625	8.1	7	2	No
Channel	73.6	12,586	168.0	8	1	No
Cobrielle	145.7	913	2.6	6.6	5	No
Frozen Ocean	246.3	11,311	23.9	6.4	20	Yes
Kejimkujik	2575.4	73,280	4.5	7.9	30	Yes
Liberty	77.5	372	5.1	11.4	5	No
Loon	84.4	76,990	447.2	6.9	2	No
Lower Silver	27.7	141	2.4	6.8	5	No
Mountain	146.6	664	4.9	6.6	14	Yes
Pebbleloggitch	35.3	146	2.8	7.4	2	No

Peskawa	421.4	4,326	3.3	6.8	8	Yes
Peskowesk	789.8	8,328	10.8	9.8	12	Yes

The molar ratio of Nitrate-Nitrogen: Phosphorus for the 16 study lakes showed that Liberty Lake had the largest Nitrate-Nitrogen: Phosphorus molar ratio (3.7), and Big Red Lake had the smallest (0.9) (Table 4.5). All Nitrate-Nitrogen: Phosphorus molar ratios were shown to be between 0.9 to 3.7 (Table 4.5)

Table 4.5 Molar Ratio of Nitrate-Nitrogen: Phosphorus for the 16 study lakes.

Lake	**Nitrate-Nitrogen: Phosphorus**
Back	1.5
Beaverskin	2.1
Big Dam East	2.2
Big Dam West	1.0
Big Red	0.9
Channel	1.2
Cobrielle	2.7
Frozen Ocean	1.0
Kejimkujik	1.5
Liberty	3.2
Loon	1.3
Lower Silver	2.2
Mountain	2.8
Pebbleloggitch	1.2
Peskawa	1.7
Peskowesk	3.2

4.3 Bacterial Community Composition

The bacterial composition of the study lakes in KNP were assessed using 16S gene sequencing techniques. The most prominent bacteria within the lakes were Proteobacteria (73%), followed by Actinobacteria (10%), Verrucomicrobia (8%), Bacteroidetes (7%), Other (1%), and Cyanobacteria (1%) (Figure 4.3). The “other” category consists of Acidobacteria, Chlamydiae, Chloroflexi, Dependentiae, Firmicutes, Patescibacteria, and Planctomycetes.

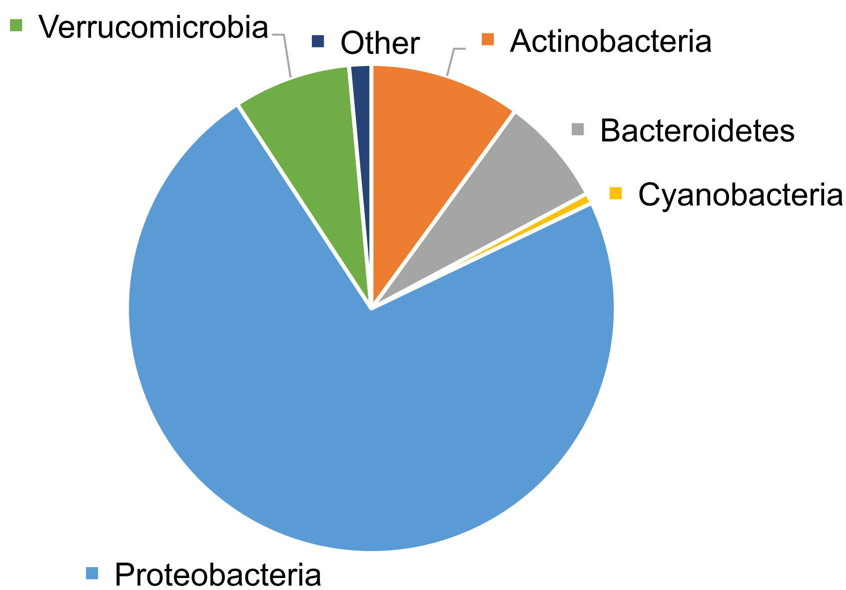


Figure 4.3 Bacterial composition (Relative Abundance %) in lakes within KNP. Based on surface samples collected in KNP lakes during August 2023.

Cyanobacteria were present in small relative abundances (RA) in ten of the 16 study lakes (Figure 4.4). Big Dam East Lake (5.7%) had the highest RA of Cyanobacteria, followed by Lower Silver Lake (3.1%), Beaverskin Lake (1.7%), Back Lake (1.2%), Cobrielle Lake (0.8%), Mountain Lake (0.6%), Liberty Lake (0.1%), Peskowesk Lake (0.05%), Big Red Lake (0.02%), and Peskawa Lake (0.01%). Synechococcales and Nostocales were the two Orders of Cyanobacteria that were detected (Figure 4.4 and Figure 4.5). Synechococcales were present in nine of the study lakes (Figure 4.5), with the highest RA in Big Dam East Lake (4.1%), followed by Lower Silver Lake (3.1%), Beaverskin Lake (1.7%), Back Lake (1.24%), Cobrielle Lake (0.8%), Mountain Lake (0.6%), Liberty Lake (0.1%), Big Red Lake (0.02%), and Peskowesk Lake (0.01%). Nostocales were present in three of the study lakes (Figure 4.5), including Big Dam East Lake (1.58%), Peskowesk Lake (0.04%), and Peskawa Lake (0.01%).

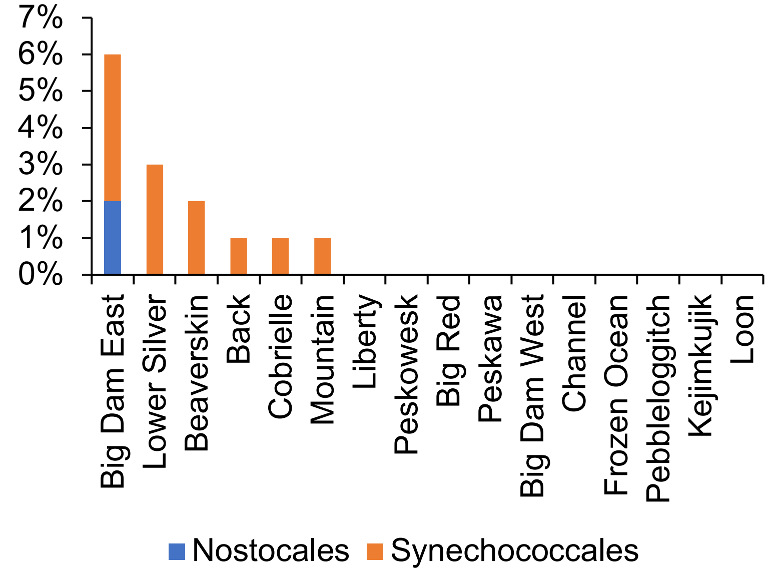
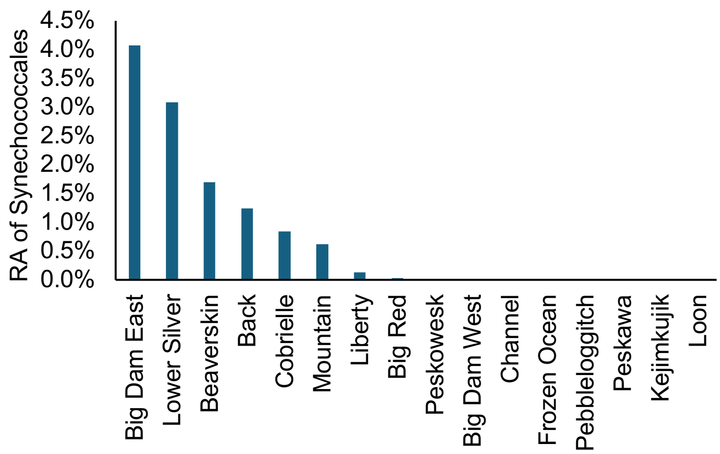


Figure 4.4 Cyanobacterial composition (Relative Abundance %) at the Order level. Based on surface samples collected in KNP lakes during August 2023.



