PALEO-ECOTOXICOLOGY OF YELLOWKNIFE (NORTHWEST TERRITORIES, CANADA) LAKES IMPACTED BY HISTORIC GOLD MINING ACTIVITIES

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A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science

Graduate Program in Geography

York University

Toronto, Ontario, Canada

August 2020

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Abstract

This thesis explores the ecological impacts of legacy arsenic contamination on Cladocera (Branchiopoda, Crustacea) from historic gold mining in Yellowknife lakes (Northwest Territories, Canada) using a paleo-ecotoxicological approach. I examined Cladocera subfossils preserved in the modern and pre-1850 lake sediments of 23 lakes in the region, and the *Daphnia* resting egg bank of Pocket Lake, a highly contaminated lake in which striking ecological changes were previously recorded. Cladocera assemblage changes since pre-1850 were dependent on local, lake-specific limnological conditions. Littoral/benthic taxa dominated the cladoceran assemblage of the most contaminated lakes, while pelagic *Bosmina* were dominant in lakes with low arsenic. No viable *Daphnia* resting eggs were found in Pocket Lake, indicating that future resurrection ecology studies from *Daphnia* populations are not feasible. My research provides new information on the ecotoxicological effects of arsenic contamination in lake ecosystems, and the potential of using Cladocera as paleo-ecotoxicological indicators.

Co-Authorship

This thesis explores the ecological impacts of legacy arsenic contamination from historical gold mining on lakes within Yellowknife (Northwest Territories, Canada) using a paleo-ecotoxicological approach, and is examined in two datachapters. Chapter 2 is based on the following manuscript and has been formatted according to the author guidelines of Hydrobiologia, the journal to which it was submitted.

Chapter 2: Persaud, A. A., C. L. Cheney, B. Sivarajah, J. M. Blais, J. P. Smol, & J. B. Korosi. Regional changes in Cladocera (Branchiopoda, Crustacea) assemblages in subarctic (Yellowknife, Northwest Territories, Canada) lakes impacted by historic gold mining activities. Submitted to Hydrobiologia.

I designed this study under the guidance of my supervisor, Prof. Jennifer Korosi (Dept. of Geography, York University), as well as Prof. Jules Blais (Dept. of Biology, University of Ottawa) and Prof. John Smol (PEARL, Dept. of Biology, Queen's University). Cynthia Cheney, Branaavan Sivarajah, Prof. Blais, and Prof. Korosi conducted the fieldwork for this chapter. I conducted Cladocera subfossil analysis under the microscope for 11 lakes, which was complemented by identifications done for an additional 15 lakes in 2015 by Prof. Korosi (unpublished data). I conducted all statistical analyses, data interpretations, produced the final figures, and wrote the manuscript, with editorial input from Prof. Korosi, Prof. Blais, Prof. Smol, Branaavan Sivarjah and Cynthia Cheney.

Acknowledgements

I wish to express my sincere gratitude and thanks to all those persons who have assisted me in the completion of this thesis.

Sincere thanks and gratitude to my supervisor, Dr. Jennifer Korosi (Dept. of Geography, York University) for not only providing me the opportunity to do research but also for all the invaluable guidance and knowledge you have imparted throughout the entire process. I am especially grateful for all your handwork in assisting me throughout the unusual circumstances in which we find ourselves.

I would also like to express my thanks to Dr. Tarmo Remmel (Dept. of Geography, York University) for being a part of my committee and providing critical feedback in improving my thesis.

Thank you as well to Grace Hoskin and Januja Jeyarajah for helping me navigate Cladocera subfossil analysis, and most of all for your support and friendship throughout the last two years. I would also like to extend special thanks to all the members of the Korosi Lab for your advice, assistance, and being the best group of people with whom I have had the pleasure of working and learning from.

Finally, a hearty thank you to my parents, grandparents and brother for going above and beyond to support me in my Master's journey. The sacrifices you have made and the encouragement you gave me have been paramount to all I have accomplished. Words cannot express the extent to which I am grateful.

List of Tables

Table 2.1. A summary of the limnological characteristics of the 23 study lakes. Lakes are arranged in descending order based on their arsenic (As) concentrations (highest to lowest). Measurements of water chemistry parameters were taken in 2010 and between 2014 and 2016. Lakes for which the cores were ^{210}Pb dated are denoted by an asterisk 46

Table 2.2. List of the most common cladoceran taxa encountered in the 23 study lakes, a summary of their distribution (abundance and number of lakes) and basic ecology. Information on basic ecology was adapted from Korosi & Smol, 2012a, 2012b 55

Table 3.1. Mass and volume of sediment from each interval of Pocket Lake and the number of empty ephippia and empty egg membranes identified 103

List of Figures

Figure 1.1. Examples of common Cladocera subfossil remains encountered in the Canadian Arctic: a) Alona affinis headshield; b) Alona circumfimbriata/guttata headshield; c) Chydorus headshield; d) Bosmina headshield; and e) Daphnia postabdominal claw. Images taken by J. Korosi 17

Figure 2.1. Map of the study region (Yellowknife, Northwest Territories, Canada) showing the locations of the 23 study lakes and their present-day lake-water arsenic concentrations. The locations of Giant Mine and Con Mine are indicated by the black and brown pentagons, and their respective mining footprints are shown. Inset map shows the study location in the context of Canada..... 43

Figure 2.2. Principal component analysis (PCA) showing: a) the distribution of the study lakes along the gradients of the measured environmental variables; b) variation in cladoceran species assemblages in the surface sediments of Yellowknife lakes; c) direction of change in cladoceran species assemblages in Yellowknife lakes between present-day (surface sediments) and the pre-industrial (bottom sediments) periods. Cladocera assemblages in the bottom sediment interval are plotted passively. The shapes of the symbols represent the present-day surface water arsenic concentrations..... 53

Figure 2.3. Simplified Cladocera relative frequency diagram of the most common cladoceran taxa in the surface sediments for our twenty-three lakes in Yellowknife, Northwest Territories. The sites are arranged in order of increasing arsenic (As) concentrations, as shown on the right. The colours of the individual bars represent

the varying arsenic concentrations at the site (red 100 - 1000 µg/l, purple 10–100 µg/l, blue 5–10 µg/l, and green < 5 µg/l)..... 58

Figure 2.4. Regression plots showing the relationship between: a) cladoceran species diversity (Hill's N2, surface sediments) and lake water arsenic concentrations for 20 of the 23 study lakes (3 lakes with the highest arsenic concentrations removed); b) species diversity (Hill's N2) regression plot with all 23 study lakes included; c) rarefied species richness (surface sediments) and lake water 61

Figure 2.5. Scatterplots showing cladoceran rarefied species richness and species diversity (Hill's N2) in present-day and pre-industrial sediment intervals. Samples that plot above the 1:1 line have higher rarefied species richness and Hill's N2 diversity in the surface interval compared to the bottom interval. Significance values are based on the Wilcoxon tests. The shapes of the symbols represent the surface water arsenic concentrations..... 63

Figure 2.6. Changes in the relative abundance of the most abundant cladoceran taxonomic between present-day and pre-industrial times for the 23 study lakes arranged by measured surface water arsenic concentrations. Kruskal-Wallis nonparametric analysis of variance for all arsenic classes: <5 µg/L (n = 7); 5 to 10 µg/L (n = 3); 10 to 100 µg/L (n = 8); and 100 to 1000 µg/L (n = 5) are provided in each plot..... 65

Figure 3.1. Map of the Yellowknife area (Northwest Territories, Canada) showing the location of Pocket Lake (yellow triangle), Giant Mine (north of Yellowknife; black pentagon), and Con Mine (south of Yellowknife; red pentagon). The respective

footprints of the two mines are shown. Inset map shows the location of the study region (red rectangle) within Canada 100

Figure 3.2 Number of empty ephippia and egg membranes corrected for volume of sediment used found in a sediment core from Pocket Lake (Yellowknife, Northwest Territories, Canada). Trends in arsenic concentrations and % Daphnia are recreated from data presented in Thienpont et al. (2016). Our analysis was conducted on a different sediment core to what was analyzed in Thienpont et al. (2016), and so trends are not directly comparable. The image presented is that of a typical Daphnia ephippia (image taken by J. Korosi)..... 104

Table of Contents

Abstract ii

Co-Authorship iii

Acknowledgements iv

List of Tables v

List of Figures vi

Table of Contents ix

Chapter 1 : General Introduction and Literature Review 1

1.1 Arsenic in surface waters 2

1.1.1 Arsenic Ecotoxicity in Surface Waters 4

1.2 Legacy arsenic from gold mining in Yellowknife 8

1.2.1 Arsenic Contamination of Yellowknife Lakes 9

1.2.2 Impacts of legacy arsenic contamination on aquatic biota in Yellowknife 11

1.2.3 Arsenic contamination in Pocket Lake 12

1.3 Paleo-ecotoxicology as a tool to assess arsenic contamination 13

1.3.1 Cladocera as "paleo-ecotoxicological" indicators 16

1.4 Resurrection Ecology – Resting eggs as paleo-ecotoxicological indicators 18

1.5 Thesis Organization and Rationale 20

1.6 References 22

Chapter 2 : Regional changes in Cladocera (Branchiopoda, Crustacea) assemblages in subarctic (Yellowknife, Northwest Territories, Canada) lakes impacted by historic gold mining activities 36

