EVALUATING BIO INDICATOR RESPONSE TO RECOVERY FROM ANTHROPOGENIC ACID DEPOSITION IN A DRINKING WATER SOURCE IN HALIFAX, NOVA SCOTIA

by

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Little is known about the biological response to anthropogenic acidification in Atlantic Canada. A multi-proxy paleolimnological investigation of Pockwock Lake, the main drinking water supply for the City of Halifax, was conducted to assess biological response to chemical water quality change and recovery trends. This thesis provides information on Cladocera and diatoms assemblages, and inferred chlorophyl *a* and TOC. It appears that Cladocera have not responded to increases in lakewater pH, ANC and alkalinity in Pockwock lake. This lack of response is most likely due to regional Ca concentration decline. Diatoms do show a response and return of two major species dominating the assemblage with low pH and high DOC optima. VNIRS-inferred chlorophyl *a* and TOC results show a chemical and biological return to pre-impact conditionunprecedented water quality trends have begun to occur.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ASV	Amplicon sequence variants
BSC	Biological safety cabinet
Chl-a	Chlorophyll a
CRS	Constant rate of supply
^{137}Cs	Cesium-137
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DI-pH	Diatom inferred pH
Dwt	Dry weight
eDNA	Environmental DNA
HTS	High-throughput sequencing
HW	Halifax Water
IMR	Integrated Microbiome Resource
JDKWSP	John Douglas Kline Water Supply Plant
KOH	Potassium hydroxide
MAR	Mass accumulation rate
MIB	2-methylisoborneol
NOM	Natural organic matter
NO_x	Nitrogen oxides
^{210}Pb	Lead-210
PCR	Polymerase chain reaction
PCoA	Principal components analysis
rRNA	Ribosomal ribonucleic acid
SO_4^{2-}	Sulfate
SO_x	Sulphur oxides
SO_2	Sulphur dioxide
TOC	Total organic carbon
VNIRS	Visible near infrared reflectance spectroscopy

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"*Knowledge is in the end based on acknowledgement*" - Ludwig Wittgenstein

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CHAPTER 1.0: INTRODUCTION

Preamble

This chapter will introduce the problem and provide the problem statement, thesis objectives, methodology and research questions. It will also provide the literature review, the originality of research and knowledge gaps.

Problem Background

Anthropogenic acid deposition has resulted in regional acidification of surface waters throughout North America and Europe (Driscoll et al. 2001; Schöpp et al. 2003). This process has negatively affected many lakes, rivers and surface waters around the world, and is considered a significant environmental stressor on freshwater ecosystems (Schindler et al. 1985; Baker and Christensen 1991; Scheuhammer 1991; Havens et al. 1993). Atlantic Canada has been profoundly affected by acid deposition, predominantly due to low buffering capacity provided by the regional bedrock (Clair et al. 2007a). Anthropogenic sulphate (SO_4^{2-}) deposition is the main driver of surface water acidification in eastern Canada (Jeffries et al. 2003a). With regulatory reductions on sulfate emission over the last few decades, brownification and recovery of freshwater lakes are now being documented across North America and Europe (Ek et al. 1995; de Wit et al. 2016; Anderson et al. 2017; Meyer-Jacob et al. 2019; Kritzberg et al. 2020a; Redden et al. 2021).

Recovery from anthropogenic acidification involves both chemical and biological transformations. Chemical water quality recovery is observed as increases in lake water pH, alkalinity, acid neutralization capacity (ANC) and natural organic matter (NOM). Few studies have documented biological recovery changes coincident with chemical recovery in acidified waters. The studies that do observe biological recovery, indicate that the biological response to

change lags behind chemical recovery (Monteith et al. 2005; Baldigo et al. 2021). It is complicated to detangle the drivers of change for biological recovery due to the complex nature of multiple-stressors in aquatic systems (Ormerod et al. 2010).

Lakes and surface waters are an important source of water for public water supplies. Approximately half of Nova Scotia's residential water supply comes from a surface water source (Nova Scotia Environment 2022). Pockwock Lake is the main water source for the city of Halifax. Over the last 8 years, Halifax Water (HW) has been faced with new water treatment challenges as surface water quality in Pockwock Lake has rapidly changed. These changes include reoccurring geosmin, and increases in organic matter and pH. The J.D. Kline Water Supply Plant (JDKWSP), which treats water from Pockwock Lake, has also experienced reduced filter run times and increases in required alum ($Al_2(SO_4)_3$) dose (Anderson et al. 2017). In 2018, a diatom bloom posed significant treatment challenges as it clogged filters and severely impacted filter run times. This source water has never experienced a noticeable algal bloom prior to this event.

By 2000 AD, the pH of fresh water systems in Nova Scotia had not returned to background conditions, nor showed noticeable indications of recovery (Clair et al. 2002; Korosi and Smol 2012a). However, evidence of recovery trends (Anderson et al. 2017; Redden et al. 2021) are now present in Nova Scotia. Despite increases in lake water pH, there is limited evidence of biological recovery in Atlantic Canada (Korosi et al. 2013a). Due to limited biological monitoring data and unknown biological recovery trends in Nova Scotia, this thesis project was initiated to gain insight into the biological signals and community diversity within Pockwock.

Problem Statement

Aquatic systems are beginning to show recovery trends from anthropogenic acid deposition. While chemical recovery trends are being documented throughout North America and Europe, biological recovery appears to be lagging. Due to multi-stressor environments, the mechanisms affecting biological recovery are not well understood. Lack of long-term monitoring data severely impedes the understanding of these environmental changes.

Objectives

The main objective of this study is to determine if a biological response is present in a water source currently showing chemical recovery trends from anthropogenic acid deposition. The second objective is to interpret the biological response and conclude what the potential drivers of change are. The third objective is to explore the community diversity of the microbiome using eDNA metabarcoding.

Methodology

A high-resolution (0.5 cm) multi-proxy paleolimnological approach is used to observe changes in cladoceran and diatom sub-fossil assemblages and changes in visible near infrared reflectance spectroscopy (VNIRS) inferred chlorophyll *a* and total organic carbon (TOC) concentrations. Environmental DNA (eDNA) metabarcoding using high-throughput sequencing (HTS) is also used to analyze microbial diversity in modern lake sediments.

Research Questions

This research approach is designed to provide clarity on the processes that might influence the observed rapidly changing water quality in Pockwock Lake by addressing the following research questions:

- Is there evidence of biological response occurring in Pockwock Lake?
- Have bio indicator assemblages recovered to pre-impact conditions?
- What mechanisms are impacting the response?
- What does the diversity of the microbiome indicate about biological response?

Hypothesis

If biological recovery trends are following chemical recovery trends, then we would expect to see a return to pre-impact conditions in the bio-indicators analyzed might be expected.

Literature Review

Acidification of surface waters

Anthropogenic acid deposition, has been a high-profile environmental issue that has affected eastern Canada since at least the 1950s, mostly in the form of acid rain (Lacoul et al. 2011). Acid deposition primarily results from emissions such as sulfur dioxide (SO_2) and nitrogen oxides (NOx) altering to sulfuric acid, ammonium nitrate and nitric acid when mixed with water. When this acidic precipitation falls, it impacts the systems in which it lands. Acidification of aquatic systems results in a reduction of pH, which deleteriously impacts surface water function. The acidification of freshwater ecosystems has a negative impact on fish (Leduc et al. 2013), waterbirds (Diamond 1989), zooplankton (Hammill et al. 2018), benthic invertebrates (Zunino et al. 2017) and algal (Rodríguez et al. 2018) populations. Aquatic organisms are affected by acidification at all trophic levels, resulting in changes in productivity and biomass (Hogsden et al. 2008). Anthropogenic acid deposition also alters soils and stresses forest vegetation (Driscoll and Wang 2019).

Atlantic Canada has some of the most acidic surface waters in Canada, even though it receives some of the lowest acid precipitation deposition (Clair et al. 2007b). This is due to the low buffering provided by regional bedrock and wetlands that produce natural acids in the soil. (Clair et al. 2007b). Parts of Nova Scotia are also significantly impacted by acid rock drainage (ARD). The Eastern Shore and Southwestern Nova Scotia are underlain by Meguma Group rock, composed of slate, greywacke/quartzite, and schist (Land Classification Nova Scotia 2017). Halifax Formation slate is high in sulphide minerals such as pyrite. When exposed and oxidized, it can generate ARD releasing sulphuric acid and metal oxides into watercourses downstream, impacting aquatic life (Land Classification Nova Scotia 2017). Regional bedrock, poor buffering capacity combined with atmospheric deposition, has resulted in stressed aquatic ecosystems that limit biological productivity, and alter food webs (Antoniades 2013).

It was expected that many acidified lakes and surface waters would recover from acidification as a result of emissions regulations and legislation (Jeffries et al. 1992; Yan et al. 1996). With reductions in acid deposition following the implementation of the Clean Air Act (1990), there has been documented recovery in acid-sensitive surface waters (Stoddard et al. 1999a; Driscoll et al. 2003).

Lake Recovery

Lake recovery, brownification, and increasing DOC have been credited to reduced anthropogenic sulfate deposition (Stoddard et al. 1999a; Evans and Monteith 2001; Futter et al. 2014; Anderson et al. 2017; Redden et al. 2021). Brownification of surface waters is a phenomenon causing changes in lakewater colour, mainly due to increases in dissolved organic matter (DOM). Brownification can have severe impacts on aquatic systems as it can affect biodiversity, biogeochemical processes (Kritzberg et al. 2020b), biological community structures

and aquatic productivity (Meyer-Jacob et al. 2019). Monitoring programs in the northern hemisphere have documented brownification of surface waters in recent decades caused by increasing dissolved organic carbon (DOC) concentrations (Monteith et al. 2007; Garmo et al. 2014; Meyer-Jacob et al. 2019).

Chemical recovery from anthropogenic acid deposition is the observed reversal of surface-water acidification, and associated chemical changes in water quality parameters including alkalinity, ANC and DOC (Monteith et al. 2007; Anderson et al. 2017; Redden et al. 2021). A surface water is considered chemically recovered once it returns to a neutral pH of > 6.0 (Jeffries et al. 2003b). The chemical recovery trends have been slow and varied in Atlantic Canada. However, the regional increases in pH, alkalinity, and ANC should be conductive to partial biological recovery (Garmo et al. 2014). Since water quality is conducive to the survival and reproduction of colonizing individuals, it is also an important prerequisite for biological recovery (Gray and Arnott 2009).

Entwined with the chemical recovery process are biological recovery processes. As lakes return to their natural chemical states, biological responses are simultaneously occurring. Biological recovery is expected to follow chemical recovery in acid-impacted areas and characterized by an increase in abundance of acid-sensitive taxa and a decline in the abundance of acid-tolerant taxa (Driscoll et al. 2001, Arseneau et al. 2011). Even though biological recovery is expected to follow chemical recovery, there appears to be a delay in the timing of biological recovery (Monteith et al. 2005; Baldigo et al. 2001). Long-term studies have detected a delay in zooplankton recovery for 3-10 years, even after water quality has reached acceptable levels (pH >6.0) (Yan et al. 2003; Frost et al. 2006; Gray and Arnott 2009).

Biological recovery is measured as a return in the communities to their pre-disturbance state (Valois et al. 2011), however most studies show limited overall recovery. Some lakes have shown improvements in chemical recovery but show no evidence of recovery biologically (Smol et al. 1998, Gray and Arnott 2009, Arseneau et al. 2001, Labaj et al. 2014, 2015). An indication of biological recovery would be an increase in species richness, species diversity, community evenness and an increase in indicator species. The slow recovery from acidification has been documented in poorly buffered lakes (Stoddard 1999; Jeffries 2003) The biological lag or muted response has been attributed to a depletion of base cations from watershed soils, counteracting reductions of acid inputs, with declines most pronounced in catchments with active timber harvesting (Jeziorski and Smol 2016).

As of 2007, there was no evidence of chemical recovery or measurable improvements in water chemistry in lakes in Nova Scotia, despite sulfate deposition reductions in Atlantic Canada (Clair et al. 2007b). This is no longer the case. After an updated assessment of data collected as recently as 2019 (Redden et al. 2021) and an examination of sulfate deposition in Nova Scotia (Anderson et al. 2017) there is evidence of chemical lake recovery in Nova Scotia.
Studies are also showing chemical recovery trends in many regions including the Adirondacks, USA (Josephson et al. 2014), Sudbury, Ontario (Keller et al. 2019) and throughout Europe (Stoddard et al. 1999b; Evans et al. 2001; Skjelkvåle et al. 2001).

A number of studies document the partial partial recovery of some bio indicators such as diatoms, while others like Cladocera remain unchanged. Cladocera in particular exhibit limited recovery in previously acidified lakes (Walseng et al. 2001, Yan et al. 2004, Labaj et al. 2014). Arseneau et al., (2011) show evidence of biological recovery in diatom and chrysophyte taxa, however no evidence of recovery or response in Cladoceran taxa occurring in the Adirondacks.

Valois et al., (2011) found that zooplankton communities often recovered once lake pH reached 5.5 but showed evidence of delays in recovery with elevated metal concentrations. Thresholds like this don't necessarily apply to all regions or lakes, which is one of the challenges of assessing recovery. Other factors also shown limiting recovery include colonist dispersal (Binks et al. 2005), DOM and UV radiation (Cooke et al. 2006), low Ca concentrations (Jeziorski and Smol 2016), predation by fish (Yan et al. 2004), elevated metal concentrations (Labaj et al. 2015), temporary fluctuations in pH (Walseng et al. 2001) and a variety of local factors (Yan et al. 2004; Binks et al. 2005).

Drivers of change

Although acid deposition has declined in many regions, it is still unclear why some lakes seem to have responded positively to the decrease in emissions, while others remain unresponsive (Smol et al. 1998). Studies indicate lakes follow different acidification trajectories as well as different patterns of recovery (Dixit et al, 1995; Cumming et al. 1992, 1994; Charles et al 1989. The drivers of change behind lake recovery and the increasing trend of colour in surface waters are debated. Some studies highlight that brownification is due to declining atmospheric sulfur deposition (Monteith et al. 2007; Krizberg and Ekström 2011), while others suggest it is driven by climate change, land-use and land management (Meyer-Jacob 2015) and also a transition from agriculture to forestry (Kritzberg 2017). It is more likely to be a result of multiple stressors impacting the systems. Stressors include environmental changes such as acidification, eutrophication, warming temperatures and calcium decline.

Analysis of climate normals in Nova Scotia show a slight warming trend from 1961-1990 followed by a more significant increase in average temperature post-1990 of 1°C (Garbary and Hill 2021). Warming air temperatures and changes in stratification and ice cover are having

profound impacts on the biota in lake systems (Rühland et al. 2015). Anthropogenic climate change as a stressor is so complex that is not fully understood. Stressors other than acidification, such as anthropogenic climate change and changes in land-use can alter the structure of different biological communities. Some ecosystems express an algal response more clearly and sensitively to climate change than others, and some regions and ecosystems respond more quickly than others (Rühland et al. 2015).

Another factor affecting response and recovery of biological communities is regional calcium (Ca) decline, a legacy of acid deposition and logging (Arnott et al. 2017). It is now common to see declining Ca concentration in lakes and their catchments. Declines in Ca concentrations are a long-term consequence of acid deposition and timber harvesting (Jeziorski and Smol 2016). Paradoxically, acid rain appears to have compounded the Ca decline problem as it has leached much of the Ca in the soil. Carbonate alkalinity and Ca concentrations have been attributed to the influence of anthropogenic acid deposition (Weyhenmeyer et al. 2019). As acid deposition has decreased, Ca concentrations have rapidly declined towards or below pre-industrial conditions (Weyhenmeyer et al. 2019). Biota with high Ca demands are particularly vulnerable to declining Ca availability in aquatic systems. Lakewater Ca concentration has been identified as a factor regulating cladoceran community composition (Jeziorski and Smol 2016). Regional climate warming may also exacerbate the effects of Ca decline (Jeziorski and Smol 2016).

Challenges in assessing biological recovery

There are many challenges in assessing biological recovery from acidification. Assessment is challenged by poor pre-acidification data and confounding effects of climate change. Assessment of biological recovery from acidification is difficult as very few acidified

lakes have been immediately subjected to annual monitoring for fish and other organisms (Holmgren 2014). Assessing recovery is extremely challenging when assessing a multi-stressor environment. The multiple-stressor framework results in a mix of pressures from climate, human and landscape, proving to be a challenge to interpret cause and effect. Barriers which might delay or prevent the return of the pre-disturbance communities once the stress is removed may be unevenly distributed across damaged sites and may obscure recovery patterns (Valois et al. 2011).

It should be anticipated that we may not see recovery trends displaying complete biological recovery back to a pre-impacted state. Due to multiple drivers of change, we may see a chemical return to pre-acidification conditions with a modified community structure allowing for the functional return of a system.

Paleolimnology

Due to lack of long-term monitoring data, paleolimnology is a useful tool to understand past limnological conditions of aquatic systems by reconstructing biotic and abiotic proxies deposited and archived in lake sediment. Paleolimnology is the scientific approach which uses biological, chemical, and physical information preserved in lake sediments to reconstruct the history of inland water bodies (Smol 2008a)(Figure 1). Lakes and surface waters are continually exposed to multiple environmental stressors including acid deposition, eutrophication, changes in land use, watershed disturbance and climate warming. Both historical and long-term water monitoring data is often limited. Due to this limitation, sediment archives are a worthy tool which allow for understanding the history of a watershed. Aquatic systems archive the chemical signals and biological activity of the water column in lake bottom sediment, preserving indirect records of previous water quality conditions. Paleolimnological assessments allow for some of

these gaps to be filled by inferring past conditions using abiotic and biotic proxies. Lake sediments are deposited chronologically through time, with the most recent layer of sediment overlying older sediments. Chronologies reconstructed from lake sediment records can portray the departure from baseline conditions. The reconstruction of ecological time series from sediment archives with paleolimnological techniques has been an effective way to enhance limited data by determining the pre-disturbance conditions of lakes and how they have changed (Smol 2010). Paleolimnology allows us to ask questions regarding pre-disturbance conditions, the range of natural variability, whether or not conditions have changed and what some of the causes of those changes are (Smol 2010). Traditional paleo indicators (diatom, chrysophate, zooplankton) identified from subfossils, pigments and other proxies have been widely applied in paleolimnology for inferring key aspects of historical water conditions (Cohen 2003).

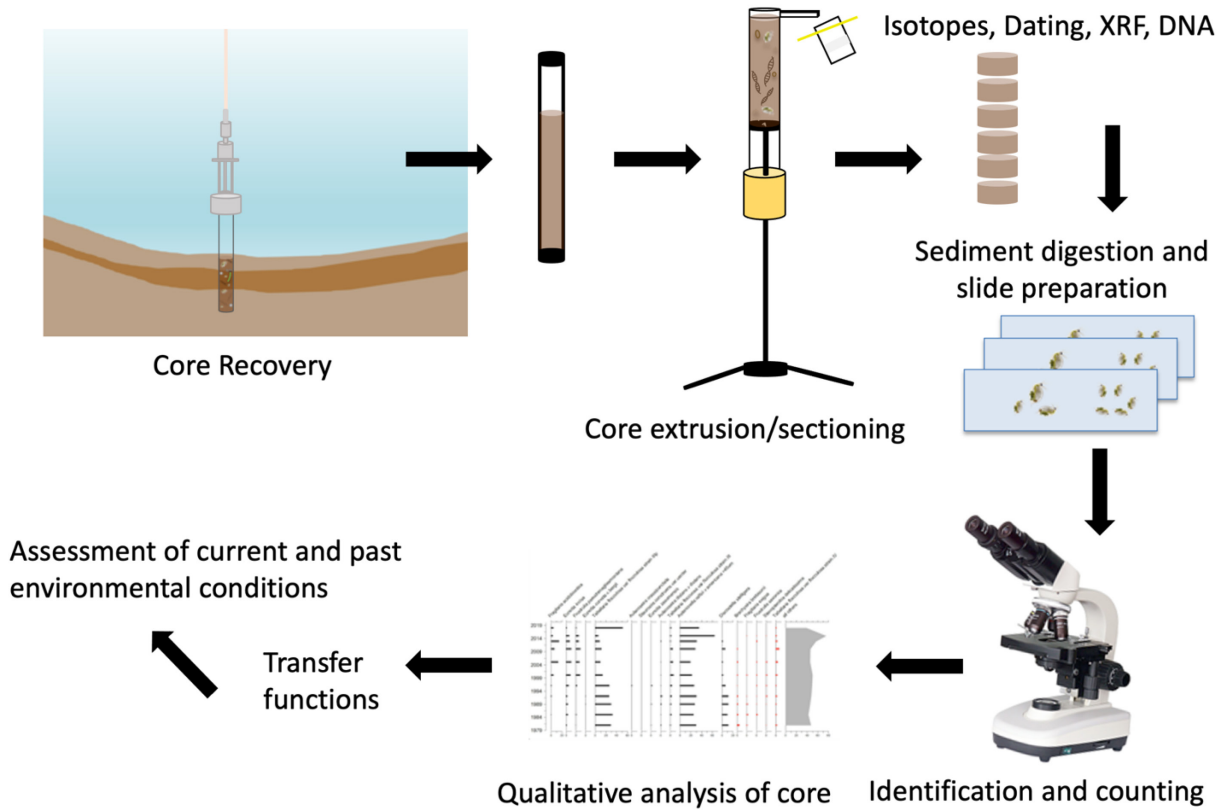

Figure 1: Schematic of paleolimnological process, modified from Smol 2003.

Cladocera

Cladocera (Crustacea, Brachipoda), an order of crustacean zooplankton, are excellent indicators of aquatic food web structure due to their role in primary production and sensitivity to predation (Korosi et al. 2013b). Paleolimnological studies of zooplankton groups like Cladocera can provide a wealth of historical ecological data because these organisms leave behind chitinous fossil remains, allowing identification of key taxa to the species level. Fossil assemblages are used to infer environmental changes to better understand long-term ecological changes occurring in lakes (Korosi et al. 2013b). Indeed, Cladocera have been used extensively to evaluate responses to environmental changes (Sweetman et al. 2008; Yan et al. 2008; Desellas et al. 2008; Kurek et al. 2011; Korosi and Smol 2012b; Shapiera et al. 2012; Barrow et al. 2014; Labaj et al. 2015; Simmatis et al. 2021). Most biological indicators have well-defined ecological optima and tolerances to limnological variables like pH and nutrients, reaching maximum abundances under the most favorable conditions (Korosi et al. 2013a). Therefore, past environmental conditions can be inferred by assessing changes in the relative abundance of cladoceran assemblages (Korosi et al. 2013a).

Lakes in acid sensitive regions have recorded drastic changes in both limnological conditions and biological taxa assemblages since pre-industrial times (Ginn et al. 2007b; Smol 2008b). The vulnerability of crustacean zooplankton to lake acidification has been studied extensively (Arnott et al. 2001; Keller et al. 2002; Korosi and Smol 2012b; Labaj et al. 2015; Jeziorski and Smol 2016; Simmatis et al. 2021). Most studies involving zooplankton responses to acidification have focused on clearwater lakes with a dynamic acidification history (Korosi and Smol 2012a). Trends in acidified lakes show cladoceran assemblages generally increasing in *Bosmina* spp. relative abundance corresponding with declines in Ca-rich *Daphnia* spp. (Locke

and Sprules 2000; Labaj et al. 2014; Simmatis et al. 2021). Evidence has also shown that cladoceran size structure has changed since pre-impact conditions, with smaller body size and shorter body length in *Daphnia* spp. and *Bosmina* spp., respectively, in modern day sediments (Korosi and Smol 2012a).

Considering a pH of approximately 6.0 as an appropriate threshold for zooplankton recovery, lake acidity remains a significant limitation to biological recovery. Gray and Arnott (2009) suggest the impediments affecting biological recovery may also differ among regions. Zooplankton species vary widely in their pH tolerance. The detection of biological recovery in a region requires knowledge of acidification threshold values for indicator species (Lacoul et al. 2011). Due to limited field-based data, a regionally specific criterion of acid sensitivity has not been proposed in Atlantic Canada (Lacoul et al. 2011). Long-term studies have detected a delay in zooplankton recovery, even after water quality has reached acceptable levels (pH >6.0) (Yan et al, 2003; Frost et al. 2006; Gray and Arnott 2009). Keller et al. (2002) suggested that successful establishment of common zooplankton species as they recover from acidification will mainly depend on biotic and abiotic interactions.

Diatoms

Diatoms (Bacillariophyceae) are unicellular, eukaryotic organisms. They have been used as indicators of water quality and to track changes in fresh water ecosystems extensively (Dixit and Smol 1994; Hall and Smol 1996; Dixit et al. 1999; Reavie and Smol 2001; Jeziorski et al. 2008; Smol and Stoermer 2010; Arseneau et al. 2011; Hawryshyn et al. 2012; Barrow et al. 2014). They can be identified to a species level and easily enumerated for paleolimnological studies. Diatom species are sensitive to a variety of environmental stressors including changes in temperature, pH, acidification, and nutrient enrichment. They are important in freshwater

environments due to their role in primary production. These microscopic algae are one of the most valuable paleo-indicators because of the wide range of lacustrine habitats in which they occur, and the excellent preservation potential of their siliceous cell walls (frustule)(Cohen 2003).

Diatoms can be used to investigate the pH history of a lake (Renberg et al. 1985), and have provided significant evidence of lake acidification in lakes across Europe and North America (Battarbee et al. 1984; Davis 1987; Korhola et al. 1999; Rühland et al. 2003). The relationship between diatom occurrence and lake water pH allows for pH to be reconstructed with a standard error (Battarbee et al. 1984), using regression models and calibration (Birks et al. 1990). Statistically robust and ecologically sound transfer function models for diatoms have been developed (Ginn et al. 2007a). The models are based on weighted averaging (WA), the assumption that species closest to their pH optima along an environmental gradient will be most abundant and can be used to infer the value (Birks 1995; Ginn et al. 2007a). These inference models are used to accurately infer lake water pH (Ginn et al. 2007b).

As we have noted previously, lakes in Europe and North America are showing evidence of chemical recovery from anthropogenic acidification (Ek et al. 1995; Stoddard et al. 1999). Studies show evidence of biological responses observed in diatom assemblage shifts, but recovery varies among sites and is usually limited compared to pre-impact reference (Battarbee et al. 2014; Rühland et al. 2015). Not all lakes chemically recovering from acidification are showing signs of biological recovery in their diatom assemblages (Dixit and Smol 2000; Arseneau et al. 2011; Greenaway et al. 2012; Sivarajah et al. 2017). Diatom inference models show pH recovery in Killarney Lakes, Ontario, have significantly different diatom assemblages compared to pre-industrial times (Dixit and Smol 2000). Diatom assemblages in Sudbury,

Ontario lakes similarly do not show signs of biological recovery despite well-documented chemical recovery trends (Sivarajah et al. 2017). It is suggested that the algal communities of those lakes studied, have crossed climate-related limnological thresholds and as the communities are not returning to pre-acidification assemblages (Sivarajah et al. 2017). Many things can influence diatom recovery, including local bedrock and the hydrological regime of the lake (Greenaway et al. 2012). However, climate change has been suggested to be the main driver, resulting in ecological shifts and the success of planktonic diatoms in many aquatic systems (Arseneau at al. 2011; Greenaway et al. 2012; Rühland et al. 2015; Sivarajah et al. 2017).