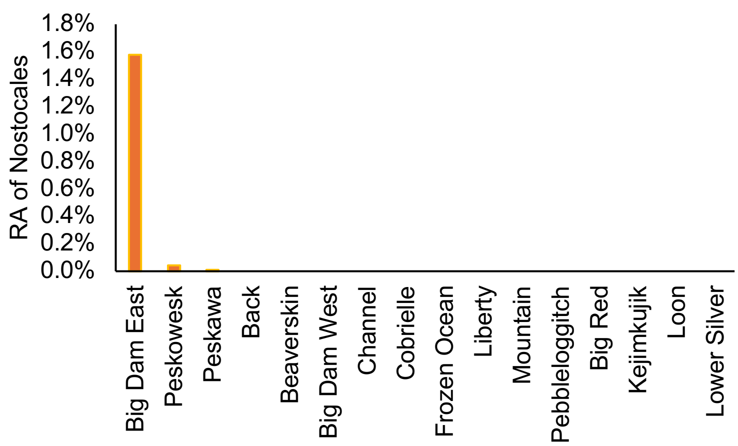


Figure 4.5 Synechococcales and Nostocales (Relative Abundance %) in lakes in KNP, Nova Scotia. Based on surface samples collected in KNP lakes during August 2023.

A Pearson's correlation coefficient was computed to assess linear relationships between water quality and hydrological parameters (pH, TP, TOC, flushing rate, maximum depth, and nitrate-nitrogen), and the RA of Cyanobacteria within the study lakes (Figure 4.6 and Figure 4.7). The mean of the water quality parameters from 2008 to 2021 was calculated for each lake. pH was shown to have the strongest linear relationship with RA of cyanobacteria of the variables evaluated (Figure 4.6). pH explained 67% of the variability in RA of cyanobacteria in the study lakes. TP explained 22% of the variability in the RA of cyanobacteria in the study lakes, and TOC 22% (Figure 4.6). The relationship between pH and RA of cyanobacteria in the study lakes indicated that as pH increased, so does the RA of cyanobacteria (Figure 4.6). The relationship between TP and TOC with RA of cyanobacteria indicated that as TP and TOC increased, the RA of cyanobacteria decreased (Figure 4.6). None of the relationships appeared to follow a strong linear trend, however certain thresholds identified within the data were shown to better explain the cyanobacteria presence (Figure 4.6). Cyanobacteria were primarily found in lakes with a pH > 5, a TP < 10 μg/L, and TOC < 5 mg/L (Figure 4.6).

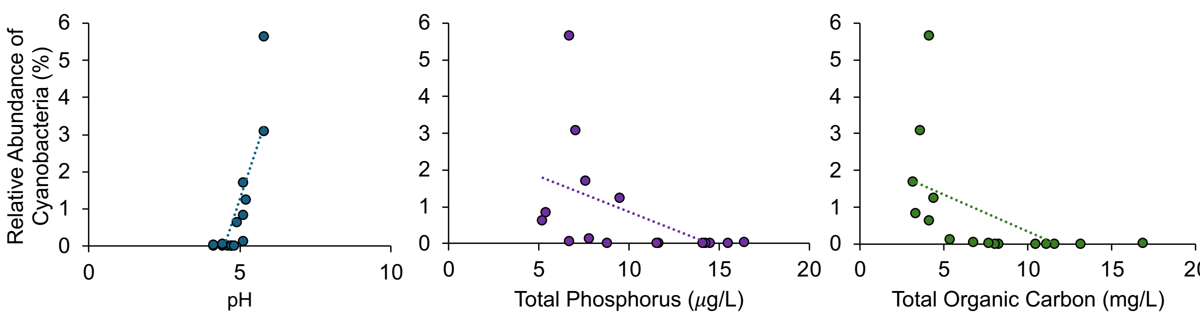


Figure 4.6 Correlations between Relative Abundance (%) of Cyanobacteria and explanatory variables: pH (left, blue, R^2 – pred = 0.67), Total Phosphorus (μg/L) (middle, purple, R^2 – pred = 0.22), and Total Organic Carbon (mg/L) (right, green, R^2 – pred = 0.22). Explanatory variables collected by Parks Canada during the summer months from 2008-2021. Cyanobacteria data was based on surface samples collected in KNP lakes during August 2023.

The flushing rate was calculated for each lake, using the outflow (m^3/yr) derived from the RStudio Script, divided by the volume (m^3) of the lake (Table 4.4). The maximum depth of each lake was determined using ArcGIS Pro 3.0.2, Arc Hydro Tools 1.0 (Table 4.4). Flushing rate did not exhibit a linear relationship with RA of Cyanobacteria (Figure 4.7). Flushing rate explained 4% of the variability in RA of Cyanobacteria in the study lakes. However, cyanobacteria were only found in lakes with flushing rates < 10 times/year. Maximum depth was also weakly linearly correlated with the RA of Cyanobacteria, explaining 11% of variability between the variables (Figure 4.7). Nitrate-nitrogen was additionally weakly correlated with the RA of Cyanobacteria (Figure 4.6), with 8% of variability being explained between the variables. However, cyanobacteria only existed between 5 μg/L and 10 μg/L nitrate-nitrogen concentrations (Figure 4.7).

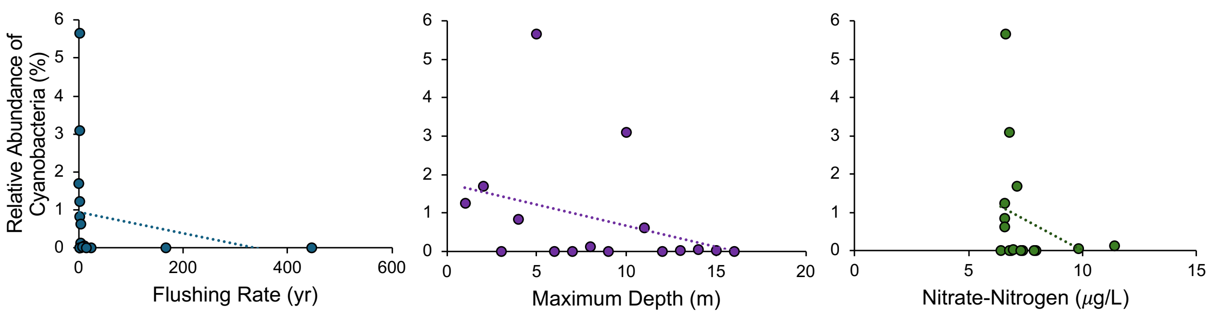


Figure 4.7 Correlations between Relative Abundance of Cyanobacteria (%) and explanatory variables: Flushing Rate (yr) (left, blue, R^2 – pred = 0.04), Maximum Depth (m) (middle, purple, R^2 – pred = 0.11), and Nitrate-Nitrogen (μg/L) (right, green, R^2 – pred = 0.08). Maximum Depth (m) derived using contour lines in ArcGIS Pro. Flushing Rate (yr) calculated by dividing the outflow by the volume. Nitrate-Nitrogen (μg/L) data collected by Parks Canada. Cyanobacteria data was based on surface samples collected in KNP lakes during August 2023.

Cyanotoxin presence was also assessed using passive sampling devices that accumulated cyanotoxins over several weeks during the summer of 2023. Anatoxins were detected in small quantities in a few of the study lakes (Figure 4.8). The highest anatoxin concentrations were found at both sampling locations for Mountain Lake (sampling location 1 = 3.26 ng/g, sampling location 2 = 2.71 ng/g). Additionally, at both sampling locations at Lower Silver Lake anatoxins were detected (sampling location 1 = 2 ng/g, sampling location 2 = 1.62 ng/g). Anatoxins were also detected at both of Back Lakes sampling locations (sampling location 1 = 0.03 ng/g, sampling location 2 = 0.06 ng/g). Kejimkujik Lake and Loon Lake had traces of anatoxins at one of the two sampling locations, at a concentration of 0.1 ng/g for both lakes. Peskowesk Lake was found to have a small concentration of anatoxins (0.03 ng/g).