2.1 Abstract 36

2.2 Introduction 37

2.3 Methods 41

2.3.1 Study Site Description 41

2.3.2 Field Methods 48

2.3.4 Laboratory Methods 49

2.3.5 Statistical Analyses 50

2.4 Results 52

2.4.1 Differences in water chemistry variables amongst Yellowknife study lakes 52

2.4.2 Differences in Cladocera assemblages from the surface sediments of the Yellowknife study lakes 54

2.4.3 Regional changes in Cladocera assemblages since pre-industrial times 62

2.5 Discussion 66

2.5.1 Differences in Cladocera species richness and diversity along a gradient of arsenic ... 66

2.5.2 Subfossil Cladocera species assemblages as paleoecological indicators of arsenic contamination 68

2.5.3 Subfossil Cladocera assemblage and diversity changes in Yellowknife lakes since pre-industrial times 71

2.5.4 Conclusions and Future Directions 73

2.6 Acknowledgments 75

2.7 References 75

Chapter 3 : Assessing the viability of *Daphnia pulex* complex resting eggs in a subarctic lake (Yellowknife, Northwest Territories, Canada) impacted by historic gold mining 93

3.1 Abstract 93

3.2 Introduction 93

3.3 Methods 98

3.3.1 Study Site Description 98

3.3.2 Field Methods 101

3.3.4 Laboratory methods 101

3.4 Results and discussion 102

3.4.1 Temporal trends in Daphnia ephippia production 105

3.4.2 Viability of resting eggs in Pocket Lake for future resurrection ecology studies 106

3.4.3 Impacts of contamination on the Daphnia resting egg bank of Pocket Lake 107

3.5 References 109

Chapter 4 : General conclusions and Future Directions 116

4.1 Thesis Summary and Conclusions 116

4.1.1 How have regional Cladocera assemblages in Yellowknife lakes changed since pre-1850? 116

4.1.2 Which cladoceran taxa are sensitive or tolerant of arsenic, and therefore candidate ecological indicators for future arsenic contamination studies? 117

4.1.3 Did arsenic contamination compromise the Daphnia resting egg bank in Pocket Lake? 118

4.1.4 Are the Daphnia pulex complex resting eggs in Pocket Lake still viable to be used in future resurrection ecology studies? 118

4.2 Future Directions 119

4.3 References 120

Glossary 121

Chapter 1 : General Introduction and Literature Review

Arsenic is a toxic element occurring in the environment, and of the natural elements, it is the 47th most abundant (Bowell et al. 2014). The amount of arsenic present in the aquatic environment has increased over the past century due to inputs from anthropogenic sources such as smelting, construction, pesticides, and metal mining (Han et al. 2003). Arsenic released into the environment directly or indirectly from mining operations have degraded freshwater ecosystems all over the world, regardless of their development status. For example, the USA (Barrett et al. 2018), Brazil (Bidone et al. 2018) and China (Zhang et al. 2009) have all experienced negative impacts from arsenic contamination near mining operations. In Canada, gold and silver mining has been a major anthropogenic source of arsenic contamination, particularly in the Northwest Territories, Nova Scotia and Northeastern Ontario (Wagemann et al. 1978; Hutchinson et al. 1982; Clark and Raven 2004; Houben et al. 2016; Willick 2019; Little et al. 2020).

The primary concern regarding arsenic pollution is the contamination of drinking water, which consequently results in severe arsenic related illnesses in humans (Han et al. 2003). Arsenic is highly toxic to humans and can result in both acute and chronic health affects. The toxicity of arsenic to humans is attributed to its ability to inactivate ~200 enzymes, including those involved in the synthesis and repair of DNA, as well as cellular energy pathways (Ratnaike 2003). It is a well-documented carcinogen capable of affecting multiple bodily organs (e.g., the lungs, bladder and prostate)(Hong et al. 2003).

In addition to its carcinogenicity, arsenic poisoning that occurs during childhood can potentially cause neurobehavioral changes in adulthood, and has also been known to cause teratogenic (affecting the development of a fetus or embryo) and genotoxic (affecting cellular

DNA) effects on humans (Hong et al. 2014; Rabieh et al. 2008; U.S. Department of Health and Human Services 2007). Of the sources of arsenic exposure, drinking water with elevated levels of arsenic can be a significant source of inorganic arsenic exposure for humans (Ahsan 2011). Arsenic contamination of surface water can therefore represent a major threat to human health as globally, approximately 144 million people depend on surface water as their primary source of drinking water. Additionally, freshwater fish that have been exposed to high concentrations of arsenic can also represent a source of inorganic arsenic exposure to humans who consume them (Jankong et al. 2007).

1.1 Arsenic in surface waters

Arsenic has four oxidation states, which are -3, 0, +3 and +5 (Kumaresan and Riyazuddin 2001). In the environment, arsenic is commonly found in the +5 and +3 oxidation states in inorganic forms as arsenate (As(V)), and arsenite (As (III)), respectively. Additionally, as aqueous species, they occur as anionic in arsenate ($H_2AsO_4^-$ and $HAsO_4^{2-}$) and neutral in arsenite ($As(OH)_3^0$) (Anderson and Bruland 1991). Organic forms of arsenic (the organoarsenicals) such as methylarsonate (MMA), and dimethylarsinate (DMA) are less prevalent, and are found within living organisms such as bacteria, fungi and algae (Anderson and Bruland 1991), zooplankton (Caumette et al. 2011), and fish (Taylor et al. 2017). Free element arsenic is rarely observed in the natural environment, with metal arsenides such as iron diarsenide and nickel diarsenide being far more common. Arsenates of metal(loids) also occur within the natural environment together with arsenic trioxide (National Research Council (US) 1977).

Inputs of arsenic into surface waters occur through a variety of means, including baseflow from groundwater, both wet and dry precipitation, snowmelt, and surface runoff. However, most of the arsenic found in surface waters is primarily from the weathering of rocks

and soils (e.g., pyrite) (Hering and Kneebone 2002), or anthropogenic mine tailings and emissions (Clark and Raven 2004). The amount of time arsenic remains in the water column (the residence time) differs depending on the characteristics of the waterbody. For example, arsenic has a residence time of approximately 2 weeks in rivers, compared to upwards of 10 years in certain lakes (Hering and Kneebone 2002). During this time, the concentrations, toxicities, and speciation of arsenic present is controlled by a variety of factors including redox controls, sorption/desorption, and dissolution/precipitation (Hering and Kneebone 2002).

With respect to redox controls, while As(V) is more thermodynamically favored and stable under oxic conditions, and As(III) in reducing conditions, the coexistence of both species has been observed in oxic hypolimnetic surface waters (Mok and Wai 1990; Anderson and Bruland 1991; Kuhn and Sigg 1993). This coexistence is attributed to the relative inertness of arsenic species to oxidants and reductants, and microbial interconversions of the two species (Eary and Schramke 1990; Manning and Goldberg 1997a). Solid phase arsenic in oxic conditions is found adsorbed to iron and manganese oxyhydroxide, but is shifted to the sulfide fraction under reducing conditions (Moore et al. 1988).

As(V) is known to strongly adsorb onto iron and aluminum oxyhydroxides, and clay minerals. In fact, the presence of oxalate-extractable aluminum and citratedithionite extractable iron have been found to promote the retention of arsenic in soils (Anderson et al. 1976; Livesey and Huang 1981; Manning and Goldberg 1997b), and iron oxides also strongly interact with As(V) in sediments (Arai et al. 2004). Adsorption of As(III) onto Fe(III) oxyhydroxides has also been observed (Leckie et al. 1980; Pierce and Moore 1982; Wilkie and Hering 1996; Raven et al. 1998). While sorption is mainly an important mechanism for controlling the occurrence and mobility of As(V) in surface waters, sorption of As(III) can be greater than that of As(V) at

higher pH (above pH 6−7.5) (Raven et al. 1998). Similarly, competition by other organic and inorganic solutes and the number of available sorbents will also limit the sorption of arsenic, both As(V) and As(III) (Hering and Kneebone 2002).

As(V) minerals are typically soluble and therefore do not tend to precipitate under natural environmental conditions. However, at low pH values, $FeAsO_{4(s)}$ and calcium arsenates have been reported when there has been an excess of Fe(III) and limestone substratum, respectively (Bowell 1994; Juillot et al. 1999). In surface waters under reducing conditions, As(III) has been observed to precipitate with sulfide, forming solid compounds like arsenopyrite (Hering and Kneebone 2002). Dissolution and precipitation are limited by the amount of dissolved oxygen, kinetic limitations and microbial activity which has been found to catalyze pyrite oxidation (Nordstrom and Southam 2019).

1.1.1 Arsenic Ecotoxicity in Surface Waters

Arsenic pollution represents a serious threat to aquatic biota because of its toxicity (dependent on speciation) and solubility in surface waters. The Canadian Council of Ministers of the Environment (CCME) has established arsenic guidelines for the protection of aquatic life in freshwater ecosystems at 5.0 $\mu g \cdot L^{-1}$, above which arsenic levels become toxic to sensitive freshwater organisms, such as *Scenedesmus obliquus* (CCME 2001). This and other CCME water quality guidelines are based on the EC_{50} (estimate of toxicant concentration that produces an effect in 50% of an exposed population) values of the most sensitive organism (for arsenic, *S. obliquus*) multiplied by a safety factor, a numerical value to account for uncertainty (CCME 2001). The interim guidelines for marine and estuary aquatic life is 12.5 $\mu g \cdot L^{-1}$(CCME 2001).

Fish exposed to arsenic experience various toxic effects, ranging from neurological dysfunction to changes in growth, plasma and hematological parameters. Zebrafish (*Danio rerio*)

exposed to arsenic in chronic toxicity bioassays have been found to exhibit an increase in oxidative stress and a reduction in locomotion, indicative of neurological dysfunction (Hallauer et al. 2016). Moreover, the progeny of these fish had substantially lower biomass (Hallauer et al. 2016). In juvenile starry flounder (*Platichthys stellatus)*, an increase in arsenic concentrations at higher temperatures resulted in decreases in growth and hematological parameters, and an increase in plasma components (Han et al. 2019). For freshwater fishes in natural surface waters, bioaccumulation factor (BAF) values can be high even when concentrations of arsenic in the environment are relatively low, and will increase with changes in arsenic levels in the environment (Williams et al. 2006).

