INTEGRATING CLASSICAL AND DNA-BASED APPROACHES TO ADVANCE THE FIELD OF PALEOLIMNOLOGY: CASE STUDIES FROM A WARM MONOMICTIC LAKE

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ABSTRACT

Paleolimnology offers insights into long-term perspective in freshwater ecosystems and is useful to fill gaps in historical limnological data. Using multiple indicators to reconstruct ecological trajectory of lakes may strengthen our comprehension of their ecology over long time periods. Past ecological changes of lakes are often based on morphological remains archived in the sediments (e.g., diatom subfossils, cladocerans remains) as well as geochemical indicators (e.g., algal pigments, carbon and nitrogen content). However, many other organisms (e.g., bacteria, protists, copepods), which might play essential roles in the aquatic food webs, are not widely studied in paleolimnology. Technical advancements in molecular methods have opened the possibility of using DNA-based approaches applied to sediment extracts, which can greatly expand the number of taxa studied in paleolimnology. My research aimed (1) to evaluate the advantages and limitations of using DNA-based approaches in paleolimnology and (2) use both classical and DNA-based approaches to reconstruct past ecological dynamics of a peri-urban lake, Cultus Lake, British Columbia. In my second chapter, I used a contemporary 36-month time series to evaluate which taxa DNA can be deposited in the sediments. The specific goals were to assess the extent to which the micro-eukaryotic communities identified with the 18S rRNA gene in the sediments reflect the biological communities present in the water column, and to assess the congruence between morphological and DNA identification of diatoms and crustaceans in water and sediment extracts. From this chapter, I identified other potential taxonomic groups of organisms that could be studied in paleolimnology, such as ciliates, dinoflagellates, chytrids and cercozoans. The results also showed that DNA-based approaches are robust enough to reconstruct ecological dynamics from sediments, when compared to morphological data. Using morphological identification of diatoms from chapter 2 and

contemporary limnological data, I evaluated the present ecological conditions of Cultus Lake to better assess how the lake deviated from its reference period. In chapter 3, I used multiple paleolimnological indicators and archival material of human history to reconstruct the ecological changes of Cultus Lake. The lake has experienced modest eutrophication since the mid-1900s, which was related to multiple stressors, such as an increase of anthropogenic use of the watershed, a warmer regional climate mainly after the 1970s, and a decrease in sockeye salmon escapement returning to the lake. This chapter indicates the importance of studying long-term perspective to understand the complexity of changes in lake ecosystems and their related drivers. In my last chapter, my objectives were to evaluate how micro-eukaryotic communities reconstructed with 18S rRNA gene changed in Cultus Lake over time and to compare the changes observed in micro-eukaryotic communities to the changes in classical paleolimnological indicators from chapter 3. Using both extracellular and intracellular DNA fractions archived in the sediments, I found that micro-eukaryotic community dynamics followed similar temporal dynamics than classical indicators. Intracellular DNA was more suitable to track long time periods as extracellular DNA seemed to have preservation issues after 30 years of burial. Overall, my doctoral thesis demonstrated that DNA-based approaches applied to sediment extracts were efficient to reconstruct past biological conditions and could increase our knowledge of ecological dynamics of lakes on a longer time scale when applied simultaneously with classical paleolimnological indicators.

RÉSUMÉ

La paléolimnologie permet d'étudier les écosystèmes aquatiques sur de longues périodes temporelles et d'obtenir de l'information additionnelle aux données limnologiques historiques existantes. Utiliser plusieurs indicateurs simultanément pour reconstruire la trajectoire écologique des lacs permet d'approfondir notre compréhension de la dynamique des lacs sur de longues périodes de temps. Les changements écologiques passés des lacs sont généralement basés sur des microfossiles morphologiques archivés dans les sédiments (e.g., diatomées et cladocères) et sur des indicateurs géochimiques (e.g., pigments algaux, carbone et azote). Toutefois, plusieurs autres organismes, pouvant avoir des rôles essentiels dans les réseaux trophiques aquatiques (e.g., bactéries, protistes, copépodes) ne sont généralement pas étudiés en paléolimnologie. Les avancements technologiques en méthodes moléculaires ont permis l'application des approches basées sur l'ADN dans les sédiments, ce qui a grandement augmenter le nombre de taxa pouvant être étudiés en paléolimnologie. Ma recherche avait pour buts (1) d'évaluer les avantages et les inconvénients d'utiliser l'ADN archivé dans les sédiments dans les études paléolimnologiques, et (2) d'utiliser les approches classiques et basées sur l'ADN pour reconstruire les dynamiques écologiques passées d'un lac péri-urbain, Cultus Lake, Colombie-Britannique. Dans mon second chapitre, une série temporelle contemporaine de 36 mois a été utilisée afin d'évaluer l'ADN des taxa pouvant être déposé dans les sédiments. Les objectifs spécifiques étaient d'évaluer à quel degré les communautés de micro-eucaryotes identifiées avec le gène 18S ARNr dans les sédiments reflètent les communautés biologiques présentes dans la colonne d'eau, et d'évaluer la congruence entre l'identification morphologique et par l'ADN des diatomées et des crustacés. Les résultats de ce chapitre ont permis d'identifier d'autres groupes taxonomiques potentiels pouvant être étudiés en paléolimnologie. Les résultats

montrent également que les l'ADN sédimentaire est robuste pour reconstruire les dynamiques écologiques. À l'aide de l'identification morphologique des diatomées du chapitre 2 et de données limnologiques contemporaines, les conditions écologiques récentes de Cultus Lake ont été évaluées afin d'estimer la déviation de son état de référence. Dans le chapitre 3, plusieurs indicateurs paléolimnologiques ont été utilisés, ainsi que des archives historiques, pour reconstruire les changements écologiques de Cultus Lake. Ce lac a subi une eutrophisation modeste depuis le milieu des années 1900, qui a été occasionné par une augmentation de l'utilisation anthropique du bassin versant, par un réchauffement du climat régional dans les années 1970, et par une diminution du retour des saumons rouges adultes. Ce chapitre indique l'importance d'avoir une perspective sur de longues périodes pour comprendre la complexité des changements des écosystèmes lacustres et les causes reliées aux changements. Dans mon dernier chapitre, les objectifs étaient d'évaluer les changements des communautés micro-eucaryotes identifiées avec le gène 18S ARNr et de comparer les changements observés avec l'ADN à ceux des indicateurs paléolimnologiques du chapitre 3. À l'aide des fractions d'ADN extracellulaire et intracellulaire archivées dans les sédiments, les communautés de micro-eucaryotes ont montré des dynamiques similaires aux indicateurs classiques. Toutefois, l'ADN intracellulaire semble être plus adéquate pour reconstruire les communautés sur de longues périodes puisque l'ADN extracellulaire semble avoir des problèmes de préservation après 30 ans d'enfouissement. Globalement, ma thèse de doctorat montre que l'ADN archivé dans les sédiments peut efficacement reconstruire les conditions biologiques passées et peut accroître notre connaissance des dynamiques écologiques des lacs sur les longues périodes en les appliquant simultanément à des indicateurs paléolimnologiques.

ACKNOWLEDGEMENTS

I decided to do a PhD as I wanted to have the ability to create and supervise my own research projects. I wanted to have the knowledge and experience to build amazing and innovative projects. Now, I believe I came to be the researcher I wished to be when I started my PhD. To get to this point, I want to acknowledge my supervisors, Dr. Irene Gregory-Eaves and Dr. David Walsh. Both brought me different aspects of the experience I have now, and they were a great pair to supervise me as a graduate student. Rene, I want to thank you for teaching me how to do great science and preparing me for the rest of my scientific career. Your support and your supervision was deeply appreciated. David, your way of thinking out of the box compare to most scientists I know, and your directness helped me to reach my goals, but mainly it gave me a perspective in science that I will remember and apply in my future projects. I know it was not always easy to deal with me for many personal issues I had to go through, but I really thank you both for being so patient with me and supported me the way you could through my chaotic life at the time. I really wish that I will collaborate with you both in the future. I also want to thank Dr. Daniel Selbie for all his efforts and work in all my projects and for helping me to develop broader thinking to have a stronger paleolimnological perspective.

During my PhD, I had a challenging time in my personal life. My research projects helped me to go through difficult time as I am very passionate about it. Many people listened to me, supported me and encouraged me to keep going and be patient that better time would come. First of all, I want to thank Mark Edlund, my diatom instructor, who became a good friend and collaborator. He supported me with his words and listened to me more than I could have wished. For the first time of my life, I went to see a psychologist and I want to tell her that she was

amazing, and I could not imagine getting through this time without her. She helped me to understand and figure out so many issues in my life, and now I feel better with myself and I am stronger than ever. Other people became amazing friends through this time, and I want to acknowledge their support: Adam Heathcote, Gabrielle Trottier, Leanne Elchyshyn and Amanda Winegardner. Specifically, I want to thank Leanne, who always helped me when my brain my foggy in the lab and supported me psychologically. Many thanks to you Gabrielle for all these kitting workshops and evenings where I could just distract my brain. I now have this passion for knitting, and I built so much self-confidence because of this hobby.

I also wish to thank many people working at Fisheries and Oceans Canada (DFO) who contributed for the sampling: Kelly Malange, Steve MacDonalds, Garrett Lidin and Lucas Pon. I want to give special thanks to Kelly Malange for managing all the samples and shipping them to McGill University as well as preparing and sharing the DFO limnological data that I needed. Part of my PhD project contained a high volume of laboratory work and many undergraduate students helped to process the samples. I want to thank each of them as they were wonderful workers and most of them also became friends. Thanks to Allene Kennedy, Kathryn Yici Han, Hannah Scanlon, Geervani Daggupati, Jenna Dilworth and Paul Mackeigan. Thanks to Brian Cummings and Kate Laird for their help to understand and apply transfer functions. I want to acknowledge the teaching and help from Heba El-Swais at the beginning of my molecular work as I was new to molecular methods. I also want to thank Stephanie Pacheco and Arthi Ramachandran for helping with developing standards for quantitative PCR and applying this method in the laboratory. Thanks to Patricia Tran who helped me for the bioinformatic processing of my sequencing data and from whom I learned a great deal about programming.

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PREFACE

Thesis format and style

This thesis is manuscript-based and includes three manuscripts. The manuscripts have been written to be submitted in scientific journals in disciplines related to paleolimnology.

Gauthier J, Walsh D, Selbie DT, Bourgeois A, Griffiths K, Domaizon I, Gregory-Eaves I (In revision) Evaluating the congruence between DNA-based and morphological taxonomic approaches in water and sediment trap samples: Analyses of a 36-month time series from a temperate monomictic lake. Submitted to *Limnology and Oceanography* in August 2020.

Gauthier J, Gregory-Eaves I, Bunting L, Leavitt PR, Tran T, Godbout L, Finney BP, Schindler DE, Chen G, Holtgrieve G, Shapley M, Selbie DT (In Press) Ecological dynamics of a peri-urban lake: A multi-proxy paleolimnological study of Cultus Lake (British Columbia) over the past ~200 years. Journal of Paleolimnology. Accepted May 2020. doi: 10.1007/s10933-020-00147-9

Gauthier J, Walsh D, Selbie DT, Domaizon I, Gregory-Eaves I. Analysis of 18S rRNA amplicons from sediment DNA of a human-impacted lake in Western Canada (Cultus Lake) reveals changes in micro-eukaryotic diversity over the past ~200 years. In preparation to be submitted to *Environmental DNA*.

The datasets generated for each chapter of this thesis were deposited in a repository as closed access and metabarcoding datasets will be publicly released upon acceptance of the manuscripts.

Gauthier J (2020) Datasets from PhD thesis: "Integrating classical and DNA-based approaches to advance the field of paleolimnology: Case studies of a warm monomictic lake". doi: 10.5281/zenodo.3994994

To make the formatting style consistent throughout my doctoral thesis, I have followed the format from the *Journal of Paleolimnology* as my third chapter was accepted in May 2020 to be published in this journal. The use of the first plural person refers to all co-authors included in the chapters. In all other sections of the thesis, the first singular person is used. The tables and figures are presented at the end of the appropriate chapter while the supplementary materials are presented at the end of the thesis.

Contribution of Authors

Although all chapters were written and developed in close collaboration with my co-authors, I was usually responsible of the project design, laboratory analyses, data management, statistical analyses and writing the first draft of the manuscripts. My supervisors, Irene Gregory-Eaves and David Walsh supervised and advised me during all steps of my projects and substantially contributed to write each manuscript. For Chapter 2, I designed the project with my co-authors, built the sampling devices (i.e., sediment traps), organize the long-term sampling and helped occasionally with the sampling. The field work was mainly conducted by staff from

Fisheries and Oceans Canada. I performed the majority of the laboratory analyses, but many undergraduate students help processing the samples. Daniel T. Selbie contributed substantially to the sampling of water and sediment trap samples, provided limnological data, helped in the interpretation of the limnology of Cultus Lake and contributed in the revision of the manuscript. Isabelle Domaizon contributed intellectually to develop the main methodological approach for DNA extraction from sediments and provided suggestions and comments during the course of the laboratory work. She also revised the manuscript and helped interpret further the results. Alyssa Bourgeois and Katherine Griffiths performed the cladoceran morphological analyses, from processing the samples to analyzing the data through microscopy. They also provided comments and suggestions on the manuscript. Chapter 3 was already designed intellectually by Daniel T. Selbie, Peter R. Leavitt, Lyse Godbout, Bruce P. Finney and Daniel E. Schindler. Daniel T. Selbie did the field work with Mark Shapley and Gordon Holtgrieve, who also provided suggestion to develop the project. Most of the laboratory analyses were previously performed by Lynda Bunting, Tanya Tran and Guangjie Chen. For Chapter 3, I identified and enumerated the diatom subfossils in the core samples, I managed all the data available for the project and structured and wrote the manuscript in close collaboration with Irene Gregory-Eaves and Daniel T. Selbie. All co-authors provided comments and suggestions for the manuscript. I conducted the fieldwork for Chapter 4 with Daniel T. Selbie. For Chapters 2 and 4, I also performed the molecular analyses and analysed the sequencing data. Daniel T. Selbie and Isabelle Domaizon contributed in Chapter 4 in a similar manner than in Chapter 2.

Statement of Originality

The research projects herein were developed in collaborative effort with my supervisors, Dr. Irene Gregory-Eaves and Dr. David Walsh. There are two main objectives in this thesis, which are (1) to evaluate the advantages and limitations of DNA-based approaches applied to sediment extracts to reconstruct past ecological trajectory of lakes; and (2) to assess the ecological trajectory of Cultus Lake in the past ~200 years using both classical and molecular approaches. Thus, my PhD projects integrated both classical indicators (i.e., geochemical and subfossils) as well as sedimentary DNA (sedDNA) to evaluate the congruency of the ecological dynamics between methods. The chapters of my thesis were developed in 2014 when I began my PhD. Although work has been achieved in the field of paleo-genetic since then, all my projects were developed to advance knowledge at that time, and my projects are still highly novel to this date. The chapters of my thesis span contemporary to centennial scale ecological dynamics and integrates archival research to better identify drivers of change in Cultus Lake.

Chapter 1

Chapter 1 introduces the main topics included in this thesis, such as paleolimnology, sedDNA, ecology of micro-eukaryotes. It also provides a description of the sampling site.

Chapter 2

DNA-based approaches are becoming popular as they can reconstruct past population and community dynamics and enhance our comprehension of ecological change in lakes. Some preliminary work with DNA-based approaches applied to lake sediment extracts has focused on evaluating the congruence between morphological and DNA taxonomical identification (e.g., Epp et al. 2011; Stoof-Leichsenring et al. 2012, 2014; Dulias et al. 2017). Fewer work has been conducted to assess whether the biological signal retrieved from the sedDNA is representative of the biological signal in the water column (e.g., Monchamp et al. 2016; Capo et al. 2017). In Chapter 2, I was interested to evaluate whether the community dynamics from the water column could be reconstructed with contemporary sediment extracts. Sediment traps were deployed at ~3 m above the sediments on a monthly basis for three years. At the same time than the sediment traps deployments, Fisheries and Oceans Canada (DFO) monitored the limnology of Culus Lake. DFO also collected samples from the water column for DNA analyses to enable the comparisons between paired samples of water and sediment traps. Chapter 2 investigated contemporary ecological dynamics using DNA-based approaches applied to water and sediment extracts to evaluate which taxa can be deposited in the sediments to be further explored in paleolimnology.

Chapter 3

Cultus Lake is a peri-urban lake near the metro city of Vancouver, and it has been moderately disturbed in the last century. It provides valuable ecosystem services for human populations, such as aesthetic value, fishing and recreational activities (e.g., boating, swimming, camping). In the last years, 2 to 3 millions people have visited Cultus Lake every year (FVRD

2011), mainly during the summer. Furthermore, Cultus Lake is a habitat for two species at risk: the endangered Cultus Lake sockeye salmon (*Oncorhynchus nerka*; COSEWIC 2003) and the endemic, threatened Cultus pygmy sculpin (*Cottus aleuticus*, Cultus Population; Rosenfield et al. 2007; COSEWIC 2010; Government of Canada 2011). Historically, the Cultus Lake sockeye salmon population has supported important commercial and subsistence fisheries, but escapement has declined substantially over the past ~50 years (Cultus Sockeye Recovery Team 2005; Shortreed 2007). In this chapter, I sought to assess the ecological trajectory of Cultus Lake in the last ~200 years and identify the major drivers of change. I used archival material, historical and contemporary limnological data as well as multiple paleolimnological indicators to answer these objectives. The use of multiple indicators in paleolimnology help to strengthen the relationship between the indicator dynamics and the drivers as some indicators can responded differently to the same driver. With this chapter, I was able to identify a reference period for the ecology of Cultus Lake and could evaluate the effects of anthropogenic activities and regional climate on Cultus Lake over the past ~200 years. This chapter stresses the fact that long-term data are necessary to better understand lake ecological trajectory to apply better management practices for our freshwater ecosystems.

Chapter 4

In Chapter 4, I further evaluate the advantages and limitations of using DNA-based approaches in paleolimnology. In Chapter 2, the main focus was to investigate which micro-eukaryotic taxa could be deposited in the sediments through their DNA while Chapter 4 focused mainly on which DNA taxa could be archived and preserved in the sediments. In Chapter 4, I

also wanted to assess whether the potential indicator taxa identified in Chapter 2 were adequately buried in the sediments. I compared the changes observed with the classical multi-proxy paleolimnological study (Chapter 3) with the biological dynamics observed with sedDNA (Chapter 4). A sediment core was collected in 2017 to apply DNA-based approaches to sediment intervals. Both extracellular and intracellular DNA fractions were targeted as most of the paleo-genetic work in lakes has focused on total DNA or on prokaryotic communities to date. Extracellular and intracellular DNA fractions were compared to evaluate which fraction can reconstruct more adequately the biological changes over the last ~200 years. This chapter provides insights into the use of extracellular and intracellular DNA. It also deepens our understanding of micro-eukaryotic community dynamics over centennial time scale in Cultus Lake.

Literature Cited

Capo, E, Debroas D, Arnaud F, Perga M-E, Chardon C, Domaizon I (2017) Tracking a century of changes in microbial eukaryotic diversity in lakes driven by nutrient enrichment and climate warming: Long-term dynamics of microbial eukaryotes. Environ Microbiol 19: 2873–2892. doi:10.1111/1462-2920.13815

COSEWIC (2003) COSEWIC assessment and status report on the sockeye salmon Oncorhynchus nerka (Cultus population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. ix + 57 pp.

COSEWIC (2010) COSEWIC assessment and status report on the Coastrange Sculpin *Cottus aleuticus*, Cultus Population in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi + 28 pp. (www.sararegistry.gc.ca/status/status_e.cfm).

Cultus Sockeye Recovery Team (2005) Conservation strategy for sockeye salmon (*Onchorhynchus nerka*), Cultus Lake population, in British Columbia. Recovery of Nationally Endangered Wildlife (RENEW).

Dulias K, Stoof-Leichsenring KR, Pestryakova LA, and Herzschuh U (2017) Sedimentary DNA versus morphology in the analysis of diatom-environment relationships. J Paleolimnol 57: 51–66. doi:10.1007/s10933-016-9926-y

Epp, LS, Stoof-Leichsenring KR, Trauth MH, and Tiedemann R (2011) Molecular profiling of diatom assemblages in tropical lake sediments using taxon-specific PCR and Denaturing High-Performance Liquid Chromatography (PCR-DHPLC). Mol Ecol Resour 11: 842–853. doi:10.1111/j.1755-0998.2011.03022.x

FVRD (2011) Official community plan for electoral area E bylaw. Fraser Valley Regional District, British Columbia, Canada

Government of Canada EC (2011) HTML Version of the Document - Species at risk public registry. http://www.registrelep-sararegistry.gc.ca/document/dspHTML_e.cfm?ocid=8366. Accessed 23 Oct 2017

Monchamp M-E, Walser J-C, Pomati F, Spaak P (2016) Sedimentary DNA reveals cyanobacteria community diversity over 200 years in two peri-alpine lakes. Appl Environ Microbiol AEM 02174-16. doi:10.1128/AEM.02174-16

Rosenfield J, Department of Fisheries and Oceans (2007) Recovery strategy for Cultus pygmy sculpin (*Cottus* sp.) in Canada. Fisheries and Oceans Canada, Ottawa, Canada

Shortreed, KS (2007) Limnology of Cultus Lake, British Columbia. Can Tech Rep Fish Aquat Sci. 2753: vi + 85p.

Stoof-Leichsenring, KR, Bernhardt N, Pestryakova LA, Epp LS, Herzschuh U, and Tiedemann R (2014) A combined paleolimnological/genetic analysis of diatoms reveals divergent evolutionary lineages of Staurosira and Staurosirella (Bacillariophyta) in Siberian lake sediments along a latitudinal transect. J Paleolimnol 52: 77–93. doi:10.1007/s10933-014-9779-1

Stoof-Leichsenring, KR, Epp LS, Trauth MH, and Tiedemann R. 2012. Hidden diversity in diatoms of Kenyan Lake Naivasha: a genetic approach detects temporal variation. Mol Ecol 21: 1918–1930. doi:10.1111/j.1365-294X.2011.05412.x

CHAPTER 1 – GENERAL INTRODUCTION

Over the last century, aquatic ecosystems have been characterized by many modifications, mainly derived from anthropogenic activities and climate change (Reid et al. 2018). These changes have intensified since the 1970s (Reid et al. 2018), and critical management is required to avoid further degradation of freshwater systems. Although freshwater ecosystems (lakes, rivers, reservoirs) account only for 2.3 % of the Earth's surface, they provide habitat for at least 9.5 % of the Earth's animal described species (Reid et al. 2018). Many other species, mainly protists, fungi and bacteria living in aquatic ecosystems, are not yet described, but could only increase the percentage of the Earth's species living in freshwater systems.

Water is the most important resource for living organisms and good water quality is required to sustain biological populations as well as human populations. Water ecosystems provide an important source of food (e.g., fish) for human populations. Without appropriate management, loss of fish habitats would negatively affect human populations depending on these resources to survive. As freshwater is an important source of fish, part of the economy of many countries is based on this resource, and present and future regional and global economy could be affected negatively without appropriate management of fish habitats. Other functions of freshwater ecosystems could be lost, such as cultural, aesthetic and recreational functions (Vörösmarty et al. 2010; Waltham et al. 2014). Applying appropriate management practices is thus necessary for water resources as populations are growing worldwide, which increase the overall disturbances on freshwater (e.g., nutrients from agricultural and septic runoffs, terrestrial and atmospheric pollutants, boat traffic, high recreational use, invasive species). Climate change is also modifying freshwater ecosystems (Battarbee 2010; Moss et al. 2010), and potential

interactive effects of multiple stressors could lead to unexpected ecological outcomes (Christensen et al. 2006).

To apply appropriate management practices on freshwater ecosystems, it is crucial to understand long-term ecological dynamics of lakes. Long-term limnological and biological data are necessary, but limnological time series usually cover only contemporary time scale (i.e., at best a few decades where data have been collected). Applying paleolimnology on lakes can help fill the gap where data are missing to better understand the ecological trajectory of lakes on a centennial scale (Smol 2008). Paleolimnology refers to the reconstruction of past environmental conditions of inland waters by using physical, chemical and biological information preserved in lake sediments (Smol 2008). The word *paleolimnology* derives from the Greek nouns "palaio" and "limnē", which mean respectively ancient and lakes or inland waters. Lake sediments are natural archives from which biological community changes can be reconstructed over centuries and even millennia. Paleolimnology has been proven a powerful field of study to better understand the effects of stressors (e.g., anthropogenic activities, climate change) on aquatic ecosystems by reconstructing past ecological dynamics (Smol 2008). Paleolimnology has also been useful to evaluate the adequacy of management practice on aquatic ecosystems (Smol 2008; Gillson and Marchant 2014; Saulnier-Talbot 2016).

Paleolimnology coupled with limnology for better management of lake ecosystems

Identifying a reference period in lake ecosystems can provide information on the deviations from their stable state, but also identify the major drivers of these changes. Combining research of archival material as well as contemporary limnological data can help understand

better the ecological trajectory of lakes (Moorhouse et al. 2014), but also assess the most appropriate management practices for aquatic ecosystems. A notion from the Huttonian Uniformitarianism analogy is that: "The present is the key to the past" (Simpsons 2012), which contributed to the key assumption in paleolimnology to reason by analogy (Birks et al. 2010). Reasoning by analogy allows to identify conservation goals to manage aquatic ecosystems by identifying undisturbed conditions of lakes (Simpsons 2012).

Paleolimnology is mostly based on geochemical indicators and morphological identification of subfossils archived in the sediments. The most common subfossils retrieved and identified in sediments are diatoms (Battarbee et al. 2001), chrysophytes scales and cysts (Zeeb and Smol 2001), remains of cladocerans (Alric and Perga 2011) and chironomids (Walker 2001). However, many other organisms without diagnostic characteristics preserved in the sediments (i.e., difficult to identify or no remains) are essential part of the aquatic food web structure in the pelagic environment and could be useful bioindicators for aquatic ecosystem changes over longer time scale. In recent years, there is an increasing interest to use molecular methods to detect environmental DNA in sediments (e.g., Coolen and Gibson 2009; Boere et al. 2011a; Domaizon et al. 2013; Savichtcheva et al. 2015) and the application of these tools to the sediment record has already shown great promises to provide insights into a much broader range of taxa (e.g., Coolen et al. 2013; Giguet-Covex et al. 2014; Capo et al. 2015).

Applying DNA-based methods in paleolimnology

Environmental DNA refers to a mixture of DNA from a single environmental sample (e.g., soil, water or sediments) where organisms are present as intact cells (e.g., bacteria or

phytoplankton cells) or as degraded DNA (e.g., extracellular DNA; Taberlet et al. 2012a). The use of molecular methods is becoming popular in biodiversity science and these approaches have proven to be effective in detecting endangered (e.g., Thomsen et al. 2012; Laramie et al. 2014) and invasive species (e.g., Jerde et al. 2011) in water bodies. DNA molecules have also been recovered from sediment extracts to complement morphological biological indicators (Coolen and Gibson 2009). To better understand how DNA-based approaches are efficient in paleolimnology and whether DNA archived in the sediments can give similar results than morphological approaches, preliminary studies have mainly focused on comparing DNA-based approaches with morphological approaches (e.g. Epp et al. 2011; Stoof-Leichsenring et al. 2012; 2014; Dulias et al. 2017). Most of studies that investigated diatoms have found high similarities between taxonomical identification from morphological and DNA-based approaches (Epp et al. 2011, Stoof-Leischenring et al., Dulias et al. 2017). Although Stoof-Leichsenring et al. (2012) have found that DNA-based approaches may uncover greater richness, these two approaches have been described as more complementary than congruent in assessing species richness (Jørgensen et al. 2012; Dulias et al. 2017).

Cyanobacterial assemblages have also been a focus in paleolimnology as their abundance increased worldwide in the last decades in aquatic ecosystems (Taranu et al. 2015), and also, because of their toxic potential for other living organisms. Monchamp et al. (2016) found that pelagic cyanobacterial assemblages identified morphologically over 30 years form water column samples were highly correlated with the paleo-genetic time series of cyanobacteria. This latter study shows the important information that can be reconstructed with DNA archived in sediments to better manage our aquatic ecosystems. In addition, an investigation of a deep subalpine lake with DNA-based approaches (quantitative PCR and sequencing) targeting

Synechococcus assemblages have found that temperature and phosphorus concentration affected cyanobacterial assemblage dynamics and structure over long time periods (Domaizon et al. 2013).

DNA-based approaches apply to sediment extracts also open up the possibility to reconstruct the dynamics of the whole microbial loop (e.g., bacteria and protists) as well as other potential essential players in aquatic food webs. Capo et al. (2015) have reconstructed past micro-eukaryotic communities from sediment records, and they retrieved 71 % of phylogenetic units from the sediments that were present in the water column. Another study from Capo et al. (2017) has shown that the modifications of micro-eukaryotic communities was correlated with phosphorus concentrations as well as air temperatures in two European lakes. Thus, DNA-based approaches in paleolimnology exhibit great promises for many other organisms that cannot easily be studied through classical methods in sediments. However, most of the studies using DNA archived in the sediments to reconstruct past biological conditions of lakes have assessed the total DNA, whereas DNA can be found as extracellular and intracellular DNA in the sediment record.

Intracellular vs extracellular DNA archived in lake sediments

DNA can be archived as extracellular DNA (exDNA) and intracellular DNA (inDNA) in the sediments. ExDNA composes a substantial fraction of the DNA pool in soil and aquatic sediments (Pietramellara et al. 2009), and can therefore represents potential genetic records to reconstruct biological communities from soil or sediment extracts (Corinaldesi et al. 2005). In marine environment, more than 90 % of the DNA content in the sediments is represented by

exDNA (Dell'Anno et al. 2002; Dell'Anno and Danovaro 2005), which usually derives from cell lysis or from active and passive extrusion mechanisms (Pietramellara et al. 2009). Organic and inorganic particles in soil or sediments can bind, adsorb, and stabilize free DNA molecules, which can reduce its degradability over long time periods (Corinaldesi et al. 2005).

Over a decade ago, DNA (both intra and extracellular fractions) was extracted with techniques that both physically and chemically lyse cells (Miller et al. 1999). However, these approaches could lead to misinterpretation of the community composition because both exDNA and inDNA pools are extracted at the same time (Corinaldesi et al. 2005). In addition, cell lysis agents could damage exDNA molecules, preventing good precision to identify an organism through its DNA. As exDNA adsorbs to soil or sediment particles via their phosphate part (England et al. 2004), a phosphate buffer can be used to selectively recover exDNA from sediments or soil. This approach was first introduced by Ogram et al. (1987) and was further developed by Corinaldesi et al. (2005). The recovery of exDNA from soil and sediments using a phosphate buffer is now a widely used approach (e.g. Corinaldesi et al. 2005; Bienert et al. 2012; Taberlet et al. 2012b; Alawi et al. 2014; Ficetola et al. 2018).

Corinaldesi et al. (2005) found that the concentration of inDNA associated with living cells in sediments is significantly lower than the exDNA concentrations in marine environments. This indicates the importance of evaluating not only inDNA, but also exDNA to recover biological communities in sediments and soil. According to Taberlet et al. (2012b), the use of exDNA to identify macro-organisms, such as plants or insects, is a preferable option to total soil DNA. Following the latter recommendation, Ficetola et al. (2018) targeted exDNA to evaluate the impact of an invasive rabbit species on the ecosystem dynamics on a sub-Arctic island over the last 600 years, and they could efficiently extract the exDNA fraction of rabbit DNA from the

sedimentary matrix. In marine environments, minimal differences have been observed between inDNA and exDNA fractions on recent and ancient sediments when evaluating prokaryotic communities (Corinaldesi et al. 2018; Torti et al. 2018; Ramírez et al. 2018). However, in lake sediments, Vuillemin et al. (2017) have observed that the signal of prokaryotic communities from the water column was only preserved in the top part of the sediment core. More work is thus needed to compare biological signal from inDNA and exDNA archived in lacustrine sediments for a broader range of organisms.

Diversity and ecological functions of micro-eukaryotes in lake ecosystems

Micro-eukaryotes from freshwater ecosystems are diverse in ecological functions, food strategies and morphology (Fig. 1). I defined micro-eukaryotes in my thesis as small unicellular and multicellular organisms. The primers used for Chapters 2 and 4 to amplify micro-eukaryotes were designed to target a fragment of the V7 region of the 18S rRNA gene. As it was previously used for paleolimnological studies (i.e., Capo et al. 2017), it has been demonstrated that these primers amplify a wide diversity of micro-eukaryotes, such as protists, fungi and also small multicellular organism (e.g., micro-crustaceans; Fig.1).

Microbial eukaryotes (i.e., unicellular eukaryotes), in particular, have received less attention than their prokaryotic counterparts (Debroas et al. 2017), even though they are diverse and can play essential ecological roles in ecosystems, such as their involvement in biogeochemical cycles (Sherr and Sherr 1998, Caron et al. 2008, Grattepanche et al. 2014). The advancement of molecular technologies has helped assess the diversity of microbial eukaryotes in environments, and researches conducted on freshwater ecosystems have revealed a great and

unexpected diversity of microbial eukaryotes (Monchy et al. 2011; Mangot et al. 2013). Network analyses have shown that microbial eukaryotic species are strongly connected in freshwater ecosystems and that Fungi, Stramenopiles and Viridiplantae seem to play central roles in planktonic communities as well as contributing to ecosystem stability (Debroas et al. 2017). Fungi can be saprotrophic as well as parasitic, and thus, can play important roles in processing resistant biochemical components such as cellulose, keratin, chitin and pollen (Powell 2017) and in controlling their host populations (Ibelings et al. 2004). The central role of Stramenopiles in freshwater ecosystems is related to their high taxonomic diversity and ecological roles including autotrophic (e.g., Diatoms), mixotrophic (e.g., Chrysophyceae), heterophic (e.g., Bicosoecida) and parasitic (some oomycetes) roles (Debroas et al. 2017). Viridiplantae are mainly represented by the Chlorophyceae, which is a diverse taxonomic group of mostly freshwater green algae involved in primary production and in the global carbon cycle (Debroas et al. 2017). Other taxonomic groups are likely of ecological importance in freshwater ecosystems, such as Alveolates, which include Ciliophora and Dinoflagellata taxa. Ciliates are considered cosmopolitan, have diverse food strategies (Lynn 2010 2017) and bacterivorous ciliates are known to decrease bacterial densities in sewage water effluents (Curds and Cockburn 1970a; b; Madoni 2003). Conversely, dinoflagellates are mainly autotrophic organisms and usually contribute substantially to primary production of aquatic ecosystems (Saldarriaga and ‘Max’ Taylor 2017). Ecological dynamics and roles of most freshwater microbial eukaryotes are understudied, but they potentially have central and essential ecological functions in ecosystems. Although more work needs to be conducted to evaluate the range and the optimal environmental conditions of the different taxonomic groups, microbial eukaryote community changes in sediment archives could help decipher how different anthropogenic and climate drivers affect

lake status changes. Investigations of microbial eukaryotes in European lakes have found that diverse groups, such as Chlorophyta, Dinophyceae, Haptophyceae, Ciliophora, Chrysophyceae, Apicomplexa and Cercozoa, can be suitable bioindicators of trophic status changes and climate change (Capo et al. 2016; 2017; 2019).

Study site: Cultus Lake, British Columbia

Cultus Lake is located in the Lower Mainland of British Columbia (49°03'11.88"N; 121°59'12.12"W; Fig.1), approximately 100 km east of Vancouver (46 m asl). The mean and maximum depths are respectively 31 m and 44 m (Shortreed 2007). The majority of the watershed (75 km^2) is contained within Canada, with a small portion in Washington State (~19 %), USA (Shortreed 2007). Eleven tributaries channel ~60 % of the total Cultus Lake watershed area, with the remainder drained by overland flow and groundwater (Putt 2014). The catchment is characterized by extensive parkland with sparse development, numerous campgrounds, and two residential development areas located at the northern and southern extents of the lake (Putt 2014). The largest tributary of Cultus Lake, Frosst Creek, accounts for almost 50 % of the water flowing into the lake and emerges directly from the Columbia Valley, which is heavily influenced by an unconstrained aquifer underlying principally agricultural lands and rural development (Putt 2014). The soil of Cultus Lake watershed is characterized by glaciofluvial outwash deposits which is rapidly drained, but differs between the two ridges, Vedder Mountain and International ridge, and the Columbia Valley (Zubel 2000).

Cultus Lake is an oligo-mesotrophic lake, and is moderately productive all year-round, with higher primary production during the stratified season (from April/May to Nov/Dec; Table

1, Chapter 2). During the mixed season (from Nov/Dec to April/May), the euphotic zone is shallower because of the enhanced input of particles from the watershed derived from higher rainfall during this period (Table 1, Chapter 2). Cultus Lake is currently a monomictic lake, but there is evidence of ice cover on the lake (i.e., 1937 from DFO archives; 1950 from Soutar 2005) in the past century, which means that the lake had been dimictic at least sporadically in the past. After the onset of thermal stratification, total chlorophyll, total nitrogen and chlorophyll *a* are highest in the metalimnion relative to the epilimnion, which suggest an important metalimnetic production in the lake (Table 1, Chapter 2). A limnological survey in 2001-2003 has shown evidence of modest eutrophication in Cultus Lake with higher nutrients, lower hypolimnetic dissolved oxygen late in the fall and higher metalimnetic production compared to the historical survey in the 1930s (Shortreed 2007).

Regional climate variability and global climate change are important influences on Cultus Lake. The regional climate is marked by quasi-periodic, inter-annual to inter-decadal variability associated with warm and cold phases of the El Niño-Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO), respectively. However, a significant directional warming has also been observed since at least 1900 (White et al. 2016). While annual air temperatures have increased significantly (resulting in a change of annual temperature of + 0.8°C between 1900-2013), the most pronounced warming has occurred in winter (+ 1.2°C; BC Ministry of Environment 2016). In addition, the precipitation has increased approximately by 14 %, with pronounced increases in the spring by 23 % (BC Ministry of Environment 2016). In 1948, there was a substantial lake level rise associated with a major flooding event in the Fraser Valley (Soutar 2005). A similar event also happened in 1950 after a colder winter when the lake was covered in ice, and with a lake water level increase during the following spring (Soutar 2005).

Numerous human-mediated disturbances within the Cultus Lake watershed have been documented. For thousands of years, the surrounding landscape of Cultus Lake has been used by First Nations (Carlson et al. 2001; Schaepe and Ts'elxwéyeqw Tribe 2017). Euro-American settlement was established in the Columbia Valley (south of Cultus Lake) in the late-1880s and a road was built around Cultus Lake in the early-1900s, which facilitated logging activities in the watershed (Soutar 2005). Many other disturbances related to anthropogenic activities occurred in the watershed during the 1900s, such as forest fires, agricultural activities, residential development and recreational activities. An extensive description of the history of anthropogenic activities and other potential drivers that affected Cultus Lake can be found in Chapter 3.

Cultus Lake as a model site to evaluate the efficiency of DNA-based method in paleolimnology

Cultus Lake is an ideal site to investigate the potential of DNA-based methods in paleolimnology as historical limnological data are available from the 1920s and the 1930s (Ricker 1937, 1938), from the early 2000s (Shortreed 2007) and a monthly limnological sampling has been developed since 2009 to the present (unpublished data, DFO). As I also conducted the multi-proxy paleolimnological study (Chapter 3), Cultus Lake was a suitable site to further explore DNA archived in the sediments. An extensive description of the ecology and historical influences on Cultus Lake can be found in all chapters of this thesis. Chapter 2 provides mainly contemporary limnological description and Chapter 3 provides and extensive reconstruction of the history of potential drivers of changes on Cultus Lake.

Objectives of the thesis

The objectives of my PhD thesis included methodological and ecological aspects. The methodological objective was to evaluate the advantages and limitations of DNA-based approaches in paleolimnology. The ecological objective was related to assess the ecological trajectory of Cultus Lake in the past ~200 years and identify the major drivers of change. In Chapter 2, the specific aims were (1) to evaluate the extent to which micro-eukaryotic communities identified from 18S rRNA gene reflect the communities present in the water column, (2) to assess the congruence between morphological and DNA-based approaches to identify diatom and crustacean assemblages from the sediments, and (3) to compare the efficiency of exDNA and inDNA to detect taxa deposited in the sediments. The objectives in Chapter 3 were to reconstruct the past ecological dynamics of Cultus Lake and to identify the major drivers (i.e., anthropogenic activities and climate change) of change. The temporal dynamics of diatom assemblages identified as subfossils in the sediment traps (Chapter 2) were used to evaluate further the diatom community changes in the sediment core. In Chapter 4, the specific goals were (1) to characterize the dynamics of micro-eukaryote communities in the past ~200 years and whether they followed similar changes than classical indicators (Chapter 3), and (2) to evaluate the similarities between the signal from inDNA and exDNA archived in the sediments.

Literature cited

Alawi M, Schneider B, Kallmeyer J (2014) A procedure for separate recovery of extra- and intracellular DNA from a single marine sediment sample. J Microbiol Methods 104: 36–42. doi:10.1016/j.mimet.2014.06.009

Alric B, Perga M-E (2011) Effects of production, sedimentation and taphonomic processes on the composition and size structure of sedimenting cladoceran remains in a large deep subalpine lake: paleo-ecological implications. Hydrobiologia 676: 101–116. doi:10.1007/s10750-011-0868-0

Battarbee RW (2010) Aquatic ecosystem variability and climate change – A palaeoecological perspective. In: Kernan M, Battarbee RW, and Moss B (eds) Climate Change Impacts on Freshwater Ecosystems. Wiley-Blackwell, pp 15-37

Battarbee, RW, Jones VJ, Flower RG, Cameron NG, Bennion H, Carvalho L, Juggins S (2001) Diatoms. In: Smol JP, Birks HJB, Last WM, Bradley RS, and Alverson K (eds) Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal and siliceous indicators. Kluwer Academic Publishers New York, pp 155-202

BC Ministry of Environment (2016) Indicators of climate change for British Columbia 2016 update. Victoria, Canada. https://www2.gov.bc.ca/assets/gov/environment/research-monitoring-and-reporting/reporting/envreportbc/archived-reports/climate-change/climatechangeindicators-13sept2016_final.pdf

Birks HJB (2012) Analysis of stratigraphical data. In: Birks HJB, Lotter AF, Juggins S, Smol JP (eds) Tracking environmental change using lake sediments. Volume 5: Data handling and numeral techniques. Springer Dordrecht, pp 355–378.

Birks HJB, Heiri O, Seppä H, Bjune AE (2010) Strengths and weaknesses of quantitative climate reconstructions based on late-Quaternary biological proxies. Open Ecol J 3: 68–110. doi:10.1016/j.quaint.2012.07.228

Boere AC, Rijpstra WIC, De Lange GJ, Sinninghe Damsté JS, Coolen MJL (2011) Preservation potential of ancient plankton DNA in Pleistocene marine sediments. Geobiology 9: 377–393. doi:10.1111/j.1472-4669.2011.00290.x

Capo E, Debroas D, Arnaud F, Domaizon I (2015) Is planktonic diversity well recorded in sedimentary DNA? Toward the reconstruction of past protistan diversity. Microb Ecol 70: 865–875. doi:10.1007/s00248-015-0627-2

Capo E, Debroas D, Arnaud F, et al (2016) Long-term dynamics in microbial eukaryotes communities: a palaeolimnological view based on sedimentary DNA. Molecular Ecology 25:5925–5943. doi:10.1111/mec.13893

Capo E, Debroas D, Arnaud F, Perga M-E, Chardon C, Domaizon I (2017) Tracking a century of changes in microbial eukaryotic diversity in lakes driven by nutrient enrichment and climate warming: Long-term dynamics of microbial eukaryotes. Environ Microbiol 19: 2873–2892. doi:10.1111/1462-2920.13815

Capo E, Rydberg J, Tolu J, et al (2019) How does environmental inter-annual variability shape aquatic microbial communities? A 40-year annual record of sedimentary DNA from a boreal lake (Nylandssjön, Sweden). Front Ecol Evol 7:245. doi:10.3389/fevo.2019.00245

Carlson K (ed), McHalsie AJ, Stó:lō Heritage Trust (2001) A Stó:lō-Coast Salish historical atlas, Douglas & McIntyre LTD. Vancouver, Canada.

Caron DA, Worden AZ, Countway PD et al (2008) Protists are microbes too: a perspective. ISME J 3: 4–12

Christensen MR, Graham MD, Vinebrooke RD, Findlay DL, Paterson MJ, Turner MA (2006) Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Glob Change Biol 12: 2316–2322. doi:10.1111/j.1365-2486.2006.01257.x

Coolen MJL, Gibson JAE (2009) Ancient DNA in lake sediment records. PAGES News 17: 104–106.

Coolen MJL, Orsi WD, Balkema C, Quince C, Harris K, Sylva SP, Filipova-Marinova M, Giosan L (2013) Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene. Proc Natl Acad Sci USA 110: 8609–8614. doi:10.1073/pnas.1219283110

Corinaldesi C, Danovaro R, Dell'Anno A (2005) Simultaneous recovery of extracellular and intracellular DNA suitable for molecular studies from marine sediments. Appl Environ Microbiol 71: 46–50. doi:10.1128/AEM.71.1.46-50.2005

Corinaldesi C, Tangherlini M, Manea ME, Dell'Anno A (2018) Extracellular DNA as a genetic recorder of microbial diversity in benthic deep-sea ecosystems. Sci Rep 8. doi:10.1038/s41598-018-20302-7

COSEWIC (2003) COSEWIC assessment and status report on the sockeye salmon *Oncorhynchus nerka* (Cultus population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. ix + 57 pp.

COSEWIC (2010) COSEWIC assessment and status report on the Coastrange Sculpin *Cottus aleuticus*, Cultus Population in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi + 28 pp. (www.sararegistry.gc.ca/status/status_e.cfm).

Cultus Sockeye Recovery Team (2005) Conservation strategy for sockeye salmon (*Onchorhynchus nerka*), Cultus Lake population, in British Columbia. Recovery of Nationally Endangered Wildlife (RENEW).

Curds CR, Cockburn A (1970a) Protozoa in biological sewage-treatment processes—I. A survey of the protozoan fauna of British percolating filters and activated-sludge plants. Water Research 4:225–236. doi:10.1016/0043-1354(70)90069-2

Curds CR, Cockburn A (1970b) Protozoa in biological sewage-treatment processes—II. Protozoa as indicators in the activated-sludge process. Water Research 4:237–249. doi:10.1016/0043-1354(70)90070-9

Debroas D, Domaizon I, Humbert J-F, et al (2017) Overview of freshwater microbial eukaryotes diversity: a first analysis of publicly available metabarcoding data. FEMS Microbiology Ecology 93:. doi:10.1093/femsec/fix023

Dell'Anno A, Bompadre S, Danovaro R (2002) Quantification, base composition, and fate of extracellular DNA in marine sediments. Limnol Oceanogr 47: 899–905. doi:10.4319/lo.2002.47.3.0899

Dell'Anno A, Danovaro R (2005) Extracellular DNA Plays a Key Role in Deep-Sea Ecosystem Functioning. Science 309: 2179–2179. doi:10.1126/science.1117475

Domaizon I, Savichtcheva O, Debroas D, Arnaud F, Villar C, Pignol C, Alric B, Perga M-E (2013) DNA from lake sediments reveals the long-term dynamics and diversity of Synechococcus assemblages. Biogeosciences 10: 3817–3838. doi:10.5194/bg-10-3817-2013

Dulias K, Stoof-Leichsenring KR, Pestryakova LA, and Herzschuh U (2017) Sedimentary DNA versus morphology in the analysis of diatom-environment relationships. J Paleolimnol 57: 51–66. doi:10.1007/s10933-016-9926-y

England LS, Vincent ML, Trevors JT, and Holmes SB (2004) Extraction, detection and persistence of extracellular DNA in forest litter microcosms. Mol Cell Probes 18: 313–319. doi:10.1016/j.mcp.2004.05.001

Epp LS, Stoof-Leichsenring KR, Trauth MH, Tiedemann R (2011) Molecular profiling of diatom assemblages in tropical lake sediments using taxon-specific PCR and Denaturing High-Performance Liquid Chromatography (PCR-DHPLC). Mol Ecol Resour 11: 842–853. doi:10.1111/j.1755-0998.2011.03022.x

Ficetola GF, Poulenard J, Sabatier P, et al (2018) DNA from lake sediments reveals long-term ecosystem changes after a biological invasion. Sci Adv 4: eaar4292. doi:10.1126/sciadv.aar4292

FVRD (2011) Official community plan for electoral area E bylaw. Fraser Valley Regional District, British Columbia, Canada

Giguet-Covex C, Pansu J, Arnaud F, et al (2014) Long livestock farming history and human landscape shaping revealed by lake sediment DNA. Nat Commun 5. doi:10.1038/ncomms4211

Gillson L, Marchant R (2014) From myopia to clarity: sharpening the focus of ecosystem management through the lens of palaeoecology. Trends Ecol Evol 29: 317–325. doi:10.1016/j.tree.2014.03.010

Government of Canada EC (2011) HTML Version of the Document - Species at risk public registry. http://www.registrelep-sararegistry.gc.ca/document/dspHTML_e.cfm?ocid=8366. Accessed 23 Oct 2017

Grattepanche J-D, Santoferrara LF, McManus GB et al (2014) Diversity of diversity: conceptual and methodological differences in biodiversity estimates of eukaryotic microbes as compared to bacteria. Trends Microbiol 22: 432–7

Ibelings BW, Bruin AD, Kagami M, et al (2004) Host Parasite Interactions Between Freshwater Phytoplankton and Chytrid Fungi (chytridiomycota)1. Journal of Phycology 40:437–453. doi:10.1111/j.1529-8817.2004.03117.x

Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) "Sight-unseen" detection of rare aquatic species using environmental DNA: eDNA surveillance of rare aquatic species. Conserv Lett 4: 150–157. doi:10.1111/j.1755-263X.2010.00158.x

Jørgensen T, Kjær KH, Haile J, et al (2012) Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA Meta-barcoding. Mol Ecol 21: 1980–1988. doi:10.1111/j.1365-294X.2011.05278.x

Laramie MB, Pilliod DS, Goldberg CS (2014) Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biol Conserv. doi:10.1016/j.biocon.2014.11.025

Lynn D (2010) The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature, 3rd ed. Springer Netherlands

Lynn DH (2017) Ciliophora. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 679–730

Madoni P (2003) Protozoa as indicators of wastewater treatment efficiency. In: Mara D, Horan N (eds) The Handbook of Water and Wastewater Microbiology. pp 361–371

Mangot J-F, Domaizon I, Taib N et al (2013) Short-term dynamics of diversity patterns: evidence of continual reassembly within lacustrine small eukaryotes. Environ Microbiol 15: 1745–58

Miller DN, Bryant JE, Madsen EL, Ghiorse WC (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol 65: 4715–4724.

Monchamp M-E, Walser J-C, Pomati F, Spaak P (2016) Sedimentary DNA reveals cyanobacteria community diversity over 200 years in two peri-alpine lakes. Appl Environ Microbiol AEM 02174-16. doi:10.1128/AEM.02174-16

Monchy S, Sanciu G, Jobard M et al (2011) Exploring and quantifying fungal diversity in freshwater lake ecosystems using rDNA cloning/sequencing and SSU tag pyrosequencing. Environ Microbiol 13: 1433–53

Moorhouse HL, McGowan S, Jones MD, Barker P, Leavitt PR, Brayshaw SA, Haworth EY (2014) Contrasting effects of nutrients and climate on algal communities in two lakes in the Windermere catchment since the late 19th century. Freshw Biol 59: 2605–2620. doi:10.1111/fwb.12457

Moss B, Battarbee RW, Kernan M (2010) Introduction. In: Kernan M, Battarbee RW, and Moss B (eds) Climate Change Impacts on Freshwater Ecosystems. Wiley-Blackwell, pp 1-15. doi: 10.1002/9781444327397.ch1

Ogram A, Sayler GS, Barkay T (1987) The extraction and purification of microbial DNA from sediments. J Microbiol Methods 7: 57–66. doi:10.1016/0167-7012(87)90025-X

Pietramellara G, Ascher J, Borgogni F, Ceccherini MT, Guerri G, Nannipieri P (2009) Extracellular DNA in soil and sediment: fate and ecological relevance. Biol Fertil Soils 45: 219–235. doi:10.1007/s00374-008-0345-8

Powell MJ (2017) Chytridiomycota. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 1523–1558

Putt AE (2014) Spatiotemporal nutrient loading to Cultus Lake: Context for eutrophication and implications for integrated watershed-lake management. Simon Fraser University

Ramírez GA, Jørgensen SL, Zhao R, D'Hondt S (2018) Minimal influence of extracellular DNA on molecular surveys of marine sedimentary communities. Front Microbiol 9. doi:10.3389/fmicb.2018.02969

Reid AJ, A. K. Carlson AK, I. F. Creed IF, et al (2018) Emerging threats and persistent conservation challenges for freshwater biodiversity: Emerging threats to freshwater biodiversity. Biol Rev. doi:10.1111/brv.12480

Ricker WE (1937) Physical and Chemical Characteristics of Cultus Lake, British Columbia. J Biol Board Can 3

Ricker WE (1938) Seasonal and Annual Variations in Quantity of Pelagic Net Plankton, Cultus Lake, British Columbia. J Fish Res Board Can 4: 34–47

Rosenfield J, Department of Fisheries and Oceans (2007) Recovery strategy for Cultus pygmy sculpin (*Cottus* sp.) in Canada. Fisheries and Oceans Canada, Ottawa, Canada

Saldarriaga JF, 'Max' Taylor FJR (2017) Dinoflagellata. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 625–678

Saulnier-Talbot É (2016) Paleolimnology as a Tool to Achieve Environmental Sustainability in the Anthropocene: An Overview. Geosciences 6: 26. doi:10.3390/geosciences6020026

Savichtcheva O, Debroas D, Perga M-E, et al (2015) Effects of nutrients and warming on Planktothrix dynamics and diversity: a palaeolimnological view based on sedimentary DNA and RNA. Freshw Biol 60: 31–49. doi:10.1111/fwb.12465

Schaepe DM, and Ts'elxwéyeqw Tribe (eds) (2017) Being Ts'elxwéyeqw: First Peoples' voices and history from the Chilliwack-Fraser Valley, British Columbia. Harbour Publishing, Madeira Park, British Columbia, Canada

Sherr EB, Sherr BF (1988) Role of microbes in pelagic food webs: a revised concept. Limnol Oceanogr 33: 1225–7

Shortreed KS (2007) Limnology of Cultus Lake, British Columbia. Can Tech Rep Fish Aquat Sci. 2753: vi + 85p

Simpson GL (2012) Analogue methods in palaeolimnology. In: Birks HJB, Lotter AF, Juggins S, Smol JP (eds) Tracking environmental change using lake sediments. Volume 5: Data handling and numeral techniques. Springer Dordrecht. pp 495–522

Smol JP (2008) Pollution of lakes and rivers: a paleolimnological perspective, 2nd edition. Blackwell Sientific Publications Publishing, Oxford, UK

Soutar M (2005) Cultus Lake: A natural paradise. Chilliwack Museum and Archives, Chilliwack, Canada

Stoof-Leichsenring KR, Bernhardt N, Pestryakova LA, Epp LS, Herzschuh U, Tiedemann R (2014) A combined paleolimnological/genetic analysis of diatoms reveals divergent evolutionary lineages of *Staurosira* and *Staurosirella* (Bacillariophyta) in Siberian lake sediments along a latitudinal transect. J Paleolimnol 52: 77–93. doi:10.1007/s10933-014-9779-1

Stoof-Leichsenring KR, Epp LS, Trauth MH, Tiedemann R (2012) Hidden diversity in diatoms of Kenyan Lake Naivasha: a genetic approach detects temporal variation. Mol Ecol 21: 1918–1930. doi:10.1111/j.1365-294X.2011.05412.x

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012a) Environmental DNA. Mol Ecol 21: 1789–1793. doi:10.1111/j.1365-294X.2012.05542.x

Taberlet P, Prud'homme SM, Campione E, et al (2012b) Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Mol Ecol 21: 1816–1820. doi:10.1111/j.1365-294X.2011.05317.x

Taranu ZE, Gregory-Eaves I, Leavitt PR, et al (2015) Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. Ecol Lett 18: 375–384. doi:10.1111/ele.12420

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E (2012) Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS ONE 7: e41732. doi:10.1371/journal.pone.0041732

Torti A, Jørgensen BB, Lever MA (2018) Preservation of microbial DNA in marine sediments: insights from extracellular DNA pools: Preservation of microbial DNA in marine sediment. Environ Microbiol 20: 4526–4542. doi:10.1111/1462-2920.14401

Vörösmarty CJ, McIntyre PB, Gessner MO, et al (2010) Global threats to human water security and river biodiversity. Nature 467: 555–561. doi:10.1038/nature09440

Vuillemin A, Horn F, Alawi M, Henny C, Wagner D, Crowe SA, Kallmeyer J (2017) Preservation and significance of extracellular DNA in ferruginous sediments from Lake Towuti, Indonesia. Front Microbiol 8. doi:10.3389/fmicb.2017.01440

Walker IR (2001) Midges: Chironomidae and Related Diptera. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. Volume 4: Zoological Indicators. Kluwer Academics Publishers New York, pp 43–66

Waltham NJ, Reichelt-Brushett A, McCann D, D. Eyre BD (2014) Water and sediment quality, nutrient biochemistry and pollution loads in an urban freshwater lake: balancing human and ecological services. Env Sci Process Impacts 16: 2804–2813. doi:10.1039/C4EM00243A

Zeeb BA, Smol JP (2001) Chrysophyte scales and cysts. In: Smol JP, Birks HJB, Last WM, Bradley RS, Alverson K (eds) Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal, and siliceous indicators. Kluwer Academics Publishers New York, pp 203–223

Zubel M (2000) Groundwater conditions of the Columbia Valley Aquifer, Cultus Lake, British Columbia, Ministry of Environment, Lands and Parks, Water Management.

Figure Chapter 1

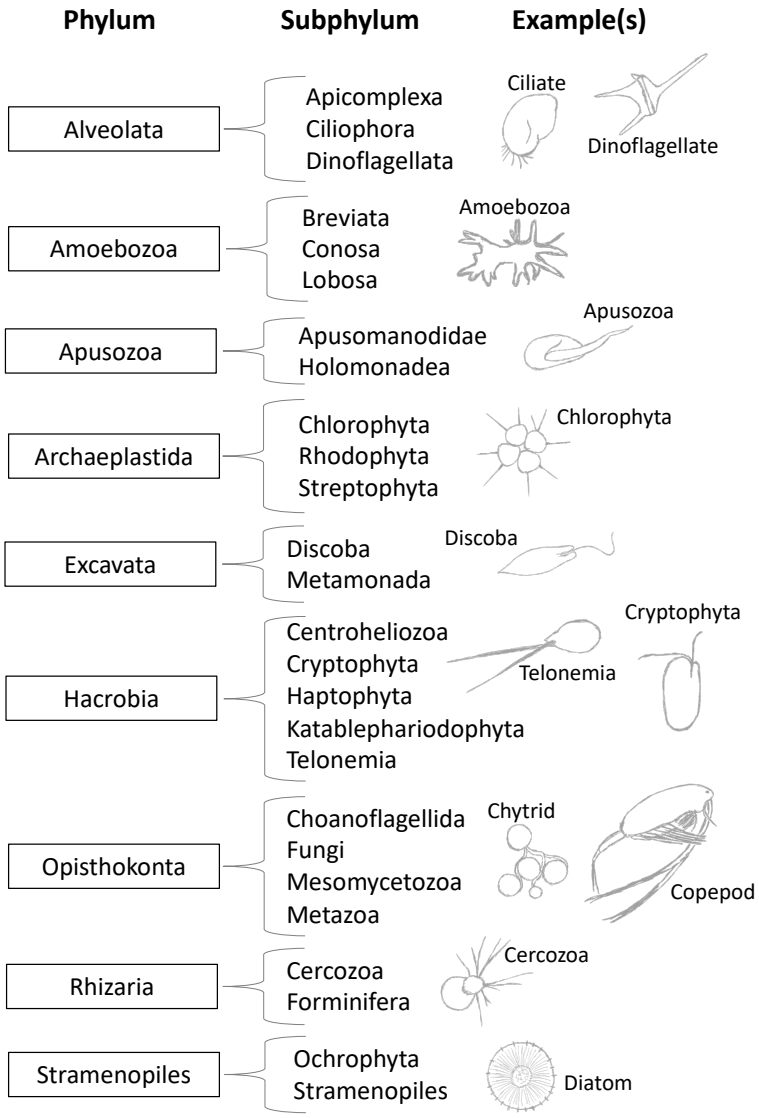

Figure 1. Phyla, subphyla and morphological examples of micro-eukaryotic organisms amplified with the primers used in Chapters 2 and 4. Drawings of examples are not on scale.

CHAPTER 2

EVALUATING THE CONGRUENCE BETWEEN DNA-BASED AND MORPHOLOGICAL TAXONOMIC APPROACHES IN WATER AND SEDIMENT TRAP SAMPLES: ANALYSES OF A 36-MONTH TIME SERIES FROM A TEMPERATE MONOMICTIC LAKE

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Abstract

Paleolimnological studies are central for identifying long-term changes, yet many studies rely on bioindicators that deposit detectable subfossils in sediments, such as diatoms and cladocerans. Emerging DNA-based approaches are expanding the taxonomic diversity that can be investigated. However, as sedimentary DNA-based approaches are expanding rapidly, calibration work is required to elucidate the advantages and limitations of these techniques. In this study, we evaluated which taxa are deposited in sediment traps from the water column to identify potential paleolimnological bioindicators of environmental variations. We also assessed the congruence between morphological and DNA-based approaches applied to sediment trap samples for diatoms and crustaceans using both intracellular and extracellular DNA methods. Based on the 18S rRNA gene amplicons, we developed and analyzed a micro-eukaryotic, monthly time series that spanned three-years and was comprised of paired water column and sediment trap samples from Cultus Lake, British Columbia, Canada. Comparisons of assemblages derived from our genetic and morphological analyses using RV coefficients revealed significant correlations for diatoms, but weaker correlations for crustaceans. Intracellular DNA reads correlated more strongly with diatom morphology, while extracellular DNA fraction correlated more strongly with crustacean morphology. Additional analyses of amplicon sequence variants shared between water and sediment trap samples revealed a wide diversity of additional taxa including Ciliophora, Dinoflagellata, Chytridiomycota, Chrysophyceae, Cryptophyceae and Cercozoa. Partial RDAs identified significant environmental predictors of these shared assemblages. Overall, our study demonstrates the effectiveness of DNA-based approaches to track community dynamics from sediment samples, an essential step for successful paleolimnological studies.

Introduction

Over the past century, anthropogenic activities and climate change have induced significant alterations to freshwater ecosystems, with an increase or intensification of stressors since the 1970s (Reid et al. 2018). Paleolimnological records have played a central role in quantifying the rate and magnitude of past ecological dynamics and have served to identify the major drivers of ecosystem change (Smol 2008; Bennion et al. 2011). In addition, paleolimnological time-series have been useful in evaluating the adequacy of management practices (e.g. Perga et al. 2010) and may inform future scenario development (e.g. Smol 2008; Gillson and Marchant 2014; Saulnier-Talbot 2016). Biological community changes in the sediment record have relied mostly on the study of a small subset of aquatic taxa that deposit detectable subfossils including diatom valves (e.g., Battarbee et al. 2001), chrysophyte cysts and scales (e.g., Zeeb and Smol 2001) and cladoceran (e.g., Alric and Perga 2011) and chironomid remains (e.g. Walker 2001). However, many pelagic organisms do not preserve as visually identifiable subfossils in the sediments (e.g., fungi, soft algae, rotifers, copepods), yet may be useful as bioindicators of environmental change.