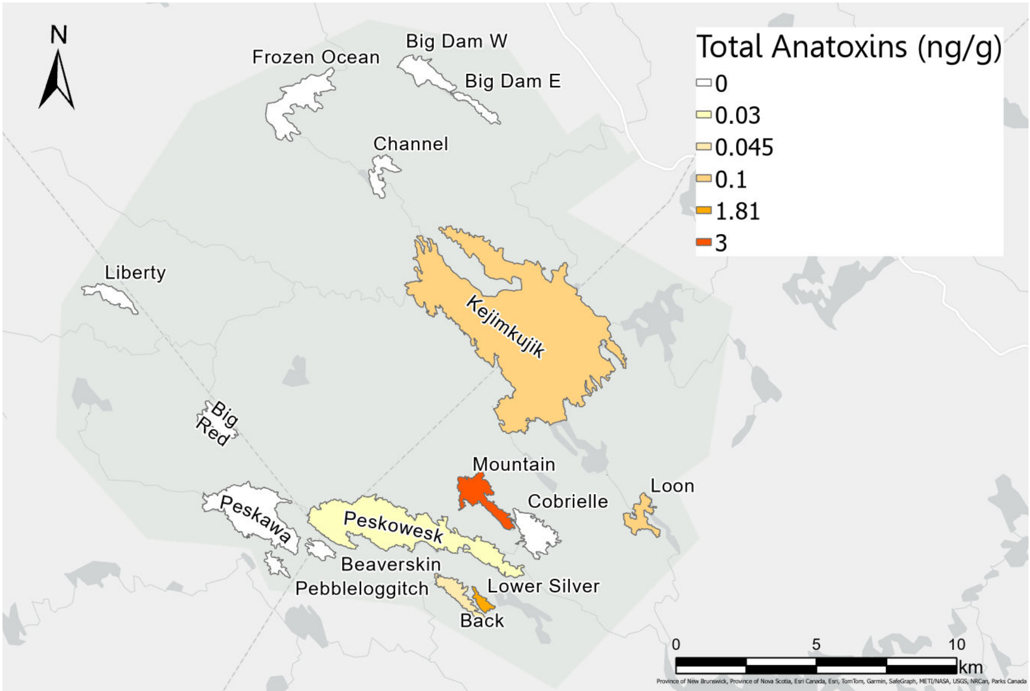


Figure 4.8 Total Anatoxins (ng/g) measured at each lake during the 2023 sampling season.

CHAPTER 5: DISCUSSION

5.0 Discussion

This was the first study which investigated the presence of cyanobacteria in KNP. The objectives of this study were to assess the occurrence, species, and factors that influenced cyanobacteria and cyanotoxin presence in the freshwater environments of KNP. The objectives of this study were met. The hypothesis of this study was that eutrophication vulnerability would be correlated with cyanobacteria presence in KNP. The results of this study do not support this hypothesis.

5.1 Prediction of Phosphorus Loading Potential

An established mass balance approach was used to classify lakes based on their eutrophic vulnerability (Johnston et al., 2021). However, the P loading model underpredicted the TP concentration in 13 out of 16 lakes (Table 4.1), and the trophic state for 11 out of the 16 study lakes (Table 4.2). In the initial study of this tool by Johnston et al., (2021), four out of the 29 lakes had predicted a trophic state less than the measured trophic state. However, the underpredicted lakes in the initial study fell within the same vulnerability level (Johnston et al., 2021); in this study, seven of the 11 underpredicted lakes fell within a different vulnerability level (Table 4.2).

5.1.1 Retention Factor

The retention factor of a lake accounts for the percentage of P that settles and does not contribute to the TP concentration in the water column (Van Heyst, 2020). A limitation of the screening approach used is that it does not account for P retention in upstream lakes (Johnston et al., 2021). This limitation is evident in the P loading model, as Loon Lake is the last lake in the series of lakes and it has the highest predicted TP concentration (Table 4.1 and Figure 5.1). Retention was only accounted for in headwater lakes. As a result of retention not being accounted for in other upstream lakes, the tool assumes all P is transferred from one lake to the following lake in the series (Figure 5.1), when that is not necessarily the case (Johnston et al., 2021). Including a P retention coefficient is an essential component in predicting an accurate P budget of lakes (Kirchner and Dillon, 1975). Therefore, including a retention factor which accounts for P retention in upstream lakes would make this tool a more accurate predictor of TP for the lakes in KNP.

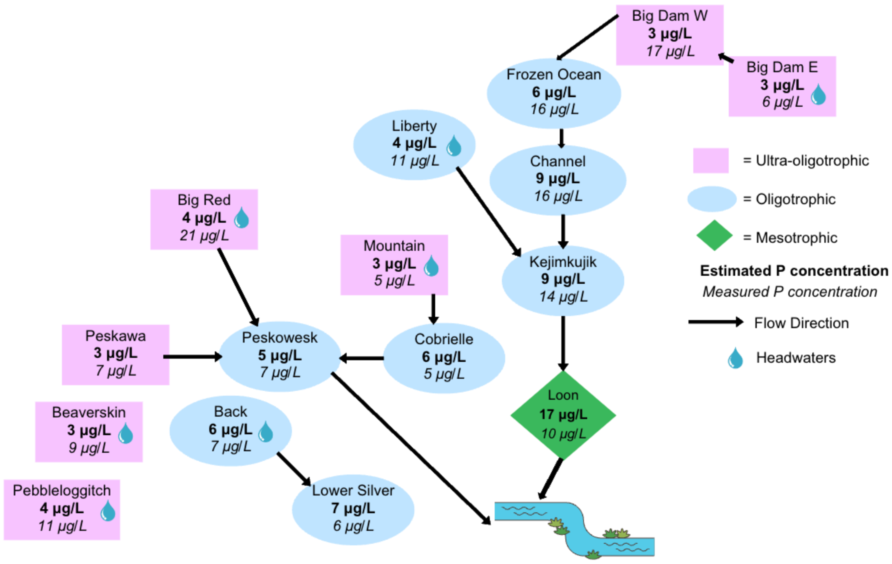


Figure 5.1 **Predicted** and measured Total Phosphorus (TP) concentrations (µg/L).

5.1.2 Calibrating the Phosphorus Loading Model for KNP

While including a retention factor might make the model a more accurate predictor of TP, it is unlikely that it would make the predicted TP concentrations more similar to the measured TP concentrations as lake retention is likely not the primary source of uncertainty. Given this, other factors influencing TP concentration within the freshwater environments of the park need to be considered in order to calibrate the P loading model to better approximate TP concentrations in KNP.

The phosphorus export coefficient of wetlands used in the screening tool was 16.0 mg/m^2/yr (Appendix A, Table A.2). This value was extracted from a sub watershed study for lakes in Shubenacadie, Nova Scotia, which accounted for wetlands containing a combination of swamps, marshlands, bogs, and fens (AECOM, 2013). However, the P retention in wetlands has the potential to be highly variable across different landscapes (Aziz and Cappellen, 2021). The wetlands in KNP primarily consist of bogs (Government of Nova Scotia, 2024). In a study conducted by Aziz and Capellen (2021), the phosphorus retention value was shown to differ between bogs, fen, marshes, and swamps, with marshes and bogs having the highest retention rates. With that being said, the P export coefficient used in the screening tool in this study may have been lower than the actual P export coefficients of the wetlands in the park. This may have contributed to the tool underpredicting TP of the lakes in the study.

Internal P loading is another factor that was not accounted for in the screening tool (Appendix B). Internal loading has the potential to contribute the same, if not more, than external P inputs, or, alternatively, relatively insignificant amounts (Orihel et al., 2017). An expansive study by Orihel et al. (2017), examined internal P loading across Canada, and found that the Canadian Shield in Ontario and Nova Scotia had the lowest internal P loading in the country. However, it is becoming increasingly important to include internal P loading into calculating the P budget of lakes, as climate change is predicted to exacerbate known factors contributing to internal P loading (Orihel et al., 2017). There are numerous biological, chemical, and physical factors that have been identified to contribute to internal P loading (Orihel et al., 2017). Specifically, pH, oxygen, and trophic state, have been classified as the primary environmental drivers of internal P loading in the freshwater environments of Canada (Orihel et al., 2017). Calibrating the model so it accounts for internal P loading of the study lakes would potentially make the tool a better predictor of TP concentrations.

In this study, the proportion of the watershed catchment underlain by sedimentary bedrock was approximated in the GIS-based analysis (Appendix B), however, other bedrock types were not accounted for. KNP is partially underlain by sedimentary and metamorphic bedrock (Nova Scotia Government, 2024.), all of which may store and release P differently (De Toledo and Baulch, 2023). Other features that were not accounted for in the tool but exist within the landscape of KNP include drumlins, erratic's, and eskers (AECOM, 2016; Government of Canada, 2022). The latter geological features additionally have the potential to impact TP in lakes (AECOM, 2016), and should be considered in the screening tool to predict TP more accurately.

There are numerous reasons the model may have underpredicted TP in this study, some of which may not have been listed in this section. Calibrating this model for KNP would make it a more effective predictor of TP for the lakes in the park. Understanding the processes contributing to P within the park will broaden our understanding of the biogeochemical processes taking place in KNP. Reassessing the runoff coefficient, free water evaporation, settling velocity, and outflow from each lake (Appendix B), are additional parameters that should be reassessed when calibrating this model for the lakes in KNP.