Environmental DNA

The use of these classical biomarkers (Cladocera and diatoms) reflects only a small part of planktonic diversity. DNA can be preserved in sediments and can be obtained from ancient and modern environments. Environmental DNA (eDNA) is genetic material collected directly from an environmental sample such as soil, water or sediment, instead of being sampled directly from an individual organism. Extracted eDNA can then be analyzed using high-throughput sequencing (HTS) and metabarcoding methods for rapid measurement and biodiversity analysis (Figure 2). eDNA metabarcoding has been identified as a promising tool for biodiversity assessment and monitoring worldwide (Lacoursière-Roussel et al. 2018; Miya 2022; ogden 2022). eDNA metabarcoding has the potential to enhance our understanding of evolutional processes in aquatic systems (Ellegaard et al. 2020). Metabarcoding is the taxonomic identification of species extracted from a mixed sample which are then PCR-amplified and sequenced (Deiner et al. 2017). To differentiate between organisms in the sample, DNA metabarcoding uses DNA libraries to determine what organisms are present. With technological

developments of HTS, rapid sequencing of DNA has become an efficient and affordable way to analyze biodiversity (Ruppert et al. 2019).

Studies have shown the importance of eDNA and how it can contribute to our understanding of aquatic systems and inland water bodies (Domaizon et al. 2017; Harrison et al. 2019; Ogden 2022). The use of eDNA as a proxy in paleolimnology analysis has only begun to gain traction recently. Findings have successfully quantified centennial to millennial-scale dynamics using DNA-based methods in paleolimnology (Coolen et al. 2013; Domazion et al. 2013; Monchamp et al. 2016). Studies have used eDNA from lake sediment to infer historical trends in cyanobacterial community diversity (Monchamp et al. 2016; Tse et al. 2018), assess the response of micro-eukaryotic diversity (Keck et al. 2020), shifts among Eukaryota, Bacteria and Archaea (Wurzbacher et al. 2017), biomonitoring and surveillance purposes (Thomsen and Willerslev 2015; Lacoursière-Roussel et al. 2018) and to track fungal community change and the return of function groups (Yan et al. 2018).

Molecular genetic approaches in paleolimnology have provided new insights in the world of lake sediment analyses. Most eDNA studies have used single-gene survey methods and consequently, the full diversity of preserved microorganisms remains unexplored (Garner et al. 2020). Shotgun metagenomic analysis is the alternative. Garner et al. (2020) tested a proof of concept that DNA from historical lake microbiomes can be recovered from sediment metagenomes. High throughput shotgun sequencing has also revealed taxonomic and derived functional shifts in benthic productivity (Broman et al. 2021).

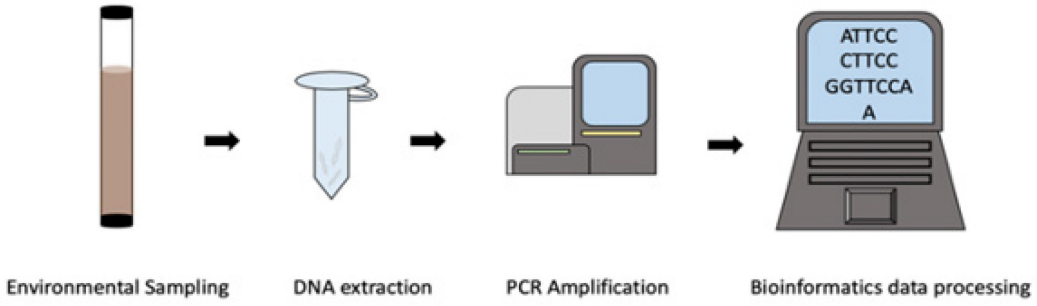

Figure 2: Schematic of eDNA process

Challenges using eDNA

When conducting eDNA research, there are still many things to consider. Firstly, there is a lack of standard methods and applications of eDNA techniques are not straightforward (Pawlowski et al. 2021), and therefore research design is important. Moving on to analysis, challenges include ensuring reliability of results (Domaizon et al. 2017), taphonomy, source area, and representation biases (Sjögren et al. 2017). The detectability of eDNA in environmental samples is limited by four main processes. These processes are eDNA production (rate of DNA shedding), degradation, removal and transport (Harrison et al. 2019). Processes affecting DNA distribution, degradation and preservation during transitions from the pelagic to benthic zones are still not fully understood, and little is known about the complex spatiotemporal dynamics of various eDNA types (Deiner et al. 2017). Other pitfalls identified include contamination of DNA during collection and extraction and limitations with the reference DNA database and primers used (Thomsen and Willerslev 2015). Errors and uncertainties can be mitigated through refined study design, appropriate primer choice and robust sampling and replication (Deiner et al. 2017).

Knowledge gaps and originality of research

Almost all aquatic systems exhibit lake-specific responses to multiple stressors . It is a challenge to determine the ultimate cause of assemblage shifts when using bioindicators and the paleolimnological approach (Smol 2010). To make meaningful interpretations using cladoceran invertebrate sub-fossils, understanding the ecological controls on cladoceran taxa in Nova Scotia is required (Korosi and Smol 2011). However, crustacean zooplankton studies in Atlantic Canada are limited (Carter et al. 1986, Korosi and Smol 2011). This research helps to address this knowledge gap.

This thesis helps address biological recovery knowledge gaps by providing information on the ecological distribution of Cladocera and diatom assemblages using subfossil remains preserved in lake sediments from an important drinking water source in Nova Scotia. With evidence of chemical recovery trends occurring in Nova Scotia (Redden et al. 2021) and specifically Pockwock Lake (Anderson et al. 2017), along with the recent diatom bloom, we re-cored Pockwock Lake to assess how the diatom assemblages have changed in the last 16 years. Due to the limited number of studies involving Cladocera in Atlantic Canada, assessing the Cladocera sub fossil record in Pockwock Lake can also help to explain the rapid changes in water quality and potentially answer questions regarding regional Ca decline.

eDNA metabarcoding is a useful tool for tracking aquatic evolution and adaptation (Ellegaard et al. 2020), There appears to be limited to no studies using eDNA metabarcoding to understand biological recovery trends. This study is the first paleolimnological study (to our knowledge) attempting to use eDNA metabarcoding to provide insight into the micro biodiversity of a freshwater lake in Atlantic Canada.

Research Summary

The overall goal of this thesis is to assess the extent of biological response and recovery that has occurred in Pockwock Lakes by examining multiple proxies using paleolimnological methods. In addition, eDNA metabarcoding is used to examine the microbiome diversity and species richness in modern deposited sediments. This research is extremely relevant to the pressing water quality challenges faced by HW and other water utilities around the world. This research intends to contribute to the growing knowledge around the complexities of biological recovery and the factors that hinder recovery from anthropogenic acid deposition.

Thesis Outline

This thesis is structured into five chapters. Chapter 1 identifies the problem and provides background and literature review for this research. Chapter 2 details methodology used in the field, lab, and for sediment analysis. Chapter 3 presents bio indicator results, discussion, and conclusions for sediment core analysis. Chapter 4 presents eDNA metabarcoding results, discussion, and conclusions for microbiome analysis. Chapter 5 provides a general summary of Chapters 3 and 4, recaps key findings, identifies research limitations and offers recommendations for further investigation.

CHAPTER 2.0: STUDY LOCATIONS AND METHODOLOGY

Preamble

This chapter provides an overview of the study locations including a background of Halifax Water. It provides methodology used for data collection in the field, laboratory and analysis methodology and explains the multi-proxy paleolimnological approach.

Study Sites

Pockwock Lake

Pockwock Lake (44°48'N, 63°52'W) is a large (903 ha), deep (max depth: 45m) lake with low-pH (pH < 6), low turbidity (<0.5 NTU) and low-alkalinity (<5 mg $CaCO_3$/L) source water (Anderson et al. 2017; Vadasarukkai et al. 2011; Knowles et al. 2012). Pockwock Lake is located in Upper Hammonds Plains, Nova Scotia, within the Pockwock Watershed Protected Water Supply Area (Figure 3). Pockwock Lake is the main drinking water source for HRM, supplying the JDKWSP, owned, and operated by HW. HW is the municipal water, wastewater, and stormwater utility serving 360,000 residents of Halifax Regional Municipality (HRM). It operates three ISO 14001 Certified water supply plants and six smaller supply plants. The J.D. Kline Water Supply Plant (JDKWSP) treats water from Pockwock Lake and supplies water to Halifax, Bedford, Sackville, Fall River, Timberlea, and Waverly. This study focuses mainly on Pockwock Lake, but we also examine the microbiome in a small lake just north of Pockwock in the watershed boundary, Island Lake. Island Lake is a known area for geosmin occurrence in the Pockwock Watershed.

The history of the Pockwock Watershed is well known and there have been a handful of previous studies at Pockwock Lake. Changing water quality trends and watershed disturbance in water source have been documented using historical monitoring data (Anderson et al. 2017), and

paleolimnological methods (Tropea et al. 2007; Dunnington et al. 2018). Bulk sediment geochemistry from Pockwock Lake reflect more than 2 centuries of anthropogenic disturbance including deforestation, urbanization, water works construction, and agriculture (Dunnington et al. 2018). Deforestation and related activity were the primary disturbances in the Pockwock catchment through analyses of the geochemical sediment record (Dunnington et al. 2018). A disturbance is apparent at ~1976, after which time Carbon (C) increased to concentrations slightly greater than prior to 1880 (Dunnington et al. 2018). Pockwock Lake has previously shown a strong diatom signal of historic pH declines compared to other regional lakes (Ginn et al. 2007; Tropea et al. 2007; Korosi et al. 2013). A two-stage assemblage signal with loss of DOC was identified using a diatom-inferred pH, (Tropea et al. 2007), the first occurring at ~1940 and the second around 1992. The first acidification trend was accompanied by a loss in DOC and water colour, typical of humic lakes in Nova Scotia, whereas the post ~1992 acidification trend and diatom assemblage shift is similar to those occurring in clearwater lakes (Tropea et al. 2007).

Island Lake

Island Lake (44°51'N, 63°50'W), is a small (50 ha), shallow (max depth: 11m) lake north of Pockwock Lake in the Pockwock Watershed. Water monitoring by Halifax Water has occurred in this headwater and has a documented history of geosmin occurrence. No other studies have involved Island Lake.

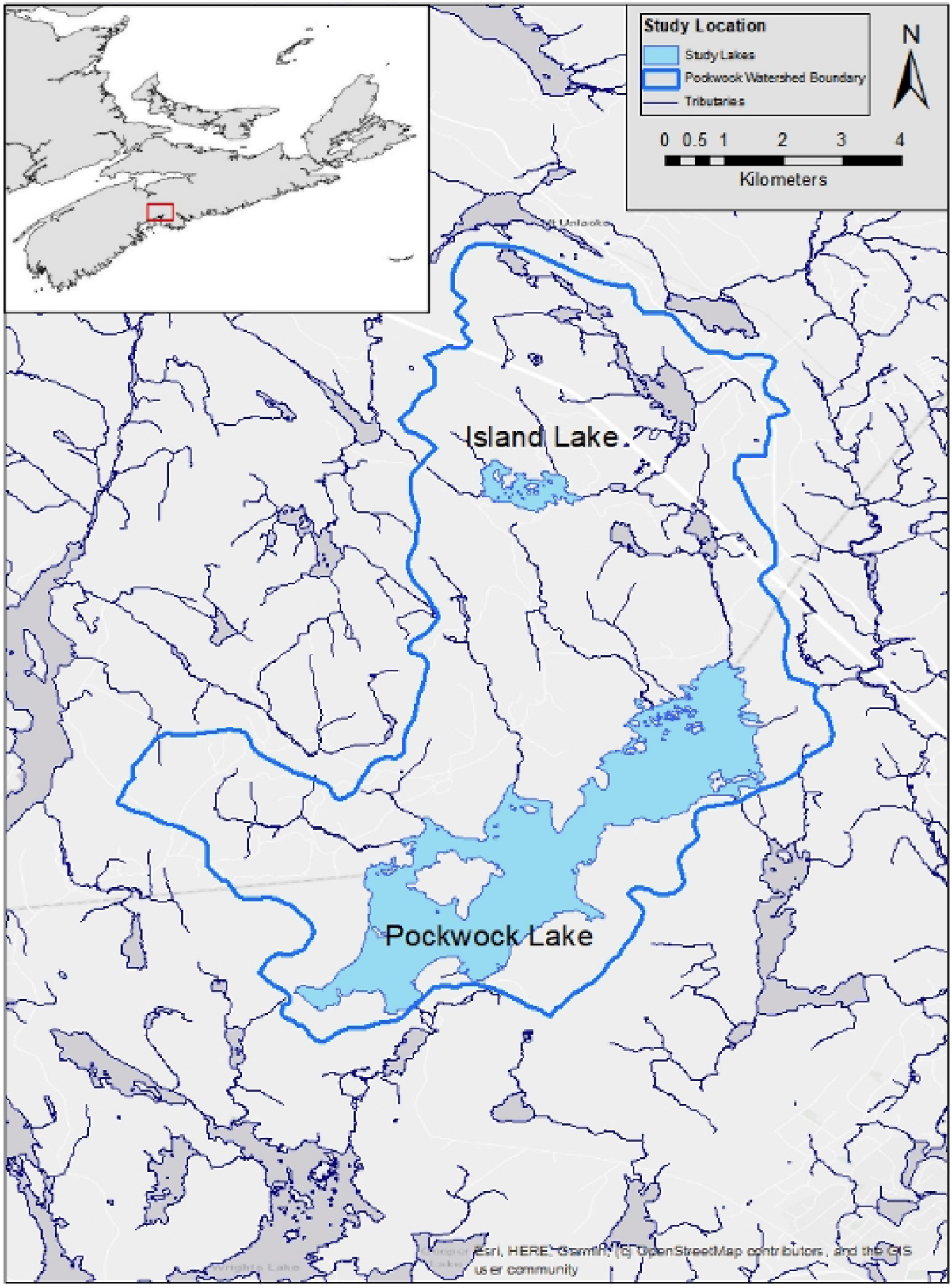

Figure 3: Pockwock Watershed boundary with study lakes

Geology

Most of the Halifax region is located on poorly buffering bedrock, including Meguma Terrane plutonic rocks (i.e., granite) and Cambrian-Devonian (i.e., quartzite, slate). Soils that are formed from this parent material contain low base cation concentrations and are thus vulnerable to acidification (Stumm et al. 1987; Clair et al. 2007). Halifax slate is also a major source of arsenopyrite that can contaminate drinking water (PNS 2017). The underlying geology (Figure 4) at Pockwock Lake is comprised of Middle-Late Devonian granodiorite, Middle-Late Devonian biotite monzogranite and Goldenville Formation consisting of sandstone turbidites and slate (PNS, 2017). Underlying geology at Island Lake consists of Halifax Formation to the north and east of the lake consisting of slate, siltstone, minor sandstone and Fe-Mn modules and Middle-Late Devonian granodiorite to the south and the west (PNS 2017). Surficial geology (Figure 5) for both lakes include glacially sourced bedrock overlain by a thin discontinuous veneer of till. Pockwock Lake also has a large amount of stoney till plain to the south.

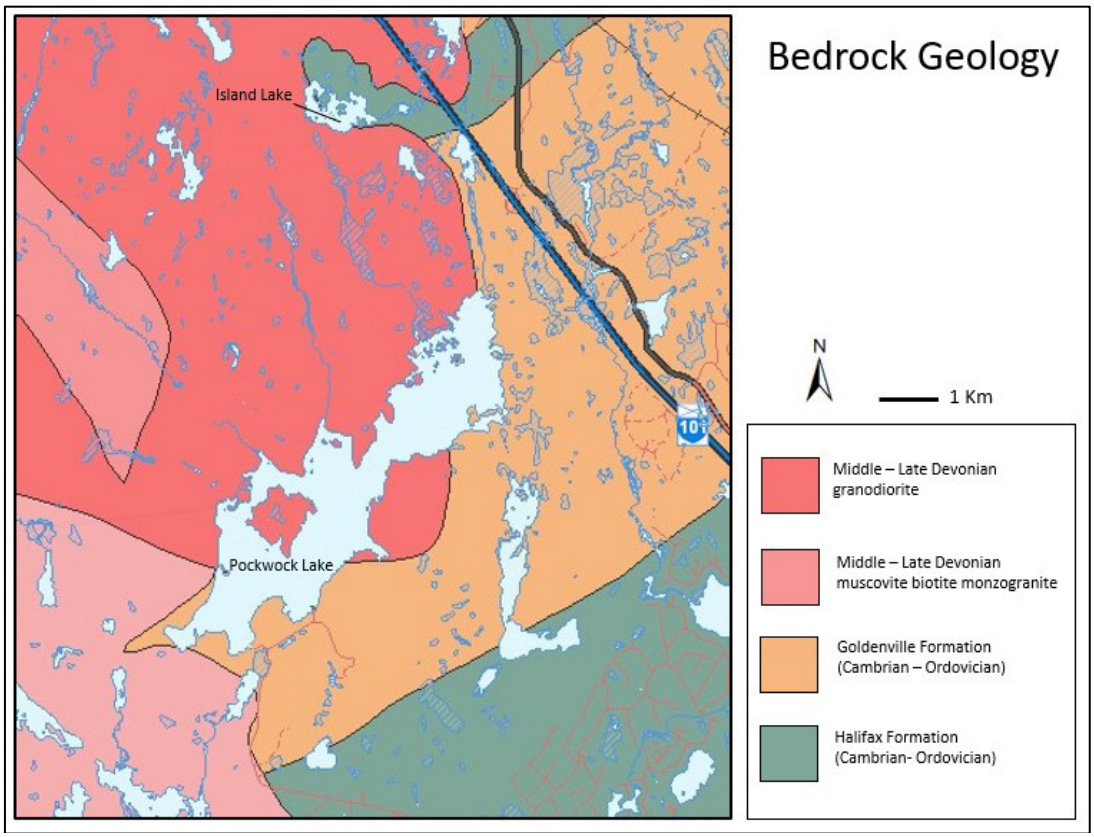

Figure 4: Bedrock geology of study location

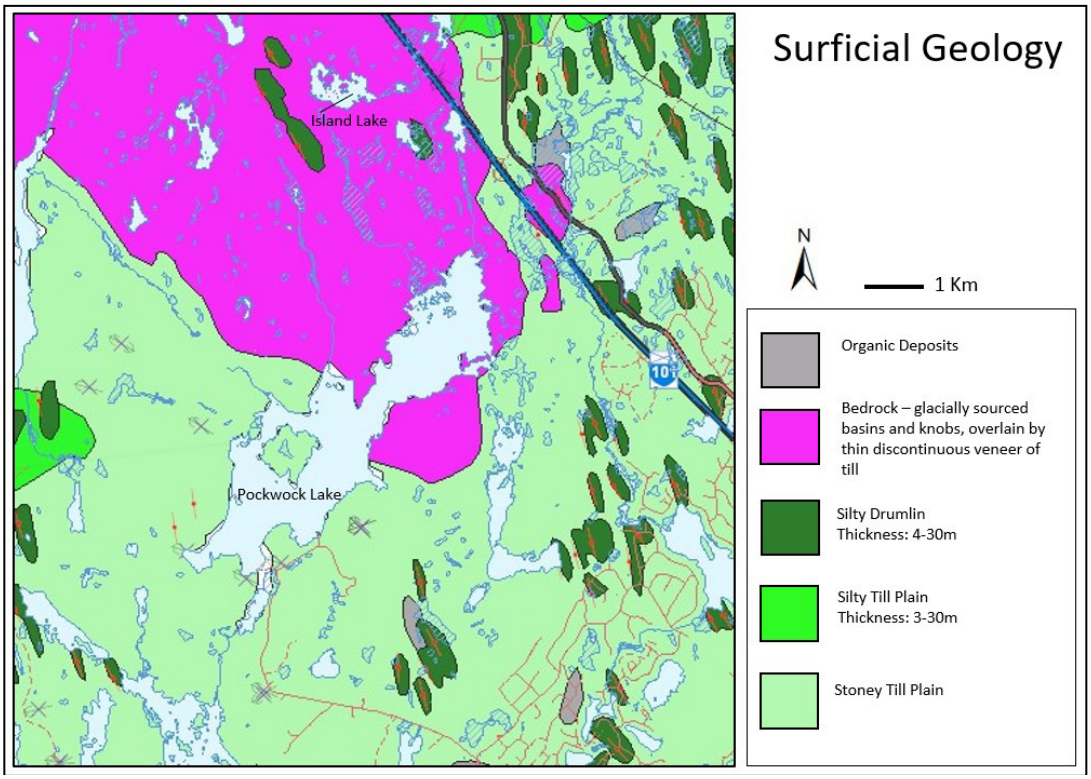

Figure 5: Surficial geology of study location

Field Methodology

Gravity Coring

Three cores (POC18-1, POC18-2 and POC18-3) were collected on October 15th, 2018, by Josh Kurek, Dewey Dunnington and Heather McGuire. Sediment cores were retrieved from the deepest part of the lake basin to capture an undisturbed sedimentary record (Cohen 2003) using a Glew gravity corer (Glew 1989, Glew et al. 2001). Core barrels were 60 cm long with a diameter of 6.3 cm. To collect the lakebed sediments, the coring device was prepped and lowered slowly into the water column from the boat. Upward tension was maintained to ensure the core barrel entered the sediment vertically (Dunnington 2018). The messenger was released down the rope to trigger the mechanism and push the core barrel further into the sediment, trapping the sediment and water interface within. After the cores were collected, they were kept vertical to ensure no sediment mixing took place. Upon returning to shore, core POC18-1 was extruded (Glew 1988) at 0.5 cm increments into pre-labelled and weighed zip lock bags. The core was dated using ^{210}Pb and analyzed for Cladocera, diatoms, VNIRS-inferred Chl-a, VNRIS-inferred TOC, and elemental geochemistry.

Core POC19-1 was collected on August 8th, 2019, by Heather McGuire and Alanna Wood. The same coring and extruding methods were performed. This core was extruded at 1-cm increments and used for DNA metabarcoding and analysis (Deiner et al. 2017) and XRF analysis (Dunnington et al. 2020). Cores IL19-1 and IL-19-2 were collected from Island Lake on July 24th, 2019, by Heather McGuire and Alanna Wood following the same field methods as above. Core IL19-1 was extruded (Glew, 1988) at 1-cm increments and was used for DNA metabarcoding and analysis (Deiner et al. 2017) and XRF analysis (Dunnington et al. 2020). A summary of sediment cores collected for this project are summarized in Table 1.

Table 1: Cores collected and used for analysis

Lake	Core ID	Date	Depth (M)	Size (ha)	Latitude	Longitude
Pockwock Lake	POC-18-1	2018-10-15	45	903	44°48'N	63°52'W
Pockwock Lake	POC-19-1	2019-08-08	45	903	44°48'N	63°52'W
Island Lake	IL-19-1	2019-07-24	11	50	44°51'N	63°50'W

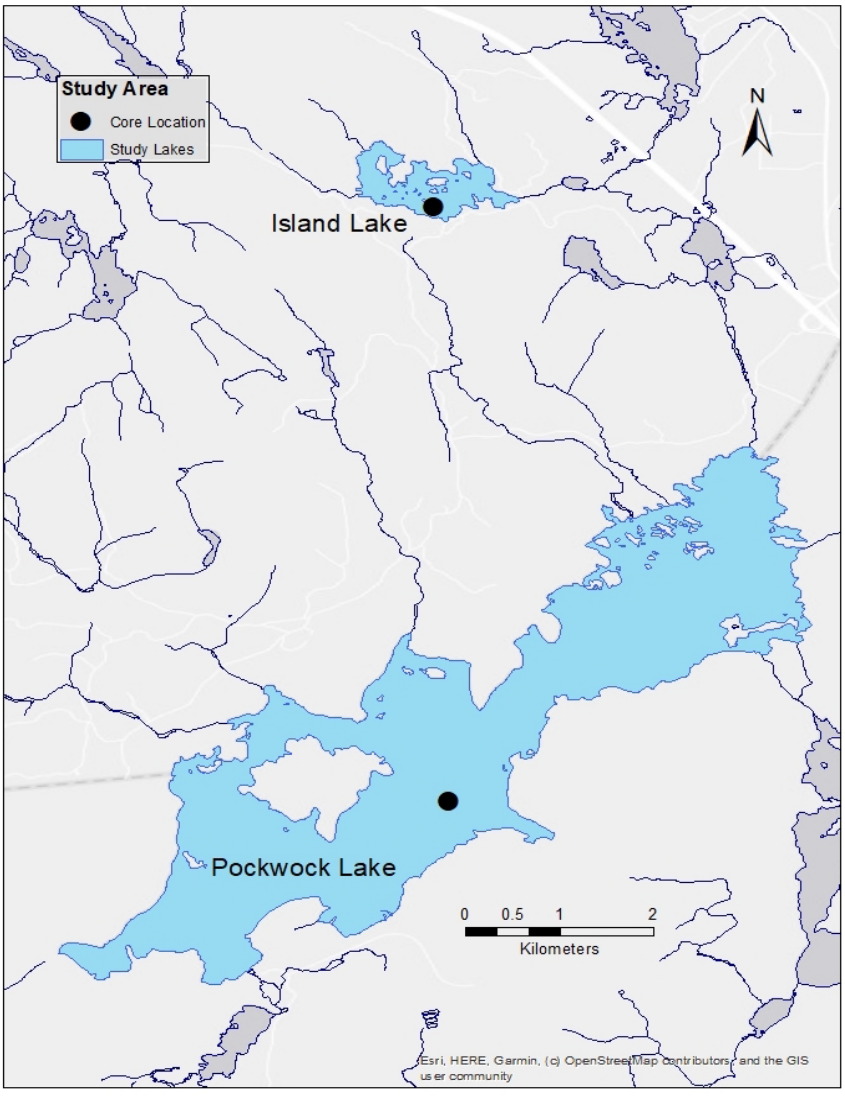

Figure 6: Core locations

Laboratory Methodology

Radiometric dating

Ages and sedimentation rates were calculated according to the constant rate of supply (CRS) model (Appleby and Oldfieldz 1983). Ages for depths prior to the ^{210}Pb background were extrapolated based on average sedimentation rate (Dunnington et al. 2018)**,** Appendix B.

Sediment core POC18-1 was radiometrically dated by analyzing ^{210}Pb using gamma spectrometry. Wet sediment samples were freeze dried prior to ^{210}Pb analysis. Samples were dried using a Labconco FreeZone 4.5 freeze drier (Kansas City, Missouri, United States). Samples were weighed before and after drying to calculate percent water. After drying, 0.5 – 1.0 gram from each core interval was placed in individual plastic tubes prior to gamma spectrometry following the procedures of Schelske et al. (1994). Gamma spectrometry was performed by the Paleoecological Environmental Assessment and Research Laboratory (PEARL), Queen's University. Resulting age-depth relationships were calculated using the constant rate of supply (CRS) dating model (Appleby and Oldfieldz 1983).

The dated core was divided into the three defined groups to determine if there was a difference in the community structure. Top of core, 0 – 7.5 cm represents the post-acidification period and is referred to as the modern period. Middle of core, 7.5 – 18.5 cm represents the acidification period. Bottom of core, 18.25 – end of core (45 cm) represents the pre-acidification period and is referred to as pre-impact.

Reflectance Spectroscopy

Sedimentary chlorophyll *a* analysis was performed on the same intervals analyzed for diatoms using visible reflectance spectroscopy (Wolfe et al. 2006, Michelutti et al. 2010, Hawryshyn et al. 2012). Visible-near-infrared reflectance spectroscopy (VNIRS) is a non-

destructive spectral technique that provides a rapid and semi-quantitative method for assessing the chlorophyll *a* content in lake sediments (Wolfe et al. 2006). Chlorophyll *a* has been used as a proxy in paleolimnology studies to track the history of primary production in lakes. VNIRS-inferred chlorophyll *a* analysis was performed on core POC18-1 by the Paleoecological Environmental Assessment and Research Laboratory (PEARL), Queen's University following the methods of Michelutti et al. 2005 and Wolfe et al. 2006.