Applying DNA-based approaches to lake sediments has the potential to expand the taxonomic diversity that can be targeted in paleolimnological studies as well as to provide an opportunity to study functional genes (Domaizon et al. 2017). DNA-based approaches in paleolimnology have proven to be effective in reconstructing the histories of some taxonomic groups such as cyanobacteria (Domaizon et al. 2013; Monchamp et al. 2016), diatoms (Epp et al. 2011; Stoof-Leichsenring et al. 2012, 2014) as well as communities of micro-eukaryotes (Capo et al. 2016, 2017, 2019). However, only a few studies have evaluated the congruence between morphological and DNA-based taxonomic approaches in sediments (Stoof-Leichsenring et al.

2012, 2014; Dulias et al. 2017). Preliminary results showed that DNA-based approaches may uncover greater richness (Stoof-Leichsenring et al. 2012), and generally, these two approaches are more complementary than congruent in assessing species richness (Jørgensen et al. 2012; Dulias et al. 2017). Additionally, there is a need to evaluate the extent to which the biological signal from the water column is captured in the sedimentary record when applying DNA-based approaches. Some promising initial studies include: 1) Capo et al. (2015) who detected 71 % of phylogenetic units (therein defined as OTUs) from the water column in the sediments and 2) Monchamp et al. (2016) who found that pelagic cyanobacteria identified morphologically over 30 years from water column samples were highly correlated when compared to paleo-genetic time series of cyanobacteria.

Sedimentary DNA (sedDNA) can be archived either as intracellular (inDNA; e.g. intact cells) or extracellular DNA (exDNA), where exDNA can be adsorbed to the sediment matrix, thereby reducing its degradability (Dell'Anno et al. 2002; Corinaldesi et al. 2005, 2008; Dell'Anno and Danovaro 2005). In marine environments, exDNA can represent greater than 90 % of the sedDNA pool (Dell'Anno et al. 2002; Dell'Anno and Danovaro 2005) and may be a significant fraction of the DNA archived for many organisms. To our knowledge, only one paleolimnological study has evaluated whether DNA from pelagic organisms is preserved as exDNA and this study was focused on bacterial assemblages (Vuillemin et al. 2017).

Since the use of DNA-based approaches in paleolimnology is expanding rapidly, careful examination of the strengths and limitations of the approach is required. In this study, we generated a sediment trap (ST) time-series spanning 36 months in order to advance our knowledge of how different components of the pelagic communities are preserved and recoverable from sedDNA by PCR-based approaches. Whereas ST are not perfect analogs for

surface sediments, analyses of ST allow one to assess the potential coherence between water column and sediment dynamics. In addition, ST provide information on the pelagic taxa that are deposited in the sediments, which is one of the key criteria for defining suitable bioindicators in paleolimnology (others include robust preservation in sediment archives and reliable indication of environmental conditions). We used general eukaryotic primers targeting the V7 region of the 18S rRNA gene, which have been previously used for paleoreconstruction (e.g., Capo et al. 2016). Previous work has demonstrated that these primers amplify protists, fungi, and larger multicellular organisms such as crustacean species; we refer to the pool of taxa identified through sequencing as micro-eukaryotes throughout the manuscript. Our specific aims were (1) to assess the extent to which micro-eukaryotic communities identified from morphological subfossils or from the amplification of the 18S rRNA gene from DNA in ST reflect the biological communities present in the water column, (2) to evaluate the congruence between morphological and DNA approaches in tracking diatom and crustacean assemblage dynamics from sediments, and (3) to compare the efficiency of exDNA versus inDNA to detect taxa in ST. Given that several studies have reported a significant congruence between the assemblages observed in the water column and those recorded in lake sediments (e.g., Winegardner et al. 2015; Capo et al. 2015; Monchamp et al. 2016), we hypothesize that analyses of ST (deployed monthly) would track similar dynamics to those reflected in the water column using similar taxonomic approaches (morphological or DNA). In addition, the few studies that have compared assemblages based on sedDNA and visual count approaches have detected significant coherence (e.g., Stoof-Leichsenring et al. 2012; Jørgensen et al. 2012; Monchamp et al. 2016). Accordingly, we hypothesize that the genetically- and morphologically derived estimates of taxonomic composition in the sediments would be significantly correlated.

Methods

Site description

Cultus Lake (49°03.3'N; 121°59.0'W) is a monomictic and oligo-mesotrophic lake located in the Lower Mainland of British Columbia (BC), Canada, at ~50 km east of the outer limit of the Greater Vancouver Regional District (Fig.1a). The surface area of Cultus Lake is 6.3 km^2 with mean and maximum depths of 31 m and 44 m, respectively (Shortreed 2007). Cultus Lake is a relatively fast-flushing lake with a water residence time of ~1.8 yr. The Cultus Lake watershed area is ~75 km^2 with a small proportion (~19 %) in the United States.

Sample collection

On a monthly basis from June 2014 (June 27) to June 2017 (June 12), water and sediment trap (ST) samples were collected and deployed at the offshore station where the DFO Lakes Research Program has been developing a limnological time series since 2009 (Fig. 1a). For each sampling occasion, the limnology of the photic zone was monitored as well as the hypolimnion following the methods described in Shortreed (2007). When the lake was thermally stratified, water samples were collected in the epilimnion (from the surface to the thermocline depth) and in the metalimnion (from the thermocline depth to the photic zone depth) (Fig. 1b). Water samples in the photic zone were also collected for the enumeration and identification of nano- and microplankton as described in Shortreed (2007). Zooplankton was collected with a vertical haul from 30 m deep to the surface. The details on the enumeration, identification and measurements are reported in Shortreed (2007).

To evaluate the congruence with DNA between the water samples and the ST samples, 1 L of water was collected from the photic zone (epilimnion and metalimnion when thermal stratification occurred; Fig. 1b) on the same day as the limnological monitoring. The water samples were then frozen at -20°C until further laboratory analyses. Within the same week as the limnological sampling, ST were deployed, and the traps deployed a month earlier were retrieved. Sediment traps were built according to Bloesch and Burns (1980) specifications, with a length of 60.96 cm and a diameter of 10.16 cm (ratio of length to diameter of ~6). Sediment traps were deployed in duplicate at ~3 m above the water-sediment interface (at a depth of ~37 m; Fig. 1b). Prior to each deployment, the traps and accumulation tubes were scrubbed and soaked in 10 % bleach for ~2 h. The accumulation tubes were also immersed for ~5 min in 10 % HCl and rinsed with deionized water. The traps themselves were sprayed with 10 % HCl on the day of the deployment, rinsed with lake water, and then sealed and secured until deployment to avoid contamination.

Sediment trap processing

Once retrieved from the lake, the accumulation tubes of the ST were oriented vertically and left at 4°C overnight to allow particles to settle. About 120 mL of water at the surface of the tubes were removed with a sterile syringe and the samples were frozen vertically at -20°C. The frozen samples (ST and water samples) were shipped overnight to McGill University every 3-6 months and were stored at -20°C. Accumulation tubes were thawed overnight and the total amount of water with sediment particles were transferred to 50 mL sterile tubes to be centrifuged at 3,750 rpm for 10 min at 4°C. After centrifugation, the water supernatants were discarded, and

the pellets of sediment particles were pooled. The sediment pellets were homogenized with a sterile spatula (which was soaked in 99 % ethanol and flamed) and, ~0.3 g of wet sediments were subsampled for DNA extraction. The sediment pellets were weighed (before and after the subsampling for DNA extraction) and then frozen prior to freeze-drying for subsequent sample processing.

Morphological analyses of cladocerans and diatoms in sediment trap samples

Cladocerans subfossils slides were prepared according to standard procedures from Korhola and Rautio (2001). The remains (headshields, carapaces, post-abdomens, post-abdominal claws and antennules) were identified and enumerated using a Leica DM2500 light microscope under 20-40X magnification. Only the most frequent remain for each taxon was used as an index of the species abundance. A minimum of 70 remains were identified and counted per sample using the keys of Witty (2004), Szeroczyñska and Sarmajo-Korjonen (2007) and Korosi and Smol (2012). From the raw counts, the density (# remains gDW^{-1}) and the biomass (ug gDW^{-1}) were calculated. Various references were used to obtain an average length for cladoceran species identified in the ST as well as the equation to calculate the biomass (Supplementary material SM1).

Microfossil diatom slides were prepared according to the standard methods described in Battarbee et al. (2001). A known concentration of microspheres (Thermo Scientific^{TM}7000 Series Copolymer Microsphere Suspension, 6 µm) were spiked in each sample and counted along the diatom valves for quantification. Diatom microfossils were identified and enumerated using a Leica DM4500 B microscope at 1000X magnification and under differential interference

contrast (DIC). The valves were counted in fields of views along parallel transects until 400 valves were counted. From the raw counts, the density (# valves gDW^{-1}) and the biovolume (um^3 gDW^{-1}) were calculated. Multiple references were used for the diatom identification as well as to estimate the biovolume (Supplementary material SM1).

DNA extraction from water samples

To minimize contamination of samples, the initial processing of ST and water samples (i.e., collection of the sediments from accumulation tubes, filtration of water samples) and the extraction of DNA were performed in a separate facility from all downstream molecular analyses (i.e., PCR amplification, library preparation, and DNA sequencing). To evaluate the potential introduction of contaminating DNA during sample processing, blank water filtrations and blank DNA extractions (both with autoclaved deionized water) were performed along with the samples. Water samples were thawed overnight and filtered onto a 3-µm pore size filter. Multiple filters were used until ~1 L of water was filtered, and the total water volume filtered was noted. Filters from each sample were pooled in a 2-mL tube and stored at -80°C until DNA extraction. DNA was extracted using a combination of chemical and physical lysis. For the lysis step, 500 mL of 25:24:1 by volume of phenol-chloroform-isoamyl alcohol (PCI), 60 mL of 20 % by weight of sodium dodecyl sulfate, and ~0.37 g of 0.7 mm zirconium beads (sterilized at 280°C for 3 hours prior to addition) were added to the filters and vortexed for 10 min. Samples were incubated at 60°C for 10 min followed by 1 min incubation at 4°C. After a 10 min centrifugation at 10,000 rpm and at 4°C, the aqueous layer was transferred to a sterile 2-mL tube. Subsequently, 500 mL of PCI was added to the supernatant, briefly vortexed and centrifuged for

10 min at 10,000 rpm and at 4°C. The PCI treatment was repeated three times or until there was no longer a white precipitate at the aqueous-organic interphase. The DNA was precipitated overnight with 1 mL of 96 % by volume ice-cold ethanol and 120 mL of 3 M sodium acetate, after which the samples were centrifuged for 60 min at 13,000 rpm and at 4°C. The supernatants were decanted, and the pellets washed with 850 mL of 80 % by volume ice-cold ethanol. The samples were vortexed briefly and centrifuged for 15 min at 13,000 rpm and 4°C. The supernatants were then removed, and the pellets were air-dried and resuspended in 50 mL of Tris EDTA buffer, and the DNA samples were stored at -20°C until further analyses.

DNA extraction from sediment trap samples

To evaluate the differential preservation of taxa in the sediments as either extracellular (exDNA) or intracellular DNA (inDNA), a phosphate buffer (NaP buffer, pH 8.0, 0.1 M) was first used to de-adsorb the exDNA from the sediment particles (Taberlet et al. 2012; Alawi et al. 2014). Specifically, 500 mL of NaP buffer was added to ~0.3 g of wet sediments, resulting in a weight:volume ratio of ~1. Samples were mixed by slow rotation for 15 min then centrifuged at 10,000 rpm for 10 min (both steps at room temperature). Only one addition of NaP buffer was used, as the quantity of exDNA was on average ~25 % of the total sedDNA (Supplementary material SM2). After centrifugation, the supernatants containing the exDNA were transferred to a new sterile 2-mL tube and the pellets were kept for the extraction of the inDNA fraction. Sediment trap DNA was extracted using the NucleoSpin® Soil kit according to the manufacturer instructions (Macherey-Nagel, Düren, Germany). The extracellular fraction of the DNA was extracted following the same commercial kit for soil DNA. However, the lysis steps of the

NucleoSpin® Soil kit were skipped to avoid further degradation of the exDNA and to ensure that no lysis occurred for potentially resuspended cells. DNA concentrations for both water and ST samples were measured using a Qubit®2.0 Fluorometer (Invitrogen) for a broad range of double-stranded DNA following the manufacturer instructions (Qubit ds-DNA BR Assays, Invitrogen). DNA concentrations in the blanks were below the detection limit (0.1 ng/µl). DNA samples were visualized on 1 % agarose electrophoresis gel that contained ethidium bromide for DNA staining and visualization.

Polymerase chain reaction (PCR) amplification and sequencing of 18S rRNA gene

A fragment of the V7 region of the 18S rRNA gene (~260 bp) was PCR amplified from water and ST DNA samples using the general eukaryotic primers 960F (5'- GGCTTAATTTGACTCAACRCG -3') (Gast et al. 2004 from Capo et al. 2016) and NSR1438 (5'-GGGCATCACAGACCTGTTAT -3') (Van de Peer et al. 2000 from Capo et al. 2016) modified with Illumina adapters and barcodes for multiplex sequencing. These primers were identified as good candidates in terms of coverage of eukaryotic diversity, length (short sequence suitable for paleogenetics), and taxonomic assignment (Capo et al. 2016). PCR was performed using Phire Hot Start II DNA Polymerase (Thermo Scientific). Each PCR reaction (total volume of 25 µL) contained 5 µL of 5X Phire reaction buffer, 0.2 µM dNTPs, 0.5 µM of each forward and reverse primers, 1.25 µL of dimethyl sulfoxide (DMSO; 5 % final concentration), and 0.5 µL of each DNA sample. The amplification conditions included an initial denaturation step at 98°C for 3 min, followed by 25 cycles of denaturation at 98°C for 5 s, an annealing step at 58°C for 5 s, and an elongation step at 72°C for 15 s, with a final elongation at

72°C for 1 min. For several water (n = 17) and ST (n = 11) samples, 35 cycles were required. All PCR reactions were performed using C1000 Touch^{TM} Thermal Cycler (Bio-Rad). To assess the performance of the PCR amplification, PCR products were visualized on 2 % agarose electrophoresis gel that contained ethidium bromide for DNA staining and visualization. The PCR amplicons were sent to Genome Quebec for barcoding (dual attach indices and sequencing adapters), library preparation, and sequencing on a MiSeq Illumina instrument (San Diego, CA). In total, 122 samples were pooled for sequencing in 1.5 libraries at equimolar concentrations, with 110 samples in one library, and 12 in the other 0.5 library (pooled with 43 samples from another project).

Bioinformatic processing and taxonomy assignment

The MiSeq reads were trimmed and filtered (no undefine bases, no sequencing error in primers, removing of primers), the paired-end reads were merged, and the chimeras removed using the package dada2 (Callahan et al. 2016) in R software (R Core Team 2018, Vienna, Austria). The taxonomy was assigned using the version 4.10.0 of the Protist Ribosomal Reference database (PR2) – SSU rRNA gene database (Guillou et al. 2013) at a minimum bootstrap confidence level of 80 %. We chose to conduct our analyses on amplicon sequence variants (ASV), and thus the sequences were not clustered into operational taxonomic units (OTUs). Each ASV was represented by a unique DNA sequence.

Statistical analyses

To assess the extent to which the micro-eukaryotic communities identified from sediments with DNA or morphological approaches preserve the biological communities identified from the water column, comparisons were made across sample matrices (e.g., epilimnion, metalimnion, sediment traps intracellular DNA (ST inDNA) and extracellular DNA (ST exDNA)). Comparisons were also applied for DNA-based approach between sample matrices for both the entire micro-eukaryotic communities and the ASVs from the pool of micro-eukaryotes that were common between the water column and the ST (but excluding Crustacea and Bacillariophyta; referred to below as the shared ASVs). To evaluate the congruence between morphological and DNA identifications, the two approaches were compared across sample matrices as well. To make the comparisons, we first performed Principal Component Analyses (PCA) on Hellinger-transformed data, which converts the data into relative abundances and then applies a square root transformation (Legendre and Gallagher 2001). For the data based on morphological identifications, we used the estimated density and biomass of each group, whereas the total number of sequences of each ASVs were used to reflect the DNA-based identifications. PCAs were applied to each sample matrix and taxonomic approach separately, and the three first axes of the site scores were extracted. We then applied an RV coefficient to correlate two matrices with corresponding rows (sites). Between two matrices of quantitative data, the RV coefficient corresponds to the square of the Pearson correlation, and the RV coefficient is thus homologous to an R^2 (Legendre and Legendre 2012). As most of the variation in the PCA was explained by the first three axes, we compared only the first axis between matrices as well as the first three axes. To have accurate comparisons between sample matrices when considering the metalimnion samples, we removed any ST samples that were deployed during the mixed period

(from Nov/Dec – April/May) from the statistical analyses. We used the term epilimnion to refer to the mixed part of the water column (regardless of whether the lake was stratified; see Fig. 1b) for simplicity in the manuscript. In addition to PCA applied to shared ASVs, we applied multivariate partial redundancy analysis (partial RDA) to identify environmental gradients associated with different potential bioindicators that could be further developed in future paleolimnological studies. The potential bioindicators were identified in the partial RDA triplot as taxa with the most distant coordinates from the centroid along the main axes of variation (axis 1 and/or axis 2, depending on significance of the axes). The community data were Hellinger-transformed prior to partial RDA, and day of year and year were used as covariates to control for temporal trends in the datasets. A suite of physico-chemical and biological variables from the photic zone in the epilimnion and the metalimnion as well as from the hypolimnion were used in partial RDAs. The epilimnetic and metalimnetic variables include nitrate (NO_3; µg L^{-1}), dissolved organic nitrogen (DON; µg L^{-1}), dissolved inorganic nitrogen (DIN; µg L^{-1}), ammonia (NH_3; µg L^{-1}), particulate nitrogen (PN; µg L^{-1}), total phosphorus (TP; µg L^{-1}), total dissolved phosphorus (TDP; µg L^{-1}); particulate phosphorus (PP; µg L^{-1}), soluble reactive phosphorus (SRP; µg L^{-1}), soluble reactive silicon (SRSi; mg L^{-1}); total dissolved solids (TDS; mg L^{-1}), pH, upper column water temperature (average of temperatures from 0 to 5 m depth; EpiTemp; °C), photic zone depth (m), dissolved oxygen (DO; mg L^{-1}), total chlorophyll (TotChl; µg L^{-1}), chlorophyll from phytoplankton > 2 µm (PhyChl; µg L^{-1}), chlorophyll from phytoplankton > 20 µm (MicroChl; µg L^{-1}) and chlorophyll from phytoplankton < = 2µm (PicoChl; µg L^{-1}). The hypolimnetic parameters include nitrate (HypoNO_3; µg L^{-1}), total phosphorus (HypoTP; µg L^{-1}), dissolved oxygen (HypoDO; mg L^{-1}) and total chlorophyll (HypoTotChl; µg L^{-1}). Other environmental parameters were measured as part of the monthly limnological monitoring but

were not included in partial RDAs as they were highly correlated (> 80%) with other variables (see supplementary material SM3 for an extensive list of all environmental variables measured in Cultus Lake). Environmental variables were normalized when possible and standardized prior to partial RDAs. A stepwise selection procedure was applied to select the best predictors of the community composition. The statistical significance of the partial RDA models and their RDA axes was tested with 999 permutations on the F-*ratio*.

The R statistical software v. 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform all statistical analyses. The library vegan (Oksanen et al. 2018) was used to perform the PCA and the function *scores()* was used to extract the PC axes site scores. To calculate the RV coefficients between sample matrices and taxonomic approaches, the function *coeffRV* was used and the statistical significance for the RV coefficients was calculated using *RV.rtest()*, from the library FactoMineR (Le et al. 2008) and ade4 (Dray et al. 2007), respectively. Partial RDA were performed using the function *ordistep()* in the library vegan (Oksanen et al. 2018). The functions *PowerTransform()* and *bcPower()* from the library car (Fox and Weisberg 2011) were used to normalize the environmental data if necessary.

Results

Contemporary limnology of Cultus Lake

Cultus Lake is an ice-free lake, characterized by a mixed water column period (typically from Nov/Dec – April/May) and a thermally stratified period (typically April/May – Nov/Dec). During this study, the stratified period was characterized by deeper light penetration, greater

phytoplankton standing stock (especially in the metalimnion) and higher zooplankton biomass (Table 1). Overall, total phosphorus and total nitrogen exhibited higher concentrations during the mixed period and lower concentrations in the epilimnion during stratification (Table 1). The hypolimnetic total chlorophyll *a*, dissolved oxygen and total phosphorus were lower during the stratified period. Crustacean biomass was high throughout the year, except for the mixed period when notable decreases occurred (from December to March; Fig. 2a). Diatom biomass in the photic zone increased substantially during two periods: in February and then over the stratified period (mainly from May to September; Fig. 2b).

Eukaryotic assemblage richness and composition based on 18S rRNA gene analyses

A total of 7,463,947 sequences were generated from the water and sediment trap (ST) samples and assigned to a total of 6,812 ASVs (Table 2). The number of ASVs per sample varied between 4 to 598 ASVs (once rarefied; Table 2). Overall, the ST extracellular DNA (ST exDNA) fraction exhibited slightly higher average richness (based on rarefied richness; Table 2) compared to other sample matrices. ASVs were mainly assigned to the phyla Opisthokonta (21 %), Stramenopiles (14 %) and Alveolata (11 %). A relatively large fraction of the total ASVs (30 %) were not assigned to a phylum (i.e., unclassified Eukaryota), but the total pool of sequences associated with this group was relatively low (i.e., on average, ~6 % and ~3 % were unclassified Eukaryota in water samples and sediment trap samples, respectively).

The micro-eukaryotic communities differed substantially between the water column (epilimnion and metalimnion) and the ST samples (Figs. 2c-f; 3). Specifically, ASVs assigned to the Hacrobia phylum dominated the epilimnetic and metalimnetic time series, except for some

summer and fall months (Figs. 2c, d; 3a). Within the Hacrobia, the most abundant ASVs in the water column communities belonged to the Cryptophyta subphylum (Fig. 3a). In total, 5 ASVs were assigned to the *Cryptomonas* genus while a sixth was within the *Geminigera* genus (Fig. 3a). Hacrobia ASVs were generally not detected in inDNA (except for May 2017) and detected in low abundance in exDNA in the ST samples (Fig. 2e, f).

Crustacea-specific comparisons across sample matrices and taxonomic approaches

Crustacean 18S rRNA gene sequences were detected in all water and ST samples. On average, crustacean represented 11 % and 4 % of all sequences in the epilimnion and metalimnion, respectively (Table 2). Crustacean sequences were more abundant in ST intracellular DNA (ST inDNA) and ST exDNA samples, representing on average 65 % and 55 % of the sequences, respectively (Table 2), but up to ~90 % of the total sequences in some ST samples (Table 2; Fig. 2c, d). The water and ST micro-eukaryotic communities were mainly dominated by ASVs assigned to crustaceans, specifically by three Maxillopoda ASVs (Figs. 3a; SM4.2).

After PCA analysis of the crustacean 18S rRNA gene and morphological datasets, the major apparent differences in the PCA biplots occurred between taxonomic approaches and were related to the dominant species in the crustacean assemblages (Figs. 4; SM4.1-SM4.4). From morphology, most of the dominant species belonged to the Branchiopoda class (Figs. 4a, b; SM4.1; SM4.3) while the dominant ASVs from DNA taxonomy belonged to the Maxillopoda class (Figs. 3a; 4c, d; SM4.2; SM4.4). The dominant ASVs in the DNA taxonomy were mainly associated with the stratification period (May to October; Figs. 2e, f; 3a; SM4.2; SM4.4).

Amongst the Branchiopoda class, *Bosmina longiremis* was detected with DNA-based approaches, but *Daphnia* spp. were not detected, even though they were dominant in morphological datasets from the water column and ST samples (Figs. 4a, b; SM4.1; SM4.3). In the water column, a third dominant species, *Diacyclops* sp. (a copepod) was detected with the morphological approach (Figs. 4a; SM4.1a, c; SM4.3a). The morphologically-identified ST assemblages were dominated by *D. longirostris*, *D. pulex* and *Bosmina* spp. (Figs. 4b; SM4.1b, d; SM4.3b), as only cladoceran remains were well-preserved enough to allow for morphological identification.

Using RV coefficients, we calculated the correlations of the PCA axes site scores between samples matrices and taxonomic approaches. We found that the strongest correlations between sample matrices and taxonomic approaches for the crustaceans were between: 1) ASVs in metalimnion and crustacean biomass from morphology in the water column (RV = 0.44 on PCA axis 1 site scores); and 2) between water column and ST samples that were both identified using morphological characters (Table 3). The two DNA fractions in the ST (exDNA and inDNA) were modestly correlated (Table 3). Another significant correlation was found between the ASVs in epilimnion and in the ST exDNA, but with a lower RV coefficient (RV = 0.18 on PCA axis 1 site scores; Table 3).

Diatoms-specific comparisons across sample matrices and taxonomic approaches

Diatom 18S rRNA gene sequences were less abundant in water samples than in ST samples (Table 2). Diatom sequences represented 16 % and 11 % of the entire micro-eukaryotic community in ST inDNA and ST exDNA, respectively (Table 2). In general, a clearer dynamic

signal was apparent in the ST compared to the water column (Figs. 2; 3), based on both morphological and DNA-based approaches (Figs. 5; SM4.5-SM4.8).

Across all samples, the diatom communities identified with DNA were dominated by two ASVs belonging to the Polar-centric-*Mediophyceae* (PCM) family and *A. subarctica* (belonging to radial-centric-basal-*Coscinodiscophyceae* (RCBC) family; Figs. 3a; 5c, d; SM4.6; SM4.8). Another RCBC was also dominant, mainly for the epilimnion and the ST inDNA (Figs. 5c, d SM4.6c; SM4.8c). For the biomass of the morphological identification approach, two PCM diatoms, *Stephanodiscus niagarae* and *Discostella stelligera* indicated variations in the community composition on the first PC axis (Figs. 5a; SM4.7a, b). Other dominant species also included *Lindavia intermedia, L. ocellata* and *L. michiganiana* in the water column (Figs. 5a; SM4.5a, b, d, e; SM4.7a, b). However, in the ST, the assemblages were mainly shaped by *S. niagarae*, *L. intermedia* and *Aulacoseira* spp. (Figs. 5b; SM4.5c, f, b; SM4.7c).

In general, all the comparisons for diatom assemblages exhibited significant RV coefficients between the three first axes of PCA site scores, except for some comparisons made with the metalimnion matrix (Table 3). Most of the strongest correlations were observed when comparing only the first PCA axis across samples or taxonomic approach matrices, which is consistent with the relatively large amount of variation explained on PC axis 1 (Fig. 5). RV coefficients greater than 0.6 were observed in cases where morphological count data were compared across sample matrices, or when the ST inDNA was compared to the morphological analyses of the ST samples (Table 3).

Structure of micro-eukaryotic communities shared between sample matrices based on 18S rRNA gene analyses

To further identify taxa deposited in the ST and explore how assemblages might be similar between water column and ST samples, we assessed the diversity and taxonomic identity of ASVs shared among the sample matrices. A total of 444 ASVs were shared among the epilimnion, ST inDNA and ST exDNA, and 221 were shared among the metalimnion, ST inDNA and STexDNA (Table SM5.1). Given that crustacean and diatom datasets were previously explored, we removed these groups, reducing the shared ASVs for the epilimnion vs ST and the metalimnion vs ST to 381 and 206, respectively (Table SM5.1). Of the remaining phyla, the most abundant shared ASVs included those assigned to Opisthokonta (proportion of shared ASVs in epi- 26 %; meta- 20 %), Alveolata (epi- 18 %; meta- 18 %), and Stramenopiles (epi- 18 %; meta- 20 %), which combined, corresponded to 62 % and 58 % of the total shared ASVs, respectively (Tables SM5.2, SM5.3). The most abundant shared ASVs belonging to the Opisthokonta phylum were mainly represented by Fungi (mainly Chytridiomycota class) and Metazoa subphyla (mainly Rotifera class) (Tables SM5.2, SM5.3). In the Alveolata phylum, the most abundant ASVs belonged to the Ciliophora sub-phylum (mainly Litostomatea and Spirotrichea classes), but the Dinoflagellata were also well represented (Tables SM5.2, SM5.3). For the shared ASVs belonging to the Stramenopiles, they were mainly assigned into the Ochrophyta subphylum (mainly Chrysophyceae) and other Stramenopiles, such as Oomycota and MAST (MArine STramenopiles) (Tables SM5.2, SM5.3). Shared ASVs belonging to the phylum Rhizaria (mainly the Cercozoa sub-phylum) were relatively abundant for both epilimnion and metalimnion vs ST samples, with 7 % and 11 % of the total shared ASVs, respectively (Tables SM5.2, SM5.3).

Ordination analyses of the shared taxa provided insight into how these assemblages varied through time and which environmental variables were associated with potential bioindicator taxa. The ordination biplots (Fig. SM5.2) and triplots (Fig. 6) of the shared ASVs for all matrices showed a clear separation of community composition during the mixed and thermal stratification periods (where present, Figs. 6a, b, c; SM5.2a, b, c). In all partial RDA analyses for the shared ASVs between the epilimnion and ST, upper water column temperature (EpiTemp) was selected as a significant driver of the communities as well as different fractions of nutrients, such as NH_3, DIN, SRSi, SRP, DON and PN (Fig. 6a, b, c). DO and indicators of algal production were also selected as significant predictors of the communities, but to a lesser extent (Fig. 6a, b, c). For the dataset focusing on the metalimnion only, the beginning of the thermal stratification (i.e., months 5, 6 and 7) were distinguished in the PCA from months at the end of this period (i.e., months 8, 9, 10 & 11; Fig. SM5.2d, e, f). However, the partial RDA triplots generally showed that the first month (i.e., month 5) of the stratification period was more similar to the last month (i.e., month 11 or 12) than the middle of the stratification period (i.e., months 8 and 9; Fig. 6d, e, f). Upper water column temperature was a significant predictor of the community composition for the metalimnion dataset (Fig. 6d), but photic zone depth was more important for both ST datasets (Fig. 6e, f). Fractions of nutrients (PN, TDP, DIN, hypolimnetic TP) and algal production (total chlorophyll, chlorophyll of phytoplankton > 2 µm, hypolimnetic total chlorophyll) were also selected in the partial RDA of the shared ASVs for the metalimnion as well as DO, but only for the inDNA and exDNA datasets (Fig. 6d, e, f). The most responsive ASVs belonged to similar taxonomic affiliations for both datasets (epilimnion vs ST and metalimnion vs ST) although the ASVs were not the same (Figs. 6; SM5.2a, b, c). For example, ASVs assigned to the classes Chrysophyceae and Cryptophyceae were found to be most

responsive across all PCA biplots and partial RDA triplots (Figs. 6; SM5.2). Dinophyceae were also a responsive component of the shared ASV assemblages (Fig. 6; SM5.2). Although well represented in ST inDNA, Chytridiomycota ASVs appeared to be more responsive to environmental conditions in the ST exDNA assemblages (Figs. 6c, f; SM5.2c, f).

To quantify the coherency among sample matrices for the shared ASVs, RV coefficients were calculated for the first axis and the three first axes of the PCA site scores for both the entire micro-eukaryotic communities and the shared ASVs after excluding shared crustaceans and diatoms (Table 4). In general, all comparisons between sample matrices for the three first PCA axes site scores were significant, with higher RV coefficients when comparing only the shared ASVs rather than the entire micro-eukaryotic communities (Table 4). When comparing the first PCA axis site scores, the correlation between metalimnion and ST matrices (inDNA and exDNA) were not significant for the entire micro-eukaryotic communities but were highly correlated and significant for the shared ASVs between metalimnion and ST matrices (Table 4). Although the RV coefficient calculated for the first PCA axis site scores between ST inDNA and ST exDNA was significant for the entire micro-eukaryotic communities, it was not significant for the shared ASVs when using the dataset including the epilimnion (Table 4).

Discussion

The development and application of DNA-based approaches in paleolimnology is rapidly expanding, in part due to technological advancements in molecular approaches (Bohmann et al. 2014). However, only a handful of studies have evaluated the congruence between morphology and sedDNA-based approaches for taxonomic identification (e.g., Stoof-Leichsenring et al. 2012,

2014; Dulias et al. 2017). Even fewer have assessed the degree to which the assemblages preserved in sediments represent the assemblages identified in the water column using DNA-based approaches (e.g., Capo et al. 2015; Monchamp et al. 2016). Our sediment trap (ST) study spanning 36 months partly fills this gap and expands our knowledge of the strengths and the weaknesses of using DNA-based approaches in paleolimnology. Overall, our study indicates that sedDNA-based approaches can be insightful, but care must be taken in drawing conclusions about water column dynamics, and in considering which groups of taxa are targeted. We found strong correlations between water and ST samples for morphological and DNA-based approaches, but mainly for diatom taxa. We also identified certain taxonomic groups that may be suitable to include in paleolimnology using DNA-based approaches, such as Ciliophora, Dinoflagellata, Chytridiomycota, Chrysophyceae, Cryptophyceae and Cercozoa.

Comparisons between water and sediment trap assemblages based on 18S rRNA gene and morphological analyses

The 18S rRNA gene analyses revealed a much wider range of taxonomic groups compared to those typically studied using morphological approaches in paleolimnology. Generally, the richness of micro-eukaryotes was greater in ST compared to water samples. Clear dynamics were also more apparent in the ST than in the water sample time series (Fig. 2c-f). The higher richness and stronger patterns associated with ST is likely due to the spatio-temporal integration (~1 month) provided by sediment trap samples compared to the single point sampling of the water column. The higher diversity in ST could also be explained by the capture of DNA

from littoral species as well as those potentially originating from the watershed (Deiner et al. 2016).

Although the average richness per sample was similar across all sample matrices based on 18S rRNA gene sequences, the observed range was higher in ST than water samples (Table 2). In the water column, the species present in the samples collected were most likely alive at the moment of the collection. The filter pore size used for water filtration (3-µm pore size) and the DNA extraction method were selected in order to mainly collect living or intact cells. On the other hand, DNA from ST samples was extracted from bulk sediment samples, which can include degraded DNA, all taxa deposited in the sediments and taxa living in the surface sediment layer. Consequently, the richness per sample and its range across samples could be more stable for the water samples than for the ST samples.

Major differences in the taxonomic composition were also found between water and ST samples. The most notable difference was the dominance of Hacrobia 18S rRNA gene sequences in the water samples (both epilimnion and metalimnion) compared to the dominance of Opisthokonta and Stramenopile DNA sequences in ST. Although Hacrobia were detected in some ST samples (mainly with exDNA; Fig. 2f), their proportion was low throughout the sampling time series. The Hacrobia sequences in the ST were mainly dominated by Cryptophyta taxa. Interestingly, Capo et al. (2015) reported similar results, where Cryptophyta were underrepresented in recently deposited sediments compared to the water column. This underrepresentation of Cryptophyta in sediments as well as the slightly higher detection in exDNA compared to inDNA (Fig. 2e, f) are likely because they are soft-bodied algae (Hoef-Emden and Archibald 2017), which can potentially result in a greater degree of cell lysis and exDNA degradation via DNAases. In addition, Cryptophyta cells might not be as efficiently

transported to sediments compared to other primary producers because of their high nutritional value and associated removal efficiency by herbivorous grazers (Brett and Müller-Navarra 1997).

The crustacean-specific results generally yielded modest to weak correlations among sample matrices using 18S rRNA gene. The strongest correlations associated with the 18S rRNA gene analyses were with ST exDNA, which suggests that exDNA preserved in sediments could be more effective at targeting crustaceans to reconstruct past ecological changes from epilimnetic environments. Crustaceans need to go through multiple moulting processes as they increase size (Sastri and Roff 2000), which could potentially lead to abundant exDNA excreted from the previous carapaces. With the primers used in this study, we identified numerous taxa within the Branchiopoda and Maxillopoda families. In particular, three ASVs of *Bosmina longirostris*, two ASVs of *Sida crystallina*, two ASVs of *Chydorus sphaericus* and two unclassified Branchiopoda. We also identified 17 ASVs assigned to more specific copepod taxa (e.g. *Cyclops* spp., *Macrocyclops* spp.), and potentially many more copepod taxa as 109 ASVs were assigned only to the Maxillopoda family. Although daphnids were quite abundant in the morphological time series, the primers we used did not detect this taxonomic group. Similarly, bosminids were quite abundant with morphological identification, but they have been detected with very low abundance of sequence numbers. The small-subunit rRNA gene in metazoan usually varies between 1,800 to 1,900 nucleotides in total length (Crease and Colbourne 1998), but can be exceptionally long in some arthropods. For example, the 18S rRNA gene in *Daphnia pulex* has a total length of 2,293 nucleotides, with particularly long hypervariable V4 and V7 regions (Crease and Colbourne 1998). Consequently, daphnids (and most likely other cladoceran taxa) are likely underrepresented in our datasets as short amplicons will be preferentially sequenced over long

amplicons. During the bioinformatic analyses, we also removed amplicons that had a length of greater than 450 bp. We recommend using primers that amplify a different region of the 18S rRNA gene (other than V4 or V7) or a different gene altogether if the goal of the study is to target crustaceans (e.g., Andújar et al. 2018). Nonetheless, the detection of copepods with 18S rRNA gene is very interesting as this is a group that does not preserve well in sediments (Korhola and Rautio 2001). In fact, the correlations comparing morphological counts between water and sediments were relatively modest and could be due to the general lack of copepods subfossils in the sediments.

In contrast to crustaceans, many significant correlations were detected for diatoms. From morphological counts, we observed a high biomass of *S. niagarae* and *Aulacoseira* spp. during the winter, which are two heavily silicified species and are usually associated with strong water column mixing (Lund 1954; Stockner and Lund 1970; Stoermer et al. 1985; Horn et al. 2011). In addition, *S. niagarae* is among the largest diatom species in freshwater ecosystems, and it peaked in biomass in both water and ST samples in the mixed period around February-March of each year (Fig. SM6.1a, b). *Aulacoseira* spp. can form long colonial chains, and also had high total biomass during the mixed period in Cultus Lake (Fig. SM6.1c, d). Therefore, both tend to sink more rapidly than other diatom species without adequate mixing conditions and could settle out through the water column to be incorporated into the sediments more quickly than the lighter species that were dominant in summer. The summer species might degrade faster in the water column once dead as higher temperatures and deeper light penetration increase bacterial activity during this period and they also have to cross the physical barrier of the thermal stratification to settle in the sediments.

Overall, DNA-based and morphological approaches were generally significantly correlated, but this was more consistent and stronger when considering diatom assemblages. Diatoms are clearly a suitable group for further comparison between taxonomic approaches in both the water column and the sediments. Furthermore, comparative taxonomic approaches could be informative when there are gaps in the curated database for freshwater diatoms as suggested by Rimet et al. (2018). For example, in our study, two ASVs belonging to Polar-centric-*Mediophyceae* (PCM) were not assigned to a species level. Based on the distribution of taxa through time, we infer that PCM6 (Fig. 5d), associated with the mixed period, is likely *S. niagarae* that were identified under the microscope (Fig. 5b). Another instance is with the species *L. intermedia*, which showed similar dynamics to the ASV PCM3 as a dominant taxon in the ST during the thermal stratification period (Fig. 5b, d).

Potential bioindicator taxa based on shared ASV analyses

Classical paleolimnological approaches are mainly based on morphological identification of certain taxonomic groups that produce adequate subfossils. However, many other groups of taxa play essential roles in lake ecosystems and could advance our understanding of the changes in lake ecology over long periods of time. Our analyses of shared ASVs identified new potential bioindicators suitable for tracking pelagic ecological dynamics in lake sediments. With our shared ASVs analyses, we evaluated which ASVs were deposited in the sediment traps from the pelagic environment and which ASVs have the potential to track environmental changes in lakes, which are two criteria for defining useful bioindicators in paleolimnology. In particular, we identified Ciliophora, Dinoflagellata, Chytridiomycota, Chrysophyceae, Cryptophyceae and

Cercozoa as groups that were both present in the water column and ST DNA samples and that showed associations with several physico-chemical and biological variables (Figs.6; SM5.2). The potential to use each of these groups as bioindicators in paleolimnology will be informed going forward by synthesizing knowledge of their ecological niches as well as ensuring that their DNA is preserved in sediment archives over longer time scales.

Ciliophora species diversity and composition have previously been used to indicate and evaluate ecosystem quality (reviewed in Lynn 2017). For example, low ciliate richness has been associated with highly oxygenated water (Šlapeta et al. 2005). Many other environmental factors can influence the species abundance and composition of ciliates (Andrushchyshyn et al. 2006). For instance, the addition of leaf litter in enclosures in a small pond led to higher abundance, but lower diversity of ciliates (Andrushchyshyn et al. 2006). Therefore, ciliates have the potential to be used in paleolimnology as bioindicators of change in water oxygen levels or organic matter concentration. According to our RDA analyses, several ciliates could be suitable bioindicators for dissolved inorganic nitrogen (DIN) level as we found a group of ciliate taxa associated with high concentration of DIN (i.e., Strombidiida, *Cyclotrichium* sp., Hypotrichia, Scuticociliatia, *Tintinnopsis* sp., *Aspidisca* sp. and Vorticellidae) (Fig. 6a-c, e). Ciliates are generally considered ubiquitous and cosmopolitan (Lynn 2010) and have diverse strategies to acquire energy (Lynn 2010, 2017). Species belonging to the genus *Mesodinium* are the only known autotrophic ciliates, but mixotrophic species of other genera can capture chloroplasts from their prey or from autotrophic endosymbionts (Lynn 2017). Ciliates can also be strictly heterotrophic feeding on bacteria, algae and other protists. Bacterivorous ciliates can maintain quality of sewage water effluents as they can efficiently decrease bacterial densities by feeding on them (Curds and

Cockburn 1970a; b; Madoni 2003). Ciliates have also previously been detected as a dominant group in sediment core archives in a few lakes (Capo et al. 2016; 2017; 2019).

Dinoflagellates are generally photosynthetic and often contribute substantially to the primary production of aquatic systems. Dinoflagellate community composition varies according to environmental factors, such as nutrient concentrations, pH, grazing intensity and surrounding vegetation (Saldarriaga and 'Max' Taylor 2017). Therefore, their historical distributions may be useful in paleolimnological studies, and they have already been used in paleoceanography using DNA-based approaches (Coolen et al. 2006, 2013; Amacher et al. 2009; Boere et al. 2011a; b). In particular, tracking grazing pressure through time may be possible by assessing the dinoflagellate assemblages in sedDNA as when grazing is intense, the dinoflagellate communities in lakes are dominated by *Ceratium* spp., while *Peridinium* sp. and *Gynmodinium* sp. are usually more abundant at lower grazing intensity (Saldarriaga and 'Max' Taylor 2017). Our RDA analyses showed that the dinoflagellate communities were separated along gradients of nutrients, such as DIN, ammonia (NH_3) and total dissolved phosphorus (TDP) (Fig. 6b, d, e). Therefore, dinoflagellates have the potential to reconstruct the past conditions of different fractions of nutrients in lakes. Ecological dynamics of freshwater dinoflagellates are understudied but are likely important components of the microbial loop as they help transfer considerable amounts of energy into planktonic food (Saldarriaga and 'Max' Taylor 2017).

Organisms from the Chytridiomycota class are common in freshwater with high organic substrates concentrations or high suitable host densities (Kagami et al. 2014). They are often found as parasites on algae and facilitate energy transfer to zooplankton (Kagami et al. 2007, 2014). They play a vital role in energy recycling as necrotrophs and biotrophs, capable of metabolizing resistant substrates, such as cellulose, keratin, chitin and pollen (Powell 2017). As

parasites, chytrids also have an essential ecological role in regulating algal blooms and zooplankton populations; the severity of these infections being directly affected by light and nutrient levels in the aquatic environments (Ibelings et al. 2004). With the growing interest in aquatic infectious diseases since the 1970s (Dudgeon et al. 2006; Reid et al. 2018), chytrids species could be powerful bioindicators of specific aquatic host-parasite dynamics in paleolimnology. According to our RDA analyses, specific taxa of chytrids were associated with particular months of the year, which could be related to the timing of host presence in the lake (Fig. 6c, f). Chytrids have also been previously detected in a sediment core archive from a few European lakes (Capo et al. 2016; 2017).

The use of Chrysophyceae as bioindicators in paleolimnology has been established and they can be microscopically identified by their scales or resting cysts (Smol 2008). However, only about 15 % of the chrysophytes species (including Chrysophyceae and Synurophyceae) have siliceous scales and the species producing many of the cysts are currently unknown (Smol 2008). As such, DNA analysis of chrysophyte taxa could enrich our understanding of this group and its potential as bioindicators of past changes in lake ecosystems. Chrysophyceae DNA has been usually detected in high proportion in sediment archives in a few lakes (Capo et al. 2016; 2017; 2019). Optimal environmental conditions of Chrysophyceae species are usually well-defined (Zeeb and Smol 2001; Kristiansen and Škaloud 2017) and thus, they are useful from a paleolimnological perspective (Zeeb and Smol 2001). Chrysophyceae usually thrive in humic and slightly acidic lakes with moderate nutrient levels (Kristiansen and Škaloud 2017). However, high richness of chrysophytes are often detected in water bodies surrounded by agricultural lands (Kristiansen and Škaloud 2017). In the partial RDA triplots, two species of chrysophytes (Chryso26 and Chryso28) were usually associated with high concentrations of NH_3 (Fig. 6a-c).

Although Chrysophyceae are generally considered to be photoautotrophic, they can also exhibit heterotrophy under specific conditions, and consequently, are strong competitors across environments (Sandgren 1988 from Zeeb and Smol 2001).

Finally, the results from the shared ASVs analyses indicated that cryptophytes assigned to *Cryptomonas* spp. were dominant in all sample matrices, even though we generally found that cryptophyte sequences were less abundant in sediment traps relative to the water column above. Therefore, the most abundant and dominant species of cryptophytes could be used as bioindicator species to track changes in lake ecological dynamics through time. For instance, the most abundant cryprophyte taxa (*Crypto*4 and *Crypto*7) in the pelagic environment of Cultus Lake was deposited in our sediment traps and was also identified as potential bioindicators according to our partial RDA analyses (Fig. 6a-e). Cryptomonads are known to be low-light specialists and can thus be important primary producers under periods of low light penetration system such as the winter period in Cultus Lake (characterized by high suspended particulate turbidity from sustained rainfall/erosion) and, thus may act as potential bioindicators of light levels in water bodies. More broadly, it is clear that DNA-based approaches are useful for identifying the presence of different cryptophyte taxa as species from the same genus can be laborious to distinguish morphologically from live or preserved cells (Hoef-Emden and Archibald 2017). However, their soft-bodied cells make them fragile to cell disruption (Hoef-Emden and Archibald 2017), and thus, metabarcoding of sediments might only detect a limited diversity of what can be found in the water column. Using the same set of primers, earlier work by Capo et al. (2015; 2016; 2019) have detected cryptophyte DNA sequences in lake sediment archives from France, Sweden and Greenland, which suggest that the potential to detect this group with molecular approaches is fairly widespread.

Although some information on the niches of potential bioindicator groups for paleolimnology are mentioned above, substantial work is still needed to identify more precisely their environmental range and optima before using them in further paleolimnological studies. Transfer functions, which are based on establishing quantitative relationships between taxa preserved in surface sediments and contemporary environmental factors, could be developed using DNA-based approaches (reviewed in Domaizon et al. 2017). In addition, these potential taxonomic groups identified in our study could be used in a multi-proxy paleolimnological study, which would help reconstruct more fully the past ecological and environmental conditions of lakes. With further studies, it will be necessary to clearly identify the preservational affinity in sediments of taxa within each potential bioindicator group. Lakes that have long-term water column records might serve as ideal sites to investigate further these new potential bioindicators.

Efficiency of intracellular and extracellular DNA to track community composition in sediments

Generally, the ecological patterns inferred from inDNA and exDNA were similar for the entire micro-eukaryotic community as well as for the diatom and crustacean assemblages, specifically. The dominant ASVs that exhibited the most change over time were the same for both inDNA and exDNA, which led to high congruence between the two different DNA fractions. For diatoms, inDNA seemed to be more suitable to identify taxa from sediment samples, as higher correlations with both morphological and DNA datasets from the water column were observed with inDNA than with exDNA. However, some Chytridiomycota taxa were more dominant in the ST with exDNA in the shared ASV matrices (Fig. 6c, f). Further comparisons between inDNA and exDNA of core samples are also needed to evaluate whether

both DNA fractions track common or distinct taxa or assemblages and to evaluate the potential for diagenesis of both DNA fractions over longer time frames.

Perspectives for future paleolimnological studies

Our sediment trap study in Cultus Lake provided insights on the advantages and limitations of using DNA-based approaches to reconstruct past biological conditions of lakes. DNA-based approaches were useful to track ecological patterns of micro-eukaryotes. The shared ASV analyses suggested potential novel bioindicators, traceable using sedDNA, that could be incorporated in future paleolimnological studies (e.g., Ciliophora, Dinoflagellata, Chytridiomycota, Chrysophyceae, Cryptophyta and Cercozoa). Although, the DNA-based approaches used in our study were adequate to track diatom assemblages, it did not perform as strongly in reconstructing the entire crustacean assemblages. Nonetheless, several crustacean taxa were clearly abundant in DNA sequences, suggesting that DNA-based approaches could be used to reconstruct copepod dynamics, and improved upon with more specific crustacean primers. Given that copepods also play essential ecological roles in lakes and are currently not accounted for in traditional morphological-based approaches, this is an area of substantial interest. Certainly, testing potential specific primers with mock communities prior to sequencing the actual samples is an effective approach to ensure that the target taxa are well-represented in the DNA sequence datasets (Zhang et al. 2018). Additionally, more work is needed to compare DNA and morphological approaches to better understand whether DNA-based approaches can reconstruct cladocerans in sediments as well as in water.

For our study, we conducted our statistical analyses on the relative abundances of ASVs. However, as our sediment samples were younger than the sediments of cores, we are aware that it could lead to different results when the sequence numbers are used in older samples. Both DNA sequence numbers as well as presence-absence should be considered in statistical analyses of sediment core DNA studies to evaluate whether the type of data provided produces similar results.

Conclusion

Our study identified a rich diversity of micro-eukaryotes in the water column of Cultus Lake and highlighted substantial temporal dynamics across the 3 years, monthly sampling period. We documented that many of the taxa present in the water column were deposited into the sediment traps. We found significant congruence between classical and molecular taxonomic approaches across both sediment trap and water samples, suggesting that DNA-based approach could greatly expand the pool of potential paleolimnological bioindicators. The relationships we identified between environmental variables and potential DNA-based bioindicator taxa represent a critical first step towards this goal. DNA burial and preservation over time was not addressed in our sediment trap work, and thus subsequent work is needed to define which taxonomic groups are adequately archived and preserved as DNA in the older sediments. Overall, our sediment trap study spanning 36-months enhances our knowledge of using DNA-based approaches in paleolimnology and provides an essential foundation for future paleolimnological work.

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References

Alawi M, Schneider B, Kallmeyer J (2014) A procedure for separate recovery of extra- and intracellular DNA from a single marine sediment sample. J Microbiol Methods 104:36–42. doi:10.1016/j.mimet.2014.06.009

Alric B, Perga M-E (2011) Effects of production, sedimentation and taphonomic processes on the composition and size structure of sedimenting cladoceran remains in a large deep subalpine lake: paleo-ecological implications. Hydrobiologia 676:101–116. doi:10.1007/s10750-011-0868-0

Amacher J, Neuer S, Anderson I, Massana R (2009) Molecular approach to determine contributions of the protist community to particle flux. Deep Sea Research Part I: Oceanographic Research Papers 56:2206–2215. doi:10.1016/j.dsr.2009.08.007

Andrushchyshyn OP, Magnusson AK, Williams DD (2006) Responses of intermittent pond ciliate populations and communities to in situ bottom-up and top-down manipulations. Aquatic Microbial Ecology 42:293–310. doi:10.3354/ame042293

Andújar C, Arribas P, Yu DW, et al (2018) Why the COI barcode should be the community DNA metabarcode for the metazoa. Molecular Ecology 27:3968–3975. doi:10.1111/mec.14844

Barnett AJ, Finlay K, Beisner BE (2007) Functional diversity of crustacean zooplankton communities: towards a trait-based classification. Freshwater Biology 52:796–813. doi:10.1111/j.1365-2427.2007.01733.x

Battarbee, RW, Jones VJ, Flower RG, Cameron NG, Bennion H, Carvalho L, Juggins S (2001) Diatoms. In: Smol JP, Birks HJB, Last WM, Bradley RS, Alverson K (eds) Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal and siliceous indicators. Kluwer Academic Publishers New York, pp 155-202

Bennion H, Battarbee RW, Sayer CD, et al (2011) Defining reference conditions and restoration targets for lake ecosystems using palaeolimnology: a synthesis. J Paleolimnol 45:533– doi:10.1007/s10933-010-9419-3

Bloesch J, Burns NM (1980) A critical review of sedimentation trap technique. Schweiz Z Hydrologie Schweizerische Zeitschrift für Hydrologie 42:15–55

Boere AC, Rijpstra WIC, De Lange GJ, et al (2011a) Preservation potential of ancient plankton DNA in Pleistocene marine sediments. Geobiology 9:377–393. doi:10.1111/j.1472-4669.2011.00290.x

Boere AC, Rijpstra WIC, de Lange GJ, et al (2011b) Exploring preserved fossil dinoflagellate and haptophyte DNA signatures to infer ecological and environmental changes during deposition of sapropel S1 in the eastern Mediterranean. PALO Paleoceanography 26:

Bohmann K, Evans A, Gilbert MTP, et al (2014) Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology & Evolution 29:358–367. doi:10.1016/j.tree.2014.04.003

Brett M, Müller-Navarra D (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483–499. doi:10.1046/j.1365-2427.1997.00220.x

Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. doi:10.1038/nmeth.3869

Capo E, Debroas D, Arnaud F, et al (2016) Long-term dynamics in microbial eukaryotes communities: a palaeolimnological view based on sedimentary DNA. Molecular Ecology 25:5925–5943. doi:10.1111/mec.13893

Capo E, Debroas D, Arnaud F, et al (2017) Tracking a century of changes in microbial eukaryotic diversity in lakes driven by nutrient enrichment and climate warming: Long-term dynamics of microbial eukaryotes. Environmental Microbiology 19:2873–2892. doi:10.1111/1462-2920.13815

Capo E, Debroas D, Arnaud F, Domaizon I (2015) Is Planktonic Diversity Well Recorded in Sedimentary DNA? Toward the Reconstruction of Past Protistan Diversity. Microbial Ecology 70:865–875. doi:10.1007/s00248-015-0627-2

Capo E, Rydberg J, Tolu J, et al (2019) How Does Environmental Inter-annual Variability Shape Aquatic Microbial Communities? A 40-Year Annual Record of Sedimentary DNA From a Boreal Lake (Nylandssjön, Sweden). Front Ecol Evol 7:245. doi:10.3389/fevo.2019.00245

Coolen MJL, Boere A, Abbas B, et al (2006) Ancient DNA derived from alkenone-biosynthesizing haptophytes and other algae in Holocene sediments from the Black Sea. Paleoceanography 21:PA1005. doi:10.1029/2005PA001188

Coolen MJL, Orsi WD, Balkema C, et al (2013) Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene. Proc Natl Acad Sci U S A 110:8609–8614. doi:10.1073/pnas.1219283110

Corinaldesi C, Beolchini F, Dell'Anno A (2008) Damage and degradation rates of extracellular DNA in marine sediments: implications for the preservation of gene sequences. Mol Ecol 17:3939–3951. doi:10.1111/j.1365-294X.2008.03880.x

Corinaldesi C, Danovaro R, Dell'Anno A (2005) Simultaneous Recovery of Extracellular and Intracellular DNA Suitable for Molecular Studies from Marine Sediments. Appl Environ Microbiol 71:46–50. doi:10.1128/AEM.71.1.46-50.2005

Crease TJ, Colbourne JK (1998) The Unusually Long Small-Subunit Ribosomal RNA of the Crustacean, *Daphnia pulex*: Sequence and Predicted Secondary Structure. J Mol Evol 46:307–313. doi:10.1007/PL00006307

Curds CR, Cockburn A (1970a) Protozoa in biological sewage-treatment processes—I. A survey of the protozoan fauna of British percolating filters and activated-sludge plants. Water Research 4:225–236. doi:10.1016/0043-1354(70)90069-2

Curds CR, Cockburn A (1970b) Protozoa in biological sewage-treatment processes—II. Protozoa as indicators in the activated-sludge process. Water Research 4:237–249. doi:10.1016/0043-1354(70)90070-9

De Cáceres M, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. Ecology 90:3566–3574. doi:10.1890/08-1823.1

Deiner K, Fronhofer EA, Mächler E, et al (2016) Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nature Communications 7. doi:10.1038/ncomms12544

Dell'Anno A, Bompadre S, Danovaro R (2002) Quantification, base composition, and fate of extracellular DNA in marine sediments. Limnology and Oceanography 47:899–905. doi:10.4319/lo.2002.47.3.0899

Dell'Anno A, Danovaro R (2005) Extracellular DNA Plays a Key Role in Deep-Sea Ecosystem Functioning. Science 309:2179–2179. doi:10.1126/science.1117475

Domaizon I, Savichtcheva O, Debroas D, et al (2013) DNA from lake sediments reveals the long-term dynamics and diversity of *Synechococcus* assemblages. Biogeosciences 10:3817–3838. doi:10.5194/bg-10-3817-2013

Domaizon I, Winegardner A, Capo E, et al (2017) DNA-based methods in paleolimnology: new opportunities for investigating long-term dynamics of lacustrine biodiversity. Journal of Paleolimnology 58:1–21. doi:10.1007/s10933-017-9958-y

Dray S, Dufour A, Chessel D (2007). "The ade4 Package II: Two-Table and K-Table Methods." _R News_, *7*(2) 47-52. URL: https://cran.r-project.org/doc/Rnews/>

Dudgeon D, Arthington AH, Gessner MO, et al (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163–182. doi:10.1017/S1464793105006950

Dulias K, Stoof-Leichsenring KR, Pestryakova LA, Herzschuh U (2017) Sedimentary DNA versus morphology in the analysis of diatom-environment relationships. J Paleolimnol 57:51–66. doi:10.1007/s10933-016-9926-y

Epp LS, Stoof-Leichsenring KR, Trauth MH, Tiedemann R (2011) Molecular profiling of diatom assemblages in tropical lake sediments using taxon-specific PCR and Denaturing High-Performance Liquid Chromatography (PCR-DHPLC). Mol Ecol Resour 11:842–853. doi:10.1111/j.1755-0998.2011.03022.x

Fox J, Weisberg S (2011) An {R} companion to applied regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gast RJ, Dennett MR, Caron DA (2004) Characterization of Protistan Assemblages in the Ross Sea, Antarctica, by Denaturing Gradient Gel Electrophoresis. Appl Environ Microbiol 70:2028–2037. doi:10.1128/AEM.70.4.2028-2037.2004

Gillson L, Marchant R (2014) From myopia to clarity: sharpening the focus of ecosystem management through the lens of palaeoecology. Trends in Ecology & Evolution 29:317–325. doi:10.1016/j.tree.2014.03.010

Guillou L, Bachar D, Audic S, et al (2013) The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:D597-604. doi:10.1093/nar/gks1160

Hoef-Emden K, Archibald JM (2017) Cryptophyta (Cryptomonads). In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 851–891

Horn H, Paul L, Horn W, Petzoldt T (2011) Long-term trends in the diatom composition of the spring bloom of a German reservoir: is Aulacoseira subarctica favoured by warm winters?: Warm winters and competition success of Aulacoseira subarctica. Freshwater Biology 56:2483–2499. doi:10.1111/j.1365-2427.2011.02674.x

Ibelings BW, Bruin AD, Kagami M, et al (2004) Host Parasite Interactions Between Freshwater Phytoplankton and Chytrid Fungi (chytridiomycota)1. Journal of Phycology 40:437–453. doi:10.1111/j.1529-8817.2004.03117.x

Jørgensen T, Haile J, Möller P, et al (2012) A comparative study of ancient sedimentary DNA, pollen and macrofossils from permafrost sediments of northern Siberia reveals long-term vegetational stability. Molecular Ecology 21:1989–2003. doi:10.1111/j.1365-294X.2011.05287.x

Kagami M, de Bruin A, Ibelings BW, Van Donk E (2007) Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. Hydrobiologia 578:113–129. doi:10.1007/s10750-006-0438-z

Kagami M, Miki T, Takimoto G (2014) Mycoloop: chytrids in aquatic food webs. Front Microbiol 5. doi:10.3389/fmicb.2014.00166

Korhola A, Rautio M (2001) Cladocera and other branchiopod crustaceans. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. Volume 4: Zoological indicators. Kluwer Academic Publishers New York, pp 5–41

Korosi JB, Smol JP (2012) An illustrated guide to the identification of cladoceran subfossils from lake sediments in northeastern North America: part 1—the Daphniidae, Leptodoridae, Bosminidae, Polyphemidae, Holopedidae, Sididae, and Macrothricidae. J Paleolimnol 48:571–586. doi:10.1007/s10933-012-9632-3

Kristiansen J, Škaloud P (2017) Chrysophyta. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 331–366

Le S, Josse J, Husson F (2008). FactoMineR: An R package for multivariate analysis. Journal of Statistical Software, 25(1), 1-18. doi:10.18637/jss.v025.i01

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280. doi:10.1007/s004420100716

Legendre P, Legendre L (2012) Numerical ecology. Elsevier, Amsterdam; Boston

Lund JWG (1954) The Seasonal Cycle of the Plankton Diatom, Melosira Italica (EHR.) Kutz. Subsp. Subarctica O. Mull. Journal of Ecology 42:151–179. doi:10.2307/2256984

Lynn D (2010) The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature, 3rd ed. Springer Netherlands

Lynn DH (2017) Ciliophora. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 679–730

Madoni P (2003) Protozoa as indicators of wastewater treatment efficiency. In: Mara D, Horan N (eds) The Handbook of Water and Wastewater Microbiology. pp 361–371

Monchamp M-E, Walser J-C, Pomati F, Spaak P (2016) Sedimentary DNA reveals cyanobacteria community diversity over 200 years in two peri-alpine lakes. Applied and Environmental Microbiology AEM.02174-16. doi:10.1128/AEM.02174-16

Oksanen, J., F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs and Helene Wagner (2018). vegan: community ecology package. R package version 2.5-3. https://CRAN.R-project.org/package=vegan

Perga M-E, Desmet M, Enters D, Reyss J-L (2010) A century of bottom-up- and top-down driven changes on a lake planktonic food web: A paleoecological and paleoisotopic study of Lake Annecy, France. Limnol Oceanogr 55:803–816. doi:10.4319/lo.2010.55.2.0803

Powell MJ (2017) Chytridiomycota. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 1523–1558

Reid AJ, Carlson AK, Creed IF, et al (2018) Emerging threats and persistent conservation challenges for freshwater biodiversity: Emerging threats to freshwater biodiversity. Biological Reviews. doi:10.1111/brv.12480

Rimet F, Abarca N, Bouchez A, et al (2018) The potential of High-Throughput Sequencing (HTS) of natural samples as a source of primary taxonomic information for reference libraries of diatom barcodes. Fottea 18:37–54. doi:10.5507/fot.2017.013

Saldarriaga JF, 'Max' Taylor FJR (2017) Dinoflagellata. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the Protists. Springer International Publishing, Cham, pp 625–678

Sandgren CD (1998) The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. In: Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Cambridge

Sastri AR, Roff JC (2000) Rate of chitobiase degradation as a measure of development rate in planktonic Crustacea. Can J Fish Aquat Sci 57:1965–1968. doi:10.1139/f00-174

Saulnier-Talbot É (2016) Paleolimnology as a Tool to Achieve Environmental Sustainability in the Anthropocene: An Overview. Geosciences 6:26. doi:10.3390/geosciences6020026

Šlapeta J, Moreira D, López-García P (2005) The extent of protist diversity: insights from molecular ecology of freshwater eukaryotes. Proceedings of the Royal Society B: Biological Sciences 272:2073–2081. doi:10.1098/rspb.2005.3195

Smol JP (2008) Pollution of lakes and rivers: a paleolimnological perspective, 2nd edition. Blackwell Sientific Publications Publishing, Oxford, UK

Stoermer EF, Wolin JA, Schelske CL, Conley DJ (1985) An Assessment of Ecological Changes During the Recent History of Lake Ontario Based on Siliceous Algal Microfossils Preserved in the Sediments1,2. Journal of Phycology 21:257–276. doi:10.1111/j.0022-3646.1985.00257.x

Stoof-Leichsenring KR, Bernhardt N, Pestryakova LA, et al (2014) A combined paleolimnological/genetic analysis of diatoms reveals divergent evolutionary lineages of *Staurosira* and *Staurosirella* (Bacillariophyta) in Siberian lake sediments along a latitudinal transect. Journal of Paleolimnology 52:77–93. doi:10.1007/s10933-014-9779-1

Stoof-Leichsenring KR, Epp LS, Trauth MH, Tiedemann R (2012) Hidden diversity in diatoms of Kenyan Lake Naivasha: a genetic approach detects temporal variation. Mol Ecol 21:1918–1930. doi:10.1111/j.1365-294X.2011.05412.x

Szeroczyñska K, Sarmaja-Korjonen K (2007) Atlas of subfossil Cladocera from central and northern Europe. Friends of the Lower Vistula Society, Świecie

Taberlet P, Prud'homme SM, Campione E, et al (2012) Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Molecular Ecology 21:1816–1820. doi:10.1111/j.1365-294X.2011.05317.x

Van de Peer Y, De Rijk P, Wuyts J, et al (2000) The European Small Subunit Ribosomal RNA database. Nucleic Acids Res 28:175–176

Vuillemin A, Horn F, Alawi M, et al (2017) Preservation and Significance of Extracellular DNA in Ferruginous Sediments from Lake Towuti, Indonesia. Frontiers in Microbiology 8. doi:10.3389/fmicb.2017.01440

Walker IR (2001) Midges: Chironomidae and related Diptera. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. Volume 4: Zoological indicators. Kluwer Academic publishers New York, pp 43–66

Winegardner AK, Beisner BE, Legendre P, Gregory-Eaves I (2015) Are the landscape-level drivers of water column and surface sediment diatoms different? Freshw Biol 60: 267–281. doi:10.1111/fwb.12478

Witty L (2004) Practical guide to identifying freshwater crustacean zooplankton, 2nd edition, Cooperative Freshwater Ecology Unit, 2004

Zeeb BA, Smol JP (2001) Chrysophyte scales and cysts. In: Smol JP, Birks HJB, Last WM, et al. (eds) Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal, and siliceous indicators. Kluwer Academic publishers New York, pp 203–223

Zhang GK, Chain FJJ, Abbott CL, Cristescu ME (2018) Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. Evolutionary Applications 11:1901–1914. doi:10.1111/eva.12694

Tables chapter 2

Table 1. Averages (± SE) of physical, chemical and biological variables for the mixed (Nov/Dec – April/May) and thermally stratified periods (April/May – Nov/Dec). (a) Physical and biological variables; (b) epilimnetic averages; (c) metalimnetic averages; (d) hypolimnetic averages. Averages were calculated with data spanning the sediment trap deployment period (from June 2014 to June 2017).