In microalgae, arsenic toxicity is dependent on several factors, including algal species, the speciation of arsenic present, and phosphate concentrations (Levy et al. 2005). Levy et al. (2005) found that *Chlorella* species and *Monoraphidium arcuatum* were both tolerant of As(III), with the former also being tolerant of As(V). Rahman et al. (2014) determined that *Chlorella* were more sensitive to As(V) than As(III); however, increases in soluble phosphate concentrations decreased the overall toxicity of As(V) to these same *Chlorella sp.* (Rahman et al. 2014) and *M. arcuatum* (Levy et al. 2005). This is likely due to uptake competition between phosphate and arsenate at the cellular level (Levy et al. 2005). Microalgae have also been found to reduce As(V) into As(III) (Hellweger et al. 2003; Levy et al. 2005; Rahman et al. 2014).

For chironomids, acute arsenic toxicity is dependent on physical, biological and chemical factors (Jeyasingham and Ling 2000). Jeyasingham and Ling (2000) found that the physical factors of temperature and season both influence the toxicity of arsenic to three Chironomid taxa *Chironomus zealandicus*, *Chironomus* sp. a and *Polypedilum pavidus.* While there is typically a positive correlation between temperature and the toxicity of many chemicals, no consistent

relationship was found between the LC_{50} (concentration of chemical at which 50% of the exposed population is killed within a given period of time) values of these species and temperature (Jeyasingham and Ling 2000). In terms of season, Jeyasingham and Ling (2000) reported that *C. zealandicus* collected in the summer were more resistant than its winter counterparts, while the opposite was true for *P. pavidus*, possibly attributed to species-specific optimal temperature. Additionally, it was found that all three species were more sensitive to As(III) than As(V) (Jeyasingham and Ling 2000). Woods et al. (1989) based on an examination of laboratory and wild *Chironomus tentans*, found that heterozygosity (two different alleles of a specific gene/genes) results in differences in sensitivity to a toxicant. Heterozygosity therefore can affect sensitivity of Chironomids (and other species) to arsenic (Jeyasingham and Ling 2000).

For zooplankton arsenic toxicity experiments, the most commonly studied species are the large-bodied cladocerans, *Daphnia*. To a lesser extent, the responses of copepods, rotifers and other cladoceran taxa, like *Bosmina* and *Ceriodaphnia cf. dubia*, have also been examined (Passino and Novak 1984; Rahman et al. 2014). In *Daphnia*, the toxic response is dependent on the form of arsenic, As(III) versus As(V) (Fan et al. 2015). In the case of As(III), there is a high affinity with sulfhydryl groups, with cellular uptake occurring via simple diffusion from extracellular to intracellular space (Kotyzová et al. 2013; Fan et al. 2015). As(V), on the other hand, has a similar structure to that of phosphate, and uptake occurring via phosphate transport systems (Hughes 2002; Yang et al. 2012; Fan et al. 2015). Following uptake, the two forms of arsenic can affect sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) activity, total superoxide dismutase (SOD) activity, malondialdehyde (MDA) production, and other important biological functions (Fan et al. 2015). Theegala et al. (2007) found that arsenic

biouptake/sequestration in *Daphnia* was negligible, and reproduction rates were also not affected by increasing arsenic concentrations. However, they did find that the *Daphnia* death rate increased (although not linearly) as arsenic concentrations increased from 0.25 to 5 mgL^{-1} (Theegala et al. 2007). Similarly, Hoang et al. (2007) also determined that mortality increased, but found that *Daphnia* reproduction was not impacted until very high concentrations (pulsed exposures to ≥ 3000 μgL^{-1} As) over a long exposure period (21 days).

In marine rotifers and copepods exposed to arsenic, Byeon et al. (2020) reported that the rotifer species *Brachionus plicatilis* and the copepod *Paracyclopina nana* were both more sensitive to As(III) than As(V); however, *B. plicatilis* was more tolerant to As(III) compared to the copepod *P. nana.* The difference in the exhibited tolerance was attributed to *B. plicatilis* having a more simplified arsenic biotransformation process and efficient antioxidant response compared to its copepod counterpart. In groundwater copepods, Hose et al. (2016) found that the 96-h EC_{50} for Budderoo cyclopoids was higher than that of Somersby copepods (i.e., the concentration of arsenic necessary to produce an effect in 50% of the population of Budderoo cyclopoids was higher than that necessary for Somersby copepods. The 96-h EC_{50} of the latter, however, were similar to those found in *Daphnia magna*. The authors noted that the results may be more accurate if the experiments were examined over a longer time period.

While *Daphnia pulex* and *Daphnia magna* are standard model organisms used in toxicity tests for contaminants like arsenic, Passino and Novak (1984) observed that the sensitivity of *Bosmina longirostris* (a small-bodied cladoceran) to As(V) was much greater than its *Daphnia* counterparts, leading them to recommend its inclusion as a zooplankton test organism for toxicity bioassays. This recommendation, however, has not yet been adopted in arsenic toxicity studies. For *Ceriodaphnia cf. dubia,* Rahman et al. (2014) found that while toxicity of the two

common freshwater arsenic species As(III) and As(V) were statistically similar, the former is more toxic to *C. cf. dubia* when compared to *D. pulex*. This also held true for *C. cf. dubia* that were exposed to dimethylarsinic acid (DMA) (Rahman et al. 2014). Similar to microalgae, it was also noted that phosphate may indirectly influence the toxicity of As(V) in Cladocera, as it has an effect on the uptake of arsenic from phytoplankton, the primary food source of Cladocera.

1.2 Legacy arsenic from gold mining in Yellowknife

The impacts of mining and other sources of anthropogenic pollution are well captured in in the lake ecosystems of remote northern regions, where few other contamination sources exist. This is particularly evident in lakes around the Yellowknife region in the Northwest Territories, Canada. Historic gold mining operations were a major source of arsenic contamination in the region (Caumette et al. 2012; Van Den Berghe et al. 2018) and has resulted in substantial impacts on aquatic biota at multiple trophic levels (Thienpont et al. 2016).

The City of Yellowknife (Northwest Territories, Canada) owes its establishment to the long history of mining in the region. Before it was established as a city, it started out as a small collection of miners' tents and has continued to grow in size since (Sandlos and Keeling 2016; City of Yellowknife 2019). As a result, mineral exploration and extraction has always played an important role in the economic development of the city, and the Northwest Territories region as a whole (Yellowknives Dene First Nation Elders Advisory Council 1997; Sandlos and Keeling 2016).

Beginning in 1938, the first large scale gold mine was established with Con Mine (1938-2003), and was quickly followed by Negus Mine (1939 - 1952), and Giant Mine (1948-2004) (Hutchinson et al. 1982). While Con Mine was operational the longest, Giant Mine had the highest total production, estimated at 7,066,750 ounces of gold (Moir et al. 2006). The gold

deposits processed at Con Mine and Giant Mine were hosted mainly in arsenopyrite ores (FeAsS), which meant that the extraction process used needed to be able to separate the gold from the arsenopyrite through the oxidation of arsenic and sulphur, a feat which was accomplished using roasters (Hutchinson et al. 1982; Canam 2006). This process resulted in the production of arsenic trioxide (As_2O_3) and sulphur dioxide (SO_2), which were vented into the atmosphere through roaster stacks at the two mines.

Between 1948 and 1999 (the year roasting ceased) As_2O_3 emissions were estimated to total approximately 20,000 tonnes (Bromstad 2011; Bromstad et al. 2017). The quantity of emissions from Con Mine were substantially lower compared to Giant Mine (Jones 1976; Schuh et al. 2018). Emissions decreased substantially after abatement technologies were implemented from 1951, starting with a Cold Cottrell Electrostatic Precipitator. In 1955, a Hot Cottrell Electrostatic Precipitator was added, which was followed by the installation of a Dracco baghouse in 1958. The combined effect of these abatement measures decreased arsenic emissions from 7,400 kg/day in 1951 to levels fluctuating around 300 kg/day by 1959 (CIRNAC 2018). The abatement measures were in part implemented due to public outcry because of the impacts on the Yellowknives Dene (Weledeh) people.

Arsenic contamination on the lakes used for sustenance by the Dene people suffered fish losses, which resulted in the Dene having to travel further for food. The greatest impact, however, were the lives of two Dene children by arsenic poisoning (Sandlos and Keeling 2016).

1.2.1 Arsenic Contamination of Yellowknife Lakes

An examination of ninety-eight (98) lakes in the Yellowknife area in the summer of 2015 showed that several lakes had arsenic concentrations exceeding the 10.0 μgL^{-1} drinking water limit established by Health Canada (Federal-Provincial-Territorial Committee on Drinking Water

2016), which was attributed to historic arsenic emissions from Giant Mine (Palmer et al. 2015). Arsenic concentrations at these levels also exceed the 5.0 $\mu g \cdot L^{-1}$ guideline for the protection of aquatic life in freshwater ecosystems (CCME 2001).

Sediment core profiles that record the history of lead, arsenic and antimony input to Yellowknife lakes showed that the concentration of these metal(loid)s peaked at the height of gold mining operations in ~1950 consistent with recorded emissions of arsenic trioxide from Giant Mine (Cheney et al. 2020). The peak concentrations of metal(loid)s decreased with increasing distance from Giant Mine (Cheney et al. 2020). Paleo-toxicity modelling based on sediment metal(loid) concentrations showed that the predicted toxicity of contaminated sediments to aquatic organisms was highest during the height of mining, and has decreased following the cessation of arsenopyrite roasting, suggesting some chemical recovery has occurred; however, in the most impacted lakes, legacy metal(loid) contamination may continue to impact aquatic organisms (Cheney et al. 2020).