Paleolimnological studies have also reconstructed past TOC/DOC trends using inference models based on VNIRS (Rosén 2005, Rouillard et al. 2011). A VNIRS model was used to infer past lake-water TOC concentrations in Pockwock Lake. VNIRS-inferred TOC analysis was performed on core POC18-1 by the Paleoecological Environmental Assessment and Research Laboratory (PEARL), Queen's University following the methods of (Meyer-Jacob et al. 2017). Using transfer functions between VNIR spectra of lake-surface sediments and corresponding TOC/DOC concentrations, the reconstruction of long-term data from sediment cores is possible (Meyer-Jacob et al. 2017).

Cladocera preparation

Cladoceran preparation and enumeration were performed by Heather McGuire. A sample was prepared for each 0.5-cm increment following the methods of Korhola and Rautio (2001) to produce subfossil Cladocera microscope slides using a 10% KOH digestion. To begin, approximately 1 g of wet sediment was weighed and placed into a clean 250 mL beaker for each sample interval. The 10% KOH solution was then prepared by dissolving 100 g of KOH pellets in 1 L of deionized water. Next, 150ml of KOH solution was added to each sample beaker containing the sediment. Samples were placed on a hot plate under a fume hood and heated to 70-80°C for 30 minutes. Samples were gently stirred using a glass rod. After digestion, the

KOH-sediment mixture was poured through a 37-µm sieve (U.S.A Standard Test Sieve No. 400). The captured remains were washed in the sieve using deionized water. This step was repeated until the water coming through the sieve was clear. Care was taken to ensure the sample was no longer slimy and completely washed of KOH and smaller sediment particles. The remaining deposit retained on the sieve screen was transferred into a glass vial. The remains were transferred into the vial using as little deionized water as possible, after which 2 – 3 drops of safranin were added to dye the remains, along with several drops of rubbing alcohol for preservation. For slide preparation, new, clean slides were placed on a small hotplate. Slides were made by pipetting a 50 µl aliquot of the well-mixed sample onto the slide. The sample was allowed to dry and permanently mounted to the slide with Entellan® (Armstrong and Kurek 2019).

Diatom preparation

Diatom preparation and enumeration were performed by Nell Libera, PhD candidate at Queen's University. Diatom subfossils were prepared following the methods of Battarbee et al. (2001). A strong acid digestion was used to isolate siliceous valves from organics in the sediment matrix using a 50:50 M combination of HNO_3 and H_2SO_4 (nitric and sulfuric acids). The acid digestion was conducted in a water bath at 85 °C for 2 hours to speed up the reaction. After allowing the sediment to settle to the bottom of the vial for 24 hours, the samples were aspirated to remove the overlying acid, and the vial refilled with water. This process was repeated daily for at least 7 days until the sample reached a neutral pH. Samples were pipetted onto coverslips, dried, and secured to glass microscope slides using Naphrax. Diatoms were enumerated and identified using a light microscope with differential inference contrast (DIC) and oil immersion at 1000x. The taxonomic guides used for identification were Krammer and Lange-Bertalot

(1986-1991), Camburn and Charles (2000), Reavie and Kireta (2015) and Reavie and Smol (1998).

Environmental DNA extraction

This study used eDNA, metabarcoding and high-throughput sequencing methods. To quantify the relative importance of the 16S rRNA and 18S rRNA sequences, real-time PCR analysis was carried out on extracellular DNA extracted from different sediment layers from both Pockwock Lake and Island Lake cores.

Environmental DNA extraction was performed by Heather McGuire. A DNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany) was used for the isolation of microbial genomic DNA. The sectioned sediment core was removed from the freezer and allowed to thaw inside a biological safety cabinet (BSC) prior to processing. The protocol for the DNeasy® PowerSoil® Kit was followed as instructed.

All laboratory procedures were carried out following strict precautions to prevent contamination. Gloves and sterile labware were used during sediment subsampling. The DNA extraction was performed in a dedicated sterile workstation within a BSC. All centrifugation steps were performed at room temperature. Twelve sediment samples were processed simultaneously during each analysis. The top 10 cm of the core was analyzed in 1 cm sections as follows: 0-1cm, 1-2 cm. 2-3 cm. 3-4 cm. 4-5 cm. 5-6 cm, 6-7 cm, 7-8 cm, 8-9 cm and 9-10 cm. Two replicate samples at 0-1 cm and 5-6 cm for both Pockwock Lake and Island Lake cores (POC19-1 and IL19-1) were also analyzed. These sediment layers were selected to investigate changes and differences in cyanobacterial and eukaryotic microbial diversity.

DNA sequencing wet-lab procedure

Extracted DNA was sent to the Integrated Microbiome Resource (IMR) laboratory at Dalhousie University for high throughput sequencing. IMR requires a nucleic acid concentration of >1 ng/μL, a volume of 10 μL aliquot of each sample and "clean" DNA coming from a commercial kit (e.g. Qiagen) or proven manual method. This is enough DNA concentration for two different targets. This study used two targets, 16S and 18S genes.

To verify the extracted samples had adequate nucleic acid concentrations for sequencing, a microplate reader (BioTeK Synergy H1 Hybrid Multi-Mode, Winooski, Vermont, United States) was used to quantify extracted sample nucleic acid and protein concentrations. A 2 μL aliquot of each extracted sample was pipetted onto the Take3 Micro-Volume plate. A total of 12 samples and two blanks were loaded onto the plate. Table 4 and Table 5 show the sample concentrations for each extracted sediment sample for Pockwock Lake and Island Lake. Following verification of sample concentrations from the microplate reader, a 10 μL aliquot of each extracted sample was pipetted into a well plate and sealed using a sealing film. Samples were kept on ice and delivered to IMR for PCR amplification using the custom sequencing targets for 16S Cyano-V3-V4 (Primers: CYA359F-CYA781R)(Nübel et al. 1997) and 18S Eukaryotes-V4 (Primers: E572F-E1009R)(Comeau et al. 2011).

The IMR sequencing procedures and library composition work-flow methods were created by Comeau et al. (2017). During this procedure, DNA gets amplified in duplicate using two separate DNA dilutions per sample via polymerase chain reaction (PCR) using high fidelity polymerase. Fusion primers were used for one run and PCR products were verified by a Hamilton Nimbus Select using Coastal Genomics Analytical Gels, normalized using a Charm Biotech Just-a-Plate 96-well Normalization Kit, pooled and then sequenced using a 300+300

base pairs (bp) V3 chemistry on an Illumina MiSeq. Samples were pooled to make one library which was then quantified fluorometrically before sequencing.

Analysis Methodology

Cladocera

All slides were counted using a compound microscope with brightfield illumination at 200x or 400x magnification (Armstrong and Kurek 2019). Each slide was examined in its entirety to avoid non-random distribution biases. All identifiable cladoceran remains including carapaces, headshields, ephippia and post abdominal claws were tabulated. Identifications followed the standard subfossil taxonomy from eastern North America (Korosi and Smol 2012c, 2012d). All remains were identified to a family level. Bosminids were grouped as one species complex, *Bosmina* sp. (*Bosmina* sp. And *Eubosmina* sp.) due to the difficulty of identifying the lateral pore (Armstrong and Kurek 2019).

In this study, we examined four main cladoceran families (Bosminidae, Daphnidae, Chydoridae and Sididae, from recent (the last ~150 years) sediments. Families Ilocryptidae, Macrothricadae, Polyphemidae constituted less than 2% of the relative abundance and were considered rare. Cladocera were counted from 15 interval depths throughout the core. Relative abundance of each cladoceran taxon was calculated by expressing the number of individuals as a percentage for each specific depth interval. Each interval was calculated based on a minimum count of 70 individuals (Armstrong and Kurek 2019).

Cladocera from the pre-acidification period (~1870 to ~1940; 26.5 cm – 18 cm) were compared to the acidification period (~1940 to ~1995; 18 cm – 7 cm) and the post-acidification period (~1995 to ~2018; 7 cm – 0 cm). To determine whether assemblages differed significantly

between pre-acidification, acidification, and post acidification periods, we conducted a permutational multivariate analysis of variance (PERMANOVA) test (Anderson 2017).

Diatoms

Each slide was examined in its entirety to avoid non-random distribution biases. All identifiable diatom remains were counted and identified to the species level. Regional diatom-based transfer functions of pH were used to understand assemblage change and response in Pockwock Lake as this method allows for the reconstruction of past environmental variables. Values were inferred using transfer functions and a previously developed diatom-inference model (Ginn et al. 2007b). From the DI-pH inference model (Ginn et al. 2007b), we can reconstruct past environmental conditions and provide trajectories of past changes in lakewater acidity, from which the time and rates of acidification and recovery can be assessed (Smol et al. 1998). RStudio (R Core Team 2020) was used to perform weighted-averaging regression and calibration functions on the diatom data. The diatom-inferred changes in pH between samples from each depth were plotted and the depths that exhibited changes that were significantly greater than the RMSE were identified. Stratigraphic changes in species diversity were inferred using Hill's N2, also calculated in R Studio.

DNA Bioinformatics

A custom and streamlined workflow for microbiome research was followed for this research (Comeau et al. 2017). The microbiome bioinformatics platform QIIME2 (Bolyen et al. 2019) was used for processing the demultiplexed microbiome data from IMR. Analysis of 16S and 18S sequencing data was carried out using the Microbiome Helper workflow (Comeau et al. 2017) Standard Operating Procedure (SOP) created for v2 qiime2 2020.8 (Bolyen et al. 2019). The scripts used to process data were produced by Comeau et al. (2017) and were bundled in an

Ubuntu 16.04 VirtualBox image. The process used a streamlined and custom approach to process samples from detailed sequencing library.

The generalized bioinformatics workflow performed on 16S and 18S data included sequence quality control, trimming primers, removing low quality reads, running DADA2 (Callahan et al. 2016) to obtain amplicon sequence variants (ASVs), constructing a phylogenetic tree in FastTree (Price et al. 2009) and assigning taxonomy to ASVs using SILVA (Quast et al. 2013) rRNA gene database and feature-classifiers. Downstream analysis including diversity metrics and plots were generated using R and RStudio (R Core Team 2020).

The phylogenetic tree was created through a multiple sequence alignment with the representative ASVs sequences using the FastTree (Price et al. 2009) software. Each leaf of the tree represents one of the ASVs, and each of the branches of the tree has a length (Wong et al. 2016)

eDNA Community Analyses

All microbiome diversity metrics were generated in RStudio using vegan (Oksanen et al. 2017), ggplot2 (Wickham 2016) and phyloseq (McMurdie and Holmes 2013) library packages. Species richness (alpha diversity) was calculated for bacteria and eukaryotes using Shannon Index (Shannon and Weaver 1964). The Shannon index accounts for both the abundance and evenness (distribution across samples) for the feature in question. Multivariate analyses were conducted in RStudio (R Core Team 2020).

Alpha diversity metrics summarize the structure of an ecological community with respect to its richness (number of taxonomic groups), evenness (distribution of abundances of groups), or both. Analyzing the alpha diversity of amplicon sequencing data is a common first approach to assessing differences between environments (Willis 2019). It is more likely to observe higher

numbers of different taxa in a sample with more microbial reads, therefore the library sizes can dominate the biology in determining the result of the diversity analysis (Lande 1996, Willis 2019).

Rarefaction is a method that adjusts for differences in library sizes across samples to aid comparisons of alpha diversity (Willis 2019). It is a preprocessing technique used when quantifying species diversity. In the case of sequencing data, the number of reads may vary greatly between samples, so it is common practice to rarefy the samples such that they all have the same number of reads as that of the smallest sample. This is done by randomly discarding reads from larger samples, such that the population distributions remain unchanged, until all samples have the same number of reads (Hurlbert 1971, Willis 2019). Replicates in microbiome experiments yield different numbers of reads, different community composition, and different levels of alpha diversity (Willis 2019). The microbiome data in this has study has been rarefied

In order to understand the relationships among microbial communities, this study uses UniFrac (Lozupone and Knight 2005) B-diversity measure to take phylogenetic information and compare environmental samples. When using Unweighted UniFrac to measure community distance, a distance of 0 means that the samples are identical, and a distance of 1 means that the two samples share no taxa in common (Wong et al. 2016). When using Weighted UniFrac, the data has been normalized to a common sequencing depth, considering the relative abundance of species shared between samples.

CHAPTER 3.0: BIO INDICATOR RESPONSE IN POCKWOCK LAKE

Preamble

This chapter summarizes the results and discusses the significance of bio indicator changes in Pockwock Lake. Paleolimnological techniques were performed to determine whether cladoceran and diatom assemblages in Pockwock Lake have recovered toward their pre-impact conditions. The goal was to assess changes in assemblages through time from present-day lake sediments to those deposited before significant human impact (~1850). A high-resolution analysis involving the examination of cladoceran and diatom assemblages at regular intervals throughout the sediment core was employed.

Sediment Age Determination

The top of core POC-18-1 corresponds to 2018, when the core was collected from Pockwock Lake. Core POC-18-1 shows ^{210}Pb activity values decreasing exponentially with depth, suggesting a relatively stable sedimentation rate between background ^{210}Pb values at 30 cm (~1840) and present (Figure **7**).

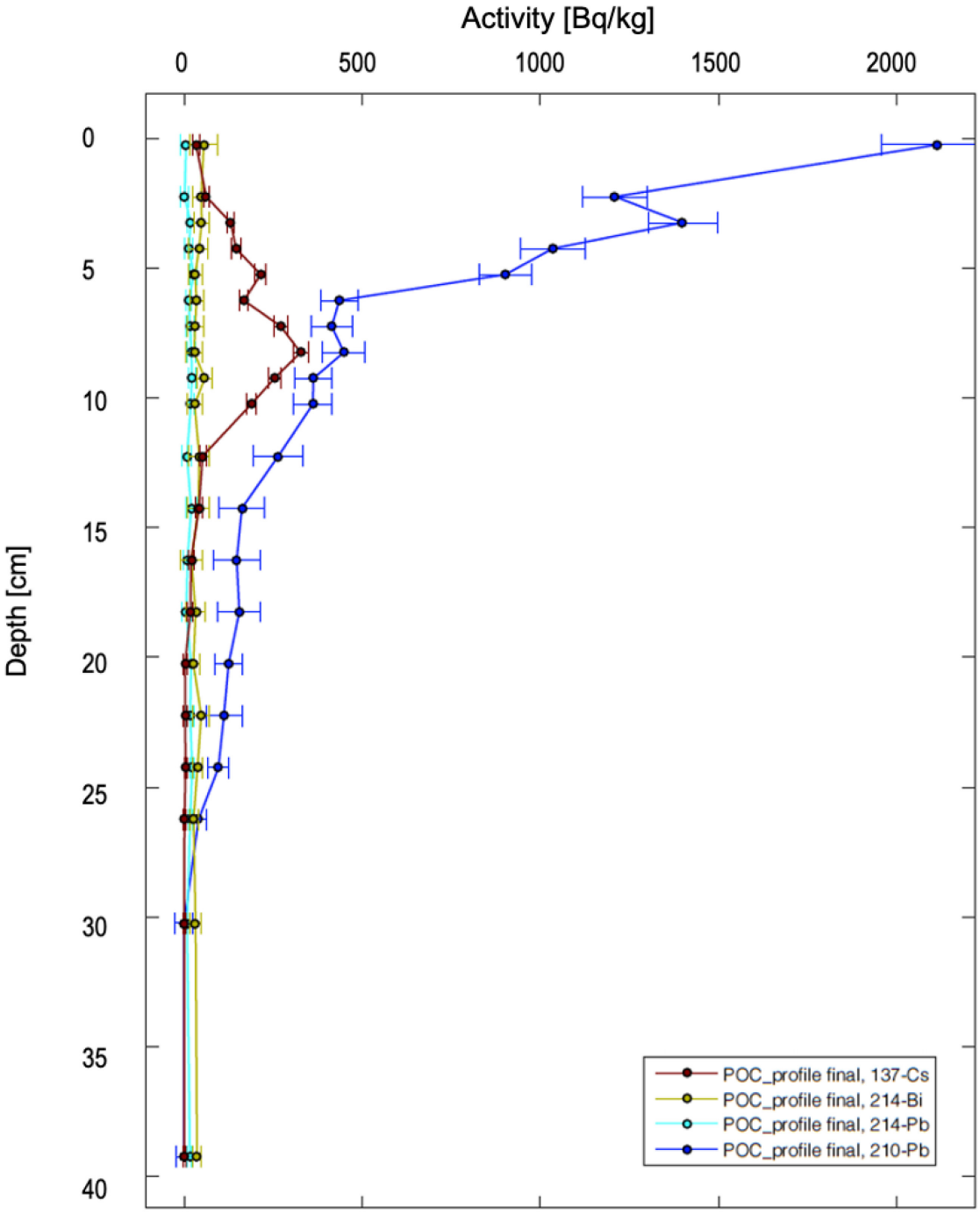

Figure 7: Radiometric activities against core depth for Pockwock Lake

Cladocera

To evaluate the biological response of cladoceran assemblages to chemical lake recovery, this study used paleolimnological techniques to reconstruct cladoceran taxa assemblages from a dated sediment core (Figure 8). Cladoceran taxa counts from 15 depth intervals at Pockwock Lake averaged 129 individuals (min.= 74, max.= 237). The cladoceran assemblages of Pockwock Lake were dominated by the planktonic taxa *Bosmina* spp. Overall, modern period assemblages (~1995 to 2018) were significantly different in relative abundance than assemblages prior to the acidification period (~1870 to ~1940). *Bosmina* spp. showed a mean increase of 10%, whereas *Daphnia* spp. decreased by an average of almost 10% between pre-impact and recovery time periods. Moreover, *Chydorid* spp. decreased by an average of 3% while Sididae increased by an average of 3%.

Sediment intervals within the pre-impact period (~1870 to ~1940; 26.5 to 18 cm) showed *Bosmina* spp. relative abundance between 54 and 74% (average 68%), whereas sediment intervals in the recovery period (~1995 to 2018; 7 to 0 cm) showed *Bosmina* spp. relative abundance between 71 and 83% (average 78%). Sediment intervals within the pre-impact period showed *Daphnia* spp. relative abundance between 11 and 22% (average 16%), and sediment intervals within the recovery period showed *Daphnia* spp. relative abundance between 1 and 4% (average 2.5%). During the pre-impact period, average relative abundances of *Chydorid* spp. and Sididae were 12 and 3%, respectively. During the recovery period *Chydorid* spp. decreased to an average of 9%, and Sididae increased to an average of 6%. All remaining families, Holopedidae, Iliocryptidae, Leptodoridea, Macrothricidae and Polyphemidae did not exceed 2% in two or more sediment intervals and were therefore considered rare.

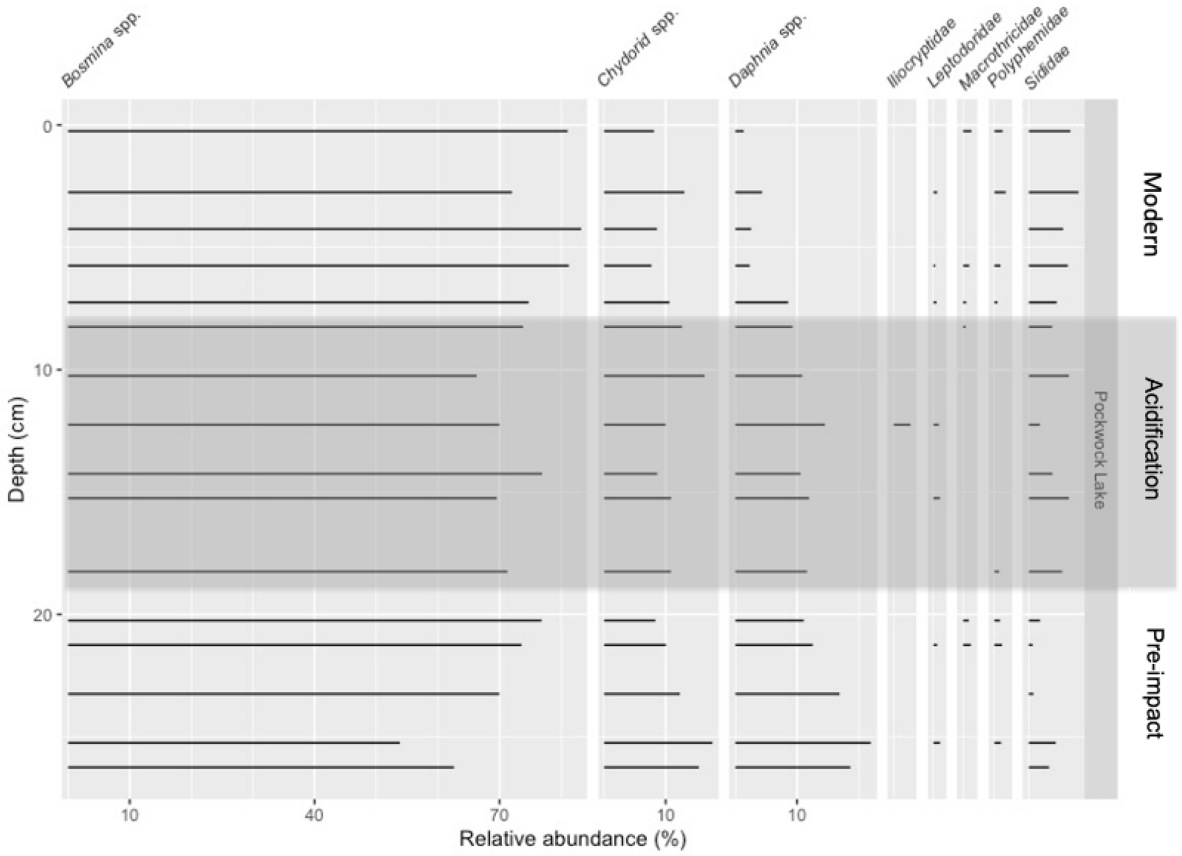

Figure 8: Stratigraphic diagram illustrating changes in relative abundance of sedimentary cladoceran remains from dated sediment core POC-18-1.

The result of PERMANOVA (Anderson 2017) between the time periods (modern, acidification, pre-impact) shows a significant difference ($p<0.001$, $R^2 = 0.43$) in cladoceran assemblages. Table 2 shows the global difference among the three groups. To determine where the difference lies between the three time periods, a PERMANOVA test was run (Table 2).

Table 2: PERMANOVA global difference in communities

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Group	2	0.06	0.03	5.0	0.43	0.002 **
Residuals	13	0.07	0.00		0.56	
Total	15	0.13			1.00	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3: PERMANOVA pairwise contrasts

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted sig
Modern vs Acidification	1	0.03	7.3	0.44	0.005	0.015
Modern vs Pre-impact	1	0.048	6.79	0.46	0.011	0.033
Acidification vs Pre-impact	1	0.010	1.62	0.15	0.202	0.606

Table 3 shows that modern cladoceran assemblages are significantly different from both acidification and pre-impact assemblages (p = 0.005 and 0.011, respectively). In contrast, acidification and pre-impact assemblages are not significantly different.

Discussion

The cladoceran assemblages of Pockwock Lake have undergone marked changes in relative abundance during the past ~150 years, largely tracking the impact of anthropogenic acidification. *Bosmina* spp. have been recognized as more disturbance tolerant than *Daphnia* spp. and can adapt quickly following environmental changes (Jiang et al. 2014; Armstrong and Kurek 2019). Most daphniids are acid-sensitive organisms, and their relative abundance in a waterbody tends to decline with acidification (Locke & Sprules 2000). Sididae is also a good acidification indicator and it tends to be more common in acidic water (Walseng et al. 2003). The most notable assemblage change that occurred is the increase in *Bosmina* spp. and the corresponding decrease in *Daphnia* spp along with an increase in Sididae. The cladoceran community exhibits low species richness, which a scarcity of acid-sensitive *Daphnia*. The data presented show that

cladoceran assemblages in Pockwock Lake have not returned to pre-impact conditions but have not responded to the chemical improvements in pH, despite the evidence of chemical water quality change in Pockwock Lake during the modern period (Anderson et al. 2017). These results align with findings from other studies, as incomplete recovery of cladoceran taxa relative to other biological groups is a trend that has been observed in other acid-impacted regions of eastern North America (Labaj et al. 2016).

Significant recovery of zooplankton, however often incomplete, have been demonstrated in lakes that reach pH > 6.0 (Gray and Arnott 2009). Even though the pH is slowly increasing in Pockwock Lake, it has not recovered to a pH greater than 6.0, suggesting that zooplankton recovery is likely to be incomplete if pH is the only factor impacting its changes. Most studies examining cladoceran assemblages show a lack of biological recovery even in chemically recovered lakes (Yan et al. 2004, Labaj et al. 2015). The list of factors limiting biological recovery is extensive, including colonist dispersal (Binks et al. 2005), DOM and UV radiation (Cooke et al. 2006), predation (Yan et al. 2004), metal toxicity (Yan et al. 2004; Labaj et al. 2015), local factors (Yan et al. 2004, Binks et al. 2005) and low Ca concentrations (Jeziorski and Smol 2016; Ross and Arnott 2021). As such, the influence of multiple stressors has likely changed Pockwock Lake from its pre-impact conditions in ways beyond those related to acidification and pH improvement (Labaj et al. 2016).

Declines in Ca concentrations in freshwater systems have been attributed to be a long-term consequence of acid deposition (Jeziorski and Smol 2016). Ecological impacts on Ca-rich members of Cladocera such as *Daphnia* spp. are severe as they are strongly affected by limited Ca in environment, development and reproduction (Giardini et al. 2015). Shapiera et al. (2011) provide strong evidence of the influence of Ca availability on cladoceran community structure.

Even though Ca-sensitive Cladocera taxa are rare even in pre-industrial sediments of most lakes, acidification and Ca decline has negatively affected *Daphnia* spp. in multiple lakes in Atlantic Canada (Korosi et al. 2013a). Mounting evidence suggests that the observed decline in *Daphnia* spp. is due to regional Ca concentration decline related to acidification and timber harvesting (Jeziorski and Smol 2016). Decreased Ca concentrations in aquatic systems have paradoxically been attributed to recovery from acidification, as base cations have been depleted within watersheds. It appears the acidification of Pockwock has negatively affected the keystone taxa Daphnia which may have greater implications for aquatic food webs in the lake. Persistent low Ca concentrations are also anticipated to impede biological recovery from acidification (Jeziorski and Smol 2016). Lake sediment bulk geochemistry from Pockwock showed decreasing Ca% concentration from ~1970 to present (Dunnington et al. 2018). *Daphnia* most likely owe their lack of recovery to the declining Ca concentrations and naturally occurring low buffering bedrock in Nova Scotia.

We document near extirpations of Ca-dependent *Daphnia* species, adding to the accumulating evidence for the threat of Ca decline in fresh water systems (Jeziorski et al. 2008). These declines in *Daphnia* species are likely exacerbated by additional stressors, including watershed disturbance and a warming climate, which may also contribute to the prevention of the recovery of the cladoceran community in Pockwock. The proliferation of *Bosmina* spp. in Pockwock may be due to earlier ice-off and stronger stratification (Nevalainen et al. 2014; Armstrong and Kurek 2019), both of which are known stressors linked to climate change. In a recent study, Armstrong and Kurek (2019) suggested that the success of *Bosmina* spp. was largely a response to indirect effects of climate warming, which potentially benefits small-bodied

filter feeders that inhabit the pelagic zone. Moreover, Locke &. Sprules (2000) also attributed the trend of increasing *Bosmina* spp. to reduced competition in the pelagic zone.