Variable	Mixed period		Thermally stratified period	
	Average	± SE	Average	± SE
(a) Physical and biological variables				
Depth of the photic zone (m)	13.5	(0.7)	17.2	(0.7)
Zooplankton biomass (mg m^{-2})	1446.7	(391.7)	2727.8	(324.3)
Epilimnetic temperature (°C; 0-5 m average)	6.8	(0.5)	18.0	(1.0)
Conductivity corrected at 25°C	127.4	(1.2)	161.8	(3.3)
(b) Epilimnion (photic zone)				
Total chlorophyll (µg L^{-1})	1.9	(0.2)	2.1	(0.3)
Total phosphorus (µg L^{-1})	8.4	(0.6)	5.4	(0.4)
Total nitrogen (µg L^{-1})	290.3	(10.5)	216.1	(11.3)
Dissolved oxygen (mg L^{-1})	10.9	(0.3)	10.2	(0.2)
pH	7.2	(0.1)	8.1	(0.1)
Nitrate (µg L^{-1})	133.0	(4.5)	26.1	(8.0)
Dissolved organic nitrogen (µg L^{-1})	127.3	(10.4)	145.5	(10.2)
Ammonia (µg L^{-1})	3.0	(0.5)	3.1	(0.3)
Dissolved inorganic nitrogen (µg L^{-1})	136.04	(4.4)	29.2	(8.2)
Particulate nitrogen (µg L^{-1})	27.0	(2.4)	41.5	(3.6)
Particulate phosphorus (µg L^{-1})	4.1	(0.2)	2.6	(0.2)
(c) Metalimnion (photic zone)				
Total chlorophyll (µg L^{-1})	-	-	4.1	(0.5)
Total phosphorus (µg L^{-1})	-	-	7.7	(0.3)
Total nitrogen (µg L^{-1})	-	-	256.8	(9.7)
Dissolved oxygen (mg L^{-1})	-	-	11.6	(0.4)
pH	-	-	7.2	(0.1)
Nitrate (µg L^{-1})	-	-	58.6	(10.1)
Dissolved organic nitrogen (µg L^{-1})	-	-	127.2	(7.7)
Ammonia (µg/L)	-	-	3.3	(0.7)
Dissolved inorganic nitrogen (µg L^{-1})	-	-	62.0	(10.6)
Particulate nitrogen (µg L^{-1})	-	-	67.7	(7.3)
Particulate phosphorus (µg L^{-1})	-	-	4.6	(0.2)
(d) Hypolimnion (35m deep)				
Total chlorophyll (µg L^{-1})	1.5	(0.2)	0.7	(0.1)
Dissolved oxygen (mg L^{-1})	10.1	(0.2)	7.3	(0.4)
Total phosphorus (µg L^{-1})	7.5	(0.5)	5.3	(0.3)
Temperature (°C)	6.1	(0.2)	6.2	(0.2)
Nitrate (µg L^{-1})	132.3	(4.3)	169.9	(2.6)
Ammonia (µg L^{-1})	1.7	(0.4)	1.2	(0.3)

Table 2. Total number of sequences and amplicon sequence variants (ASV) for micro-eukaryotic taxa, diatoms, and crustaceans in the photic zone of the water column (epilimnion and metalimnion) and in the sediment traps (ST; intracellular (in) and extracellular (ex) DNA fractions). The percentage of sequences amplified, unique ASVs and the number of single and doubleton are presented for crustaceans and diatoms.

Site	Total amplified sequences*	# Seq. / sample	Total unique ASV	Rarefied richness / sample (range)†	Crustaceans			Diatoms		
					% seq. (range)	Unique ASV	Single-doubleton	% seq. (range)	Unique ASV	Single-doubleton
Epi. (n=36)	2,182,177	60,616	2,037	220 (116-302)	11% (0-56.3)	37	0	8% (0.11-53.1)	97	0
Meta (n=19)	1,144,096	60,216	1,391	238 (151-349)	4% (0.05-10.9)	10	0	8% (0.15-22.5)	47	2
STin (n=34)	2,219,871	65,290	3,393	231 (66-559)	65% (0.8-98.1)	110	1	16% (0.02-83)	131	2
ST ex (n=32)	1,917,803	59,931	3,190	244 (4-598)	55% (2.7-99.6)	91	2	11% (0-60.5)	119	0

*Number of sequences after filtering, trimming and removing chimeras

†The minimum sample size used to calculate the rarefied richness was 16,032 sequences.

Table 3. RV coefficients quantifying the congruence between PCA site scores of different taxonomic and sample matrices for crustacean and diatom ASVs. The significant correlations are indicated in bold.

			Crustaceans		Diatoms	
Comparisons	**Matrix A**	**Matrix B**	**RV coefficient of 1st PCA axis of sites scores**	**RV coefficient of 3 first PCA axes site scores**	**RV coefficient of 1st PCA axis of sites scores**	**RV coefficient of 3 first PCA axes site scores**
Morpho vs DNA - Water epilimnion (n = 36)	Site scores from species density	Site scores from ASV - DNA	0.07 (0.21)	0.1 (0.63)	0.03 (0.37)	**0.36 (<0.0001*)**
	Site scores from species biomass	Site scores from ASV - DNA	0.01 (0.64)	0.1 (0.67)	**0.37 (0.0001*)**	**0.26 (0.0003*)**
Morpho vs DNA - Water metalimnion (n = 19)	Site scores from species density	Site scores from ASV - DNA	0.32 (0.07)	0.23 (0.45)	**0.5 (0.001*)**	**0.53 (0.0003*)**
	Site scores from species biomass	Site scores from ASV - DNA	**0.44 (0.03*)**	0.29 (0.22)	**0.31 (0.01*)**	0.21 (0.24)
Morpho vs inDNA – ST (n = 33)	Site scores from species density	Site scores from ASV - inDNA	0.01 (0.59)	0.11 (0.68)	**0.62 (<0.0001*)**	**0.27 (0.0005*)**
	Site scores from species biomass	Site scores from ASV - inDNA	0.01 (0.59)	0.12 (0.56)	**0.53 (<0.0001*)**	**0.27 (0.001*)**
Morpho vs exDNA – ST (n = 31)	Site scores from species density	Site scores from ASV - exDNA	0.11 (0.19)	0.26 (0.072)	**0.3 (0.001*)**	**0.23 (0.008*)**
	Site scores from species biomass	Site scores from ASV - exDNA	0.11 (0.18)	0.27 (0.058 **..**)	**0.28 (0.002*)**	**0.2 (0.04*)**
Water epilimnion vs ST – DNA (n = 33)	Site scores from ASV - water epilimnion	Site scores from ASV - ST inDNA	0.002 (0.82)	0.05 (0.70)	0.08 (0.12)	**0.34 (<0.0001*)**
	Site scores from ASV - water epilimnion	Site scores from ASV - ST exDNA	**0.18 (0.02*)**	0.16 (0.10)	**0.13 (0.046*)**	**0.33 (0.002*)**
Water metalimnion vs ST – DNA (n = 16)	Site scores from ASV - water metalimnion	Site scores from ASV - ST inDNA	0.002 (0.80)	0.14 (0.67)	0.16 (0.12)	0.31 (0.061)
	Site scores from ASV - water metalimnion	Site scores from ASV - ST exDNA	0.14 (0.15)	0.28 (0.16)	0.004 (1)	0.26 (0.18)
Water epilimnion vs ST – Morpho (n = 34)	Site scores from species density - water epilimnion	Site scores from species density -ST	**0.34 (0.007*)**	**0.32 (0.01*)**	**0.21 (0.005*)**	**0.31 (<0.0001*)**
	Site scores from species biomass - water epilimnion	Site scores from species biomass - ST	**0.43 (0.002*)**	**0.35 (0.005*)**	**0.66 (<0.0001*)**	**0.26 (0.0006*)**
Water metalimnion vs ST – Morpho (n = 19)	Site scores from species density - water metalimnion	Site scores from species density -ST	-	-	**0.58 (0.0004*)**	**0.38 (0.002*)**
	Site scores from species biomass - water metalimnion	Site scores from species biomass - ST	-	-	0.11 (0.16)	0.25 (0.12)
ST inDNA vs ST exDNA (n = 32)	Site score from ASV - ST inDNA	Sites score from ASV - ST exDNA	**0.2 (0.004*)**	**0.3 (0.004*)**	**0.36 (0.0004*)**	**0.42 (<0.0001*)**

*For the crustaceans when comparing morphology to DNA in the epilimnion or the metalimnion, note that the morphology was performed on the entire water column (30 m deep net haul).

Table 4. RV coefficients quantifying the congruence between PCA site scores of different combinations of DNA sample matrices for the entire micro-eukaryotic communities and for the shared ASVs excluding shared crustacean and diatom ASVs. The significant correlations are indicated in bold.

			Micro-eukaryotic ASVs		Shared micro-eukaryotic ASVs	
Dataset	**Matrix A**	**Matrix B**	**RV coefficient of 1st axis of sites scores**	**RV coefficient of 3 first PCA axes site scores**	**RV coefficient of 1st axis of sites scores**	**RV coefficient of 3 first PCA axes site scores**
Epilimnion - STinDNA - STexDNA (381 shared ASVs)	Site scores from ASV - Epilimnion	Site scores from ASV - STinDNA	**0.48 (<0.0001*)**	**0.48 (<0.0001*)**	**0.67 (<0.0001*)**	**0.56 (<0.0001*)**
	Site scores from ASV - Epilimnion	Site scores from ASV - STexDNA	**0.19 (0.01*)**	**0.37 (<0.0001*)**	**0.38 (0.0004*)**	**0.49 (<0.0001*)**
	Site scores from ASV - STinDNA	Site scores from ASV - STexDNA	**0.72 (<0.0001*)**	**0.54 (<0.0001*)**	0.04 (0.27)	**0.55 (<0.0001*)**
Metalimnion - STinDNA - STexDNA (206 shared ASVs)	Site scores from ASV - Metalimnion	Site scores from ASV - STinDNA	0.09 (0.3)	**0.34 (0.04*)**	**0.85 (0.0001*)**	**0.5 (0.0003*)**
	Site scores from ASV - Metalimnion	Site scores from ASV - STexDNA	0.03 (0.6)	**0.41 (0.009*)**	**0.48 (0.004)**	**0.56 (<0.0001*)**
	Site scores from ASV - STinDNA	Site scores from ASV - STexDNA	**0.48 (0.004*)**	0.29 (0.08)	**0.64 (0.0006*)**	**0.53 (0.0001*)**

Figures chapter 2

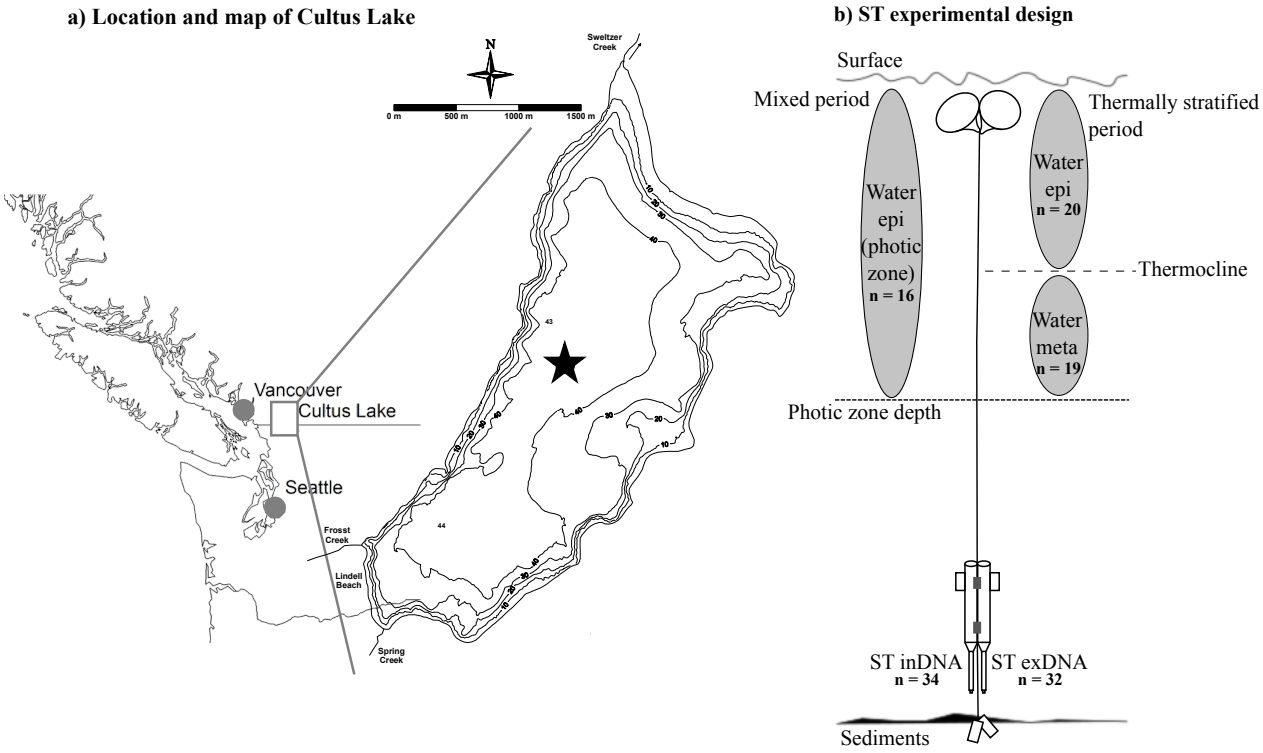


Figure 1. a) Location and map of Cultus Lake (modified from Shortreed 2007). The star represents the approximate location of the limnological sampling site and where the sediment traps were deployed. b) Sediment trap (ST) experimental design with sample types collected each sampling occasions and the number (n) of samples of each type (water epilimnion (epi); water metalimnion (meta), sediment trap intracellular DNA (ST inDNA) and sediment trap extracellular DNA (ST exDNA)).

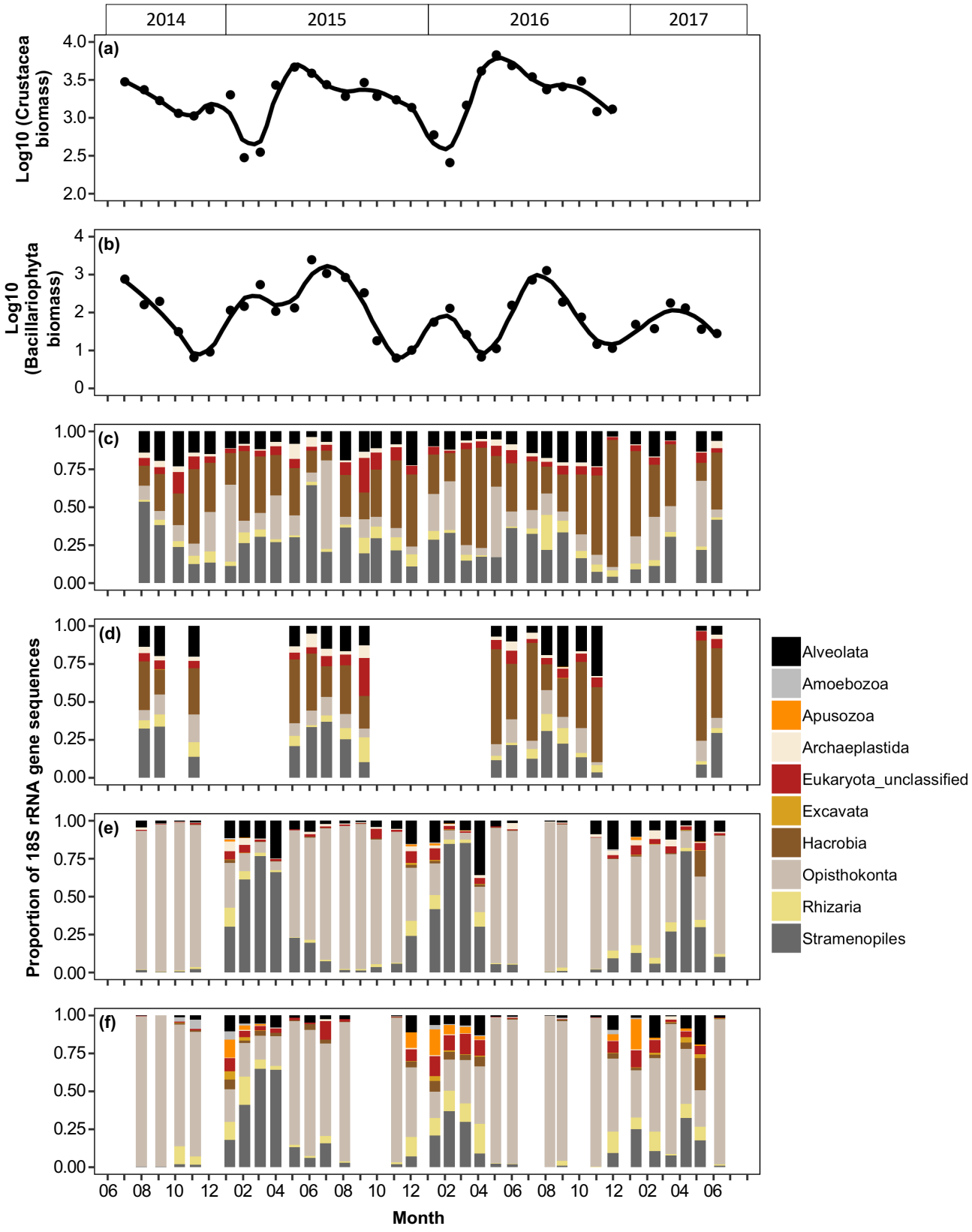

2014
2015
2016
2017
(a)
Log10 (Crustacea biomass)
4.0
3.5
3.0
2.5
2.0
(b)
Log10 (Bacillariophyta biomass)
4
3
2
1
0
(c)
(d)
(e)
(f)
Proportion of 18S rRNA gene sequences
1.00
0.75
0.50
0.25
0.00
06 08 10 12 02 04 06 08 10 12 02 04 06 08 10 12 02 04 06
Month
Alveolata
Amoebozoa
Apusozoa
Archaeplastida
Eukaryota_unclassified
Excavata
Hacrobia
Opisthokonta
Rhizaria
Stramenopiles

Figure 2. Time series from July 2014 to July 2017 demonstrating the biomass of (a) diatoms from the photic zone and (b) crustaceans from water column (net hauls from 30 m deep). Proportion of sequences identified through 18S rRNA gene sequencing for different phyla of micro-eukaryotes represented as barplots in (c) the epilimnion; (d) the metalimnion; (e) the intracellular DNA fraction of the sediment trap samples; and (f) the extracellular DNA fraction in the sediment trap samples.

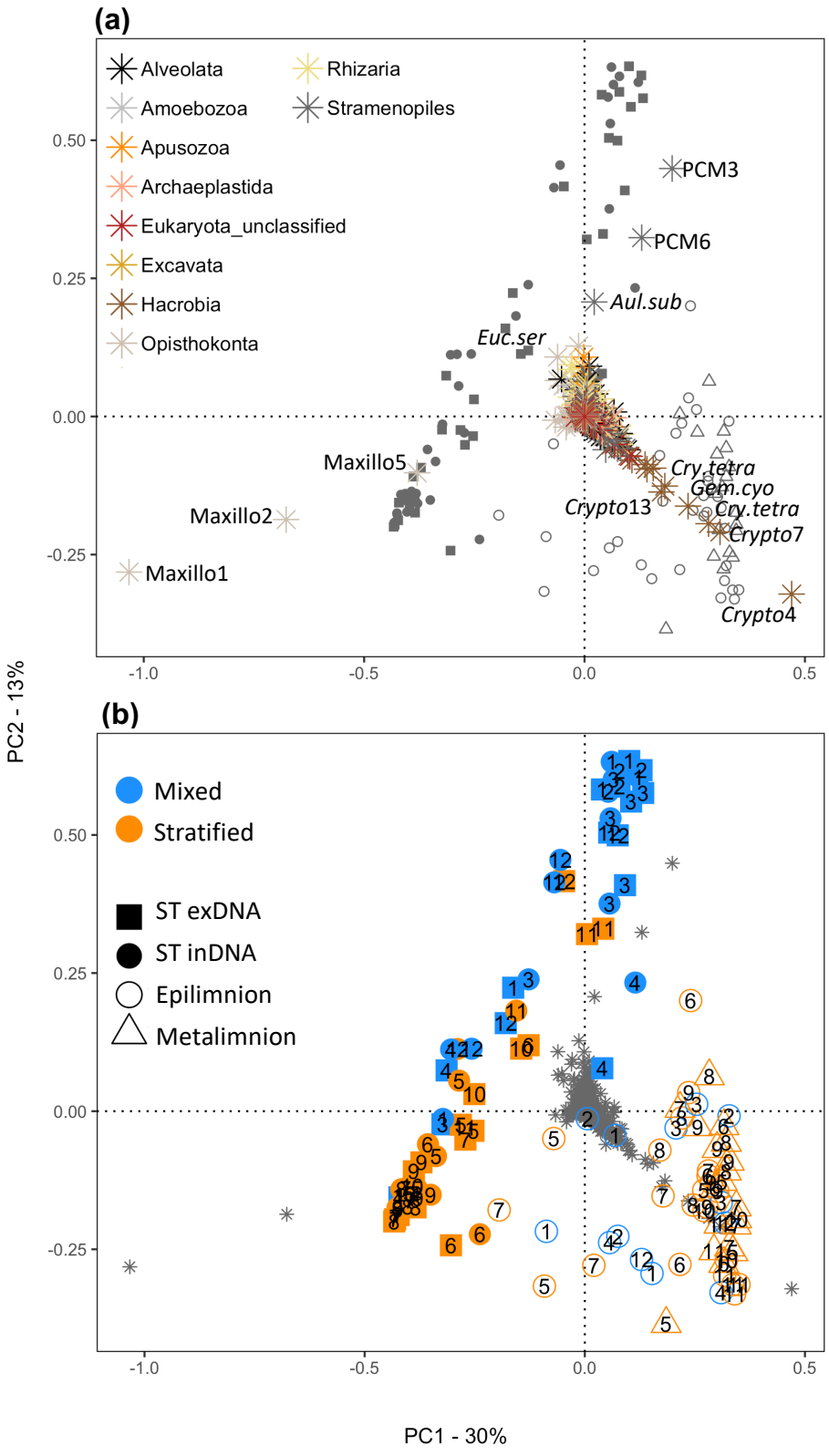
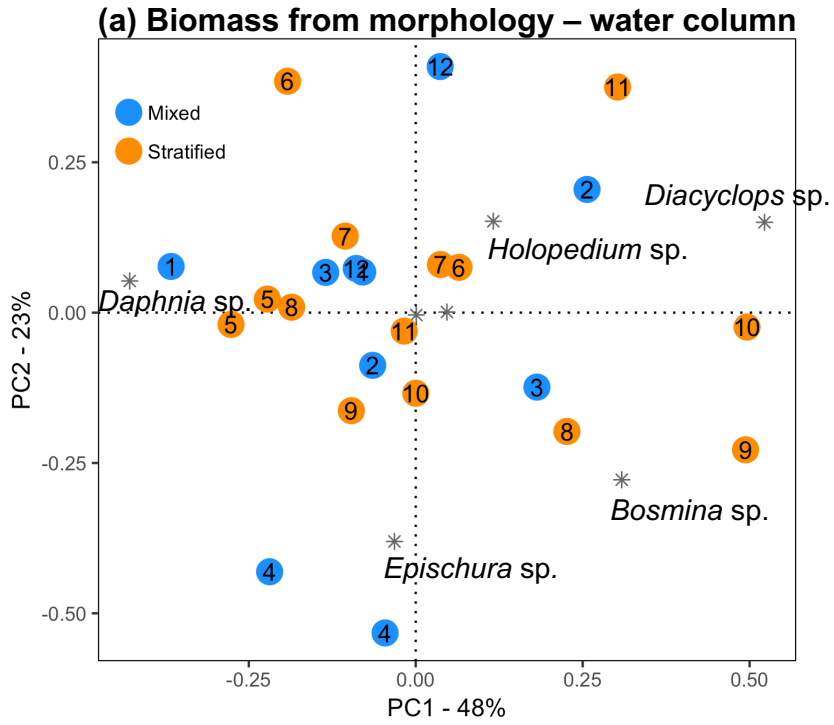
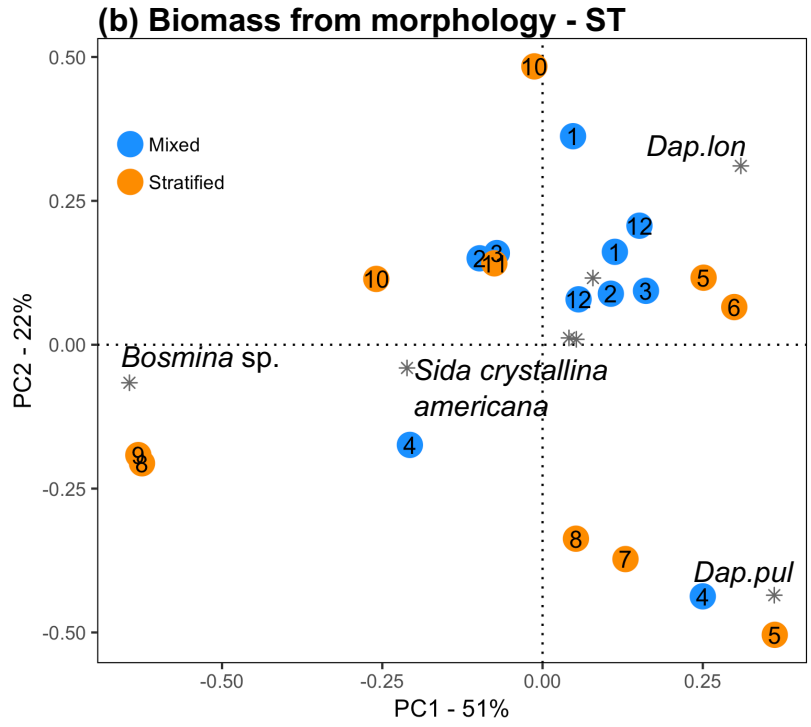
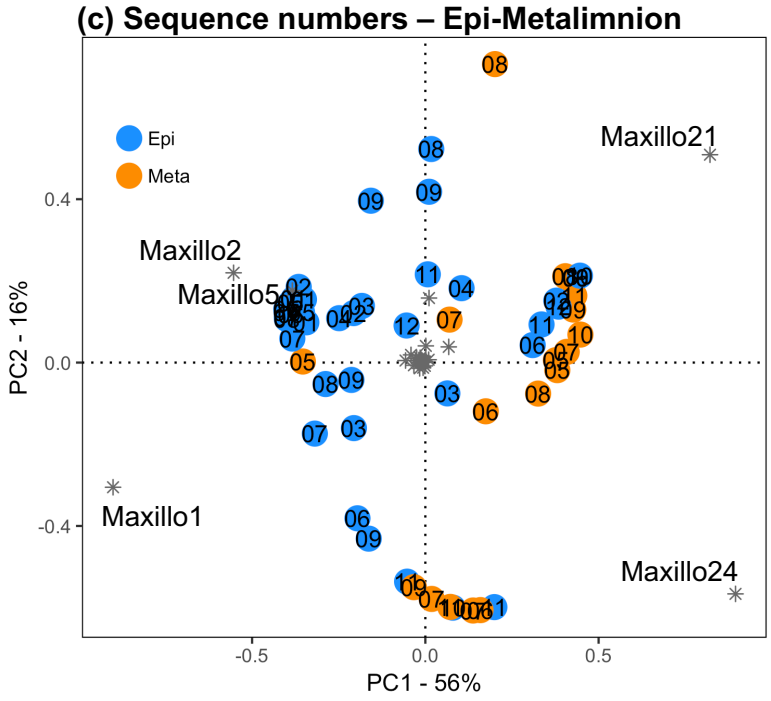

(a)
Alveolata
Amoebozoa
Apusozoa
Archaeplastida
Eukaryota_unclassified
Excavata
Hacrobia
Opisthokonta
Rhizaria
Stramenopiles
PCM3
PCM6
Aul.sub
Euc.ser
Maxillo5
Maxillo2
Maxillo1
Cry.tetra
Gem.cyo
Cry.tetra
Crypto13
Crypto7
Crypto4
(b)
Mixed
Stratified
ST exDNA
ST inDNA
Epilimnion
Metalimnion
0.50
0.25
0.00
-0.25
-1.0
-0.5
0.0
0.5
PC2 - 13%
PC1 - 30%

Figure 3. PCA of the photic zone (epilimnion and metalimnion) and sediment trap (intracellular DNA (ST inDNA) and extracellular DNA (ST exDNA)) samples for the micro-eukaryotes identified through 18S rRNA gene sequencing. Number of sequences per ASVs were Hellinger-transformed prior to ordination. In (a), the PCA shows the species scores per phylum and identifies the dominant taxa. In (b), PCA shows sites scores for epilimnion (light circles), metalimnion (light triangles) and sediment trap samples (dark squares as ST exDNA and dark circles as ST inDNA) and are colour-coded to identify the period at the time of sampling: mixed in blue or thermally stratified in orange. The number in the circles indicates the sampling month. Taxa abbreviations in panel (a) are as follows: *Aulacoseira subarctica* (*Aul.sub*); *Cryptomonas* sp. (*Crypto*); *Cryptomonas tetrapyrenoidosa* (*Cry.tetra*); *Eucyclops serrulatus* (*Euc.ser*); *Geminigera cyophyla* (*Gem.cyo*); Maxillopoda (Maxillo); and Polar-centric-Mediophyceae (PCM).


(a) Biomass from morphology – water column
Mixed
Stratified
Diacyclops sp.
Holopedium sp.
Daphnia sp.
Bosmina sp.
Epischura sp.
PC2 - 23%
PC1 - 48%
0.25
0.00
-0.25
-0.50
-0.25
0.00
0.25
0.50


(b) Biomass from morphology - ST
Mixed
Stratified
Dap.lon
Bosmina sp.
Sida crystallina americana
Dap.pul
PC2 - 22%
PC1 - 51%
0.50
0.25
0.00
-0.25
-0.50
-0.50
-0.25
0.00
0.25


(c) Sequence numbers – Epi-Metalimnion
Epi
Meta
Maxillo21
Maxillo2
Maxillo5
Maxillo1
Maxillo24
PC2 - 16%
PC1 - 56%
0.4
0.0
-0.4
-0.5
0.0
0.5

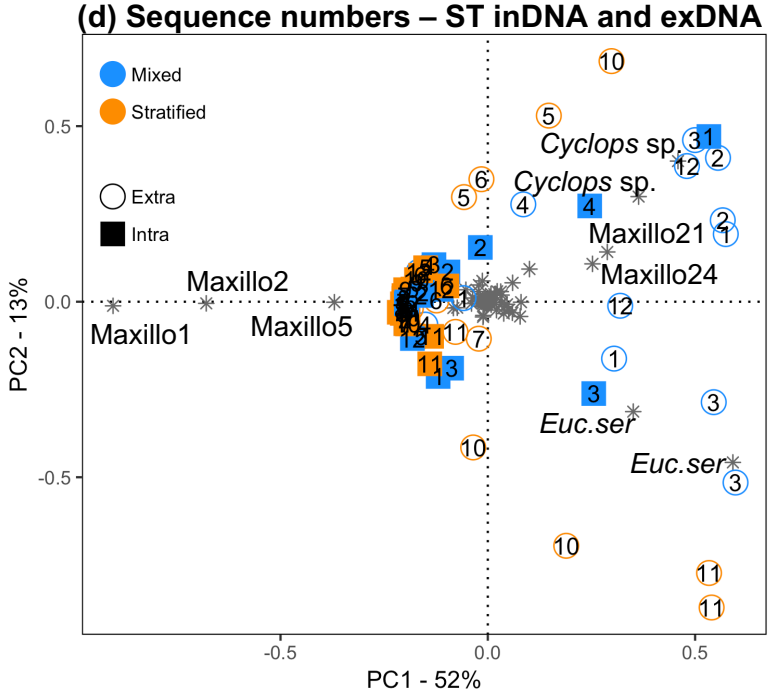
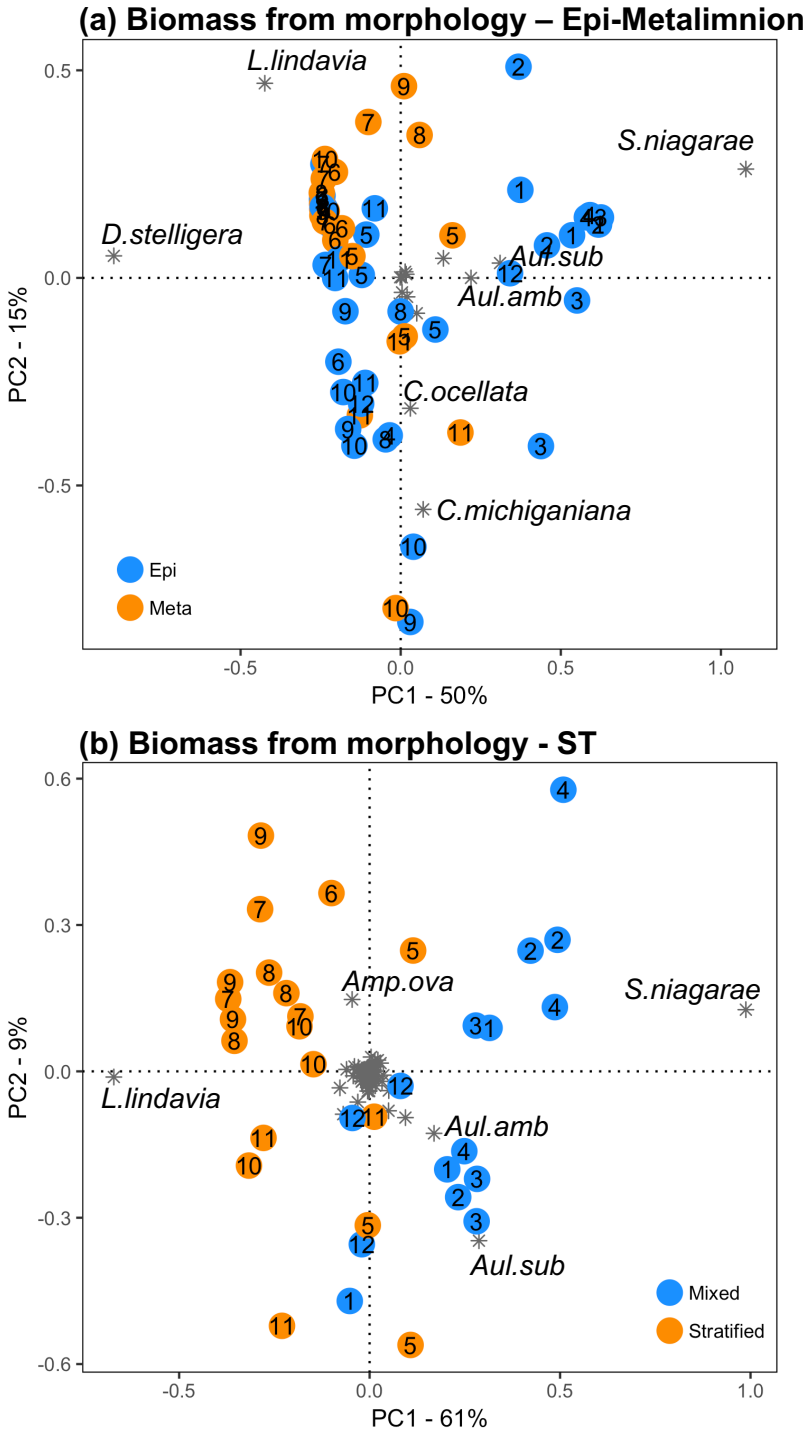
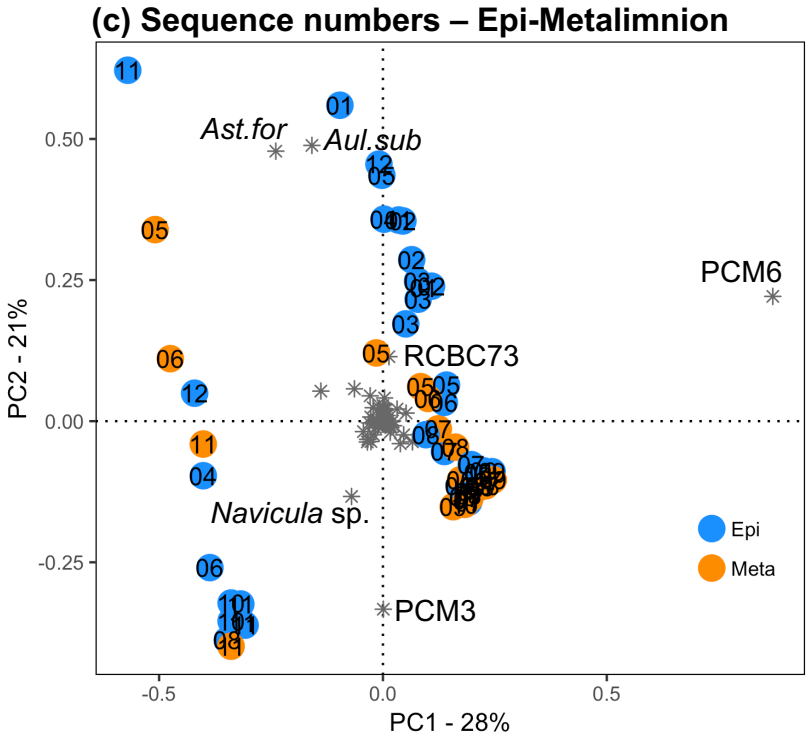

(d) Sequence numbers – ST inDNA and exDNA
Mixed
Stratified
Extra
Intra
Cyclops sp.
Cyclops sp.
Maxillo21
Maxillo24
Maxillo2
Maxillo1
Maxillo5
Euc.ser
Euc.ser
PC2 - 13%
PC1 - 52%
0.5
0.0
-0.5
-0.5
0.0
0.5

Figure 4. Crustacean PCA biplots based on different sample matrices and taxonomic approaches: (a) biomass of morphologically identified specimens from water samples (net hauls from 30 m deep); (b) biomass of morphologically identified specimens from sediment traps samples; (c) ASVs from 18S rRNA gene analyses from epilimnion and metalimnion; and (d) ASVs from 18S rRNA gene analyses from ST inDNA and ST exDNA. Biomass and number of sequences were Hellinger-transformed prior to ordination. The blue and orange circles represent the mixed and the stratified periods, respectively; except in (c) where they represent the epilimnion and metalimnion, respectively. The number in the circles indicates the sampling month. Taxa abbreviations are as follows: *Daphnia longispina* (*Dap.lon*); *Daphnia pulex* (*Dap.pul*); *Eucyclops serrulatus* (*Euc.ser*); and Maxillopoda (Maxillo).


(a) Biomass from morphology – Epi-Metalimnion
L.lindavia
S.niagarae
D.stelligera
Aul.sub
Aul.amb
C.ocellata
C.michiganiana
Epi
Meta
PC2 - 15%
PC1 - 50%
(b) Biomass from morphology - ST
Amp.ova
S.niagarae
L.lindavia
Aul.amb
Aul.sub
Mixed
Stratified
PC2 - 9%
PC1 - 61%


(c) Sequence numbers – Epi-Metalimnion
Ast.for
Aul.sub
PCM6
RCBC73
Navicula sp.
PCM3
Epi
Meta
PC2 - 21%
PC1 - 28%
0.50
0.25
0.00
-0.25
-0.5
0.0
0.5

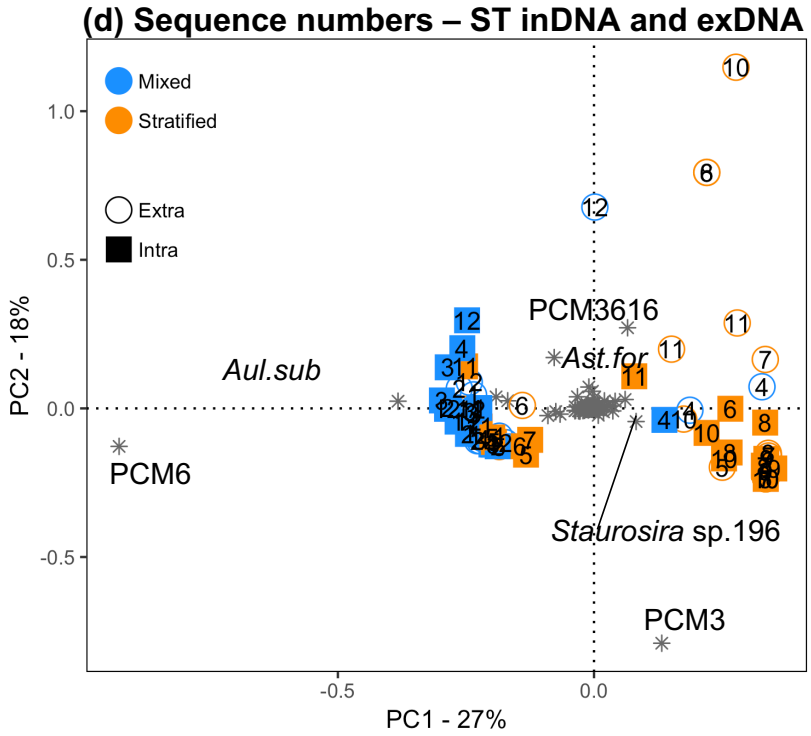
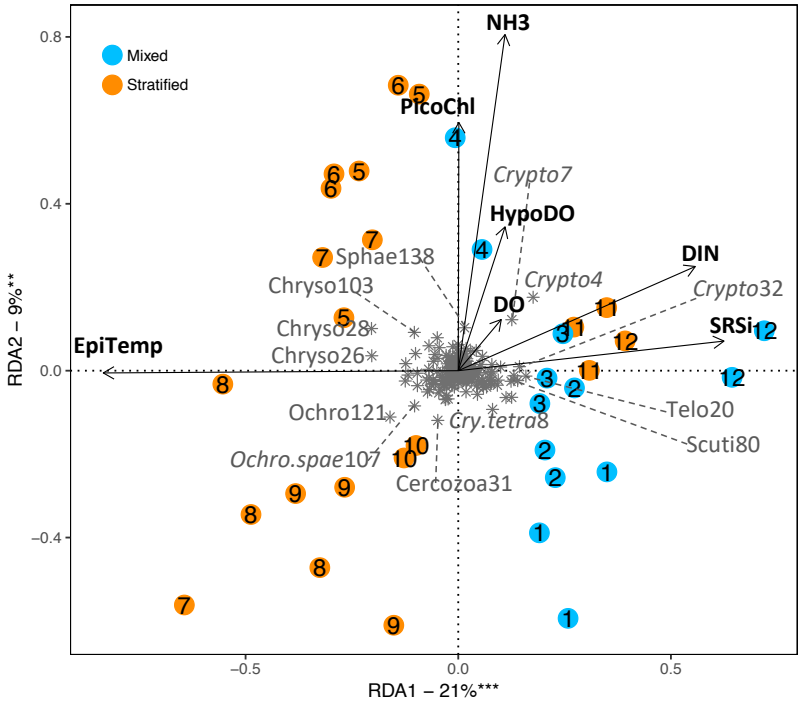
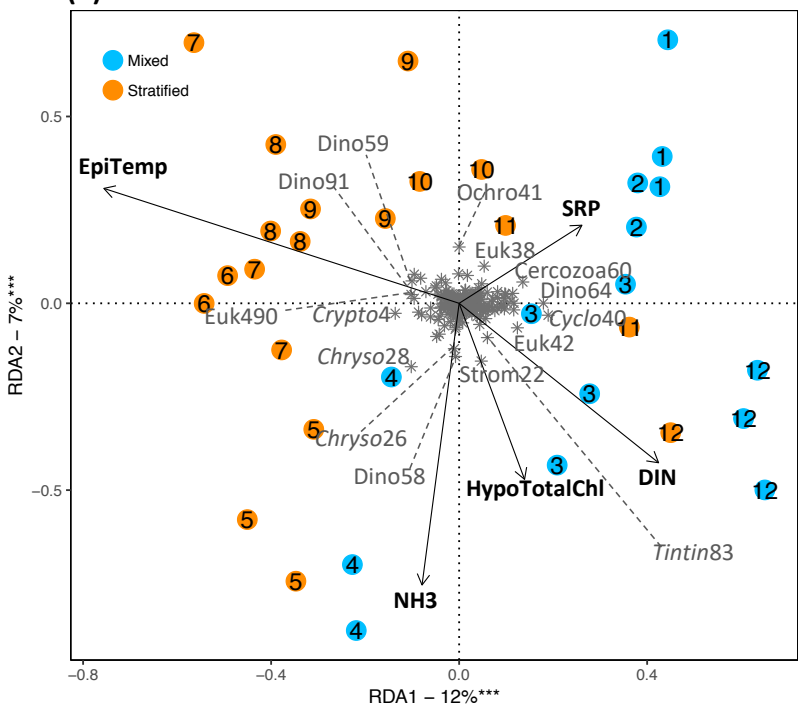

(d) Sequence numbers – ST inDNA and exDNA
Mixed
Stratified
Extra
Intra
PCM3616
Ast.for
Aul.sub
PCM6
Staurosira sp.196
PCM3
PC2 - 18%
PC1 - 27%
1.0
0.5
0.0
-0.5
-0.5
0.0

Figure 5. Diatom PCA biplots based on different sample matrices and taxonomic approaches: (a) biomass of morphologically identified specimens from water samples (depth weighted averages of epilimnion and metalimnion); (b) biomass of morphologically identified specimens from sediment traps samples; (c) ASVs from 18S rRNA gene analyses from epilimnion and metalimnion; and (d) ASVs from 18S rRNA gene analyses from ST inDNA and ST exDNA. Biomass and number of sequences were Hellinger-transformed prior to ordination. The blue and orange circles in (a) and (c) represent the epilimnion and metalimnion, respectively; in (b) and (d), they represent the mixed and the stratified periods, respectively. The number in the circles indicates the sampling month. Taxa abbreviations are as follows: *Amphora ovalis* (*Amp.ova*); *Asterionella formosa* (*Ast.for*); *Aulacoseira ambigua* (*Aul.amb*); *Aulacoseira subarctica* (*Aul.sub*); *Discostella stelligera* (*D.stelligera*); *Lindavia michiganiana* (*L.michiganiana*); *Lindavia ocellata* (*L.ocellata*) *Lindavia intermedia* (*L.intermedia*); Polar-centric-Mediophyceae (PCM); Radial-centric-basal-Coscinodiscophyceae (RCBC); and *Stephanodicus niagarae* (*S.niagarae*).

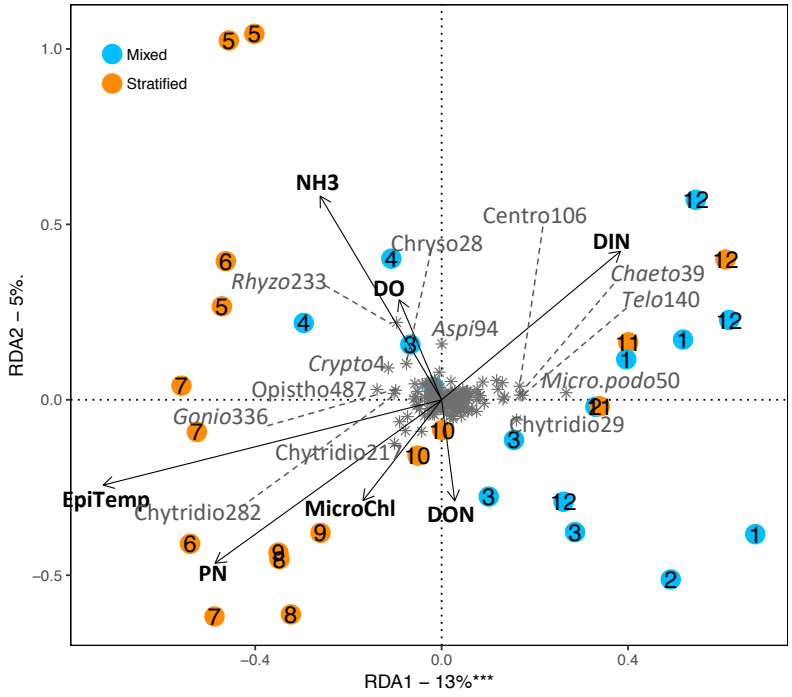
(a) Water epilimnion – 381 shared ASVs



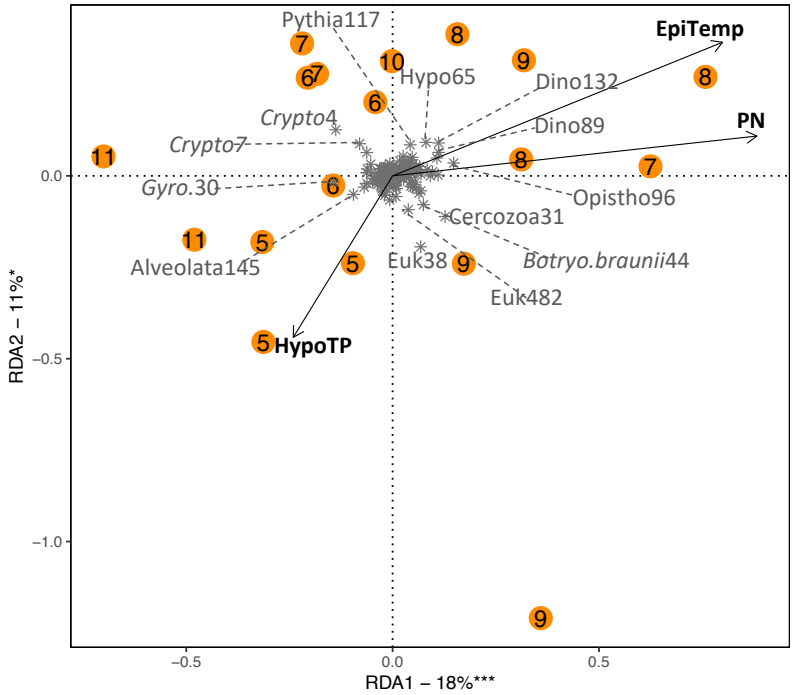
(b) ST inDNA – 381 shared ASVs



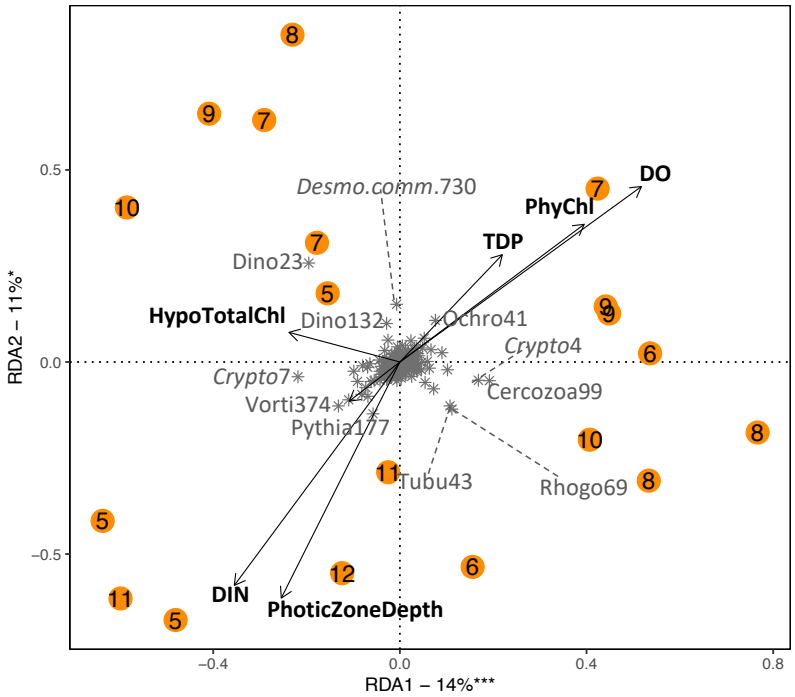
(c) ST exDNA – 381 shared ASVs



(d) Water metalimnion – 206 shared ASVs



(e) ST inDNA – 206 shared ASVs



(f) ST exDNA – 206 shared ASVs

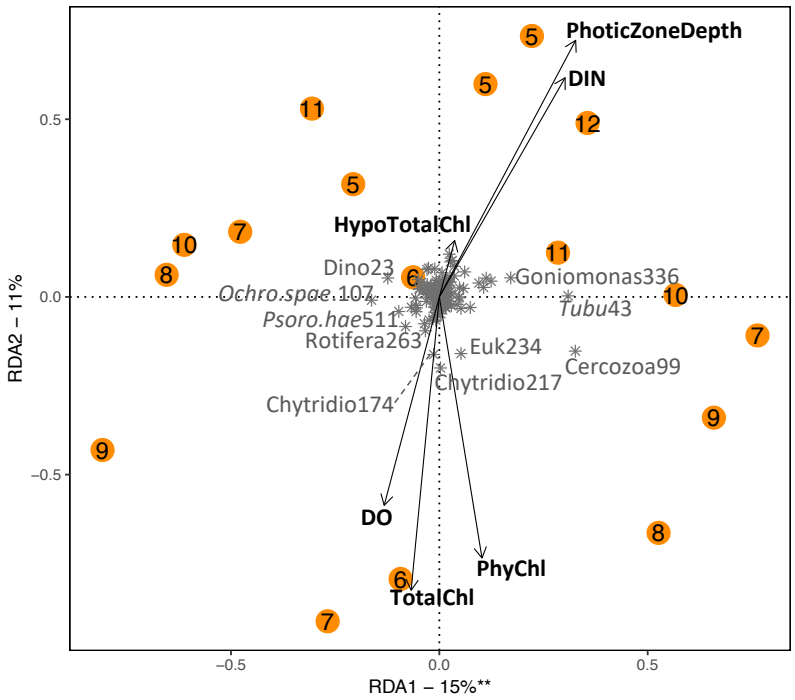


Figure 6. RDA triplots with shared ASV dataset from 18S rRNA gene analyses for the combination of matrices of (a) epilimnion, (b) ST intracellular DNA (ST inDNA), (c) ST extracellular DNA (ST exDNA), and for the combination of (d) metalimnion, (e) ST inDNA, and (f) ST exDNA. Crustacean and diatom ASVs were excluded from the shared ASV dataset. Number of sequences per ASVs were Hellinger-transformed and environmental variables were normalized and standardized prior to ordination. The blue and orange circles represent the mixed and the stratified periods, respectively. The number in the circles indicates the sampling month. Environmental variables are as follows: dissolved inorganic nitrogen (DIN); dissolved oxygen (DO); dissolved organic nitrogen (DON); average water temperature from 0 to 5 m deep (EpiTemp); hypolimnetic total chlorophyll (HypoTotalChl); hypolimnetic total phosphorus (HypoTP); chlorophyll from phytoplankton > 20 µm (MicroChl); depth of the euphotic zone (PhoticZoneDepth); chlorophyll from phytoplankton > 2 µm (PhyChl); chlorophyll from phytoplankton < = 2 µm (PicoChl); particulate nitrogen (PN); ammonia (NH_3); soluble reactive phosphorus (SRP); soluble reactive silicon (SRSi); total dissolved phosphorus (TDP); total chlorophyll (TotalChl). Taxa abbreviations are as follows: *Aspidisca* (*Aspi*); *Botryococcus braunii* (*Botry.braunii*); Centroheliozoa (Centro); *Chaetonotus* sp. (*Chaeto*); Chrysophyceae (Chryso); Chytridiomycota (Chyrtridio); Cryptophyceae (Crypto); *Cryptomonas tetrapyrenoidosa* (*Cry. tetra*); *Cyclotrichium* sp. (*Cyclo*.); *Desmodesmus communis* (*Desmo. comm.*); Dinophyceae (Dino); *Goniomonas* sp. (*Gonio*); *Gyrodinium* sp. (*Gyro.*); Hypotrichia (Hypo); Eukaryota unclassified (Euk); *Micronuclearia podoventralis* (*Micro. podo*); Ochrophyta (Ochro); *Ochromonas sphaerocystis* (*Ochro. sphae*); Opisthokonta (Opistho); *Psorospermium haeckeli* (*Psoro. hae*); Pythiaceae (Pythia); Rhogostoma (Rhogo); *Rhyzophidiales* (*Rhyzo.*);

Scuticociliatia (Scuti); Sphaeropleales (Sphae); Strombidiida (Strom); *Tintinnopsis* (*Tintin*); Telonemia (Telo); *Tubulinea* (*Tubu*); Vorticellidae (Vorti).

CONNECTING STATEMENT 1

For my PhD thesis, I wanted to explore DNA-based approaches in paleolimnology. The field of environmental DNA (eDNA) is growing since the last decades due to technological advancements (Cristescu and Hebert 2018), and has shown great promises to detect endangered species (e.g., Thomsen et al. 2012), invasive species (i.e., earlier detection; e.g., Jerde et al. 2011), and communities (e.g., Mangot et al. 2013) in water bodies. DNA preserved in sediments can also be extracted to reconstruct past community and population dynamics (e.g., Monchamp et al. 2016; Capo et al. 2017). As sedDNA is starting to be widely used in paleolimnology, there is a need for calibration work to evaluate the advantages and limitations of sedDNA in paleolimnology. Two main objectives were described for my PhD thesis: one as methodological and one as ecological. First, I wanted to evaluate the advantages and limitations to using DNA-based approaches in paleolimnology (methodological objective; Chapters 2 and 4) and assess the ecological trajectory of Cultus Lake in the last ~200 years and relate these changes to potential drivers (ecological objective; Chapters 3 and 4). These two main objectives of my PhD are interconnected as I applied DNA-based approaches on contemporary (water and sediment traps; Chapter 2) and on sediment core samples (Chapter 4) in Cultus Lake. I also used paleolimnological, historical and contemporary data from Cultus Lake to better understand the ecological modifications that occurred over the last ~200 years in the lake (Chapter 3).

To evaluate the advantages and limitations to using DNA-based approaches in paleolimnology, I first focused on contemporary data (Chapter 2). Sediment traps were deployed on a monthly for about three years, and water samples were also collected simultaneously. Similar DNA-based approaches were applied to both sediment and water samples. In chapter 2, I

wanted to evaluate which DNA taxa were deposited in the sediments from the water column and whether the biological signal from the water column can be reconstructed from the sediments. In addition, I wanted to assess the congruence between morphological and DNA taxonomic identification of diatoms and crustaceans in both water and sediments. The results of chapter 2 demonstrated the effectiveness of DNA-based approaches applied to sediment samples to reconstruct past community dynamics of particular focal groups. The results of this chapter also revealed a wide variety of additional taxa that could be used as potential bioindicators in future paleolimnological work.

In Chapter 2, as I wanted to evaluate the congruency between morphological and DNA taxonomic identification, I could also explore the contemporary seasonal dynamics of diatom communities in Cultus Lake from morphology. I used one year of the seasonal dynamics of diatom subfossils collected with the sediment traps to help identify mechanisms of changes in Cultus Lake over the past ~200 years (Chapter 3). The seasonal dynamics of limnological variables were also used in Chapter 3 to identify the status of the lake in the present time and understand how Cultus Lake has deviated from its reference status (ca. 1800-1900). With contemporary limnological and paleolimnological data as well as the history of the Cultus Lake watershed based on archival material, the ecological trajectory of Cultus Lake could be reconstructed, and several major drivers of change could be identified, such as logging, residential development and agriculture in the watershed and airshed as well as regional climate warming. Four periods of major changes in the ecological trajectory of Cultus Lake were identified as well as a reference period. Chapter 3 showed the importance of contemporary data and paleolimnological data to better evaluate the magnitude of change in lake ecology and to

better assess the most appropriate management practices to be applied for conservation of our valuable aquatic ecosystems.