Palmer et al. (2019) examined seasonal arsenic cycling in Yellowknife lakes, and concluded that under-ice processes are important for arsenic speciation and toxicity. During the winter season, ice cover leads to the development of anoxia resulting in reducing conditions at the sediment water interface which remobilize inorganic As(III) from the sediment into porewaters and back into the water column. This occurs as a result of the reduction of As(V) to As(III), and changes to the mobility of As(III) in sediment porewaters (Palmer et al. 2019). The mobilization of arsenic during the winter months could in part be responsible for hindering biological recovery in shallow lakes within the region (Palmer et al. 2019). Additionally, it was found that higher temperatures during the summer months led to another increase in As(III) concentrations (Palmer et al. 2019), an issue that could be exacerbated by climate warming.

1.2.2 Impacts of legacy arsenic contamination on aquatic biota in Yellowknife

Almost two decades following the cessation of mining in Yellowknife, the subarctic fish species in the region continue to be affected by arsenic contamination. For example, Chételat et al. (2019) reported that the arsenic concentrations within the muscles of burbot (*Lota lota*), lake whitefish (*Coregonus clupeaformis*), and northern pike (*Esox lucius*) in Yellowknife Bay were almost two times greater than those in the rest of Great Slave Lake. Yellowknife Bay has historically received arsenic run-off from Giant Mine and has a higher present-day surface water arsenic concentration compared to unimpacted areas of the greater Great Slave Lake. Littoral fish taxa generally had lower concentrations of arsenic in muscle tissue compared to their pelagic counterparts (Chételat et al. 2019).

Arsenic concentrations in zooplankton tissues have also been reported in Yellowknife lakes, primarily as inorganic forms of arsenic, As^{III}–S and As^{V}–O, but some zooplankton were also found to have contained organic arsenic species such as methylarsonate (MMA), and dimethylarsinate (DMA) (Caumette et al. 2011). In Grace Lake, which has a relatively low concentration of surface water arsenic (7 ± 2 μg/L), arsenobetaine (AB) was also measured in zooplankton.

The major difference between zooplankton in heavily contaminated lakes compared to lakes with lower arsenic concentrations appears to be the proportion of organic arsenic: zooplankton from less contaminated lakes have a higher proportion of organic arsenic compared to those in heavily contaminated lakes (Caumette et al. 2011). Toxicity tests using *Daphnia pulex* from Rat Lake in Yellowknife pointed to the possibility of *Daphnia* synthesizing the organoarsenical compounds, as only inorganic forms of arsenic (AsIII–S and AsV–O) were found in the lake sediments and phytoplankton consumed as food (Caumette et al. 2012).

1.2.3 Arsenic contamination in Pocket Lake

One of the most heavily impacted lakes in the Yellowknife region is Pocket Lake, a small headwater lake located approximately one kilometer from Giant Mine's roaster stack, the source of arsenic trioxide emissions. Striking ecological impacts to diatoms (siliceous algae), zooplankton (Cladocera), and benthic macroinvertebrates (the Chironomidae) corresponding to arsenic contamination were observed based on subfossil remains analyzed in a sediment core from this lake (Thienpont et al. 2016). The Pocket Lake sediments that represent materials deposited during the period of unregulated arsenic trioxide emissions from Giant Mine consisted of more than 3% arsenic by dry weight (Thienpont et al. 2016). Several other metal(loid)s associated with roaster operations also increased during this period, including iron, antimony and mercury.

Changes to the plankton and macroinvertebrate community were inferred from their subfossil remains preserved in a sediment core from Pocket Lake. Planktonic diatom taxa were found to have disappeared from the assemblage following the onset of arsenic concentrations and have not recovered (Thienpont et al. 2016). Additionally, the chironomid subfossil record showed that as concentrations increased, *Tanytarsus* decreased, while *Cricotopus* (metal tolerant species) and *Psectrocladius* increased. However, in contrast to the diatoms, there was evidence of potential recovery for the benthic (sediment associated) chironomids (Thienpont et al. 2016).

For the Cladocera, a striking shift occurred with significant changes observed in the sediment record coincident with the onset of arsenic contamination in the region. Specifically, the subfossil record revealed the appearance of *Daphnia* at the time of onset of arsenic enrichment, a taxa which was previously absent from the sediment records of Pocket Lake. As arsenic levels peaked, all Cladocera (including *Daphnia*), were functionally extirpated similar to

the response of planktonic diatoms. As it currently stands, there has been no evidence of recovery for these taxa (Thienpont et al. 2016). The results of Thienpont et al. (2016) led the authors to hypothesize that (1) *Daphnia* was initially favoured by arsenic contamination due to a competitive advantage over other zooplankton species that may be more sensitive to arsenic, and (2) that the eventual extirpation of *Daphnia* was due to its critical threshold for arsenic exposure being exceeded (i.e., the lake became too toxic for *Daphnia*).

In lakes further removed from Pocket Lake, Sivarajah et al. (2019) found that the planktonic *Discostella stelligera/pseudostelligera* complex dominating in lakes with arsenic concentrations within the guideline for the protection of aquatic life, whereas in those lakes that exceeded the threshold, benthic diatoms (small fragilarioid taxa and *Achnanthidium minutissimum*) were in abundance. The main finding was that diatom composition, species richness and diversity were not primarily influenced by long term arsenic contamination, but rather by a myriad of limnological variables and climatic conditions - mainly nutrients, ions and contaminants.

1.3 Paleo-ecotoxicology as a tool to assess arsenic contamination

To address temporal knowledge gaps encountered during traditional ecotoxicological studies, a newly emerging subfield of paleolimnology, “paleo-ecotoxicology”, is starting to be become more popular. Paleo-ecotoxicology combines traditional approaches in paleolimnology and ecotoxicology (Korosi and Smol 2012a, b). Paleolimnology is a field in which lake sediment cores are analyzed to understand the past lake conditions and trajectories of ecosystem change (Frey 1988). Sediments that have been either produced within the lake or from external sources (deposition from the atmosphere, run-off from the watershed), concentrate at the bottom of the lake in an “accumulation zone” (Smol 2008). Due to the low hydrodynamics commonly

associated with the accumulation zone, these sediments are not easily removed and build up as a stratigraphic record (Wetzel 2001; Cohen 2003; Smol 2008). The resulting sediment profiles hold information about both short-term and long-term natural and human-induced changes in the aquatic ecosystem (Wetzel 2001). The chemical, biological and physical indicators stored in these records are the objects of analyses in paleolimnological inquiry.

Paleolimnological studies can be conducted using a "top-bottom" approach, and/or a downcore approach. The top-bottom approach is a paleolimnological technique in which the environmental changes between two defined time periods across multiple lakes are examined (Smol 2008). In contrast to downcore analysis, only two sediment intervals are analysed, the top centimeter (or 0.5 cm) interval and the bottom centimeter interval. These intervals are indicative of modern day changes (typically the last few years prior to sampling), and pre-industrial conditions (ca. 1850 prior to major anthropogenic stressors), respectively (Cumming 1992; Heiskary 2009). The technique therefore provides a snapshot of change, and because analysis is limited to only two sediment intervals, it allows for rapid assessment of many lakes (Heiskary 2009). In contrast, the downcore approach involves a detailed analysis of sediment cores at high temporal resolution (i.e., analysing the indicators in several intervals). This approach allows us to determine the timing of changes, however, owing to its time and resource-intensive nature, a fewer number of lakes (in comparison to the "top-bottom" approach) are selected for analysis (Wetzel 2001; Heiskary 2009).

The paleo-ecotoxicological approach begins with the collection of a sediment core, which is then sectioned into intervals (0.25, 0.50 or 1.00 cm depending on sedimentation), and dated using ^{210}Pb by gamma spectrometry (Appleby 2001), supplemented by ^{137}Cs (Jaakkola et al. 1983) to establish a chronology. The sediments are subsampled to isolate biological proxy

records (e.g., Cladocera subfossils) and geochemical indicators (contaminant concentration record), which are analyzed analogous to the “dose-response” framework of ecotoxicological approaches. The data obtained is used to infer how an aquatic ecosystem is responding to a given stressor, such as arsenic contamination (Korosi, et al. 2017b). This approach also provides the opportunity to test laboratory predictions, for instance those from a toxicity bioassay, over a long term period in a natural ecosystem (Korosi, et al. 2017a).

The paleo-ecotoxicological approach has mainly been adapted in studies examining metal contamination (Chen et al. 2015; Labaj et al. 2015), and has provided critical insight as to the responses of aquatic biota to this type of contamination. For example, Labaj et al. (2015) used sediment cores to track the extent of recovery of Cladocera in two lakes near Sudbury and Killarney Provincial Park (Ontario, Canada). Both lakes experienced lake acidification, but differed in their metal contamination, and multiproxy paleolimnological records indicated that metal contamination impeded the recovery of Cladocera. Chen et al. (2015) provided some of the first evidence on the long-term impacts of arsenic on lakes. The authors examined two lakes in China, both of which had a history of arsenic contamination attributed to the discharge of industrial tailings. They noted high losses of crustacean zooplankton and algae concurrent with increasing arsenic concentrations, and among the most striking changes were the reductions in daphniid and bosminid fluxes despite continuous nutrient enrichment that would be expected to increase the abundances of these taxa. These types of changes are not readily apparent from short-term mesocosm or lab studies. Moreover, they demonstrated the particular utility of the Cladocera as paleo-ecotoxicological indicators of long-term metal contamination.