Other factors which could be contributing to the lack of biological recovery of Cladocera in Pockwock include shifts in thermal regimes (observed in lakes with changing stratification) and decreased ice cover (Smol et al. 2005), dispersal limitations and altered predation dynamics (Kurek et al. 2011).

Cladocera do show that there was a significant change in assemblages since pre-impact, as assemblages are significantly reduced relative to their pre-acidified state. The PERMANOVA test shows that the change in assemblages happened after ~1994. There was not a significant difference between the pre-impact assemblage and the acidification period. This insight supports the Ca decline argument. While acidification was being addressed by emission controls, other stressors continued affecting recovery. Due to the acidification of Pockwock, Cladocera may be more sensitive to environmental changes because of different baseline water chemistry.

Conclusion

The change in cladoceran assemblage composition identified in sediments from Pockwock suggests that a shift in structure has occurred from pre-impact to modern limnological conditions. The most prominent trends were a significant increase in relative abundance of the planktonic grazer *Bosmina* spp., a decrease in Ca-rich *Daphnia* spp., an increase in acid loving *Sididae*, and an overall decline in assemblage species richness. The cladoceran assemblages in Pockwock show evidence of response to acidification, most likely a result of regional Ca concentration decline from acidification and timber harvesting (Jeziorski and Smol 2016). Multiple stressor environments are a challenge to de-tangle when assessing casual relationships between biotic and abiotic proxies. With mounting evidence, it appears that lakes undergoing

chemical recovery may not experience complete biological recovery to pre-impact conditions. This proves to be a challenge for the management of freshwater aquatic systems and reinforces the importance of multi-proxy studies. This research aims to contribute to the understanding of ecosystem change and provide useful information for future environmental inferences. The cladoceran assemblages provide a snapshot of a single bioindicator in Pockwock. To further understand the biological response to chemical water quality change in Pockwock, we also assessed the diatom assemblage record.

Diatoms

To evaluate the biological response of diatom assemblage to acidification, this study uses paleolimnological techniques and DI-pH inference models to observe assemblage changes during three defined periods, pre-impact, acidification, and modern. The sedimentary diatom assemblage is species rich, with ~175 distinct diatom taxa identified. The 11 most common taxa are plotted as histograms (Figure 9). Prior to anthropogenic acidification in Pockwock, *Asterionella ralfsii v americana*, *Tabellaria flocculosa var flocculosa strain IIIp* and *Discostella stelligera* dominated the diatom assemblage.

The diatom bloom which occurred in Pockwock Lake in summer of 2018, clogging water treatment filters is unmistakable in the paleo record. The relative abundance of *Asterionella ralfsli v americana* increased from an average of 25% throughout the sediment record to as much as 68% when it bloomed in modern sediments (~4 years, 2015 to 2018). *Tabellaria flocculosa var flocculosa strain IIIp* also increased from an average of 26% throughout the sediment record to as much as 52% in modern sediments. These low pH optima species also made up most of the relative abundance during the acidification period from ~1940 to ~1990. These species both begin to decrease during the noted chemical water quality change period (~1994 to 2018; 7 – 0

cm), while acid loving *Fragilaria acidobiontica* increased in abundance to 15%. However, as the bloom of planktonic *Asterionella ralfsli v americana* and *Tabellaria flocculosa var flocculosa strain IIIp* occurs, *Fragilaria acidobiontica* decreases to a relative abundance of 3% in the modern sediments. *Eunotia incisa* also dominates in relative abundance while *Asterionella ralfsli v americana and Tabellaria flocculosa var flocculosa strain IIIp* are decreasing just before they bloom during the modern period. We observe a steady decline in *Discostella stelligera* from the bottom to the top of the core. *No Discostella stelligera* is observed in the top 1 cm of the core (~4 years). The top three most abundant diatom species assemblages representing the top of the core (1 – 0 cm, ~2014 to 2018) include *Asterionella ralfsii v americana* (35%), *Tabellaria flocculosa var flocculosa strain IIIp* (52%), and *Fragilaria acidobiontica* (4%). Compared to pre-impact conditions (prior to ~1900), the same species represented 23%, 23% and less than 1%.

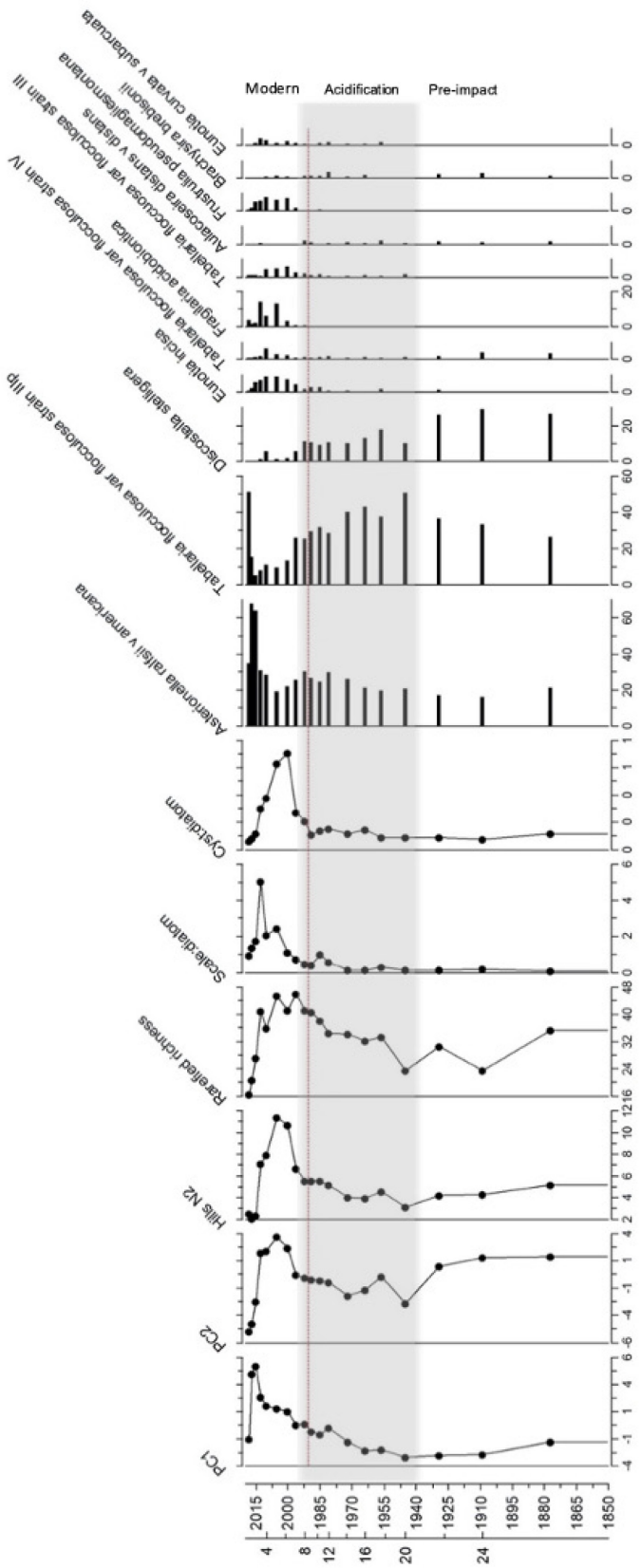

Figure 9: Relative percent abundance of dominant diatom taxa in Pockwock Lake

The DI-pH model was highly significant (r^2 = 0.82; RMSE = 0.49). Pockwock showed a decrease (range ~ -0.03 to -1.2 pH units) in diatom-inferred pH (Figure 10). The mean diatom inferred background pH for Pockwock Lake is ~5.5 Constant with the decline, diatom assemblages exhibited an increase in relative abundance of acidophilous *Fragilariforma acidobiontica.* Species diversity of diatoms also decline markedly with the Hill's N2 index, from ~11 to 1. We do not see an increase in the relative abundance of acidophilous *Fragilariforma acidobiontica* (pH optima ~4.9) during the defined acidification period, but we do during the modern period (~1995 to 2018; 7 cm – 0 cm). This is of interest because the two main diatoms of interest that clogged the filters at the JDKWSP were *Asterionella ralfsii v americana* and *Tabellaria flocculosa var flocculosa strain IIIp.*

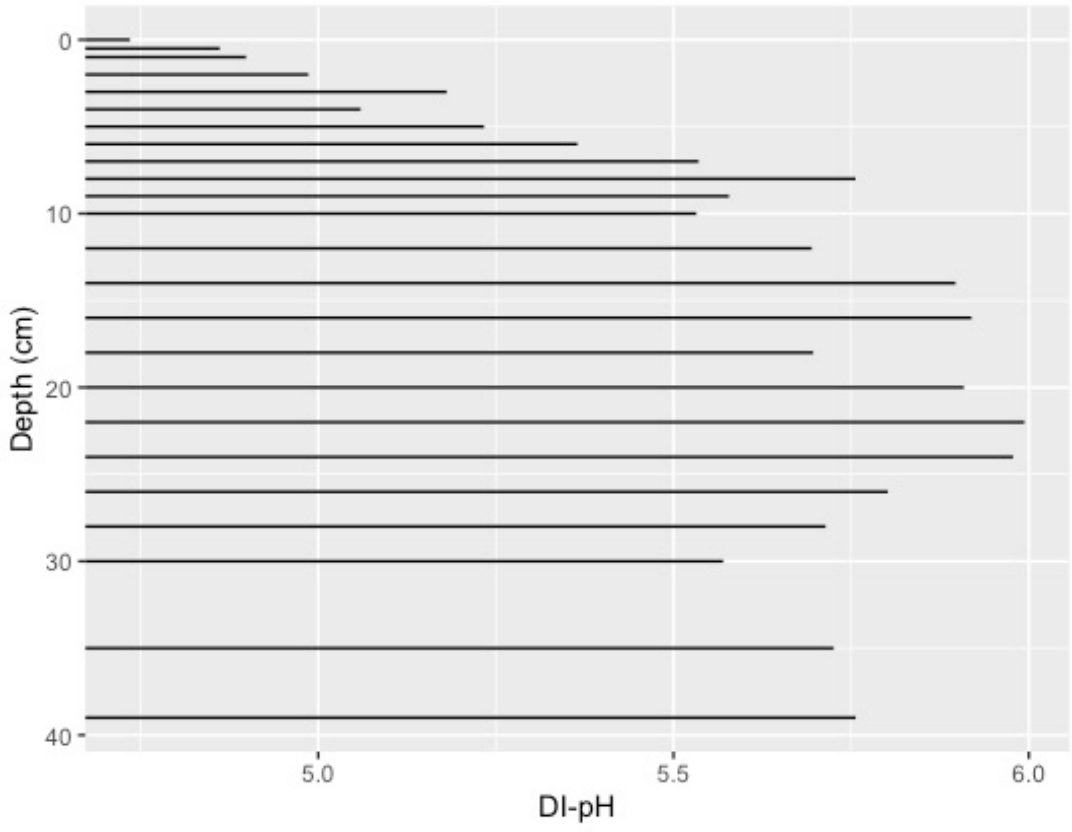

Figure 10: Diatom inferred pH in Pockwock Lake

Discussion

Comparable to Tropea et al. (2007), POC-18-1 core shows two distinct changes in diatom assemblages that resulted in marked decreases in diatom-inferred pH, or evidence of two distinct acidification periods. Between ~1850 and ~1940, the diatom assemblage was dominated by *Asterionella ralfsii v americana*, *Tabellaria flocculosa var flocculosa strain IIIp* and *Fragilaria acidobiontica*, also the same as Tropea et al. (2007), however, our results indicate a lower DI-pH compared to the ~ 6.3 Tropea et al. (2007) presents. The first major sign of acidification is the loss of planktonic diatoms at pH values between 5.5 and 5.8 (Battarbee et al. 1984).

Interestingly, the two distinct acidification trends observed by Tropea et al. (2007), usually occur in chemically different lake conditions. The first trend (~1940 to ~1992) follows that of humic (high-DOC) lakes, and the second trend (post ~1992) follows that of clearwater low-DOC lakes. Tropea et al. (2007) concluded the acidification signal observed in Pockwock likely indicates a loss of DOC and the sudden drop in DI-pH suggests the weak buffering system of humic DOC had been exceeded. With the POC-18-1 core representing 16 additional years of sediment deposition since Tropea et al. (2007), we now see a third acidification trend in the lake. It is dominated by the blooming *Asterionella ralfsii var. americana* and *T. flocculosa strain III.* Both species with a low pH and high DOC optima. This diatom assemblage is similar to pre-impact conditions, however both dominating species have a higher relative abundance and there is no presence of *Discostella stelligera*. The trend of *Discostella stelligera* being notably absent from acidic lakes (pH < or equal to 5.4) which we see in Pockwock, is consistent with other studies showing the loss of this genus at low pH (Battarbee et al. 1999; Barrow et al. 2014).

Stratification and ice changes are linked to increases in planktonic diatoms (Rühland et al. 2015). The modern-day diatom assemblages in Pockwock contain higher relative abundances of planktonic diatoms with lower silicified and benthic taxa, suggesting shifts consistent with changes stratification (thermal stability) and ice-cover changes as a result of warming temperatures (Barrow et al. 2014; Rühland et al. 2015). Diatom blooms in Pockwock co-occur with a period of increasing (but still relatively low) pH, increasing DOC, most likely more stable stratification, most likely decreased duration of ice cover. Therefore, the diatoms that are competitive in stable/less ice conditions must also be diatoms that can tolerate relatively low pH and increasing DOC. Both *Asterionella ralfsii v americana* and *Tabellaria flocculosa var flocculosa strain IIIp* fit this description and we see their bloom in the sediment record.

Core POC-18-1 shows an acidification signal that is similar to those observed in other lakes in southwestern Nova Scotia, with low diatom-inferred background pH (Ginn et al. 2007; Cao et al. 2014). The change in diatom assemblage to dominance by *Asterionella ralfsii* and *Tabellaria flocculosa* is similar to that observed in other acidification-impacted lakes in Nova Scotia which have a naturally acidic background pH (Ginn et al. 2007c, 2007c).

Diatom assemblages from the modern sediments at the top of the core contain higher relative abundances of planktonic diatom taxa and lower relative abundances or heavily silicified diatoms and benthic taxa. Rühland et al. (2015) show that unparalleled warming has resulted in widespread increases in planktonic diatoms across the northern hemisphere. The limnological context dictates which species respond to changing climatic conditions. The diatoms assemblage shifts in Pockwock suggest that the diatoms blooms we see in Pockwock are climate-mediated alterations due to changes in water column properties, stratification and shifts in ice cover (Rühland et al. 2015).

Post-1995 declines in the relative abundance of diatom taxa with low pH-optima and increases in taxa with higher pH-optima indicate biological recovery in a sediment core analyzed from Big Moose Lake in the Adirondacks, New York, USA (Arseneau et al. 2011).

Lake water pH is an important limnological variable governing the distribution of diatom taxa and driving ecological change in this region, however not the only one. Seeing low pH optima diatoms blooming during increases in pH suggests other environmental stressors such as regional climate warming are responsible for the shifts in diatom assemblages. The use of diatom-inferred pH from the diatom assemblages provides an indication of what the important stressors are. To aid in the examination, past pH values were inferred based on a diatom-inference model from a modern diatom dataset of 494 lakes (Ginn et al. 2007b). The shifts in diatom assemblages and changes in diatom-inferred pH that we see in Pockwock up to 2002 are indicative of acidification.

Multiple stressors have likely affected the trajectory of biological response in Pockwock. The marked difference in composition between modern and pre-impact diatom assemblages suggests this as complete diatom species recovery is not observed. A higher scale:diatom value index suggests the regional warming has influenced the observed algal re-organization (Sivarajah et al. 2017).

Conclusion

Our results indicate that diatom species composition have changed since pre-impact conditions. Unlike Cladocera, we are seeing a response and change in the diatom assemblage because of chemical water quality change due to acidification in Pockwock. The two blooming diatom species, *Asterionella ralfsii* and *Tabellaria flocculosa*, are both greater than their relative abundances prior to impact. D*iscostella stelligera* appears to not be showing any sign of return to

pre-impact relative abundance numbers. We are not seeing complete biological assemblage recovery to pre-impact conditions, but a partial return, consistent with other diatom studies.

Chlorophyl a and TOC

In addition to the Cladocera and diatom assemblage data, two other proxies, VNIRS-inferred chlorophyll *a* and TOC were used. Our paleolimnological findings demonstrate a decline in VNIRS-inferred Chl *a* concentration coincident with the acidification trends and then increase again as thermal stability/ice cover changes take effect in more recent decades. Chl *a* concentration remained stable during the pre-impact period (Figure 11). Levels declined and remained consistently low throughout the acidification period. With the onset of the post-acidification period, Chl *a* increased back to a level of ~.018 $mg \cdot g^{-1}$ dwt and appears to have an increasing trend with one outlier at ~0.035 $mg \cdot g^{-1}$ dwt.

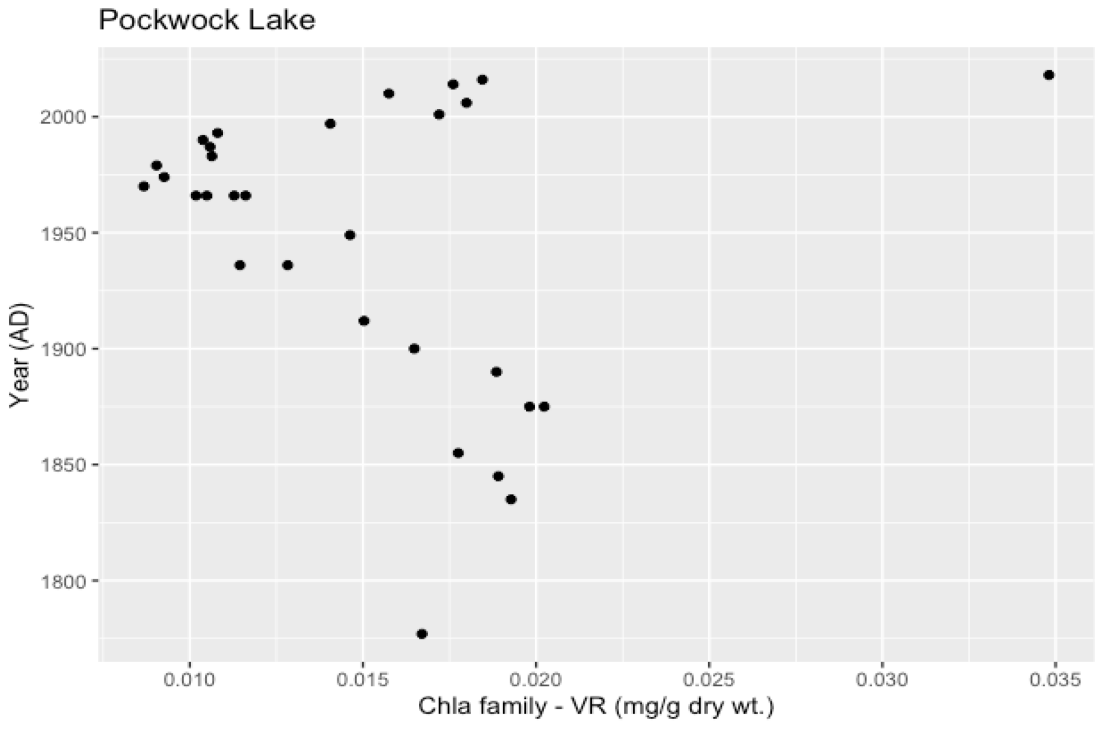

Figure 11: Spectrally inferred Chlorophyl *a* in Pockwock

VNRIS-inferred Chl *a* was used to reconstruct primary aquatic production (Michelutti et al. 2010). We observe increases in chlorophyll in the sediment record which reflect an increase in phytoplankton abundance. A pronounced increase in spectrally inferred chlorophyll *a* concentration in Pockwock after 2000 is observed. Michelutti et al. (2005) infer primary production increases as a result of climate warming.

Similar trends for spectrally inferred TOC are observed (Figure 12). Stable concentration pre-impact, followed be a steady decline during the acidification period, below 8 mg L-1, then increase back to during the recovery period.

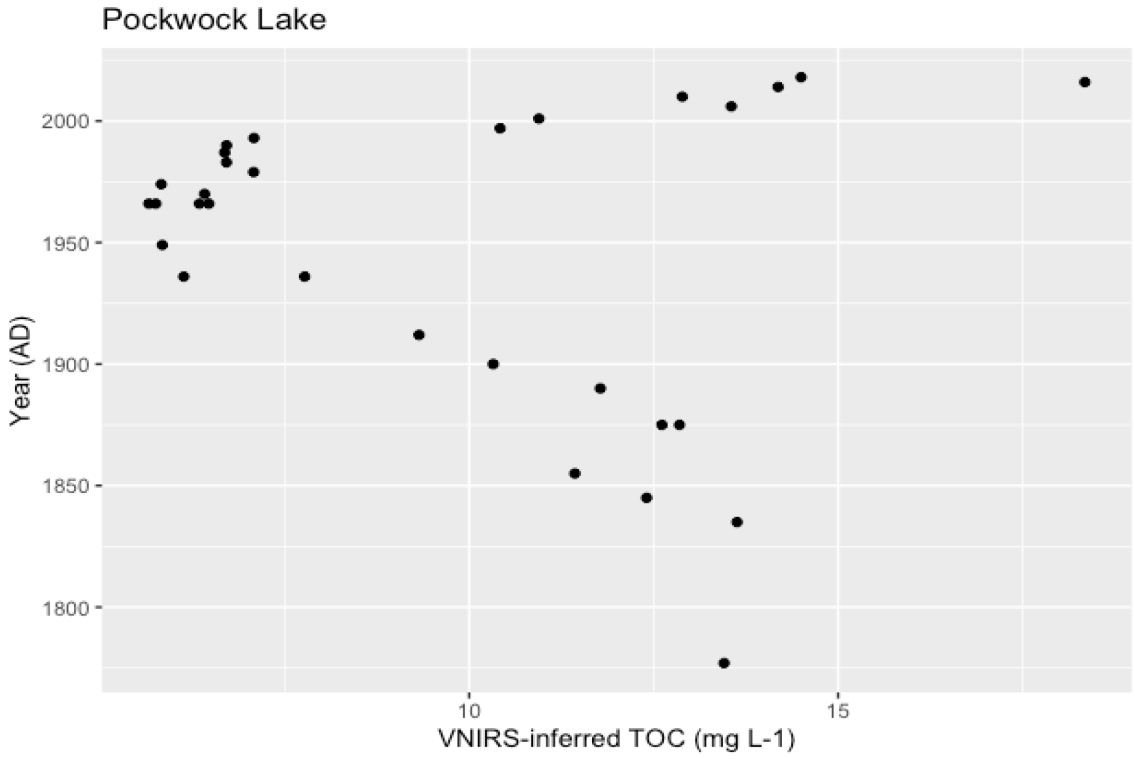

Figure 12: Spectrally inferred TOC in Pockwock

Sediment-inferred TOC dynamics examined by Meyer-Jacob et al. (2017), closely follow changes in sulfate deposition. They suggest that the observed TOC increase is a response to reduced acid deposition. Inferred TOC concentrations in Pockwock suggest that TOC has

returned to and even possibly exceeded pre-impact levels. The VNRIS-inferred TOC for Pockwock adds to the growing evidence that Pockwock is chemically recovering from anthropogenic acid deposition due to reductions in sulphate emissions.

Discussion

Figure 12 shows the changing TOC levels in Pockwock Lake and allows for the interpretation of the trajectory of where TOC levels may be headed. The JDKWSP was designed and built when water quality looked very different than it does today. In forty years, the water quality has changed in Pockwock Lake so significantly that the plant has reached its threshold for treating the water from Pockwock Lake. What was once a clear, low TOC water has now become coloured with more organics present, which must be removed for treatment. The JDKWSP needs to be retrofitted and upgraded.

Conclusion

VNIRS-inferred chlorophyl *a* and TOC results show a chemical and biological return to and exceedance of pre-impact conditions, suggesting that unprecedented water quality trends have begun to occur. Only having one data point significantly higher than the rest suggests that TOC trends could be increasing beyond levels that have ever been experienced before in Pockwock Lake and more exploration of sediment should take place to have a larger data set to make future predictions and to track climate driven changes.

CHAPTER 4.0: THE MICROBIOME (EDNA)

Preamble

In this study, we investigate the lake sediment microbiome and diversity of two lakes in the Pockwock Watershed. Sediment cores collected from Pockwock and Island Lake were collected and sectioned at 1-cm increments. Taxonomic differences in the sediment microbiome were compared through profiling different regions of the 16S rRNA gene of bacteria and the 18S rRNA gene of eukaryotes using eDNA metabarcoding and high-throughput sequencing.

Environmental DNA metabarcoding

This study used a custom and streamlined workflow for microbiome research (Comeau et al. 2017). We analyzed the 16S rRNA gene using primers targeting the V3-V4 region. Sequencing data was extracted from 12 sediment depths in each core. eDNA was extracted from the first 10 cm of each core, with two replicate samples from the top (0 cm) and from the middle (5 cm). Some samples had to be discarded from further analysis due to failed or low sequencing depth. All twelve 16S samples from Pockwock Lake returned weak/failed results. Pockwock Lake returned two 18S weak/failed samples, POCK5-6R and POCK9-10. Island Lake returned three 16S weak/failed samples, IL6-7, IL 8-9 and IL 9-10 and three 18S weak/failed samples, IL6-7, IL 8-9 and IL 9-10.

The quantity and quality of DNA extracted sediments were found to meet the requirements for reconstruction of the micro-eukaryotic and bacteriome diversity (Table 4 and Table 5) required by IMR. Concentrations of extracted eDNA ranged from 1.84 ng/µL to 6.01 ng/µL for Pockwock Lake and 1.78 ng/µL to 29.32 ng/µL for Island Lake.

Table 4: Pockwock Lake nucleic acid and protein concentrations from microplate reader

Sample Name	Depth of Core (cm)	Sample Concentrations (ng/µL)	16S	18S
POCK0-1	0-1	4.019	Failed	
POCK0-1R	0-1	7.498	Failed	
POCK1-2	1-2	6.008	Failed	
POCK2-3	2-3	4.645	Failed	
POCK3-4	3-4	3.277	Failed	
POCK4-5	4-5	2.474	Failed	
POCK5-6	5-6	2.151	Failed	
POCK5-6R	5-6	2.463	Failed	Failed
POCK6-7	6-7	2.072	Failed	
POCK7-8	7-8	1.881	Failed	
POCK8-9	8-9	1.843	Failed	
POCK9-10	9-10	2.539	Failed	Weak

Table 5: Island Lake nucleic acid and protein concentrations from microplate reader

Sample Name	Depth of Core (cm)	Sample Concentrations (ng/µL)	16S	18S
IL0-1	0-1	28.089		
IL0-1R	0-1	29.321		
IL1-2	1-2	28.630		
IL2-3	2-3	19.177		
IL3-4	3-4	14.075		
IL4-5	4-5	11.119		
IL5-6	5-6	9.488		
IL5-6R	5-6	2.938		
IL6-7	6-7	4.670	Failed	Failed
IL7-8	7-8	1.789		
IL8-9	8-9	2.815	Failed	Failed
IL9-10	9-10	2.106	Failed	Failed

POCKWOCK LAKE

The original intent of this study was to compare eDNA of eukaryotic microbes and prokaryotic cyanobacteria among both lakes. All 16S rRNA samples from Pockwock failed the sequencing step at IMR. Therefore, there is no 16S alpha or beta diversity data for Pockwock Lake.