5.2 Water Quality Parameters in KNP Lakes

Water quality parameters were assessed to determine if there was a relationship between these parameters and the RA of cyanobacteria in the freshwater environments of KNP. The cyanobacteria levels being assessed were taken during a snapshot in time and were relatively low (Figure 4.4), making it difficult to draw any conclusions to the relationship between cyanobacteria abundance and the water quality parameters. Additionally, with the lack of historical cyanobacteria data in the park, there is nothing to compare the current data to in order to assess if there have been increases or decreases in cyanobacteria populations over time.

5.2.1 pH in KNP

Lakes in KNP were found to be relatively acidic, with pH values ranging from 4.1 to 5.8 from 2008 to 2021 (Table 4.3). These findings are consistent with other findings for lakes in Atlantic Canada, which have historically shown surface waters to be particularly acidic, due to their vulnerability to acidification because of the poorly buffered soils and bedrock in the region (Clair et al., 2011; Shilts, 1981). Long-term studies in the Atlantic provinces have shown that surface water pH values were between 4.5 to 5.5 (Clair et al., 2007). However, due to decreasing atmospheric deposition in the Atlantic provinces, some studies have shown pH to have increased in many surface waters (Clair et al., 1995; Jefferies et al., 2005). The pH of Beaverskin Lake (6.1) and Kejimkujik Lake (5.2) in 2021 were shown to be less acidic compared to 1982-1983 findings, where Beaverskin Lake had a pH of 5.2, and Kejimkujik Lake had a pH of 4.8 (Kerekes et al., 1989). However, this relationship was not found for Pebbleloggitch Lake, which was the other lake studied in Kerekes et al. (1989) research, where the pH value was recorded to be 4.6 in the 1982-1983 findings, which is the same as the pH value recorded in 2021. Given that Pebbleloggitch Lake is surrounding by bogs, pH recovery would be less likely to occur since bogs are naturally acidic and contribute organic acids (Perdue et al., 1984). Similarly to this finding, a study by Houle et al., (2022) which assessed the impact of declining atmospheric deposition on lakes, found that lakes with high dissolved organic carbon (DOC) concentrations are less likely to see a large increase in pH because the organic acids may be slowing down pH recovery to some degree.

The observed increase of pH in KNP by a value of approximately one half of a pH unit from 2008 to 2021 (Figure 4.1) indicates that the area could be recovering from acidification due to decreased SO_4^{2-} deposition (Findlay et al., 2003; Sutherland et al., 2015; Houle et al., 2022). However, this finding is not consistent among studies, as the Jefferies et al. (2003) study suggests there has been no change in the pH of aquatic ecosystems in the Maritime Provinces at the time the Jefferies et al. (2003) study was conducted. This finding is partially supported by the Houle et al. (2022) study, as a majority of lakes which experienced significant declines in SO_4^{2-}, did experience increased pH, but not all of them. Additionally, the average pH from 2008 to 2021 for seven of the study lakes were similar to the observations made by Kerekes et al. (1990) from samples collected in the summer of 1979 to 1981 (Figure 5.2). This likely shows that the lakes in this region are naturally acidic due to the widespread presence of peatlands (Ginn et al., 2007; Aziz and Cappellen, 2021).

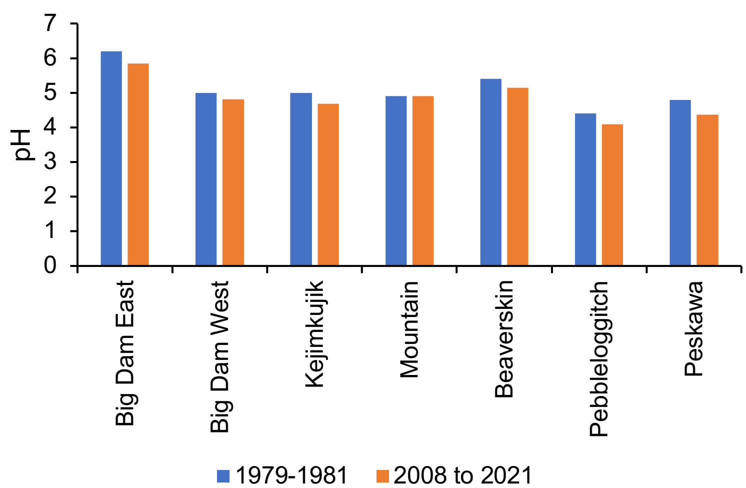


Figure 5.2 pH measured in 1979-1981 (Kerekes et al., 1990) and pH measured in 2008 to 2021 (representing averaged value).

Our findings suggest that lakes with the highest pH (for example Big Dam East Lake and Upper Silver Lake), have the lowest TOC, and likewise for lakes with the lowest pH having the highest TOC (Figure 4.2). This relationship is supported in the study conducted by Bowman et al. (2014) in KNP, where pH and TOC were shown to be negatively correlated. With that being said, our study does not indicate a notable linear relationship between the collective pH and TOC of the study lakes in KNP ($R^2 = 0.03$) (Figure 5.3), this relationship was only prevalent for individual lakes (Figure 4.2). Contrasting relationships have been found in the literature, where reductions in SO_4^{2-} have led to increased organic acids and pH (Evans and Monteith, 2001).

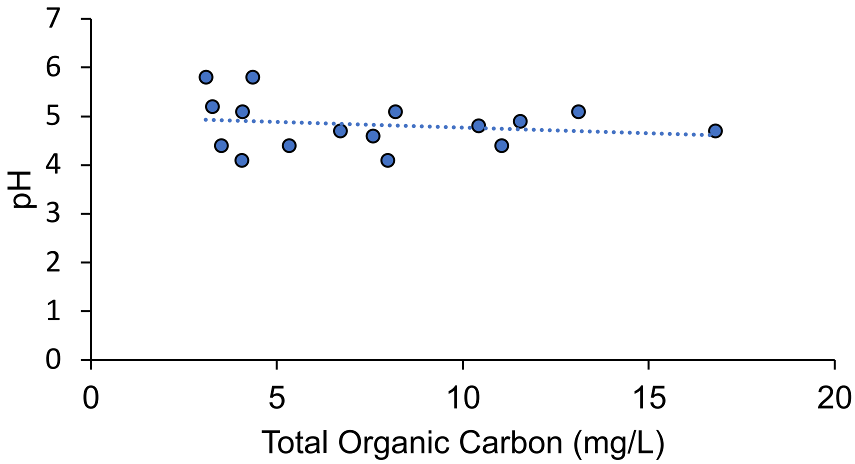


Figure 5.3 Correlation between pH and explanatory variable: Total Organic Carbon (mg/L) (R^2 = 0.03).

Lakes with higher pH were shown to have a strong relationship with the total RA of cyanobacteria (Figure 4.6, R^2 = 0.67). This relationship parallels those found within the literature, which demonstrates the positive relationship between increasing pH and phytoplankton (Findlay et al., 1999; Sutherland et al., 2015; Turner et al., 1995). Cyanobacteria have been documented to be significantly reduced in lakes with pH values below 5.1 (Findlay et al., 1999). In the lakes where cyanobacteria were present, pH values ranged from 4.1 (being Big Red Lake, which had a marginal RA of cyanobacteria) to 5.8 (being Big Dam East Lake, which had the highest RA of cyanobacteria of the study lakes, however, the RA was still quite low) (Figure 4.2 and Figure 4.4). There have been many studies that have found cyanobacteria to prefer neutral or alkaline conditions (Brock, 1973; Fogg, 1956), and this is true of the primary findings in our study (Figure 4.2 and Figure 4.4). However, to contrast this finding, our study did identify cyanobacteria in lakes with pH values lower than five (Figure 4.2 and Figure 4.4). Cyanobacteria presence in lakes with a pH below five is a rare phenomenon (Prasanna, 2007; Kim et al., 2014). Cyanobacteria presence within these acidic lakes may be indicative of other driving factors contributing to the cyanobacteria presence.