1.3.1 Cladocera as "paleo-ecotoxicological" indicators

Cladocera (commonly known as water fleas) are a superorder of small, typically herbivorous, crustaceans (Class Branchiopoda) ranging in size from 0.2 - 3.0 mm. They occupy various niches in freshwater ecosystems worldwide and occur across a wide range of environmental conditions (Korhola and Rautio 2001; Wetzel 2001). They are considered to be highly important in aquatic food webs because of their role as regulators of primary production, and a food source for higher organisms (Wetzel 2001). They are sensitive to ecosystem change, and respond rapidly to changing environmental conditions (Pociecha et al. 2019). Due to many of their body parts (e.g., post abdominal claw, antennules, carapaces) being composed of chemically-inert chitin, they can be preserved in lake sediments for millennia (Korhola and Rautio 2001). From their subfossil remains (Figure 1.1), their responses to changes in their ecosystems can be inferred over centennial to millennial timescales (Korhola and Rautio 2001), including changes due to heavy metal contamination (e.g., Labaj et al. 2015; Thienpont et al. 2016; Leppänen et al. 2018).

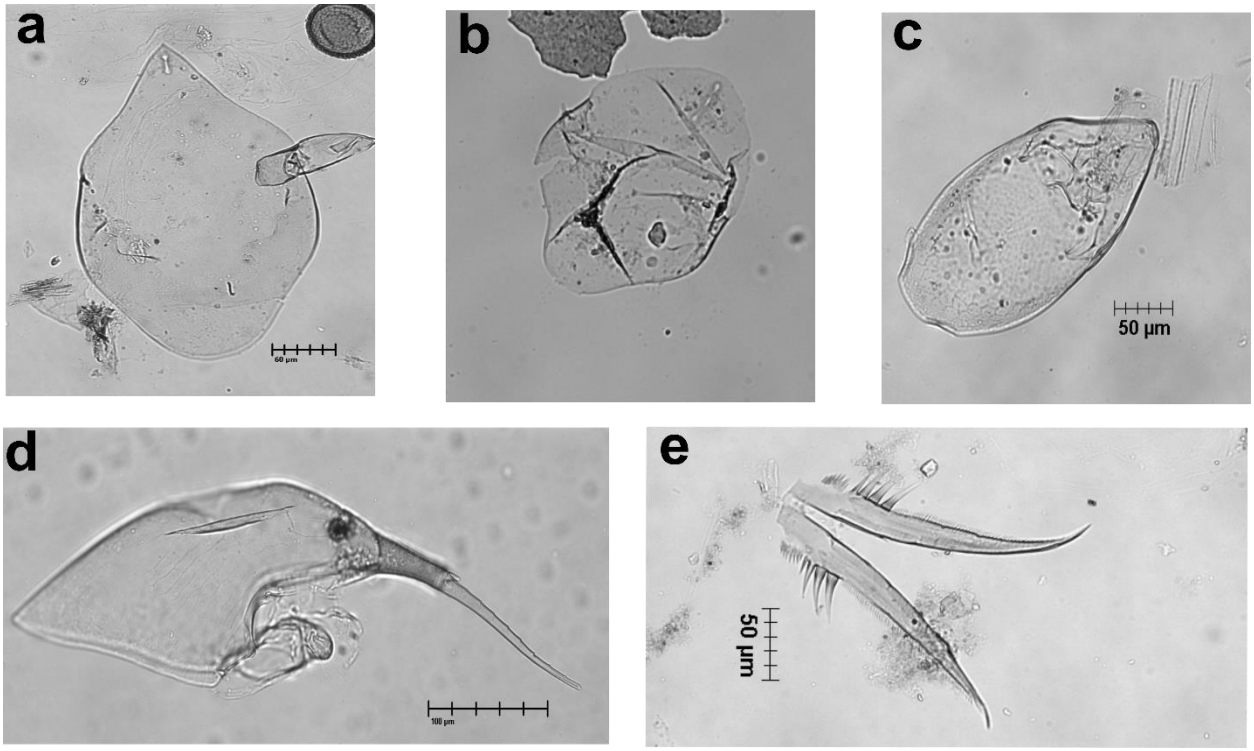

Figure 1.1. Examples of common Cladocera subfossil remains encountered in the Canadian Arctic: a) *Alona affinis* headshield; b) *Alona circumfimbriata/guttata* headshield; c) *Chydorus* headshield; d) *Bosmina* headshield; and e) *Daphnia* postabdominal claw. Images taken by J. Korosi.

While limited research has been conducted on the long-term responses of Cladocera to arsenic (e.g., Chen et al. 2015; Thienpont et al. 2016), several paleolimnological studies have noted striking changes in Cladocera assemblages coincident with periods of heavy metal enrichment. For example, Pociecha et al. (2019) found that metal contamination (copper and cadmium) from a mine discharge in southern Poland resulted in significant decreases in the relative abundances of *Alonella nana*, *Alona affinis*, *Alona sp.* and *Pleuroxus sp.* and an increase in more tolerant species such as *Chydorus sphaericus*. Another study conducted in Canada by Winegardner et al. (2017) found that heavy metal contamination impacted Cladocera species richness. Species richness is the number of species in a defined spatial unit and is one of the

simplest measures of biodiversity (Veech 2017). Winegardner et al. (2017) noted that increased metal contamination (e.g., Al, As and Fe) led to decreased species diversity and cladoceran richness in Lake Dauriat, in addition to a high turnover in the species assemblage during the period of mining.

In the field of ecotoxicology, cladocerans are widely used in lab bioassays as biological indicators to examine the toxicological impacts of contaminants. While several different taxa are used for these analyses, *Daphnia magna* and *Daphnia pulex* are the most common (Dodson and Frey 2001; Dumont and Negrea 2002; Sarma and Nandini 2006). They are selected for various reasons, including their sensitivity to common toxic substances, and the availability of well-developed culture techniques (Koivisto 1995). While these types of traditional ecotoxicological studies provide some insight on responses to arsenic, they are conducted on relatively short timescales. As such, these studies may not account for potential long term effects that have been noted from paleo-ecotoxicology studies on metal contaminants (e.g., Rogalski 2017; Thienpont et al. 2016). Consequently, techniques that combine the long-term perspective provide vital information regarding the long-term responses and possible (mal)adaptations of taxa. Resurrection ecology is one such method that allows for the examination of living organisms that are genetically representative of populations from multiple time periods.

1.4 Resurrection Ecology – Resting eggs as paleo-ecotoxicological indicators

Many living creatures adopt dormancy strategies during periods of adverse environmental disturbances to ensure the survival of their populations (Dahms, 1995; Radzikowski et al., 2018). One such strategy is the formation of resting stages, which is a state of arrested development (Danks 1987; Radzikowski 2013). For Cladocera, including *Daphnia*, the production of subitaneous eggs (eggs with no dormancy stage) shift to the production of diapausing eggs (eggs

that have a dormancy/resting stage) under adverse environmental conditions (Radzikowski et al. 2018). The resting eggs are found within ephippia, which is a chitinous shell (a modification of the carapace) that encapsulates the eggs (Schultz 1977).

Daphnia resting eggs can remain viable (able to hatch) for several decades (Radzikowski 2013) and even centuries (Frisch et al. 2014), and can end up trapped within the sediment matrix of a lake basin (Burge et al. 2018). Assuming the sediment profile is not perturbed, the sediment layers in which the resting eggs are trapped can be dated to determine when the eggs were produced. Because of their long-lived nature, the ephippial eggs can be extracted from the sediment matrix and stimulated to hatch clonal lines of past populations. To stimulate the hatching process, a combination of two stimuli are used, light/photoperiod and temperature regimes (Radzikowski et al. 2018; Cuenca Cambronero and Orsini 2018).

Resurrected populations provide insight into the evolutionary response of the species to certain contaminants (Kerfoot et al. 1999; Kerfoot and Weider 2004; Smol 2008b). The model organism selected for resurrection ecology has mainly been *Daphnia*, primarily because of their cyclical parthenogenetic life cycle, the availability of well-established genomic resources, as well as the ability of their diapausal eggs to survive over an extended period of time (Burge et al. 2018).

Studies have been able to show the response of resurrected *Daphnia* populations to pesticides (Simpson et al. 2015), and heavy metal contamination (Rogalski 2017). Simpson et al. (2015) assessed the responses of different genotypes of *Daphnia pulicaria* from a single population, resurrected from three time periods, to an organophosphate insecticide. Test organisms were resurrected using resting eggs collected from South Center Lake, Minnesota, USA. In this study, eggs from as far back as 1301–1646 were successfully hatched. It was found

that the organisms with genotypes from the oldest time period (1301-1646) were more sensitive to the pesticide than those from 1967-1977. The authors suggested that this difference was caused by microevolutionary shifts leading to decreasing sensitivity, in response to increasing cultural eutrophication.

In contrast to the previous study, Rogalski (2017) observed the opposite trend when studying metal contamination, where ancient *Daphnia ambigua* clones were less sensitive to cadmium and copper exposure compared to clones from periods of increased cadmium and copper concentrations. This study provided evidence for potential maladaptation of *Daphnia* to increases in heavy metal contamination. Thus far resurrection methods have not been used to study the long-term response of *Daphnia* to arsenic exposure, therefore the long-term adaptation/maladaptation of this taxa to arsenic is unknown.