Pockwock's 18S eukaryotic eDNA fingerprint consists of 46.7% protozoa, 25.5% metazoan, and 15.1% fungal phylogenies. Of the 46.7% protozoa, 14.8% were Ciliophora

(flagellated phagocytic protozoa), 12.4 % dinoflagellates, 10.3% Ochrophyta (red algae), and 6.5% Circozoa (single-celled phagics). Of the 25.5% metazoan, 11.2% are copepods, 3.8% Rotifera, and 1.5% Gastrotricha. A significant amount of worms are present, 2.2% Annelida (earthworms), 1.4% Nematoda (roundworms), and 0.8% Platyhlminthes (flatworms). Fungi in Pockwock constitutes 5.8% Chytridomycota, 4.9% Cryptomycota, and 4.4% Ascomcota.

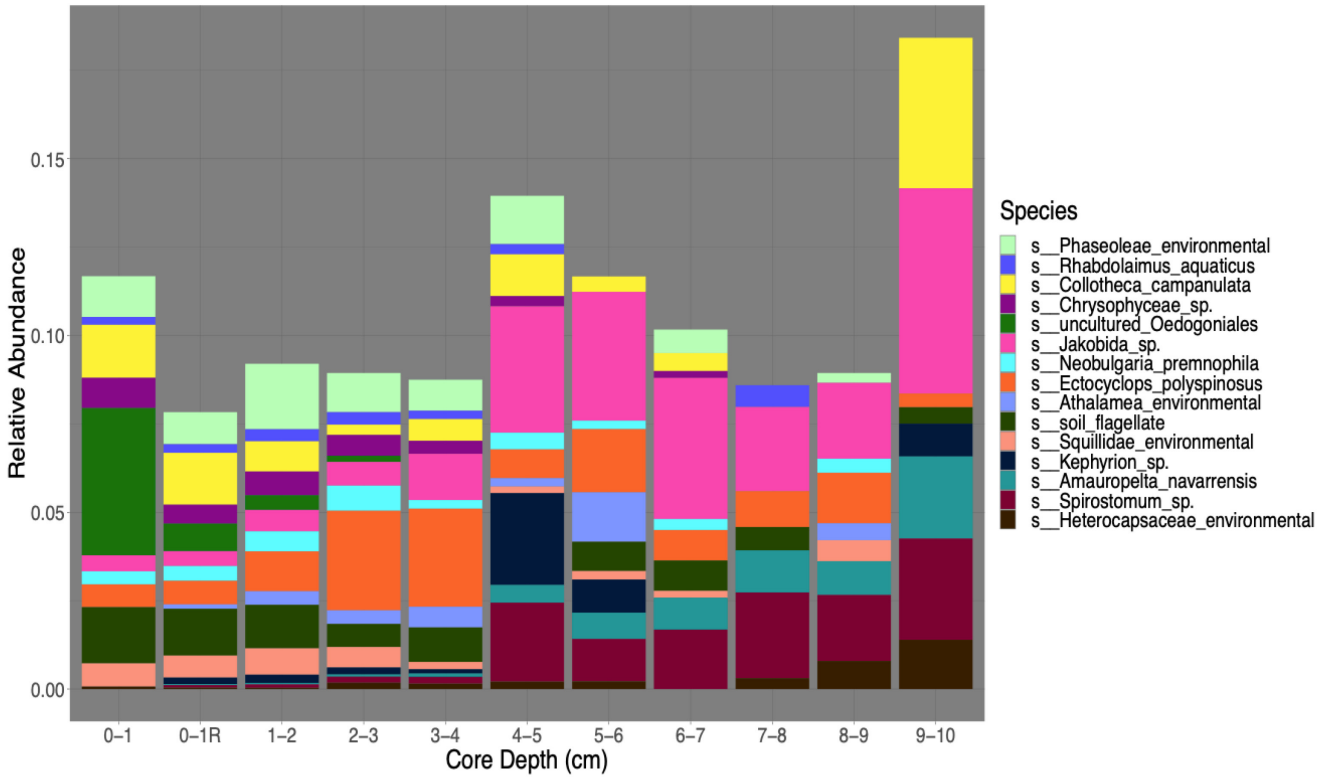

Figure 13: Pockwock Lake 18S Ribosomal ribonucleic acid (rRNA) most abundant species

The taxonomic histogram (Figure 13) shows the most abundant 10% of species in Pockwock. Both Spirstomum sp. and Jakobid sp. appear to decline in relative abundance as you move from the bottom 10 cm to the top 0 cm.

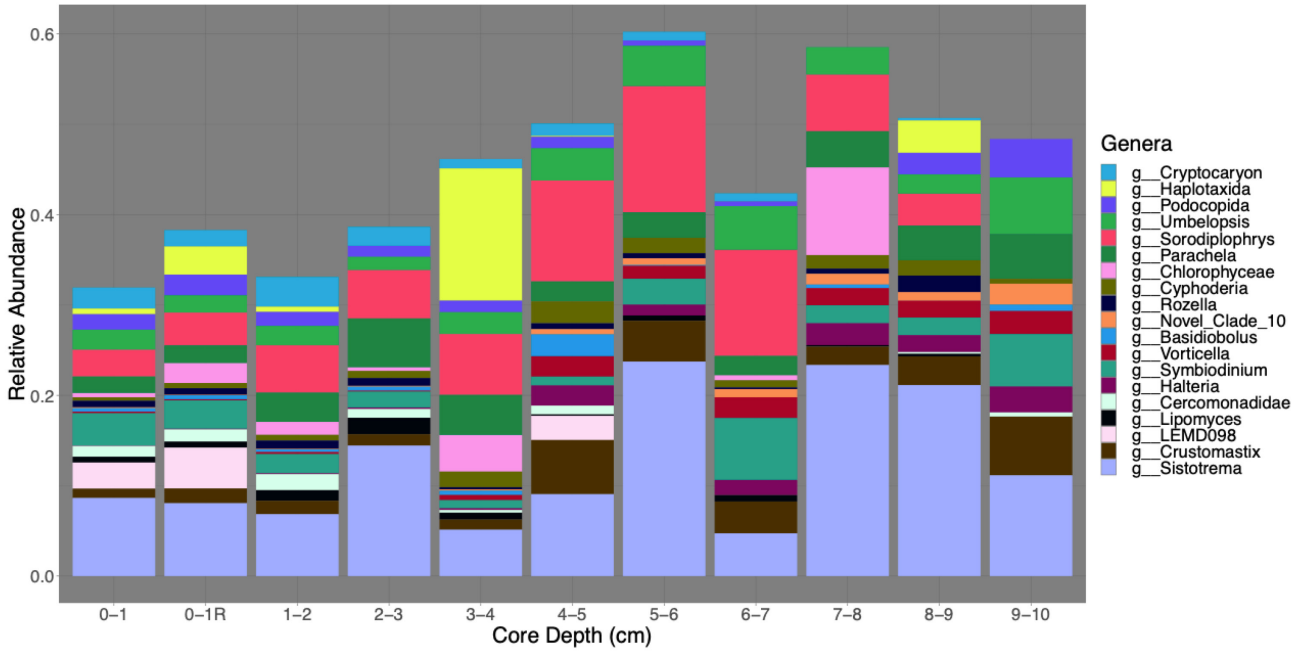

Figure 14: Pockwock Lake 18S rRNA most abundant genera

The taxonomic histogram (Figure 14) shows the 10% most abundant gene assigned genera in Pockwock. Both Sorodiplophrys and Crustomastix appear to decline in relative abundance as you move from the bottom 10 cm to the top 0 cm. The general trends show that the bottom, middle and top of the 10 cm examined are quite different in species and genera occurrence. To fully try and capture differences in depth intervals, alpha and beta diversity were examined.

Alpha Diversity

When looking at the alpha diversity, the sediment samples were group into three groups. These groups are 0-3 cm, 3-6 cm and 6-10 cm (Figure 15), or Top, Middle and Bottom of the 10 cm that was used for eDNA metabarcoding.

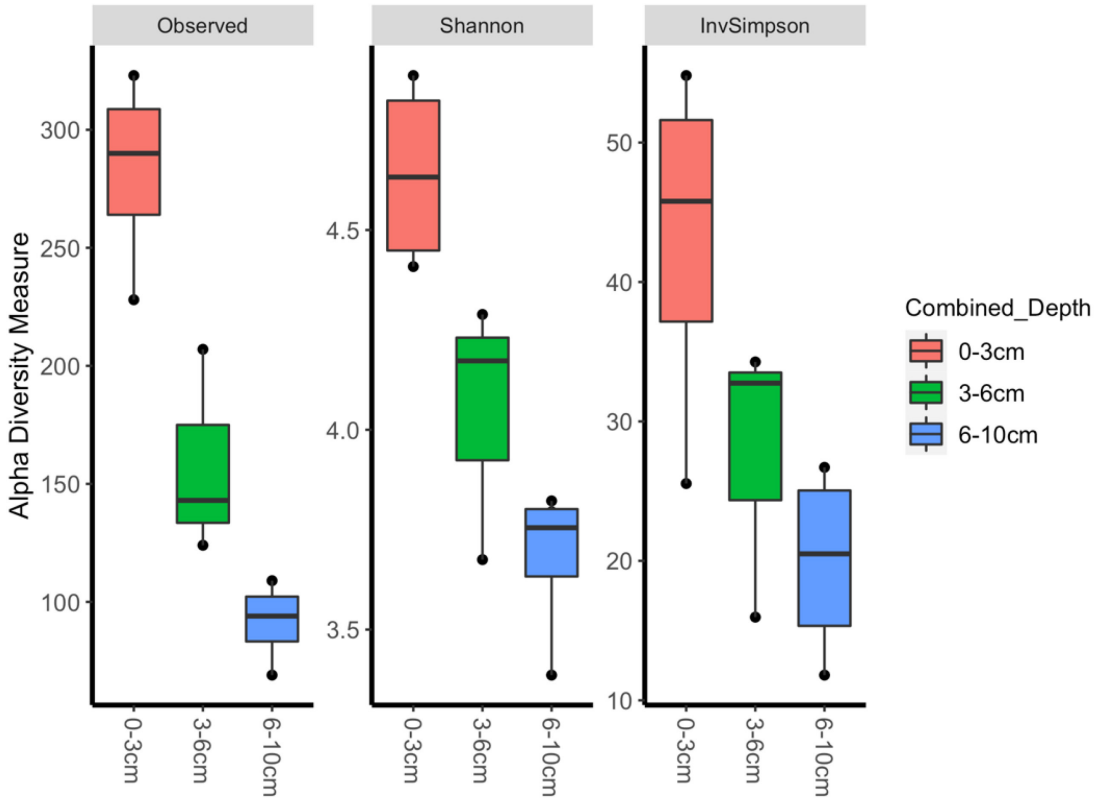

Figure 15: Alpha diversity of 18S rRNA ASVs in Pockwock Lake

We see a consistent increase of diversity from the bottom, 6 – 10 cm to the top, 0 – 3 cm. Observed, Shannon and Inverse Simpson all show the same trend with a greater alpha diversity in the top of the core, showing greater species richness in the top sediments.

Beta Diversity

UniFrac, the phylogenetic distance measure, was used to determine which of the microbial communities represented by the 12 different samples were significantly different. The results show biologically meaningful patterns that reveal striking features of the microbial communities in Pockwock Lake. To understand how the sediment depths related to one another, we used the Unweighted UniFrac distance matrix to perform PCoA (Figure 16). Axis.1 shows a percent variation of 25.4% and Axis.2, 11.4%.

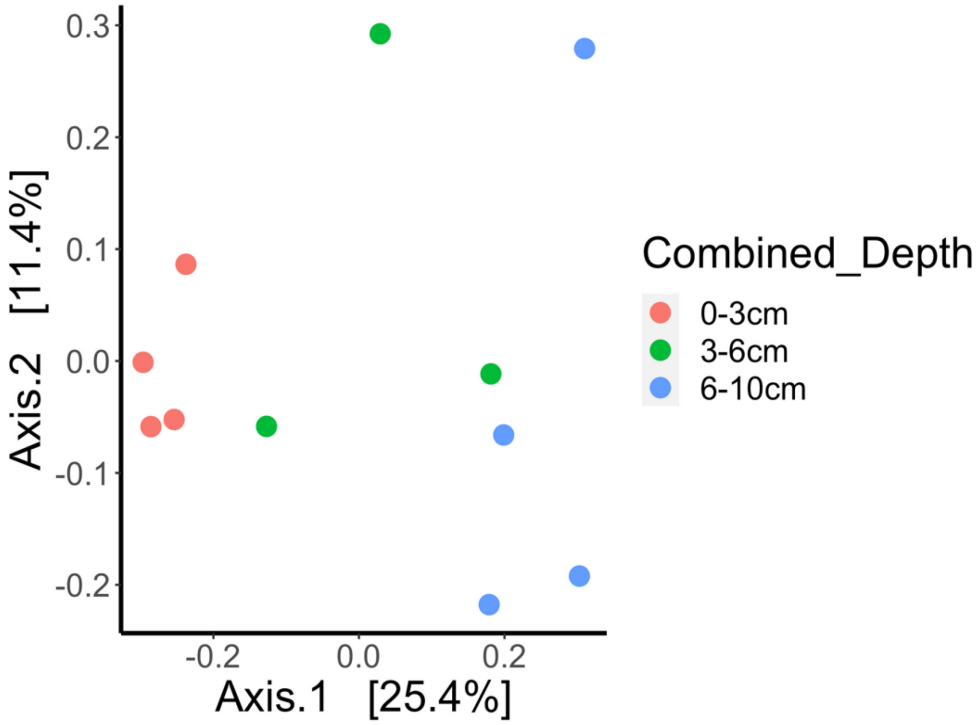

Figure 16: PCoA/MDS with unweighted UniFrac of the 18S gene in Pockwock Lake

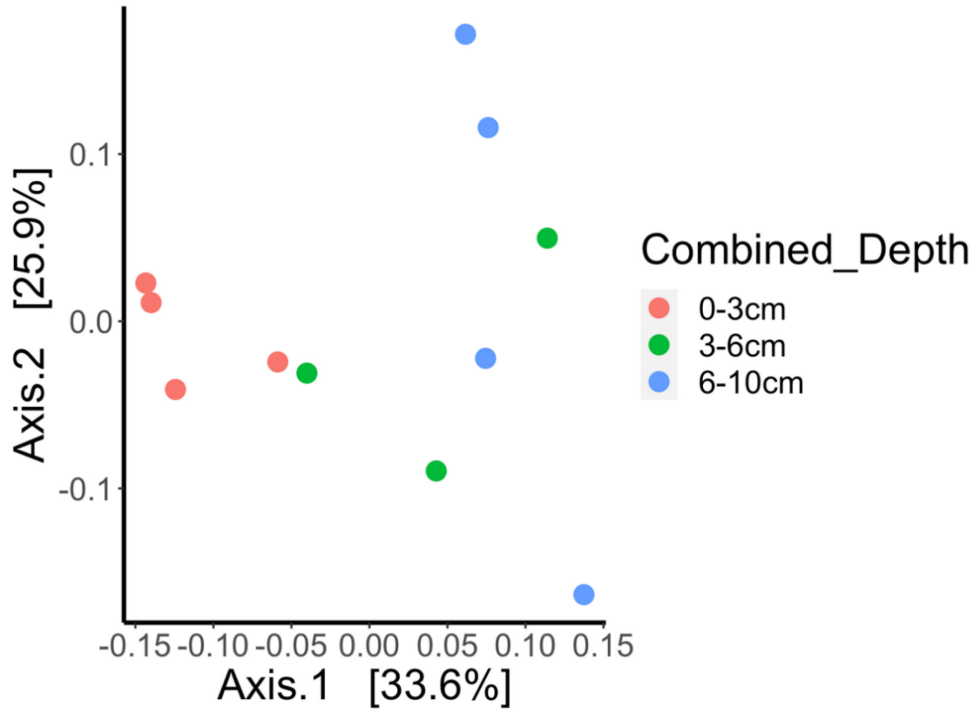

Figure 17: PCoA/MDS with weighted UniFrac of the 18S gene in Pockwock Lake.

The weighted UniFrac (Figure 17) shows the phylogenetic differences according to the abundance of each lineage (Lozupone and Knight 2005). Axis.1 shows a percent variation of 33.6% and Axis.2 25.9%.

ISLAND LAKE

Island Lake's 18S eukaryotic eDNA fingerprint consists of 49% protozoa, 42.6.% metazoan, and 5.1% fungal phylogenies. Of the 49% protozoa, 19.8% were Ciliophora (flagellated phagocytic protozoa), 7.0 % dinoflagellates, 4.4% Ochrophyta (red algae), and 4.8% Circozoa (single-celled phagics). Metazoans are dominated in Island Lake by a unique bloom of Protalveolata (unicellular myzozoan). Of the 42.6% metazoan, 29.7% are the Protalveolata, 5.2% are copepods, 3.7% Rotifera, and 0.3% Gastrotricha. Annelida (earthworms) are not present in Island Lake, while it contains 2.8% Nematoda (roundworms), and 0.6% Platyhlminthes (flatworms). There are no mollusk species in significant number in Island Lake, Walker's Pea Clam is notably absent. Fungi in Island Lake constitutes 3.8% Chytridomycota, 0% Cryptomycota, and 0.5% Ascomcota.

18S rRNA

The taxonomic histogram (Figure 14) shows the 10% most abundant gene assigned genera in Island Lake. The most abundant A31 and Paramicrosporidium both decline in relative abundance significantly as you move from the bottom 10 cm to the top 0 cm. Similarly, to Pockwock, the general trends show that the bottom and top of the 10 cm examined are quite different in genera occurrence.

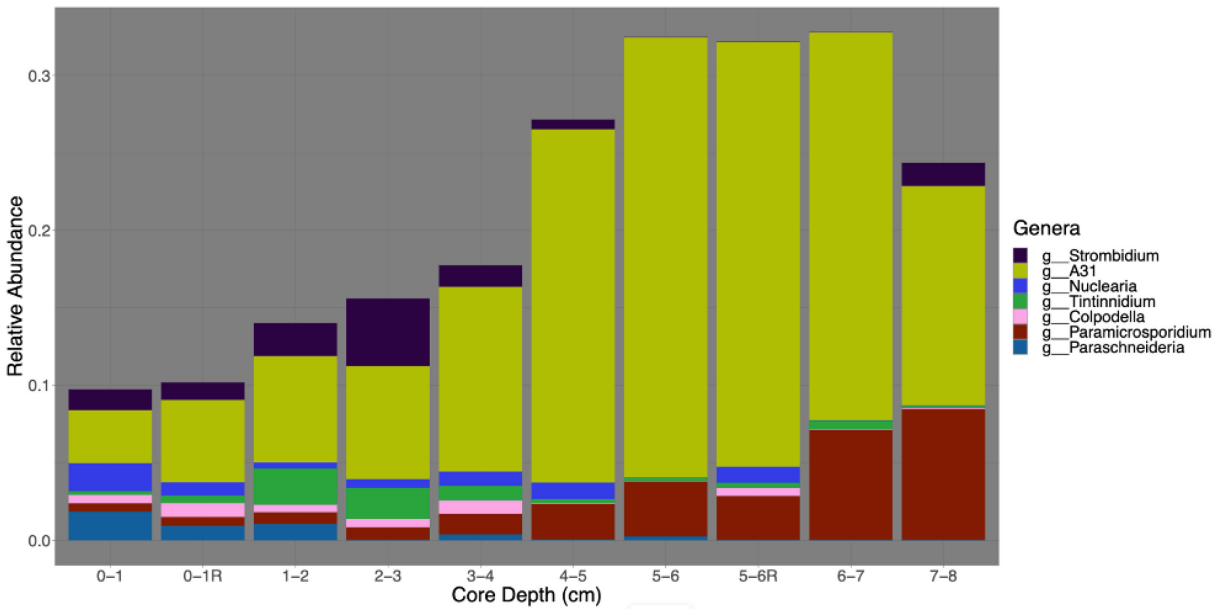

Figure 18: Island Lake 18S rRNA most abundant genera

We see a consistent increase in alpha diversity from the bottom, 6 – 10 cm to the top, 0 – 3 cm (Figure 18). Observed and Shannon show the same trend with a greater alpha diversity in the top of the core, showing greater species richness in the top sediments.

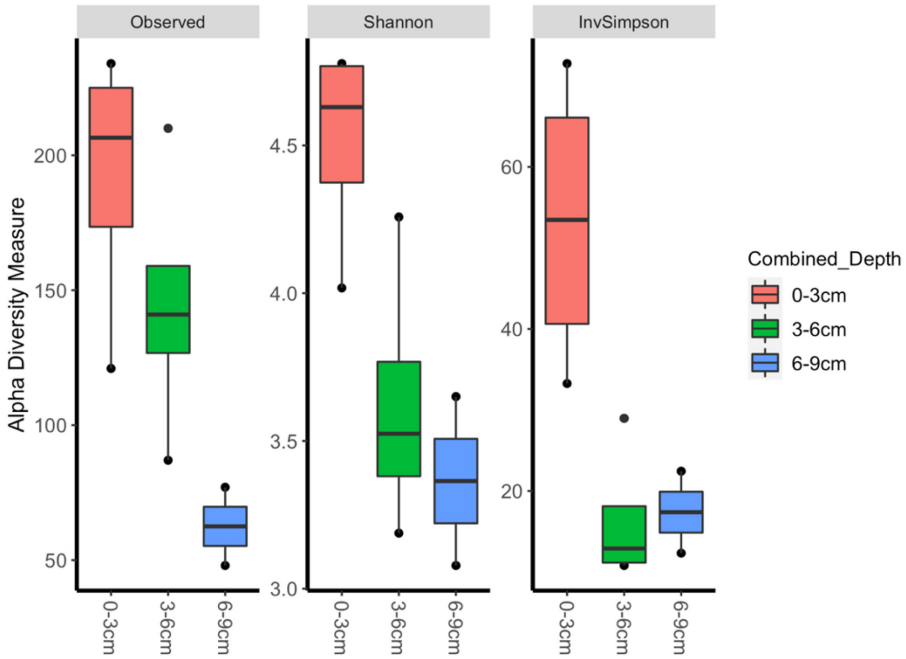

Figure 19: Alpha diversity of 18S rRNA ASVs in Island Lake

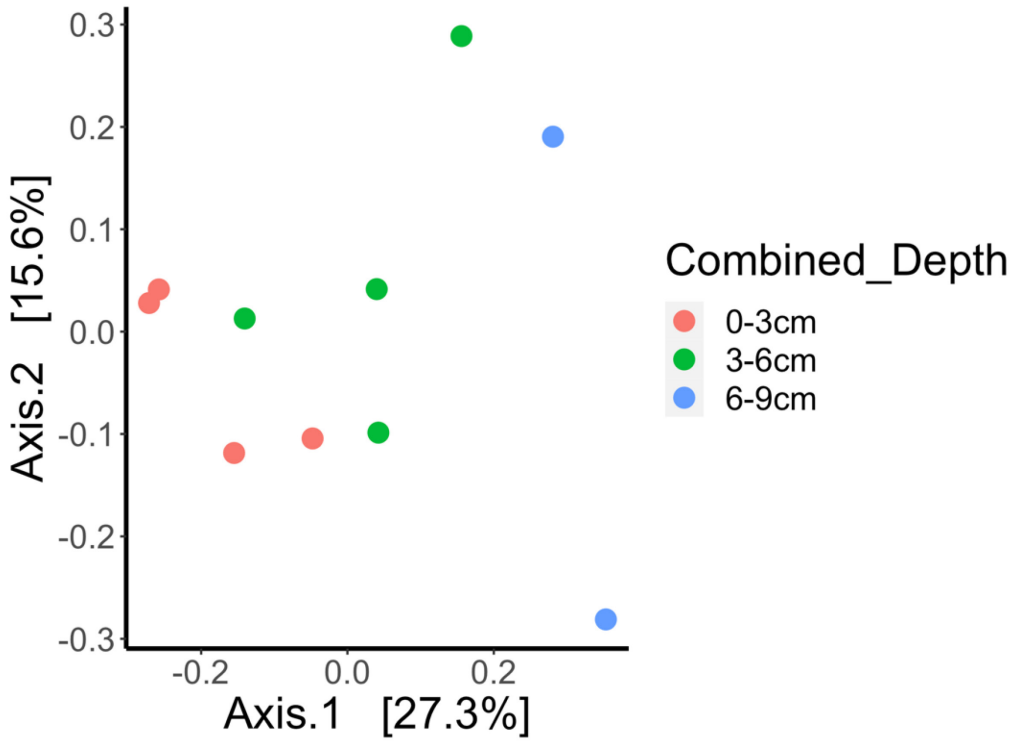

Figure 20: PCoA/MDS with unweighted UniFrac of the 18S gene in Island Lake

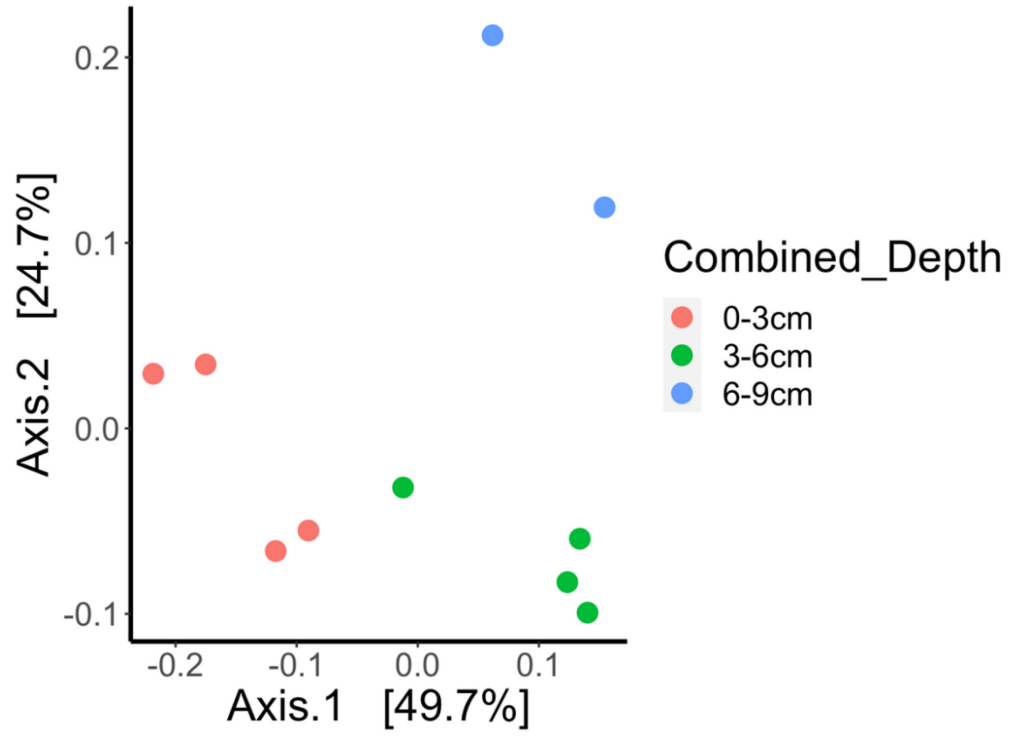

Figure 21: PCoA/MDS with weighted UniFrac of the 18S gene in Island Lake

Figure 20 shows ordination of community points by PCoA with the unweighted UniFrac metric on the 18S gene from Island Lake. Axis.1 shows a percent variation of 27.3% and Axis.2, 15.6%. The weighted UniFrac (Figure 21) shows the phylogenetic differences according to the abundance of each lineage (Lozupone and Knight 2005). Axis.1 shows a percent variation of 49.7% and Axis.2 24.7%.