Literature cited

Capo E, Debroas D, Arnaud F, et al (2017) Tracking a century of changes in microbial eukaryotic diversity in lakes driven by nutrient enrichment and climate warming: Long-term dynamics of microbial eukaryotes. Environmental Microbiology 19:2873–2892. doi:10.1111/1462-2920.13815

Cristescu ME, Hebert PDN (2018) Uses and misuses of environmental DNA in biodiversity science and conservation. Annu Rev Ecol Evol Syst 49: 209–230. doi:10.1146/annurev-ecolsys-110617-062306

Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) "Sight-unseen" detection of rare aquatic species using environmental DNA: eDNA surveillance of rare aquatic species. Conserv Lett 4: 150–157. doi:10.1111/j.1755-263X.2010.00158.x

Mangot J-F, Domaizon I, Taib N, Marouni N, Duffaud E, Bronner G, Debroas D (2013) Short-term dynamics of diversity patterns: evidence of continual reassembly within lacustrine small eukaryotes: Short-term dynamics of small eukaryotes. Environ Microbiol 15: 1745–1758. doi:10.1111/1462-2920.12065

Monchamp M-E, Walser J-C, Pomati F, Spaak P (2016) Sedimentary DNA reveals cyanobacteria community diversity over 200 years in two peri-alpine lakes. Applied and Environmental Microbiology AEM.02174-16. doi:10.1128/AEM.02174-16

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E (2012) Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol 21: 2565–2573. doi:10.1111/j.1365-294X.2011.05418.x

CHAPTER 3

ECOLOGICAL DYNAMICS OF A PERI-URBAN LAKE: A MULTI-PROXY PALEOLIMNOLOGICAL STUDY OF CULTUS LAKE (BRITISH COLUMBIA) OVER THE PAST ~200 YEARS

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Abstract

Peri-urban lakes offer many valued ecosystem services, but their vulnerability to climate change and anthropogenic disturbances increases with expanding human populations. As the effects and interactions of multiple stressors on lakes can lead to unexpected outcomes, affecting societal and ecological values, it is necessary to evaluate ecosystem trajectories and respective drivers in peri-urban lakes. Better management practices could thus be applied to preserve ecosystem services of peri-urban lakes. We conducted a multi-proxy paleolimnological study on Cultus Lake, British Columbia, a Canadian peri-urban lake experiencing cultural eutrophication, to reconstruct a comprehensive ecological trajectory of the lake over the past ~200 years. We also integrated historical data as well as historical archival information to identify the potential drivers of the changes. We identified ca. 1800-1900 CE as a reference period, reflected in muted variations across most paleo-indicators. Minor increases in sedimentary $\delta^{15}N$ ca. 1880-1940 CE coincided with the onset of anthropogenic modifications to the Cultus Lake watershed. Signs of early eutrophication were evident by ca. 1940 CE, as indicated by increases in all sedimentary pigments. By ca. 1970-1990 CE, elevated concentrations of sedimentary cyanobacterial pigments and changes in diatom species assemblages highlighted the potential interactive effects of multiple stressors, including cultural eutrophication, climate warming and declines in the endangered Cultus Lake sockeye salmon population. Recent (ca. 1990-2008 CE) declines in sedimentary pigments and increases in cladoceran fluxes suggested an increase in top-down control of the lake food web. From the collection of changes observed in the past ~200 years in our study, it is clear that Cultus Lake and its associated ecosystem services would benefit from abatement of nutrient loadings from terrestrial and atmospheric sources. Our study emphasizes the complexity and interactivity of drivers in peri-urban lake ecosystems and the necessity of

long-term perspectives to contextualize modern ecological conditions to inform lake and watershed management.

Introduction

Peri-urban environments are experiencing increasing pressures from urban population growth as they are subjected to complex interactions between rural, urban and natural systems (Allen 2003), and rapid modifications of associated landscapes (Douglas 2008). Peri-urban lakes are particularly vulnerable to multiple anthropogenic stressors owing to residential development, recreation and agriculture within their watersheds as well as the deposition of atmospheric contaminants (Huang et al. 2007; Melymuk et al. 2011). Intensifying anthropogenic activities in urban centers and within peri-urban environments impose a suite of stresses that can degrade important habitats for aquatic species and critical ecosystem services (Chu et al. 2015). Climate change is concurrently modifying lake ecosystems (Adrian et al. 2009), and the potential interactive effects of multiple stressors can modify the ecological structure and functioning of affected ecosystems (Jackson et al. 2016). For instance, climate change can amplify lake ecological responses to cultural eutrophication, which is a pervasive stressor on freshwater ecosystems worldwide (Moss et al. 2011). The effect of multiple stressors on lakes can lead to unexpected ecological outcomes (Christensen et al. 2006), which may translate into unanticipated changes in ecosystem services that these lakes offer, such as water supplies, fishing and recreational activities (Vörösmarty et al. 2010; Waltham et al. 2014).

Evaluating the effects of multiple drivers on peri-urban lake ecosystems over centennial time scales provides an insightful perspective for understanding contemporary ecological

conditions and developing effective lake and watershed management practices. With the expected global intensification of urbanization (United Nations et al. 2014), it is critical to conduct such studies, so that targeted management efforts can minimize further degradation of peri-urban lakes. Herein, we focus on a Canadian peri-urban lake, Cultus Lake, which is located ~50 km from the outer limit of the Greater Vancouver Regional District in British Columbia (BC; population of ~2.5 million; Province of BC 2018) and ~10 km from the city of Chilliwack (population of ~85,000; Province of BC 2018). Cultus Lake currently experiences strong recreational activities and residential development pressures (Shortreed 2007; Chiang et al. 2015; Sumka 2017). Recent limnological work at Cultus Lake has shown elevated metalimnetic phytoplankton biomass in summer and autumn relative to other regional lakes, as well as seasonal depletion of hypolimnetic oxygen (Shortreed 2007; Putt et al. 2019).

Effective lake and watershed management strategies are essential to halt or reverse the water quality degradation of Cultus Lake, which is a critical habitat for two species at risk, the endangered Cultus Lake sockeye salmon (*Onchorynchus nerka* Walbaum in Artedi; COSEWIC 2003) and the endemic, threatened Cultus pygmy sculpin (*Cottus aleuticus* Gilbert, Cultus Population; Rosenfield et al. 2007; COSEWIC 2010; Government of Canada 2011). Historically, the Cultus Lake sockeye salmon population has supported important indigenous, subsistence and commercial fisheries, but escapement has declined substantially over the past ~50 years (Cultus Sockeye Recovery Team 2005; Shortreed 2007). Fisheries management of Cultus Lake sockeye salmon for conservation objectives has been particularly challenging as it is part of the Fraser River sockeye salmon mixed-stock fishery and co-migrates with other abundant sub-populations of sockeye salmon (Fisheries and Oceans Canada 2017).

Despite historical fisheries assessment and limnological investigations on Cultus Lake (Ricker 1937, 1938; Shortreed 2007), we only have a fragmented perspective on the historical trajectory of ecosystem structure and functioning, as well as the principal drivers of lake change. Paleolimnology can be used to understand natural ecosystem variability over centennial scales and to identify the influences of more recent anthropogenically induced changes (Smol 2008; Battarbee 2010; Bennion et al. 2011). As such, our study combines a multi-proxy paleolimnological study with analyses of regional instrumental time series of environmental change and a review of historical information (Moorhouse et al. 2014) to establish a history of lake ecology and potential drivers of changes. Our main objective was to evaluate the complexity of anthropogenic and climate drivers of ecological changes in a model Canadian peri-urban lake system using a long-term approach. Specifically, we aimed (1) to quantify the chemical and biological changes in Cultus Lake over the past ~200 years and (2) to relate these observed changes to suspected anthropogenic and climate drivers.

Site description

Cultus Lake has a surface area of 6.3 km^2, and mean and maximum depths of 31 m and 44 m, respectively (Shortreed 2007). Cultus Lake is currently a warm monomictic system, but there is sporadic historical evidence of ice cover (1937 from DFO archives; 1950 from Soutar 2005), as well as periodic dimixis in the past. The Cultus Lake watershed has an area of ~75 km^2 (Shortreed 2007) and contains two recreational parks (municipal and provincial), numerous campgrounds (~3 % of the watershed), areas of rural agriculture (~9 % of the watershed), and two residential developments (~2 % of the watershed; Fig. 1; Putt et al. 2019). Eleven primary

tributaries channel surface runoff from ~60 % of the total watershed area, with the remainder of the water entering the lake by overland flows and groundwater influxes (Putt et al. 2019). The largest tributary to Cultus Lake, Frosst Creek, accounts for almost 50 % of the water flowing into the lake. Frosst Creek emerges directly from the Columbia Valley (Fig. 1), which is seasonally influenced by a large, unconstrained aquifer underlying principally agricultural lands and rural development (Putt et al. 2019).

The regional climate of the lower mainland of British Columbia is strongly influenced by quasi-periodic, inter-annual to inter-decadal variability associated with the El Niño-Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO), respectively (BC Ministry of Environment 2016). In addition, this area has experienced significant directional warming since at least 1900 CE (BC Ministry of Environment 2016). While annual air temperatures have significantly increased (+ 0.8°C between 1900-2013 CE), the most pronounced warming has occurred in winter (+ 1.2°C; BC Ministry of Environment 2016). Regional air temperatures exhibited two periods of warming since the 1900s, with one from the beginning of the 1920s to the mid-1940s and another one since the 1970s (Fig. 2). Air temperatures greatly influence the upper water column temperatures (average from 0 to 5 m lake depth) in Cultus Lake as indicated by their linear regression of monthly data ($R2 = 0.89$; p-value < 0.001, SM1). In addition, the annual mean precipitation has increased ~14 % since the 1900s, with the most pronounced increases occurring in spring (BC Ministry of Environment 2016). Flood records, available for the past 100 years, show that the area is susceptible to large flooding event. Specifically, there was a substantial lake level rise associated with a major flooding event in the Fraser Valley in 1948 CE (Soutar 2005). A similar event also happened in 1950 CE after a very cold winter when the lake froze and, then water levels rose substantially the following spring (Soutar 2005).

Enumeration of sockeye salmon spawners (adults returning from the Pacific Ocean to spawn) and emigration of smolts to the Pacific Ocean has been conducted since 1925 CE (Foerster 1930, 1934, 1936; Shortreed 2007). The Cultus Lake sockeye salmon population has experienced a long-term decline since the 1970s in the off-cycle brood lines and pronounced declines across all brood lines occurring in the 1990s (Peterman et al. 2012).

History of anthropogenic and natural disturbances in the Cultus Lake region

Radiocarbon dating and indigenous oral history indicates modest use of the surrounding landscape of Cultus Lake by Coast Salish People (Stó:lō) for the past ~5000 years (Carlson et al. 2001; Schaepe and Ts'elxwéyeqw Tribe 2017). Euro-American settlement was established in the Columbia Valley (south of Cultus Lake; Fig. 1) in the late 1880s and a road was built around Cultus Lake in the early 1900s, which facilitated logging activities within the watershed (Soutar 2005). Road access promoted a modest development of permanent lakeshore residences in the 1920s (Soutar 2005), which has greatly expanded in the subsequent period. Logging activities occurred between 1900 and ~1940 CE, with a peak in the 1920s (Cramer 2005). The area logged was primarily in the Columbia Valley and secondarily on Vedder Mountain (Cramer 2005). As logging declined, small-scale farming was established in the Columbia Valley (Cramer 2005). Clearing of land for agriculture with fire was prevalent, leading to several substantial forest fires (Cramer 2005). An extensive fire occurred in 1951 CE and burnt ~27 % of the watershed area (~20.4 km^2; Province of BC 2013; SM2). Much of the farming post-1940 CE focussed on dairy production, leading to further land clearing in the Columbia Valley (Cramer 2005). Post-1970s farming in the Columbia Valley has largely converged towards pastoral grazing, pork

production, and more recently an extensive expansion of berry crops (Zubel et al. 2000; FVRD 2011).

Nutrient influxes to Cultus Lake are mainly derived from anthropogenic activities within its watershed as well as atmospheric deposition of regional pollutants (Putt et al. 2019). According to the steady-state water quality model developed for Cultus Lake by Putt et al. (2019), watershed runoff contributes to 53 % and 73 % of the total phosphorus (P) and nitrogen (N) loadings, respectively (Table 1). Substantial inputs of total N (41 %) and P (26 %) from the watershed to the lake arise from the Columbia Valley (Putt et al. 2019), which has been affected by agricultural activities (Zubel 2000; Putt et al. 2019). Further nutrient loadings to Cultus Lake include septic leachate (19 % of P and 9 % of N; Table 1) that largely arises from campgrounds and residential areas (Putt et al. 2019). Currently, residential and campground sewage collection systems mainly consist of individual or communal septic tanks, which disperse waste via leaching fields (FVRD 2014). A sewer service was established in 1979, but it serves a limited number of people within the northern catchment (FVRD 2015). None of the existing sewage systems in the watershed carry waste to a treatment facility (FVRD 2014). Guano from migratory gulls has also been identified as a significant source of total P (22 %; Table 1) to Cultus Lake (Putt et al. 2019), as an expansion of the bird population has been occurring since at least the late 1970s (National Audubon Society, 2017). Finally, substantial nutrients loadings to the lake are attributable to atmospheric deposition from the nutrient-contaminated regional airshed. Direct atmospheric deposition on the lake surface contributed to 17 % and 5 % of the total N and P loadings to the lake, respectively (Table 1). Putt et al. (2019) estimated that ~66 % of N and ~70 % of P in the watershed runoff ultimately originated from atmospheric deposition. Thus, atmospheric deposition is considered the largest source of nutrient loadings to Cultus Lake

with cumulative contributions of 42 % and 63 % of total N and P loaded to the lake, respectively. The Cultus Lake airshed is mainly influenced by emissions from agricultural and urban landscapes of the Greater Vancouver Regional District, Fraser Valley Regional District (total population of ~296,000; Province of BC 2018) and Whatcom County (population of ~221,000; U.S. Census Bureau 2018) in northwestern Washington State, USA (Metro Vancouver 2013).

Cultus Lake has been a popular recreational site since the beginning of the 1900s with two regional parks, the municipal Cultus Lake Park (established in 1924 CE; Chwk. Prog. Aug. 6, 1924) and the Cultus Lake Provincial Park (established in 1948 CE; BC Parks 2017). As early as 1960 CE, an estimated 500,000 people visited Cultus Lake Park during the summer months (Chwk. Prog. Aug. 2, 1960); these numbers increased to over 1 million in 1973 CE (Chwk. Prog. Oct 15, 1973). Today, it is estimated that 2 to 3 million people visit Cultus Lake annually (FVRD 2011), largely during the summer for recreational activities, including camping, boating and fishing.

Major modifications to the littoral zone have occurred with the introduction of the invasive Eurasian milfoil (*Myriophyllum spicatum* (L.) Bonnier & Layens) after 1977 CE. By 1988 CE, *M. spicatum* covered ~60 % of the littoral zone to 6 m deep (Truelson 1988) expanding to 73 % littoral coverage by 2004 CE (Shortreed 2007). A detailed history on the anthropogenic development of Cultus Lake and its watershed are presented in SM2.

Methods

Field and laboratory analyses

Limnological conditions of Cultus Lake have been monitored in 1923-1924, 1927-1929 and 1932-1936 CE (Ricker 1937, 1938) and from 2001 to 2003 CE (Shortreed 2007). Since 2009 CE, Cultus Lake has been monitored monthly by Fisheries and Oceans Canada (DFO Lakes Research Program, unpublished data). Only three limnological parameters (temperature, dissolved oxygen and Schmidt stability index) were comparable among the different sampling periods due to variation in methodology (SM3). No major temporal changes were detected between the comparable limnological variables, except for a decline in hypolimnetic dissolved oxygen in the 2000s compared to the 1920s-1930s (Shortreed 2007; SM3).

From July 2014 CE to June 2015 CE, a suite of physical, chemical, and biological limnological parameters were measured following the methods described in Shortreed (2007) and sediment traps were deployed monthly to assist in understanding seasonality in diatom species composition, which was also examined in the sediment core. Sediment traps were deployed at ~3 m above the sediment-water interface near the core collection site. The aspect ratio (ratio of length to diameter) of the sediment traps was ~6 as suggested by Bloesch and Burns (1980), with a length of 61.0 cm and diameter of 10.2 cm. Diatom species assemblages were identified and enumerated from the sediment trap samples.

A 33-cm sediment core was collected in April 2008 CE from a deep depositional basin (N 49°03.45'; W 121°59.046') in Cultus Lake using a Glew Maxi Gravity Corer (diameter of 7.6 cm). The core was sectioned at 0.5 cm intervals from the surface to 2.5 cm and at 0.25 cm intervals thereafter. The samples were frozen (-20°C) until further analyses. Sediment samples

were then lyophilized (~48 h, 0.01 Pa) prior to analyses for geochronology (^{210}Pb, ^{137}Cs activities), geochemistry (% dry mass carbon (C) and nitrogen (N); C and N stable isotopes δ^{15}N and δ^{13}C), algal abundance, and assemblage composition (sedimentary pigments, diatoms), and cladoceran zooplankton composition, sub-fossil size, and abundance.

The sediment core chronology was established by gamma spectroscopy of ^{210}Pb and ^{137}Cs activities on 18 sediment samples distributed across the core (one sample every ~1.5 cm) following the methods of Appleby (2001). A constant rate of supply (CRS) model was used to calculate the sediment age and mass accumulation rates (g cm^{-1} yr^{-1}) as well as the age error for each sediment interval (Binford 1990; SM4).

At the Institute of Environmental Change and Society, University of Regina, a ThermoQuest (F-MAT) Delta ^{PLUS} XL isotope ratio mass spectrometer was used to determine bulk sediment C and N content (% dry mass) and stable isotope values for nitrogen (δ^{15}N) and carbon (δ^{13}C) following Savage et al. (2004). Isotopic values were expressed in conventional δ-notation relative to atmospheric N_2 and a local reference for the Vienna Pee Dee Belemnite standard, respectively. Sample reproducibility was ± 0.2 ‰. Elemental content and isotope values of bulk sediments were used to provide an indication of the source of organic matter (allochthonous, autochthonous, or admixture) to the sediments (Meyers and Teranes 2001).

Sedimentary pigments were extracted and purified following the standard procedures of Leavitt and Hodgson (2001). Pigments and their derivatives were isolated and quantified using an Agilent model 1100 high-performance liquid chromatography (HPLC) system, equipped with a photo-diode array and fluorescence detectors. The HPLC system was calibrated with authentic standards from DHI Lab (Denmark) prior to pigment quantification. Pigment concentrations

were expressed as nmoles pigment g^{-1} of dry sediment C. The pigments analysed through the HPLC system are indicative of all phototrophs (β-carotene, Chl *a* and its derivative pheophytin *a*), siliceous algae and some dinoflagellates (fucoxanthin, diatoxanthin), cryptophytes (alloxanthin), chlorophytes (chlorophyll *b*, pheophytin *b*), Nostocales cyanobacteria (canthaxanthin) and total cyanobacteria (echinenone) following Leavitt and Hodgson (2001). Two other pigments, lutein from chlorophytes and zeaxanthin from cyanobacteria were inseparable in this HPLC system and were presented together as lutein-zeaxanthin (Leavitt and Hodgson 2001). An index of UV radiation penetration into Cultus Lake (UV index) was estimated using standard procedures from photoprotective pigments as the ratio of UV-absorbing compound A to the sum of the carotenoid pigments alloxanthin, lutein+zeaxanthin and diatoxanthin (Leavitt et al. 1997). All pigments detected were used in our statistical analyses as they are known to be relatively stable once buried in lake sediments (Leavitt and Hodgson 2001).

Microfossil diatom slides were prepared following the standard methods of Battarbee et al. (2001). Identification and enumeration of diatom microfossils were performed at 1000X magnification under differential interference contrast (DIC) optics on a Leica DM4500 B microscope. Diatom microfossils were counted along parallel slide transects to reach a minimum sample size of at least 400 diatom valves and were expressed in relative abundance. The references used for diatom identification are presented in SM5.

Cladoceran subfossil slides were also prepared according to standard procedures (Korhola and Rautio 2001). Briefly, the slides were counted using a Leica DM4500 B microscope under 200X and 400X magnification. The identification of bosminids (*Eubosmina* sp. and *Bosmina* sp.) and *Daphnia* spp. along with the sizes of their subfossil remains (carapace for bosminids and post-abdominal claws for *Daphnia* spp.) were quantified following Sweetman and Finney (2003)

and Korosi et al. (2008, 2010). At least 50 individuals per interval were enumerated and the carapace of bosminids and the post-abdominal claws of *Daphnia* spp. were measured to infer relative size changes of cladoceran community through time. The abundance of individuals was integrated with our chronology to calculate sedimentary fluxes (number of individuals cm^{-2} y^{-1}) to better infer changes in secondary production within Cultus Lake. The sedimentation rates used to calculate cladoceran fluxes were estimated from the age-depth model and the calculations for the cladoceran fluxes are presented in SM4.

Data and statistical analyses

To identify different stratigraphic periods and detect temporal patterns that could indicate environmental changes in the lake, a constrained hierarchical clustering analysis, CONISS (constrained incremental sum-of-squares), followed by a broken-stick model (Birks 2012), was applied on chord-transformed data (Grimm 1987) using rioja package (Juggins 2017) in R software. This analysis was performed on four different datasets, which were sedimentary geochemistry (molar C:N ratios, $\delta^{15}N$ and $\delta^{13}C$), pigments, diatom and cladoceran (*Daphnia* spp. and bosminid fluxes and remain sizes) data. Prior to the chord transformation, z-scores were calculated for the geochemical and cladoceran data because the units differed for each indicator within the datasets. To further identify the major changes in multivariate datasets (pigments and diatoms), we applied a principal component analysis (PCA) to the data following a Hellinger-transformation (Legendre and Legendre 2012) using vegan package (Oksanen et al. 2018) in R software. The first principal component axis was then plotted against time to detect the major assemblage shifts over time. For the diatom community data, a total of 18 taxonomic groups

were used in the statistical analyses. The rare species (< 1 % of relative abundance or taxa occurring only in 1 or 2 samples) were grouped and only the dominant species (> 1 % of relative abundance) were kept separate in our analyses (SM5). All statistical analyses were performed using R statistical software v. 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Modern limnology of Cultus Lake (2014-2015)

Contemporary limnological monitoring of Cultus Lake indicated clear seasonal differences in both physicochemical conditions and biological communities (Table 2, Fig. 3, SM3). Chlorophyll *a* concentrations in Cultus Lake varied seasonally with epilimnetic concentrations averaging ~2 µg L^{-1} throughout the year, reaching higher mean concentrations (~4 µg L^{-1}) in the metalimnion during the thermally-stratified period (Table 2). During thermal stratification, total phosphorus (TP) and total nitrogen (TN) reached maximal values in the metalimnion relative to the epilimnion (Table 2). The euphotic zone depth varied seasonally, with a shallower light penetration through the water column during the mixed period (Table 2). Given that it is unclear whether individual nitrogen fractions were bioavailable (e.g., dissolved organic N, DON), we have focussed on the dissolved inorganic nitrogen:total phosphorus mass ratio (DIN:TP) as an indicator of nutrient deficiencies (Bergström 2010). Euphotic zone mass DIN:TP indicated potential P-deficient growth for most of the year (Fig. 3a, Table 2), with the exception of the summers 2014 and 2015 CE when mass DIN:TP were more suggestive of co-limitation of N and P. This co-limitation was largely coincident with larger biomasses of cyanobacteria and diatoms (Fig. 3b, c).

Diatom assemblages analyzed from our sediment traps indicated a strong seasonality in species dominance (Fig. 3d). From late autumn to early spring, the diatom community was composed principally of Aulacoseira species, mainly A. subarctica (Otto Müller) Haworth. Lindavia intermedia (Manguin ex Kociolek and Reviers) Nakov et al. ex Daniels et al. (previously Cyclotella bodanica var. intermedia) also exhibited elevated relative abundance for much of the year with the exception of the months of February and March 2015 CE (Fig. 3d). Lindavia michiganiana (Skvortzov) Nakov, Guillory, ML Julius, EC Ther. and AJ Alverson (previously C. michiganiana), a predominant species throughout most of the sediment record (Fig. 6), was low in relative abundance throughout the period of the sediment trap deployment. Another Lindavia species, L. comensis (Grunow) Nakov, Guillory, ML Julius, EC Ther. and AJ Alverson (previously C. comensis), was also present at a low relative abundance for the entire sediment trap deployment period. Stephanodiscus species, including S. parvus Stoermer & Håkansson, S. minitulus (Kützing) Cleve & Möller, S. medius H. Håkansson and S. niagarae Ehrenberg, were mostly present from February to June 2015 CE. The main Stephanodiscus taxon, S. niagarae, appears to have emerged in Cultus Lake since the collection of the sediment core in 2008 CE (Fig. 3d).

Sediment chronostratigraphy

Sedimentary ^{210}Pb unsupported activity showed a strong negative relationship to cumulative dry mass and reached background concentrations (supported ^{210}Pb) within the top 15 cm of the sediment core (SM4). Second and third order polynomial regressions were explored to develop age-depth models within the dated intervals (0-15 cm). The two polynomial

regressions were mainly consistent in the period from 1900 to 2008 CE, an interval which includes most of the major changes identified by the broken-stick models. Although the third order polynomial model had a better fit based on Bayesian Information Criterion (BIC) values, the relationship seemed to rely substantially on the oldest data point. As such, we calculated the average age from the second and third order models to extrapolate the dates beyond the background of supported ^{210}Pb using a conservative approach (SM4). This average age-depth model was then used to determine ages for all intervals of the core. The sediment chronological analyses also exhibited a ^{137}Cs peak at 12.25 cm indicating the date of 1963 CE (Appleby 2001), which corresponds well with the date of that level derived from the ^{210}Pb model (SM4).

Sediment geochemistry

From the CONISS analysis, interpretable shifts in the geochemical data were identified at ca. 1858 / 1862, 1965 / 1966 and 1984 / 1985 CE (Fig. 4a, b). According to the constrained cluster analysis, the largest shift occurred in 1984 / 1985, followed by the one in 1858 / 1862 (SM6). The first chronological break at ca. 1858 / 1862 CE detected very subtle changes in C:N and δ^{15}N, which we have not over-interpreted (Fig. 4a, b). Sediment values of molar C:N generally followed an increasing trend prior to the mid-1940s, after which it briefly exhibited substantially higher values (Fig. 4a). Following this event, molar C:N decreased toward values lower than those prior to the mid-1940s, which was identified as a shift in ca. 1965 / 1966 CE by the CONISS analysis (Fig. 4a). Generally, δ^{15}N exhibited low values and only modest variation ($\pm$ 1 ‰) prior to ca. 1900 CE, with the exception of a 2 ‰ drop ca. 1890 CE. Thereafter, δ^{15}N

exhibited an increasing trend with a more pronounced increase since the shift identified in ca. 1984 / 1985 CE, coincident with the decreases in molar C:N (Fig. 4b).

Sedimentary pigments

CONISS analysis of the pigment data identified interpretable shifts ca. 1946 / 1950 and ca. 1992 / 1993 CE (Figs. 4c, 5). Prior to the shift in ca. 1946 / 1950 CE, sedimentary pigments remained at relatively low concentrations and were mostly stable, suggesting a low but consistent algal abundance in Cultus Lake (Fig. 5). All pigments increased markedly after the shift in ca. 1946 / 1950 CE, with most pigments reaching their highest total concentrations between the shifts in ca. 1946 / 1950 and ca. 1992 / 1993 CE. By contrast, echinenone (indicator for cyanobacteria) exhibited a more gradual increase after the shift in 1946 / 1950 relative to other sedimentary pigments, followed by an abrupt increase at the beginning of the ca. 1970s. Echinenone concentrations have increased during the recent period (1977-2008 CE; Figs. 5, SM7), when temperatures were warmer, sockeye salmon escapements were lower, and nutrient run-off from the watershed was elevated relative to historical model hindcasts (Putt et al. 2019). From the shift in ca. 1992 / 1993 CE onwards, there was a general decline across all pigments (Figs. 5, SM8.2). The UV index showed high variability during ca. 1806 to the shift in 1946 / 1950 CE (Fig. 5). The high variability in the UV index was followed by a decrease reflecting less UVR exposure (Leavitt et al. 1997), which coincided with higher sedimentary pigment concentrations. After the shift in ca. 1992 / 1993 CE, the UV index decreased further, exhibiting the lowest values of the entire sediment record (Fig. 5).

Sedimentary diatom assemblages

The first ~150 years of the sediment record (ca. 1824 to the shift in 1964 / 1973 CE) exhibited a relatively stable diatom assemblage predominated by *Aulacoseira ambigua* (Grunow) Simonsen and *L. michiganiana* (Fig. 6). No major taxonomic shifts in the diatom assemblages occurred until the shift in ca. 1964 / 1973 CE as shown by the first PCA axis (Fig. 4d) and CONISS analysis (Figs. 4d, 6). The transition in ca. 1964 / 1973 CE was marked by a shift in species composition from *A. ambigua* to *A. subarctica*, as well as a decrease in relative abundance of *L. michiganiana*. Two other centric species, *L. intermedia* and *L. comensis*, appeared in the sediment record following the shift in 1964 / 1973 increasing in relative abundance mainly after ca. 1980 CE (Fig. 6).

Sedimentary cladocerans

Both cladoceran groups showed limited variation and low fluxes to the sediments prior to the shift identified by CONISS in ca. 1973 / 1981 CE but increased in deposition thereafter (Fig. 4e). The broken-stick analysis following CONISS for both cladoceran fluxes and size measurements indicated only this one interpretable shift in ca. 1973 / 1981 CE. Fluxes of both cladocerans tended to exhibit increase in the most recent period (from the shift in 1973 / 1981 to 2008 CE; Figs. 4e, SM7). The average length of bosminid remains showed a modest decline in size during the most recent periods, while *Daphnia* spp. claw length increased relative to earlier periods (Figs. 4f, g, SM7).

Discussion

With the intensification of urbanization and climate change projected for the coming century, a wide range of ecosystem services offered by peri-urban lakes are likely to be compromised without proactive stewardship. As such, it is important to understand the complex interactions of human and natural drivers influencing ecological changes in these valued ecosystems. Our paleolimnological record from Cultus Lake indicates that it was a relatively nutrient poor ecosystem prior to Euro-American occupation in the 1900s, which is consistent with the pre-development conditions inferred from steady-state lake nutrient modeling (Putt et al. 2019). Following this period of relative stability, four major periods of change were identified within our sedimentary record and cultural eutrophication was identified as a major driver of the changes. Analysis of contemporary limnological data shows that Cultus Lake is currently oligo-mesotrophic (chl-*a* of ~2.2 μm L^{-1} ± 0.8 and TP of 8.0 μg L^{-1} ± 1.3; Putt et al. 2019), and these values, considered in isolation, do not suggest substantial water quality degradation. However, our centennial scale study clearly shows how multiple environmental stressors can affect peri-urban lakes and provide context to frame management of the modern ecological conditions. Given the success of nutrient management strategies in similar fast-flushing temperate lakes (Jeppesen et al. 2005; Perga et al. 2010), we suggest that Cultus Lake could benefit from efforts to reduce nutrients from its water- and airshed (Putt et al. 2019).

Ecosystem stability in Cultus Lake (ca. 1800 to 1900 CE)

During the early to late 1800s, the Cultus Lake sediment record exhibited relatively low temporal variability across all sedimentary time series (Figs. 4, 5, 6), including the relatively low

algal and cyanobacterial pigment concentrations and cladoceran fluxes characteristic of oligotrophic conditions. The source of organic matter during this period was inferred to be principally autochthonous (molar C:N ~9-10) and fairly consistent, suggesting relative stability in the catchment and lake (Meyers and Teranes 2001; Selbie et al. 2009). Only a small change in molar C:N and $\delta^{15}N$ was identified by a break in ca. 1858 / 1862 CE by CONISS analysis and no apparent change was observed in the diatom assemblage. Thus, we consider ca. 1800-1900 CE to be a reference period for Cultus Lake, largely indicative of pre-disturbance conditions. Based on our analyses of the historical archives, anthropogenic influences within the Cultus Lake watershed were likely minimal prior to Euro-American settlement in the late 1800s (Soutar 2005; Schaepe and Ts'elxwéyeqw Tribe 2017), which is consistent with the ecological stability inferred from the sediment record.

Onset of Anthropogenic Watershed Changes (ca. 1900 to 1940 CE)

From the ca. 1900s to 1940s, sedimentary $\delta^{15}N$ increased slowly and consistently from the reference state (Fig. 4b) suggesting a change in N loading sources or cycling within Cultus Lake. While several within-lake processes (e.g. nitrification, denitrification, ammonia volatilization; Talbot 2002) and salmon carcass loading (Finney et al. 2000; Selbie et al. 2009) can increase $\delta^{15}N$ values within the sedimentary record, the changes in $\delta^{15}N$ observed from ca. 1900 to 1940 CE are most likely related to an increase in anthropogenic influences including wastewater from humans and livestock (Bunting et al. 2007, 2016; Botrel et al. 2014). Within the Cultus Lake catchment, we inferred that the enrichment in $\delta^{15}N$ was more consistent with the effects of increases in anthropogenic activities, such as agriculture and increases in human

occupation (i.e., sewage; SM2). The first European families settled in the Columbia Valley at the end of the 1800s and the population expanded in the area with the increase in logging, which reached a peak in the 1920s. Climate warming was observed from the 1920s to the mid-1940s, and was comparable in magnitude to that observed in 1977-2008 CE (Fig. 2b). Although some stressors (forest clearance and fires) could have influenced Cultus Lake during the period from the ca. 1880s to the ca. 1940s, our records indicate that it is not the most dynamic interval (i.e., slight increase in $\delta^{15}N$, no distinct changes in other proxies; Fig. 4) relative to other periods over the last ~200 years. These results are consistent with observations from other studies showing that effects of forest clearance and fires do not generally lead to persistent changes in sedimentary indicators (Paterson et al. 1998; Laird et al. 2001; Bredesen et al. 2002), although Scully et al. (2000) reported that forest clearcutting affected a lake's pigment record, likely caused by changes in lake mixing.

Early Eutrophication of Cultus Lake (ca. 1940 to 1970 CE)

The mid-1940s were marked by a brief increase of the molar C:N (Fig. 4a), which suggests a period of increased terrestrial organic matter delivery to the lake (Meyers and Teranes 2001; Selbie et al. 2009). The cumulative effect of two flooding events (1948 and 1950 CE) as well as a major forest fire (1951 CE) within the catchment (Soutar 2005) could have resulted in substantial allochthonous organic matter delivery to the lake. Following this period, greater concentrations of almost all sedimentary pigments were observed, a pattern suggesting increased influxes of nutrients to the lake from the watershed (Fig. 5). Increased nutrient delivery may have arisen from the dairy industry in the Columbia Valley and its associated spreading of

manure as fertilizer on pasture lands, as well as the expansion of tourism, which intensified septic inputs within the watershed. As the septic systems were probably limited and localized during this period, the flooding events (Soutar 2005) and the forest fire (Province of BC 2013) may have also enhanced the delivery of nutrients to the lake from livestock and human waste. Molar C:N (ca. 1950 CE) subsequently decreased to values more indicative of organic matter derived from aquatic production (Meyers and Teranes 2001; Selbie et al. 2009).

Atmospheric deposition of nutrients from the regional airshed influenced by urbanization, industry, transportation, and agricultural emissions, may have also contributed to nutrient loadings to Cultus Lake, as it is known to be a regional driver of aquatic enrichment (Putt et al. 2019). The sedimentary record of nearby Loon Lake, which in contrast to Cultus Lake has been protected from development within its watershed, has exhibited a significant decline in sedimentary $\delta^{15}N$ over the 1900s, tracking deposition of isotopically-depleted reactive nitrogen from the atmosphere (Holtgrieve et al. 2011). As atmospheric fluxes of nutrients to Cultus Lake were likely comparable during this period, the increase in sedimentary $\delta^{15}N$ and pigment concentrations in the Cultus Lake sediment record suggest early nutrient enrichment from locally-sourced, isotopically-enriched nutrient sources (i.e., cattle manure and human sewage), as observed elsewhere in agriculturally-dominated landscapes (Elliott and Brush 2006; Bunting et al. 2007, 2016; Botrel et al. 2014). Although increases in almost all sedimentary pigments were observed during this period, the diatom assemblages and cladoceran fluxes did not record any substantial changes in composition.

Multiple stressors underlying recent changes in Cultus Lake (ca. 1970 to 1990 CE)

Inferred changes in lake ecology from the ca. 1970s to 1990s were characterized by higher abundances of echinenone (a ubiquitous cyanobacterial pigment), reduced UV light penetration (based on pigment indicator), and an increase in cladoceran fluxes to the sediments. These changes coincided with measured declines in sockeye salmon escapement to the lake and the appearance of Eurasian watermilfoil in 1977 CE (Shortreed 2007). The decline in Cultus Lake sockeye salmon escapement certainly resulted in reduced in-lake juvenile densities, thereby reducing planktivory on zooplankton, which could enhance grazing pressures and be recorded in the sediments with higher fluxes of large-bodied zooplankton and lower concentrations of sedimentary pigments (Hume et al. 1996; Kyle 1996). Moreover, a decrease in sedimentary molar C:N indicated coeval increases in autochthonous organic matter contributions to the sediments (Meyers and Teranes 2001; Leng et al. 2006; Selbie et al. 2009). Further enrichment in the sedimentary $\delta^{15}N$ signature at this time suggest an enhanced contribution of organic wastes to the lake (i.e., septic leachate, manure runoff, avian guano; Putt et al. 2019), supporting continued cultural eutrophication over this period.

A common symptom of cultural eutrophication in lakes is the development of greater cyanobacterial biomass and the appearance of surface bloom-forming species (Downing et al. 2001). The increase in total cyanobacteria (as echinenone) observed in our sediment record coincided with elevated nutrient loading from increases in residential development and an increase of recreational activities in the Cultus Lake watershed, which could have led to higher P export to the lake and N limitation. Modifications in limiting nutrient stoichiometry can result in a structural reorganization of the phytoplankton community (Dolman et al. 2012). Based upon the contemporary euphotic zone DIN:TP (Fig. 3a), Cultus Lake was generally P-limited, but

exhibited the potential for co-limitation of N and P in summer, when cyanobacteria are more common. In addition, warmer water temperatures could favour cyanobacterial growth relative to other algal groups (Paerl et al. 2011) and warmer temperatures have been identified as a significant predictor explaining centennial-scale cyanobacterial trends in temperate and boreal lakes (Taranu et al. 2015). These inferred physical and biological changes may have, in turn, altered the depth of photosynthetic activity, and light penetration in the water column leading to changes in the inferred UV index (Fig. 5). The growth of the contemporary observed metalimnetic phytoplankton community may have also altered underwater light conditions.

A diatom shift from *A. ambigua* to *A. subarctica* was observed following the 1970s, and could have been influenced, in part, by the development of a warmer thermal regime in Cultus Lake, particularly in winter. Our sediment trap data indicate that *A. subarctica* is most common during the winter and is largely absent during the summer months. *A. subarctica* can tolerate very low light environments (Foy and Gibson 1993; Kilham et al. 1996), which would make it highly competitive under the recent winter conditions in Cultus Lake including negligible winter ice cover, strong water column mixing, and shallow light penetration (Table 2). The observed pattern of *A. subarctica* is consistent with seasonal water column studies of other deep mixing lacustrine environments (Lund 1954; Stockner and Lund 1970; Horn et al. 2011), as well as other shallow lakes (Gibson et al. 2003) from the Northern Hemisphere. A similar pattern of increase in *A. subarctica* was also observed in a paleolimnological study, which was associated to a faster flushing rate as a consequence of increase in spring rainfall (Bennion et al. 2012). However, in the case of Cultus Lake, the diatom shift might have been the result of an interaction between human and climatic stressors. Whereas the magnitude of atmospheric warming after the 1970s was comparable to that recorded during the 1920s to the mid-1940s, water temperatures at 20-40

m have been warmer during the mixed season and DO concentrations have been lower at 30 m during both mixed and stratified seasons in recent years at Cultus Lake (compared to the 1920s and 1930s; SM3). With the expansion of the human population and agriculture in the lower mainland of BC during the 1970s and the expected increase in nutrient loading to the lake (Putt et al. 2019), Cultus Lake could have been more vulnerable to climate warming during this recent period.

Additional variations in diatom species composition were observed in the recent sediment record, in particular the rise of two centric diatom taxa, *L. intermedia* and *L. comensis,* after ca. 1980 CE (Fig. 6). These two centric species were predominant in the contemporary diatom communities from the sediment trap samples (especially *L. intermedia*) between spring and autumn. The appearance of *L. intermedia* supports inferences of increasing water column stability during the summer, as indicated by physical modeling of Cultus Lake (Sumka 2017), as *Lindavia* sp. has been previously reported to benefit from its positive buoyancy to persist in nutrient depleted and well-illuminated surface waters during calm summer periods (Interlandi et al. 2003). Together, the combined inferences drawn from the sedimentary pigments and diatom assemblages strongly suggest that the recent history of Cultus Lake was subject to complex forcing resulting from cultural eutrophication and climate warming (Fig. 2).

Recent lake conditions (ca. 1990 CE to present) and management strategies

The Cultus Lake paleolimnological record following the 1990s was similar in trajectory to that observed ca. 1970 to 1990 CE. Diatom assemblages did not exhibit additional changes compared to the previous period (ca. 1970-1990 CE). However, the sedimentary record in the

1990s was marked by a sharp increase in $\delta^{15}N$, which could be associated with an increase of recreational activities as well as residential development in the Cultus Lake watershed during the recent period. A general decline in sedimentary pigment concentration was also observed, likely as a result of an increase grazing pressure from zooplankton inferred from an increase of cladoceran fluxes to the sediments.

Cladoceran fluxes continued to increase following ca. 1990 CE, while at the same time an increase in *Daphnia* spp. body size and a decline in bosminid size was observed (Fig. 4, SM7). The changes in cladoceran abundances could be a response to declines in sockeye salmon abundances (Fig. 4h), as juvenile salmonids are key predators of cladocerans (Hume et al. 1996; Kyle 1996). An inverse relation between cladoceran body sizes and temperature have been noted elsewhere (Havens et al. 2014), while changes in subfossil fluxes and modern *Daphnia* spp. size can be associated with variation in zooplankivory (Leavitt et al. 1989). Although the *Daphnia* spp. size increased in Cultus Lake following the decrease of sockeye salmon population, Mushet et al. (2019) have reported an increase of large bodied daphnids following the introduction of rainbow trout, which they explained with an increase of a deep, low oxygen refugia caused by the increase of nutrients and lake production. Given that multiple stressors influenced the changes in Cultus Lake during the recent periods, together with the limited taxonomic resolution of the cladoceran subfossils, it is difficult to disentangle all potential mechanisms. Interestingly, contemporary zooplankton collections (since 2009 CE) have shown an expansion of *Leptodora kindtii* Focke (DFO, unpublished data), a known predator on smaller-bodied cladocerans such as bosminids, and thus analyses of these water column time series could help advance our mechanistic understanding in the future. The overall increase in cladoceran fluxes during this period, suggests an inferred increase in herbivory that could have induced lower algal

abundance, eliciting more pronounced grazing regulation of phytoplankton communities (Carpenter and Kitchell, 1993).

The recent ecological drivers acting upon Cultus Lake appear to involve interactions between excess nutrient loadings and a warming climate. Climate model predictions for the British Columbia's Fraser Valley indicate substantial warmer annual temperatures (+1.7 ^{o}C) by 2050 CE, with marked reductions in summer precipitation (-14 %; PCIC 2013). Such climatic changes will likely make Cultus Lake more prone to enhanced algal production, particularly if nutrient loading continues unabated (Moss et al. 2010). Land development and recreational use of the Cultus Lake watershed are rapidly expanding, as are intensive agriculture operations in the Columbia and Fraser valleys. Therefore, it is highly plausible that nutrient loadings to the lake will be sustained or increase without targeted intervention, consistent with predictions and steady-state lake nutrient models for Cultus Lake (Putt et al. 2019). Our paleolimnological study indicates that Cultus Lake has already experienced cultural eutrophication since the beginning of the 1900s and that future degradation of water quality and habitat could negatively impact the persistence of two species at risk, the Cultus Lake sockeye salmon and the endemic Cultus pygmy sculpin, as well as the diverse services that this lake ecosystem offers. The current water quality in Cultus Lake, combined with the potentially rapid recovery of such fast-flushing coastal lakes in response to targeted nutrient management (Hampton et al. 2006), highlights that Cultus Lake may respond positively and rapidly to nutrient abatement efforts. Time is of the essence in averting continued lake eutrophication from intensifying sources, and avoiding reinforcing nutrient loadings, such as internal nutrient loading from the lake sediments (Nguyen and Maeda 2016; Putt et al. 2019).

In concert with the quantitative hindcast modeling of Putt et al. (2019), the long-term ecological context afforded by our paleolimnological record, provides system-specific reference conditions and quantitative recovery targets (e.g., ca. 1800-1900 CE) for lake nutrient management. It is of great importance to apply sustainable land use practices, focused on the abatement of nutrient loading from water- and airshed sources to preserve Cultus Lake ecosystem services and its species at risk.

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References

Allen A (2003) Environmental planning and management of the peri-urban interface: perspectives on an emerging field. Environ. Plan. Manag. 15: 135–147

Appleby PG (2001) Chronostratigraphic techniques in recent sediments. In: Last WM, Smol JP (eds) Tracking environmental change using lake sediments. Volume 2: Basic analysis, coring and chronological techniques. Kluwer Academic publishers New York, pp 171–203

Adrian R, O'Reilly CM, Zagarese H, et al (2009) Lakes as sentinels of climate change. Limnol Oceanogr 54: 2283–2297

Battarbee RW (2010) Aquatic ecosystem variability and climate change – A palaeoecological perspective. In: Kernan M, Battarbee RW, Moss B (eds) Climate change impacts on freshwater ecosystems. Wiley-Blackwell, pp 15–37

Battarbee RW, Jones VJ, Flower RJ, et al (2001) Diatoms. In: Smol JP, Birks HJB, Last WM, et al. (eds) Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal ad siliceous indicators. Kluwer Academic Publisher New York, pp 155–202

BC Ministry of Environment (2016) Indicators of climate change for British Columbia (2016) Update. Victoria, Canada. https://www2.gov.bc.ca/assets/gov/environment/research-monitoring-and-reporting/reporting/envreportbc/archived-reports/climate-change/climatechangeindicators-13sept2016_final.pdf

BC Parks (2017) Cultus Lake Provincial Park. http://www.env.gov.bc.ca/bcparks/explore/parkpgs/cultus_lk/. Accessed on April 28, 2017

Bennion H, Battarbee RW, Sayer CD, et al (2011) Defining reference conditions and restoration targets for lake ecosystems using palaeolimnology: a synthesis. J Paleolimnol 45:533–544. doi:10.1007/s10933-010-9419-3

Bennion H, Carvalho L, Sayer CD, et al (2012) Identifying from recent sediment records the effects of nutrients and climate on diatom dynamics in Loch Leven. Freshw Biol 57: 2015-2029. doi:10.1111/j.1365-2427.2011.02651.x

Bergström A-K (2010) The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. Aquat Sci 72:277–281. doi:10.1007/s00027-010-0132-0

Binford MW (1990) Calculation and uncertainty analysis of 210Pb dates for PIRLA project lake sediment cores. J Paleolimnol 3:253–267. doi:10.1007/BF00219461

Birks HJB (2012) Analysis of stratigraphical data. In: Birks HJB, Lotter AF, Juggins S, Smol JP (eds) Tracking environmental change using lake sediments. Volume 5: Data handling and numerical techniques. Springer Dordrecht, pp 355–378

Bloesch J, Burns NM (1980) A critical review of sedimentation trap technique. Schweiz Z Hydrol Schweiz Z Für Hydrol 42:15–55

Botrel M, Gregory-Eaves I, Maranger R (2014) Defining drivers of nitrogen stable isotopes (δ15N) of surface sediments in temperate lakes. J Paleolimnol 52:419–433. doi:10.1007/s10933-014-9802-6

Bredesen EL, Bos DG, Laird KR, Cumming BF (2002) A cladoceran-based paleolimnological assessment of the impact of forest harvesting on four lakes from the central interior of British Columbia, Canada. J Paleolimnol 28:389–402. doi:10.1023/A:1021887915458

Bunting L, Leavitt PR, Gibson CE, et al (2007) Degradation of water quality in Lough Neagh, Northern Ireland, by diffuse nitrogen flux from a phosphorus-rich catchment. Limnol Oceanogr 52:354–369. doi:10.4319/lo.2007.52.1.0354

Bunting L, Leavitt PR, Simpson GL, et al (2016) Increased variability and sudden ecosystem state change in Lake Winnipeg, Canada, caused by 20th century agriculture. Limnol. Oceanogr. 61: 2090–2107. doi:10.1002/lno.10355.

Carlson K (ed), McHalsie AJ, Stó:lō Heritage Trust (2001) A Stó:lō-Coast Salish historical atlas, Douglas & McIntyre LTD. Vancouver, Canada.

Carpenter SR, Kitchell JF (eds) (1993) The trophic cascade in lakes, 1st edn. Cambridge: Cambridge University Press. doi:10.1017/CBO9780511525513

Chiang E, Pon L, Selbie DT, et al (2015) Identification of critical habitat for Coastrange Sculpin (Cultus Population) (Cottus aleuticus). DFO Can. Sci. Sec. Res. Doc. 2015/033

Christensen MR, Graham MD, Vinebrooke RD, et al (2006) Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Glob Change Biol 12:2316–2322. doi:10.1111/j.1365-2486.2006.01257.x

Chu C, Minns CK, Lester NP, et al (2015) An updated assessment of human activities, the environment, and freshwater fish biodiversity in Canada. Can J Fish Aquat Sci 72:135–148. doi:10.1139/cjfas-2013-0609

[Chwk. Prog.] Chilliwack progress, August 6, 1924. Chilliwack Museum and Archives.

[Chwk. Prog.] Chilliwack progress, August 2, 1960. Chilliwack Museum and Archives.

[Chwk. Prog.] Chilliwack progress, October 15, 1973. Chilliwack Museum and Archives.

COSEWIC (2003) COSEWIC assessment and status report on the sockeye salmon Oncorhynchus nerka (Cultus population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. ix + 57 pp.

COSEWIC (2010) COSEWIC assessment and status report on the Coastrange Sculpin *Cottus aleuticus*, Cultus Population in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi + 28 pp. (www.sararegistry.gc.ca/status/status_e.cfm).

Cramer FH (2005) The valley beyond: a history of Columbia Valley & Lindell Beach. Columbia Valley Ratepayers Association, Lindell Beach, Canada

Cultus Sockeye Recovery Team (2005) Conservation strategy for sockeye salmon (Onchorhynchus nerka), Cultus Lake population, in British Columbia. Recovery of Nationally Endangered Wildlige (RENEW), Ottawa, Ontario. https://www.pac.dfo-mpo.gc.ca/science/habitat/cultus/docs/conservstrat.pdf

Dolman AM, Rücker J, Pick FR, et al (2012) Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. PLoS ONE 7:e38757. doi:10.1371/journal.pone.0038757

Douglas I (2008) Environmental change in peri-urban areas and human and ecosystem health. Geogr. Compass 2: 1095–1137. doi:10.1111/j.1749-8198.2008.00122.x

Downing JA, Watson SB, McCauley E (2001) Predicting cyanobacteria dominance in lakes. Can J Fish Aquat Sci 58:1905–1908. doi:10.1139/f01-143

Elliott EM, Brush GS (2006) Sedimented organic nitrogen isotopes in freshwater wetlands record long-term changes in watershed nitrogen source and land use. Environ Sci Technol 40:2910–2916

ESRI Canada (2017) Provinces and Territories of Canada, Natural Earth Vector. Retrieved online via ArcGIS ESRI on January 15, 2018.

ESRI, Tomtom North America Inc., U.S. Census Bureau, U.S. Department of Agriculture (USDA), National Agricultural Statistics Service (NASS) (2017) USA States. Retrieved online via ArcGIS ESRI on January 15, 2018.

Finney BP, Gregory-Eaves I, Sweetman J, et al (2000) Impacts of climatic change and fishing on pacific salmon abundance over the past 300 years. Science 290:795–799. doi:10.1126/science.290.5492.795

Fisheries and Oceans Canada (2017) 2017/2018 Salmon integrated fisheries management plan - Southern BC, Canada. https://waves-vagues.dfo-mpo.gc.ca/Library/40601006.pdf

Foerster RE (1930) An investigation of the life history and propagation of the sockeye salmon (*Oncorhynchus Nerka*) at Cultus Lake, British Columbia. No3 The down-stream migration of the young in 1926 and 1927. Contrib Can Biol Fish 55–82

Foerster RE (1934) An investigation of the life history and propagation of the sockeye salmon (*Oncorhynchus Nerka*) at Cultus Lake, British Columbia. No4 The life history cycle of the 1925 year class with Natural Propagation. Contrib Can Biol Fish 345–355

Foerster RE (1936) An investigation of the life history and propagation of the sockeye salmon (*Oncorhynchus Nerka*) at Cultus Lake, British Columbia. No5 The life history cycle of the 1926 year class with artificial propagation involving the liberation of free-swimming fry. J Biol Board Can 311–333

Foy RH, Gibson CE (1993) The influence of irradiance, photoperiod and temperature on the growth kinetics of three planktonic diatoms. Eur J Phycol 28:203–212. doi:10.1080/09670269300650311

FVRD (2011) Official community plan for electoral area E bylaw. Fraser Valley Regional District, British Columbia, Canada

FVRD (2014) Cultus Lake sanitary sewer infrastructure assessment. Engineering and Land Services, British Columbia, Canada

FVRD (2015) Sewer & Septic. http://www.fvrd.ca/EN/main/services/sewer-septic/sewer-systems.html. Accessed June 22, 2018.

Geo BC (2017) Data Catalogue. https://apps.gov.bc.ca/pub/dwds-rasp. Accessed April 28, 2017

Gibson CE, Anderson NJ, Haworth EY (2003) *Aulacoseira subarctica* : taxonomy, physiology, ecology and palaeoecology. Eur J Phycol 38:83–101. doi:10.1080/0967026031000094102

Government of Canada EC (2011) HTML Version of the Document - Species at risk public registry. http://www.registrelep-sararegistry.gc.ca/document/dspHTML_e.cfm?ocid=8366. Accessed 23 Oct 2017

Grimm EC (1987) CONISS: a FORTRAN 77 program for stratigraphically constrained cluster analysis by the method of incremental sum of squares. Comput Geosci 13:13–35. doi:10.1016/0098-3004(87)90022-7

Hampton SE, Scheuerell MD, Schindler DE (2006) Coalescence in the Lake Washington story: Interaction strengths in a planktonic food web. Limnol Oceanogr 51:2042–2051. doi:10.4319/lo.2006.51.5.2042

Havens KE, Pinto-Coelho RM, Beklioğlu M, et al (2015) Temperature effects on body size of freshwater crustacean zooplankton from Greenland to the tropics. Hydrobiologia 743:27–35. doi:10.1007/s10750-014-2000-8

Holtgrieve GW, Schindler DE, Hobbs WO, et al (2011) A coherent signature of anthropogenic nitrogen deposition to remote watersheds of the Northern Hemisphere. Science 334:1545–1548. doi:10.1126/science.1212267

Horn H, Paul L, Horn W, Petzoldt T (2011) Long-term trends in the diatom composition of the spring bloom of a German reservoir: is *Aulacoseira subarctica* favoured by warm winters?: Warm winters and competition success of *Aulacoseira subarctica*. Freshw Biol 56:2483–2499. doi:10.1111/j.1365-2427.2011.02674.x

Huang B, Zhao Y, Shi X, et al (2007) Source identification and spatial variability of nitrogen, phosphorus, and selected heavy metals in surface water and sediment in the riverine systems of a peri-urban interface. J Environ Sci Health Part A Tox Hazard Subst Environ Eng 42:371–380. doi:10.1080/10934520601144675

Hume JM, Shortreed KS, Morton KF (1996) Juvenile sockeye rearing capacity of three lakes in the Fraser River system. Can J Fish Aquat Sci 53:719–733. doi:10.1139/f95-237

Interlandi SJ, Kilham SS, Theriot EC (2003) Diatom—chemistry relationships in Yellowstone Lake (Wyoming) sediments: Implications for climatic and aquatic processes research. Limnol Oceanogr 48:79–92. doi:10.4319/lo.2003.48.1.0079

Jackson MC, Loewen CJG, Vinebrooke RD, Chimimba CT (2016) Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob Change Biol 22:180–189. doi:10.1111/gcb.13028

Jeppesen E, Sondergaard M, Jensen JP, et al (2005) Lake responses to reduced nutrient loading: an analysis of contemporary long-term data from 35 case studies. Freshw Biol 50:1747–1771. doi:10.1111/j.1365-2427.2005.01415.x

Juggins S (2017) rioja: Analysis of quaternary science data, R package version (0.9-21). (http://cran.r-project.org/package=rioja).

Kilham S, Theriot EC, Fritz S (1996) Linking planktonic diatoms and climate change in the large lakes of the Yellowstone ecosystem using resource theory. Limnol Oceanogr 41(5):1052-1062

Korhola A, Rautio M (2001) Cladocera and other branchiopod crustaceans. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. Volume 4: Zoological indicators. Kluwer Academic Publishers New York, pp 5–41

Korosi JB, Paterson AM, Desellas AM, Smol JP (2008) Linking mean body size of pelagic Cladocera to environmental variables in Precambrian Shield lakes: A paleolimnological approach. J Limnol 67:22–34. doi:10.4081/jlimnol.2008.22

Korosi JB, Paterson AM, DeSellas AM, Smol JP (2010) A comparison of pre-industrial and present-day changes in *Bosmina* and *Daphnia* size structure from soft-water Ontario lakes. Can J Fish Amp Aquat Sci 67:754–762. doi:10.1139/F10-013

Kyle GB (1996) Stocking sockeye salmon (*Oncorhynchus nerka*) in barren lakes of Alaska: effects on the macrozooplankton community. Fish Res 28:29–44. doi:10.1016/0165-7836(96)00486-9

Laird K, Cumming B, Nordin R (2001) A regional paleolimnological assessment of the impact of clear-cutting on lakes from the west coast of Vancouver Island, British Columbia. Can J Fish Aquat Sci 58:479–491. doi:10.1139/f00-26

Leavitt PR, Hodgson DA (2001) Sedimentary pigments. In: Smol JP, Birks HJB, Last WM, et al. (eds) Tracking environmental change using lake sediments. Volume 2: Physical and geochemical methods. Kluwer Academic Publishers New York, pp 295–325

Leavitt PR, Carpenter SR, Kitchell JF (1989) Whole-lake experiments: The annual record of fossil pigments and zooplankton. Limnol. Oceanogr. 34: 700-717. doi:10.4319/lo.1989.34.4.0700

Leavitt PR, Vinebrooke RD, Donald DB, et al (1997) Past ultraviolet radiation environments in lakes derived from fossil pigments. Nature 388:457–459. doi:10.1038/41296

Legendre P, Legendre L (2012) Numerical ecology. Elsevier, Amsterdam; Boston

Lehner B, Verdin K, Jarvis A (2008) New global hydrography derived from spaceborne elevation data. Eos Trans AGU 89:93. doi:10.1029/2008EO100001

Leng MJ, Lamb AL, Heaton THE, et al (2006) Isotopes in lake sediments. In: Isotopes in palaeoenvironmental research. Springer, Dordrecht, pp 147–184

Lund JWG (1954) The seasonal cycle of the plankton diatom, *Melosira italica* (EHR.) Kutz. Subsp. *subarctica* O. Mull. J Ecol 42:151–179. doi:10.2307/2256984

Melymuk L, Robson M, Diamond ML, et al (2011) Wet deposition loadings of organic contaminants to Lake Ontario: Assessing the influence of precipitation from urban and rural sites. Atmos Environ 45:5042–5049. doi:10.1016/j.atmosenv.2011.02.007

Metro Vancouver (2013) 2010 Lower Fraser Valley air emissions inventory and forecast and backcast: Final report and summarized results. Air Quality Policy and Management Division, Vancouver, Canada. http://www.metrovancouver.org/services/air-quality/AirQualityPublications/2010LowerFraserValleyAirEmissionsInventoryandForecastandBackcast.pdf

Meyers PA, Teranes JL (2001) Sediment organic matter. In: Last WM, Smol JP (eds) Tracking environmental change using lake sediments. Volume 2: Physical and geochemical indicators. Kluwer Academic Publishers New York, pp 239–269

Moorhouse HL, McGowan S, Jones MD, et al (2014) Contrasting effects of nutrients and climate on algal communities in two lakes in the Windermere catchment since the late 19th century. Freshwater Biology 59:2605–2620. doi:10.1111/fwb.12457

Moss B, Battarbee RW, Kernan M (2010) Introduction. In: Climate change impacts on freshwater ecosystems. Wiley-Blackwell, pp 1–14

Moss B, Kosten S, Meerhoff M, et al (2011) Allied attack: climate change and eutrophication. Inland Waters 1:101–105. doi:10.5268/IW-1.2.359

Mushet GR, Laird KR, Leavitt PR, et al (2019). Bottom-up forces drive historical increases in the abundance of large daphniids in four small lakes stocked with rainbow trout (*Oncorhynchus mykiss*), interior British Columbia, Canada. Ecosystems. doi:10.1007/s10021-019-00443-0.

National Audubon Society. 2017. Audubon Christmas bird count. http://www.audubon.org/conservation/science/christmas-bird-count/. Accessed on July 19, 2017

Nguyen HV, Maeda M (2016) Effects of pH and oxygen on phosphorus release from agricultural drainage ditch sediment in reclaimed land, Kasaoka Bay, Japan. J of Wat & Envir Tech 14:228–235. doi:10.2965/jwet.15-027

Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2018) vegan: Community ecology package. R package version 2.5-3. https://CRAN.R-project.org/package=vegan

Paerl HW, Hall NS, Calandrino ES (2011) Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. Sci Total Environ 409:1739–1745. doi:10.1016/j.scitotenv.2011.02.001

Paterson AM, Cumming BF, Smol JP, et al (1998) Assessment of the effects of logging, forest fires and drought on lakes in northwestern Ontario: a 30-year paleolimnological perspective. Can J For Res 28:1546–1556. doi:10.1139/x98-138

[PCIC] The Pacific Climate Impacts Consortium (2013) Climate summary: South Coast. University of Victoria, Victoria. https://www.pacificclimate.org/sites/default/files/publications/Climate_Summary-South_Coast.pdf

Perga M-E, Desmet M, Enters D, Reyss J-L (2010) A century of bottom-up- and top-down driven changes on a lake planktonic food web: A paleoecological and paleoisotopic study of Lake Annecy, France. Limnol Oceanogr 55:803–816. doi:10.4319/lo.2010.55.2.0803

Peterman RM, Dorner B, Rosenfeld JS (2012) A widespread decrease in productivity of sockeye salmon (*Oncorhynchus nerka*) populations in western North America. Can J Fish Aquat Sci 69:1255–1260. doi:10.1139/f2012-063

Province of BC (2013) Data from iMapBC application. https://maps.gov.bc.ca/ess/hm/imap4m/?catalogLayers=1756,1757,1758&scale=400000.0&center=-13161319.5843,6668201.90948. Accessed on April 28, 2017

Province of BC (2018) 2016 total population results – Municipalities by Regional District. BC Stats organization. http://www.bcstats.gov.bc.ca/StatisticsBySubject/Census/2016Census.aspx. Accessed on May 10, 2018

Putt AE, MacIsaac EA, Herunter HE, et al (2019) Eutrophication forcings on a peri-urban lake ecosystem: Context for integrated watershed to airshed management. PLoS ONE 14:1–21

Ricker WE (1937) Physical and chemical characteristics of Cultus Lake, British Columbia. J Biol Board Can 3(4):363-402

Ricker WE (1938) Seasonal and annual variations in quantity of pelagic net plankton, Cultus Lake, British Columbia. J Fish Res Board Can 4:34–47

Rosenfield J, Department of Fisheries and Oceans (2007) Recovery strategy for Cultus pygmy sculpin (*Cottus* sp.) in Canada. Fisheries and Oceans Canada, Ottawa, Canada

Savage C, Leavitt PR, Elmgren R (2004) Distribution and retention of effluent nitrogen in surface sediments of a coastal bay. Limnol Oceanogr 49:1503–1511. doi:10.4319/lo.2004.49.5.1503

Schaepe DM, Ts'elxwéyeqw Tribe (eds) (2017) Being Ts'elxwéyeqw: First Peoples' voices and history from the Chilliwack-Fraser Valley, British Columbia. Harbour Publishing, Madeira Park, British Columbia, Canada

Scully NM, Leavitt PR, Carpenter SR (2000) Century-long effects of forest harvest on the physical structure and autotrophic community of a small temperate lake. Can. J. Fish. Aquat. Sci. 57 (Suppl. 2): 50-59. doi:10.1139/cjfas-57-S2-50

Selbie DT, Finney BP, Barto D, et al (2009) Ecological, landscape, and climatic regulation of sediment geochemistry in North American sockeye salmon nursery lakes: Insights for paleoecological salmon investigations. Limnol Oceanogr 54:1733–1745

Shortreed KS (2007) Limnology of Cultus Lake, British Columbia. Can Tech Rep Fish Aquat Sci 2753:vi + 85p

Smol JP (2008) Pollution of lakes and rivers: a paleolimnological perspective, 2nd edition. Blackwell Sientific Publications Publishing, Oxford, UK

Soutar M (2005) Cultus Lake: A natural paradise. Chilliwack Museum and Archives, Chilliwack, Canada

Stockner JG, Lund JWG (1970) Live algae in postglacial lake deposits. Limnol Oceanogr 15:41–58. doi:10.4319/lo.1970.15.1.0041

Sumka MG (2017) Climate change impacts on a eutrophying lake: Cultus Lake, British Columbia. University of British Columbia. https://open.library.ubc.ca/cIRcle/collections/ubctheses/24/items/1.0354396

Sweetman JN, Finney BP (2003) Differential responses of zooplankton populations (*Bosmina longirostris*) to fish predation and nutrient-loading in an introduced and a natural sockeye

salmon nursery lake on Kodiak Island, Alaska, USA. J Paleolimnol 30:183–193. doi:10.1023/A:1025543421436

Talbot MR (2002) Nitrogen isotopes in palaeolimnology. In: Last WM, Smol JP (eds) Tracking environmental change using lake sediments. Volume 2: Physical and geochemical methods. Kluwer Academic Publishers New York, pp 401–439

Taranu ZE, Gregory-Eaves I, Leavitt PR, et al (2015) Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. Ecol Lett 18:375–384. doi:10.1111/ele.12420

Truelson R (1988) Cultus Lake, survey of *Myriophyllum spicatum* and other aquatic plants. Province of British Columbia, Canada

United Nations, Department of Economic and Social Affairs, Population Division (2014) World urbanization prospects: the 2014 revision. https://www.un.org/en/development/desa/publications/2014-revision-world-urbanization-prospects.html

U.S. Census Bureau (2018) Quick facts: Whatcom County, Washington. https://www.census.gov/quickfacts/fact/table/whatcomcountywashington/PST045216. Accessed on May 10, 2018.