5.2.2 Total Organic Carbon in KNP

Many of the lakes in KNP were shown to contain high TOC concentrations (Figure 4.2), compared to lakes in other regions of North America (Clair et al., 2007). Kerekes (1975) findings suggest that numerous lakes in the park appear dark-coloured due to the high concentrations of dissolved organic substances. There were no obvious trends showing increased or decreased levels of TOC over time (Figure 4.1), as shown from assessing these variables from 2008 to 2021. TOC for Beaverskin Lake, Liberty Lake, Mountain Lake, and Upper Silver Lake increased from data collected by Vaidya & Howell., (2002) in 1996 to 1998. Beaverskin Lake increased from 2.5 mg/L (1996-1998) to 3.2 mg/L (2021), Liberty Lake increased from 5.8 mg/L (1996-1998) to 5.9 mg/L (2021), Mountain Lake increased from 4.1 mg/L to 4.5 mg/L, and Upper Silver Lake increased from 3.4 mg/L (1996-1998) to 3.5 mg/L (2021). The increase in TOC in these surface waters may be due to recovery from acidification (due to decreased atmospheric deposition), as was found in a study conducted across eastern North America (Monteith et al., 2007). Garmo et al. (2014) observed a similar trend across North America and Europe, where 22% of lakes experiencing decreased SO_4^{2-} encountered increased DOC concentrations. However, 76% of these lakes did not see a change in DOC, and 2% of the lakes were observed to have decreased DOC (Garmo et al., 2014).

Pebbleloggitch Lake had decreased TOC concentrations observed in 2021, in comparison to the 1996-1998 observations collected by Vaidya & Howell., (2002). Pebbleloggitch Lake experienced a substantial decline, from an initial TOC concentration of 13.4 mg/L in 1996-1998 to 6.4 mg/L in 2021. It was also observed that the TOC concentration of Pebbleloggitch Lake declined from 2008 to 2021. Given the distinctive location of Pebbleloggitch Lake, being surrounded by bogs, it is likely primarily impacted by the organic acidity of the bog-surrounding habitat (Kerekes, 1989). The findings of Pebbleloggitch Lake are consistent with the 2% of lakes which were observed to have a decreased DOC in the study by Garmo et al., (2014). However, assessing the collective TOC in the freshwater environments of KNP, indicate there is no substantial change in TOC concentrations over time (Figure 4.1), which supports the findings of Jefferies et al., (2003).

Observations of the TOC from 1981 collected by Kerekes., (1990) suggest that Pebbleloggitch Lake was not the only lake to experience a notable decline in TOC over time

(Figure 5.4). TOC has decreased in Beaverskin Lake and Kejimkujik Lake since 1981 as well (Figure 5.4).

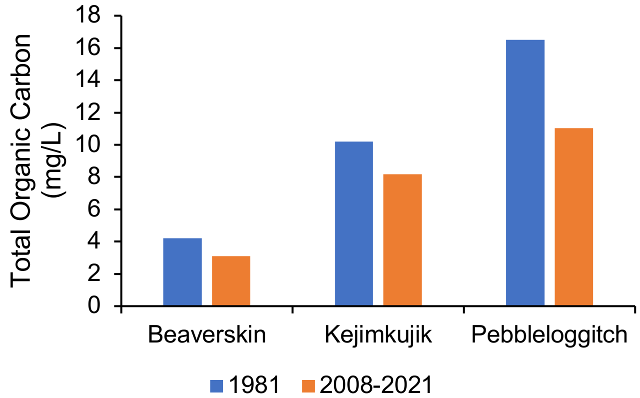


Figure 5.4 Total Organic Carbon measured in 1981 (Kerekes et al., 1990) and Total Organic Carbon measured in 2008 to 2021 (representing averaged value).

It is important to note that TOC decreased in 2016, while TP increased during this time (Figure 4.1). This contrasting trend could be due to the wildfire in KNP that occurred in 2016 (McMillan, 2016). Other studies have found similar trends, indicating wildfires alter the nutrient composition within receiving waters (Waters et al., 2023; Rhoades et al., 2018; Writer et al., 2014). A study which evaluated the impacts of a wildfire in Colorado, found a sudden decrease in TOC during the fire, which was followed by an increase in TOC the following year (Writer et al., 2014), these findings support our findings which exhibited similar trends (Figure 4.1). Another study, which investigated the impacts of a prescribed fire on an ecosystem, found P to increase substantially during the fire (Waters et al., 2023); this study aligns with our findings which indicated P increased in 2016 (Figure 4.1). While data from 2016 on the RA cyanobacteria in the park has not been recorded, it would be interesting to further explore these findings. TOC can also be impacted by hydrological features, such as runoff and precipitation, which increase TOC loading and might explain the annual variability in the TOC data (Yanni et al., 2000; Köhler et al., 2009).

5.2.3 Total Phosphorus in KNP

The measured TP concentrations did not display any strong trends (Figure 4.1). While TP and TOC exhibited relatively similar progressions, there were noteworthy divergences in the observations (Figure 4.1). The observed P concentrations from 2008 to 2021 differ from the measured P concentrations in 1974 (Kerekes et al., 1990) (Figure 5.5).

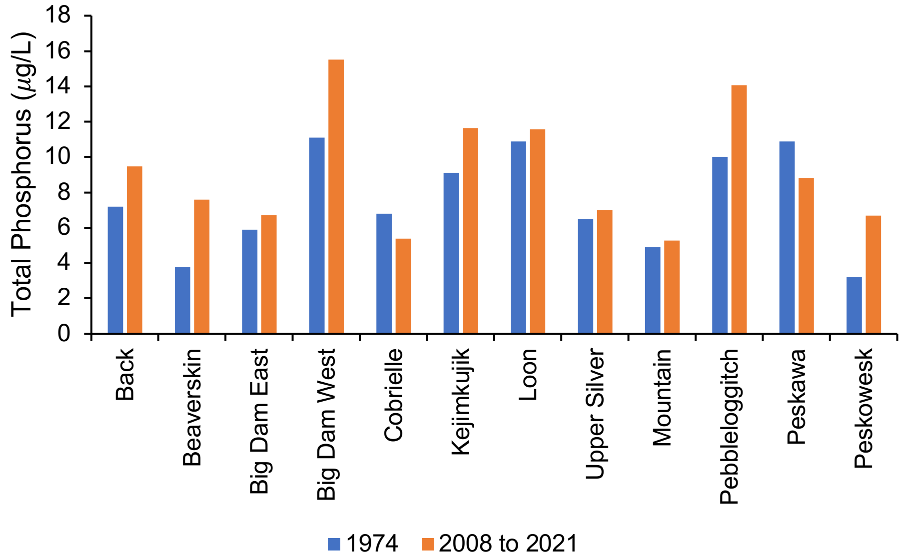


Figure 5.5 Total Phosphorus (μg/L) measured in 2008 to 2021 (representing averaged value), and average Total Phosphorus (μg/L) measured near surface in 1974 (Kerekes et al., 1975).

The 2008 to 2021 TP concentrations indicate that eight of the lakes were oligotrophic, seven were mesotrophic, and one was meso-eutrophic (Table 4.1), which is different than the findings by Kerekes & Freedman. (1989), which characterized all the lakes in the park to be oligotrophic. The reason for the apparent TP increases are not clear, however, it is likely that differences in sampling may explain some of the change. The samples taken from 2008 to 2021 were only surface samples, while samples taken by Kerekes & Freedman. (1989) were taken at different depths of the lakes.

The dominance of N-fixing cyanobacteria in low N:P ratios has been demonstrated in mesocosm and ecosystem-scale experiments across Canada (Orihel et al., 2012; Schindler et al., 2018). The Nitrate-Nitrogen: Phosphorus molar ratio for this study did not include ammonia nitrogen or organic nitrogen (Table 4.5), however nitrate-nitrogen is usually the predominant form in aerobic surface waters (Allan & Castillo, 2007). The defined ratio for phytoplankton biomass is N: P = 16:1, when the ratio is greater than 16, P is typically the limiting nutrient, and when it is lower, N (nitrogen) is the limiting nutrient (Allan & Castillo, 2007). In our results, the Nitrate-Nitrogen: Phosphorus molar ratio was much lower than 16 (Table 4.5). Cyanobacteria were only present in lakes with a Nitrate-Nitrogen: Phosphorus molar ratio between 1.5 to 3.2 (Figure 5.6), with the highest RA of cyanobacteria found at a molar ratio around 2 (Figure 5.6). However, given that the concentrations of nitrate-nitrogen were relatively low (Table 4.4), this relationship cannot be verified.