16S rRNA

The three phyla that constitutes the dominant 81.8% of detected amplicons are Cyanobacteria, Chloroflexi, and Actinobacteriota. Island Lake's 16S prokaryotic eDNA fingerprint consists of 47.7% Cyanobacteria with 13 species.

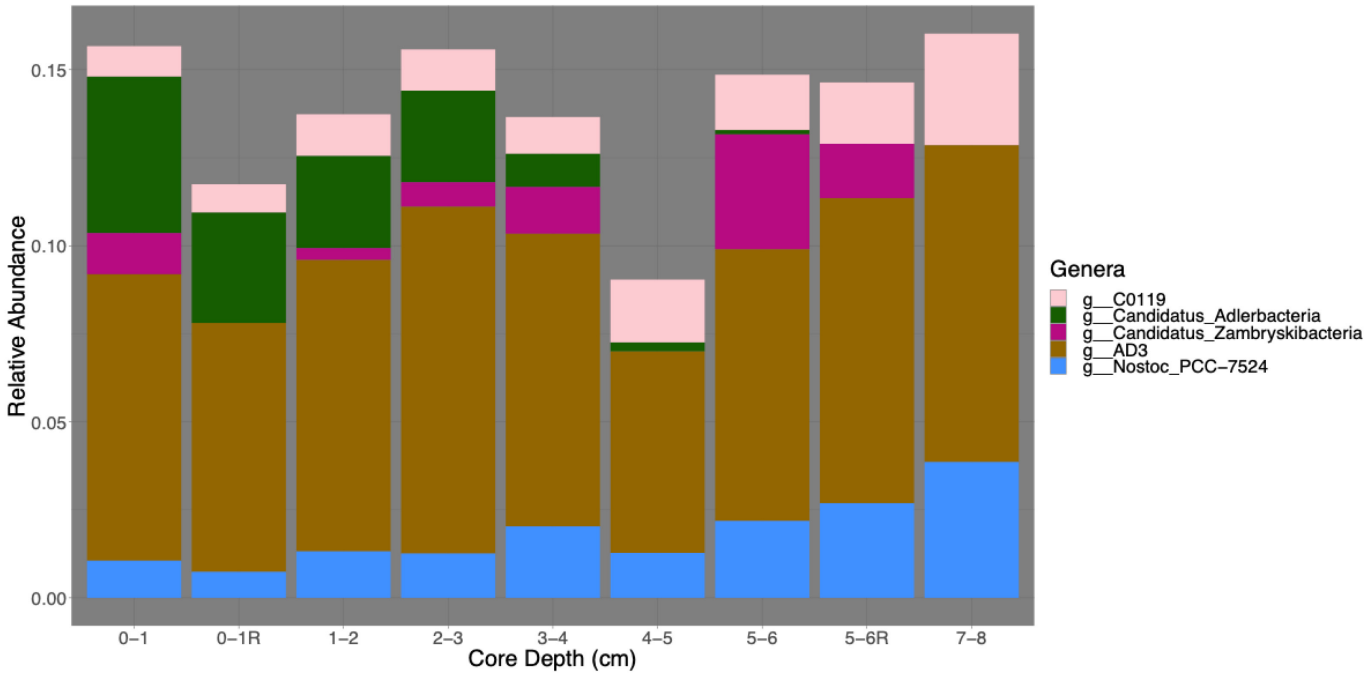

Figure 22: Island Lake 16S rRNA most abundant genera

As there is no 16S data for Pockwock, we cannot compare the alpha diversity between the two lakes. For Island Lake, it appears that the middle of the examined section (3 – 6 cm) is more diverse compared to the top and bottom sections (Figure 22). The bottom (6 – 9 cm) was

also lacking more information because three of the samples were weak/failed and never added to the analysis.

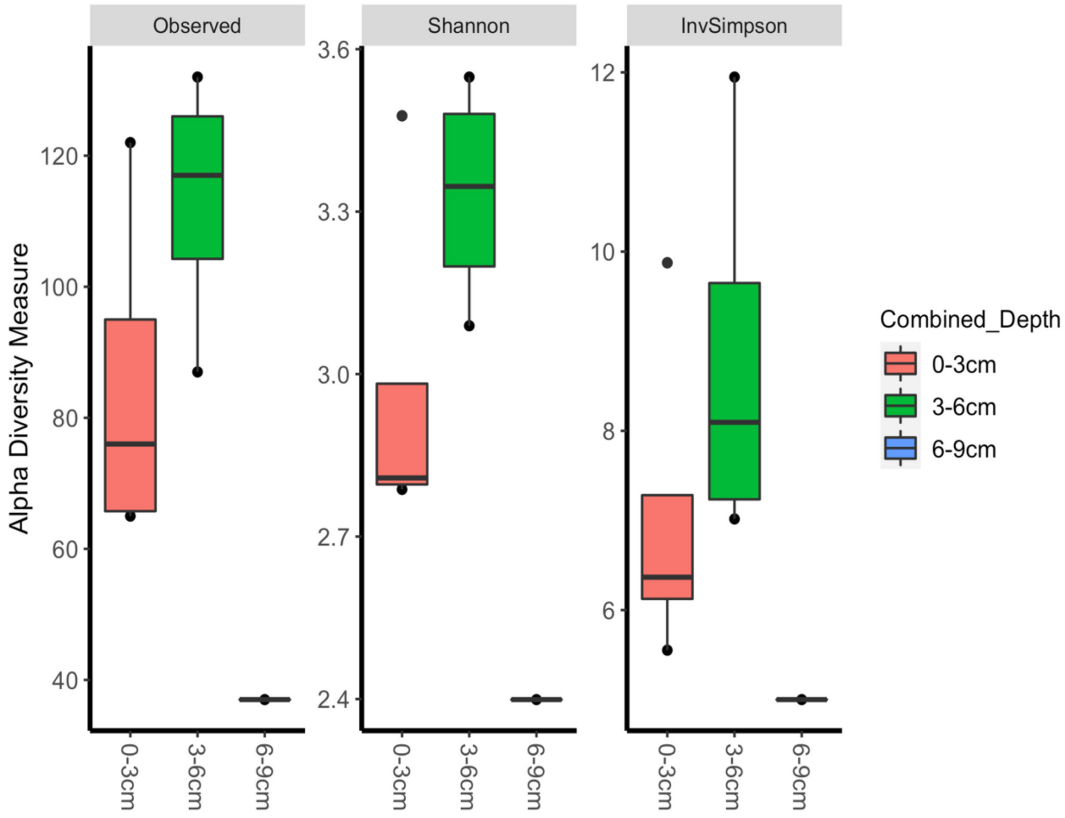

Figure 23: Alpha diversity of 16S rRNA ASVs in Island Lake

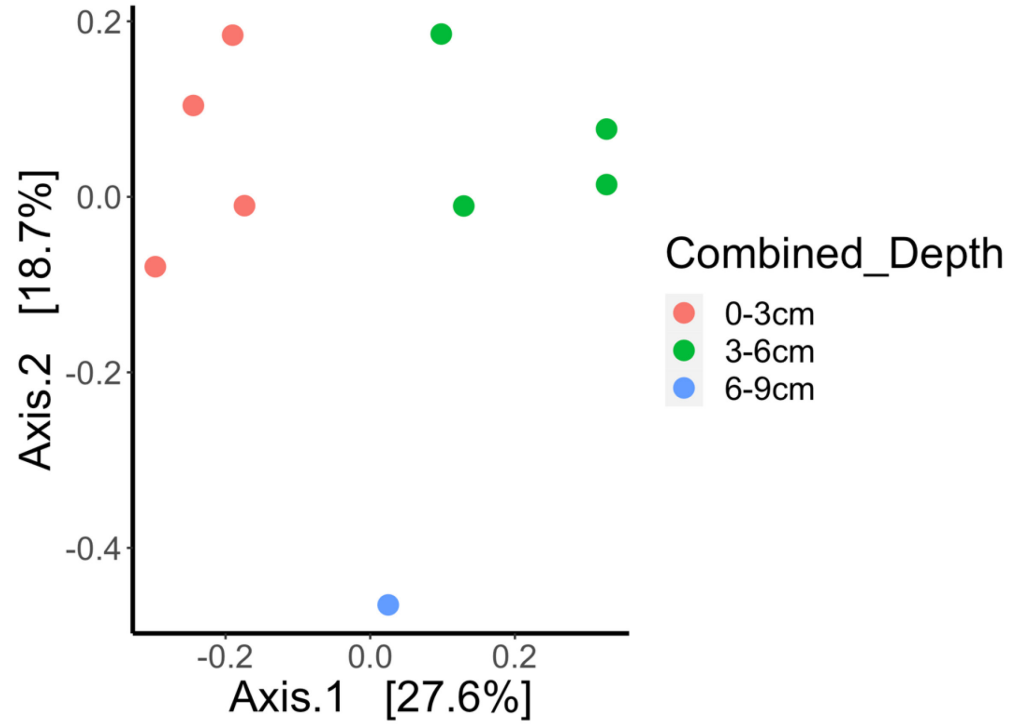

Figure 24*:* 16S unweighted UniFrac beta diversity Island Lake

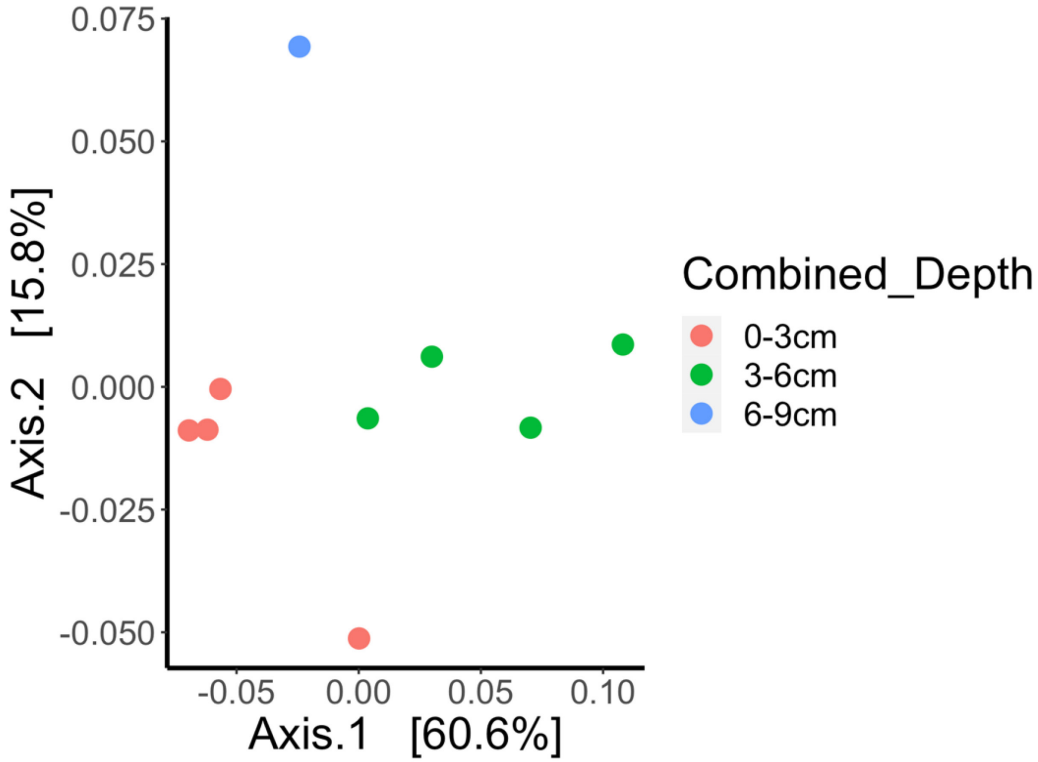

Figure 25: 16S weighted UniFrac beta diversity Island Lake

Figure 24 shows ordination of community points by PCoA with the unweighted UniFrac metric on the 16S gene from Island Lake. Axis.1 shows a percent variation of 27.6% and Axis.2, 18.7%. The weighted UniFrac (Figure 25), Axis.1 shows a percent variation of 60.6% and Axis.2 15.8%.

Discussion

To make sense of the metabarcoding data, we compare the differences in 18S rRNA ASVs between Pockwock and Island Lake to see how similar or different their microbiomes are. As there is only 16S rRNA data for Island Lake, 16S bacterial interpretation follows 18S eukaryote interpretation.

Comparing Pockwock and Island Lake with their alpha diversity measures, Pockwock is the more diverse lake when it comes to eukaryotic life forms and has a greater number of species present. Comparing Pockwock and Island Lake with their beta diversity, Island Lake is the more diverse lake when it comes to eukaryotic life forms and the differences between the sediment groups. Weighted UniFrac considers abundances, and the percent variation is explained by factors that are present, and not due to error variance. The higher percentage of explained variance indicates a stronger strength association (Rosenthal & Rosenthal 2011). By comparing the Weighted UniFrac percentage variation, Island Lake shows a greater percent variation 49.7% 33.6

The alpha diversity for Island Lake 16S rRNA shows a greater abundance in the middle of the samples. The beta diversity shows significant percent variation 60.6% suggesting high diversity between community members. HW has been faced with challenges involving the taste and odour compound geosmin since 2012. Cyanobacteria have been associated with the organic compounds geosmin and 2-methylisoborneol (MIB). Geosmin and MIB give water an earthy, musty taste that is difficult to remove with conventional treatment processes. Both compounds

are metabolites produced by cyanobacteria, actinomycetes and proteobacteria. Interesting things to note which are present in Island Lake in very small amounts include Aphanizomenon NIES-81, a toxic filamentous cyanobacterium that causes water blooms in freshwaters across the globe (Cao et al. 2014), Pseudanabaena sp., a common and harmful species in freshwater cyanobacteria blooms (Gao et al., 2018), Microcystaceae (Elenkin 1933), a family that contains the harmful algal bloom Microcystis aeruginosa and the genus Nostoc, usually known for producing secondary metabolites which are highly toxic to humans and other animals (Nowruzi et al. 2012). Additional research is highly recommended to better examine the cyanobacterial community in Island and Pockwock Lakes. Sequencing the full 16S gene is recommended as it provides better taxonomic resolution than just the sequencing part of the gene (Johnson et al. 2019).

There are a few potential reasons why the 16S metabarcoding failed for Pockwock Lake. The first reason is due to the primers that were selected to target the gene. The polymerase choice can affect both occurrence and relative abundance estimates (Nichols et al. 2018). A second reason is due to DNA degradation or limited production. eDNA production can be affected by the type of organism/species and by the season (Sales et al. 2019). The season could have an impact on what species are present in both lakes as the cores were collected during different seasons, (Pockwock in the fall and Island Lake in the summer).

One of the biggest challenges when examining the microbiome through eDNA metabarcoding is making sure you have good representation. Interestingly neither zooplankton nor diatoms leave much of an eDNA record in this study. It begs the question; how much other stuff are we not seeing? To clarify, the phylogenies percent presented in the results section are not relative abundances. They are occurrence percentages of ASVs. One study replicated different PCR samples and showed more than 10 replicate PCRs were needed to get full taxa

representation (Nichols et al. 2018). Due to this, we cannot confidently say we have full representation to quantify community abundances, especially where we targeted only limited regions of the 16S and 18S genes.

When looking at the DNA concentration extracted, there is a general decrease in concentration as you move down core. This decrease in concentration is most likely due to DNA degradation, which means there would be less DNA to amplify down core if the DNA is less. Interestingly, Island Lake has greater DNA concentration from the extraction kit, however, has lower species richness compared to Pockwock.

Cyano-specific primer targets used by IMR have a low coverage percent for the SILVA database. In hindsight this was a study design flaw. Instead of trying to target the Cyano-specific region of the gene (V3-V4), we should have used Bacteria-specific primer targets focused on the (V6-V8) gene region.

Conclusion

HTS of eDNA allows for fast and efficient way to fingerprint ecological communities. Incorporating eDNA into paleolimnological studies helps to fill analysis gaps not possible by using classical bio indicators. Although the submission of 16S rRNA gene metabarcodes failed for Pockwock rendering cyanobacterial comparison with Island Lake impossible, we were still able to compare alpha and beta diversity for 18S rRNA genes and make observations that Pockwock is the more species rich and diverse lake. The uniqueness of the eDNA representation for each lake cannot be overemphasized. eDNA metabarcoding represents an astonishing new avenue of comparison for one site to another, going forward to monitoring of biodiversity or looking back into the paleolimnology past. The eDNA species richness and diversity analysis in

this study is just scratching the surface of possibility, and therefore future work is highly recommended.

CHAPTER 5.0: SUMMARY AND RECOMMENDATIONS

The purpose of this study is to identify and assess the biological response to chemical water quality change occurring in Pockwock Lake by conducting a detailed multi-proxy paleolimnological investigation using invertebrate assemblages, reflectance spectra and eDNA. As lakes recover from anthropogenic acid deposition, they become more productive and organisms such as algae proliferate. Due to lack of long-term monitoring data in Atlantic Canada, characterizing the ecology of aquatic systems can be challenging. Currently, there is little known about the ecology and biological diversity present in Pockwock Lake. With recent evidence of chemical recovery trends in Pockwock, biological response to changing water quality trends is investigated.

Summary

The paleolimnological approach is useful for providing the historical context on multiple stressors. While increases in pH, ANC and alkalinity are evidence of chemical water quality change, the Cladocera and diatom subfossil assemblages indicate that bio indicator response to these changes reflect differently in the assemblages.

We hypothesized that cladoceran and diatom subfossil assemblages will reflect water quality changes in pH indicative of responses to chemical recovery from anthropogenic acid deposition in Pockwock Lake. The cladoceran assemblage did not exhibit increases in diversity or assemblage changes indicative of chemical recovery, however it did show a response to water quality changes. Cladocera exhibited increases in *Bosmina spp*. and decreases in acid-sensitive *Daphnia spp*. indicating something is inhibiting assemblage response in the modern period, most likely Ca decline. This was not evident in the diatom assemblage. Diatoms showed clear evidence of a change in lakewater pH since ~1992, evident as two assemblage changes with

increases in *Asterionella ralfsii* and *Tabellaria flocculosa* and a decrease in Hills N2 diversity. Two of the three dominate pre-impact species have returned after the two acidification periods. We interpret these diatom shifts in overall assemblage structure and richness as responses to indirect effects of climate warming, stratification, and shorter-ice cover.

Since eDNA metabarcoding only occurred for the first 10 cm of core, we are not able to make conclusions on response or change using eDNA from the pre-impact benchmark. However significant changes in diversity were observed in the eDNA analyzed. Even though we did not capture the fingerprint of eDNA during pre-impact conditions, community insight was still valuable. eDNA metabarcoding for Pockwock and Island Lake highlights the many technical factors impacting eDNA analyses which results in many limitations with this study. eDNA metabarcoding complements traditional paleolimnology analyses as it helps add another dimension to the biological story of Pockwock.

Limitations of this study

The first major limitation of this research is study design for the eDNA metabarcoding. We could have used a "top-bottom" approach to attempt to recover DNA from pre-impact to better understand changes between modern and pre-impact sediments. The second limitation, due to a weaker study design then resulted in a poor choice of primer targets for DNA sequencing. The Cyano-specific primers used by the IMR tend to perform less well in PCR sequencing than other primer sets they offer. Due to the choice of primers, we ended up with limited DNA coverage from both lakes. We were able to collect a snapshot or fingerprint of diversity, however with major gaps present. This resulted in having no 16S data for Pockwock Lake. With a more thorough research design, recommended Universal and Bacteria-specific targets would have been used. A third limitation of this study is the use of different cores for eDNA analysis and the

other proxies. To make sound interpretations about species diversity and richness from eDNA metabarcoding, the proxies should all come from the same sediment core. A fourth limitation is the Cladocera is not identified to the species level.

Suggestions for future research

Further detailed core analyses of cores from multiple lakes in the region would contribute to a better understanding of the biological response to recovery from anthropogenic acidification. This research is a starting point in trying to characterize the microbiome and diversity of Pockwock Lake. We would recommend the following future work:

- Use a reference lake approach to compare Cladocera and diatom assemblages. Using a reference lake approach would allow recovery benchmarks to be set in order to estimate the rate of change in bio indicator communities (Gray and Arnott 2009).
- Refinement of methods used for eDNA sampling design. Take samples from top, middle and bottom of core to try and capture pre-impact time periods to compare against other biotic proxies
- Use Bacteria-specific (V4) region instead of the Cyano-specific (V3-V4) region to as the Cyano-specific primers used by the IMR tend to perform less well in PCR and sequencing than other primer sets
- Examine the diversity archived in contemporary and pre-industrial sediments using a shotgun metagenomic analysis to increase phylogenetic coverage by targeting entire 16S gene and not just regions.
- Compare DNA from water column to sediment surface. Even though sediment shows higher DNA concentration and longer detectability than surface water (Turner et al.

2015), have a baseline for what is detectable in the water is a good starting point to understand what is potentially missing from the sediment.

- Instead of examining eDNA throughout an entire sediment core, collect multiple surface samples throughout a lake to monitor biodiversity and lake health.

Final thoughts

The goal of this work is to help with the understanding of water quality changes in drinking water sources and aid in the implementation of management protocols by lake managers. The results of this study opens the door to new questions about biological response and changing aquatic systems. As more research is published around these topics, we must understand that complete biological recovery, or recovery back to a pre-impacted state will most likely not occur. Aquatic systems are changing so rapidly within multiple stressed environments, it may be unrealistic to expect pre-impact conditions to ever be reestablished. Functional ecosystem recovery of impacted systems is the most probable path forward. eDNA metabarcoding offers a new dimension to paleolimnological analysis and with refined study design, appropriate primer choice and intentional sampling and replication, it is a robust tool to understand ecosystem recovery.

This research illustratates just how quickly water quality is changeing. As the climate continues to warm and chemical water quality continues to change in Pockwock, HW will be faced with more challenges as increased productivity can result in harmful algal blooms and increases the potential for cyanotoxins. This research helps to characterize the ecosystem of a drinking water source. As lake managers make projections about future water quality, this research can be used by water utilities to aid in bio monitoring programs to track biological responses in changing source waters to help make predictions about future treatment necessities.

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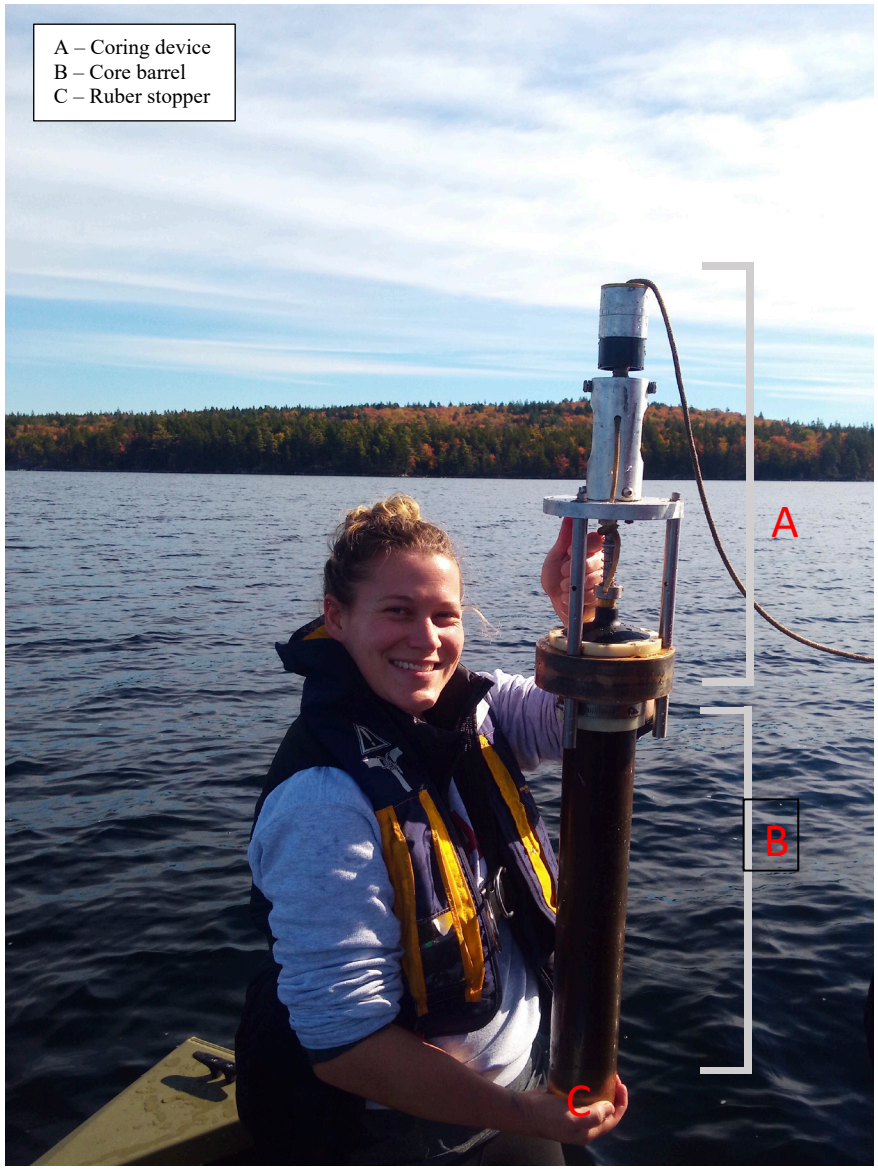
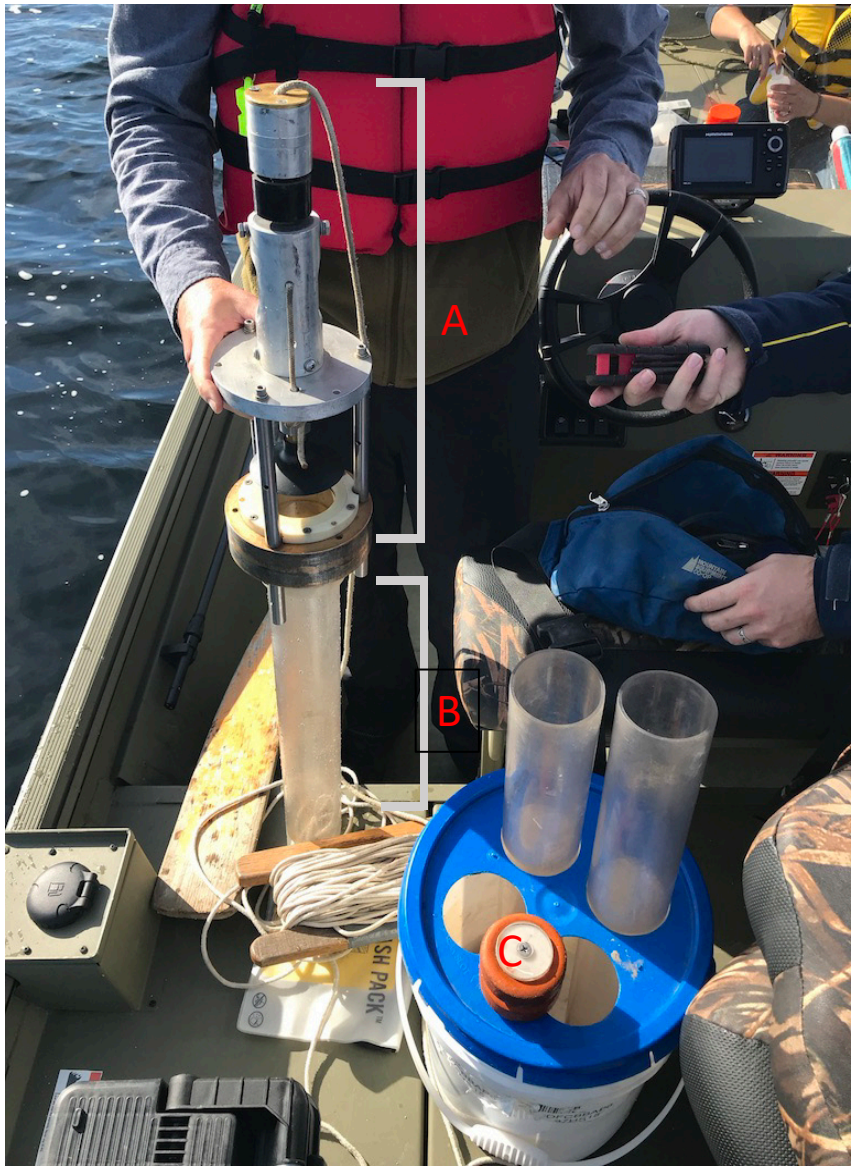
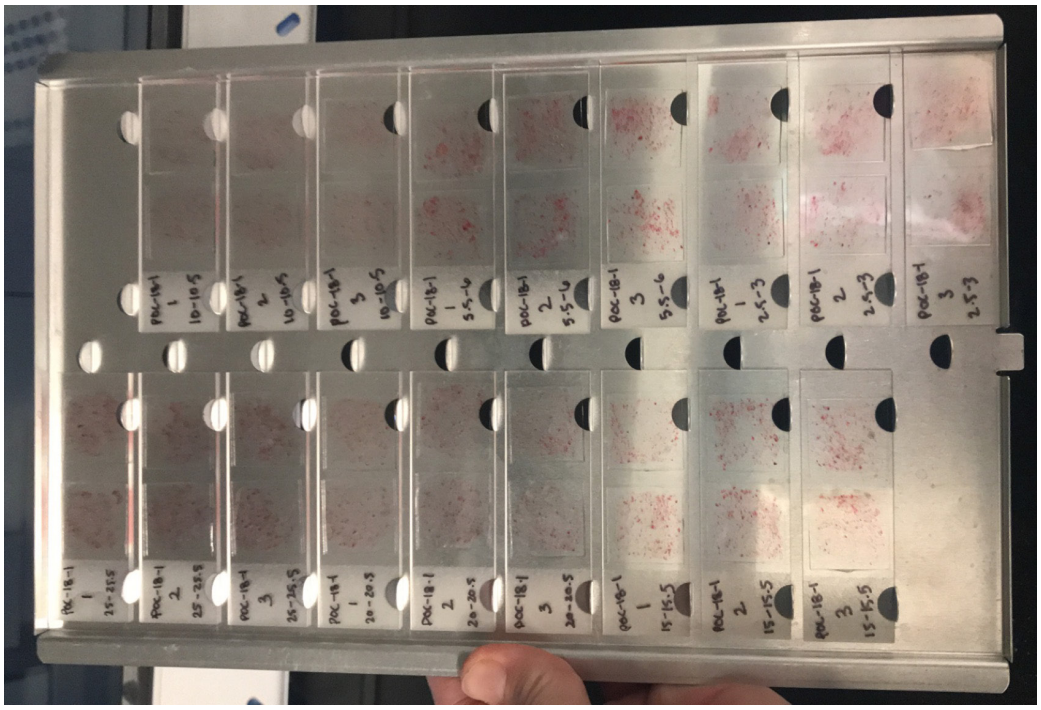
APPENDIX A: PHOTOGRAPHIC LOG