Vörösmarty CJ, McIntyre PB, Gessner MO, et al (2010) Global threats to human water security and river biodiversity. Nature 467:555–561. doi:10.1038/nature09440

Waltham NJ, Reichelt-Brushett A, McCann D, Eyre BD (2014) Water and sediment quality, nutrient biochemistry and pollution loads in an urban freshwater lake: balancing human

and ecological services. Env Sci Process Impacts 16:2804–2813. doi:10.1039/C4EM00243A

Zubel M (2000) Groundwater conditions of the Columbia Valley aquifer, Cultus Lake, British Columbia. Ministry of Environment, Lands and Parks, Water Management, Surrey, BC, Canada. http://a100.gov.bc.ca/appsdata/acat/documents/r6477/1287_1143682706883_8b7182b483ed458aad9da29ff7cef555.pdf

Tables Chapter 3

Table 1. Contributions of different sources of total phosphorus (TP) and nitrogen (TN) to Cultus Lake*.

Sources	TP	TN
Watershed runoffs	53.2 %	72.7 %
From Columbia Valley subwatershed	26.4 %	40.7 %
From other subwatersheds	26.8 %	32.0 %
Direct atmospheric deposition	4.5 %	17.1 %
Septic leachate	19.1 %	9.0 %
Migratory gulls	22.4 %	0.9 %
Sockeye salmon carcasses	0.8 %	0.4 %

*Data from Putt et al. 2019

Table 2. Average (± SE) of contemporary (2014-2015) physico-chemical and biological variables of Cultus Lake, British Columbia for mixed (December-April) and stratified (May-November) periods; a) Water column parameters: Schmidt stability index (SSI), surface temperature, hypolimnetic dissolved oxygen (DO) and Secchi depth; b) Epilimnetic and c) Metalimnetic nutrients (total phosphorus (TP), total nitrogen (TN) and dissolved inorganic nitrogen (DIN)), mass DIN:TP, chlorophyll *a* and cyanobacterial biomasses for the euphotic zone.

Variables	Mixed	Stratified
a) Water column variables		
SSI (J m^{-2})	192.3 (9.9)	1862.1 (125.1)
Surface temp. (°C)	6.0 (0.3)	18.3 (0.6)
Hypolimnetic DO at 30 m depth (mg L^{-1})	10.5 (0.2)	8.0 (0.3)
Secchi depth (m)	6.4 (0.2)	8.5 (0.2)
b) Epilimnion – euphotic zone		
TP (µg L^{-1})	7.3 (0.3)	4.8 (0.5)
TN (µg L^{-1})	259.36 (5.6)	186.1 (8.1)
DIN (µg L^{-1})	131.7 (5.1)	32.3 (10.9)
Mass DIN:TP	18.2 (0.8)	8.7 (3.4)
Chl *a* (µg L^{-1})	2.1 (0.3)	2.0 (0.3)
Cyano biomass (µg L^{-1})	1.0 (0.5)	32.2 (7.5)
c) Metalimnion – euphotic zone		
TP (µg L^{-1})	-	7.3 (0.5)
TN (µg L^{-1})	-	229.4 (9.2)
DIN (µg L^{-1})	-	58.3 (11.8)
Mass DIN:TP	-	9.6 (2.3)
Chl *a* (µg L^{-1})	-	4.1 (0.6)
Cyano biomass (µg L^{-1})	-	36.2 (9.3)

Figures Chapter 3

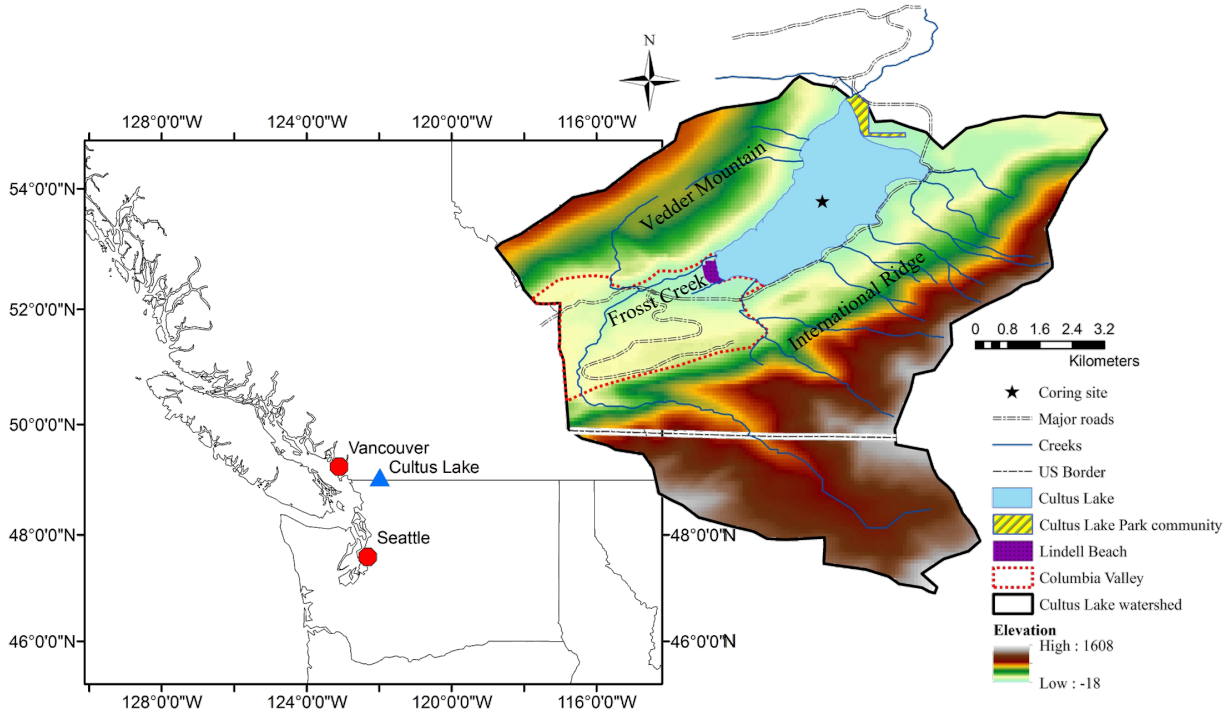

Figure 1. Location of Cultus Lake and its watershed. Cultus Lake, indicated by the blue triangle, is located at about ~50 km east of the outer limit of the Greater Vancouver Regional District, British Columbia, Canada. The star in the lake indicates the coring site. Major creeks, roads, residential communities are shown on the map as well as other relevant sites. Geographic features were retrieved from Geo BC (2017), the digital elevation map from Lehner et al. (2008), and the basemaps from ESRI Canada (2017) and ESRI et al. (2017) for Canada and USA, respectively.

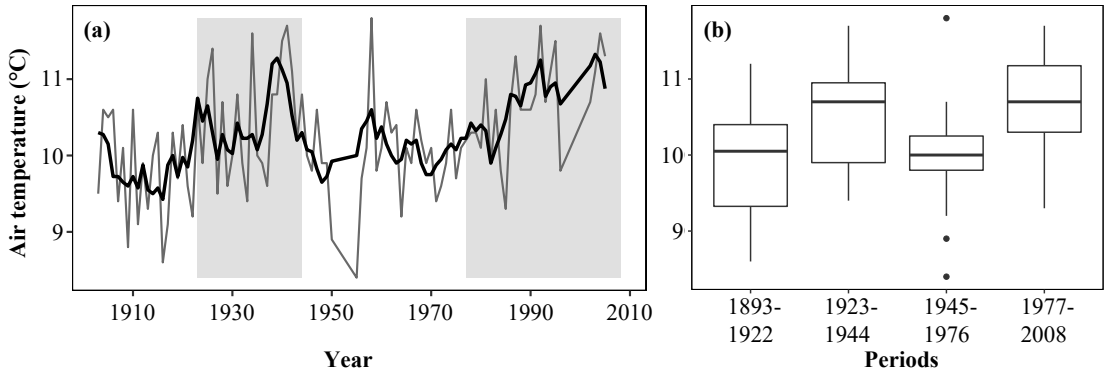

Figure 2. (a) Time series of annual average of regional air temperatures. The grey line and the black line represent the actual air temperatures and the 4-year moving average, respectively. The light grey zones represent the two periods of regional climate warming: 1923-1944 CE and 1977-2008 CE. (b) Boxplots of annual average of regional air temperatures for different periods showing the two regional climate warming periods.

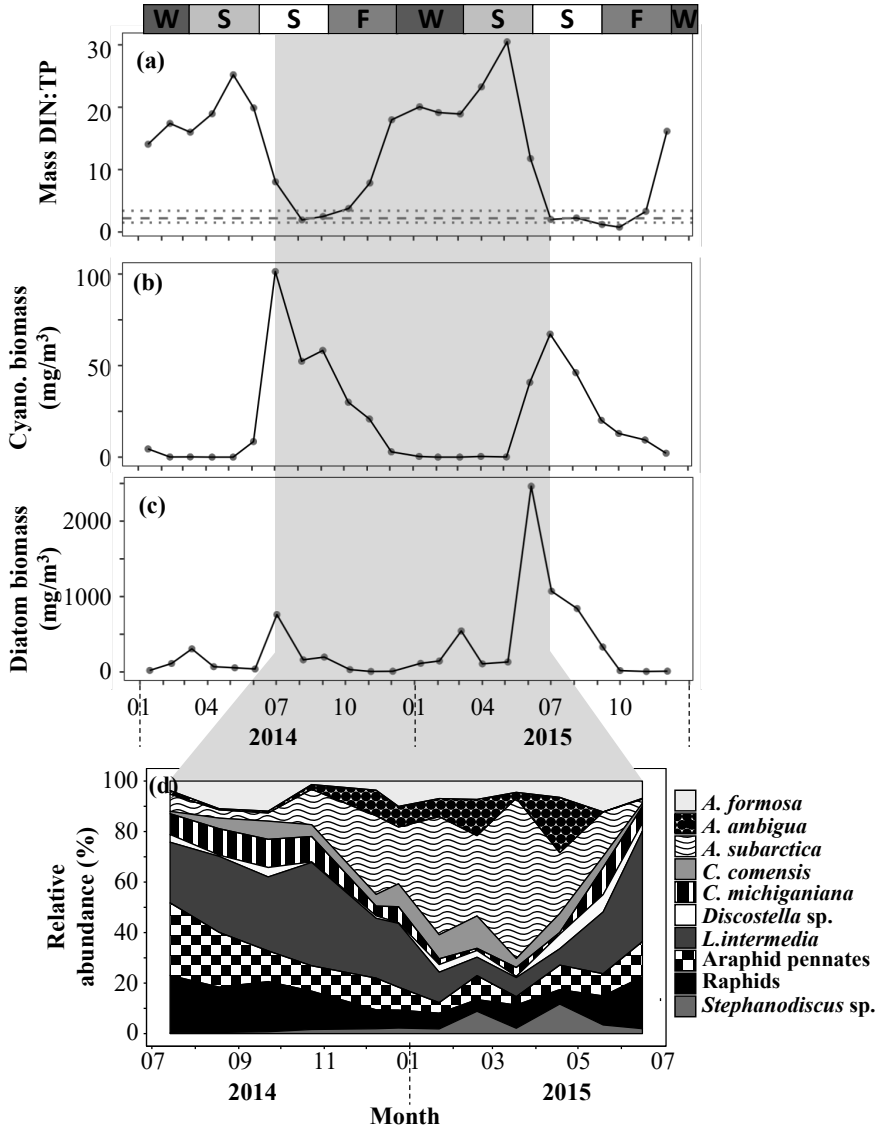

Figure 3. Contemporary time series for 2014 and 2015 of (a) mass dissolved inorganic nitrogen:total phosphorus ratio (DIN:TP), (b) biomasses of cyanobacteria, (c) biomasses of diatoms and, (d) relative abundance of subfossils diatoms in sediment traps (from July 2014 to June 2015 CE). Probabilities of P limitation are indicated in (a) by a dashed line for 50 % threshold (i.e., DIN:TP mass ratio of 2.2) and dotted lines for 25 % and 75 % (i.e., DIN:TP mass ratio of 1.5 and 3.4, respectively).

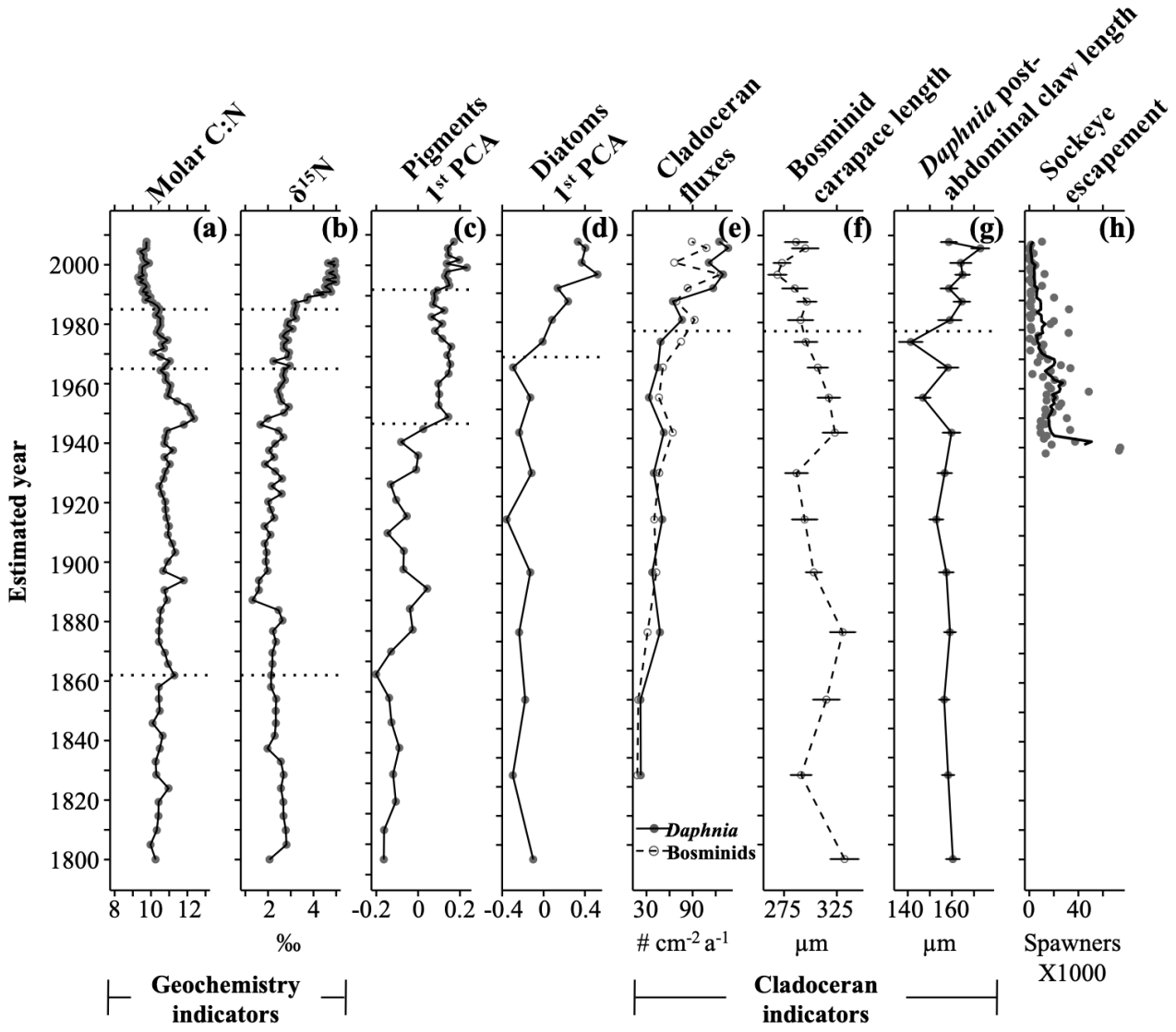

Figure 4. Centennial time series for sedimentary (a) carbon:nitrogen (C:N) molar ratio, (b) $\delta^{15}N$, (c) 1st PCA axis of sedimentary pigment data (explaining 55 % of the total variation), (d) 1st PCA axis of diatom community (explaining 38 % of the total variation), (e) cladoceran fluxes, (f) bosminid carapace length, (g) *Daphnia* spp. post-abdominal claw length, and (h) sockeye salmon historical escapement. The dashed lines represent the breaks identified by CONISS followed by broken-stick analyses, which were done on each indicator group separately. The dates older than 1879 (± 66) CE should be interpreted with care as they are beyond the unsupported ^{210}Pb.

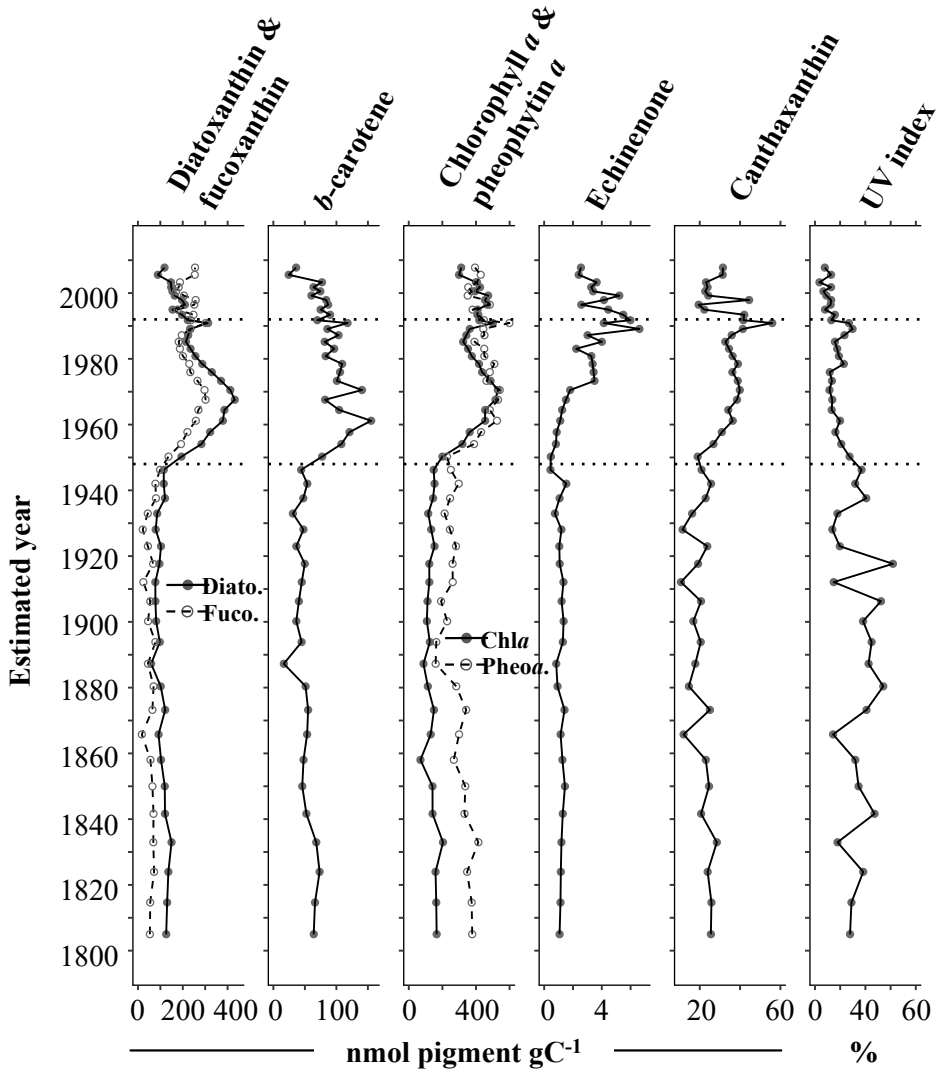

Figure 5. Centennial time series of sedimentary pigment concentrations (nmol of pigment g^{-1} sediment C) representing siliceous algae and some dinoflagellates (fucoxanthin, diatoxanthin), all algae (*b*-carotene, Chl *a*, pheophytin *a*), total cyanobacteria (echinenone), Nostocales cyanobacteria (canthaxanthin), and UV index (ratio of fossil UV-radiation-specific pigment (compound A) and fossil carotenoid pigments (alloxanthin, lutein+zeaxanthin and diatoxanthin); Leavitt and Hodgson 2001). The dashed lines represent the breaks identified by CONISS followed by a broken-stick analyses. The dates older than 1879 (± 66) CE should be interpreted with care as they are beyond the unsupported ^{210}Pb.

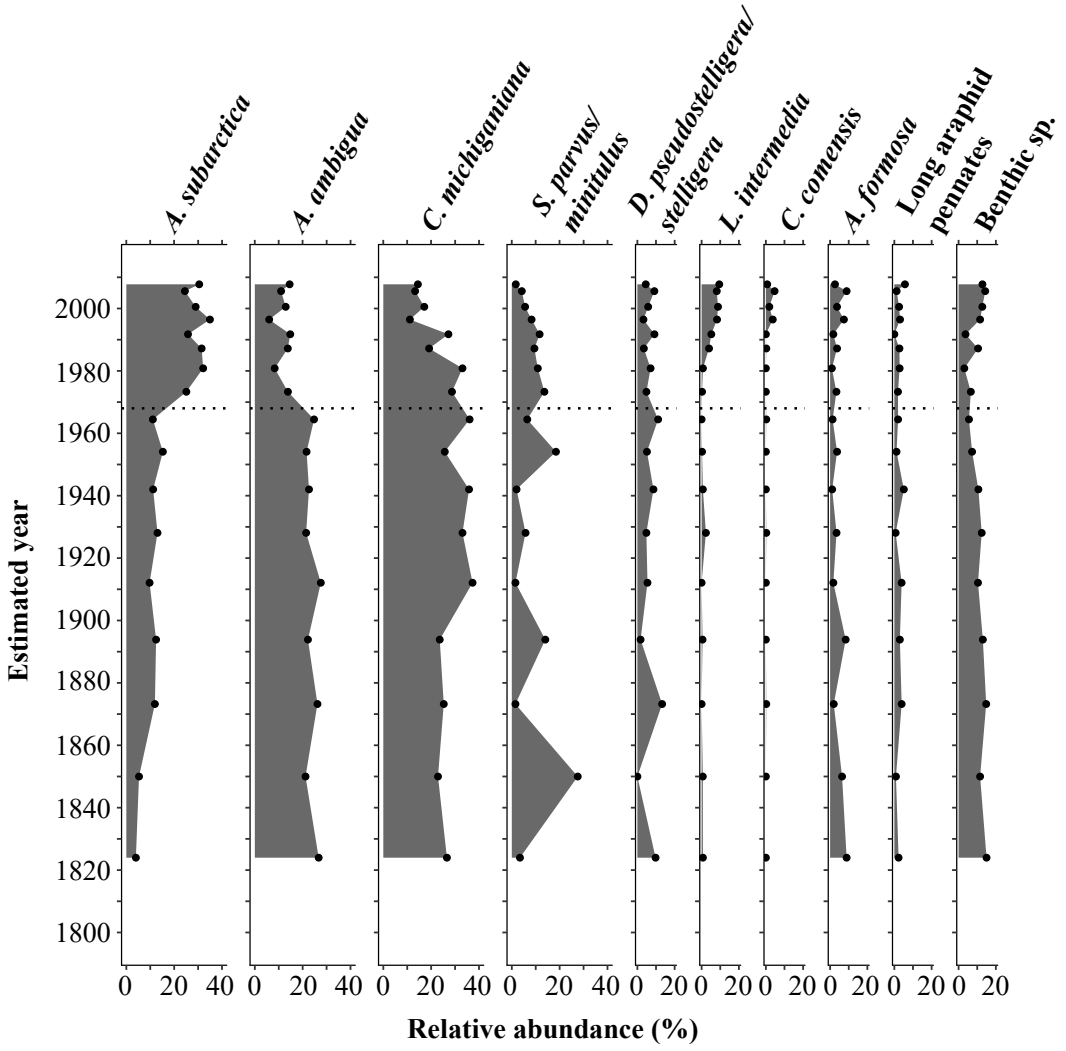

Figure 6. Centennial time series of predominant diatom subfossils (> 5 % relative abundance). The dashed lines represent the breaks identified by CONISS followed by a broken-stick analyses. The dates older than 1879 (± 66) CE should be interpreted with care as they are beyond the unsupported ^{210}Pb.

CONNECTING STATEMENT 2

Chapter 3 presented the importance of long-term evaluation of aquatic ecosystems to apply better management practices to freshwater ecosystems. A reference period (ca. 1800-1900) and four main periods of change were identified, which showed modest eutrophication of Cultus Lake, mainly since the mid-1900s. The drivers of change were complex and involved increase of watershed development (i.e., residential development, agriculture, recreational activities), regional climate warming and decline in sockeye salmon. Although changes in Cultus Lake ecology were identified in Chapter 3, only classical paleolimnological indicators were used (i.e., geochemistry, sedimentary pigments, diatoms and cladoceran subfossils). To further explore DNA-based approaches in paleolimnology, the goal of Chapter 4 was to explore which micro-eukaryotic groups can be archived and preserved in sediments. Using micro-eukaryotic communities identified through 18S rRNA gene, I evaluated whether these communities followed similar ecological dynamics than classical paleo-indicators. I used the knowledge acquired in Chapter 3 about the history of anthropogenic development and the period identified through the multi-proxy paleolimnological study to compare DNA-based methods to classical paleolimnological approach.

In addition, the goal of Chapter 2 was to explore which DNA taxa could be deposited in the sediments, and several taxonomic groups were identified as potential bioindicators for future paleolimnological studies. However, a suitable bioindicator for paleolimnology should, not only be deposited in the sediments, but also be properly archived and preserved in sediments. In Chapter 4, the objective was then to evaluate which DNA taxa is archived and preserved in older sediment samples. I also evaluated whether the taxa associated with specific period of changes

were similar than the taxa identified as potential bioindicators from Chapter 2. Furthermore, I targeted exDNA and inDNA fractions to evaluate whether both DNA fractions could exhibit similar results. The results in Chapter 4 showed that exDNA seemed to have preservation issues in samples older than 30 years and should be treated and interpreted with care. However, inDNA was efficient to detect similar ecological trajectory than classical paleolimnological approaches observed in Chapter 3. Chapter 4 showed that sedDNA is efficient to reconstruct past ecological dynamics of micro-eukaryotic communities and offer a wider taxa diversity to study in paleolimnology. As for classical paleo-indicators, sedDNA should be used in multi-proxy paleolimnological studies or in well-known aquatic systems, mainly when targeting taxonomical groups that are not widely used in paleolimnology.

CHAPTER 4

ANALYSIS OF 18S rRNA AMPLICONS FROM SEDIMENT DNA OF A HUMAN-IMPACTED LAKE IN WESTERN CANADA (CULTUS LAKE) REVEALS CHANGES IN MICRO-EUKARYOTIC DIVERSITY OVER THE PAST ~200 YEARS.

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Abstract

Although the use of sedimentary DNA (sedDNA) has increased over the last decade, more work is still needed to evaluate the efficiency of sedDNA to track past ecological conditions. Even more work is needed to assess whether extracellular (exDNA) and intracellular DNA (inDNA) archived in the sediments yield to similar information. Cultus Lake was chosen as a model site as a previous classical multi-proxy paleolimnological study was conducted and limnological monitoring has occurred sporadically since the early 20th century, with monthly sampling since 2009. DNA was extracted from samples of a sediment core collected in 2017 to characterize whether the changes in micro-eukaryotic communities over the past ~200 years were similar to those observed with the classical paleolimnological methods. We also evaluated whether the inDNA and exDNA fractions yield to similar information. Amplicons from the V7 region of the 18S rRNA gene showed that exDNA and inDNA provided different insights into ecological dynamics. However, preservation of exDNA in sediment archives ~30 years after burial seemed to be an issue, and thus, we focused our analyses on the inDNA fraction. PCoA and indicator species analyses based on inDNA amplicons showed that micro-eukaryotic community diversity was rich and dynamic, with many community changes occurring at similar time periods identified with the previous paleolimnological study. For instance, a decrease of Opisthokonta 18S rRNA gene amplicons in ca. 1926, ca. 1939 and ca. 1954 might be associated with an increase of herbivory by juvenile sockeye salmon as a relatively elevated numbers of adult spawners were observed during these years. Furthermore, two diatom species identified morphologically exhibited similar temporal dynamics to two diatom taxa identified with sedDNA in both the core and sediment trap samples, suggesting that sedDNA can track past diatom species dynamics as well as broader micro-eukaryotic community changes. Overall, our

study provides insights into the use of exDNA and inDNA in sedimentary records and showed that sedDNA enriches our understanding of micro-eukaryotic community dynamics over centennial time scales.

Introduction

Over the last decade, there has been an increase in the use of sedimentary DNA (sedDNA) to reconstruct past lake conditions (e.g., Epp et al. 2010; Domaizon et al. 2013; Capo et al. 2017). Several paleolimnological studies have shown the congruency between taxa described from sedDNA data and other classical indicators (e.g. ,Stoof-Leichsenring et al. 2014; Monchamp et al. 2016; Dulias et al. 2017). For example, a previous study with sediment trap samples has shown that the ecological dynamics quantified from DNA deposited in the sediments are significantly correlated with plankton community changes in the water column (Gauthier et al. In revision). Certainly, sedDNA is gaining in popularity as it provides information on the presence and on the dynamics of many taxa for which subfossils are difficult to identify or absent in sediment archives (Domaizon et al. 2017). Yet, more work is needed to fully identify the conditions which favor DNA burial in the sediments, so we can assess a priori where sedDNA can be effectively used to reconstruct past biological conditions in lakes.

Most sedDNA studies have focused on the total DNA archived in the sediments. However, total DNA can be archived as two fractions: intracellular DNA (inDNA; i.e., intact cells, dormant cells) and extracellular DNA (exDNA). Substantial fraction of the DNA pool in aquatic sediments is comprised of exDNA (Pietramellara et al. 2009), which can represent more than 90 % of the total sedDNA in certain marine environments (Dell'Anno et al. 2002;

Dell'Anno and Danovaro 2005). ExDNA usually derives from cell lysis or from active and passive extrusion mechanisms (Pietramellara et al. 2009). Organic and inorganic particles in soil or sediments can bind, adsorb and stabilize free DNA molecules (Dell'Anno et al. 2002), which might reduce its degradability over long periods of time. Thus, the exDNA pool represents potential genetic records to reconstruct biological communities from aquatic sediment extracts (Corinaldesi et al. 2005). To date, research conducted in marine sedimentary environments have observed minimal differences between exDNA and inDNA fractions on recent and ancient sedimentary prokaryotic communities (Corinaldesi et al. 2018; Torti et al. 2018; Ramírez et al. 2018). Vuillemin et al. (2017) have observed that exDNA in lacustrine sediments preserved prokaryotic community signature from the water column, but only in the shallower section of the sediment core. Ficetola et al. (2018) targeted exDNA to track past changes in macrofauna, as initially recommended by Taberlet et al. (2012), and could efficiently reconstruct the invasive rabbit dynamics on a sub-Antarctic island over many centuries. However, much of the previous work on exDNA has focused on prokaryotes, and thus there is a need to assess the efficiency to track past biological changes of the co-extraction of exDNA and inDNA on micro-eukaryotic communities from lake sediments.

To evaluate the efficiency of sedDNA to track past biological conditions and compare inDNA and exDNA signal, we chose to conduct our study on Cultus Lake because it has been monitored periodically over the last century (Ricker 1937, 1938; Shortreed 2007; DFO unpublished data) and a multi-proxy paleolimnological study was recently conducted to identify the major periods of change and related drivers (Gauthier et al. In press). Cultus Lake was characterized by oligotrophic conditions prior to the 1900s, which was identified as a reference period. Starting in the 20th century, anthropogenic and regional climate changes led to a modest

cultural eutrophication of Cultus Lake (Gauthier et al. In press). From ca. 1880 to ca. 1940, a minor increase of sedimentary $\delta^{15}N$ was observed, which coincided with the onset of anthropogenic watershed changes. Increases in the concentration of all algal pigments in the sediments from ca. 1940 to ca. 1970 indicated signs of early eutrophication, which was followed by an increase in cyanobacterial pigments ca. post 1970, reflecting the influence of both eutrophication and climate warming in the region (Gauthier et al. In press). The major anthropogenic influences on Cultus Lake were related to increase of human population in the watershed and related activities, such as deforestation, agriculture and recreation and incident forest fires. Regional climate events also affected Cultus Lake with two major floods in the mid-1900s and an overall warming climate mainly since the 1970s (Gauthier et al. In press). As the major periods of change and related drivers were identified with a classical multi-proxy paleolimnological study (Gauthier et al. In press), Cultus Lake is an ideal site to explore and validate sedDNA-based approaches in paleolimnology.

Our overall objectives were to characterize the changes in micro-eukaryotes in the past ~200 years using sedDNA and whether changes in micro-eukaryotic communities occurred during similar periods than the changes observed with classical paleo-proxies. Furthermore, we evaluated whether the signal from inDNA and exDNA archived in the sediments led to similar results. As diatom subfossils were morphologically identified in the previous paleolimnological study, we also aimed to evaluate the congruence between diatom DNA and morphological identification in the sediment core.

Methods

Sampling site

Cultus Lake is located at ~50 km of the outside limit of the Greater Vancouver and ~10 km of the city of Chilliwack in British Columbia, Canada. The maximum depth of Cultus Lake is ~40 m and the lake and the watershed surface area are 6.3 km^2 and ~75 km^2, respectively. Most of its watershed is located in Canada, but a small portion (~19 %) is located in Washington State, USA (Fig. 1). Cultus Lake is presently a warm monomictic lake, but periodic dimixis was indicated by evidence of ice cover in the past (in 1937 from DFO archives and in 1950 from Soutar 2005). Details of the history of the watershed and responses of multiple paleo-indicators are provided in Gauthier et al. (In press). Modest limnological monitoring of Cultus Lake occurred in the 1920s and the 1930s (Ricker 1937, 1938) as well as at the beginning of the 2000s (Shortreed 2007). Since 2009, Cultus Lake has been monitored monthly by Fisheries and Oceans Canada (DFO unpublished data).

Sediment core collection, geochronological and geochemical analyses

A 30 cm sediment core was collected at the beginning of May 2017 from a deep depositional basin (41 m deep) in Cultus Lake (49˚3’12.841’’N, 121˚59’11.688’’W) using the Aquatic Research Instruments Universal Percussion corer with no weight (consequently functioning as a gravity corer; internal core diameter of 6.67 cm). The core was sectioned in 0.5 cm intervals using a vertical extruder from the top to the bottom. For each interval, a sterile spatula was used to remove a thin layer of the sediments at the surface of the interval, which was

deposited in a sterile whirl-pak bag. Another sterile spatula was used to subsample for sedDNA analyses at the center of the interval avoiding going through the 0.5 cm. Between 0.5 g to 1.1 g of sediments were collected in a sterile 2-mL tube. The remaining sediments of the interval was collected in the whirl-pak bag for geochronological and geochemistry analyses. All sediment samples were then frozen at -20˚C until further analyses. Only the sediment collected in whirl-pak bags were lyophilized (~48 h, 0.01 Pa) prior to geochronological (^{210}Pb, ^{137}Cs activities) and mass percent elemental carbon (C) and nitrogen (N) content analyses. Once the sediment samples were freeze-dried, the water percentage was calculated from the wet and dry weight. The sediment weight that was subsampled for sedDNA analyses could then be added to total sediment weight to accurately calculate the mass accumulation rate (g cm^{-1} yr^{-1}).

The chronology of the sediment core was established by measuring ^{210}Pb and ^{137}Cs activities (using a gamma spectrometer at the Geotop, UQAM, Montreal) on 18 sediment samples every ~1.5 cm across the core and then applying the methods described in Appleby (2001). A constant rate of supply (CRS) model was used to calculate the sediment age, the sediment age error and the mass accumulation rate (Binford 1990), which was the same method applied to the core retrieved and analyzed in 2008 (Gauthier et al. In press). Background level of ^{210}Pb were identified below the interval 22-22.5 cm as the unsupported ^{210}Pb reached negative values beyond that point, and thus, the four deepest sediment intervals were removed from the sediment age calculations. As done with the 2008 sediment core (Gauthier et al. In press), 2nd and 3rd polynomial fits were applied and an average between the two polynomials was calculated to determine the sediment age for each interval of the core. The dates of the two sediment cores were then compared to evaluate which interval of the 2017 core should be used for sedDNA

analyses (Fig. 2). To evaluate the congruence between morphological and DNA-based identification for diatoms, comparable dates were necessary.

DNA extraction from sediment samples and downstream analyses

To evaluate the congruence in biological signal from exDNA and inDNA archived in the sediments, both DNA fractions were extracted from the sediment samples. A phosphate buffer (NaP buffer, pH 8.0, 0.1 M) was used to de-adsorb the exDNA fraction from the sediment particles (Corinaldesi et al. 2005; Taberlet et al. 2012; Alawi et al. 2014) prior to DNA extraction. For each sample, the volume of NaP buffer added was adjusted to a final weight:volume ratio of ~1. The samples were rotated by slow rotation for 15 min at room temperature to homogenize the NaP buffer and the sediments to ensure appropriate de-adsorbtion of the exDNA fraction. The samples were then centrifuged at 10,000 rpm for 10 min at room temperature and the supernatants containing the exDNA were transferred to sterile 2 mL tubes. Sediment pellets were used to extract the inDNA fraction. Both DNA fractions were extracted using the NucleoSpin® Soil kit according to the manufacturer instructions (Macheray-Nagel, Düren, Germany). However, for exDNA, the first steps of the commercial kit involving lysis were skipped to avoid further degradation of this DNA fraction and lysis of potential resuspended cells. To avoid DNA contamination, samples were processed under a UV hood sterilized for 30 min prior to DNA extraction. Blank extractions using autoclaved deionized water to evaluate cross-contamination between samples were also performed following the same procedures for each occasion of DNA extractions.

A Qubit®2.0 Fluorometer (Invitrogen) was used to measure DNA concentrations for a broad range of double-stranded DNA following the manufacturer instructions (Qbit ds-DNA BR Assays, Invitrogen). DNA concentrations in all blanks were below the detection limit of the Qubit®2.0 Fluorometer (0.1 ng μl^{-1}). DNA quality of the samples were visualized on 1 % agarose electrophoresis gel containing ethidium bromide for DNA staining and visualization (Supplementary Material SM1).

Polymerase chain reaction (PCR) amplification were performed on sedDNA targeting a fragment of the V7 region of the 18S rRNA gene (~260 bp) using the primers 960F (5'-GGCTTAATTTGACTCAACRCG -3'; Gast et al. 2004 from Capo et al. 2017) and NSR1438 (5'-GGGCATCACAGACCTGTTAT -3'; Van de Peer et al. 2000 from Capo et al. 2017). The primers were modified with Illumina adapters and barcodes for multiplex sequencing. The Phire Hot Start II DNA Polymerase (Thermo Scientific) was used to perform PCR amplification. In a total volume of 25 µL, the PCR reactions contained 1X Phire reaction buffer (5 µl of concentration 5X), 0.2 µM dNTPs, 0.5 µM of each forward and reverse primers, 5 % dimethyl sulfoxide (DMSO, volume of 1.25 µl in each reaction) and 0.5 µL of each DNA sample. Using a C1000 Touch™ Thermal Cycler (Bio-Rad), the amplification conditions were set to an initial denaturation step at 98°C for 3 min, followed by 35 cycles of denaturation at 98°C for 5 s, annealing step at 58°C for 5 s and elongation step at 72°C for 15 s with a final elongation at 72°C for 1 min. To assess the performance of the PCR amplification, PCR products were visualized on 2 % agarose electrophoresis gel that contained ethidium bromide for DNA staining and visualization. Barcoding (dual attach indices and sequencing adapters), library preparation and sequencing on a MiSeq Illumina instrument (San Diego, CA) of the PCR amplicons was

achieved at Genome Quebec. A total of 43 samples was pooled in 0.5 library with 12 samples from another project.

The package dada2 (Callahan et al. 2016) in R software (R Core Team 2018, Vienna, Austria) was used to trimmed and filtered (no undefined bases, no sequencing error in primers, removing of primers) the MiSeq reads, to merge the paired-end reads and to remove the chimeras. Taxonomic assignment was performed using version 4.10.0 of the Protist Ribosomal Reference database (PR2) – SSU rRNA gene database (Guillou et al. 2013) at a minimum bootstrap confidence level of 80 %.

Statistical analyses

All statistical analyses were performed using the R statistical software v. 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). To evaluate temporal dynamics of the micro-eukaryotic communities identified with 18S rRNA gene over the last ~200 years, we performed PCoA using presence-absence data, applying a Jaccard dissimilarity index to sedDNA time series. We used presence-absence data to account for DNA degradation between taxa and sediment age. We chose to use PCoA to analyze our data because the dissimilarities are not distorted in the ordination plot as they can be in the Nonmetric Multidimensional Scaling (NMDS; Borcard et al. 2018). We used the PCoA biplot to identify clusters of sediment intervals to be used in the indicator species analyses, which corresponded to similar periods of change identified in the classical paleolimnological multi-proxy study (ca. 1791-1926; ca. 1939-1954; ca. 1964-1979; ca. 1987-2017; Gauthier et al. In press). Indicator species analysis was performed using the latter periods to evaluate which ASVs were associated with each of these time periods.

To perform the PCoA, the function *cdmscale()* was used in the library vegan (Oksanen et al. 2019). The indicator species analysis was performed using the function *multipatt()* in the library indicspecies (De Càceres and Legendre 2009) with an argument of 1000 permutations. Our threshold to select the indicators ASVs for each time period was an indicator value greater than 0.6. We defined primary indicators as indicator ASVs with a relative abundance greater than 5 % across all the samples in which the ASVs were present. We used the threshold of 5 % to identify ASVs that could be biologically meaningful and common in a given time period. To evaluate the relationship of the micro-eukaryotic communities identified with 18S rRNA gene amplicons between sediment core samples and sediment trap samples (deployed from June 2014 to July 2017; Gauthier et al. In revision), we also applied a Principal Coordinate Analysis (PCoA) on the presence-absence data, with a Jaccard dissimilarity transformation.

Given that we previously conducted a diatom analysis of a core collected from Cultus Lake (Gauthier et al. In press), we compared the similarity in diatom assemblages between morphological and sedDNA approaches (both inDNA and exDNA). PCoA were performed on relative abundance using a Bray-Curtis dissimilarity matrix prior to the ordination on both approaches. The first three axes of the PCoA site scores were then extracted using the function *scores()* in the library vegan prior to applying an RV coefficient to correlate the two taxonomical approaches. RV coefficient is homologous to the square of the Pearson correlation between two vectors and to an R^2 between two matrices (Legendre and Legendre 2012). We used only the first three axes as most of the variation was explained by these axes in the PCoAs. From the library FactoMineR (Le et al. 2008), the function *coeffRV()* from the library FactoMineR (Le et al. 2008) was used to calculate the correlations between morphological and sedDNA-based

approaches and the statistical significance of the RV coefficients was calculated using *RV.rtest()* from the library ade4 (Dray et al. 2007).

Results

Sediment chronostratigraphy and geochemistry

The constant rate of supply (CRS) model was used to determine sediment ages throughout the core. Sedimentary ^{210}Pb unsupported activity showed a strong negative relationship to cumulative dry mass (adjusted R^2 of 0.91; Supplementary Material SM2) and reached background concentrations (supported ^{210}Pb) within the top 22 cm of the sediment core (Fig. 2). The ^{210}Pb dates corresponded well with the peak of ^{137}Cs occurring at 12.25 cm (Fig. 2a), which corresponded to 1960 ± 5 years (Table SM2.1). Comparison of the ^{210}Pb dates between the sedDNA core (retrieved in 2017) and the earlier core (retrieved in 2008) showed a similar trend with core depth (Fig. 2b), which enabled us to select intervals from the 2017 that yield robust comparisons with the 2008 core (Table SM2.2). Based on visual inspection of the core after collection, colour changes were noted distinguishing the top 6 cm from the rest of the core (full details provided in Supplementary Material SM3).

Diversity and composition of microbial eukaryotes based on 18S rRNA gene

We generated a total of 2,135,839 18S rRNA gene sequences that were assigned to 3,264 ASVs. More 18S rRNA gene sequences and ASVs were generated for the intracellular DNA

(inDNA) samples compared to the extracellular DNA (exDNA) samples (Table 1). The number of sequences per inDNA sample was comparable to our sediment trap study (65,931 sequences per sample for inDNA in Gauthier et al. In revision), but was lower for exDNA (59,931 sequences per sample for exDNA in Gauthier et al. In press) (Table 1). Within the core, the rarefied richness per sample was twice as high in the inDNA fraction compared to the exDNA, although the range of the rarefied richness was greater with exDNA. The range of rarefied richness followed the same pattern as detected in our sediment trap study, where the range of the rarefied richness was higher with exDNA than with inDNA (Gauthier et al. In revision).

The number of sequences varied from sample to sample for the inDNA fraction, but there was a clear drop in the number of sequences across the three oldest samples (ca. 1843, 1822 and 1791; Fig. 3a). The main phyla that changed in number of sequences were Opisthokonta, Alveolata, Archeaplastida, Stramenopiles and Rhizaria (Fig. 3a). For the exDNA fraction, the number of sequences declined with the age of the samples, but more intensely from ca. 1987 to the oldest samples (Fig. 3b). PCR amplification was low for exDNA sample dated to ca. 1822 as the DNA was at a low concentration and was possibly degraded. The phyla which varied the most in number of sequences for exDNA were Opisthokonta and Stramenopiles, although unclassified eukaryotic sequences also varied significantly (Fig. 3b). In exDNA, signal from different phyla than inDNA was retrieved, such as Amoebozoa, Apusozoa, Excavata and Hacrobia (Fig. 3). In the samples pre- ca. 1987, the distribution in sequences across phyla varied more greatly from sample to sample than ca. post-1987 (Fig. 3b). When comparing inDNA and exDNA fractions on the entire micro-eukaryotic communities using RV coefficient, we found that both DNA fractions were significantly correlated for both the first PCoA axis ($RV = 0.45$; p-value < 0.001) and the first three PCoA axes ($RV = 0.54$; p-value < 0.001).

Shared ASVs amongst inDNA and exDNA fractions

Across all sediment intervals, only 336 of 3263 ASVs were shared between inDNA and exDNA matrices. The total number of unique ASVs across the whole core for the exDNA and inDNA fractions was 1437 and 2162 ASVs, respectively (Table 1). The highest number of shared ASVs was found for the more recent samples and generally decreased with age of the sediments (Fig. 4a; Table SM4.1). From ca. 2017 to ca. 1987, the phyla that generally contributed the most to the shared ASVs were the Stramenopiles, Opisthokonta and Alveolata (Fig. 4b). In the samples dating prior to ca. 1979, exDNA fraction had a very dynamic composition and may reflect poor preservation. From ca. 1987 to ca. 2017, a large number of unique ASVs in exDNA were not assigned to any phylum (Fig. 4b). The contribution of the Excavata phylum to unique ASVs was also apparent throughout these samples for exDNA, which was not observed in inDNA fraction (Fig. 4b). The major phyla contributing to unique ASVs in inDNA were Opisthokonta, Alveolata, Rhizaria and Stramenopiles (Fig. 4b). Amplicons not assigned to any phylum also represented a substantial proportion of the unique ASVs in both inDNA and exDNA matrices (Fig. 4b). Similar contribution of each phylum was observed throughout the core for inDNA matrix, with a slight shift from Opisthokonta dominance towards Alveolata dominance towards past time (Fig. 4b).

Indicator ASVs of main periods of change

Because of the limited number of sequences and ASVs generated from the older exDNA samples, we chose to perform an indicator species analysis on inDNA fraction only. Four major time periods were apparent in the PCoA biplot (Fig. 5), which concords approximately to the

periods identified in the classical multi-proxy paleolimnological study (Gauthier et al. In press). These time periods are the following: 1) ca. 1800 to 1930 (7 samples); 2) ca. 1930 to 1960 (2 samples); 3) ca. 1960 to 1980 (3 samples); and 4) ca. 1980 to 2017 (9 samples). The indicator analysis was performed using these time periods as categories. We identified a total of 369 ASVs as indicators for all time periods (Table SM5.1). We further investigated the indicator ASVs that were present at greater than 5% of relative abundance across all samples in which the ASV was present. We refer to these ASVs as the primary indicators. In the period from ca. 1800-1930, 10 ASVs were identified as indicators (Table SM5.1), eight of which were primary indicators. The three most abundant primary indicators belonged to the phyla Stramenopile (Oomycota class), Alveolata (unclassified Alveolata) and Archaeplastida (Embryophyceae class; Fig. 6). 170 ASVs were identified as indicators for the period ca. 1930-1960 (Table SM5.1), 16 of which were primary indicators. The three most abundant primary indicators in this period belonged to the phyla Alveolata (one unclassified and one Ciliophora) and Chlorophyta (assigned to the species *Chlamydomonas monadina*) (Fig. 6). In contrast to the prior period, only 84 ASVs were identified as indicator species in the period ca. 1960-1980 (Table SM5.1), although there were more primary indicators ASVs (i.e., 33). The three most abundant primary indicators belonged to the phylum Opisthokonta, with one 1 Ostracoda (*Limnocythere* sp.) and one Metazoa unclassified, and Alveolata (Ciliophora – Hypotrichia; Fig. 6). The most recent period (ca. 1980-2017) was characterized by 105 indicator ASVs (Table SM5.1) with 36 primary indicators. Amongst these 36 ASVs, the three most dominant belonged to the phyla Stramenopiles and Alveolata. The Stramenopiles were represented by diatom species belonging to the Polar-Centric *Mediophyceae* (PCM) class and the Alveolata phylum by a species belonging to Ciliophora (Strombidiida; Fig. 6).

Congruence between morphological and sedDNA identification of diatoms

Focusing on just the diatom ASVs, we found some similarities in assemblages over time between both DNA fractions, such as the dominance of centric taxa in recent years (Fig. SM6.1). Earlier time periods were more associated with araphid pennate and raphid pennate taxa (Fig. SM6.1). Based on the RV coefficients, we found that morphology and sedDNA-based methods were significantly correlated with inDNA (Table 2). For the correlation between morphology and exDNA, only the RV coefficient between the first three axes was significant (Table 2). Similarly, only the RV coefficient between the first three axes was significant when comparing both DNA fractions (exDNA vs inDNA) with only diatom taxa (Table 2).

Discussion

Our study indicates that micro-eukaryotic community dynamics were effectively reconstructed using DNA archived in the sediments from our focal monomictic temperate lake. Furthermore, our analyses of community composition as well as indicator ASVs from the sedDNA archives strengthen the interpretations that can be drawn from other paleolimnological proxies. However, the inDNA and exDNA fractions identified substantially different communities, with the results of older intervals of exDNA suggesting preservation issues. As such, we chose to focus on the inDNA fraction to study the past ecological dynamics of Cultus Lake and related these changes with the prior classical multi-proxy paleolimnological study (Gauthier et al. In press). The periods of changes observed with the micro-eukaryotic communities concurred with the periods that were previously identified with the multi-proxy paleolimnological study conducted on Cultus Lake, which corresponded approximately to the

following time periods: 1) prior ca. 1930, 2) from ca. 1930 to 1960, 3) from ca. 1960 to 1980, and 4) ca. 1980 to 2017.

Should inDNA and/or exDNA be targeted to reconstruct past biological conditions in lakes?

Our results indicate that both DNA fractions can provide insights into biological community changes over time. However, exDNA must be interpreted with care as preservation over long periods of time seemed to be an issue, at least in Cultus Lake. We observed a much higher decline as a function of sediment age in the number of sequences from exDNA compared to inDNA. In addition, the dynamics of the ASV proportion for each phylum in the inDNA fraction (Fig. 4b) was consistent over time, but the pattern with exDNA was much more stochastic, mainly pre- ca. 1987. In Vuillemin et al. (2017), analyses of 16S rRNA gene dataset provided similar pattern in Lake Towuti, Indonesia, where sediments initially reflected environmental changes, but older sediments were modified by the subsurface microbial community. Interestingly, the sediment intervals of Cultus Lake corresponding to ca. 1987-2017 were characterized by a clear brown color (Supplementary Material SM3) and a relatively high percentage of carbon (Fig. SM7.1), which collectively indicate a higher concentration of organic matter in the shallower section of the sediment core. The greater amount of organic matter in the core post- ca. 1987 may be due to enhanced production in the water column and/or less diagenesis in the sediments. Certainly, sedimentation rates and mass accumulation rates have increased since the end of the 1990s (Fig. SM7.2), which might result in more rapid DNA burial, and then, less prone to degradation. Although exDNA can be adsorbed to sediment particles reducing its degrability (Dell'Anno et al. 2002; Corinaldesi et al. 2005; Dell'Anno and Danovaro

2005), exDNA is more exposed to secreted DNAases as well as uptake by bacteria than inDNA. ExDNA can play a key role in ecosystem functioning in the first cm of the sediment layers as a P-rich molecule (~10 % weight to weight; Dell'Anno and Danovaro 2005). Thus, exDNA can stimulate the production of prokaryotes present in the sediments. However, results from Dell'Anno and Danovaro (2005) indicate that exDNA can be rapidly degraded as it is selectively remineralized in the organic P pool within the first 10 cm of the deep-sea sediments. In addition, for both DNA fractions, we observed a decrease in their concentrations with the age of the sediments, with relatively lower DNA concentrations in older sediments (i.e., corresponding to pre- ca. 1990, Figs. SM1.1; SM1.2).

Although exDNA seemed to be more unstable than inDNA, unique ASVs for both DNA fractions was usually higher than the shared ASVs (between inDNA and exDNA). The use of exDNA and inDNA in parallel targeting prokaryotic communities can help discriminate between sources of exogeneous and endogeneous DNA, which could address in-lake and post-depositional processes (Vuillemin et al. 2017). Thus, exDNA could be informative for specific bioindicators from different environments (i.e., pelagic, littoral or terrestrial), particularly under environmental conditions where it is well preserved. More work will be required to evaluate which taxonomic groups (beyond micro-eukaryotes) should be targeted with exDNA. Overall, our results echo our findings from our earlier sediment trap time series study from Cultus Lake (Gauthier et al. In revision), where we also found that inDNA more accurately tracked the dynamics of diatom communities. As inDNA exhibited more consistency in temporal patterns as well as higher richness and number of sequences generated, we suggest that exDNA should be treated and interpreted with care, particularly when the core samples are older than ~30 years.

What new insights are provided by our sedDNA study of micro-eukaryotic community dynamics over the last ~200 years?

The main apparent changes in micro-eukaryotic communities throughout the core were from the phyla Opisthokonta, Alveolta, Stramenopiles and Rhizaria. All these phyla changed the most in number of sequences as well as in their ASV proportions in inDNA. Using the previously established periods of change based on the analysis of classical subfossils and geochemical indicators (from Gauthier et al. In press), we found numerous indicator ASVs from the sedDNA analyses that characterized each period. For example, from ca. 1800 to ca. 1930, the primary indicators identified were an Oomycota, an Alveolata and an Embryophyceae ASVs. Oomycota, known as "water mold", can be devastating plant pathogens, although some are completely harmless (Fry and Grünwald 2010). However, as this indicator ASVs could not be assigned to a more specific taxonomic level, it is difficult to hypothesize what ecological role this taxon could have played during the period ca. 1800 to ca. 1930. The Embryophyceae ASVs could be indicative of a system that received more terrestrial inputs in the past than in the present as Embryophyceae are terrestrial plants including ferns, moss and liverworts. During this period, Cultus Lake received a mixed input of terrestrial and aquatic matter to the sediments (Fig. SM7.1) according to the molar C:N ratio (Meyers and Teranes 2001; Selbie et al. 2009). The Embryophyceae ASVs identified as a primary indicator could also be related to well-preserved DNA present as spores or pollen in the sediments.

The end of the period ca. 1800 to ca. 1930 exhibited a decrease of Opisthokonta 18S rRNA gene sequences compared to other time periods. The number of sequences from the phylum Opisthokonta were lowest in ca. 1926, ca. 1939 and ca. 1954. During these years, we also observed an increase in the number of sequences assigned to the Alveolata phylum. A

program was put into place in the beginning of the 1910s (Chwk. Prog. Nov. 27, 1912) to enhance the production and return of sockeye salmon spawners in Cultus Lake (Soutar 2005; Shortreed 2007). An increase of sockeye salmon spawners returning to Cultus Lake was observed in the following years, with a highest number of spawners recorded in 1939 and 1940 (73,189 and 74,121, respectively; Shortreed 2007). Adult sockeye salmon spawners returning to their lake of origin is a good proxy for herbivorous pressure on zooplankton from juvenile sockeye (Chen et al. 2011). Opisthokonta 18S rRNA gene sequences in our study were dominated mainly by Maxillopoda class, which include copepods that can be a suitable resource for young sockeye juveniles rearing in the lake. Unfortunately, as our primers did not detect *Daphnia* sp. because of the longer V7 region of the 18S rRNA gene of *Daphnia* sp. (Crease and Colbourne 1998), which is the preferable food for juvenile sockeye salmon but depends upon availability (Burgner 1991), we cannot conclude definitively that the decrease of Opisthokonta 18S rRNA gene sequences was directly related to sockeye salmon dynamics in the lake. However, this would be an interesting line of investigation to follow up on whether sockeye salmon could be targeted with a different marker (e.g., 12S rRNA gene). The period ca. 1930 to ca. 1960 was mainly characterized by an increase of terrestrial organic matter to the lake most likely caused by a combination of an increase of anthropogenic activities in the watershed and regional climate (floods in 1948 and 1950 and a forest fire in 1951; Gauthier et al. In press), which could have influenced an increase of Alveolata over Opisthokonta taxa. Alveolata are a very diverse group, and ciliates and dinoflagellates have previously been identified as potential indicators for future paleolimnological study (Gauthier et al. In revision) and have been identified as bioindicators of nutrient status of lakes and climate warming (Capo et al. 2016; 2017; 2019). For instance, ciliate abundance has been shown to increase in enclosures with leaf

litter additions (Andrushchyshyn et al. 2006), and may be the mechanism behind the increase of Alveolata 18S rRNA gene sequences during this period. We also detected an increase of all algal pigments in the classical paleolimnological study (Gauthier et al. In press), which concords with an increase of 18S rRNA gene sequences of a Chlorophyta, *C. monadina*. This species was also identified as a primary indicator for the period ca. 1930 to ca. 1960.

In the period ca. 1960 to ca. 1980, major changes occurred in Cultus lake and its watershed. Returning sockeye salmon spawners declined drastically below 12,000 spawners during the 1970s, with a minimum of 353 in 1977 (Shortreed 2007). In addition, the invasion of a macrophyte, *Myriophyllum spicatum*, was observed post-1977 that subsequently has become pervasive in the littoral zone in the following years (Shortreed 2007) and to the present time (DFO, data not shown). At the watershed and broader regional scale, there was pronounced climate warming and expansion of residential development. All these drivers led to substantial changes in the lake, which were observed with the classical paleolimnological studies with increase of sedimentary cyanobacterial pigments and changes in diatom subfossil assemblages. The indicator ASVs identified for this period with inDNA were an Ostracoda, a Metazoa and a Hypotrichia. Ostracods mostly live in littoral zone of lakes (Szlauer-Lukaszewska 2015) and an increase of an ostracod taxon might indicate substantial changes in the littoral zone of Cultus Lake. As *M. spicatum* started to invade the littoral zone of Cultus Lake in the late 1970s, it could have provided more refuge for the ostracods and might have contributed to the increase of this primary indicator during ca. 1960 to ca. 1980.

Finally, the period ca. 1980 to ca. 2017 was characterized by an increase of two diatom taxa in the sediment core. With the morphological identification, *Lindavia intermedia* appeared in the sedimentary profiles in the beginning of the 1990s (Gauthier et al. In press). In addition,

Stephanodiscus niagarea was observed to be abundant in the sediment trap samples (Gauthier et al. In revision), but was not observed based on morphological analyses of subfossils extracted from the sediment core collected a decade earlier, suggesting that these taxon has only recently become abundant in Cultus Lake (Gauthier et al. In revision). With sedDNA, we observed an increase of two Polar Centric *Mediophyceae* (PCM) since ca. 1980, PCM3 and PCM6. Both *L. intermedia* and *S. niagarea* are classified as PCM, and based on our earlier sediment trap study, we observed similar temporal dynamics between PCM3 and *L. intermedia* and between PCM6 and *S. niagarea*, suggesting that these ASVs could be assigned to these morphological-identified taxa (Gauthier et al. In revision). Although subfossils of *S. niagarea* were not observed in the 2008 sediment core, subfossils of *L. intermedia* appeared at a similar time than the DNA of the ASV PCM3 in the sedimentary record. Consequently, sedDNA tracked similar temporal dynamics than morphological approach for diatoms, both in recent and older sedimentary deposit in lakes. In addition, ordination analyses comparing the core samples to the sediment traps samples collected and processed in a similar manner (see Gauthier et al. In revision for details) showed that the recent core samples for inDNA seemed to be more associated with the sediment trap samples that were deployed when the water column was mixed (Dec – May; Fig.7). The stronger association between the recent core samples (from ca. 1987 to ca. 2017) and the mixed water column sediment trap samples might be attributed to the higher mass and carbon accumulation rates in the sediment trap samples collected during the mixed period relative to the months when the lake was stratified (Fig. SM7.3). As such, the total sediment and carbon fluxes likely contributed to a greater proportion of the total sediments that were then preserved in the sediment core archive.

Conclusion

Based on our analyses of the Cultus Lake sediment core archives, inDNA seems to be more appropriate to track changes over long time periods than exDNA. Comparing inDNA temporal dynamics with the previous paleolimnological study conducted on Cultus Lake (Gauthier et al. In press), we found novel indicator groups that changed congruently with the previously identified periods of change. We found that exDNA can track different community dynamics of micro-eukaryotes over ~30 years old sedimentary records and could be a substantial addition to better understand changes in lake ecosystems. However, more work is still needed to compare inDNA and exDNA with different primers and in other sediment core archives to understand better the preservation of these different DNA fractions and their potential to yield paleoecological information. We recommend targeting more specific groups that are potential bioindicators to further study exDNA signal in paleolimnology, such as dinoflagellates, ciliates, chrysophytes and chytrids specifically (Gauthier et al. In revision). Overall, sedDNA enrich our understanding of micro-eukaryotic community dynamics, but we encourage future research to target more specific taxonomic groups (e.g., zooplankton with COI and fish with 12S rRNA gene marker) to further understand the preservation and burial of sedDNA.