Resurrection ecology is predicated on there being viable resting eggs within the sediment matrix. However, viable restings eggs can be extremely rare. For example, in examining the resting egg bank of six lakes in the High Tatra Mountains, Marková et al. (2006) only encountered 94 potentially viable eggs from a total of 4,948 ephippia. This extreme rarity in viable resting eggs can be attributed to resting egg abortion (failure to deposit resting eggs in ephippia) which frequently occurs naturally (Conde-Porcuna et al. 2011), successful hatching of eggs in the lake (Winegardner et al. 2017), or due to the degradation of eggs (DeStasio 1989).

1.5 Thesis Organization and Rationale

Ecotoxicological studies have been conducted in Yellowknife to understand the effects of arsenic pollution on zooplankton (Caumette et al. 2011; Caumette et al. 2012) but were all conducted on short timescales. Therefore, they are not designed to examine legacy and cumulative effects of arsenic exposure on aquatic biota (Chen et al. 2015; Korosi et al. 2017a;

Korosi et al. 2017b). My research examines the long-term impacts of arsenic contamination on Cladocera in Yellowknife lakes using a paleo-ecotoxicological framework. It builds on the findings of the paleo-ecotoxicological study of Pocket Lake (Thienpont et al. 2016), which documented striking ecological changes and eventual extirpation of Cladocera in response to arsenic contamination. Pocket Lake is situated on the Giant Mine lease territory and is one of the most impacted lakes in the Yellowknife region. Little is known about how Cladocera in less impacted lakes have responded to historic arsenic contamination. This thesis is composed of two related studies, written as independent manuscripts (Chapter 2 and 3) that collectively address knowledge gaps arising from the Pocket Lake study and provide direction for future research to better understand the ecological impacts of historic gold mining and arsenic contamination on Yellowknife lakes (summarized in Chapter 4: General conclusions and Future Directions).

In Chapter 2, I assess regional changes in Cladocera in twenty-three (23) lakes in the Yellowknife region along a gradient of arsenic contamination and distance from Giant Mine using a "top-bottom" paleolimnological approach. There were two primary objectives for this study: to 1) infer regional changes in cladoceran species assemblages from pre-mining conditions to present day; and 2) characterize possible cladoceran species responses to arsenic contamination in impacted lakes that can be explored in future studies. In addition, my "top-bottom" study of Yellowknife lakes also identified lakes with abundant *Daphnia* populations that are potential candidate lakes for resurrection ecology studies (Chapter 3).

In Chapter 3, I examine the viability of *Daphnia pulex* resting eggs in Pocket Lake to determine if they can be used in future arsenic toxicity bioassays using resurrected *Daphnia* to test hypotheses derived from the Cladocera-based paleolimnological study of Pocket Lake by Thienpont et al. (2016): (1) that *Daphnia* was initially favoured by arsenic contamination due to

a competitive advantage over other zooplankton species that may be more sensitive to arsenic, and (2) that the eventual extirpation of *Daphnia* was due to its critical threshold for arsenic exposure being exceeded. The specific objectives for this study were to: 1) investigate if sediment arsenic toxicity compromised the integrity of the *Daphnia* resting egg bank in Pocket Lake; and 2) assess the viability of resting eggs in Pocket Lake sediments for use in future resurrection ecology studies.

Daphnia clones obtained from a reference lake may not be representative of the *Daphnia* clones that historically colonized Pocket Lake, which is why the resurrection of *Daphnia* from the resting egg bank of Yellowknife lakes has potential value for future study. Pocket Lake was examined for resting egg viability due to the striking shifts in the *Daphnia pulex* population observed by Thienpont et al. (2016).

Long-term environmental data is vital for the development of ecosystem management plans, to understand the magnitude of environmental changes, and to develop informed interventions (Saulnier-Talbot 2016; Smol 1992). My study aims to apply paleo-ecotoxicological methods to understand the impacts of arsenic contamination on Yellowknife lakes and other sensitive environments, by providing the missing long-term perspective. Moreover, the study will provide information to the Yellowknives Dene First Nation, for whom the region is of great cultural significance, as to detrimental effects of the metal(loid) contamination on aquatic habitats that resulted from gold mining.

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Chapter 2 : Regional changes in Cladocera (Branchiopoda, Crustacea) assemblages in subarctic (Yellowknife, Northwest Territories, Canada) lakes impacted by historic gold mining activities

2.1 Abstract

In Yellowknife (Northwest Territories, Canada), roaster stack emissions from historic gold mining activities, particularly Giant Mine (1948-2004), have left a legacy of arsenic contamination in lakes. We examined Cladocera (Branchiopoda, Crustacea) subfossil remains in the recent and pre-industrial sediments of 23 lakes (lake-water arsenic gradient of 1.5 to 750 µg/L) within a 40 km radius of Giant Mine to provide a snapshot of regional change in Cladocera since pre-1850. We found that littoral and benthic taxa dominated the recent assemblages in high-arsenic lakes ([As] > 100 µg/L), while pelagic *Bosmina* was dominant in lakes with lower arsenic. Cladocera richness and diversity were positively correlated with arsenic (p = 0.004, R^2 = 0.39; and p = 0.002, R^2 = 0.46, respectively), except for four lakes with [As] >100 µg/L. The lakes that showed the most pronounced changes in Cladocera since pre-1850 were those affected by urban development in Yellowknife, where complete shifts in the dominant taxa occurred. Lakes that were heavily impacted by arsenic emissions did not experience notable shifts in Cladocera assemblages. Our study shows that changes in Cladocera assemblages in subarctic lakes are dependent on local, lake-specific limnological conditions rather than being driven by regional forcing factors.

**The raw data for this chapter is available under a creative commons licence in YorkSpace, accessible at:* http://hdl.handle.net/10315/37806

2.2 Introduction

Cladocera are a superorder of small crustaceans (Class Branchiopoda) that are useful bioindicators of metal contamination, owing to their rapid responses to changing environmental conditions (Leppänen, 2018a; Pociecha et al., 2019). Many cladoceran species are considered to be keystone species, and impacts to their communities have the potential to cascade through the aquatic food web (Wetzel, 2001). Furthermore, Cladocera leave identifiable subfossil remains (primarily post abdominal claws, headshields, antennules and carapaces) composed of chemically inert chitin that preserve in lake sediments over centuries to millennia (Korhola & Rautio, 2001). Cladocera are therefore useful paleoecological indicators to track aquatic ecosystem changes over timescales not otherwise feasible through contemporary sampling. Importantly, in mining regions where pre-impact ecological data are limited or unavailable, Cladocera subfossil remains provide an opportunity to assess the environmental impacts of mining on nearby aquatic ecosystems within a temporal context that includes an assessment of natural variability and baseline conditions (Saulnier-Talbot, 2016).

The response of Cladocera to metal pollution have been documented in paleolimnological studies globally. In the Canadian province of Québec, increases in the concentrations of iron, aluminium, copper, cobalt and nickel from wastewater inputs and atmospheric deposition from local mining operations resulted in a decrease in Cladocera species diversity and richness (Winegardner et al., 2017). In southern Poland, sediment records from subsidence ponds downstream of mine discharge exhibited a negative correlation between copper concentration and the occurrence of the cladoceran taxa *Alona*, *Alonella*, *Daphnia* and *Graptoleberis* (Pociecha et al., 2019). In addition, the subsidence ponds showed a negative correlation between sedimentary zinc and lead concentrations and the abundance of *Alona* (Pociecha et al., 2019).

Cladocera-based paleolimnological studies have also documented biological recovery from mining-associated metal pollution. In northwest Russia, *Daphnia* and *Leptodora kindtii* were extirpated in Lake Imandra due in part to nickel and copper pollution; however, decreases in anthropogenic stressors in the region, including the introduction of stricter pollution control measures, led to the recolonization and recovery of these species (Moiseenko et al., 2009).

The long-term sensitivity of cladocerans to arsenic pollution has received less attention compared to copper, aluminum, cadmium, nickel and zinc pollution. Arsenic is a naturally occurring element in the environment that in some cases is a significant stressor on water quality from widespread urbanization and industrialization (Smedley & Kinniburgh, 2002). *Daphnia*, one of the most commonly used model organisms in ecotoxicology, are relatively tolerant of high arsenic, but can still experience high mortality in highly contaminated waters (Suhendrayatna et al., 1999; Okamoto et al., 2015). The smaller pelagic taxon, *Bosmina*, may be more sensitive than *Daphnia* to arsenic pollution (Passino & Novak, 1984), but despite this possibility, *Daphnia* is used in arsenic toxicity studies rather than *Bosmina*. Paleolimnological studies of highly arsenic-contaminated lakes support the findings documented in lab bioassays on *Daphnia* and *Bosmina*. In two arsenic-contaminated lakes in China, a >10-fold decline in the abundance of *Daphnia* and *Bosmina* subfossil remains occurred concurrent with an increase in sediment arsenic concentrations, which peaked at 479 and 949 μg/g dry weight, compared to pre-industrial baseline levels of 34.4 ± 5.1 (±1 SD) μg/g and 44.2 ± 7.1 (±1 SD) μg/g dry weight, respectively (Chen et al., 2015). The reductions in daphniid and bosminid abundances occurred despite increases in nutrient concentrations that would otherwise be expected to increase cladoceran abundances (Chen et al., 2015).

In the subarctic city of Yellowknife (Northwest Territories, Canada), historic gold mining has resulted in substantial long-term contamination of freshwater ecosystems by arsenic and other metal(loid)s (Palmer et al., 2015). While arsenic concentrations increased during the period of mining, elevated pre-mining concentrations of arsenic have been noted in some lakes, indicating naturally elevated geogenic arsenic in Yellowknife lakes (Galloway et al., 2012; Cheney et al., 2020). Paleotoxicity modelling based on sediment arsenic concentrations suggested that some lakes therefore may have been naturally above the biological effects probable threshold (Cheney et al., 2020).