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APPENDIX B: AGE DATA

location	depth_start	depth_end	depth_mid	drybulkdensity	Water content	cumulative_dry_mass	pb210	pb210err	pb214	pb214err	age	age_sd	mar	mar_sd	inventory	inventory_sd	year
POC 18-1	0	0.5	0.25	19.3	0.9809	0.048	2112.88	154.23	5.73	17.15	0.35745077	3.3916126	0.13353627	0.01726654	8972.13823	950.111218	2018.43255
POC 18-1	0.5	1	0.75	NA	NA	0.22675	NA	NA	NA	NA	1.42536301	NA	0.14474337	NA	8677.91042	NA	2017.36464
POC 18-1	1	1.5	1.25	NA	NA	0.4055	NA	NA	NA	NA	2.53011481	NA	0.15902655	NA	8383.6826	NA	2016.25989
POC 18-1	1.5	2	1.75	NA	NA	0.58425	NA	NA	NA	NA	3.67433894	NA	0.17785372	NA	8089.45478	NA	2015.11566
POC 18-1	2	2.5	2.25	52.1	0.9495	0.763	1209.29	90.52	1.36	10.74	4.86096094	3.49449355	0.20380346	0.02715382	7795.22697	850.441072	2013.92904
POC 18-1	2.5	3	2.75	NA	NA	1.0735	NA	NA	NA	NA	6.54918147	NA	0.1791167	NA	7394.97392	NA	2012.24082
POC 18-1	3	3.5	3.25	72.2	0.9309	1.384	1398.94	97.07	18.47	9.56	8.33136578	3.24344591	0.15781307	0.01950223	6994.72087	708.123135	2010.45863
POC 18-1	3.5	4	3.75	NA	NA	1.7535	NA	NA	NA	NA	10.4317267	NA	0.17021677	NA	6550.72977	NA	2008.35827
POC 18-1	4	4.5	4.25	75.6	0.9277	2.123	1034.37	90.23	13.42	12.75	12.679555	4.06428414	0.18705681	0.0289968	6106.73867	774.565643	2006.11044
POC 18-1	4.5	5	4.75	NA	NA	2.5655	NA	NA	NA	NA	14.9711307	NA	0.18627289	NA	5685.06947	NA	2003.81887
POC 18-1	5	5.5	5.25	101.4	0.9046	3.008	901.59	71.99	27.25	9.37	17.4393903	3.74035319	0.18537157	0.02641948	5263.40027	614.026926	2001.35061
POC 18-1	5.5	6	5.75	NA	NA	3.5585	NA	NA	NA	NA	19.7064556	NA	0.23433223	NA	4903.71473	NA	1999.08354
POC 18-1	6	6.5	6.25	118.7	0.8894	4.109	435.28	51.27	12.58	8.91	22.1463012	5.57625579	0.33762297	0.07185694	4544.02918	790.532374	1996.6437
POC 18-1	6.5	7	6.75	NA	NA	4.6305	NA	NA	NA	NA	23.6893507	NA	0.32989112	NA	4330.29515	NA	1995.10065

POC 18-1	7	7.5	7.25	90	0.9147	5.152	414.52	58.3	16.95	10.95	25.3105216	6.65429202	0.32175745	0.08175187	4116.56112	854.673355	1993.47948
POC 18-1	7.5	8	7.75	NA	NA	5.631	NA	NA	NA	NA	26.9009327	NA	0.293764	NA	3917.13679	NA	1991.88907
POC 18-1	8	8.5	8.25	101.5	0.9045	6.11	448.26	58.86	20.48	11.09	28.5744647	6.20130298	0.26795082	0.06341743	3717.71245	719.08128	1990.21554
POC 18-1	8.5	9	8.75	NA	NA	6.619	NA	NA	NA	NA	30.3344884	NA	0.2813272	NA	3518.92518	NA	1988.45551
POC 18-1	9	9.5	9.25	102.2	0.9039	7.128	362.94	51.33	21.82	9.96	32.1968835	6.73189736	0.2979842	0.07656423	3320.1379	697.072165	1986.59312
POC 18-1	9.5	10	9.75	NA	NA	7.6665	NA	NA	NA	NA	34.052113	NA	0.28193499	NA	3133.28124	NA	1984.73789
POC 18-1	10	10.5	10.25	113.1	0.8943	8.205	361.16	53.86	19.11	10.21	36.0214514	7.09676155	0.26580324	0.07199252	2946.42457	652.045531	1982.76855
POC 18-1	10.5	11	10.75	NA	NA	8.7885	NA	NA	NA	NA	37.9672117	NA	0.2689676	NA	2772.75179	NA	1980.82279
POC 18-1	11	11.5	11.25	NA	NA	9.372	NA	NA	NA	NA	40.038871	NA	0.27264721	NA	2599.079	NA	1978.75113
POC 18-1	11.5	12	11.75	NA	NA	9.9555	NA	NA	NA	NA	42.2538559	NA	0.27697908	NA	2425.40621	NA	1976.53614
POC 18-1	12	12.5	12.25	120.4	0.8879	10.539	264.23	68.04	8.2	13.48	44.6334816	12.403762	0.28215351	0.13371567	2251.73343	871.498378	1974.15652
POC 18-1	12.5	13	12.75	NA	NA	11.165	NA	NA	NA	NA	46.4483577	NA	0.29701442	NA	2127.68543	NA	1972.34164
POC 18-1	13	13.5	13.25	NA	NA	11.791	NA	NA	NA	NA	48.3722851	NA	0.31570118	NA	2003.63744	NA	1970.41771
POC 18-1	13.5	14	13.75	NA	NA	12.417	NA	NA	NA	NA	50.4192118	NA	0.33990948	NA	1879.58944	NA	1968.37079
POC 18-1	14	14.5	14.25	130	0.8796	13.043	162.2	64.72	19.88	14.13	52.6059453	19.959216	0.37251078	0.28419358	1755.54145	1093.65127	1966.18405
POC 18-1	14.5	15	14.75	NA	NA	13.699	NA	NA	NA	NA	54.3257382	NA	0.36193369	NA	1663.76066	NA	1964.46426
POC 18-1	15	15.5	15.25	NA	NA	14.355	NA	NA	NA	NA	56.1431449	NA	0.35080965	NA	1571.97987	NA	1962.64686

POC 18-1	15.5	16	15.75	NA	NA	15.011	NA	NA	NA	NA	58.0699165	NA	0.33909509	NA	1480.19908	NA	1960.72008
POC 18-1	16	16.5	16.25	132.5	0.8775	15.667	147.73	66.57	9.61	14.23	60.1200631	22.7648597	0.32674171	0.2843215	1388.4183	986.527078	1958.66994
POC 18-1	16.5	17	16.75	NA	NA	16.32425	NA	NA	NA	NA	62.2573658	NA	0.30145126	NA	1298.78929	NA	1956.53263
POC 18-1	17	17.5	17.25	NA	NA	16.9815	NA	NA	NA	NA	64.5475599	NA	0.27684607	NA	1209.16028	NA	1954.24244
POC 18-1	17.5	18	17.75	NA	NA	17.63875	NA	NA	NA	NA	67.0142167	NA	0.25289865	NA	1119.53127	NA	1951.77578
POC 18-1	18	18.5	18.25	130.4	0.8793	18.296	155.12	60.11	5.98	13.23	69.6868169	19.4837622	0.22958298	0.1709298	1029.90226	626.13552	1949.10318
POC 18-1	18.5	19	18.75	NA	NA	18.98425	NA	NA	NA	NA	72.4777854	NA	0.22248378	NA	943.953779	NA	1946.31221
POC 18-1	19	19.5	19.25	NA	NA	19.6725	NA	NA	NA	NA	75.5353839	NA	0.21452133	NA	858.0053	NA	1943.25462
POC 18-1	19.5	20	19.75	NA	NA	20.36075	NA	NA	NA	NA	78.9159894	NA	0.205528	NA	772.056821	NA	1939.87401
POC 18-1	20	20.5	20.25	144.9	0.867	21.049	124.75	40.19	19.66	8.93	82.6959972	16.6704837	0.19528973	0.12432709	686.108342	356.68758	1936.094
POC 18-1	20.5	21	20.75	NA	NA	21.68025	NA	NA	NA	NA	85.8916854	NA	0.1821239	NA	620.95404	NA	1932.89831
POC 18-1	21	21.5	21.25	NA	NA	22.3115	NA	NA	NA	NA	89.4419391	NA	0.16813151	NA	555.799737	NA	1929.34806
POC 18-1	21.5	22	21.75	NA	NA	22.94275	NA	NA	NA	NA	93.4353709	NA	0.15323218	NA	490.645435	NA	1925.35463
POC 18-1	22	22.5	22.25	107.5	0.8991	23.574	111.79	49.89	17.25	10.78	97.9986203	23.4283305	0.13733478	0.12292465	425.491132	310.978882	1920.79138
POC 18-1	22.5	23	22.75	NA	NA	24.13925	NA	NA	NA	NA	102.030289	NA	0.12611001	NA	375.164247	NA	1916.75971
POC 18-1	23	23.5	23.25	NA	NA	24.7045	NA	NA	NA	NA	106.643555	NA	0.11391449	NA	324.837362	NA	1912.14644
POC 18-1	23.5	24	23.75	NA	NA	25.26975	NA	NA	NA	NA	112.034967	NA	0.1006166	NA	274.510477	NA	1906.75503

POC 18-1	24	24.5	24.25	118.6	0.8895	25.835	96.39	27.97	22.67	6.92	118.521374	15.7165589	0.0860 5978	0.0515 7012	224.183592	109.702778	1900.26863
POC 18-1	24.5	25	24.75	NA	NA	26.40725	NA	NA	NA	NA	123.214699	NA	0.0897 4009	NA	193.625592	NA	1895.5753
POC 18-1	25	25.5	25.25	NA	NA	26.9795	NA	NA	NA	NA	128.715849	NA	0.0953 4568	NA	163.067593	NA	1890.07415
POC 18-1	25.5	26	25.75	NA	NA	27.55175	NA	NA	NA	NA	135.361936	NA	0.1049 2244	NA	132.509594	NA	1883.42806
POC 18-1	26	26.5	26.25	110.2	0.8968	28.124	40.52	22.69	17.4	5.78	143.758253	40.7166765	0.1250 0498	0.1945 1031	101.951594	129.530735	1875.03175
POC 18-1	26.5	27	26.75	NA	NA	28.653625	NA	NA	NA	NA	148.741524	NA	NA	NA	87.2612282	NA	1870.04848
POC 18-1	27	27.5	27.25	NA	NA	29.18325	NA	NA	NA	NA	153.724795	NA	NA	NA	74.6876202	NA	1865.06521
POC 18-1	27.5	28	27.75	NA	NA	29.712875	NA	NA	NA	NA	158.708065	NA	NA	NA	63.9257632	NA	1860.08193
POC 18-1	28	28.5	28.25	NA	NA	30.2425	NA	NA	NA	NA	163.691336	NA	NA	NA	54.7145991	NA	1855.09866
POC 18-1	28.5	29	28.75	NA	NA	30.772125	NA	NA	NA	NA	168.674606	NA	NA	NA	46.8306862	NA	1850.11539
POC 18-1	29	29.5	29.25	NA	NA	31.30175	NA	NA	NA	NA	173.657877	NA	NA	NA	40.0827788	NA	1845.13212
POC 18-1	29.5	30	29.75	NA	NA	31.831375	NA	NA	NA	NA	178.641148	NA	NA	NA	34.3071879	NA	1840.14885
POC 18-1	30	30.5	30.25	101.6	0.9044	32.361	0	24.26	8.23	6.43	183.624418	NA	NA	NA	29.3638111	NA	1835.16558
POC 18-1	30.5	31	30.75	NA	NA	32.9783111	NA	NA	NA	NA	189.432733	NA	NA	NA	24.4935764	NA	1829.35727
POC 18-1	31	31.5	31.25	NA	NA	33.5956223	NA	NA	NA	NA	195.241047	NA	NA	NA	20.431111	NA	1823.54895
POC 18-1	31.5	32	31.75	NA	NA	34.2129334	NA	NA	NA	NA	201.049362	NA	NA	NA	17.0424397	NA	1817.74064
POC 18-1	32	32.5	32.25	NA	NA	34.8302446	NA	NA	NA	NA	206.857676	NA	NA	NA	14.2158079	NA	1811.93232

POC 18-1	32.5	33	32.75	NA	NA	35.4475557	NA	NA	NA	NA	212.66599	NA	NA	NA	11.8579968	NA	1806.12401
POC 18-1	33	33.5	33.25	NA	NA	36.0648669	NA	NA	NA	NA	218.474305	NA	NA	NA	9.8912484	NA	1800.3157
POC 18-1	33.5	34	33.75	NA	NA	36.682178	NA	NA	NA	NA	224.282619	NA	NA	NA	8.25070178	NA	1794.50738
POC 18-1	34	34.5	34.25	NA	NA	37.2994892	NA	NA	NA	NA	230.090934	NA	NA	NA	6.88225359	NA	1788.69907
POC 18-1	34.5	35	34.75	NA	NA	37.9168003	NA	NA	NA	NA	235.899248	NA	NA	NA	5.74077403	NA	1782.89075
POC 18-1	35	35.5	35.25	NA	NA	38.5341115	NA	NA	NA	NA	241.707562	NA	NA	NA	4.78861844	NA	1777.08244
POC 18-1	35.5	36	35.75	NA	NA	39.1514226	NA	NA	NA	NA	247.515877	NA	NA	NA	3.99438585	NA	1771.27412
POC 18-1	36	36.5	36.25	NA	NA	39.7687338	NA	NA	NA	NA	253.324191	NA	NA	NA	3.33188341	NA	1765.46581
POC 18-1	36.5	37	36.75	NA	NA	40.3860449	NA	NA	NA	NA	259.132506	NA	NA	NA	2.77926256	NA	1759.65749
POC 18-1	37	37.5	37.25	NA	NA	41.0033561	NA	NA	NA	NA	264.94082	NA	NA	NA	2.31829852	NA	1753.84918
POC 18-1	37.5	38	37.75	NA	NA	41.6206672		NA	NA	NA	270.749134	NA	NA	NA	1.93378924	NA	1748.04087
POC 18-1	38	38.5	38.25	NA	NA	42.2379784	NA	NA	NA	NA	276.557449	NA	NA	NA	1.61305405	NA	1742.23255
POC 18-1	38.5	39	38.75	NA	NA	42.8552895	NA	NA	NA	NA	282.365763	NA	NA	NA	1.34551549	NA	1736.42424

APPENDIX C: XRF DATA

location	depth_start	Na	Mg	Al	Si	P	S	Cl	K	Ca
POC18-1	0	16073.79	15868.46	-59441.3	-16110.2	1149.556	2509.829	96.381	2352.637	3286.801
POC18-1	0.5	2066.806	2501.082	49942	564.537	902.847	2956.348	67.687	5218.629	2761.787
POC18-1	1	62636.31	61901.91	50376.08	-29788.6	1299.48	3903.272	74.24	7923.167	3785.086
POC18-1	1.5	8355.248	11955.61	-110861	-24734.1	1273.972	4922.419	64.903	9052.246	3694.875
POC18-1	2	19085.87	14996.13	18222.33	-10619.2	1021.522	3464.476	45.773	8250.38	3068.564
POC18-1	2	3958.341	59221.52	-237407	-64015.6	986.981	3211.022	40.024	8081.372	3266.004
POC18-1	2	49502.52	48586.6	-202319	-38219.7	999.018	3190.356	47.178	6441.611	3436.399
POC18-1	2.5	4048.712	1709.148	51377.97	1359.262	1380.75	6382.705	42.186	10472.07	3703.439
POC18-1	3	3157.102	786.547	40286.24	1570.646	932.15	4533.072	39.711	10600.94	3345.939
POC18-1	3.5	4063.491	1820.673	50086.07	1243.11	1358.902	7588.388	31.514	10166.15	3470.106
POC18-1	4	3181.473	1958.962	41693.01	877.951	1030.554	5883.642	36.732	8927.237	3380.424
POC18-1	4.5	3846.258	1767.675	46956.83	1065.746	1270.612	11585.97	43.3	12889.71	3503.456
POC18-1	5	57929.14	51996.46	-551505	-73690.7	1102.631	6395.842	17.798	11472.89	3049.165
POC18-1	5.5	3565.173	4011.877	58668.04	1445.139	1361.731	9605.13	26.487	13406.93	3286.52
POC18-1	6	-7.939	6900.417	-64085.4	-16884.1	1018.32	5966.786	35.823	9413.61	2751.582
POC18-1	6.5	12125.2	18751.66	163342.4	1574.534	1340.256	10451.03	21.742	12010.57	2894.687
POC18-1	7	3921.621	2237.252	48516.05	2236.151	1199.385	6646.618	31.025	7896.332	2958.288
POC18-1	7.5	12616.43	17902.1	163183.5	2699.129	1138.471	10689.9	30.349	7853.688	2978.638
POC18-1	8	3978.254	1890.652	42897.43	1696.859	1154.789	5237.412	33.953	8684.095	2644.139
POC18-1	8	3930.914	2005.316	44068.7	1728.375	1033.991	5385.646	25.848	8908.997	2569.442
POC18-1	8	3953.236	2236.513	46826.08	2195.13	1112.676	5432.202	22.989	8203.593	2712.474
POC18-1	8.5	4059.151	1794.088	45715.1	1642.792	1141.672	6785.997	26.812	9370.202	3055.455
POC18-1	9	4069.212	2036.406	42360.09	1487.816	1162.075	3776.591	31.095	10348.49	2819.341

POC18-1	9.5	4026.213	2014.306	47357.32	2216.245	1198.457	4416.487	36.403	6778.502	3250.37
POC18-1	10	1634.061	-5663.89	-17729	64.759	1048.335	2687.138	29.089	7102.031	2743.047
POC18-1	10.5	4071.108	2010.403	44928.92	1890.005	1199.252	3385.574	33.625	6191.242	3005.219
POC18-1	11	4087.856	1854.517	43948.94	1711.14	1325.733	3594.512	36.629	9115.128	3091.334
POC18-1	11.5	4094.892	1923.934	45477.1	1793.531	1353.671	3362.114	31.29	8259.527	3138.81
POC18-1	12	4071.117	1959.45	40728.61	1173.39	1177.837	2284.982	29.915	8637.35	2808.506
POC18-1	12	3744.846	746.394	34001.67	1556.303	1064.183	2503.315	29.815	7245.664	2772.354
POC18-1	12	3970.068	1505.175	41365.02	1865.929	1116.61	2704.504	27.738	9088.266	2846.853
POC18-1	12.5	4070.316	1761.018	42692.25	1695.846	1214.633	3566.078	37.679	8221.841	2913.01
POC18-1	13	4033.984	2044.901	46713.71	1970.1	1266.62	3243.131	32.24	8797.024	2970.065
POC18-1	14	3922.285	2139.026	42716.93	1604.583	1015.407	2301.767	34.866	7866.649	2720.881
POC18-1	14.5	4028.552	1637.377	43947.59	2070.81	1345.854	2880.247	31.501	7888.885	2950.067
POC18-1	15	3389.919	300.877	37170.66	2790.535	1303.873	2701.975	24.845	9035.905	2957.251
POC18-1	15.5	4027.401	1984.006	47162.93	2300.251	1245.233	2463.069	33.313	8954.023	2912.279
POC18-1	16	3970.944	2027.156	41832.6	1596.628	1042.6	1801.296	31.073	9525.253	2877.112
POC18-1	16.5	4017.048	2074.747	46068.9	2102.46	1193.011	2284.891	36.219	8315.795	3085.175
POC18-1	17	4011.739	1901.5	43234.63	1861.605	1113.994	2504.632	35.388	8996.58	2958.64
POC18-1	18	3968.554	1981.367	42122.24	1930.404	1075.698	2110.198	31.373	7926.117	2593.08
POC18-1	20	4059.633	2001.517	38656.88	996.084	1071.055	2379.127	39.336	5524.551	2323.487
POC18-1	22	3979.373	1983.363	40704.98	1163.311	1131.081	1955.463	37.951	7830.821	3031.495
POC18-1	22	4105.072	2004.335	42908.7	2023.769	1220.204	1881.401	31.3	7886.003	2862.986
POC18-1	22	4063.77	2136.969	43422.2	1918.141	1084.417	1859.735	37.743	5578.491	2940.427
location	depth_start	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu
POC18-1	0	7.111	1308.221	50.979	38.372	1294.38	127049.3	17.865	13.21	35.827
POC18-1	0.5	6.92	1568.053	49.206	36.459	883.63	83838.7	17.201	9.524	22.467
POC18-1	1	6.89	2539.5	76.3	31.385	1036.836	74557.67	16.643	19.047	35.58

POC18-1	1.5	6.94	3032.348	74.303	34.161	782.457	56610.25	15.85	37.477	41.599
POC18-1	2	6.882	2748.306	82.416	27.973	657.356	46524.93	16.398	24.408	35.684
POC18-1	2	6.981	3008.007	79.605	31.084	725.077	51395.95	16.904	27.851	40.161
POC18-1	2	6.999	2616.237	77.755	30.771	688.397	46822.78	16.074	27.694	34.421
POC18-1	2.5	6.856	3613.845	94.036	37.598	803.746	49768.17	16.102	36.487	33.76
POC18-1	3	6.969	2981.651	74.693	34.339	652.746	46401.5	16.155	25.097	34.41
POC18-1	3.5	7.034	3382.051	131.072	38.679	679.613	48082.6	16.22	30.361	34.613
POC18-1	4	6.978	2802.736	96.166	32.511	594.317	42485.39	15.747	23.932	38.062
POC18-1	4.5	7.032	3659.496	118.953	38.651	749.851	54514.06	16.397	19.404	35.278
POC18-1	5	6.991	3218.335	78.266	34.703	617.426	45570.9	16.154	21.157	27.079
POC18-1	5.5	6.956	4108.411	103.729	37.79	631.314	46460.93	15.833	32.558	26.821
POC18-1	6	6.967	3159.242	70.383	30.811	529.981	38416.14	15.985	28.591	30.448
POC18-1	6.5	7.015	3291.049	99.08	31.863	620.411	44024.64	16.236	37.172	18.99
POC18-1	7	7.072	3266.716	89.425	32.415	647.851	42086.98	15.724	18.482	17.656
POC18-1	7.5	6.999	3334.821	110.496	35.406	578.794	40811.36	15.949	31.503	19.901
POC18-1	8	6.985	2999.27	54.307	29.593	561.707	38518.22	15.697	20.872	23.133
POC18-1	8	6.964	3003.705	89.751	30.53	562.264	38427.1	15.88	20.089	21.822
POC18-1	8	6.945	2995.96	65.518	29.515	559.226	37328.44	15.882	19.93	13.088
POC18-1	8.5	6.915	3119.389	99.102	33.231	608.157	40666.46	15.667	20.201	22.384
POC18-1	9	6.915	2818.52	96.404	26.322	608.822	37226.51	15.256	13.612	25.097
POC18-1	9.5	6.913	2754.024	62.31	32.826	576.43	34688.66	15.2	22.091	26.345
POC18-1	10	6.921	2714.96	64.16	26.846	617.107	36674.25	15.741	19.623	18.78
POC18-1	10.5	6.876	2968.313	76.051	26.753	576.82	35202.16	15.402	19.86	15.992
POC18-1	11	6.91	3014.429	40.552	26.649	558.342	36092.52	15.537	16.978	22.783
POC18-1	11.5	6.971	3198.812	74.523	30.01	553.888	36251.1	15.42	14.691	23.113
POC18-1	12	6.988	2905.219	97.217	23.596	607.993	34593.38	15.857	11.895	13.934