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References

Alawi M, Schneider B, Kallmeyer J (2014) A procedure for separate recovery of extra- and intracellular DNA from a single marine sediment sample. J Microbiol Methods 104:36–42. doi:10.1016/j.mimet.2014.06.009

Andrushchyshyn OP, Magnusson AK, Williams DD (2006) Responses of intermittent pond ciliate populations and communities to in situ bottom-up and top-down manipulations. Aquatic Microbial Ecology 42:293–310. doi:10.3354/ame042293

Appleby PG (2001) Chronostratigraphic techniques in recent sediments. In: Last WM, Smol JP (eds) Tracking environmental change using lake sediments. Volume 1: Basin Analysis, coring and chronological techniques. Kluwer Academic Publishers New York, pp 171–203

Binford MW (1990) Calculation and uncertainty analysis of 210Pb dates for PIRLA project lake sediment cores. J Paleolimnol 3:253–267. doi:10.1007/BF00219461

Borcard D, Gillet F, Legendre P (2018) Numerical ecology with R. Springer International Publishing, Cham

Burgner RL (1991) Life history of sockeye salmon. In: Pacific salmon life histories, UBC Press. C. Groot and L. Margolis, Vancouver, pp 1–117

Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. doi:10.1038/nmeth.3869

Capo E, Debroas D, Arnaud F, et al (2016) Long-term dynamics in microbial eukaryotes communities: a palaeolimnological view based on sedimentary DNA. Molecular Ecology 25:5925–5943. doi:10.1111/mec.13893

Capo E, Debroas D, Arnaud F, Perga M-E, Chardon C, Domaizon I (2017) Tracking a century of changes in microbial eukaryotic diversity in lakes driven by nutrient enrichment and climate warming: Long-term dynamics of microbial eukaryotes. Environ Microbiol 19: 2873–2892. doi:10.1111/1462-2920.13815

Capo E, Rydberg J, Tolu J, et al (2019) How does environmental inter-annual variability shape aquatic microbial communities? A 40-year annual record of sedimentary DNA from a boreal lake (Nylandssjön, Sweden). Front Ecol Evol 7:245. doi:10.3389/fevo.2019.00245

Chen G, Selbie DT, Finney BP, et al (2011) Long-term zooplankton responses to nutrient and consumer subsidies arising from migratory sockeye salmon (Oncorhynchus nerka). Oikos 120:1317–1326. doi:10.1111/j.1600-0706.2011.19042.x

[Chwk. Prog.] Chilliwack progress, November 27, 1912. Chilliwack Museum and Archives.

Corinaldesi C, Danovaro R, Dell'Anno A (2005) Simultaneous recovery of extracellular and intracellular DNA suitable for molecular studies from marine sediments. Appl Environ Microbiol 71:46–50. doi:10.1128/AEM.71.1.46-50.2005

Corinaldesi C, Tangherlini M, Manea E, Dell'Anno A (2018) Extracellular DNA as a genetic recorder of microbial diversity in benthic deep-sea ecosystems. Scientific Reports 8: doi:10.1038/s41598-018-20302-7

Crease TJ, Colbourne JK (1998) The unusually long small-subunit ribosomal RNA of the crustacean, *Daphnia pulex*: Sequence and predicted secondary structure. J Mol Evol 46:307–313. doi:10.1007/PL00006307

Dell'Anno A, Bompadre S, Danovaro R (2002) Quantification, base composition, and fate of extracellular DNA in marine sediments. Limnology and Oceanography 47:899–905. doi:10.4319/lo.2002.47.3.0899

Dell'Anno A, Danovaro R (2005) Extracellular DNA Plays a Key Role in Deep-Sea Ecosystem Functioning. Science 309:2179–2179. doi:10.1126/science.1117475

Domaizon I, Savichtcheva O, Debroas D, et al (2013) DNA from lake sediments reveals the long-term dynamics and diversity of *Synechococcus* assemblages. Biogeosciences 10:3817–3838. doi:10.5194/bg-10-3817-2013

Domaizon I, Winegardner A, Capo E, et al (2017) DNA-based methods in paleolimnology: new opportunities for investigating long-term dynamics of lacustrine biodiversity. Journal of Paleolimnology 58:1–21. doi:10.1007/s10933-017-9958-y

Dulias K, Stoof-Leichsenring KR, Pestryakova LA, Herzschuh U (2017) Sedimentary DNA versus morphology in the analysis of diatom-environment relationships. J Paleolimnol 57:51–66. doi:10.1007/s10933-016-9926-y

Epp LS, Stoof KR, Trauth MH, Tiedemann R (2010) Historical genetics on a sediment core from a Kenyan lake: intraspecific genotype turnover in a tropical rotifer is related to past environmental changes. J Paleolimnol 43:939–954. doi:10.1007/s10933-009-9379-7

Ficetola GF, Poulenard J, Sabatier P, et al (2018) DNA from lake sediments reveals long-term ecosystem changes after a biological invasion. Science Advances 4:eaar4292. doi:10.1126/sciadv.aar4292

Fry WE, Grünwald NJ (2010) Introduction to Oomycetes. Plant Health Instructor. doi:10.1094/PHI-I-2010-1207-01

Gast RJ, Dennett MR, Caron DA (2004) Characterization of Protistan Assemblages in the Ross Sea, Antarctica, by Denaturing Gradient Gel Electrophoresis. Appl Environ Microbiol 70:2028–2037. doi:10.1128/AEM.70.4.2028-2037.2004

Gauthier J, Gregory-Eaves I, Bunting L, et al (In press) Ecological dynamics of a peri-urban lake: A multi-proxy paleolimnological study of Cultus Lake (British Columbia) over the past ~200 years. Journal of Paleolimnology. Accepted May 2020. doi: 10.1007/s10933-020-00147-9

Gauthier J, Walsh D, Selbie DT, et al (In revision) Evaluating the congruence between DNA-based and morphological taxonomic approaches in water and sediment trap samples: Analyses of a 36-month time series from a temperate monomictic lake. Limnology and Oceanography. Submitted August 2020

Guillou L, Bachar D, Audic S, et al (2013) The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:D597-604. doi:10.1093/nar/gks1160

Le S, Josse J, Husson F (2008) FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software, 25(1), 1-18. doi: 10.18637/jss.v025.i01

Legendre P, Legendre L (2012) Numerical ecology. Elsevier, Amsterdam

Meyers PA, Teranes JL (2001) Sediment organic matter. In: Last WM, Smol JP (eds) Tracking environmental change using lake sediments. Volume 2: Physical and geochemical indicators. Kluwer Academic Publishers New York, pp 239–269

Monchamp M-E, Walser J-C, Pomati F, Spaak P (2016) Sedimentary DNA reveals cyanobacteria community diversity over 200 years in two peri-alpine lakes. Applied and Environmental Microbiology AEM.02174-16. doi:10.1128/AEM.02174-16

Oksanen J, Blanchet FG, Friendly M, et al (2019) vegan: Community Ecology Package. Version 2.5-4URL https://CRAN.R-project.org/package=vegan

Pietramellara G, Ascher J, Borgogni F, et al (2009) Extracellular DNA in soil and sediment: fate and ecological relevance. Biology and Fertility of Soils 45:219–235. doi:10.1007/s00374-008-0345-8

Ramírez GA, Jørgensen SL, Zhao R, D'Hondt S (2018) Minimal Influence of Extracellular DNA on Molecular Surveys of Marine Sedimentary Communities. Frontiers in Microbiology 9:. doi:10.3389/fmicb.2018.02969

Ricker WE (1937) Physical and Chemical Characteristics of Cultus Lake, British Columbia. J Biol Board Can 3:

Ricker WE (1938) Seasonal and Annual Variations in Quantity of Pelagic Net Plankton, Cultus Lake, British Columbia. J Fish Res Board Can 4:34–47

Selbie DT, Finney BP, Barto D, et al (2009) Ecological, landscape, and climatic regulation of sediment geochemistry in North American sockeye salmon nursery lakes: Insights for paleoecological salmon investigations. Limnol Oceanogr 54:1733–1745

Shortreed KS (2007) Limnology of Cultus Lake, British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences 2753:vi + 85p

Soutar M (2005) Cultus Lake: A Natural Paradise. Chilliwack Museum and Archives, Chilliwack

Stoof-Leichsenring KR, Bernhardt N, Pestryakova LA, et al (2014) A combined paleolimnological/genetic analysis of diatoms reveals divergent evolutionary lineages of Staurosira and Staurosirella (Bacillariophyta) in Siberian lake sediments along a latitudinal transect. Journal of Paleolimnology 52:77–93. doi:10.1007/s10933-014-9779-1

Szlauer-Lukaszewska A (2015) Substrate type as a factor affecting the ostracod assemblages in groyne fields of the Oder River (Poland). North-Western Journal of Zoology 11:247–287

Taberlet P, Prud'homme SM, Campione E, et al (2012) Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Molecular Ecology 21:1816–1820. doi:10.1111/j.1365-294X.2011.05317.x

Torti A, Jørgensen BB, Lever MA (2018) Preservation of microbial DNA in marine sediments: insights from extracellular DNA pools: Preservation of microbial DNA in marine sediment. Environmental Microbiology 20:4526–4542. doi:10.1111/1462-2920.14401

Van de Peer Y, De Rijk P, Wuyts J, et al (2000) The European Small Subunit Ribosomal RNA database. Nucleic Acids Res 28:175–176

Vuillemin A, Horn F, Alawi M, et al (2017) Preservation and Significance of Extracellular DNA in Ferruginous Sediments from Lake Towuti, Indonesia. Frontiers in Microbiology 8:. doi:10.3389/fmicb.2017.01440

Tables Chapter 4

Table 1. Total number of 18S rRNA gene sequences and amplicon sequence variants (ASV) for micro-eukaryotic taxa and diatoms present in the sediment core of Cultus Lake as intracellular DNA (inDNA) and extracellular DNA (exDNA). The minimum sample size used to calculate the rarefied richness was 42,089 and 3,374 sequences for inDNA and exDNA, respectively. The percentage of sequences amplified with their range and the number of single and doubleton are presented for diatoms.

Site	**Total amplified sequences***	**# Seq. / sample**	**Total ASV**	**Rarefied richness / sample (range)**	**Diatoms**		
					% seq. (range)	Unique ASV	Single-doubleton
inDNA (n=21)	1,284,315	61,158	2,162	201 (46-296)	4 (0-19)	81	1
exDNA (n=20)	851,524	42,576	1,437	89 (4-330)	9 (0-34)	6	0

*Number of sequences after filtering, trimming and removing chimeras

Table 2. RV coefficients to quantify the congruence between PCoA site scores of different taxonomic methods and intracellular DNA (inDNA) and extracellular DNA (exDNA) for diatom taxa in the core samples. PCoAs were performed on Bray-Curtis dissimilarity matrix of relative abundance data. The significant correlations are indicated in bold.

Comparisons	Matrix A	Matrix B	RV coefficient for core comparisons	
			1st PCoA axis of site scores	3 first PCoA axes of site scores
Morpho vs inDNA Diatoms	Morpho	inDNA	**0.29 (0.03*)**	**0.39 (0.006*)**
Morpho vs exDNA Diatoms	Morpho	exDNA	0.19 (0.15)	**0.41 (0.03*)**
inDNA vs exDNA Diatoms	inDNA	exDNA	0.0001 (0.81)	**0.35 (0.03*)**

Figures Chapter 4

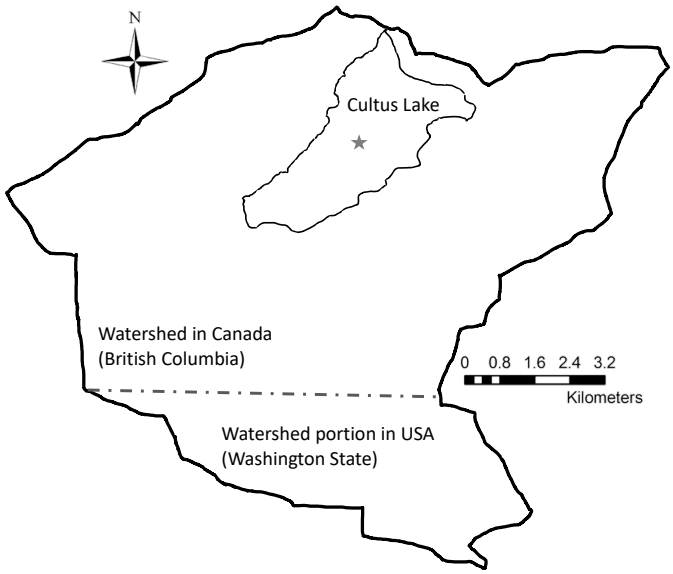


Figure 1. Representation of Cultus Lake and its watershed. The boundaries of the watershed are represented by the thicker black line. The grey star indicates the approximate location of the sediment core collection.

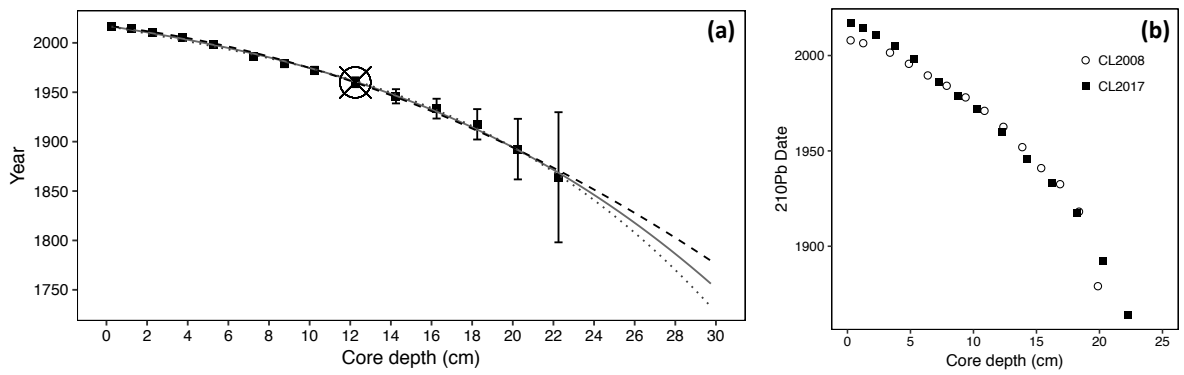


Figure 2. (a) The radioisotopic ^{210}Pb age model for the gravity core of Cultus Lake, British Columbia. The sediment core age-depth model was based on both 2nd (dashed line) and 3rd polynomial (dotted line) fits. The grey line represents the average between the two polynomial models and the black square are the ^{210}Pb dates from the CRS model. The ^{137}Cs peak is represented by the circled X (corresponding to ca. 1960 ± 5, occurring at 12.25 cm). (b) ^{210}Pb dates calculated from CRS for both cores collected in 2008 (open circles; Gauthier et al. In press) and 2017 (black squares).

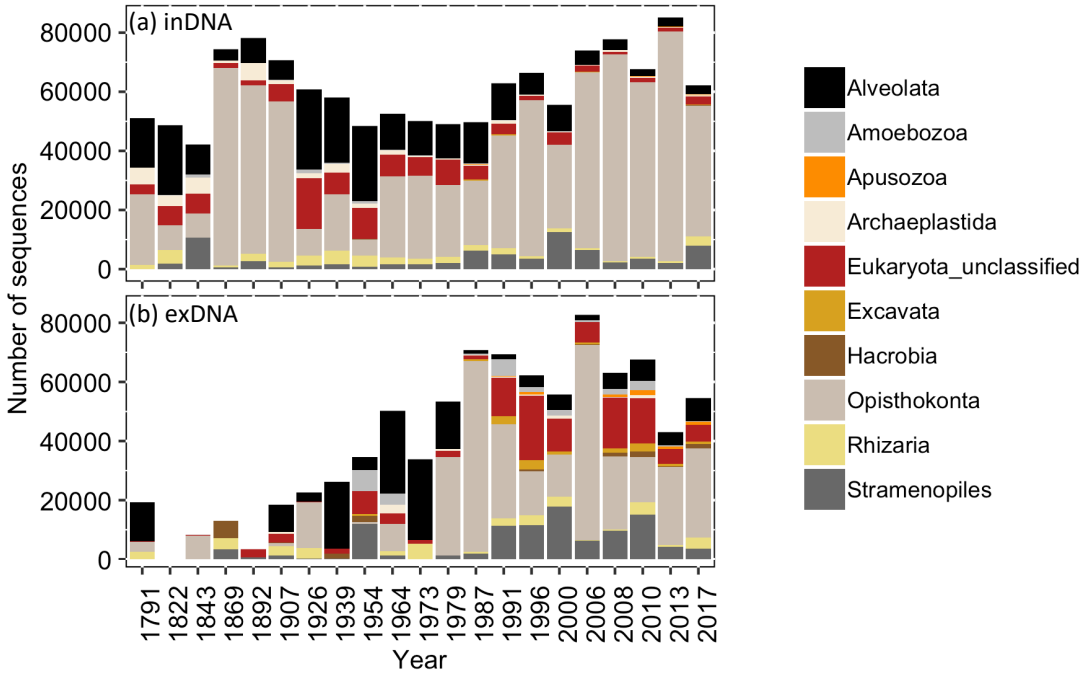


Figure 3. Number of sequences per phyla for intracellular (a) and extracellular (b) DNA fractions for each sediment interval. The dates older than 1864 ± 66 should be interpreted with care as they are beyond the unsupported ^{210}Pb.

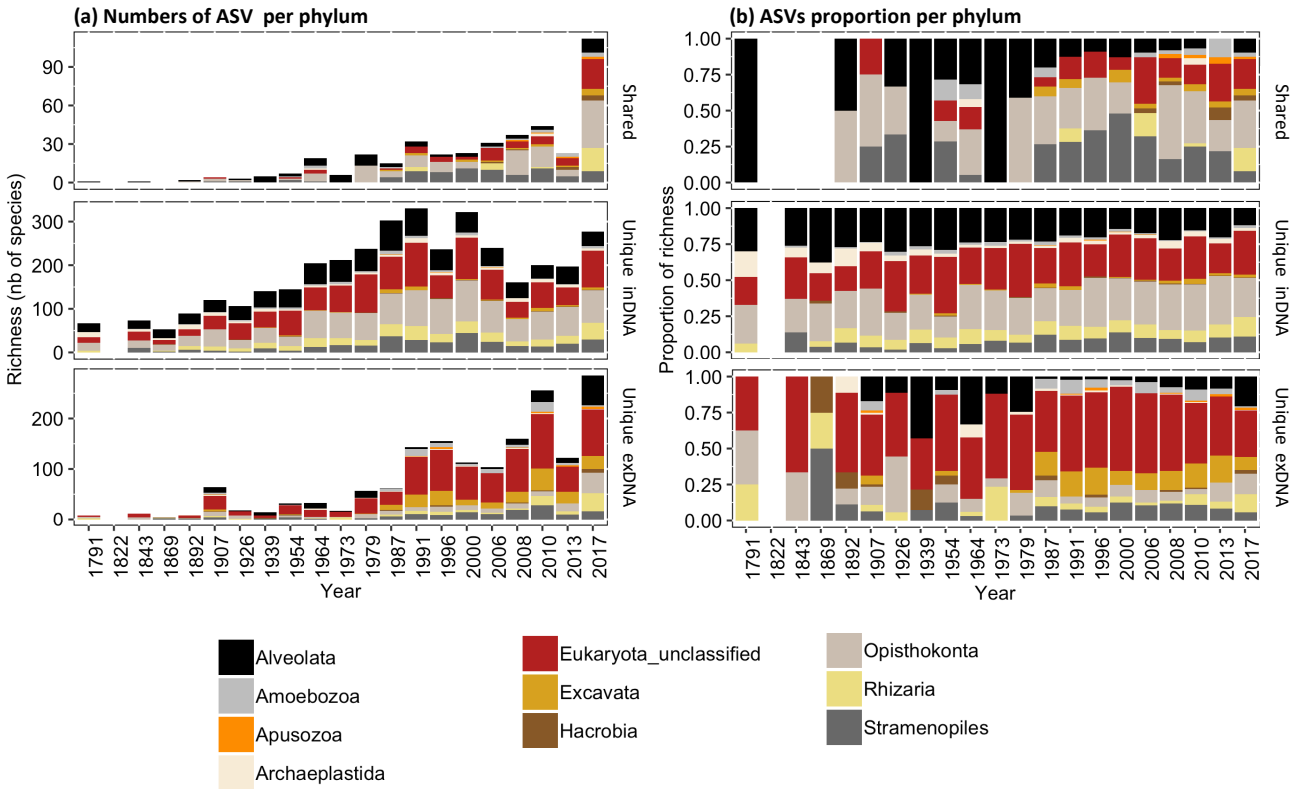


Figure 4. ASV numbers (a) and ASV proportion (b) for each phylum of shared ASVs between both DNA fractions and unique ASVs for intracellular (inDNA) and extracellular DNA (exDNA) fractions. The dates older than 1864 ± 66 should be interpreted with care as they are beyond the unsupported ^{210}Pb.

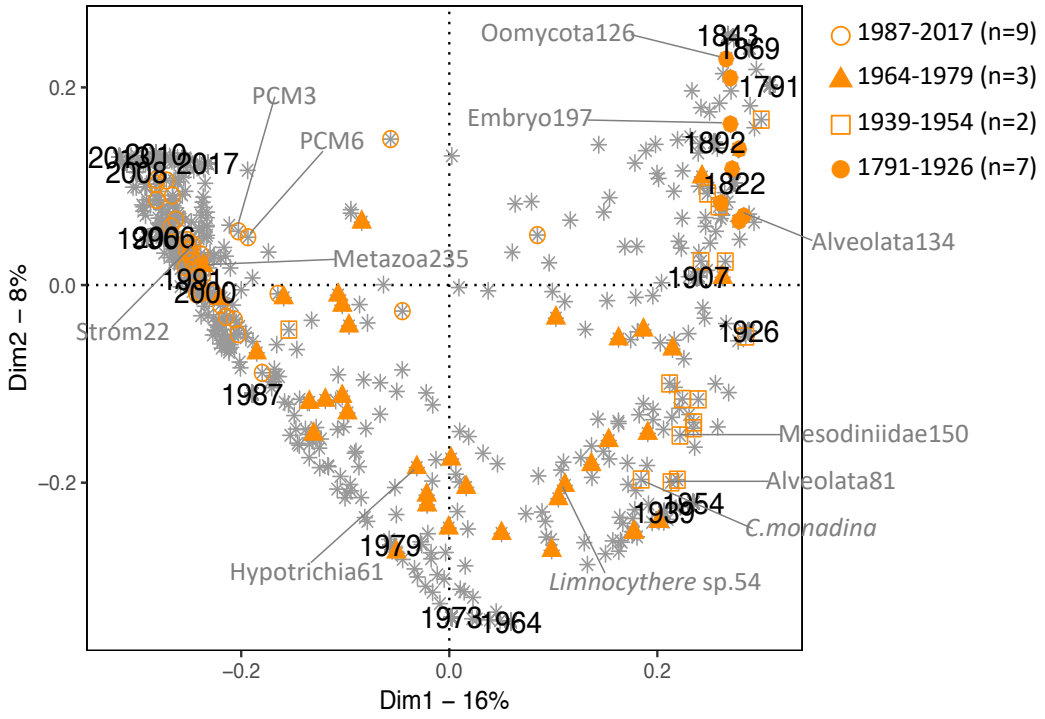


Figure 5. PCoA biplot of intracellular DNA of the sediment core samples. Identified primary indicator ASVs for the four time periods are indicated with: 1) close circles for ~1791-1926; 2) open squares for ~1939-1954; 3) close triangles for ~1964-1979; 4) open circles for ~1987-2017. The name of the three most abundant primary indicators for each time periods are indicated in the biplot. The abbreviations are as follows: *Chlamydomonas monadina* (C.monadina); Embryophyceae (Embryo); Polar Centric *Mediophyceae* (PCM); Strombidiida (Strom). The dates older than 1864 ± 66 should be interpreted with care as they are beyond the unsupported ^{210}Pb.

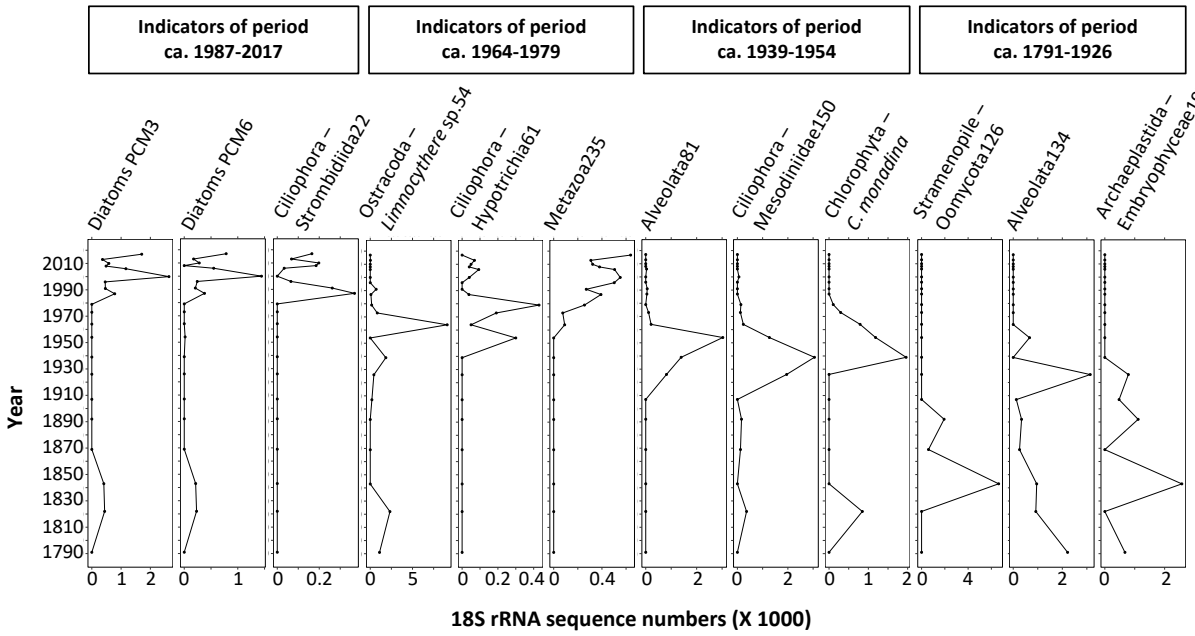


Figure 6. Stratigraphies of 18S rRNA gene sequence numbers from intracellular DNA for three primary indicators identified for each time period. The dates older than 1864 ± 66 should be interpreted with care as they are beyond the unsupported ^{210}Pb.

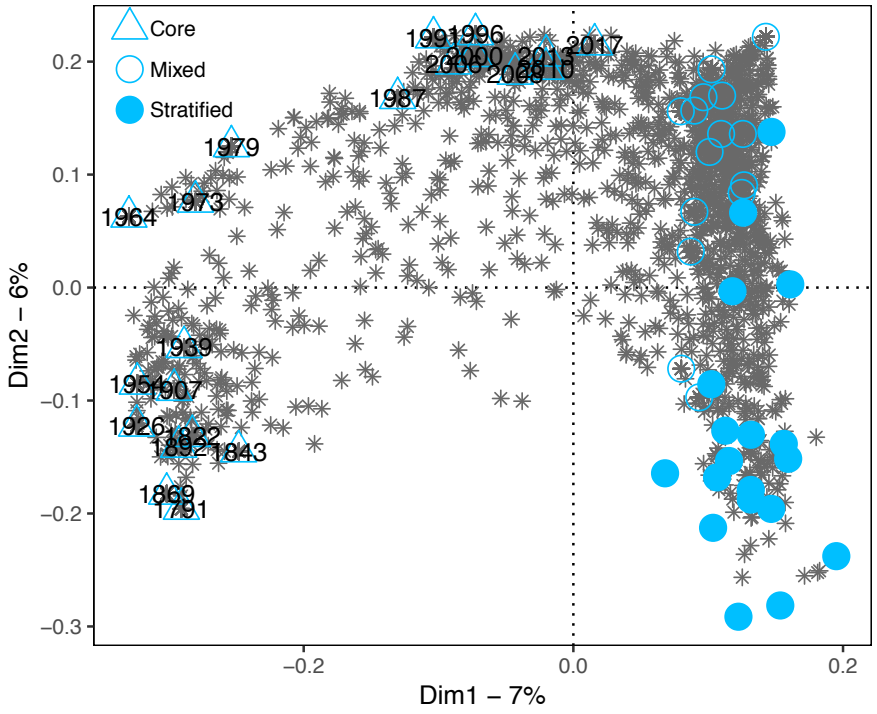


Figure 7. PCoA biplot of intracellular DNA fraction for the sediment trap samples (circles) and core samples (triangles). For the sediment trap samples, the stratification period is represented by the close circles and the mixed season by the open circles. For the sediment core samples, the dates older than 1864 should be interpreted with care as they are beyond the unsupported ^{210}Pb.

GENERAL CONCLUSION

The main objectives of my PhD thesis were to evaluate the advantages and limitations of DNA-based approaches in paleolimnology (methodological objective) and deepen our knowledge of the ecological trajectory of Cultus Lake, a peri-urban lake in British Columba, in the last ~200 years (ecological objective). I found that DNA-based approaches applied to sediment extracts were robust to reconstruct similar signal from the water column dynamics (Chapter 2), but also from a paleolimnological perspective (Chapter 4). Furthermore, the classical multi-proxy paleolimnological study (Chapter 3) and the paleo-genetic study (Chapter 4) provided insights on the ecological trajectory of Cultus Lake. I could identify major drivers of changes in Cultus Lake using contemporary limnological, historical limnological and classical paleolimnological data as well as archival material (Chapter 3). With DNA-based approaches applied on a sediment core, I could also deepen the understanding of the biological changes in Cultus Lake for taxonomical groups that are not widely used in paleolimnology (Chapter 4).

Using amplicons from the 18S rRNA gene, a wide variety of taxa could be identified as potential bioindicators in paleolimnology, including taxa that are not usually studied as such because they lack distinguishable characteristics in the sediments (Chapter 2). These potential bioindicators for future paleolimnological studies are the phyla Ciliophora, Chytridiomycota, Chrysophyceae, Cryptophyta and Cercozoa. The diversity of ecological functions between and within these taxonomical groups (e.g., feeding strategies, environmental optima) could deepen our knowledge on ecological changes in lake and help identify more precisely drivers of changes over longer time scale. Comparisons between morphological and DNA-based approaches to identify diatoms and crustaceans in water and sediment trap samples showed that DNA-based

approaches are suitable to reconstruct signal from water and sediment extracts, mainly for diatoms. Stronger correlations were found between diatom morphology and inDNA than with exDNA, which indicates that inDNA fraction is more efficient to reconstruct diatom assemblages in water and sediment extracts. On the other hand, exDNA seemed to be more efficient than inDNA to detect crustacean assemblages as stronger correlations were found between morphology and exDNA for this taxonomic group. During some sampling months, crustaceans were the dominant group represented with the DNA approach. However, the primers used in my projects did not detect some abundant cladoceran taxa (daphnids and bosminids) in Cultus Lake most likely because the V7 region of the 18S rRNA gene of *Daphnia* species (and potentially all cladocerans) is particularly long (Crease and Colbourne 1998). The correlations between morphology and DNA were thus weaker for crustaceans than for diatoms. Consequently, more work is needed to evaluate whether sedDNA is efficient to track past assemblages for crustacean with primers that can efficiently amplify this taxonomic group. In Chapter 2, results from the morphological identification of diatoms in sediment trap samples exhibited strong seasonal dynamics, which was used to better understand the contemporary ecology of Cultus Lake and how the state of the lake has deviated from its reference period (Chapter 3).

A reference period of Cultus Lake was identified from ca. 1800-1900 when most of paleo-indicators were stable and showed oligotrophic conditions in the lake (Chapter 3). Following this period of stable conditions, a slow increase of $\delta^{15}N$ was detected from the ca. 1900s to ca. 1940s, which coincided with the establishment of a permanent human settlement in the Cultus Lake watershed. The period from the 1940s to the 1970s was characterized with an increase of terrestrial matter and an increase of all sedimentary algal pigments to the sediments. These changes in the sedimentary stratigraphies coincided with an increase of agricultural

practices in the watershed, a major forest fire and two major flood events. This early eutrophication of Cultus Lake was followed by an increase of cyanobacterial pigments and modifications in diatom communities, which occurred at a time of a directional regional climate warming, increase of anthropogenic activities in the watershed as well as a decrease of sockeye salmon escapement to the lake. Two diatom species also appeared in the recent sediment core samples and were present in the sediment trap samples, but they were not present in older core samples, which indicates changes in nutrient status and ecology of Cultus Lake. *Stephanodiscus niagarea* was abundant mainly in the sediment trap samples, which suggests that Cultus Lake received more nutrients in the present period than in the past as *S. niagarea* is usually found in more nutrient replete environments (Cumming et al. 2015). The diatom species that appeared in the sediment core samples was *Lindavia intermedia*, which supports inferences of increasing water column stability during the summer in Cultus Lake as this species was found to use its positive buoyancy to persist in nutrient depleted and high light surface waters during calm summer periods (Interlandi et al. 2003). These two diatom species identified morphologically were likely associated with two amplicon sequence variants (ASV3 and ASV6) identified in Chapter 2 as they exhibited similar seasonal dynamics. They were also detected in recent samples of the sediment core with DNA-based methods in Chapter 4 and appeared at a similar time than with morphological approach.

Responses of the micro-eukaryotic community dynamics demonstrated that sedDNA can efficiently track past biological communities on a longer time scale (Chapter 4). With indicator species analyses, I could identify indicator ASVs that responded accordingly to the period of changes identified in Chapter 3, which further indicates the efficiency of DNA-based approaches in paleolimnology. InDNA was mainly used to draw the ecological trajectory of micro-

eukaryotic communities in Chapter 4 as it seemed to be more stable in sediments over longer time periods than exDNA. Preservation issues seemed to appear in exDNA fraction on samples buried for more than 30 years and care should be taken when treating and interpreting results from this DNA fraction in older samples. I also found that exDNA and inDNA seemed to exhibit different ecological patterns, but more work is needed in more specific taxonomic groups to better understand these differences.

Significance of findings and perspectives in paleolimnology

With my PhD thesis, I was able to identify several advantages and limitations of DNA-based approaches in paleolimnology. DNA-based approaches increase the diversity of taxa that can be studied in paleolimnology (Chapters 2 and 4), such as groups without apparent diagnostic features in the sediments (e.g., Ciliophora and Chytridiomycota). However, contemporary work on specific taxonomic groups is necessary to be able to use them as bioindicators in a paleolimnological perspective. For instance, transfer functions, which are used to infer past environmental conditions based on contemporary relationships between taxa preserved in surface sediments and environmental parameters, could be developed using sedDNA of specific taxonomic groups. Developing such transfer functions using sedDNA could be particularly useful for future paleolimnological studies (reviewed in Domaizon et al. 2017). The DNA deposited in the sediments identified similar seasonal dynamics than the DNA signal in the water column for micro-eukaryotic communities as well as for diatoms (Chapter 2). Therefore, sedDNA is efficient to track part of the water column dynamics, at least in Cultus Lake. SedDNA was also accurate to reconstruct micro-eukaryotic communities on a longer time period

(Chapter 4) and the response of these communities concorded with the time periods identified in the previous multi-proxy paleolimnological study (Chapter 3). In general, most of paleolimnological studies have targeted total DNA archived in the sediments, except a study that targeted both DNA fractions to amplify prokaryotes in sediment samples (Vuillemin et al. 2017). As DNA can be archived in the sediments as exDNA and inDNA, both fractions needed to be assessed for micro-eukaryotes to evaluate their efficiency to track past biological conditions. From my PhD projects, I found that inDNA was more suitable to target diatoms in paleolimnology as correlations between morphology and DNA were higher with inDNA than with exDNA for this biological group (Chapters 2 and 4). For crustaceans, correlations between morphology and exDNA were stronger than with inDNA, which indicates the potential of exDNA to target multicellular organisms (Chapter 2), as suggested by Taberlet et al. (2012). However, when targeting ancient DNA (old core sediment samples), exDNA should be treated and interpreted with care as it seemed to degrade substantially after 30 years of burial (Chapter 4). More work is needed on other systems and with other taxonomic groups, such as macro-organisms (i.e., fish, macro-invertebrates, mammals from terrestrial environments) to better evaluate how exDNA should be used in paleolimnological studies. To evaluate the advantages and limitations of DNA-based approaches in paleolimnology, I chose to target a wide variety of taxa with general micro-eukaryotic primers for exploration purposes. Those primers were efficient to amplify most of the groups of microbial eukaryotes (i.e., unicellular eukaryotes), but did not properly amplify some abundant cladocerans taxa in Cultus Lake. The longer V7 region of the 18S rRNA gene of *Daphnia* species is most likely responsible for the absence of this taxonomic group in our genetic dataset. Therefore, the choice of primers should be made with care and it has been suggested to test the potential specific primers with mock communities prior

to sequencing actual samples to ensure a good representation of the target taxa in the DNA sequence datasets (Zhang et al. 2018).

Finally, my PhD thesis also showed the importance of a longer time perspective to reconstruct more accurately the ecological trajectory of lakes, but also using multiple indicators as some taxonomic groups can detect different changes in lake ecology (Chapters 3 and 4). Therefore, sedDNA simultaneously used with classical paleolimnological indicators has a great potential to enhance our knowledge of modifications in lakes over long time periods and to understand more precisely the drivers and ecological mechanisms of these changes.

Literature cited

Crease TJ, Colbourne JK (1998) The unusually long small-subunit ribosomal RNA of the crustacean, *Daphnia pulex*: Sequence and predicted secondary structure. J Mol Evol 46:307–313. doi:10.1007/PL00006307

Cumming BF, Laird KR, Gregory-Eaves I, et al (2015) Tracking past changes in lake-water phosphorus with a 251-lake calibration dataset in British Columbia: tool development and application in a multiproxy assessment of eutrophication and recovery in Osoyoos Lake, a transboundary lake in Western North America. Frontiers in Ecology and Evolution 3. doi:10.3389/fevo.2015.00084

Domaizon I, Winegardner A, Capo E, et al (2017) DNA-based methods in paleolimnology: new opportunities for investigating long-term dynamics of lacustrine biodiversity. Journal of Paleolimnology 58:1–21. doi:10.1007/s10933-017-9958-y

Interlandi SJ, Kilham SS, Theriot EC (2003) Diatom—chemistry relationships in Yellowstone Lake (Wyoming) sediments: Implications for climatic and aquatic processes research. Limnol Oceanogr 48: 79–92. doi:10.4319/lo.2003.48.1.0079

Taberlet P, Prud'homme SM, Campione E, et al (2012) Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Molecular Ecology 21: 1816–1820. doi:10.1111/j.1365-294X.2011.05317.x

Vuillemin A, Horn F, Alawi M, et al (2017) Preservation and Significance of Extracellular DNA in Ferruginous Sediments from Lake Towuti, Indonesia. Frontiers in Microbiology 8. doi:10.3389/fmicb.2017.01440

SUPPLEMENTARY MATERIAL

Chapter 2 – Supplementary Material

Chapter 2 – Supplementary Material SM1. List of taxa and references used for morphological identification

References for cladoceran length and equations for biomass

Artsdatabanken. Alona guttata Sars. Accessed on February 15, 2019. https://www.artsdatabanken.no/Pages/214483

Barnett AJ, Finley K, Beisner BE (2007) Functional diversity of crustacean zooplankton communities: towards a trait-based classification. Freshwater Biology, 52, 796-813.

Gillooly JF (2000) Effect of body size and temperature on generation time in zooplankton. Journal of Plankton Research, 22: 241–251.

Lynch M (1980) The evolution of cladoceran life histories. The Quarterly Review of Biology, 55: 23-42.

Witty LM (2004) Practical Guide to Identifying Freshwater Crustacean Zooplankton. Cooperative Freshwater Ecology Unit, Sudbury, Ontario.

References for morphological identification of diatoms

Cumming BF, Wilson SE, Hall RI, Smol JP (1995) Diatoms from British Columbia (Canada) lakes and their relationship to salinity, nutrients and other limnological variables. Cramer in der Gebr.-Borntraeger-Verl.-Buchh, Berlin

Hofmann G, Werum M, Lange-Bertalot H (2011) Diatomeen im Süßwasser - Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten Arten und ihre Ökologie. Koeltz Scientific Books, Königstein

Houk V (2003) Atlas of freshwater centric diatoms with a brief key and descriptions. 1: Melosiraceae, Orthoseiraceae, Paraliaceae and Aulacoseiraceae. Czech Phycological society, Praha

Houk V, Klee R, Tanaka H (2010) Atlas of freshwater centric diatoms with a brief key and descriptions: Part 3: Stephanodiscaceae A, *Cyclotella*, *Tertiarius*, *Discostella*. Czech Phycological Society, Praha

Houk V, Klee R, Tanaka H (2014) Atlas of freshwater centric diatoms with a brief key and descriptions. Stephanodiscaceae B : *Stephanodiscu*s, *Cyclostephanos*, *Pliocaenicus*,

Hemistephanos, *Stephanocostis*, *Mesodictyon* & *Spicaticribra*. Czech Phycological society, Praha

Houk V, Klee R (2007) Atlas of freshwater centric diatoms with a brief key and descriptions. Part II. Melosiraceae and Aulacoseiraceae (Supplement to Part I). Koeltz

Krammer K, Lange-Bertalot H (1986–1991). Bacillariophyceae 1. Teil: Naviculacea. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K, Lange-Bertalot H (1986–1991) Bacillariophyceae 2. Teil: Bacillariaceae, Epithemiacea, Surirellaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K, Lange-Bertalot H (1986–1991) Bacillariophyceae 3. Teil: Centrales, Fragilariacea, Eunotiaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K, Lange-Bertalot H (1986–1991) Bacillariophyceae 4. Teil: Achnanthaceae, Kristische Ergänzumgen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis Teil 1-4. Teil: Centrales, Fragilariacea, Eunotiaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K (2002) Diatoms of Europe: Diatoms of the european inland waters and comparable habitats. Volume 3: *Cymbella*. ARG Ganter Verlag KG

Lange-Bertalot H (2001) Diatoms of Europe: diatoms of the european inland waters and comparable habitats. Volume 2: *Navicula* sensu stricto, 10 genera separated from *Navicula* sensu lacto, *Frustulia*. ARG Ganter Verlag KG

Lange-Bertalot H, Bak M, Witkowski A (2002) Diatoms of Europe: Diatoms of the european inland waters and comparable habitats. Volume 6: *Eunotia* and some related genera. ARG Ganter Verlag KG

Lavoie I, Hamilton PB, Campeau S, Grenier M, Dillon PJ (2008) Guide d'identification des diatomées des rivières de l'Est du Canada. Presse de l'Université du Québec, Québec

Patrick R, Reimer CW (1966) The diatoms of the United States. Monographs of The Academy of Natural Sciences of Philadelphia, vol 1, no 13

Spaulding SA, Lubinski DJ, Potapova M (2010) Diatoms of the United States. http://westerndiatoms.colorado.edu

References for length and biovolume of diatoms

Diatoms of North America: The source for diatom identification and ecology. Retrieved in June 2018. https://diatoms.org/species

Phycology Section, Patrick Center for Environmental Research, Academy of Natural Sciences of Drexel University (2011) Diatom Taxa Names. Retrieved in April 2018. https://ansp.org/research/environmental-research/sections/phycology/

Table SM1.1. Crustacean taxa identified through morphological approach in the water column and in the sediment trap samples.

Taxa in water samples	Taxa in sediment trap samples
1. *Bosmina* sp.	1. *Alona guttata*
2. *Diacyclops* sp.	2. *Alonella nana*
3. *Daphnia* sp.	*3. Bosmina* sp.
4. *Epischura* sp.	4. *Chydorus spaericus*
5. *Holopedium* sp.	5. *Daphnia longispina*
6. *Leptodiaptomous* sp.	6. *Daphnia pulex*
	7. *Sida crystallina Americana*

Table SM1.2. Diatom taxa identified through morphological approach in the water (epilimnion and metalimnion) and in the sediment trap samples.

Taxa in water samples	Taxa in sediment trap samples
1. *Asterionella formosa*	**Dominant taxa:**
2. *Aulacoseira subarctica*	1. *Achnanthidium minutissimum*
3. *Aulacoseira ambigua*	2. *Amphora pediculus*
4. *Discostella stelligera*	3. *Asterionella formosa*
5. *Eunotia lunaris*	4. *Aulacoseira ambigua*
6. *Eunotia pectinalis*	5. *Aulacoseira subarctica*
7. *Fragilaria crotonensis*	6. *Lindavia comensis* (previously *Cyclotella comensis*)
8. *Lindavia intermedia*	7. *Lindavia michiganiana* (previously *Cyclotella michiganiana*)
9. *Lindavia ocellata*	8. *Discostella stelligera* / *Discostella pseudostelligera* complex
10. *Staurosira construens*	9. *Lindavia intermedia* (previously *Cyclotella bodanica* var. *intermedia*)
11. *Stephanodiscus niagarae*	10. *Pseudostaurosira brevistriata*
12. *Synedra acus*	11. *Stephanodiscus medius*
13. *Synedra ulna*	12. *Stephanodicus niagarae*
14. *Tabellaria fenestrata*	13. *Stephanodiscus minutulus* / *Stephanodiscus parvus* complex
15. *Tabellaria flocculosa*	

Taxa in water samples	Taxa in sediment trap samples
	Other taxa:
	14. Centrics: *Aulacoseira canadensis* / *Lindavia ocellata* / *Melosira* sp.
	15. Assymetrical biraphids: *Amphora copulata* / *Amphora minutissima* / *Amphora ovalis* / *Amphora pellucida* / *Amphora* sp. / *Cymbella cymbiformis* / *Cymbella. frequens* / *Cymbella proxima* / *Cymbella c.f. turgida* / *Cymbella* sp. / *Cymbopleura amphicephala* / *Encyonema caespitosum* / *Encyonema minutus* / *Encyonema silesiacum* / *Encyonema* sp. / *Encyonema* sp2 / *Encyonema* sp3 /*Encyonopsis hustedtii* / *Encyonopsis microcephala* / *Encyonopsis subminuta* / *Entomoneis paludosa* / *Gophonema angustatum* / *Gomphonema coronatum* / *Gomphonema kobayasii* / *Gomphonema olivaceum* var. *olivaceum* / *Gomphonema* sp. / *Gomphonema truncatum* / *Gyrosigma acuminatum* / *Halamphora* sp. / *Halamphora thumensis* / *Hippodonta capitata* / *Hippodonta* sp. / *Hippodonta* sp.
	16. Symmetrical biraphids: *Aneumstus rostratus* / *Brachysira neoexilis* / *Caloneis bacillum* / *Caloneis silicula* / *Cavinula pseudoscutiformis* / *Cavinula scutelloides* / *Craticula halophila* / *Craticula riparia* / *Diploneis elliptica* / *Diploneis onblogella* / *Eolimna* sp1 / *Eolimna* sp2 / *Frustulia vulgaris* / *Geissleria acceptata* / *Geissleria decussis* / *Luticola* sp. / *Navicula capitatoradiata* / *Navicula cincta* / *Navicula crytotenella* / *Navicula menisculus var. upsaliensis* / *Navicula onbloga* / *Navicula praeterita* / *Navicula radiosa* / *Navicula schadei* / *Navicula c.f. uternoehlii* / *Navicula viridula* / *Navicula vulpina* / *Navicula* sp. / *Neidium ampliatum* / *Neidium* c.f. *binodeformis* / *Placoeis gastrum* / *Sellaphora pupula* / *Stauroneis* sp.
	17. Monoraphids: *Achnanthidium exiguum* / *Achnanthidium gracillimum* / *Achnanthidium pyrenaicum* / *Achnanthidium rivulare* / *Achnanthidium c.f. rosenstockii* / *Achnanthidium subhudsonis* / *Achnanthidium* sp. / *Cocconeis neothumensis* / *Cocconeis placentula* / *Cocconeis pseudothumensis* / *Karayevia clevei* / *Karayevia laterostrata* / *Karayevia sp.* / *Karayevia c.f. suchlandtii* / *Planothidium* sp. / *Planothidium frequentissimum* / *Planothidium haynaldii* / *Planothidium hustedtii* / *Planothidium joursacence* / *Platessa c.f. stewartii* / *Platessa zeigleri* / *Platessa conspicua* / *Psammothidium c.f. curtissiumum* / *Rossithidium anastasiae* / *Rossithidium pusillum*
	18. Small araphids: *Diatoma mesodon / Meridion circulare* var. *constrictum* / *Pseudostaurosira neoelliptica* / *Pseudostaurosira parasitica* / *Pseudostaurosira robusta* / *Pseudostaurosirospsis* sp. / *Punctastriata c.f. pinnata* / *Staurosira construens* / *Staurosira construens var. binodis* / *Staurosira construens var. venter* / *Staurosirella lapponica* / *Staurosirella leptostauron* / *Staurosirella martyi* / *Staurosirella pinnata*
	19. Long araphids: *Fragilaria bicapitata* / *Fragilaria crotonensis* / *Fragilaria gracilis* / *Fragilaria mesolepta* / *Fragilaria radians* / *Fragilaria* sp. / *Fragilaria synegrotesca* / *Fragilaria tenera* / *Fragilaria vaucheriae* / *Hannea arcus* / *Synedra cyclopum* / *Synedra c.f. famelica* / *Tabularia fasciculate* / *Tabellaria fenestrate*
	20. Epithemioid: *Epithemia adnata* / *Epithemia c.f. sorex* / *Rhopalodia gibba*
	21. Nitzschioid: *Denticula tenuis* / *Nitzschia angustata / Nitzschia c.f. gracilis* / *Nitzschia communis* / *Nitzschia fonticola* / *Nitzschia recta* / *Nitzschia* sp. / *Nitzschia paleo var. tenuirostris* / *Nitzschia sinuata var. tabellaria* / *Tryblionella* sp.
	22. Surirelloid: *Surirella amphioxys* / *Surirella* sp1 / *Surirella* sp.

Chapter 2 - Supplementary Material SM2. DNA concentration for each sample of sediment traps and percentage of intracellular and extracellular DNA.

Table SM2.1. DNA concentration (ng μl^{-1} and μg of DNA g^{-1} sediment dry weight) for intracellular DNA (inDNA) and extacellular DNA (exDNA) for each sample of sediment traps. The total is the sum of both DNA portions measured separately. The percentage of each portion is also presented.

Sediment trap retrival Date	Thermal Stratification	inDNA (ng µl^{-1})	inDNA (µgDNA g^{-1} DW)	exDNA (ng µl^{-1})	exDNA (µgDNA g^{-1} DW)	Total DNA (µgDNA g^{-1} DW)	% inDNA	% exDNA
2014-08-01	Stratified	4.82	9.08	1.02	1.92	11.00	82.53	17.47
2014-09-05	Stratified	12.30	15.14	NA	0.00	15.14	100.00	0.00
2014-10-09	Stratified	14.50	77.17	49.60	263.96	341.13	22.62	77.38
2014-11-07	Stratified	28.90	166.96	26.20	151.36	318.32	52.45	47.55
2015-01-08	Stratified	81.20	32.94	49.60	20.12	53.07	62.08	37.92
2015-01-08	Mixed	75.10	52.26	41.60	28.95	81.21	64.35	35.65
2015-02-04	Mixed	5.01	6.66	14.70	19.54	26.20	25.42	74.58
2015-03-04	Mixed	9.72	11.45	28.50	33.57	45.02	25.43	74.57
2015-04-01	Mixed	22.50	38.59	2.24	3.84	42.44	90.95	9.05
2015-05-05	Mixed	60.50	84.98	3.00	4.21	89.19	95.28	4.72
2015-06-02	Stratified	19.20	51.58	2.05	5.51	57.09	90.35	9.65
2015-06-30	Stratified	18.30	23.79	12.30	15.99	39.78	59.80	40.20
2015-08-04	Stratified	20.70	79.57	3.70	14.22	93.80	84.84	15.16
2015-09-02	Stratified	47.90	106.77	NA	0.00	106.77	100.00	0.00
2015-09-30	Stratified	14.50	43.66	NA	0.00	43.66	100.00	0.00
2015-11-06	Stratified	47.00	71.70	18.70	28.53	100.22	71.54	28.46
2015-12-02	Stratified	92.30	34.29	95.30	35.41	69.70	49.20	50.80
2016-01-14	Mixed	1.36	0.53	12.80	4.99	5.52	9.60	90.40
2016-02-09	Mixed	81.20	40.99	49.80	25.14	66.12	61.98	38.02
2016-03-09	Mixed	1.72	0.54	NA	0.00	0.54	100.00	0.00
2016-04-04	Mixed	99.10	56.20	24.50	13.78	69.99	80.31	19.69
2016-05-03	Mixed	14.40	23.65	1.83	3.01	26.66	88.72	11.28
2016-06-02	Stratified	4.95	15.24	1.88	5.79	21.03	72.47	27.53
2016-08-09	Stratified	32.00	112.86	2.56	9.03	121.89	92.59	7.41
2016-08-31	Stratified	34.80	129.71	4.24	15.80	145.51	89.14	10.86
2016-10-04	Stratified	16.70	144.52	NA	0.00	144.52	100.00	0.00
2016-10-31	Stratified	46.60	60.76	2.67	3.48	64.24	94.58	5.42
2016-12-01	Stratified	53.40	154.76	18.20	52.75	207.51	74.58	25.42
2017-01-12	Mixed	92.60	38.11	NA	0.00	38.11	100.00	0.00
2017-02-15	Mixed	44.50	27.77	8.28	5.17	32.94	84.31	15.69
2017-03-16	Mixed	27.30	30.94	9.73	11.03	41.97	73.72	26.28
2017-04-12	Mixed	95.20	45.01	22.90	10.83	55.83	80.61	19.39
2017-05-08	Mixed	35.90	53.92	24.10	36.20	90.12	59.83	40.17
2017-06-12	Stratified	2.28	3.74	NA	0.00	3.74	100.00	0.00
2017-07-04	Stratified	NA	NA	NA	NA	NA	NA	NA

Table SM2.2. Average (and range) of DNA concentration (ng μl^{-1} and μg of DNA g^{-1} sediment dry weight) and percentage for intracellular DNA and extracellular DNA in (a) all samples, (b) mixed period samples and (c) the stratified period samples. Data summarized from table SM2.1.

	inDNA (ng μl^{-1})	**inDNA (μgDNA g^{-1} DWsed)**	**exDNA (ng μl^{-1})**	**exDNA (μgDNA g^{-1} DWsed)**	**Total DNA (μgDNA g^{-1} DWsed)**	**% inDNA**	**% exDNA**
(a) All samples	37.01 (1.36-99.10)	54.3 (0.53-166.96)	19.7 (1.02-95.3)	24.24 (0-263.96)	78.53 (0.54-342.13)	74.69 (9.60-100)	25.31 (0-90.39)
(b) Mixed period	44.41 (1.36-99.1)	34.11 (3.74-84.98)	18.77 (1.02-49.8)	13.35 (0-36.20)	47.46 (0.54-90.12)	69.37 (9.61-100)	30.63 (0-90.40)
(c) Stratified period	31.18 (2.28-92.3)	70.22 (3.74-166.96)	20.57 (1.02-95.3)	32.84 (0-263.96)	103.06 (3.74-341.13)	78.88 (22.62-100)	21.12 (0-77.38)

Chapter 2 – Supplementary Material SM3. Physico-chemical and biological environmental variables measured in Cultus Lake.

Table SM3. Physico-chemical and biological environmental variables measured in Cultus Lake. (a) General water column variables, (b) photic zone variables within the epilimnion and the metalimnion and, (c) hypolimnetic variables. The asterisks show the variables that were included in the partial RDA analyses.

Variables	Abbreviations	Units
(a) General water column environmental variables		
Upper column water temperature (average of 0 to 5m deep)	EpiTemp*	°C
Surface temperature	SurfaceTemp	°C
Schmidt stability index	Stability35	kJ m^{-2}
Depth of the photic zone	PhoticZoneDepth*	m
(b) Photic zone environmental variables (epilimnion and metalimnion)		
Turbidity	Turb	NTU
Conductivity corrected at 25°C	Cond25	µS cm^{-1}
Dissolved oxygen	DO*	mg L^{-1}
Particulate carbon	PC	µg L^{-1}
Nitrate	NO_3*	µg L^{-1}
Ammonia	NH_3*	µg L^{-1}
Dissolved organic nitrogen	DON*	µg L^{-1}
Particulate nitrogen	PN*	µg L^{-1}
Dissolved inorganic nitrogen	DIN*	µg L^{-1}
Soluble reactive phosphorus	SRP*	µg L^{-1}
Total phosphorus	TP*	µg L^{-1}
Total dissolved phosphorus	TDP*	µg L^{-1}
Particulate phosphorus	PP*	µg L^{-1}
Soluble reactive silicon	SRSi*	mg L^{-1}
Total dissolved solids	TDS*	mg L^{-1}
Total chlorophyll	TotChl*	µg L^{-1}

Chlorophyll from phytoplankton > 2 µm	PhyChl*	µg L^{-1}
Chlorophyll from phytoplankton > 20 µm	MicroChl*	µg L^{-1}
Chlorophyll from phytoplankton < = 2 µm	PicoChl*	µg L^{-1}
Chlorophyll from phytoplankton > 2 <= 20 µm	NanoChl	µg L^{-1}
pH	pH*	
(c) Hypolimnetic environmental variables		
Nitrate	HypoNO_3*	µg L^{-1}
Ammonia	HypoNH_3	µg L^{-1}
Total phosphorus	HypoTP*	µg L^{-1}
Total chlorophyll	HypoTotChl*	µg L^{-1}
Dissolved oxygen	HypoDO*	mg L^{-1}
Temperature (at 35m deep)	Temp35m	°C
Soluble reactive phosphorus	HypoSRP	µg L^{-1}
Total dissolved phosphorus	HypoTDP	µg L^{-1}

Chapter 2 – Supplementary Material SM4. Proportion barplots and PCA biplots for crustaceans and diatoms for each matrix separately

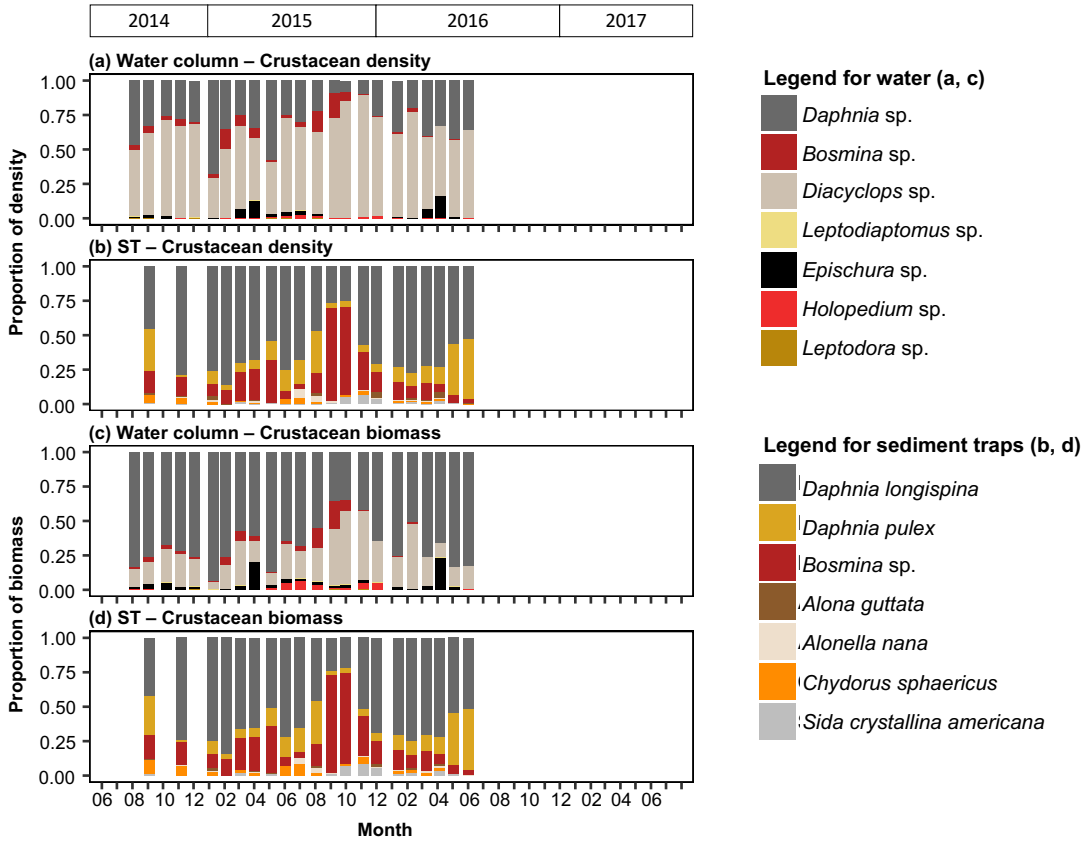


Figure SM4.1. Proportion of different crustacean taxa identified through morphological approach represented as barplots for density data in (a) the water column (net haul from 30 m deep); (b) the sediment traps (ST); and from biomass data in (c) the water column; and (d) the sediment traps.

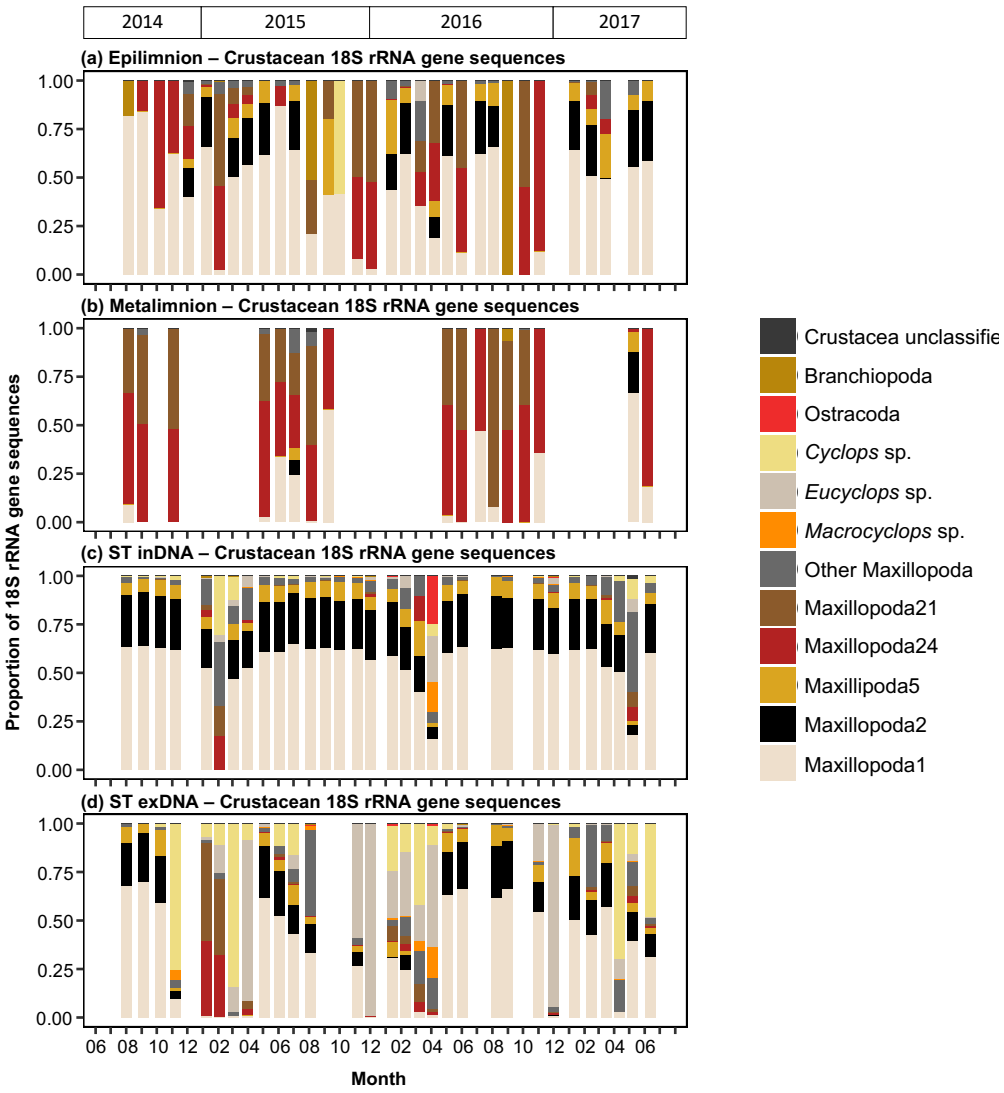


Figure SM4.2. Proportion of sequences identified through 18S rRNA gene sequencing for different crustacean taxa represented as barplots in (a) the epilimnion; (b) the metalimnion; (c) the intracellular DNA fraction of the sediment trap samples (ST inDNA); and (d) the extracellular DNA fraction in the sediment trap samples (ST exDNA).