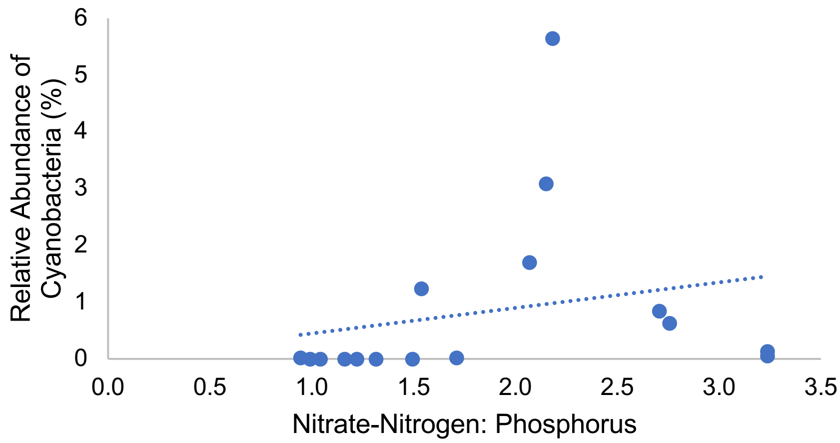


Figure 5.6 Correlation between Relative Abundance of Cyanobacteria (%) and explanatory variable: Nitrate-Nitrogen: Phosphorus (R^2 – pred = 0.05). Cyanobacteria data is based on surface samples collected in KNP lakes during August 2023.

5.3 Cyanobacteria in Oligotrophic Lakes

Many studies have shown that cyanobacteria prevalence is associated with the nutrient state of lakes (MacKeigan et al., 2023; Downing et al., 2001; Schindler et al., 2008). However, the findings of this study suggest that higher nutrient levels were not the primary driver of cyanobacteria (Figure 4.6). Measured and predicted TP concentrations for Peskowesk Lake, Peskawa Lake, and Big Dam East Lake, (the three lakes containing potentially toxin-producing Nostocales), were less than 10 μg/L (Table 4.3 and Figure 4.2). The measured and predicted TP concentrations for the lakes containing Synechococcales were also primarily within the oligotrophic range (Table 4.3 and Figure 4.2). Other studies assessing the promotion of cyanobacteria in oligotrophic waters have found that increased air temperatures, longer ice-free seasons, and increased low-wind periods allow lakes to form thermal and oxygen gradients, which escalates the flux of reduced-Fe from sediments into the water column (Jabbari et al., 2019). Increased reduced-Fe has been shown to potentially promote cyanobacteria growth (Reinl et al., 2021); however, data on iron concentrations was not collected for the study lakes. Other studies have shown that parameters such as water colour and pH might be better predictors of cyanobacteria than trophic state (Carvalho et al., 2011).

5.3.1 Findings Related to Water Quality Parameters

The findings of our data suggest that the presence of cyanobacteria is aligned with thresholds of water quality parameters (Figure 4.6 and Figure 4.7). Cyanobacteria were not found to exist in water bodies containing TP concentrations greater than 10 μg/L (Figure 4.6). This is not a common finding in the literature, as typically lakes with higher TP concentrations have higher RA of cyanobacteria as opposed to lakes with low TP (MacKeigan et al., 2023; Schindler et al., 2008; Orihel et al., 2012).

Our study also showed that cyanobacteria did not exist in lakes with TOCs greater than ~ 5 mg/L (Figure 4.6), aside from Big Red Lake where there was a particularly low RA of cyanobacteria (Figure 4.4). A study by MacKeigan et al. (2023), corroborated this finding, indicating lakes with high TOC had less cyanobacteria than lakes with low TOC. Additionally, a study assessing organic matter (OM) in a mesocosm, which resembled eutrophic, shallow lakes, found that phytoplankton biomass decreased with DOC concentrations > 10 mg/L, but increased in DOC concentrations < 7 mg/L (Feuchtmayr et al., 2019). While none of the study lakes are eutrophic, these findings may be more relevant to lakes with high TP concentrations and intermediate TOC concentrations.

Our study showed that the presence of cyanobacteria in lakes with low flushing rates is higher than in lakes with high flushing rates (Figure 4.7). This finding is supported by literature which has also found this relationship (Carvalho et al., 2011; Elliott, 2010). Other studies have found that since cyanobacteria are relatively slow-growing, marginal flushing rates are favourable towards cyanobacterial growth (Carvalho et al., 2011). Kerekes et al. (1975) study also found that lakes with low flushing rates have low TP, which was also observed within our study (Figure 4.2 and Table 4.4). Kerekes et al. (1975) highlighted this relationship between variables in Big Dam East Lake and Big Dam West Lake, in which both lakes have quite different flushing rates, which is further reflected in their TP concentrations, and can also be seen from the findings of our study (Figure 4.2 and Table 4.4). Lakes with low TP, were also shown to be the lakes with the highest RA of cyanobacteria (Figure 4.6), indicating that low TP and low flushing rates were correlated with cyanobacteria in KNP. The Pearson's correlation analysis only informed the presence of a linear relationship between the variables, which means there may be other non-linear or multivariate relationships that have not been considered between the water quality parameters and RA of cyanobacteria.

5.3.2 Nostocales

Nostocales were present in three out of the 16 study lakes (Big Dam East Lake, Peskawa Lake, and Peskowesk Lake) (Figure 4.5). Nostocales is a potentially toxin-producing order of cyanobacteria (WHO, 2021), with nitrogen-fixing capabilities (Ballot et al., 2011). The lakes Nostocales were present in were oligotrophic (Table 4.2), which parallels the findings of a study on lakes in Germany, which concluded that Nostocales were dominant in lakes with low trophic states (Ballot et al., 2011). There are several genera that have been identified under the order Nostocales, many of which have been involved in the formation of cyanobacteria blooms (Cirés & Ballot, 2015). Nostocales is a particularly threatening order of cyanobacteria, due to its competitive advantages over other competitors. Global warming will have advantageous impacts on Nostocales because they prefer higher temperatures and have the ability to withstand harsh weather due to their akinetes (dormant cells which form during resting stages) (Cirés & Ballot, 2015). To further understand Nostocales presence in the park, a qualitative approach of assessing akinetes within sediments can provide data on the diversity of Nostocales presence within the lakes (Legrand et al., 2017).

The RA of Nostocales in Peskowesk Lake was 0.04% during August 2023; following Hurricane Lee, a cyanobacteria bloom was reported in this lake (Figure 5.7). The lake was resampled, and the RA of Nostocales in Peskowesk Lake in October 2023 was 1.7%. The excessive rainfall from hurricanes can result in amplified nutrient loads, which has been shown to result in cyanobacterial blooms (Phillips et al., 2005), as found in Peskowesk Lake (Figure 5.7). It is also possible that the winds from the hurricane promoted mixing of the lake, potentially bringing nutrient rich water from the hypolimnion to the surface water layers.

Figure 5.7 Cyanobacteria bloom in KNP taken at the end of September 2023. Image taken by Parks Canada, 2023.

5.3.3 Synechococcales

Synechococcales were detected in nine of the study lakes (Figure 4.5), with its highest abundance in Big Dam East Lake, Lower Silver Lake, and Beaverskin Lake. Synechococcales were previously reported in lakes in Nova Scotia, as demonstrated by a study on the cyanobacterial presence for lakes in the HRM (Betts., 2018). Another study assessing the presence of cyanobacteria in an oligotrophic lake in New Brunswick found that Synechococcales were the dominant order, with their RA varying from 38% to 85% during the sampling period (Brown et al., 2021). An important observation from this study informs the consensus that cyanobacteria taken during a single snapshot in time cannot fully inform the abundance of cyanobacteria in a given lake. Given that the RA of cyanobacteria fluctuated from 38% to above 85% from June to October at the same sampling location (Brown et al., 2021), indicates that further studies on the cyanobacterial composition in KNP need to be conducted for an improved comprehension of the cyanobacterial abundance within the park.

5.3.4 Anatoxins

Anatoxins are a group of alkaloids produced by neurotoxins, which are produced by cyanotoxins (Carmichael, 1992). Anatoxins have been linked to the death of dogs in Nova Scotia and New Brunswick, and in both cases the anatoxins were produced by benthic cyanobacterial mats (Johnston et al., 2024; McCarron et al., 2023). Anatoxins were present in small quantities in six of the study lakes (Figure 4.8). The highest concentration of anatoxins (which was still considered relatively low) was found in Mountain Lake (Figure 4.8). While literature regarding toxin-producing cyanobacteria in acidic lakes in Nova Scotia is limited, a study by Hushchyna et al., (2019) found that the presence of anatoxins in acidic lakes in the province was associated with cold water temperatures. However, the anatoxin data being assessed in this study was only representative of SPATT samples taken in August 2023, when the water was still warm. A study by Kramer et al., (2022) found that anatoxins were observed under limited P conditions, and this is likely a stress response due to the nutrient-limited environments. The findings in this study do suggest that anatoxins were highest in lakes with low P (Figure 5.7), however given that anatoxins were detected in so few lakes and in such small quantities, drawing conclusions to this would not be fully substantiated. Another finding by Gagnon & Pick (2012), found that anatoxins were most abundant at moderate N stress (~18 μg/L). In our findings, anatoxins were present in lakes with low nitrate-nitrogen concentrations (Figure 5.7), however, given that the levels of anatoxins were so low, more research needs to be done to determine what is driving the anatoxins in KNP.