POC18-1	12	6.997	2782.025	77.386	31.137	596.725	35316.34	15.581	24.872	24.051
POC18-1	12	6.941	2839.057	74.369	31.76	653.813	36272.5	15.379	19.774	18.772
POC18-1	12.5	6.979	2879.657	74.406	34.813	622.334	36410.76	15.281	19.701	18.68
POC18-1	13	6.991	3054.251	71.742	27.563	668.977	35740.76	15.428	16.823	15.333
POC18-1	14	6.863	2557.016	60.731	34.978	558.719	33218.63	15.322	21.851	19.652
POC18-1	14.5	6.959	3048.558	84.914	27.419	607.297	33931.06	15.226	19.62	19.632
POC18-1	15	6.902	2847.696	71.078	30.026	544.516	31811.4	15.095	18.538	13.529
POC18-1	15.5	6.999	3362.355	85.889	30.703	667.999	36340.03	15.554	21.17	16.034
POC18-1	16	6.997	3185.671	54.52	30.468	723.86	34380.04	15.265	23.434	16.485
POC18-1	16.5	6.969	3215.207	70.17	31.302	660.584	35189.02	15.644	18.976	10.212
POC18-1	17	6.973	3034.5	59.492	28.537	673.13	33506.33	15.008	18.254	10.809
POC18-1	18	6.964	2915.832	71.788	28.008	697.05	32528.28	15.315	22.221	23.548
POC18-1	20	6.975	2277.635	73.217	29.51	768.291	32290.38	15.156	16.127	16.869
POC18-1	22	7.007	2800.284	79.783	25.525	762.896	36352.65	15.444	18.114	12.557
POC18-1	22	6.975	2534.66	78.534	28.323	728.599	34525.22	15.509	16.461	19.776
POC18-1	22	6.902	2474.16	51.236	30.226	685.781	33670.09	15.652	21.172	17.138
location	depth_start	Zn	Ga	As	Rb	Sr	Y	Zr	Nb	Mo
POC18-1	0	73.196	15.892	20.256	8.619	24.546	13.476	15.068	0.767	3.516
POC18-1	0.5	87.368	14.078	18.43	10.486	21.436	14.547	19.823	3.988	4.666
POC18-1	1	72.36	17.961	13.686	20.15	26.298	14.721	23.84	1.928	3.116
POC18-1	1.5	174.532	20.323	22.834	24.205	36.103	15.894	27.342	3.891	2.439
POC18-1	2	91.57	13.613	14.397	46.635	49.259	17.032	33.264	1.757	2.462
POC18-1	2	89.876	16.302	18.735	51.274	61.002	18.999	42.583	6.293	6.614
POC18-1	2	102.288	20.221	10.121	43.598	52.274	18.697	34.43	3.564	2.026
POC18-1	2.5	130.56	18.975	15.496	28.708	47.476	17.382	30.414	0.182	1.164
POC18-1	3	81.352	20.824	19.888	50.876	58.275	18.6	40.711	4.435	4.229

POC18-1	3.5	90.181	20.172	19.672	36.363	42.918	14.942	31.784	1.837	2.165
POC18-1	4	127.34	20.587	18.816	47.244	51.82	17.941	34.717	4.708	2.444
POC18-1	4.5	127.104	15.773	13.168	47.493	45.515	16.535	35.074	3.524	2.048
POC18-1	5	147.407	16.062	21.91	56.785	67.441	19.847	42.45	4.684	5.291
POC18-1	5.5	129.43	26.387	23.329	54.482	52.904	15.586	32.436	2.513	0.82
POC18-1	6	181.182	14.823	12.392	43.991	48.846	16.748	33.771	2.423	3.151
POC18-1	6.5	177.662	20.758	10.407	40.32	54.42	19.33	36.87	3.184	1.347
POC18-1	7	138.539	15.773	25.629	38.823	54.237	18.699	41.262	3.617	2.627
POC18-1	7.5	160.014	16.169	17.988	23.005	44.591	15.333	31.943	4.606	3.525
POC18-1	8	129.09	20.575	21.899	34.145	53.758	18.594	38.593	1.972	3.763
POC18-1	8	129.185	19.044	23.773	38.332	53.434	19.411	42.785	2.744	2.837
POC18-1	8	194.55	22.876	32.016	36.381	52.255	19.231	43.696	3.89	4.833
POC18-1	8.5	128.243	15.564	21.515	26.378	45.237	17.993	36.212	2.454	1.612
POC18-1	9	92.365	18.942	16.331	37.169	55.227	18.626	39.502	2.537	4.787
POC18-1	9.5	141.869	17.075	19.804	22.052	35.508	15.592	30.721	1.34	2.602
POC18-1	10	119.199	18.629	23.487	40.435	58.644	19.874	40.379	0.98	3.298
POC18-1	10.5	107.534	13.568	17.331	22.317	38.076	14.671	30.417	2.313	2.306
POC18-1	11	138.855	20.429	16.136	25.088	38.743	15.044	30.083	3.452	1.332
POC18-1	11.5	133.895	20.745	25.991	25.769	44.029	13.564	31.498	2.406	2.784
POC18-1	12	115.661	18.925	12.431	31.45	51.109	15.449	37.584	4.714	3.769
POC18-1	12	153.395	22.421	22.361	23.508	46.838	15.422	35.538	-0.278	2.413
POC18-1	12	107.802	19.061	17.89	29.498	58.335	16.973	39.485	4.598	3.092
POC18-1	12.5	109.217	20.717	13.374	24.74	44.094	15.669	30.587	1.323	2.669
POC18-1	13	101.073	18.149	27.948	26.071	39.121	15.839	32.988	1.312	3.22
POC18-1	14	97.888	19.062	12.665	28.316	49.928	18.356	36.143	3.937	3.835
POC18-1	14.5	98.626	17.649	22.321	22.295	40.069	15.861	34.093	1.709	3.51

POC18-1	15	87.044	17.565	13.902	18.048	34.916	15.153	28.293	0.175	3.486
POC18-1	15.5	92.907	18.25	17.011	28.788	43.572	17.33	32.405	0.982	4.261
POC18-1	16	98.105	21.639	28.305	29.678	49.829	17.101	36.286	-0.01	2.498
POC18-1	16.5	111.796	16.259	17.054	19.813	41.472	16.205	32.429	1.893	1.178
POC18-1	17	82.3	17.109	20.94	21.82	40.274	15.327	33.15	1.014	2.403
POC18-1	18	92.049	23.059	13.289	25.014	40.545	16.196	33.85	3.682	3.198
POC18-1	20	85.812	17.157	18.992	23.981	43.777	16.498	31.247	2.08	4.416
POC18-1	22	63.134	16.37	14.503	29.084	44.799	17.3	37.286	4.414	5.47
POC18-1	22	91.584	24.572	19.355	28.16	44.773	15.963	34.126	1.723	2.087
POC18-1	22	83.661	18.726	21.552	22.713	45.38	16.246	31.827	2.718	2.162
location	depth_start	Cd	In	Sn	Sb	Te	Ba	La	Ce	Ta
POC18-1	0	1.045	96.854	6.134	2.832	7534.279	151.997	15.538	54.164	-2586.96
POC18-1	0.5	1.056	2333.971	5.966	2.998	-19831	251.243	29.231	55.281	4783.339
POC18-1	1	1.003	-2171.46	6.318	1.665	1102.314	150.682	24.923	59.721	-2619.74
POC18-1	1.5	1.047	1176.614	5.985	2.966	7232.059	165.706	25.811	56.988	229.015
POC18-1	2	0.988	-5673.57	6.314	1.372	-6792.57	227.07	26.13	51.713	2601.369
POC18-1	2	0.927	-11293.5	6.334	1.461	-1838.02	175.448	28.064	64.525	3833.599
POC18-1	2	0.978	-3427.85	6.284	1.469	2984.188	376.043	29.144	41.591	1181.776
POC18-1	2.5	0.963	-5209.59	6.247	1.656	5058.404	162.627	29.143	48.737	1582.857
POC18-1	3	1.048	1284.454	5.986	3.537	1571.075	592.202	28.198	24.362	367.045
POC18-1	3.5	1.053	753.449	6.136	2.758	11561.49	261.386	29.165	40.843	-530.404
POC18-1	4	1.002	-3790.79	6.327	1.827	-1505.47	204.386	33.445	55.125	-202.711
POC18-1	4.5	0.986	-5526.85	6.299	1.463	14856.59	242.258	27.614	52.677	2068.237
POC18-1	5	0.993	-3904.15	6.315	1.47	8037.618	235.912	29.05	46.826	354.341
POC18-1	5.5	1.057	580.584	6.061	3.287	-911.285	213.466	31.575	56.537	7094.557
POC18-1	6	1.051	637.9	6.071	2.897	-984.254	66.141	21.864	71.73	2936.04

POC18-1	6.5	1.051	1586.991	6.136	2.522	13559.25	192.08	18.272	64.434	7252.575
POC18-1	7	0.945	-7862.5	6.423	1.127	6740.507	196.426	22.414	51.974	1392.281
POC18-1	7.5	1.006	-2869.38	6.296	2.042	4451.883	164.479	31.05	59.715	1162.475
POC18-1	8	0.978	-4467.35	6.283	1.511	-629.589	222.28	27.948	60.089	-985.73
POC18-1	8	0.95	-6099.15	6.185	1.917	433.96	227.886	30.514	53.702	1560.984
POC18-1	8	0.963	-6491.66	6.379	1.546	2267.388	133.502	24.601	57.971	1224.201
POC18-1	8.5	0.989	-3991	6.292	1.476	3198.121	321.506	28.521	45.325	3143.414
POC18-1	9	0.977	-6016.25	6.097	1.575	-2238.71	283.34	27.53	58.573	371.013
POC18-1	9.5	0.997	-3332.18	6.239	2.042	-5957.27	439.509	30.679	47.282	-1086.17
POC18-1	10	1.049	1184.778	5.927	2.941	-5151.12	299.821	26.202	51.981	4392.104
POC18-1	10.5	0.974	-5136.71	6.262	1.594	-13526.3	253.557	26.43	56.76	3219.464
POC18-1	11	0.976	-4637.85	6.216	1.611	-6729.05	308.511	22.624	48.582	2138.37
POC18-1	11.5	0.998	-3265.67	6.272	1.868	-2465.6	214.067	27.811	56.044	2316.1
POC18-1	12	0.978	-4454.84	6.266	1.794	1376.318	143.965	29.479	59.639	3646.419
POC18-1	12	1.058	1740.375	5.942	3.151	4960.29	241.015	30.568	52.241	-812.704
POC18-1	12	0.97	-4095.94	6.42	1.522	1383.128	270.548	33.536	49.263	785.194
POC18-1	12.5	0.973	-4185.99	6.266	1.697	-3098.85	333.077	29.639	43.696	-1282.81
POC18-1	13	0.972	-4244.52	6.24	1.773	-332.292	325.739	19.901	46.052	3183.956
POC18-1	14	1.077	1281.362	6.109	2.884	-9291.11	513.743	32.907	33	1043.851
POC18-1	14.5	1.005	-2074.74	6.255	1.93	-4105.31	306.225	29.953	50.349	1115.114
POC18-1	15	0.98	-3192.17	6.337	1.81	-11907.1	435.531	23.513	41.135	1679.192
POC18-1	15.5	0.96	-4555.55	6.483	1.511	1858.132	217.059	29.626	51.41	-470.421
POC18-1	16	1.037	1262.904	6.112	2.794	10560.34	236.929	27.52	49.513	181.961
POC18-1	16.5	0.989	-4168	6.271	1.996	1212.498	263.994	27.452	57.172	2737.355
POC18-1	17	0.976	-3046.79	6.371	2.057	-7574.99	298.274	22.911	58.974	2391.781
POC18-1	18	1.042	1041.849	6.061	2.66	5085.53	219.373	29.758	49.495	1240.184

POC18-1	20	1.073	1751.407	6.008	3.151	-712.737	269.631	24.327	43.925	1065.016
POC18-1	22	0.977	-5241.98	6.42	1.647	5949.434	177.673	23.863	49.552	2627.461
POC18-1	22	0.982	-4006.92	6.267	1.838	-2734.11	401.778	25.853	41.441	358.745
POC18-1	22	0.958	-2888.72	6.382	1.836	-11706.8	407.233	29.987	38.996	-1413.75
location	depth_start	W	Pb	Bi	Th	U				
POC18-1	0	2.805	20.399	1.701	-996.078	5.561				
POC18-1	0.5	2.791	26.397	1.768	496.338	6.034				
POC18-1	1	2.922	34.096	1.739	-3141.33	6.956				
POC18-1	1.5	2.775	28.788	1.739	-7986.89	6.304				
POC18-1	2	2.905	49.301	1.766	2086.125	6.972				
POC18-1	2	2.86	43.999	1.785	24446.65	6.506				
POC18-1	2	2.838	56.302	1.771	-3559.48	7.301				
POC18-1	2.5	3.141	50.829	1.57	6844.142	9.418				
POC18-1	3	3.079	55.606	1.495	924.697	6.4				
POC18-1	3.5	2.907	45.225	1.791	-2720.4	6.41				
POC18-1	4	2.893	43.551	1.694	-8784.56	5.434				
POC18-1	4.5	2.85	46.341	1.552	1858.004	6.125				
POC18-1	5	2.99	57.161	1.512	-2395.55	9.916				
POC18-1	5.5	2.83	66.381	1.878	6492.429	9.427				
POC18-1	6	2.833	85.885	1.448	-98.31	9.011				
POC18-1	6.5	2.893	86.499	1.638	-6959.11	7.688				
POC18-1	7	2.802	94.041	1.534	12224.73	5.482				
POC18-1	7.5	3.06	78.85	1.672	731.842	9.033				
POC18-1	8	2.884	86.223	1.637	-3088.96	4.742				
POC18-1	8	2.82	98.057	1.685	-238.211	7.126				
POC18-1	8	2.962	78.651	1.755	-3037.96	6.887				

POC18-1	8.5	2.813	86.158	1.405	6882.567	7.452				
POC18-1	9	2.675	102.599	1.681	4854.596	3.4				
POC18-1	9.5	2.983	67.163	1.573	-9122.66	6.148				
POC18-1	10	2.895	80.548	1.623	1532.973	6.732				
POC18-1	10.5	2.971	75.886	1.577	199.617	5.494				
POC18-1	11	2.832	65.729	1.486	-3700.41	7.26				
POC18-1	11.5	2.911	65.519	1.67	-7668.04	6.304				
POC18-1	12	2.837	73.875	1.965	-8392.24	5.515				
POC18-1	12	2.941	61.952	1.557	-6905.65	5.407				
POC18-1	12	2.921	82.966	1.584	3437.722	5.429				
POC18-1	12.5	2.899	64.499	1.72	3036.003	5.729				
POC18-1	13	2.842	48.854	1.561	-4618.79	4.426				
POC18-1	14	2.838	67.475	1.906	-5479.73	9.236				
POC18-1	14.5	2.924	57.908	1.565	-3589.26	5.766				
POC18-1	15	2.716	48.878	1.624	-4314.91	5.881				
POC18-1	15.5	2.913	55.674	1.713	6119.058	6.09				
POC18-1	16	2.942	40.48	1.646	-11284.8	4.353				
POC18-1	16.5	2.909	50.608	1.586	178.437	6.482				
POC18-1	17	3.077	47.572	1.625	-2439.73	6.71				
POC18-1	18	2.881	60.61	1.695	-6317.45	4.77				
POC18-1	20	2.939	42.088	1.805	5841.288	10.248				
POC18-1	22	2.788	44.492	1.572	613.164	6.135				
POC18-1	22	3.01	36.459	1.693	-2457.47	7.711				
POC18-1	22	2.912	45.371	1.78	-623.526	7.471				

APPENDIX D: CLADOCERA RELATIVE ABUNDANCE DATA

core	location	sample_depth	taxon	count	rel_abund	date
POC18-1	Pockwock	0.25	Bosminidae	60	0.81	2018.43255
POC18-1	Pockwock	2.75	Bosminidae	116	0.72	2012.24082
POC18-1	Pockwock	4.25	Bosminidae	194	0.83	2006.11044
POC18-1	Pockwock	5.75	Bosminidae	243	0.81	1999.08354
POC18-1	Pockwock	7.25	Bosminidae	148	0.75	1993.47948
POC18-1	Pockwock	8.25	Bosminidae	175	0.74	1990.21554
POC18-1	Pockwock	10.25	Bosminidae	61	0.66	1982.76855
POC18-1	Pockwock	12.25	Bosminidae	77	0.7	1974.15652
POC18-1	Pockwock	14.25	Bosminidae	80	0.77	1966.18405
POC18-1	Pockwock	15.25	Bosminidae	64	0.7	1962.64686
POC18-1	Pockwock	18.25	Bosminidae	92	0.71	1949.10318
POC18-1	Pockwock	20.25	Bosminidae	83	0.77	1936.094
POC18-1	Pockwock	21.25	Bosminidae	117	0.74	1929.34806
POC18-1	Pockwock	23.25	Bosminidae	91	0.7	1912.14644
POC18-1	Pockwock	25.25	Bosminidae	49	0.54	1890.07415
POC18-1	Pockwock	26.25	Bosminidae	57	0.63	1875.03175
POC18-1	Pockwock	0.25	Daphniadae	1	0.01	2018.43255
POC18-1	Pockwock	2.75	Daphniadae	7	0.04	2012.24082
POC18-1	Pockwock	4.25	Daphniadae	6	0.03	2006.11044
POC18-1	Pockwock	5.75	Daphniadae	7	0.02	1999.08354
POC18-1	Pockwock	7.25	Daphniadae	17	0.09	1993.47948
POC18-1	Pockwock	8.25	Daphniadae	22	0.09	1990.21554
POC18-1	Pockwock	10.25	Daphniadae	10	0.11	1982.76855
POC18-1	Pockwock	12.25	Daphniadae	16	0.15	1974.15652
POC18-1	Pockwock	14.25	Daphniadae	11	0.11	1966.18405
POC18-1	Pockwock	15.25	Daphniadae	11	0.12	1962.64686
POC18-1	Pockwock	18.25	Daphniadae	15	0.12	1949.10318
POC18-1	Pockwock	20.25	Daphniadae	12	0.11	1936.094
POC18-1	Pockwock	21.25	Daphniadae	20	0.13	1929.34806
POC18-1	Pockwock	23.25	Daphniadae	22	0.17	1912.14644
POC18-1	Pockwock	25.25	Daphniadae	20	0.22	1890.07415
POC18-1	Pockwock	26.25	Daphniadae	17	0.19	1875.03175
POC18-1	Pockwock	0.25	Chydoridae	6	0.08	2018.43255
POC18-1	Pockwock	2.75	Chydoridae	21	0.13	2012.24082
POC18-1	Pockwock	4.25	Chydoridae	20	0.09	2006.11044
POC18-1	Pockwock	5.75	Chydoridae	23	0.08	1999.08354
POC18-1	Pockwock	7.25	Chydoridae	21	0.11	1993.47948

POC18-1	Pockwock	8.25	Chydoridae	30	0.13	1990.21554
POC18-1	Pockwock	10.25	Chydoridae	15	0.16	1982.76855
POC18-1	Pockwock	12.25	Chydoridae	11	0.1	1974.15652
POC18-1	Pockwock	14.25	Chydoridae	9	0.09	1966.18405
POC18-1	Pockwock	15.25	Chydoridae	10	0.11	1962.64686
POC18-1	Pockwock	18.25	Chydoridae	14	0.11	1949.10318
POC18-1	Pockwock	20.25	Chydoridae	9	0.08	1936.094
POC18-1	Pockwock	21.25	Chydoridae	16	0.1	1929.34806
POC18-1	Pockwock	23.25	Chydoridae	16	0.12	1912.14644
POC18-1	Pockwock	25.25	Chydoridae	16	0.18	1890.07415
POC18-1	Pockwock	26.25	Chydoridae	14	0.15	1875.03175
POC18-1	Pockwock	0.25	Sididae	5	0.07	2018.43255
POC18-1	Pockwock	2.75	Sididae	13	0.08	2012.24082
POC18-1	Pockwock	4.25	Sididae	13	0.06	2006.11044
POC18-1	Pockwock	5.75	Sididae	19	0.06	1999.08354
POC18-1	Pockwock	7.25	Sididae	9	0.05	1993.47948
POC18-1	Pockwock	8.25	Sididae	9	0.04	1990.21554
POC18-1	Pockwock	10.25	Sididae	6	0.07	1982.76855
POC18-1	Pockwock	12.25	Sididae	2	0.02	1974.15652
POC18-1	Pockwock	14.25	Sididae	4	0.04	1966.18405
POC18-1	Pockwock	15.25	Sididae	6	0.07	1962.64686
POC18-1	Pockwock	18.25	Sididae	7	0.05	1949.10318
POC18-1	Pockwock	20.25	Sididae	2	0.02	1936.094
POC18-1	Pockwock	21.25	Sididae	1	0.01	1929.34806
POC18-1	Pockwock	23.25	Sididae	1	0.01	1912.14644
POC18-1	Pockwock	25.25	Sididae	4	0.04	1890.07415
POC18-1	Pockwock	26.25	Sididae	3	0.03	1875.03175
POC18-1	Pockwock	0.25	Holopedidae	0	0	2018.43255
POC18-1	Pockwock	2.75	Holopedidae	0	0	2012.24082
POC18-1	Pockwock	4.25	Holopedidae	0	0	2006.11044
POC18-1	Pockwock	5.75	Holopedidae	0	0	1999.08354
POC18-1	Pockwock	7.25	Holopedidae	0	0	1993.47948
POC18-1	Pockwock	8.25	Holopedidae	0	0	1990.21554
POC18-1	Pockwock	10.25	Holopedidae	0	0	1982.76855
POC18-1	Pockwock	12.25	Holopedidae	0	0	1974.15652
POC18-1	Pockwock	14.25	Holopedidae	0	0	1966.18405
POC18-1	Pockwock	15.25	Holopedidae	0	0	1962.64686
POC18-1	Pockwock	18.25	Holopedidae	0	0	1949.10318
POC18-1	Pockwock	20.25	Holopedidae	0	0	1936.094
POC18-1	Pockwock	21.25	Holopedidae	0	0	1929.34806
POC18-1	Pockwock	23.25	Holopedidae	0	0	1912.14644

POC18-1	Pockwock	25.25	Holopedidae	0	0	1890.07415
POC18-1	Pockwock	26.25	Holopedidae	0	0	1875.03175
POC18-1	Pockwock	0.25	Iliocryptidae	0	0	2018.43255
POC18-1	Pockwock	2.75	Iliocryptidae	0	0	2012.24082
POC18-1	Pockwock	4.25	Iliocryptidae	0	0	2006.11044
POC18-1	Pockwock	5.75	Iliocryptidae	0	0	1999.08354
POC18-1	Pockwock	7.25	Iliocryptidae	0	0	1993.47948
POC18-1	Pockwock	8.25	Iliocryptidae	0	0	1990.21554
POC18-1	Pockwock	10.25	Iliocryptidae	0	0	1982.76855
POC18-1	Pockwock	12.25	Iliocryptidae	3	0.03	1974.15652
POC18-1	Pockwock	14.25	Iliocryptidae	0	0	1966.18405
POC18-1	Pockwock	15.25	Iliocryptidae	0	0	1962.64686
POC18-1	Pockwock	18.25	Iliocryptidae	0	0	1949.10318
POC18-1	Pockwock	20.25	Iliocryptidae	0	0	1936.094
POC18-1	Pockwock	21.25	Iliocryptidae	0	0	1929.34806
POC18-1	Pockwock	23.25	Iliocryptidae	0	0	1912.14644
POC18-1	Pockwock	25.25	Iliocryptidae	0	0	1890.07415
POC18-1	Pockwock	26.25	Iliocryptidae	0	0	1875.03175
POC18-1	Pockwock	0.25	Leptodoridae	0	0	2018.43255
POC18-1	Pockwock	2.75	Leptodoridae	1	0.01	2012.24082
POC18-1	Pockwock	4.25	Leptodoridae	0	0	2006.11044
POC18-1	Pockwock	5.75	Leptodoridae	1	0	1999.08354
POC18-1	Pockwock	7.25	Leptodoridae	1	0.01	1993.47948
POC18-1	Pockwock	8.25	Leptodoridae	0	0	1990.21554
POC18-1	Pockwock	10.25	Leptodoridae	0	0	1982.76855
POC18-1	Pockwock	12.25	Leptodoridae	1	0.01	1974.15652
POC18-1	Pockwock	14.25	Leptodoridae	0	0	1966.18405
POC18-1	Pockwock	15.25	Leptodoridae	1	0.01	1962.64686
POC18-1	Pockwock	18.25	Leptodoridae	0	0	1949.10318
POC18-1	Pockwock	20.25	Leptodoridae	0	0	1936.094
POC18-1	Pockwock	21.25	Leptodoridae	1	0.01	1929.34806
POC18-1	Pockwock	23.25	Leptodoridae	0	0	1912.14644
POC18-1	Pockwock	25.25	Leptodoridae	1	0.01	1890.07415
POC18-1	Pockwock	26.25	Leptodoridae	0	0	1875.03175
POC18-1	Pockwock	0.25	Macrothricidae	1	0.01	2018.43255
POC18-1	Pockwock	2.75	Macrothricidae	0	0	2012.24082
POC18-1	Pockwock	4.25	Macrothricidae	0	0	2006.11044
POC18-1	Pockwock	5.75	Macrothricidae	3	0.01	1999.08354
POC18-1	Pockwock	7.25	Macrothricidae	1	0.01	1993.47948
POC18-1	Pockwock	8.25	Macrothricidae	1	0	1990.21554
POC18-1	Pockwock	10.25	Macrothricidae	0	0	1982.76855

POC18-1	Pockwock	12.25	Macrothricidae	0	0	1974.15652
POC18-1	Pockwock	14.25	Macrothricidae	0	0	1966.18405
POC18-1	Pockwock	15.25	Macrothricidae	0	0	1962.64686
POC18-1	Pockwock	18.25	Macrothricidae	0	0	1949.10318
POC18-1	Pockwock	20.25	Macrothricidae	1	0.01	1936.094
POC18-1	Pockwock	21.25	Macrothricidae	2	0.01	1929.34806
POC18-1	Pockwock	23.25	Macrothricidae	0	0	1912.14644
POC18-1	Pockwock	25.25	Macrothricidae	0	0	1890.07415
POC18-1	Pockwock	26.25	Macrothricidae	0	0	1875.03175
POC18-1	Pockwock	0.25	Polyphemidae	1	0.01	2018.43255
POC18-1	Pockwock	2.75	Polyphemidae	3	0.02	2012.24082
POC18-1	Pockwock	4.25	Polyphemidae	0	0	2006.11044
POC18-1	Pockwock	5.75	Polyphemidae	3	0.01	1999.08354
POC18-1	Pockwock	7.25	Polyphemidae	1	0.01	1993.47948
POC18-1	Pockwock	8.25	Polyphemidae	0	0	1990.21554
POC18-1	Pockwock	10.25	Polyphemidae	0	0	1982.76855
POC18-1	Pockwock	12.25	Polyphemidae	0	0	1974.15652
POC18-1	Pockwock	14.25	Polyphemidae	0	0	1966.18405
POC18-1	Pockwock	15.25	Polyphemidae	0	0	1962.64686
POC18-1	Pockwock	18.25	Polyphemidae	1	0.01	1949.10318
POC18-1	Pockwock	20.25	Polyphemidae	1	0.01	1936.094
POC18-1	Pockwock	21.25	Polyphemidae	2	0.01	1929.34806
POC18-1	Pockwock	23.25	Polyphemidae	0	0	1912.14644
POC18-1	Pockwock	25.25	Polyphemidae	1	0.01	1890.07415
POC18-1	Pockwock	26.25	Polyphemidae	0	0	1875.03175