Thienpont et al. (2016) examined the long-term biological effects of arsenic on lake biota in a paleolimnological study of Pocket Lake, located within a 1 km radius of the Giant Mine roaster stack (the largest gold mine in Yellowknife). This study showed that despite the naturally elevated arsenic concentrations, the biological assemblages were stable prior to mining. However, a striking ecological shift from epiphytic and benthic taxa like *Alona* to pelagic *Daphnia* occurred following the onset of mining (Thienpont et al., 2016), supporting lab toxicity studies that indicate *Daphnia* are relatively tolerant of arsenic (Passino & Novak, 1984; Suhendrayatna, 1999). At the peak of arsenic emissions, when arsenic concentrations in the Pocket Lake sediments reached greater than 30,000 µg/g (more than 3% arsenic by dry weight), all Cladocera (including *Daphnia*) were functionally extirpated with no evidence of recovery since emissions were reduced in 1951 and mining operations ceased in 2004 (Thienpont et al., 2016). Similarly, planktonic diatom (siliceous algae) taxa disappeared from the sediment assemblage at the height of arsenic contamination and have not recovered (Thienpont et al., 2016).

Although Pocket Lake represents an extreme example of arsenic contamination in Yellowknife lakes, several lakes within a ~15 km radius of Giant Mine's operations currently have arsenic concentrations exceeding the 10 μg/L drinking water limit established by Health Canada (Health Canada, 2017), and the CCME probable effects level threshold of 41.6 mg As/kg (CCME, 1999). These exceedances are attributed to legacy arsenic from mining emissions (Palmer et al., 2015; Houben et al., 2016). Sediment core reconstructions of the history of arsenic contamination of Yellowknife lakes confirm this, as post-mining sediment enrichment of arsenic, antimony, and lead are highest in lakes closer to Giant Mine, and decreased with increasing distance from the roaster stack (Cheney et al., 2020). Paleotoxicity modelling based on dated sediment core intervals deposited at different periods in the history of the mine found that the predicted toxicity to aquatic organisms was highest during the height of mining, and decreased following the cessation of roasting, suggesting some potential for biological recovery (Cheney et al., 2020). For some lakes, however, legacy metal(loid) contamination of lake sediments may continue to have ecosystem effects, as arsenic concentrations exceed the CCME interim sediment quality guidelines (ISQG) (Cheney et al., 2020).

Despite the extent of arsenic contamination, examination of subfossil diatom assemblages in the surface sediments of thirty-three Yellowknife lakes spanning a gradient of arsenic concentrations found that diatom assemblages were primarily influenced by nutrients and ionic compounds while mining related variables played a limited role in explaining the variation in species assemblages, richness, and diversity (Sivarajah et al., 2019). Stewart et al. (2018) found that recent climate warming has exacerbated water quality issues associated with eutrophication in Niven Lake, a Yellowknife lake that received raw sewage inputs from 1948 to 1981. The combined stressors of urbanization, eutrophication, and climate warming are also related to shifts

in subfossil diatom assemblages in paleolimnological studies of Yellowknife lakes (Sivarajah et al. 2020).

Clearly, multiple stressors complicate efforts to understand the ecological legacy of arsenic pollution from historic gold mining activities on Yellowknife lakes. The purpose of this study is to investigate the potential of subfossil Cladocera assemblages as bioindicators of arsenic pollution in Yellowknife lakes in a multi-stressor framework, expanding on the findings from Pocket Lake (Thienpont et al., 2016) to include lakes along a gradient of impact from mining activities. We used a "top-bottom" paleolimnological approach (Smol, 2008) to provide a snapshot of present-day (surface or "top" sediments) and pre-industrial (sediment interval representative of the pre-industrial period, ca. 1850, or "bottom" sediments) cladoceran assemblages in twenty-three Yellowknife-area lakes. The primary objectives of our study were to: 1) reconstruct regional changes in cladoceran species assemblages since pre-industrial times; and 2) identify cladoceran taxa that appear to be sensitive or tolerant of arsenic, which can be explored as candidate ecological indicators of arsenic pollution in future research. This study provides insights into ecological change in Yellowknife lakes impacted by historic gold mining, urban development, and climate warming, as well as the use of Cladocera as paleo-ecotoxicological indicators of arsenic contamination in freshwater ecosystems.

2.3 Methods

2.3.1 Study Site Description

The City of Yellowknife is located on the northern shore of Great Slave Lake (Figure 2.1). It is the capital of the Northwest Territories (Canada), and has a population of 18,884 people (Statistics Canada, 2017). Yellowknife, and the surrounding area, is underlain by the

Slave Structural Province of the Canadian Shield, and located within the Great Slave Uplands and Lowlands of Taiga Shield High Boreal ecoregions (Ecosystem Classification Group, 2008). The regional landscape is dominated by exposed bedrock plains, hilly bedrock uplands, and an abundance of lakes and streams (Ecosystem Classification Group, 2008). Vegetation is characterized by lichen woodlands and moss forests, which are comprised primarily of black spruce (*Picea mariana*), white spruce (*Picea glauca*), lichen on exposed bedrock, paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), and jack pine (*Pinus banksiana*). Variable-textured Brunisols, Organic Cryosols, Organic, and Gleysols are the dominant soil types in the region (Ecosystem Classification Group, 2008). Climate in the region is subarctic continental, and the permafrost is discontinuous (Wolfe, 1998). Mean annual temperature is -4.38℃ and mean annual precipitation is 289 mm, based on meteorological data recorded by Yellowknife Airport averaged over the period of 1981 to 2010 (Environment and Climate Change Canada, 2019). The Yellowknives Dene (Weledeh) First Nation have a long history of habitation and traditional subsistence use of the land and surface water resources in the north Great Slave Lake region (Sandlos & Keeling, 2016).

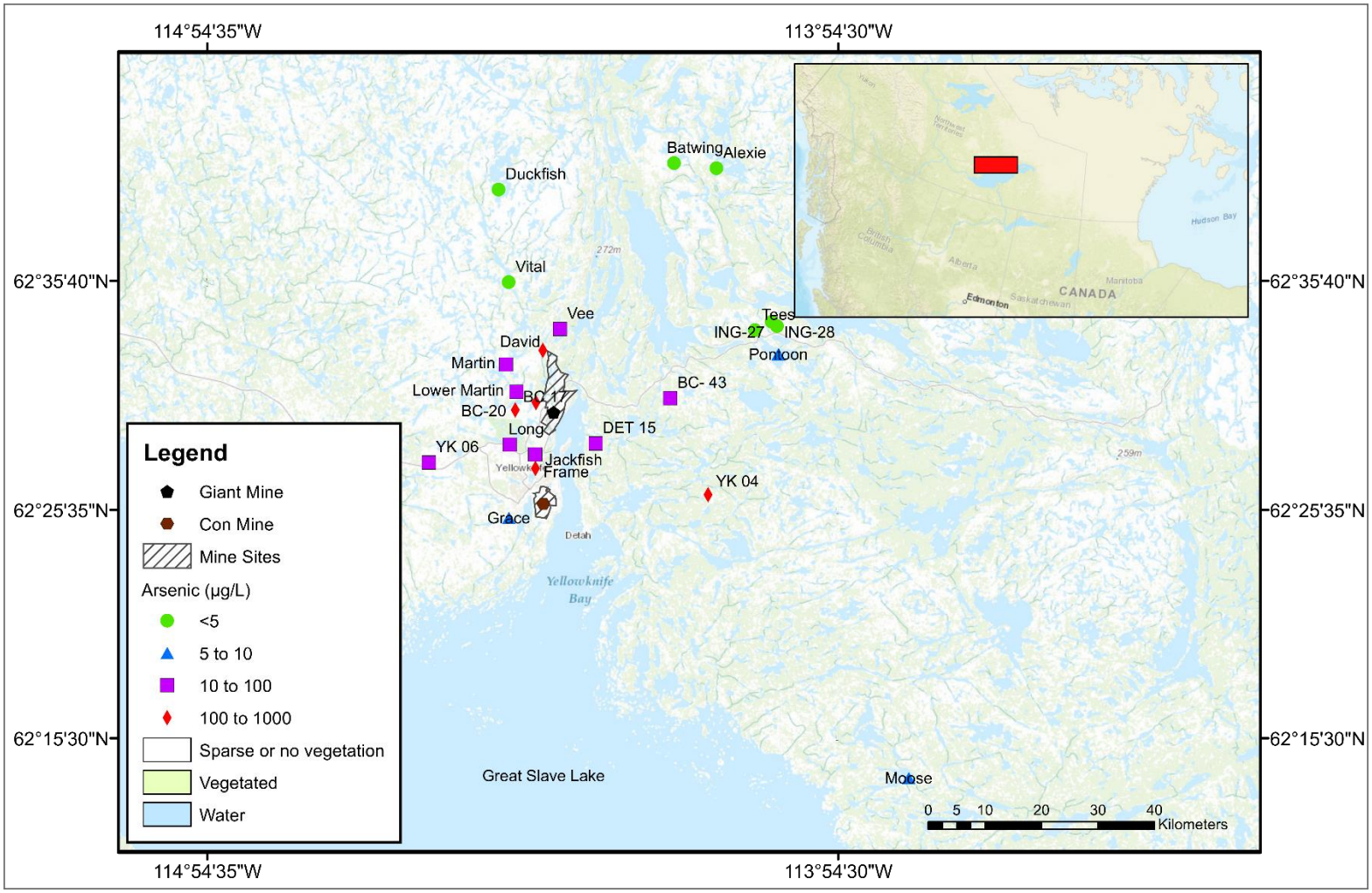

Figure 2.1. Map of the study region (Yellowknife, Northwest Territories, Canada) showing the locations of the 23 study lakes and their present-day lake-water arsenic concentrations. The locations of Giant Mine and Con Mine are indicated by the black and brown pentagons, and their respective mining footprints are shown. Inset map shows the study location in the context of Canada