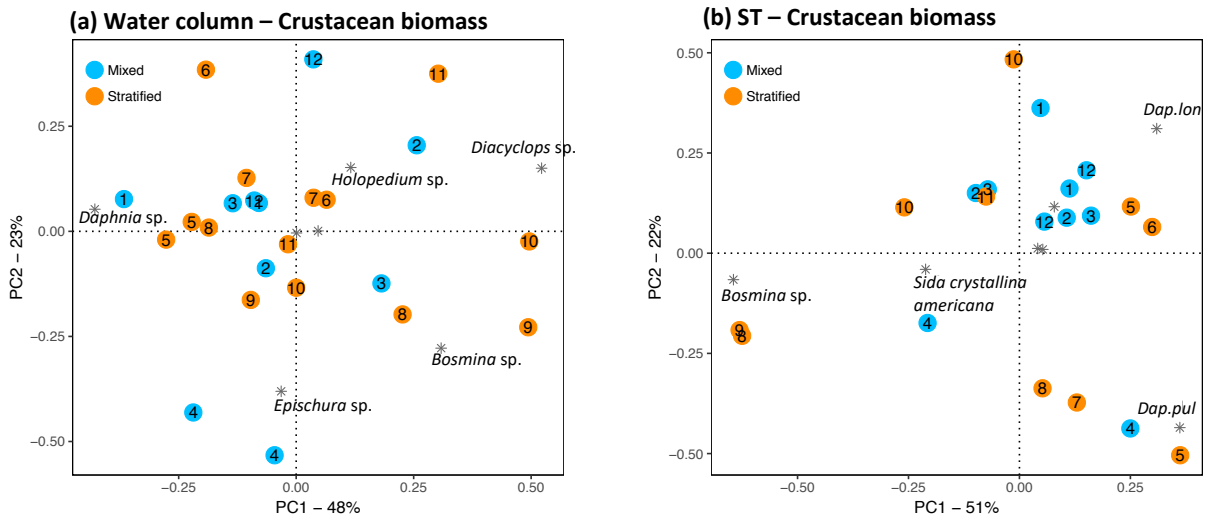


Figure SM4.3. PCA biplots of crustacean morphological approach for (a) biomass in water samples (30 m deep net haul); and (b) biomass in sediment traps samples (ST). Biomasses were Hellinger transformed prior to ordination. Taxa abbreviations are as follows: *Daphnia longispina* (*Dap.lon*); *Daphnia pulex* (*Dap.lon*).

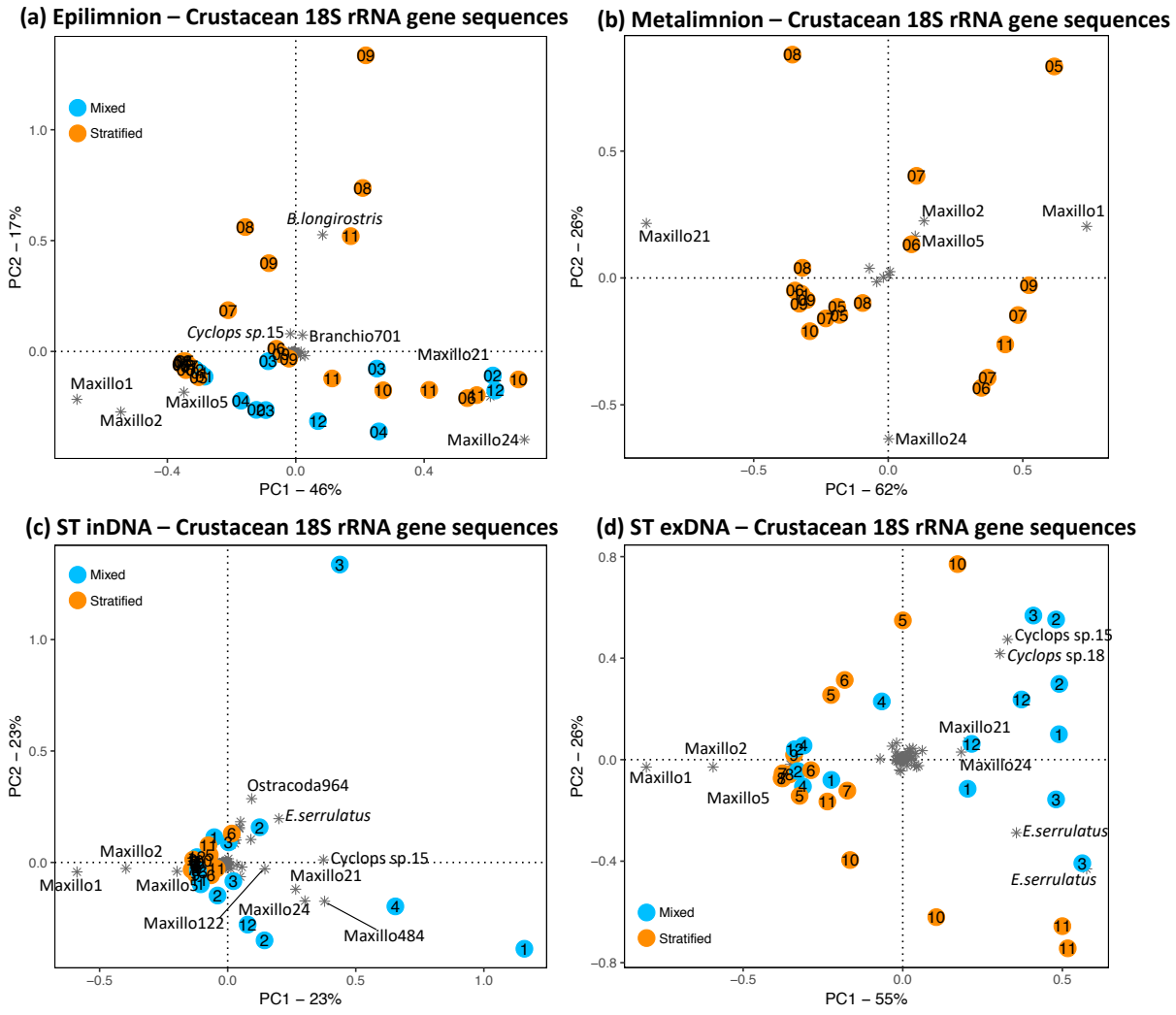


Figure SM4.4. PCA biplots of crustacean identified through 18S rRNA gene sequencing (a) epilimnion; (b) metalimnion; (c) intracellular DNA in sediment traps (ST inDNA); and (d) extracellular DNA in sediment traps (ST exDNA). Sequence numbers were Hellinger transformed prior to ordination. Taxa abbreviations are as follows: *Bosmina longirostris* (*B. longirostris*); Branchiopoda (Branchio); *Eucyclops serrulatus* (*E.serrulatus*); and Maxillopoda (Maxillo).

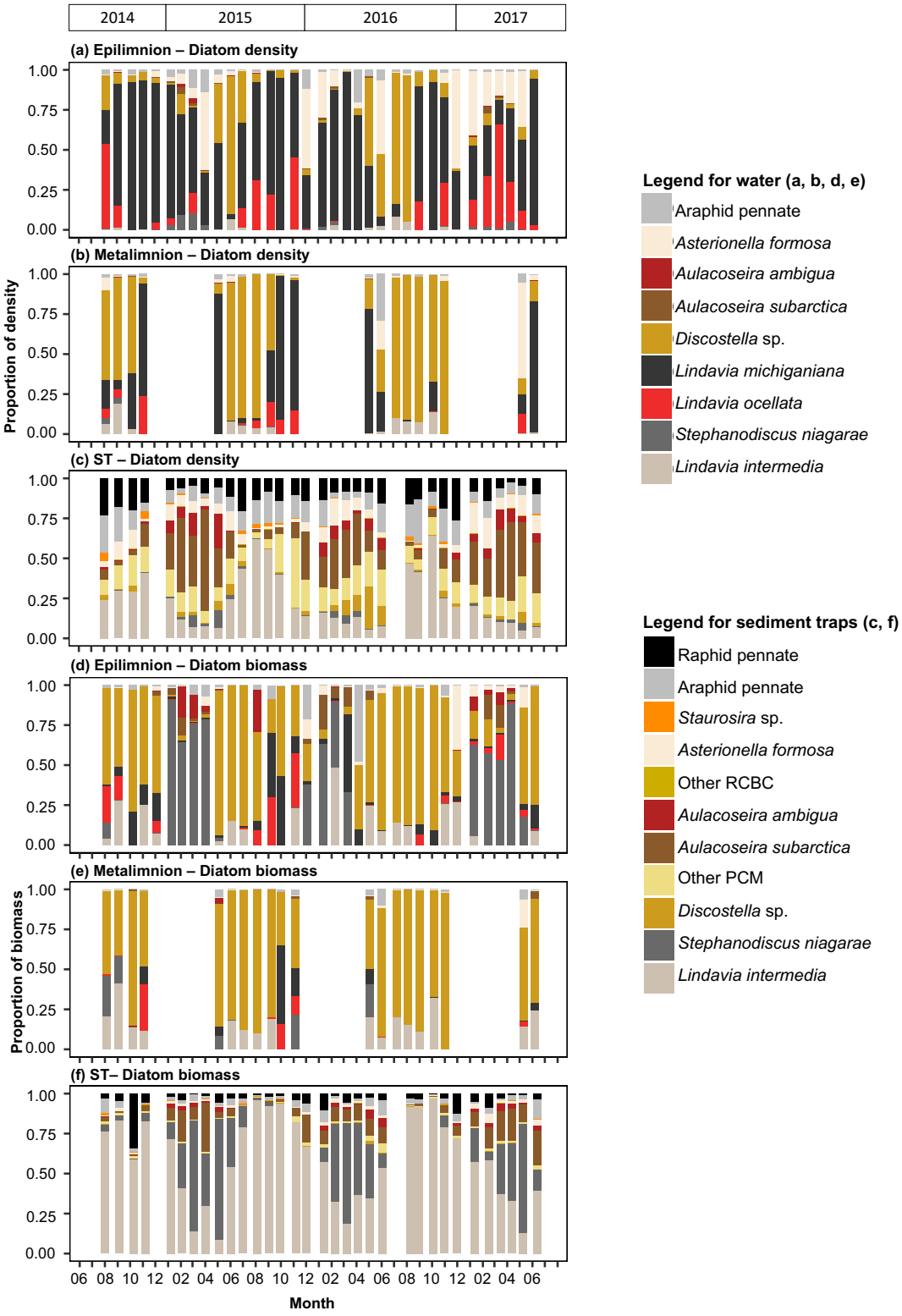

2014
2015
2016
2017
(a) Epilimnion – Diatom density
(b) Metalimnion – Diatom density
(c) ST – Diatom density
(d) Epilimnion – Diatom biomass
(e) Metalimnion – Diatom biomass
(f) ST– Diatom biomass
Proportion of density
Proportion of biomass
1.00
0.75
0.50
0.25
0.00
06 08 10 12 02 04 06 08 10 12 02 04 06 08 10 12 02 04 06
Month
Legend for water (a, b, d, e)
Araphid pennate
Asterionella formosa
Aulacoseira ambigua
Aulacoseira subarctica
Discostella sp.
Lindavia michiganiana
Lindavia ocellata
Stephanodiscus niagarae
Lindavia intermedia
Legend for sediment traps (c, f)
Raphid pennate
Araphid pennate
Staurosira sp.
Asterionella formosa
Other RCBC
Aulacoseira ambigua
Aulacoseira subarctica
Other PCM
Discostella sp.
Stephanodiscus niagarae
Lindavia intermedia

Figure SM4.5. Proportion of different diatom taxa identified through morphological approach represented as barplots for density data in (a) the epilimnion; (b) the metalimnion; (c) the sediment traps (ST); and from biomass data in (d) the epilimnion; (e) the metalimnion; and (f) the sediment traps. PCM: Polar centric Mediophyceae; RCBC: Radial-centric-basal-Coscinodiscophyceae.

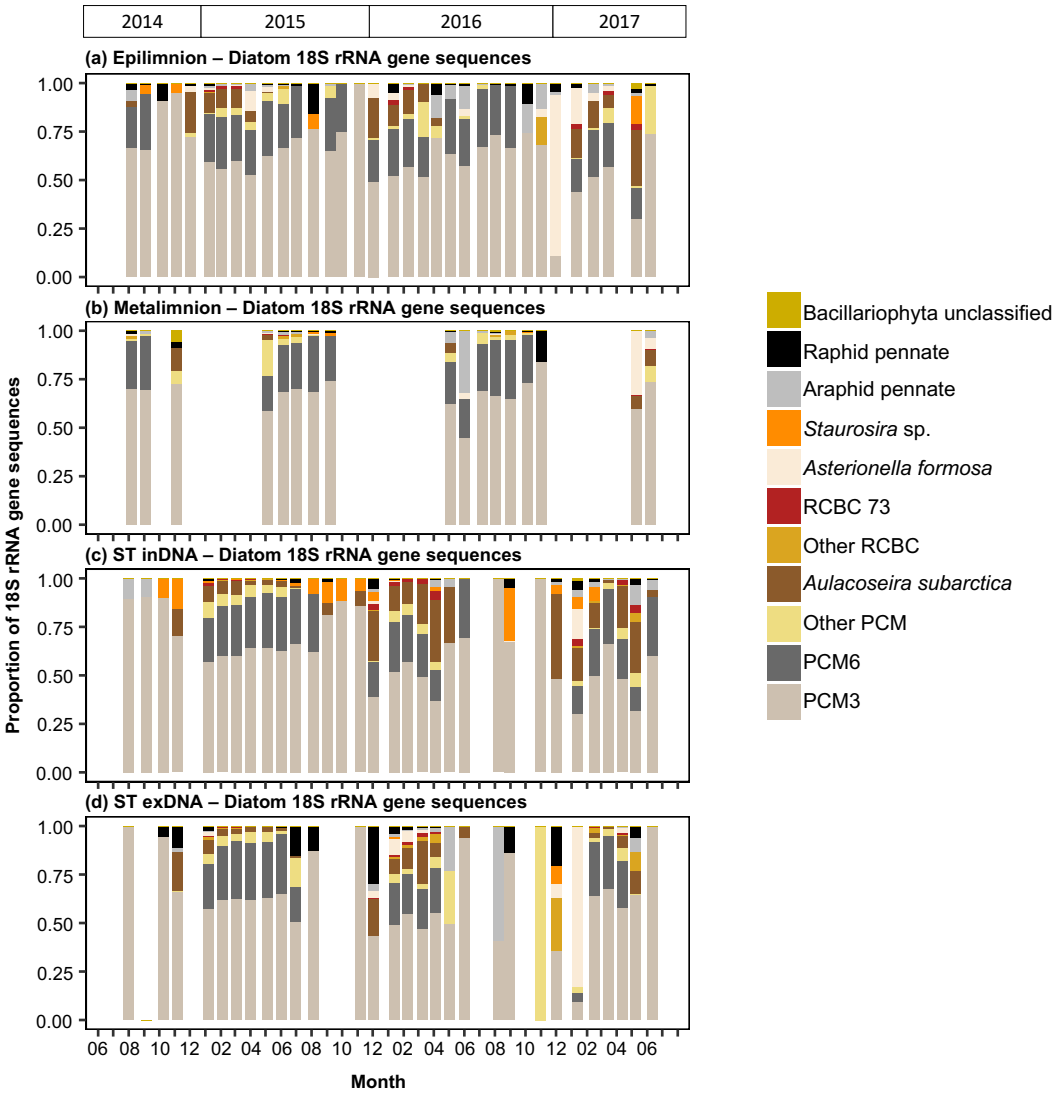


Figure SM4.6. Proportion of sequences identified through 18S rRNA gene sequencing for different diatom taxa represented as barplots in (a) the epilimnion; (b) the metalimnion; (c) the intracellular DNA fraction of the sediment trap samples (ST inDNA); and (d) the extracellular DNA fraction in the sediment trap samples (ST exDNA). PCM: Polar centric Mediophyceae; RCBC: Radial-centric-basal-Coscinodiscophyceae.

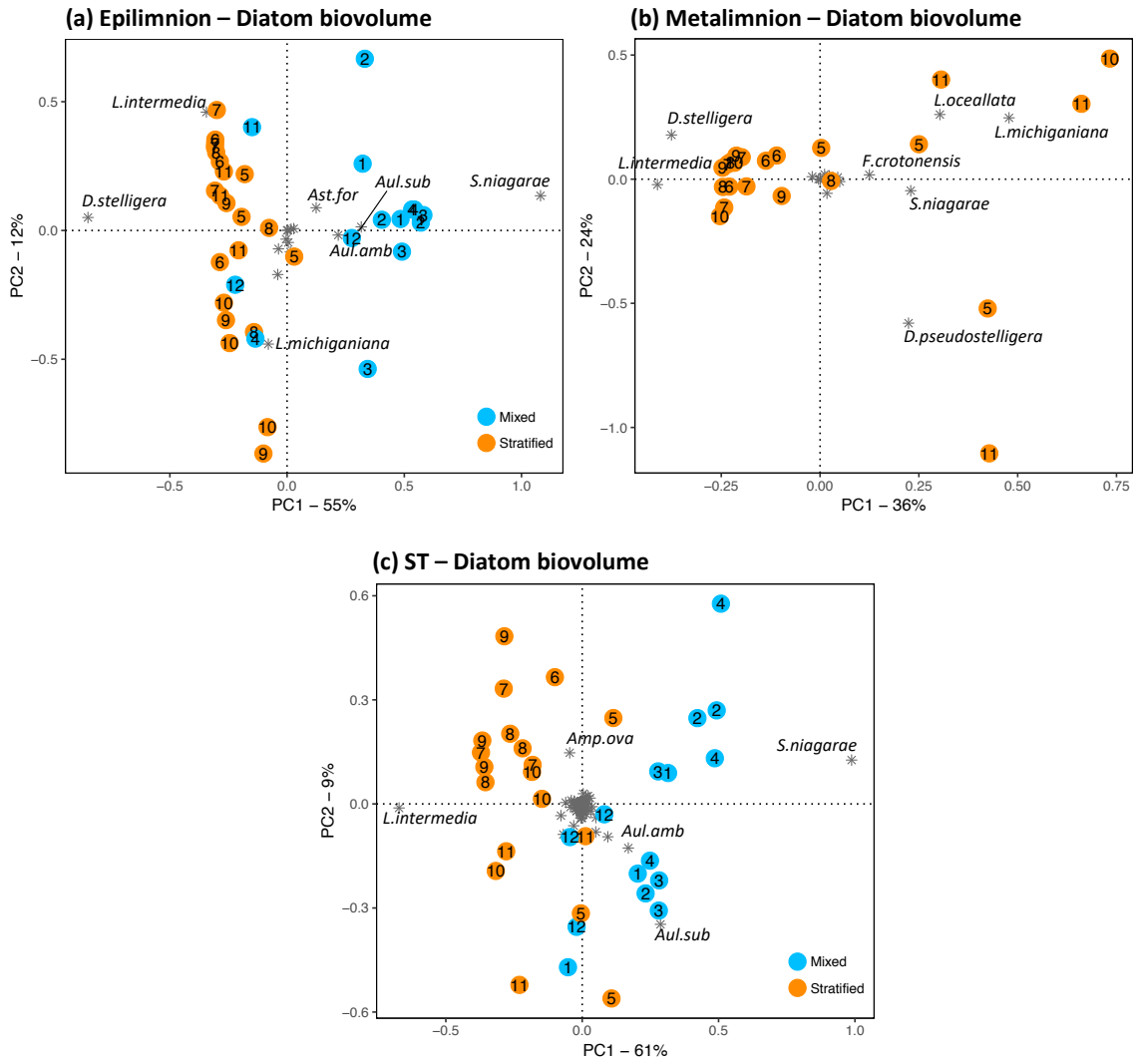


Figure SM4.7. PCA biplots or diatoms identified through morphological approach for (a) biovolume in epilimnion; (b) biovolume in metalimnion; and (c) biovolume in sediment trap (ST) samples. Biovolumes were Hellinger transformed prior to ordination. Taxa abbreviations are as follows: *Amphora ovalis* (*Amp.ova*); *Asterionella formosa* (*Ast.for*); *Aulacoseira ambigua* (*Aul.amb*); *Aulacoseira subarctica* (*Aul.sub*); *Discostella stelligera* (*D.stelligera*); *Discostella pseudostelligera* (*D.pseudostelligera*); *Fragilaria crotonensis* (F.crotonensis); Lindavia *intermedia* (*L.intermedia*); *Lindavia michiganiana* (*L.michiganiana*); *Lindavia oceallata* (*L.oceallata*); and *Stephanodicus niagarae* (*S.niagarae*).

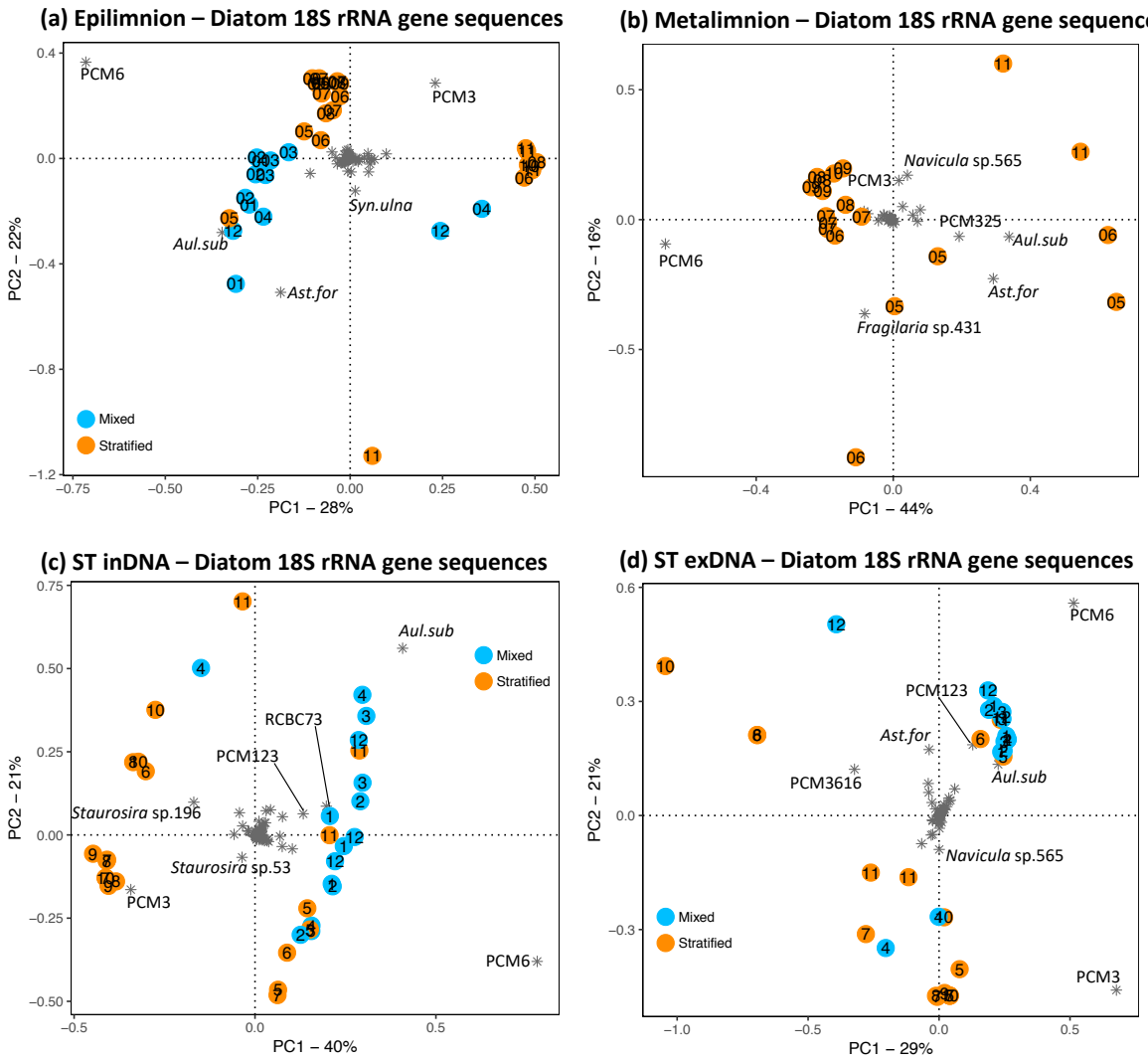


Figure SM4.8. PCA biplots of diatoms identified through 18S rRNA taxonomical approach for (a) epilimnion; (b) metalimnion; (c) intracellular DNA in sediment traps (ST inDNA); and (d) extracellular DNA in sediment traps (ST exDNA). Sequence numbers were Hellinger transformed prior to ordination. Taxa abbreviations are as follows: *Asterionella formosa* (*Ast.for*); *Aulacoseira ambigua* (*Aul.amb*); *Aulacoseira subarctica* (*Aul.sub*); Polar-centric-Mediophyceae (PCM); Radial-centric-basal-Coscinodiscophyceae (RCBC); and *Synedra ulna* (*S.ulna*).

Chapter 2 – Supplementary Material SM5. Shared ASVs between 18S rRNA gene sequence sample matrices

The highest proportion of total shared ASVs was found between the epilimnion and the metalimnion, which had a shared pool of ASVs representing 36 % of their assemblages (Fig. SM5.1a). The second highest proportion of shared ASVs was found between ST inDNA and ST exDNA, which shared 24 % of their shared pool (Fig. SI5.1b). However, the total number of shared ASVs was higher between ST inDNA and ST exDNA (1294 versus 910 shared ASVs between the epilimnion and the metalimnion). Comparisons between water samples and ST samples showed that there was a greater number of shared ASVs between the epilimnion and ST samples, with the highest proportion of shared ASVs between the epilimnion and ST inDNA (16 %; Fig. SI5.1c). In contrast, the lowest number of shared ASVs was found when comparing the water samples and ST exDNA (12 % for epilimnion and 11 % for metalimnion; Fig. SI5.1c, d).

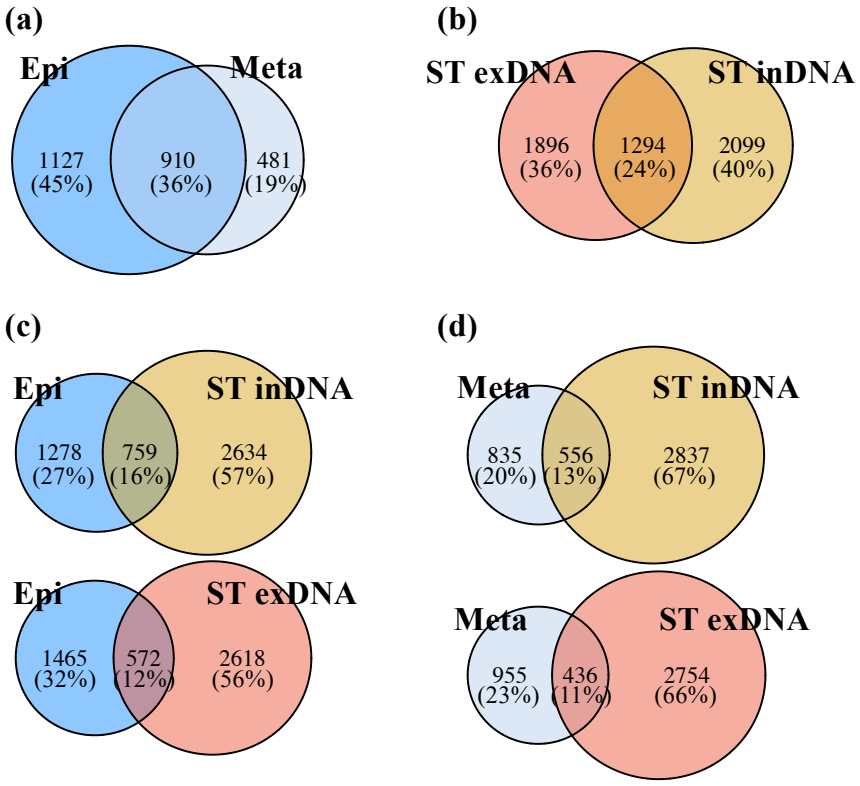


Figure SM5.1. Number and proportion of shared micro-eukaryotic ASVs among DNA sample matrices. (a) Epilimnion versus metalimnion; (b) ST exDNA versus ST inDNA; (c) Epilimnion versus ST inDNA and exDNA; (c) Metalimnion versus ST inDNA and ST exDNA.

Table SM5.1. Number of shared ASVs with and without shared crustacean and diatom ASVs between different DNA sample matrix combinations.

Matrice combinations	Shared ASVs	Shared ASVs without shared crustacean and diatom
Water column* – ST inDNA – ST exDNA	492	428
Epilimnion – ST inDNA – ST exDNA	444	381
Metalimnion – ST inDNA – ST exDNA	221	209
Epilimnion – Metalimnion – ST inDNA – ST exDNA	291	255

*Water column: epilimnion and metalimnion were combined together

Table SM5.2. RV coefficients to quantify the congruence between PCA site scores of the water column matrices (epilimnion and metalimnion combined), ST inDNA and ST exDNA for the entire micro-eukaryotic communities and for the shared ASVs (excluding shared diatoms and crustacean ASVs). The significant correlations are indicated in bold.

		Micro-eukaryote ASVs				**Shared micro-eukaryote ASVs**			
Matrix A	**Matrix B**	**RV coefficient of 1st axis of sites scores**	**P-value**	**RV coefficient of 3 first PCA axes site scores**	**P-value**	**RV coefficient of 1st axis of sites scores**	**P-value**	**RV coefficient of 3 first PCA axes site scores**	**P-value**
Site scores from ASV - Water Column	Site scores from ASV - STinDNA	**0.52**	**<0.0001***	**0.5**	**<0.0001***	**0.63**	**<0.0001***	**0.57**	**<0.0001***
Site scores from ASV - Water Column	Site scores from ASV - STexDNA	**0.2**	**0.01***	**0.37**	**<0.0001***	0.11	0.066	**0.51**	**<0.0001***
Site scores from ASV - STinDNA	Site scores from ASV - STexDNA	**0.72**	**<0.0001***	**0.54**	**<0.0001***	0.02	0.49	**0.56**	**<0.0001***

Table SM5.3. Taxonomy of the 381 shared ASVs for the dataset epilimnion, ST inDNA and ST exDNA. All identified phyla are included with only the subphyla and classes with the majority of ASVs within each phylum. The phyla, sub-phyla and classes containing the most abundant shared ASVs are indicated in bold.

Phylum	Nb of ASVs	% of ASVs	Subphylum	Nb of ASVs	% of ASVs	Class	Nb of ASVs	% of ASVs
Alveolata	**67**	**17.6%**	**Ciliophora**	**38**	**10.0%**	Litostomatea	13	3.4%
						Spirotricea	14	3.7%
			Dinoflagellata	16	4.2%	Dinophyceae	15	3.9%
Amoebozoa	3	0.8%						
Apusozoa	3	0.8%						
Archaeplastida	25	6.6%	Chlorophyta	15	3.9%	Chlorophyceae	8	2.1%
			Streptophyta	10	2.6%	Embryophyceae	8	2.1%
Eukaryota unclassified	**59**	**15.5%**						
Excavata	2	0.5%						
Hacrobia	28	7.4%	Cryptophyta	16	4.2%	Cryptophyceae	16	4.2%
Opisthokonta	**98**	**25.7%**	Chanoflagellida	8	2.1%			
			Fungi	**50**	**13.1%**	**Chytridiomycota**	**23**	**6.0%**
						Fungi unclass.	10	2.6%
			Metazoa	24	6.3%	Rotifera	15	3.9%
Rhizaria	29	7.6%	Cercozoa	28	7.4%	Cercozoa unclass.	13	3.4%
						Filosa-Imbricatea	7	1.8%
Stramenopiles	**67**	**17.6%**	**Ochorophyta**	**40**	**10.5%**	**Chyrsophyceae**	**22**	**5.8%**
						Ochrophyta unclass.	11	2.9%
			Stramenopiles	24	6.3%	Bioecea	4	1.0%
						MAST	7	1.8%
						Oomycota	9	2.4%

Table SM5.4. Taxonomy of the 206 shared ASVs for the dataset metalimnion, ST inDNA and ST exDNA. Only the dates when there was thermal stratification in the lake were included. All identified phyla are included with only the subphyla and classes with the majority of ASVs within each phylum. The phyla, sub-phyla and classes containing the most abundant shared ASVs are indicated in bold.

Phylum	Nb of ASVs	% of ASVs	Subphylum	Nb of ASVs	% of ASVs	Class	Nb of ASVs	% of ASVs
Alveolata	**37**	**18.0%**	**Ciliophora**	**17**	**8.3%**	Litostomatea	6	2.9%
						Spirotricea	5	2.4%
			Dinoflagellata	13	6.3%	**Dinophyceae**	**12**	**5.8%**
Amoebozoa	2	0.6%						
Apusozoa	4	1.3%						
Archaeplastida	11	5.3%	Chlorophyta	8	3.9%	Chlorophyceae	4	1.9%
						Trebouxiophyceae	4	1.9%
Eukaryota unclassified	**28**	**13.6%**						
Hacrobia	18	8.7%	Cryptophyta	10	4.9%	Cryptophyceae	10	4.9%
Opisthokonta	**41**	**19.9%**	**Fungi**	**22**	**10.7%**	**Chytridiomycota**	**15**	**7.3%**
			Metazoa	6	2.9%	Rotifera	5	2.4%
			Opisthokonta unclass.	9	4.4%			
Rhizaria	23	11.2%	**Cercozoa**	**23**	**11.2%**	Cercozoa unclass.	10	4.9%
						Filosa-Imbricatea	5	2.4%
						Filosa-Thecofilosea	4	1.9%
Stramenopiles	**42**	**20.4%**	**Ochorophyta**	**25**	**12.1%**	**Chyrsophyceae**	**15**	**7.3%**
			Stramenopiles	15	7.3%	MAST	4	1.9%
						Oomycota	6	2.9%

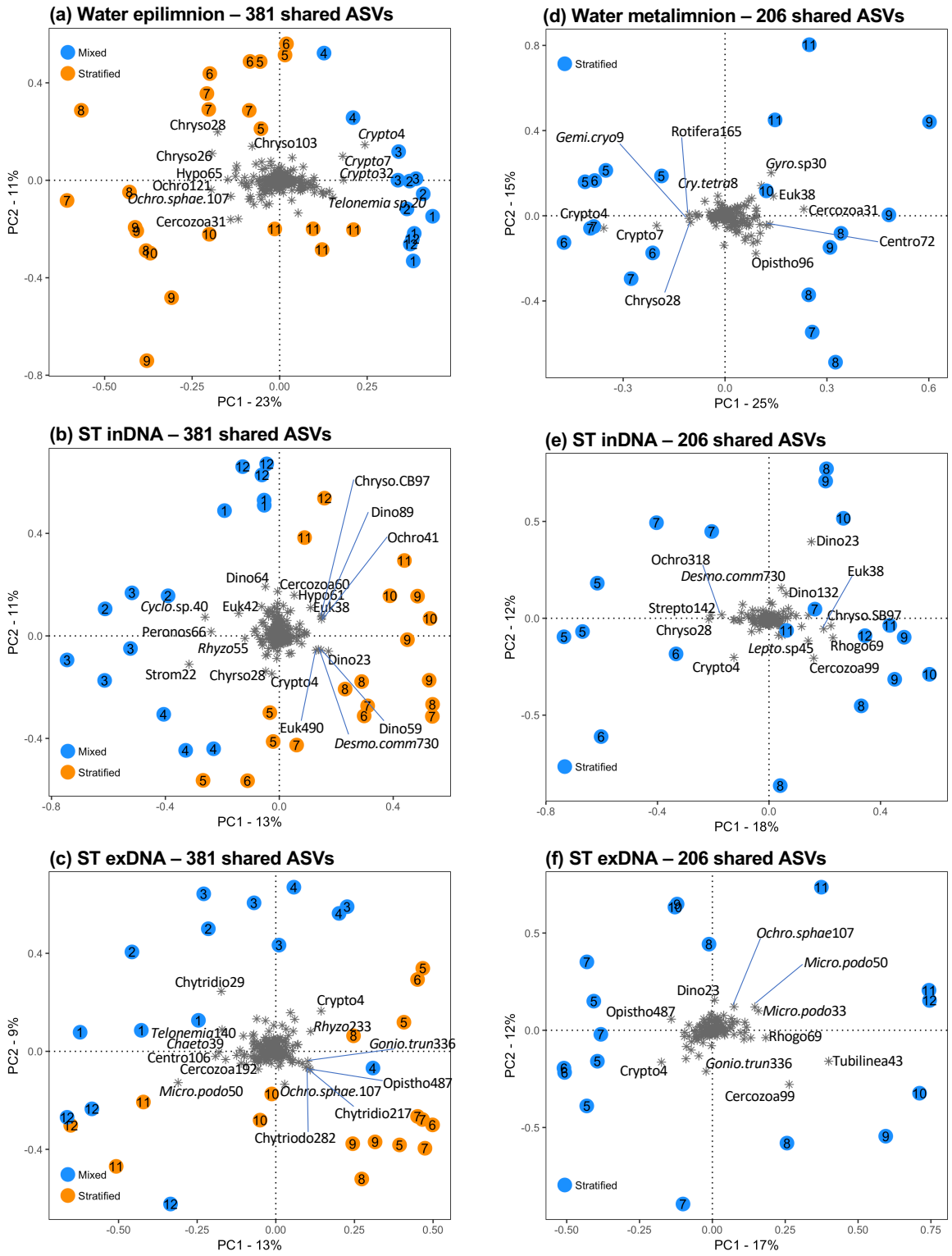

(a) Water epilimnion – 381 shared ASVs
Mixed
Stratified
Chryso28
Chryso103
Crypto4
Chryso26
Crypto7
Hypo65
Crypto32
Ochro121
Ochro.sphae.107
Telonemia.sp.20
Cercozoa31
PC1 - 23%
PC2 - 11%
(d) Water metalimnion – 206 shared ASVs
Stratified
Gemi.cryo9
Rotifera165
Gyro.sp30
Cry.tetra8
Euk38
Crypto4
Cercozoa31
Crypto7
Centro72
Opistho96
Chryso28
PC1 - 25%
PC2 - 15%
(b) ST inDNA – 381 shared ASVs
Chryso.CB97
Dino89
Ochro41
Dino64
Cercozoa60
Hypo61
Euk38
Cyclo.sp.40
Euk42
Peronos66
Rhyzo55
Dino23
Strom22
Chyrso28
Crypto4
Euk490
Dino59
Desmo.comm730
Mixed
Stratified
PC1 - 13%
PC2 - 11%
(e) ST inDNA – 206 shared ASVs
Dino23
Ochro318
Desmo.comm730
Euk38
Dino132
Strepto142
Chryso.SB97
Chryso28
Lepto.sp45
Rhogo69
Crypto4
Cercozoa99
Stratified
PC1 - 18%
PC2 - 12%
(c) ST exDNA – 381 shared ASVs
Chytridio29
Crypto4
Rhyzo233
Telonemia140
Chaeto39
Gonio.trun336
Centro106
Cercozoa192
Opistho487
Micro.podo50
Ochro.sphae.107
Chytridio217
Chytriodo282
Mixed
Stratified
PC1 - 13%
PC2 - 9%
(f) ST exDNA – 206 shared ASVs
Ochro.sphae107
Micro.podo50
Dino23
Opistho487
Micro.podo33
Rhogo69
Crypto4
Gonio.trun336
Tubilinea43
Cercozoa99
Stratified
PC1 - 17%
PC2 - 12%

Figure SM5.2. Shared ASV PCA biplots for the combination of matrices of (a) epilimnion, (b) ST intracellular DNA (ST inDNA), (c) ST extracellular DNA (ST exDNA), and for the combination of (d) metalimnion, (e) ST inDNA, and (f) ST exDNA. Diatom and crustacean ASVs were excluded from the shared ASV analyses. Number of sequences per ASVs were Hellinger transformed prior to ordination. Taxa abbreviations are as follows: Centroheliozoa (Centro); *Chaetonotus* sp. (*Chaeto*); Chrysophyceae (Chryso); Chytridiomycota (Chyrtridio); Cryptophyceae (Crypto); *Cryptomonas tetrapyrenoidosa* (*Cry. tetra*); *Cyclotrichium* sp. (*Cyclo*.); *Desmodesmus communis* (*Desmo. comm.*); Dinophyceae (Dino); *Geminigera cryophila* (*Gemi. cryo*); *Goniomonas truncata* (*Giono.trun.*); *Gyrodinium* sp. (*Gyro.*); Hypotrichia (Hypo); Eukaryota unclassified (Euk); *Leptolegnia* sp. (*Lepto*.); *Micronuclearia podoventralis* (*Micro. podo*); Ochrophyta (Ochro); *Ochromonas sphaerocystis* (*Ochro. sphae*); Opisthokonta (Opistho); Peronosporales (Peronos); Rhogostoma (Rhogo); *Rhyzophidiales* (*Rhyzo.*); Streptophyta (Strepto); Strombidiida (Strom).

Table SM5.5. Taxonomy of the 59 shared ASVs identified as potential bioindicators for both datasets: (1) epilimnion, ST inDNA and ST exDNA; (2) metalimnion, ST inDNA and ST exDNA.

Phylum	Nb of ASVs	Subphylum	Nb of ASVs	Class	Nb of ASVs
Alveolata	17	Alveolata unclassified	1		
		Ciliophora	7	Litostomatea	1
				Oligohymnophora	2
				Spirotricea	4
		Dinoflagellata	9	Dinophyceae	9
Amoebozoa	1	Lobosa	1	Tubulinea	1
Apusozoa	1	Hylonomadea	1	Planomonadidae	1
Archaeplastida	3	Chlorophyta	3	Chlorophyceae	2
				Trebouxiophyceae	1
Eukaryota unclassified	5				
Hacrobia	9	Centroheliozoa	1		
		Cryptophyta	6	Cryptophyceae	6
		Telonemia	2		
Opisthokonta	10	Fungi	5	Chytridiomycota	5
		Mesomycetozoa	1	Ichthyosporea	1
		Metazoa	2	Gastrotricha	1
				Rotifera	1
		Opisthokonta unclass.	2		
Rhizaria	5	Cercozoa	5	Cercozoa unclass.	3
				Filosa-Thecofilosea	2
Stramenopiles	8	Ochorophyta	7	Chyrsophyceae	5
				Ochrophyta unclassified	2
		Stramenopiles	1	Oomycota	1

Chapter 2 – Supplementary Material SM6. Biomass time-series of *A. subarctica*, *S. niagarae* and cryptophytes

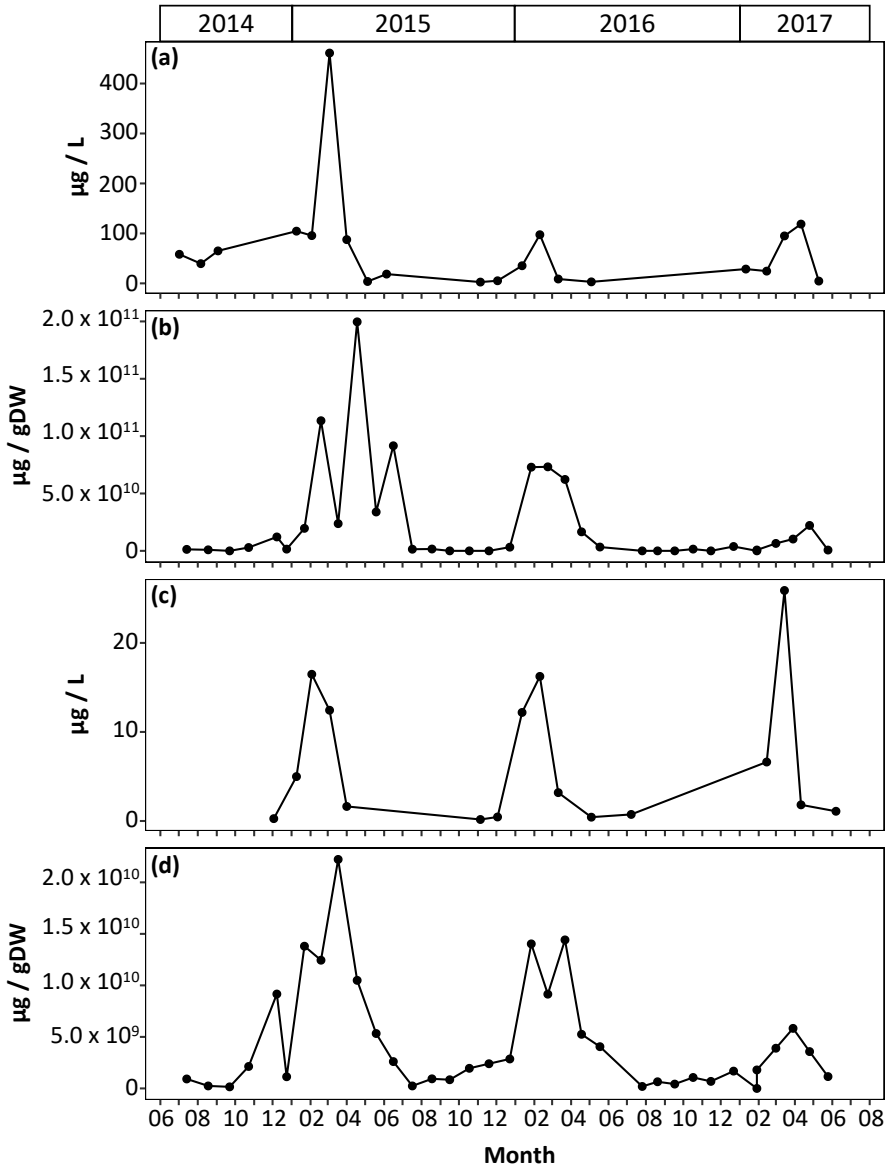


Figure SM6.1. Biovolume time-series of (a) *S. niagarae* in the photic zone of the water column, and (b) in the sediment traps; and (c) *A. subarctica* in the photic zone of the water column, and (d) in the sediment traps.

Chapter 3 – Supplementary Material

Chapter 3 – Supplementary Material SM1. Reconstruction of Cultus Lake surface water temperature

To reconstruct the epilimnetic temperatures of Cultus Lake since the late 1800s, air temperature from Agassiz meteorological station (ID 1100120; (http://climate.weather.gc.ca/historical_data) were used. These latter climate data were used because the records of Cultus Lake station do not go back as far in time. Agassiz is located ~20 km north-east of Cultus Lake and average temperatures from both meteorological stations are highly correlated ($R^2 = 0.99$). A linear regression between the contemporary upper column (0-5 m) water temperatures (2001-2002 CE from Shortreed and 2009-2016 CE unpublished data, DFO Lakes Research Program) and corresponding air temperatures was applied and used to estimate the upper water column temperatures of the entire record. As the lake heat budget is affected by other factors than local air temperature (i.e., radiative and non-radiative heat exchange processes), the upper column water temperatures is likely to respond with a delay in relation to air temperatures (Livingstone and Lotter 1998). Consequently, to reconstruct the water temperatures, the air temperatures were associated with the water temperatures a month later in the linear regression (data on a monthly basis; $R^2 = 0.89$; p-value < 0.001; Fig. SM1).

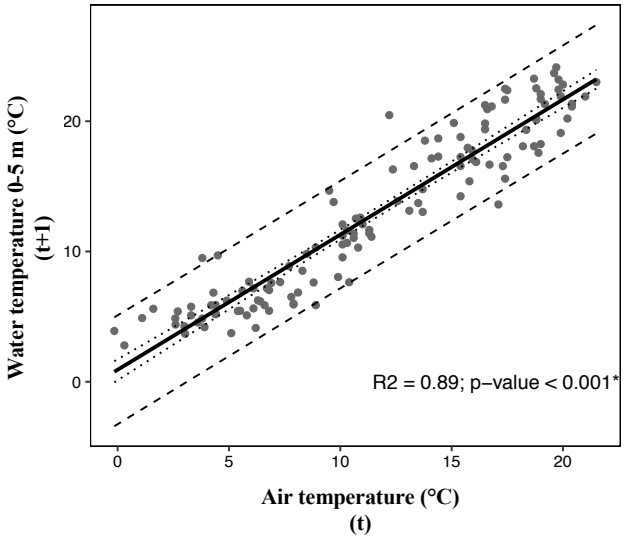


Figure SM1. Upper column (0-5 m) water temperatures in relation to air temperatures. The data of the air temperatures were associated with the water temperatures a month later. The dotted lines represent the 95 % confidence intervals and the dashed lines represent the 95 % prediction intervals.

References

M. Livingstone D, Lotter A (1998) The relationship between air and water temperatures in lakes of the Swiss Plateau. J Paleolimnol 19:181–198. doi: doi:10.1023/A:1007904817619

Chapter 3 – Supplementary Material SM2. History of the Cultus Lake watershed

Table SM2.1 Time of important historical events and disturbances (anthropogenic and climate) in Cultus Lake watershed from ~1850 to present. Anthropogenic disturbances are classified as agriculture, mining, recreation, logging, forest fire, development (related to human expansion in the area). The anthropogenic activities related to salmon research and manipulation are also included in the table.

Year(s)	Event/Disturbance	Category	Ref.
~5000 years ago	Evidence of Chilliwack and Cultus Lake watershed use by First Nations		1
1858	Beginning of Gold rush Use of the trails from Washington state to Cultus Lake.	Mining	2
1859 / 1860	US/Canada border laid out by Royal Engineers.	Development	3
1870 - 1880	Camping at Cultus Lake	Recreation	4
1887	Establishment of the first families in the Columbia Valley	Development	3
1900	Start of logging in the area surrounding Cultus Lake. A road was built for logging that opened the access to Cultus Lake.	Logging	2
1901	Columbia Valley first school established	Development	5
1906	First post office in Columbia Valley – Lindell post office	Development	5
1910	The British Columbia Electric Railway (BCER) completed the construction of a railway line that followed the base of Vedder Mountain along the shore of Sumas Lake.	Development	3
1910	Access to Columbia Valley via Maple Fall (WA, USA) Maple Fall as a booming logging and mining town	Mining / Logging	6
1910	First BC Electric Railway train arrived in Chilliwack (Sardis station). From October 1910 to September 1950, the railway between Chilliwack and Vancouver was running.	Development	7
Prior to 1912	From oral history: “The road was built prior to June 1912” (see p. 35 in Cramer 1992).	Development	5
1912	Provincial government reported that a salmon hatchery would be established around Cultus Lake. Cultus Lake was considered one of the best spawning ground in the Fraser River. - 60,000 sockeye salmon fry released in Cultus Lake - 75,000 sockeye fry released in Sumas lake	Salmon	2, 8

1912	7 additional hatcheries in operation at the end of the year for a total of 58 in operation in BC Millions of sockeye and coho eggs were collected from Sweltzer creek for the Bon Accord and Harrison Lake hatcheries.	Salmon	9
1916	Opening of the salmon hatchery. The hatchery was located on eastern corner of Sweltzer Creek bridge along Columbia Valley Hwy (main hatchery building now the community hall).	Salmon	2
1917	Columbia Valley first school burnt	Fire	5
1919	Second school built in the Columbia Valley established by Campbell River Co. for the children of the workers	Development	5
1920s	Logging reached its peak in the 1920s Connections between Cultus Lake and Chilliwack was established in 1916 when a wagon road was pushed along the lakeside (logging railroad). Campbell River Logging Company commenced logging in the Valley early in the 1920s.	Logging	5, 10
1920s	By the early 1920s, a timber lease of several thousand acres of timbered land in Columbia Valley was purchased. Logging operations in the valley began in 1922 under the name "The Campbell River Timber Co."; company that logged most of the lower regions of the Columbia Valley.	Logging	5
1920	First permanent housing started to appear around Cultus Lake	Development	2
1924	Park of Cultus Lake created and officially opened on Aug. 6 – park board acquired 63.8 acres Brought establishment of general store, bath house, boats	Recreation	2, 11
1924	The old wagon road became unsuitable for the new cars and the railroad on the Canadian side was abandoned.	Development	5
1924	Forest fire breaking out on the logged-off areas of the timber limits of the Campbell Mills Ltd that destroyed the school at Lindell beach	Fire	5, 12
1925	R. Earl Foerster came to run the hatchery and proposed to establish a research field station.	Salmon	2
1926	Establishment of the research station proposed by Foerster	Salmon	2
1926	Thousands of sockeye salmon marked in Cultus Lake.	Salmon	13
1926	2,000 acres land logged off lands in the Columbia Valley, which burned early in the previous last fall. Opportunity to seed the land for agriculture.	Logging / Fire	14
Late 1920s – early 1930s	Stump farming starts in the Columbia Valley: Opportunity for farming emerged and people removed the stumps by setting them on fire. Major cause of fire in the region.	Fire	5
1930	Golf course built; abandoned in 1933	Recreation	2
1931	Cultus Lake considered as favorite playground of British Columbia	Recreation	15
1932	Cultus Lake Park Board formed	Recreation	4
1932	Vedder Logging Company logged from 1932 to 1940 on Vedder Mountain and along Chilliwack River	Logging	5
1934	Sockeye eggs taken as salmon enter Cultus Lake.	Salmon	16

	Harrison lake hatchery has been reopened and eggs are taken daily by truck from Cultus to a maximum of 20 million eggs (capacity of the Harrison hatchery).		
1934	Devil's corner on Cultus Lake road was abandoned and a new road was constructed.	Development	4, 17
Mid-1930s	By the mid-1930s, several acres of land were cleared from stumps and small farms were developed.	Agriculture	5
1937	Space for additional cars in the parking lot provided and Maple trees planted adjacent to the recreational playground.	Recreation	18
1937	Preparations for cutting of 150 million feet of timber in Vedder Mountain limits by Vedder River Logging Co. Logging railway line being built on the north side of the hill of Vedder mountain.	Logging	19
1938	Roller skating rink built (where the tennis courts are today).	Recreation	2
1939	Pavilion built for stores and dance hall; dismantled in 1990 280 cottage owners, almost all summer residents	Recreation	2
1940s	As the community of Lindell Beach established, Frosst Creek was diverted from the middle of the beach to the west side of the beach.	Development	20
1940s	Increasing of permanent populations in Cultus Lake	Development	2
1940s	Growth of the dairy industry in the Columbia Valley Clearing of the land for the expansion of the dairy industry The valley prospered from that industry in the 1950s.	Agriculture	5
1942	During 2nd World War, training base established at Vedder Crossing; receiving over 6,000 troops Cultus Lake evolved from a summer resort to a community	Development	2, 4
1942	Logging around Teapot Hill; camp located near Frosst Creek	Logging	5
1948	Cultus Park board acquired 35 acres from a property called Sunnyside because Westminster Mills failed and closed.	Recreation	2
1948	Fraser Valley flood – Vedder Crossing particularly affected	Climate	2
1948	Creation of Cultus Lake provincial park	Recreation	2, 21
1949	$25,000 invested into the trout hatchery at Smith falls	Salmon	22
1950s	During the 1950s and 1960s, the dairy industry was the major source of income of the Columbia Valley until 1969 when the farm storage of milk had to be in stainless steel tanks. By 1974, only 2 dairy farms in Columbia Valley	Agriculture	5
1950	Lake froze over the winter. Subsequent melt waters flooded part of the lake.	Climate	2
1951	Forest fire from Columbia Valley (starting point) to north and west along the mountain; burnt west to north-west of Lindell beach, high above Cultus Lake	Fire	23
1951	A little gravel road on the side of the lake went through the Columbia Valley.	Development	24
1953	3 room school built in Cultus Lake	Development	2

1955	10 miles of light and power line extension from Lindell Beach into the Columbia Valley	Development	24
1956	Hydro power reached the Columbia Valley	Development	5
1956	167,000 visitors to Cultus Lake during the year	Recreation	26
1957	Road improvements in the Columbia Valley	Development	5
1957	Marked trout returned to Sweltzer Creek Fish were released from Sweltzer Creek in March 1954, 1955 and 1956; a total of 20,000 fish were released	Salmon	27
1957	Recreational demand in Cultus Lake growing rapidly 180 units addition to Cultus campsites	Recreation	28
1958	Telephone reached the Columbia Valley	Development	5
1958	Brush fire swept through 15 acres of hay field surrounding Sardis	Fire	29
1960	~3,000 fish were accidentally killed by the poison released to combat swimmer's itch The fish killed were part of a scientific experiment conducted by Fisheries and Oceans Canada (DFO) and they lost essential data for their experiment.	Salmon	30
1961	34 acres of bush transformed into campsites and fishing area around Cultus Lake	Recreation	31
1962	Cultus Lake Salmon Research lab built – deal with all salmon species	Salmon	2
1970	Lakeside Marina built on site of former provincial trout hatchery	Development	2
1974	The former hatchery became the community hall after renovation.	Development	32
1979	Twister-like wind wrecks road and houses in Cultus Lake.	Climate	33
1983	Public participated in lake milfoil control campaign.	Invasive species	34
1985	Cultus Lake Water Park and Waterslides built	Recreation	2
1988	New plaza built, burnt down and rebuilt the same year	Development	2
1990	Golf course established; 40 acres golf course opened near Sunnyside campground	Recreation	2, 35
2001	Cultus Lake Salmon Research lab turned into a fish hatchery. In 2000-2001, *Parvicapsula minibicornis* disease attacking fish kidney caused mortalities in 2/3 of the Cultus Lake sockeye salmon spawners. The sockeye run was earlier that year, which let the time for the disease to kill spawners before they spawned.	Salmon	36
2002	Fish came back early and died before spawning from the same disease than the previous years; problem everywhere in the Fraser Valley	Salmon	37
2002	DFO officials approved native commercial fisheries	Salmon	38, 39
2003	Earlier and earlier run of sockeye coming back to Cultus Lake	Salmon	40
2004	Tagging pikeminnow to know their abundance in the lake; pikeminnow are predators of sockeye fry	Salmon	41
2004	Sockeye fishery had to be closed to protect the Cultus Lake run.	Salmon	42

2004	Cultus Lake sockeye salmon population designated as endangered by COSEWIC, but not federally protected under the Species at Risk Act (SARA) because of high socio-economic cost.	Salmon	43
2005	460 permanent residents and seasonal owners around Cultus Lake	Development	2
2005	Activities at the Vedder Mountain quarry approved by the Ministry of Energy and Mines the previous summer. Amendment to the mining license allows removal of up to 245,000 tonnes/year.	Development	44
2006	Milfoil removal program – $40,000 invested for this project	Invasive species	45
2007	Northern pikeminnow removal – goal of 18,000 fish removal because they prey on sockeye fry	Invasive species	46
2007	Plan for expansion of Cultus Lake water park approved by Cultus Lake park board	Recreation	47

References

1. Schaepe DM, Ts'elxwéyeqw Tribe (eds) (2017) Being Ts'elxwéyeqw: First Peoples' voices and history from the Chilliwack-Fraser Valley, British Columbia, Harbour Publishing, Madeira Park, British Columbia, Canada
2. Soutar M (2005) Cultus Lake: A natural paradise. Chilliwack Museum and Archives, Chilliwack, Canada
3. Chilliwack Museum and Archives, Subject Files, Columbia Valley, 971.137. From Chilliwack museum exhibit.
4. Chilliwack Museum and Archives, Subject Files, Cultus Lake, 971.137.
5. Cramer FH (2005) The valley beyond: a history of Columbia Valley & Lindell Beach. Columbia Valley Ratepayers Association, Lindell Beach, Canada
6. Chilliwack Progress, January 26, 1910, p.5. Chilliwack Museum and Archives.
7. Chilliwack Museum and Archives, Subject Files, Chilliwack Archives Photograph Database, 778.3.
8. Chilliwack progress, May 15, 1912, p.1. Chilliwack Museum and Archives.
9. Chilliwack progress, November 27, 1912, p.1. Chilliwack Museum and Archives.
10. Chilliwack progress, January 30, 1974, p.4. Chilliwack Museum and Archives.
11. Chilliwack progress, August 6, 1924. Chilliwack Museum and Archives.
12. Chilliwack progress, May 28, 1924, p.6. Chilliwack Museum and Archives.
13. Chilliwack progress, March 31, 1926. Chilliwack Museum and Archives.
14. Chilliwack progress, April 14, 1926, p.1. Chilliwack Museum and Archives.
15. Chilliwack progress, June 4, 1931. Chilliwack Museum and Archives.
16. Chilliwack progress, November 22, 1934. Chilliwack Museum and Archives.
17. Chilliwack progress, April 19, 1934. Devil's Corner on lake road to be abandoned. Chilliwack Museum and Archives.
18. Chilliwack progress, January 20, 1937, p.1. Chilliwack Museum and Archives.
19. Chilliwack progress, June 9, 1937, p.1. Chilliwack Museum and Archives.
20. Cultus Sockeye Recovery Team. 2005. Conservation strategy for sockeye salmon (*Oncorhynchus nerka*), Cultus Lake population, in British Columbia. Recovery of Nationally Endangered Wildlife (RENEW). Ottawa, Ontario, 48 pp.
21. British Columbia Parks. Cultus Lake Provincial Park. Accessed on May 5, 2017. http://www.env.gov.bc.ca/bcparks/explore/parkpgs/cultus_lk/
22. Chilliwack progress, March 30, 1949, p.7. Chilliwack Museum and Archives.
23. Chilliwack progress, April 18, 1951, p.1. Chilliwack Museum and Archives.
24. Chilliwack progress, October 22, 1975. Chilliwack Museum and Archives.
25. Chilliwack progress, January 5, 1955, p.5. Chilliwack Museum and Archives.
26. Chilliwack progress, December 11, 1957. Chilliwack Museum and Archives.
27. Chilliwack progress, April 3, 1957, p.2. Chilliwack Museum and Archives.
28. Chilliwack progress, January 5, 1955, p.5. Chilliwack Museum and Archives.
29. Chilliwack progress, July 16, 1958, p.5. Chilliwack Museum and Archives.
30. Chilliwack progress, July 5, 1960. Poison Freak kills 3,000 Fish. Chilliwack Museum and Archives.
31. Chilliwack progress, June 6, 1961, p.5. Chilliwack Museum and Archives.
32. Chilliwack progress, February 3, 1974, p.8b. Chilliwack Museum and Archives.

33. Chilliwack progress, February 21, 1979, p.7c. Chilliwack Museum and Archives.
34. Chilliwack progress, August 10, 1983, Section C. Chilliwack Museum and Archives.
35. Chilliwack progress, January 17, 1990. Chilliwack Museum and Archives.
36. Chilliwack times, November 12, 2001, p.7. Chilliwack Museum and Archives.
37. Chilliwack times, February 1, 2002, p.4. Chilliwack Museum and Archives.
38. Chilliwack progress, August 13, 2002, p.3. Chilliwack Museum and Archives.
39. Chilliwack times, August 13, 2002, p.2. Chilliwack Museum and Archives.
40. Chilliwack progress, July 4, 2003, p.6. Chilliwack Museum and Archives.
41. Chilliwack progress, May 21, 2004, p.14. Chilliwack Museum and Archives.
42. Chilliwack times, August 17, 2004, p.7. Chilliwack Museum and Archives.
43. Chilliwack progress, October 26, 2004, p.7. Chilliwack Museum and Archives.
44. Chilliwack times, February 11, 2005. Chilliwack Museum and Archives.
45. Chilliwack progress, March 10, 2006, p.3. Chilliwack Museum and Archives.
46. Chilliwack progress, May 22, 2007, p.7. Chilliwack Museum and Archives.
47. Chilliwack progress, March 2, 2007. Chilliwack Museum and Archives.