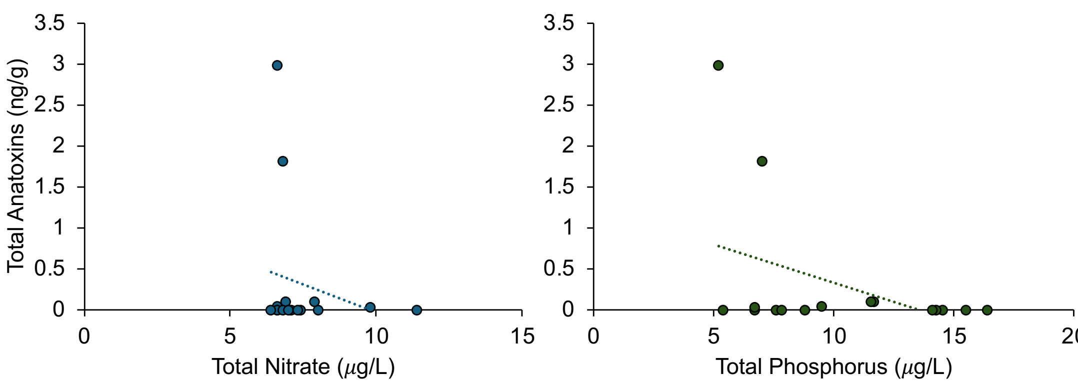


Figure 5.8 Correlations between Total Anatoxins (ng/g) and explanatory variables: Nitrate-Nitrogen (μg/L) (left, blue, R^2 – pred = 0.04), and Total Phosphorus (μg/L) (right, green, R^2 – pred = 0.18). Anatoxin data collected using SPATT samplers.

5.2.1.3 Historical Cyanobacteria Findings in KNP

While this is the first broad scale study on the presence of cyanobacteria in KNP, a study conducted by Kerekes & Freedman (1989) characterized the chemical and biological characteristics of Beaverskin, Pebbleloggitch and Kejimkujik lakes. They analyzed the overall phytoplankton composition of the three lakes (Kerekes & Freedman, 1989). Cyanobacteria was present in both Beaverskin and Kejimkujik lakes, however its presence in Beaverskin Lake was much more pronounced as it was 94% of the total cell density (Kerekes & Freedman, 1989). *Agmenellum thermale,* of the order Synechococcales were present in Beaverskin and Kejimkujik lakes, and *Chroococcus dispersus,* of the order Chroococcales were also present in Beaverskin Lake in a very small abundance (Kerekes & Freedman, 1989). Similarly, Beaverskin Lake was found to contain Synechococcales in our study (Figure 4.5), however, a more detailed DNA sequencing analysis is needed to further validate the genera of cyanobacteria present.

Kerekes and Freedman (1989) supported the Kwiatkowski and Roffs (1976) finding that cyanobacteria dominates the phytoplankton community in clearwater acidic lakes. This is shown in a study of lakes near Sudbury, Ontario (Kwiatkowski and Roffs, 1976), and in Kerekes and Freedman (1989) study in KNP. While our findings cannot currently affirm that cyanobacteria is the dominating phytoplankton in Beaverskin Lake, our findings do show that cyanobacteria of the same order are still present (Figure 4.5).

CHAPTER 6: CONCLUSION

Given that this was the first study on cyanobacteria in KNP, it revealed crucial information about the water quality parameters and cyanobacteria presence in the park. Among the findings identified in this study, the most notable was the presence of cyanobacteria, which was identified in ten of the 16 study lakes. While the RA of cyanobacteria was observed to be particularly low in each of the study lakes, it was interesting that the cyanobacteria presence in the park did not follow conventional predictors. The highest RA of cyanobacteria were present in low-nutrient lakes, as opposed to the common assumption that cyanobacteria would be highest in high-nutrient lakes (MacKeigan et al., 2023; Trimbee & Prepas, 1987; Johnston et al., 2021). However, the cyanobacteria data was collected during a single snapshot in time, so fluctuations in the data have not been accounted for, and there is no historical data to evaluate temporal trends. Further research is needed to identify the specific factors driving cyanobacteria proliferation in the park.

The GIS-based assessment underpredicted the TP for 13 out of 16 of the study lakes, as well as the trophic state for 11 out of 16 of the study lakes. Calibrating the tool to make it a better predictor of TP for lakes in KNP is essential to understanding the biogeochemical processes taking place in the aquatic environments of the park. While TP has been shown to be a weak predictor of cyanobacteria, determining what factors influence TP might provide more insight on other factors that could potentially be promoting cyanobacterial growth in the park. Additionally, the variance between the data collected by Dr. Joseph Kerekes in the park in the 1970s and 1980s and the data collected between 2008 to 2021, indicates that water quality parameters such as TP and TOC, have changed over time.

Overall, I propose that additional research be undertaken to further understand the factors driving cyanobacteria presence within the park. Specifically, I suggest that water quality parameters in the park be further analyzed. TP concentrations should be measured and recorded at multiple depths within the lakes. Once this data has been collected it can be compared to Kerekes (1975) findings, which suggest TP concentrations are highest in the hypolimnion. The results of these findings can determine the potential for internal P loading, which will provide more insight on the benthic environment of the lakes (Orihel et al., 2017). Temperature and dissolved oxygen profiles should also be measured throughout the water column to determine if the lake stratifies and if it goes anoxic (Johnston et al., 2021). Additionally, assessing phytoplankton assemblages in the lakes is pivotal to understanding the role cyanobacteria plays in its community (Kerekes and Freedman, 1989). I also recommend water samples which assess the concentration of Fe be taken in the lakes during future sampling seasons. Since oligotrophic cyanobacterial blooms have been linked to low Fe environments (Sorchetti et al., 2013), it is important to assess the Fe concentrations in the park.

Metagenomic sequencing techniques should be used to determine the genera and species of Nostocales and Synechococcales present in the lakes. This information is especially important for the lakes that Nostocales were detected in. Determining if there are toxin-producing genera in these lakes is crucial because there are many organisms that rely on the freshwater environments found in KNP (Parks Canada, 2023), and toxin-producing cyanobacteria can impact the ecosystem health (Chorus & Bartram, 1999). Many people camp in KNP, and toxin-producing cyanobacteria has the potential to have detrimental impacts on their health if consumed (WHO, 2021). Additionally, more measurements of the cyanotoxin concentrations in the park should be collected and analyzed. Measuring anatoxin concentrations from ice-out to ice-in would be beneficial to fully understand the threat of cyanotoxins in KNP. Microcystin data, along with additional anatoxin data, would be advantageous in determining the presence and abundance of cyanotoxins in the park.

Pearson's correlation was used in this study to determine the linear relationship between water quality parameters and the RA of cyanobacteria. However, in the future more advanced statistics should be used to evaluate other relationships between the explanatory variables and RA of cyanobacteria. For example, exponential regression can be used to determine relationships between changing variables over time and cyanobacteria abundance, as was used in the Sorchetti et al., (2014) study. Since many of the water quality parameters had non-linear relationships with the RA cyanobacteria, generalized additive models should be used to further explore these non-linear relationships, as was used in the study by Carvalho et al., (2011). Principal component analysis is another statistical analysis that can be used, as shown in the study by Ou et al., (2013). Advanced statistical tests should be used to further investigate the relationships cyanobacteria have with other variables in KNP.

I recommend that management practices which do not primarily focus on managing eutrophication be assessed (Reinl et al., 2021). The drivers of the cyanobacteria presence are critical to informing any kind of remediation approach. The potential unintended environmental impacts of remediation approaches need to be inspected prior to their implementation to avoid changing physical and biological characteristics of the lake. Some remediations approaches that have been used to manage cyanobacterial proliferation in oligotrophic waters include artificial mixing, which would decrease light availability and impede phytoplankton growth (Visser et al., 2016). Additionally, a magnetic-coagulation method has been shown to be an effective method of removing algal cells from bodies of water (Liu et al., 2013). The management techniques mentioned have all shown to be successful at controlling cyanobacterial growth in freshwater environments and should be considered if the cyanobacterial propensity is shown to increase in future sampling events in the park, however, remediation approaches can make problems worse if not properly informed. Further studies on the cyanobacteria presence within the park need to be conducted to determine how to properly manage it.

To conclude, this study contributes to the body of knowledge of cyanobacteria in oligotrophic waters in Atlantic Canada. Additionally, this study is the first study of cyanobacteria in KNP, making it relevant to future water management initiatives. We should work towards ensuring the water bodies in KNP are unharmed from anthropogenic sources which might contribute to the proliferation of cyanobacteria.

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APPENDICES

Appendix A: Input variables to RStudio Script.

Table A.1 The spatial datasets used for the GIS-based assessments. The table is taken from Johnston et al. (2021) supplementary RStudio script.

Dataset	Description	Source
Enhanced Digital Elevation Model	Hydrologically correct grid of elevation for Nova Scotia.	Nova Scotia Department of Lands and Forestry (2006)
Nova Scotia Hydrographic Network	Points, polylines, and polygons of inland surface waters in Nova Scotia.	Province of Nova Scotia (2015)
Geology Mapping of Southwestern Nova Scotia	Geological data of the southwestern portion of Nova Scotia.	Natural Resources and Renewables, Nova Scotia (2015)
Forest Inventory Layer for Nova Scotia	Polygon layers for all lands in the province (water, forested and non-forested, and additional freshwater wetlands and coastal habitat area classifications).	Nova Scotia Department of Lands and Forestry (2017)
Digital Property Layer for Nova Scotia	Geometry and attribute information for unique parcel identification numbers.	Province of Nova Scotia (2018)

Table A.2 Land use categories with designated P coefficients. The table is taken from Johnston et al. (2021).

Land use category	P export coefficient ($mg/m^2/yr$)	Literature source
Forest	6.9 $mg/m^2/yr$	Scott et al., 2000
Cleared	10.6 $mg/m^2/yr$	CWRS, 2017
Burned/Dead	10.6 $mg/m^2/yr$	CWRS, 2017
Low-density residential	13.0 $mg/m^2/yr$	AECOM, 2013
Wetland	16.0 $mg/m^2/yr$	AECOM, 2013
Inland water	17.3 $mg/m^2/yr$	AECOM, 2013
Roads (paved and unpaved)	83.0 $mg/m^2/yr$	AECOM, 2013
Agriculture	44.6 $mg/m^2/yr$	Brylinksy, 2004
Green space	15.0 $mg/m^2/yr$	Brylinksy, 2004

Table A.3 CCME classification scheme based on total phosphorus (μg/L) (Canadian Council of Ministers of the Environment [CCME], 2004).

Total Phosphorus (TP)	**Trophic Status**	**Eutrophication Vulnerability**
< 4 μg/L	Ultra-oligotrophic	Low vulnerability
4 to < 10 μg/L	Oligotrophic	Low vulnerability
10 to < 20 μg/L	Mesotrophic	Moderate vulnerability
20 to <35 μg/L	Meso-eutrophic	High vulnerability
35 to 100 μg/L	Eutrophic	High vulnerability
> 100 μg/L	Hypereutrophic	High vulnerability

Appendix B: RStudio Script

RStudio Supplementary Script, for Tier 1. The script is taken from Johnston et al. (2021).

```
# script to run lake vulnerability screening tool: tier 1 (GIS-based
analysis)

# required packages

library(tidyverse)
library(raster)
library(sf)

### in this section we bring in our GIS layers:
### catchment shapefile, lake shapefile, landuse raster, geology raster,
property shapefile

# pull in lake watershed shapefile

lake_catch <- read_sf("c_catch_p.shp")

# pull in lake shapefile

lakes <- read_sf("CC_lakes.shp")

# format table for use in function (lake area and lake name)

lakes_table <- lakes %>% as.data.frame() %>% dplyr::select(SHAPE_AREA,
Name)

# pull in landuse raster

landuse <- raster("landuse.tif")

# pull in geology raster

geo <- raster("geo_cc")

# pull in residential centroids (we use residential parcels within the
watershed to estimate population)

resident <- read_sf("res_pt.shp")


### now we will apply the land use phosphorus export coefficient from our
literature review
### to our land use raster

# example export coefficients by land use

ex_co <- tribble(~landuse, ~class, ~coefficient,
                 "forest", 1, 6.9,
                 "cleared", 2, 10.6,
```

```
                 "burned", 3, 10.6,
                 "residential", 4, 2.7,
                 "wetland", 5, 16.0,
                 "water", 6, 17.3,
                 "barren", 7, 107.1,
                 "agricultural", 8, 44.6,
                 "green", 9, 15.0
)


# create reclassification matrix

matrix <- c(0, 1, 6.9,
            1, 2, 10.6,
            2, 3, 10.6,
            3, 4, 2.7,
            4, 5, 16.0,
            5, 6, 17.3,
            6, 7, 107.1,
            7, 8, 44.6,
            8, 9, 15.0)

# transform into matrix

matrix <- matrix(matrix, ncol = 3, byrow = TRUE)


# reclassify landuse raster by export coefficients

new_landuse <- reclassify(landuse, matrix)


## all possible classes for geology layer


class <- tribble(~ class,
                 1,
                 2,
                 3,
                 4,
                 5)


# the following function is used to calculate the final p conc and trophic
status of a given lake

screen_func <- function(row) {

  # select chosen catchment which will be used as a mask for the following
operations
  catch <- lake_catch %>% slice(row)

  # name of lake
  name <- catch$Name
```

```
  # area-weighted (by land use) phosphorus export coefficient in mg/m2/yr
  coeff <- raster::extract(new_landuse, as(catch %>% st_zm(), "Spatial"),
                           na.rm = TRUE,
                           weights = TRUE,
                           fun = mean)[[1]]

  # give a dataframe of the different geology types within the catchment
  geo_values <- raster::extract(geo, as(catch %>% st_zm(), "Spatial"),
                                na.rm = TRUE, df = TRUE)

  # calculate the proportion of catchment that is underlain by sedimentary
bedrock
  sed_prop <- geo_values %>%
    group_by(geo_cc) %>%
    summarize(count = n()) %>%
    right_join(., class, by = c("geo_cc" = "class")) %>%
    mutate(count = ifelse(is.na(count), 0, count),
           sum = sum(count),
           prop = count/sum) %>%
    filter(class == 1) %>% # in our case, class 1 is sedimentary
    pull(prop)

  # adjust for sedimentary geology and convert mg to g
  x <- sed_prop*0.20
  new_coeff <- (1 + x)*coeff/1000

  # catchment and lake areas
  catch_area <- catch$Shape_Area
  lake_area <- lakes_table %>% filter(Name == name) %>% pull(SHAPE_AREA)

  # catchment area (excluding lake area)
  catch_exc <- catch_area - lake_area

  # estimate the number of residences in the catchment
  no_res <- st_join(catch, resident) %>% count() %>% pull(n)

  # to calculate the total load coming from runoff, multiply the adjusted
export coefficient
  # by the catchment area
  runoff <- new_coeff*catch_area

  # to calculate the total load coming from on-site wastewaster systems,
  # multiple the number of residences by the average annual contribution
(660)
  # by the average number of inhabitants per residence
  res <- no_res*660*2.56

  # sum all loads to come up with total p load
    p_load <- runoff + res

  # loading rate in ug/L --> multiple by 1000 (m3 to L) divide by 1000000
(g to ug)
  p_rate <- (p_load/lake_area)*1000
```

```
  # annual precipitation is a variable in the catchment shapefile
  # calculated using thiessen polygons and nearby met stations
  precip <- as.numeric(catch$Precip)

  # calculate outflow from the lake
  # using runoff coefficient of 0.7 (applied to catchment area excluding
lake surface)
  # and free water evap of 500 mm (applied to lake surface)
  Q <- precip/1000*0.7*catch_exc + (precip/1000*lake_area - 0.5)

  # calculate the
  qs <- Q/lake_area

  # settling velocity of 0.07 m/d converted to m/yr
  vs <- 0.07*365

  # final P conc calculation
  P <- p_rate/(qs + vs)

  tibble(
    lake = catch$Name,
    pre = precip,
    coeff = coeff,
    sed_prop = sed_prop,
    no_res = no_res,
    res = res,
    runoff = runoff,
    p_load = p_load,
    Q = Q,
    p_conc = P
  )

  }
```