Table SM2.2. Year and burnt area (m^2) of each forest fire occurred in the Cultus Lake watershed from 1920 to 2013 along with the cause and geographic coordinates. Data accessed iMapBC application online*.

Fire ID§	Year	Cause	Area burned (m^2)	Latitude	Longitude
16	1935	Human	54520.27	49.03670	122.03423
21	1931	Human	4251715.41	49.02094	122.03442
48	1935	Human	76545.54	49.01202	122.03171
49	1935	Human	11263.82	49.02868	122.02415
79	1938	Human	7669919.54	49.02716	122.99853
85	1934	Human	93137.02	49.02717	122.02687
137	1941	Human	693629.36	49.06421	122.00918
227	1934	Human	3525593.28	49.04256	122.06361
252	1947	Human	3350.64	49.03316	122.03882
266	1941	Human	2757985.42	49.05415	122.02527
381	1938	Human	45335.25	49.01391	122.03989
425	1946	Human	699076.53	49.04530	121.92938
651	1938	Human	25341.82	49.06539	122.01846
55a	1932	Human	27040.52	49.02406	122.02183
V00009	1956	Human	1388892.33	49.01374	122.07086
V00009	1956	Human	1531585.93	49.01680	121.94346
V00056	1951	Human	2745442.25	49.07910	121.95421
V00088	1951	Human	20356520.51	49.03715	122.06015
V00309	1958	Human	250799.77	49.01258	121.97270
V00427	1965	Human	1397545.23	49.01306	122.10718
V10027	1982	Human	130856.39	49.04033	122.01999
V10048	1987	Human	1120116.05	49.03999	122.02503
V10050	1987	Human	312602.27	49.04507	122.03544
V10050	1987	Human	612680.40	49.02883	122.08921
V10217	2013	Human	20017.26	49.04504	122.00760

*Province of BC. Data from iMapBC application. https://maps.gov.bc.ca/ess/hm/imap4m/?catalogLayers=1756,1757,1758&scale=400000.0&center=-13161319.5843,6668201.90948. Accessed on April 28, 2017.
§ Fire IDs are from the iMapBC application.

Chapter 3 – Supplementary Material SM3. Historical and contemporary limnological parameters

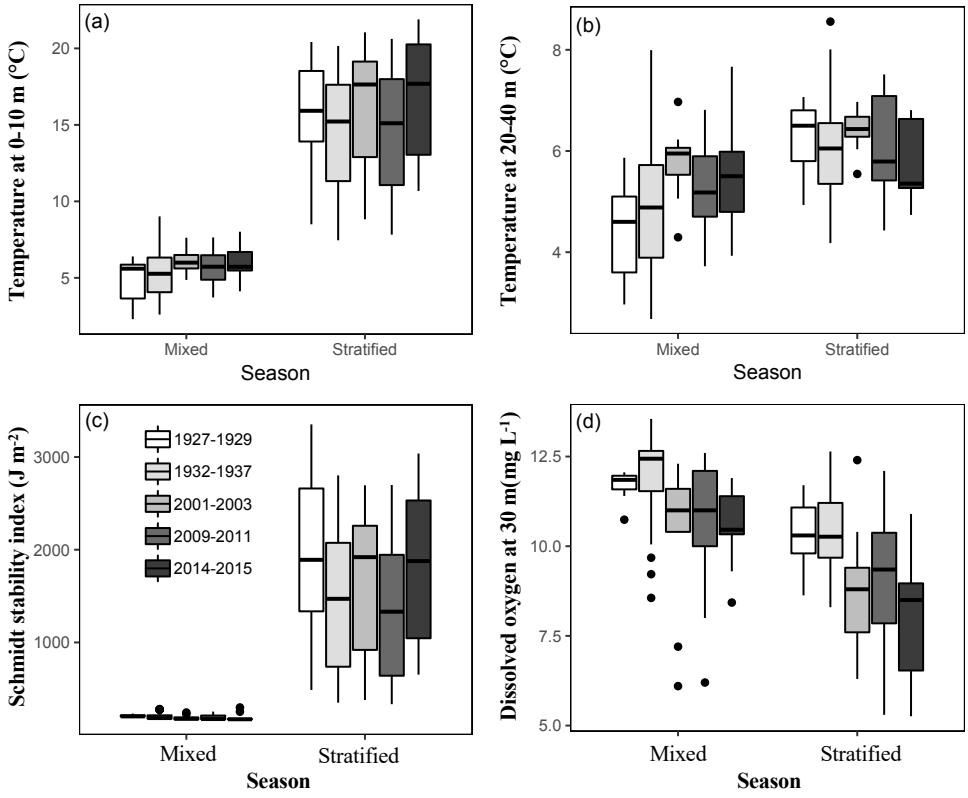


Figure SM3. Boxplots of comparable limnological variables between historical and contemporary monitoring of Cultus Lake. (a) Average water temperature at 0-10 m; (b) Average water temperature at 20-40 m; (c) Schmidt stability index; (d) Dissolved oxygen at 30 m. The periods of limnological monitoring compared are: 1) 1927-1929 CE; 2) 1932-1937 CE; 3) 2001-2003 CE; 4) 2009-2011 CE, and 5) 2014-2015 CE.

Table SM3. Seasonal average (± SE) of contemporary (2014-2015 CE) physico-chemical and biological parameters of Cultus Lake, British Columbia for winter (December to February), spring (March to May), summer (June to August) and autumn (September to November); a) Water column parameters: Schmidt stability index (SSI), surface temperature, temperature at 30 m, hypolimnetic dissolved oxygen (DO) and euphotic zone depth; b) Epilimnetic and c) Metalimnetic nutrients (total phosphorus (TP), total nitrogen (TN) and dissolved inorganic nitrogen (DIN)), chlorophyll *a* and cyanobacterial biomasses for the photic zone (measured with a Secchi disk). NA in the table means that the SE could not be calculated as only one measurement was available for the given period.

Parameters	Winter		Spring		Summer		Autumn	
	2014	2015	2014	2015	2014	2015	2014	2015
a) Water column parameters								
SSI (J m^{-2})	170.9 (0.9)	171.6 (2.8)	359.5 (148.7)	424.9 (193.4)	796.3 (459.7)	673.9 (389.1)	1838.7 (490.6)	1649.4 (359.5)
Surface temp. (°C)	5.3 (0.6)	6.3 (0.7)	8.2 (2.5)	8.9 (2.0)	20.7 (2.1)	22.3 (1.4)	17.6 (2.4)	16.4 (1.8)
Temp. 30m (°C)	5.3 (0.6)	6.2 (0.8)	4.4 (0.2)	6.0 (0.2)	5.0 (0.1)	6.6 (0.1)	5.3 (0.1)	6.7 (0.1)
Hypo. DO (mg L^{-1})	10.4 (0.6)	9.7 (1.1)	11.5 (0.3)	10.2 (0.6)	9.3 (0.6)	8.3 (0.3)	7.6 (1.0)	5.9 (0.4)
Euphotic zone depth (m)	5.4 (0.2)	7.0 (0.4)	8.2 (1.2)	7.4 (1.1)	8.1 (0.7)	7.5 (0.8)	8.9 (0.2)	8.6 (0.7)
b) Epilimnion – photic zone								
TP (µg L^{-1})	7.6 (0.4)	7.9 (0.1)	5.4 (1.7)	5.8 (1.0)	4.2 (0.5)	4.0 (0.6)	4.9 (0.7)	7.1 (1.0)
TN (µg L^{-1})	240.2 (0.3)	270.8 (7.5)	235.3 (14.7)	253.8 (26.1)	159.3 (12.5)	211.1 (6.7)	155.9 (14.3)	206.5 (12.2)
DIN (µg L^{-1})	124.8 (4.1)	144.7 (8.2)	108.4 (12.5)	135.0 (3.7)	38.9 (24.8)	19.6 (11.7)	9.7 (4.2)	8.8 (4.3)
Chl *a* (µg L^{-1})	1.5 (0.1)	1.6 (0.4)	2.3 (1.0)	2.5 (0.2)	1.9 (0.5)	2.8 (1.6)	1.8 (0.2)	1.8 (0.2)
Cyano biomass (mg m^{-3})	2.5 (1.3)	0.8 (0.7)	0	0.1 (0.1)	49.0 (9.8)	66.3 (16.5)	24.0 (1.8)	11.1 (1.0)
c) Metalimnion – photic zone								
TP (µg L^{-1})	-	-	3.8 (NA)	5.7 (NA)	7.0 (0.7)	7.2 (0.7)	7.5 (0.9)	9.2 (0.6)
TN (µg L^{-1})	-	-	192.0 (NA)	281.9 (NA)	217.3 (7.9)	241.0 (12.9)	223.5 (29.7)	230.5 (24.1)
DIN (µg L^{-1})	-	-	85.8 (NA)	155.4 (NA)	64.1 (26.4)	39.1 (23.2)	56.48 (19.4)	32.1 (19.8)
Chl *a* (µg L^{-1})	-	-	2.19 (NA)	2.7 (NA)	4.1 (1.3)	7.2 (1.4)	3.6 (1.2)	2.8 (0.9)
Cyano biomass (mg m^{-3})	-	-	0 (NA)	0.6 (NA)	58.0 (34.2)	42.1 (5.1)	49.1 (21.3)	19.2 (7.3)

Chapter 3 – Supplementary Material SM4. Sediment age calculation

The constant rate of supply (CRS) was used to determined age of every interval measured with ^{210}Pb following Binford (1990). The unsupported ^{210}Pb reached a negative value at the interval 22.75-23 cm, which defined the background level and subsequent intervals were removed from the sediment age calculation (the three deepest sediment intervals were removed). The confidence intervals of the sediment age were determined following Binford (1990). Two different polynomial fits were applied through the top of the core to calculate the sediment age, one of 2nd order and one of 3rd order. To select the best sediment core age-depth model to calculate the age through the core, the Bayesian Information Criterion (BIC) was calculated for each model. Even though the 3rd order polynomial fit was identified as the best model (103.05 for the 2nd order polynomial versus 96.37 for the 3rd order polynomial), an average of both models was calculated to accurately extrapolate the sediment age beyond the background level. The age of each interval was then calculated with this model from the top to the bottom of the core.

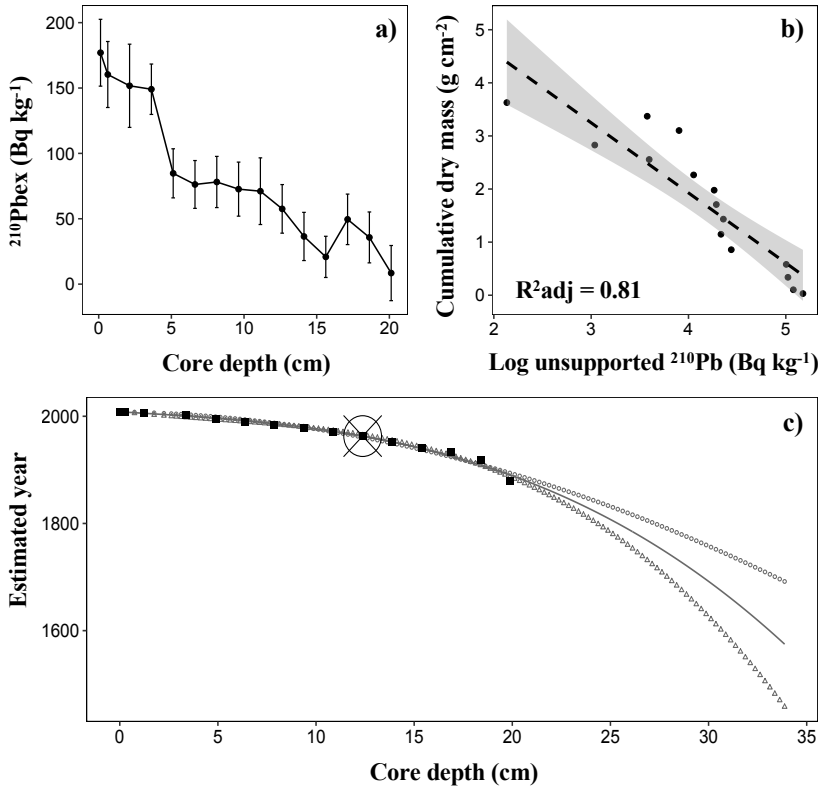


Figure SM4. The radioisotopic ^{210}Pb age model for the gravity core of Cultus Lake, British Columbia, Canada. (a) Unsupported ^{210}Pb activity with core depth. All the data shown here were used for the sediment age calculations. The sediment intervals below 20 cm are not illustrated in the figure as they are beyond the ^{210}Pb background level. (b) Correlation between cumulative dry mass and unsupported ^{210}Pb. (c) Sediment core age-depth model based on both 2nd polynomial fit (BIC = 103.05; R_{adj}^2 = 0.98; open circle) and 3rd polynomial fit (BIC = 96.37; R_{adj}^2 = 0.99; open triangle). The line represents the average between the two polynomial models and the black square are the ^{210}Pb dates from the CRS model. The calculated ^{210}Pb date corresponds well with the ^{137}Cs peak (occurring at 12.375 cm; shown by the circled X).

Table SM4. CRS model estimated sediment age and age error of each dated interval throughout the core.

Interval (cm)	Estimated year	Age error (year)
0-0.5	2007.96	3.39
1-1.5	2006.48	3.44
3.25-3.5	2001.52	3.69
4.75-5	1995.66	4.06
6.25-6.5	1989.57	4.51
7.75-8	1984.15	4.95
9.25-9.5	1978.05	5.55
10.75-11	1971.00	6.41
12.25-12.5	1962.62	7.72
13.75-14	1951.97	9.88
15.25-15.5	1940.96	12.77
16.75-17	1932.55	15.29
18.25-18.5	1918.14	21.73
19.75-20	1879.07	65.96

Cladoceran remain flux calculations

1- Calculation of sediment rate

Sedimentation rate (cm yr^{-1}) = (Depth sediment interval x + 1 - Depth sediment interval x) / (Date sediment interval x - Date sediment interval x +1)

To calculate the sedimentation rate, the average date from the 2nd and 3rd order polynomial models was used.

2- Calculation of cladoceran remain concentration

Remain concentration (# cm^{-3}) = # remains / (g sediment analyzed / sediment density (g cm^{-3}))

3- Calculation of remain flux

Remain flux (# cm^{-2} yr^{-1}) = remain concentration (# cm^{-3}) / sed. rate (cm yr^{-1})

Chapter 3 – Supplementary Material SM5. Diatom taxa grouped for Principal Component Analysis

Table SM5. Taxa groups prior to Principal Component Analysis (PCA); a) Species with a relative abundance ≥ 1% in at least three different intervals; b) Other species grouped by morphological and functional groups to have a relative abundance ≥ 1% in at least three different intervals.

Groups

a) Species abundance ≥ 1% in at least 3 different intervals

23. *Achnanthidium minutissimum* (Kützing) Czarnecki 1994
24. *Amphora pediculus* (Kützing) Grunow in Schmidt et al. 1875
25. *Asterionella formosa* Hassall 1850
26. *Aulacoseira ambigua* (Grunow) Simonsen 1979
27. *Aulacoseira subarctica* (Otto Müller) Haworth 1990
28. *Lindavia comensis* (Grunow) Nakov, Guillory, M.L. Julius, E.C.Ther. and A.J.Alverson 2015 (previously *Cyclotella comensis* Grunow in Van Heurck 1882
29. *Lindavia michiganiana* (Skvortzov) Nakov, Guillory, M.L. Julius, E.C.Ther. and A.J.Alverson 2015 (previously *Cyclotella michiganiana* Skvortzow 1937)
30. *Discostella stelligera* (Cleve and Grunow) Houk and Klee 2004 / *Discostella pseudostelligera* (Hustedt) Houk and Klee 2004
31. *Lindavia intermedia* (Manguin ex Kociolek and Reviers) Nakov et al. ex Daniels et al. 2016 (previously *Cyclotella bodanica* var. *intermedia* Manguin ex Kociolek and Reviers 1996)
32. *Pseudostaurosira brevistriata* (Grunow) D.M.Williams and Round 1987
33. *Stephanodiscus medius* H. Håkansson 1986
34. *Stephanodiscus minutulus* (Kützing) Cleve & Möller 1882 / *Stephanodiscus parvus* Stoermer & Håkansson 1984

b) Grouped taxa to reach ≥ 1% in at least 3 different intervals

35. Assymetrical biraphids (*Amphora pediculus* excluded)
    Amphora copulata / *Amphora minutissima* / *Amphora ovalis* / *Amphora* sp. / *Cymbella cymbiformis* / *Cymbella. frequens* / *Cymbella c.f. turgida* / *Encyonema minutus* / *Encyonema* sp. /*Encyonopsis descripta* / *Encyonopsis subminuta* / *Gophonema angustatum* / *Halamphora thumensis* / *Hippodonta capitata* / *Hippodonta hungarica* / *Hippodonta* sp. / *Rhoicosphenia* sp.
36. Symmetrical biraphids
    Amphipleura pellucida/ *Brachysira neoexilis* / *Cavinula scutelloides* / *Craticula riparia* / *Diploneis elliptica* / *Diploneis onblogella* / *Eolimna* sp. / *Geissleria acceptata* / *Navicula crytocephala* / *Navicula cincta* / *Navicula crytotenella* / *Navicula menisculus var. upsaliensis* / *Navicula onbloga* / *Navicula praeterita* / *Navicula radiosa* / *Navicula c.f. uternoehlii* / *Navicula viridula* / *Navicula vulpina* / *Navicula* sp. / *Pinnularia* sp. / *Stauroneis c.f. anceps* / *Stauroneis* sp.
37. Monoraphids (*Achnanthidium minutissimum* excluded)
    Achnanthidium exiguum / *Achnanthidium c.f. kriegeri* / *Achnanthidium pyrenaicum* / *Achnanthidium rivulare* / *Achnanthidium c.f. rosenstockii* / *Achnanthidium subhudsonis* / *Cocconeis neothumensis* / *Cocconeis placentula* / *Cocconeis pseudothumensis* / *Gliwiczia c.f. calcar* / *Karayevia clevei* / *Karayevia laterostrata* / *Karayevia sp.* / *Karayevia c.f. suchlandtii* / *Planothidium* sp. / *Planothidium conspicua* / *Planothidium dubium* / *Planothidium frequentissimum* / *Planothidium haynaldii* / *Planothidium hustedtii* / *Planothidium joursacence* / *Planothidium c.f. stewartii* / *Planothidium zeigleri* / *Psammothidium c.f. curtissiumum* / *Rossithidium pusillum*
38. Small araphids
    Diatoma mesodon / *Pseudostaurosira neoelliptica* / *Pseudostaurosira parasitica* / *Pseudostaurosira robusta* / *Punctastriata c.f. pinnata* / *Staurosira construens* / *Staurosira construens var. binodis* / *Staurosira construens var. venter* / *Staurosirella lapponica* / *Staurosirella leptostauron* / *Staurosirella martyi* / *Staurosirella pinnata*
39. Long araphids
    Fragilaria capucina / *Fragilaria crotonensis* / *Fragilaria gracilis* / *Fragilaria mesolepta* / *Fragilaria nanana* / *Fragilaria radians* / *Fragilaria* sp. / *Fragilaria synegrotesca* / *Fragilaria tenera* / *Fragilaria vaucheriae* / *Hannea arcus* / *Synedra cyclopum* / *Synedra c.f. famelica* / *Tabularia fasciculate* / *Tabellaria fenestrate*
40. Other benthics
    Epithemioid: *Epithemia adnate* / *Epithemia c.f. sorex* / *Rhopalodia gibba* / *Denticula tenuis*
    Nitzschioid: *Nitzschia c.f. gracilis* / *Nitzschia communis* / *Nitzschia fonticola* / *Nitzschia recta* / *Nitzschia paleo var. tenuirostris* / *Nitzschia sinuata var. tabellaria* / *Tryblionella* sp.
    Surirelloid: *Entomoneis paludosa* / *Surirella amphioxys* / *Surirella* sp.

References used for diatom identification

Cumming BF, Wilson SE, Hall RI, Smol JP (1995) Diatoms from British Columbia (Canada) lakes and their relationship to salinity, nutrients and other limnological variables. Cramer in der Gebr.-Borntraeger-Verl.-Buchh, Berlin

Hofmann G, Werum M, Lange-Bertalot H (2011) Diatomeen im Süßwasser - Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten Arten und ihre Ökologie. Koeltz Scientific Books, Königstein

Houk V (2003) Atlas of freshwater centric diatoms with a brief key and descriptions. 1: Melosiraceae, Orthoseiraceae, Paraliaceae and Aulacoseiraceae. Czech Phycological society, Praha

Houk V, Klee R, Tanaka H (2010) Atlas of freshwater centric diatoms with a brief key and descriptions: Part 3: Stephanodiscaceae A, *Cyclotella*, *Tertiarius*, *Discostella*. Czech Phycological Society, Praha

Houk V, Klee R, Tanaka H (2014) Atlas of freshwater centric diatoms with a brief key and descriptions. Stephanodiscaceae B : *Stephanodiscu*s, *Cyclostephanos*, *Pliocaenicus*, *Hemistephanos*, *Stephanocostis*, *Mesodictyon* & *Spicaticribra*. Czech Phycological society, Praha

Houk V, Klee R (2007) Atlas of freshwater centric diatoms with a brief key and descriptions. Part II. Melosiraceae and Aulacoseiraceae (Supplement to Part I). Koeltz

Krammer K, Lange-Bertalot H (1986–1991). Bacillariophyceae 1. Teil: Naviculacea. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K, Lange-Bertalot H (1986–1991) Bacillariophyceae 2. Teil: Bacillariaceae, Epithemiacea, Surirellaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K, Lange-Bertalot H (1986–1991) Bacillariophyceae 3. Teil: Centrales, Fragilariacea, Eunotiaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K, Lange-Bertalot H (1986–1991) Bacillariophyceae 4. Teil: Achnanthaceae, Kristische Ergänzumgen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis Teil 1-4. Teil: Centrales, Fragilariacea, Eunotiaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart

Krammer K (2002) Diatoms of Europe: Diatoms of the european inland waters and comparable habitats. Volume 3: *Cymbella*. ARG Ganter Verlag KG

Lange-Bertalot H (2001) Diatoms of Europe: diatoms of the european inland waters and comparable habitats. Volume 2: *Navicula* sensu stricto, 10 genera separated from *Navicula* sensu lacto, *Frustulia*. ARG Ganter Verlag KG

Lange-Bertalot H, Bak M, Witkowski A (2002) Diatoms of Europe: Diatoms of the european inland waters and comparable habitats. Volume 6: *Eunotia* and some related genera. ARG Ganter Verlag KG

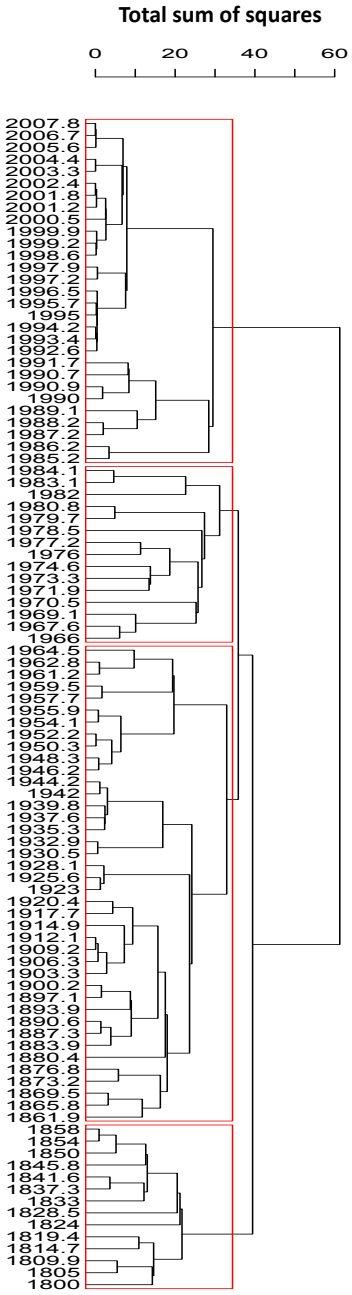
Lavoie I, Hamilton PB, Campeau S, Grenier M, Dillon PJ (2008) Guide d'identification des diatomées des rivières de l'Est du Canada. Presse de l'Université du Québec, Québec

Patrick R, Reimer CW (1966) The diatoms of the United States. Monographs of The Academy of Natural Sciences of Philadelphia, vol 1, no 13

Spaulding SA, Lubinski DJ, Potapova M (2010) Diatoms of the United States. http://westerndiatoms.colorado.edu

Chapter 3 – Supplementary Material SM6. Results from CONISS analysis on geochemical data

Figure SM6. Dendogram from CONISS analysis on geochemical data (molar C:N ratios, $\delta^{15}N$ and $\delta^{13}C$). The red boxes indicate the zones identified by the broken-stick model.



Chapter 3 – Supplementary Material SM7. Boxplots of paleolimnological indicators for different time periods

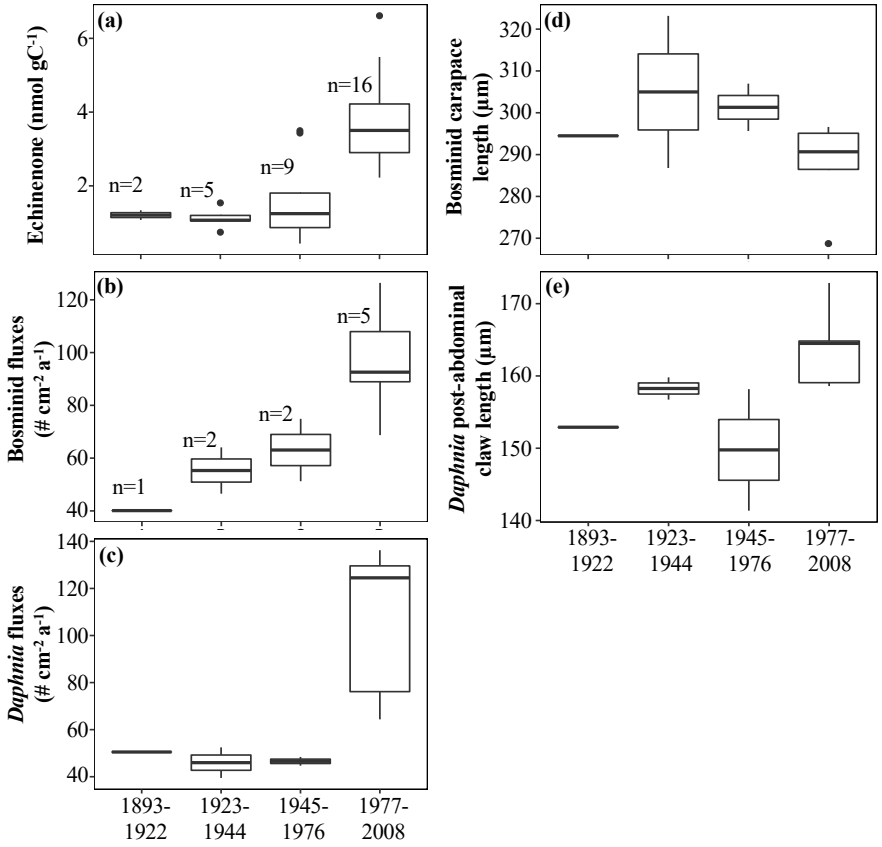


Figure SM7. Boxplots of paleolimnological indicators for different time periods: 1) 1892-1922 CE, 2) 1923-1944 CE; 3) 1945-1976 CE; 4) 1977-2008 CE. (a) Sedimentary pigment echinenone; (b) Bosminid fluxes; (c) *Daphnia* fluxes; (d) Bosminid carapace length; (e) *Daphnia* post-abdominal claw length. The numbers besides each box represent the sample size. The sample size for cladoceran data are only indicated in (b) as they are the same for all the cladoceran data.

Chapter 3 – Supplementary Material SM8. Entire sediment record for indicators included in the study

Figure SM8.1 Entire sediment record for geochemical indicators. (a) % carbon, (b) % nitrogen, (c) molar carbon:nitrogen, (d) $\boldsymbol{\delta}^{13}$C, (e) $\boldsymbol{\delta}^{15}$N. The dashed lines represent the breaks identified by CONISS analyses. The dates older than 1879 (± 66) CE should be interpreted with care as they are beyond the unsupported Pb^{210}.

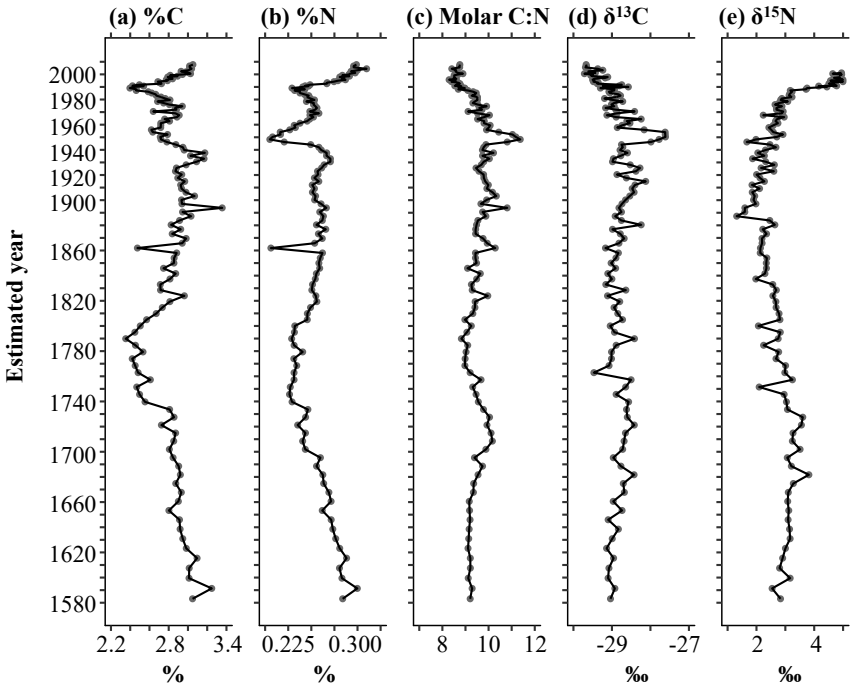
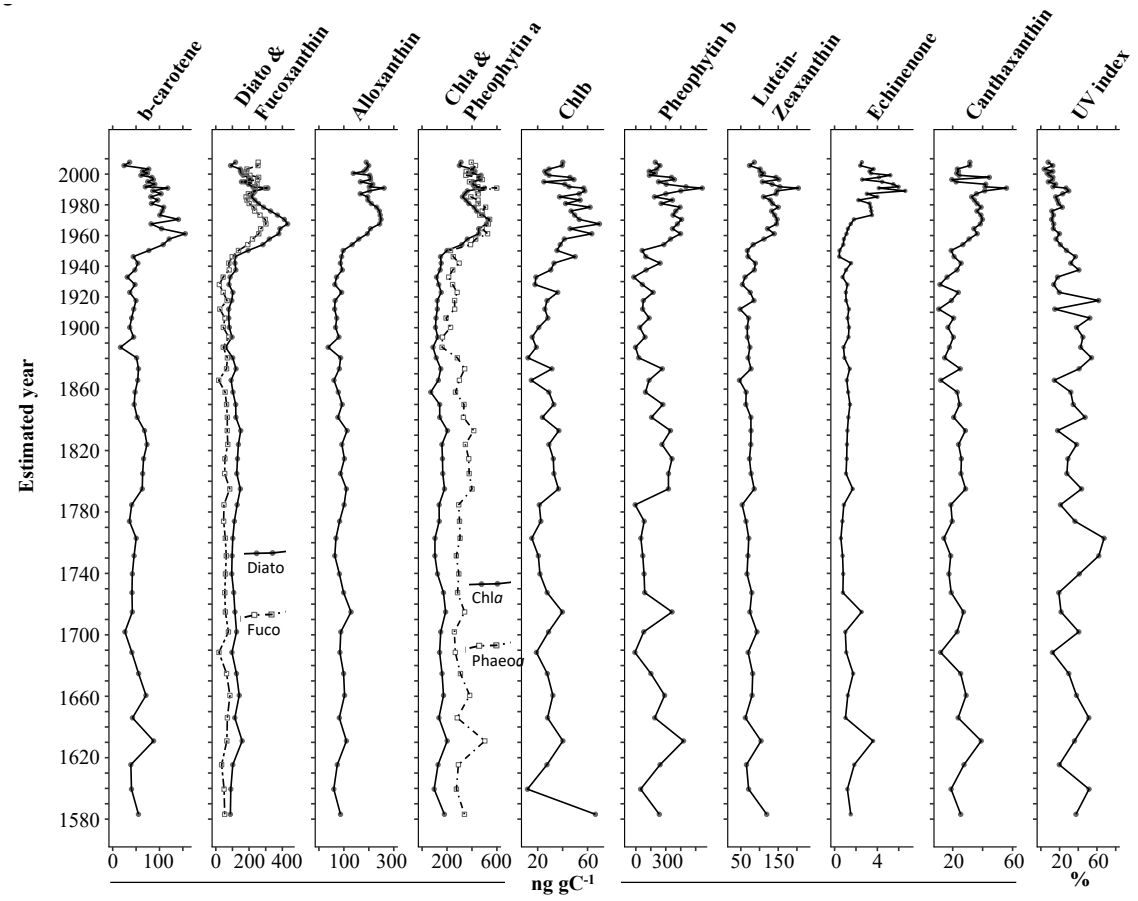


Figure SM8.2 Entire sediment record of all measured sedimentary pigment concentrations (nmol of pigment g^{-1} sediment C) and UV index (ratio of fossil UV-radiation-specific pigment (compound A) and fossil carotenoid pigments (alloxanthin, lutein+zeaxanthin and diatoxanthin)). The dates older than 1879 (± 66) CE should be interpreted with care as they are beyond the unsupported Pb^{210}.



Chapter 4 – Supplementary Material

Chapter 4 – Supplementary Material SM1. Electrophoresis gels for intracellular and extracellular DNA fractions in core samples.

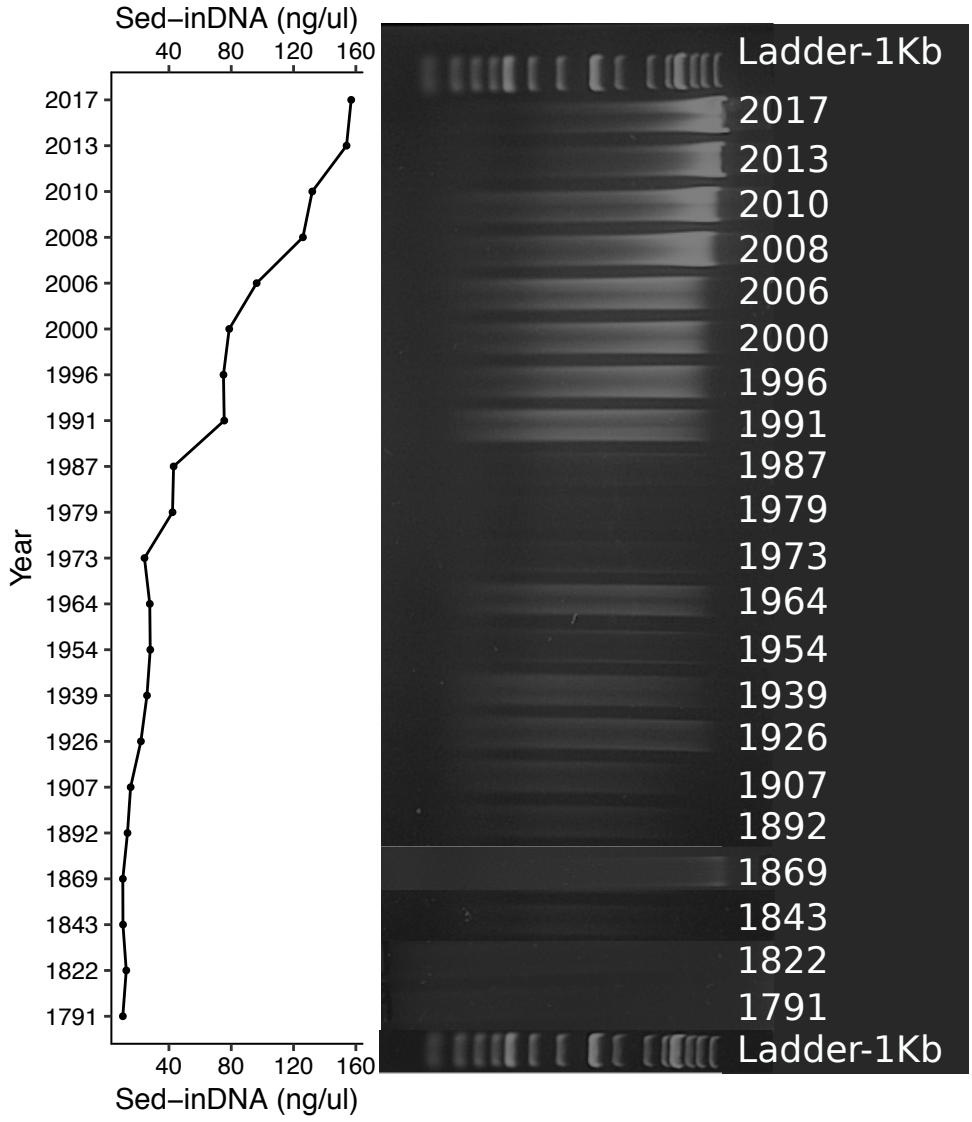


Figure SM1.1. Sedimentary intracellular DNA (ng/µl) concentration and gel electrophoresis pictures for core samples in Cultus Lake.

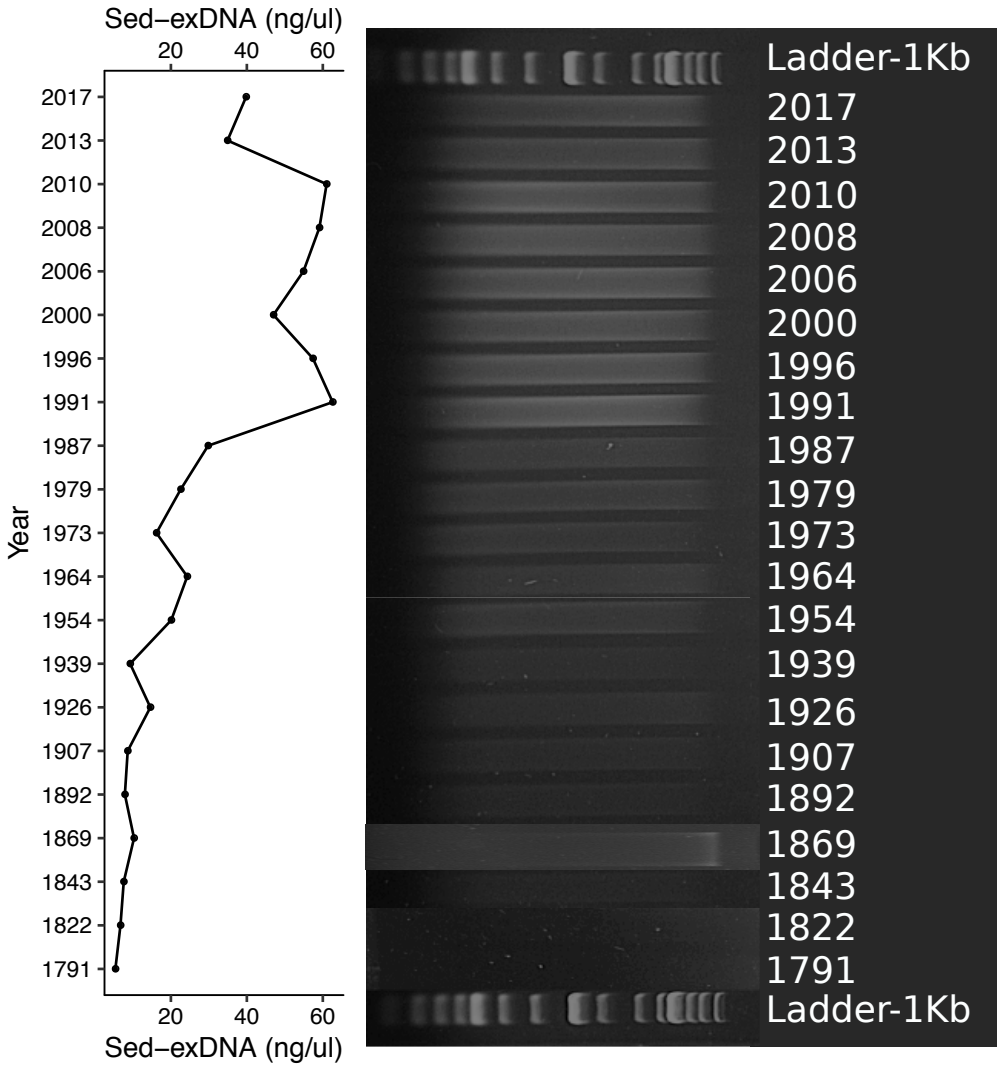


Figure SM1.2. Sedimentary intracellular DNA (ng/µl) concentration and gel electrophoresis pictures for core samples in Cultus Lake.

Chapter 4 – Supplementary Material SM2. Age-depth model and sediment age comparison for two cores collected in Cultus Lake.

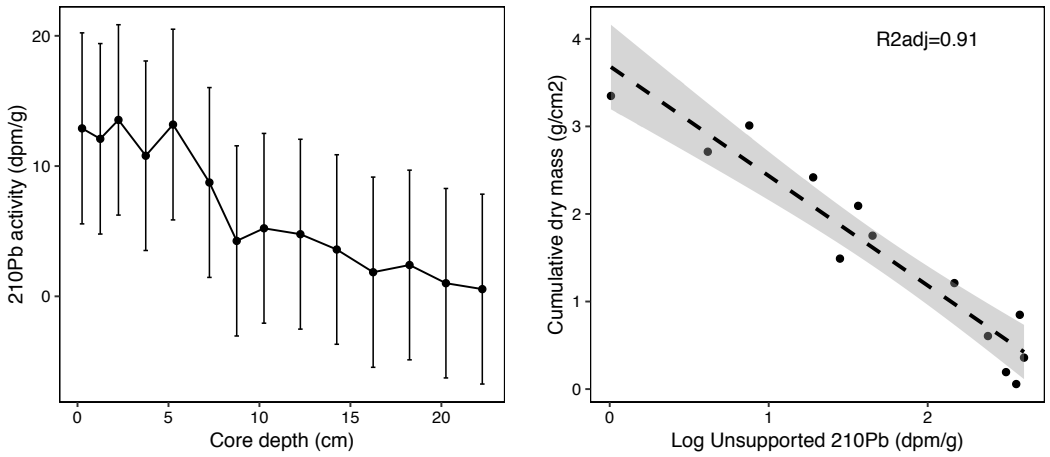


Figure SM2.1. Unsupported ^{210}Pb activity in the gravity core collected in April 2017 in Cultus Lake, British Columbia. (a) Unsupported ^{210}Pb activity with core depth. All the data shown here were used for the sediment age calculations. The sediment intervals below 22 cm are not illustrated in the figure as they were beyond the ^{210}Pb background level. (b) Correlation between cumulative dry mass and log unsupported ^{210}Pb activity.

Table SM2.1. Sediment age and age error of each ^{210}Pb dated interval throughout the sediment core.

Interval (cm)	^{210}Pb dated year	Age error
0-0.5	2016.82	1.63
1-1.5	2014.26	1.68
2-2.5	2010.74	1.76
3.5-4	2005.00	1.92
5-5.5	1998.22	2.16
7-7.5	1986.12	2.79
8.5-9	1978.62	3.24
10-10.5	1972.08	3.71
12-12.5	1960.15	4.96
14-14.5	1945.91	7.20
16-16.5	1933.41	10.02
18-18.5	1917.55	15.38
20-20.5	1892.44	30.66
22-22.5	1863.94	65.86

Table SM2.2. Selected intervals of the core collected in 2017 to compare with core collected in 2008 (Gauthier et al. In press).

Core collected in 2017		Core collected in 2008	
Interval (cm)	**Calculated Year**	**Interval (cm)**	**Calculated Year**
0 - 0.5	2017	-	-
1.0 - 1.5	2013	-	-
2.0 - 2.5	2010	-	-
2.5 - 3.0	2008	0 - 0.5	2008
3.0 - 3.5	2006	0.5 - 1.0	2007
4.5 - 5.0	2000	3.25 - 3.5	2001
5.5 - 6.0	1996	5.0 - 5.25	1996
6.5 - 7.0	1991	6.25 - 6.5	1992
7.5 - 8.0	1987	7.75 - 8.0	1986
9.0 - 9.5	1979	9.5 - 9.75	1979
10.0 - 10.5	1973	10.5 - 10.75	1973
11.5 - 12.0	1964	12.0 - 12.25	1965
13.0 - 13.5	1954	13.5 - 13.75	1954
15.0 - 15.5	1939	15.5 - 15.75	1937
16.5 - 17.0	1926	16.75 - 17.0	1926
18.5 - 19.0	1907	18.5 - 18.75	1906
20.0 - 20.5	1892	19.75 - 20.0	1891
22.0 - 22.5	1869	21.25 - 21.5	1870
24.0 - 24.5	1843	23.0 - 23.25	1842
25.5 - 26.0	1822	24.25 - 24.5	1819
27.5 - 28.0	1791	25.75 – 26.0	1790

Chapter 4 - Supplementary Material SM3. Visual inspection of sediment core collected in 2017 from Cultus Lake.

A visual inspection was performed on the sediment core collected in 2017 prior to be sectioned in sediment intervals. From the top (0 cm) to ~6 cm deep (corresponding to ca. 1990-2017), the sediments were characterized with clear brown color (Fig. SM3.1). From ~6 cm to the bottom of the core (30 cm), the sediments were darker with a color grey-brown (Fig. SM3.1). Presence of charcoal layers were apparent through the core from ~9 cm to the bottom of the core, with more distinct layers at ~9.5 cm (ca. 1980), ~13 cm (ca. 1950) and ~25 cm (ca. 1830) (Fig. SM3.1).

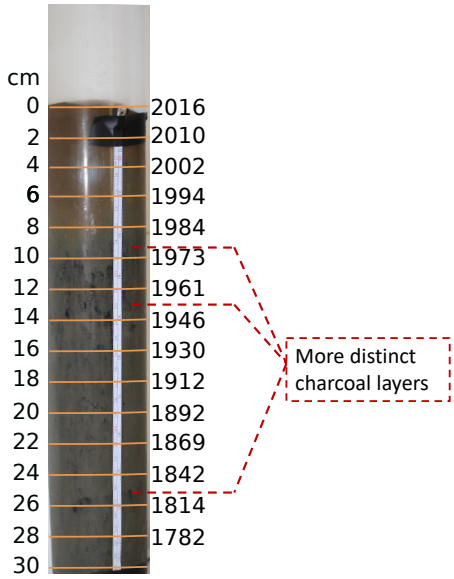


Figure SM3.1. Picture of the sediment core collected in April 2017 from Cultus Lake. The left represents the depth of the core (cm) and the right the approximate year of the sediment section. Three distinct charcoal layers are indicated by the dashed red lines.

Chapter 4 – Supplementary Material SM4. Shared and unique ASVs between intracellular and extracellular DNA

Table SM4.1. Number of ASVs and sequence numbers per samples for shared ASVs between intracellular and extracellular DNA and unique ASVs from both intracellular and extracellular fractions.

	Shared ASVs		Unique ASVs IntraDNA		Unique ASVs ExtraDNA	
Year	**Richness**	**Seq. Nb.**	**Richness**	**Seq. Nb.**	**Richness**	**Seq. Nb.**
2017	112	224	277	17548	285	20488
2013	23	46	197	82591	122	17824
2010	44	88	200	10064	256	36063
2008	37	74	161	9281	160	32135
2006	31	62	240	18480	104	10632
2000	23	46	322	30184	113	29537
1996	22	44	236	21822	155	38983
1991	32	64	330	29471	143	25251
1987	15	30	303	33545	61	26967
1979	22	44	238	26890	57	14505
1973	6	12	212	45315	17	12116
1964	19	38	205	35757	33	15522
1954	7	14	145	45331	32	18763
1939	5	10	140	55010	14	9751
1926	3	6	106	59588	18	12954
1907	4	8	121	47253	63	17514
1892	2	4	89	22536	9	3347
1869	0	0	53	74333	4	12918
1843	1	2	73	41882	12	8178
1791	1	2	67	50573	8	6102

Chapter 4 – Supplementary Material SM5. Number of indicator ASVs per phylum per time periods.

Table SM5.1. Number of indicator ASVs per phylum, sub-phylum and class for the four time periods used.

Taxonomy levels			Time periods				
Phylum	Subphylum	Class	1987-2017 (n=9)	1964-1979 (n=3)	1939-1954 (n=2)	1791-1926 (n=7)	Grand Total
Alveolata			**17**	**28**	**40**	**5**	**90**
	Alveolata_unclassified			4	4	2	10
		Alveolata_unclassified		4	4	2	10
	Apicomplexa		1	9	8		18
		Apicomplexa	1	9	8		18
	Ciliophora		7	9	11		27
		Ciliophora_unclassified			2		2
		Litostomatea	3	5	9		17
		Oligohymenophorea	1				1
		Prostomatea		1			1
		Spirotrichea	3	3			6
	Dinoflagellata		9	6	17	3	35
		Dinophyceae	9	6	17	3	35
Amoebozoa			**2**		**6**		**8**
	Conosa				4		4
		Archamoebea			2		2
		Variosea			2		2
	Lobosa		2		2		4
		Lobosa			1		1
		Tubulinea	2		1		3
Apusozoa			**1**				**1**
	Hilomonadea		1				1
		Planomonadida	1				1
Archaeplastida			**4**	**1**	**4**	**1**	**10**
	Chlorophyta		2	1	3		6
		Chlorophyceae	1		2		3
		Chlorophyta_unclassified	1				1
		Trebouxiophyceae		1	1		2
	Streptophyta		2		1	1	4
		Embryophyceae			1	1	2
		Streptophyta_unclassified	2				2
Eukaryota_unclassified			**16**	**19**	**67**	**2**	**104**
Excavata			**1**		**2**		**3**
	Discoba		1		2		3
		Euglenozoa	1		1		2
		Heterolobosea			1		1
Hacrobia					**1**		**1**
	Haptophyta				1		1
		Prymnesiophyceae			1		1
Opisthokonta			**37**	**23**	**30**	**1**	**91**
	Fungi		2	3	18	1	24
		Ascomycota		2	1	1	4
		Basidiomycota			7		7
		Chytridiomycota	1	1	3		5
		Cryptomycota	1		3		4
		Fungi_unclassified			2		2
		Microsporidiomycota			2		2
	Mesomycetozoa				1		1
		Ichthyosporea			1		1
	Metazoa		28	18	3		49
		Arthropoda	10	12			22
		Craniata		1			1
		Gastrotricha	9				9
		Metazoa_unclassified	3	3	1		7
		Nematoda	4				4
		Rotifera	2	2	2		6
	Opisthokonta_unclassified		7	2	8		17
Rhizaria			**7**	**7**	**12**		**26**
	Cercozoa		7	7	12		26
		Cercozoa_unclassified	1	1	5		7
		Endomyxa			2		2
		Endomyxa-Phytomyxea		4	2		6
		Filosa-Imbricatea	1				1
		Filosa-Sarcomonadea	3	1	3		7
		Filosa-Thecofilosea	2				2
		Novel-clade		1			1
Stramenopiles			**20**	**6**	**8**	**1**	**35**
	Ochrophyta		16	2	5		23
		Bacillariophyta	10	2	3		15
		Chrysophyceae	4				4
		Eustigmatophyceae			1		1
		Ochrophyta_unclassified	2		1		3
	Stramenopiles		4	4	3	1	12
		Bicoecea			1		1
		Oomycota	4	3	2	1	10
		Pirsonia_Clade		1			1
Grand Total			**105**	**84**	**170**	**10**	**369**

Chapter 4 – Supplementary Material SM6. Diatom PCoA biplots of morphological and sedDNA-based methods

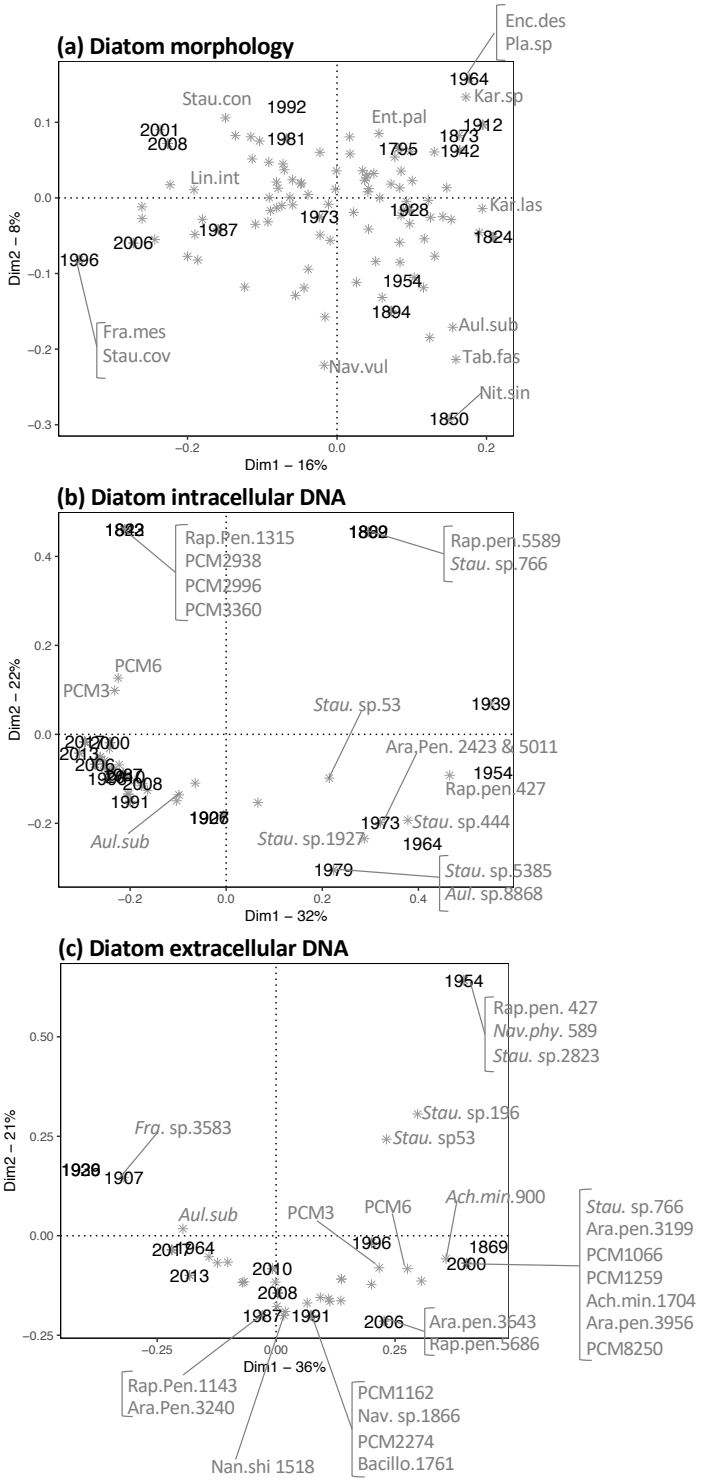


Figure SM6.1. PCoA biplots with diatom taxa for (a) morphological; (b) sedimentary intracellular DNA; and (c) sedimentary extracellular DNA approaches. Bray-Curtis dissimilarity matrices from relative abundance were calculated prior to PCoAs. Abbreviations are as follows: *Achnanthidium minutissmum* (Ach.min), Araphid pennate (Ara.Pen), *Aulacoseira* (Aul), *Aulacoseira subarctica* (Aul.sub), Bacillaryophyta (Bacillo), *Encyonopsis descripta* (Enc.des), *Entomoneis paludosa* (Ent.pal), Fragilaria (Fra), *Fragilaria* mesolepta (Fra.mes), *Karayevia lasterostrata* (Kar.las), *Karayevia* sp. (Kar.sp), *Nanofrustulum shiloi* (Nan.shi), Navicula (Nav), *Navicula vulpina* (Nav.vul), *Navicula phyllepta* (Nav.phy), *Nitzschia sinuata* var. *tabellaria* (Nit.sin), Polar Centric *Mediophyceae* (PCM), *Planothidium* sp. (Pla.sp), Raphid pennate (Rap.Pen), *Staurosira* (Stau), *Staurosira contruens* (Stau.con), *Staurosira construens* var. *venter* (Stau.cov), *Tabularia fasciculata* (Tab.fas).

Chapter 4 – Supplementary Material SM7. Geochemical paleolimnological indicators of the cores collected in 2008 and 2017.

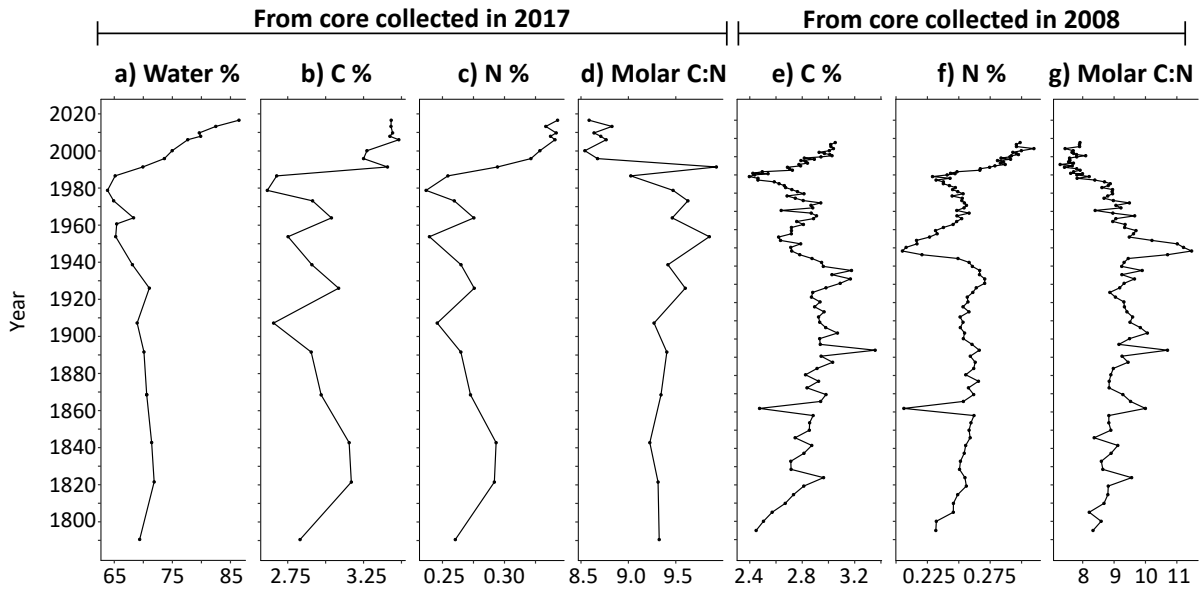


Figure SM7.1. Cultus Lake sediment record of geochemical indicators from the core collected in 2017 for (a) water percentage; (b) carbon percentage; (c) nitrogen percentage; (d) molar carbon:nitrogen; and from the core collected in 2008 for (e) carbon percentage; (f) nitrogen percentage; and (g) molar carbon:nitrogen. The dates older than ~1880 should be interpreted with care as they are beyond the unsupported ^{210}Pb background. The entire sediment record from the core collected in 2008 can be found in Gauthier et al. (In press).

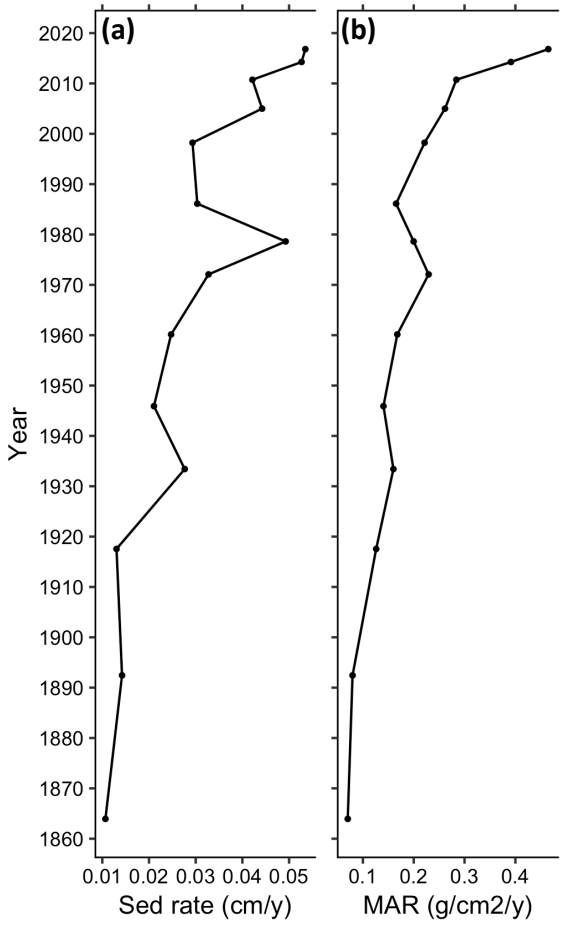


Figure SM7.2. Stratigraphies of (a) sedimentation rate (Sed rate; cm yr^{-1}); and (b) mass accumulation rate (MAR; g cm^{-2} y^{-1}) calculated from the constant rate of supply (CRS) model of the sediment core collected in 2017 in Cultus Lake.

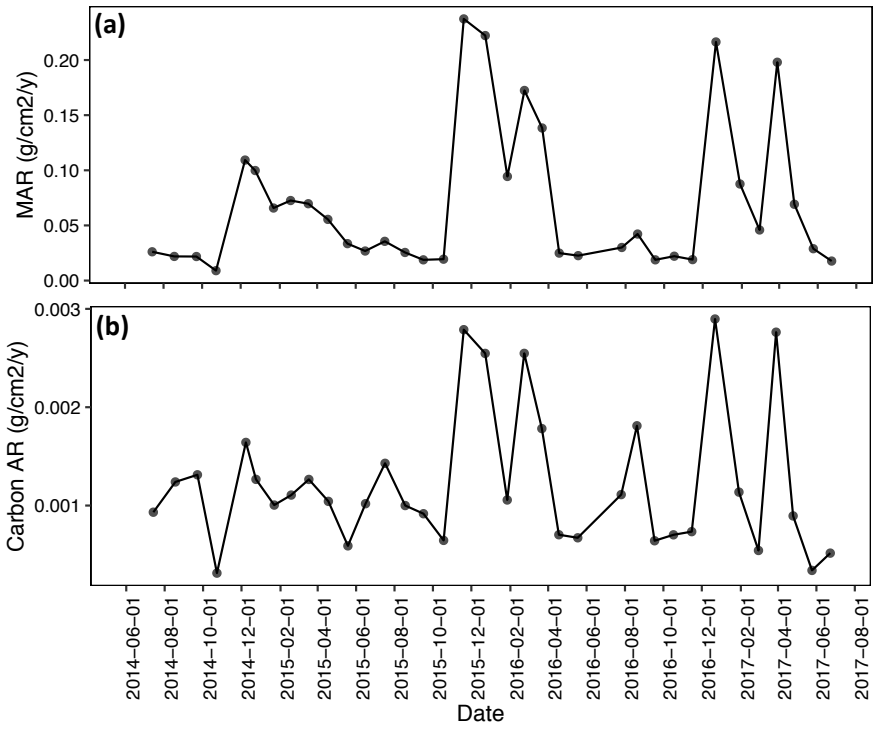


Figure SM7.3. Time series of (a) mass accumulation rate (MAR; g cm $^{-2}$ y^{-1}); and (b) carbon accumulation rate (Carbon AR; g cm $^{-2}$ y^{-1}) from the sediment trap samples.