Mining in Yellowknife began in 1938 (Hutchinson et al., 1982), starting with Con Mine (operational from 1938 to 2003). Two other large-scale mines were established and operational from 1939 to 2004, namely Negus Mine (1939 - 1952), and Giant Mine (1948-2004). The gold deposits of Con Mine and Giant Mine were hosted in arsenopyrite ores (FeAsS), necessitating an extraction process that separated the gold and arsenopyrite through the oxidation of arsenic and sulphur, which was accomplished using roasters (Hutchinson et al., 1982; Canam, 2006). This process resulted in the production of arsenic trioxide (As_2O_3) dust, a highly toxic, bioavailable and water-soluble arsenic compound (Liu et al., 2008), which was free-vented (unregulated emission) into the atmosphere in the early years of the mine (Wagemann et al., 1978). Emissions decreased after 1951 when an electrostatic precipitator and baghouses were used to reduce emissions following public pressure (Jamieson, 2014). From 1948 to the cessation of roasting in 1999, As_2O_3 emissions to the surrounding environment were estimated to be approximately 20,000 tonnes, with most emissions occurring prior to the implementation of emission controls more than 50 years ago (Bromstad, 2011; Bromstad et al., 2017).

The selection of the 23 study lakes (Figure 2.1; Table 2.1) was based on multiple criteria. These included present-day arsenic concentrations in water and sediment, the distance from Giant Mine and Con Mine, and the position from the mine relative to the prevailing northwest wind direction (Tanamal et al., 2018). Arsenic concentrations ranged from 1.5 μg/L to 750 μg/L, and distance from Giant Mine ranged from 1.52 km to as far as 42.2 km. The estimated zone of impact based on present-day surface arsenic concentrations is within a 15 km radius of the mine (Palmer et al., 2015), and within a ~40 km radius based on historic arsenic concentrations inferred from lake sediment cores (Cheney et al., 2020). Sediment core profiles for arsenic and

other metals have been published (Cheney et al., 2020) for the following study lakes: Alexie, BC-17, David, Duckfish, Lower Martin, Vee, and Vital (Figure 2.1).

Table 2.1. A summary of the limnological characteristics of the 23 study lakes. Lakes are arranged in descending order based on their arsenic (As) concentrations (highest to lowest). Measurements of water chemistry parameters were taken in 2010 and between 2014 and 2016. Lakes for which the cores were ^{210}Pb dated are denoted by "*"

Lake Name	Latitude	Longitude	Distance from Giant Mine	Lake Area	Depth	pH	DOC	SO4	Ca	Al	As	Sb
	Decimal Degrees		km	km^2	m	-	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L
BC 17*	62.49985	-114.421	3.10	0.16	0.50	8.25	35.80	13.00	19.10	101.00	750.00	12.30
BC 20*	62.50542	-114.388	1.52	0.36	0.50	9.76	52.20	22.00	23.60	98.60	697.00	12.20
Frame*[2]	62.45712	-114.389	5.35	0.93	5.00	7.59	42.80	174.00	120	9.70	343.00	5.10
David*	62.54320	-114.378	4.55	0.13	2.00	8.25	37.20	67.00	42.20	54.20	184.00	5.60
YK 04[§]	62.43778	-114.115	14.58	0.02	4.00	6.93	150.00	1.00	53.20	84.50	117.00	0.30
Jackfish	62.46703	-114.392	4.25	0.52	7.50	9.07	9.70	31.00	35.10	6.50	74.50	1.50
Lower Martin[1]*	62.51312	-114.419	3.27	1.35	7.00	7.88	15.90	3.00	14.90	7.80	40.60	1.20
Long[1]*	62.47439	-114.430	4.69	1.15	6.00	7.63	9.10	14.00	29.40	1.50	39.70	1.20
Vee*	62.55253	-114.348	5.79	0.77	5.20	8.00	20.60	14.00	42.40	41.70	34.20	1.00
YK 06	62.46120	-114.558	12.10	0.03	0.90	7.41	29.90	2.00	8.50	97.00	32.30	0.60
DET-15	62.47524	-114.294	4.50	0.02	0.86	7.36	33.40	2.00	11.20	39.00	26.60	0.90
Martin*	62.53313	-114.436	5.49	3.05	3.50	7.83	14.30	5.00	14.90	14.20	25.80	1.00
BC 43	62.50851	-114.175	9.75	0.40	5.00	8.64	33.70	4.00	29.90	11.70	16.40	0.30
Grace[1] *	62.42068	-114.431	10.23	0.63	16.00	7.49	15.30	14.00	18.50	3.30	9.70	0.40
Moose *	62.22893	-113.796	42.20	3.68	1.00	7.36	130.00	1.00	96.60	34.20	7.40	0.20
Pontoon	62.54077	-114.003	18.95	3.17	5.70	8.45	24.20	4.00	32.50	5.20	5.70	0.20
Vital *	62.59334	-114.432	10.74	1.52	10.00	7.44	15.90	3.00	13.70	9.30	4.80	0.30
Tees *	62.55828	-114.041	17.69	0.17	6.00	7.52	16.70	6.00	18.80	9.20	2.90	0.10
Batwing *[§]	62.68025	-114.169	22.05	0.22	8.00	7.13	35.40	1.00	17.50	5.40	2.60	0.10
ING-28	62.56122	-114.005	18.50	0.18	2.10	8.23	12.60	1.00	10.10	20.00	2.50	0.10
Duckfish[1] *	62.66088	-114.448	18.24	6.28	3.00	8.12	28.6	3.00	22.2	7.90	2.20	0.50

Lake Name	Latitude	Longitude	Distance from Giant Mine	Lake Area	Depth	pH	DOC	SO_4	Ca	Al	As	Sb
	Decimal Degrees	km	km^2	m	-	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L
ING-27	62.56450	-114.014	18.30	0.12	11.50	7.94	8.60	3.00	9.00	20.00	2.00	0.20
Alexie *[§]	62.67648	-114.102	23.52	4.56	21.00	7.69	13.40	4.00	13.80	4.50	1.50	0.10

[§]Lakes were sampled in the winter, and therefore water chemistry parameters will be influenced by solute exclusion during ice formation.

[1]Data from Palmer et al. (2015)

[2]Data from Sivarajah et al. (2019)

The selected lakes are underlain by either Archean metasedimentary bedrocks, volcanic rocks or Precambrian granitoids (Kerr & Wilson, 2000; Houben et al., 2016), are generally shallow (median depth of 5.00 m with a range of 0.5 m to 21 m) and small (median surface area of 0.52 km^2, with a range of 0.02 km^2 to 6.28 km^2; Table 2.1). Generally, the pH of the lakes was circumneutral to alkaline, with calcium concentrations ranging from 8.5 mg/L to 120 mg/L and dissolved organic carbon (DOC) concentrations from 8.6 mg/L to 150 mg/L (Table 2.1). Apart from gold mining and arsenic trioxide deposition, other stressors acting on the lakes include municipal development in Yellowknife (Gavel et al., 2018) and climate warming (Sivarajah et al., 2020). In order to better explore the changes in Cladocera assemblages across different arsenic concentrations, our study lakes were sorted into four groups based on present-day lakewater arsenic concentrations: <5 μg/L (n = 7), 5 to 10 μg/L (n = 3), 10 to 100 μg/L (n = 8) and 100 to 1000 μg/L (n = 5).

2.3.2 Field Methods

Sediment cores were collected from each of the study lakes using a UWITEC gravity corer (Mondsee, Austria) over numerous sampling seasons conducted during the period of March 2014 to July 2016. Lake bathymetries were unavailable for most of the study lakes, and as such sediment coring locations were selected from the centre of each of the lakes. The depths presented in Table 2.1 were measured at the coring locations. Water samples were collected from the surface waters at the coring locations. Water chemistry variables for YK 04, Batwing and Alexie were analyzed based on under-ice water samples and are therefore influenced by solute exclusion. Cores were sectioned into 0.5 cm intervals using a modified Glew (1988) vertical extruder and stored cold (4°C) or frozen until analysis at York University.

2.3.4 Laboratory Methods

Water chemistry variables were analyzed at the Taiga Environmental Laboratory located in Yellowknife, a Canadian Association for Laboratory Accreditation Inc. (CALA) certified institute, using Standard US Environmental Protection Agency methods. Water chemistry variables taken in 2010 were analyzed for trace metals (EPA Method 208–1) at the Taiga Environmental Laboratory, with sulphates, calcium, and dissolved organic carbon analyses conducted at Environment Canada's National Laboratory for Environmental Testing (NLET, a CALA accredited institute) following standard operating procedures (SOP #s 1080, 1061, 1021) by Environment Canada, and pH was measured in situ with a Thermo Orion Model 106 meter (Houben et al., 2016).

Chronologies for sediment cores from 15 of the 23 study lakes (Table 2.1) were established using gamma spectroscopy techniques to measure ^{210}Pb and ^{137}Cs activities, and the constant rate of supply (CRS) model (Appleby, 2001), at the University of Ottawa. The results of ^{210}Pb dating were used to select bottom intervals that would represent sediments that were deposited at least 150 years ago, in the pre-industrial period (please see Cheney et al., 2020 for details). For the 8 lakes that were not ^{210}Pb-dated, bottom intervals were chosen based on regional estimates of sedimentation rates from similar lakes that had established ^{210}Pb chronologies.

Cladoceran subfossils were isolated from the sediment matrix in the top 0.5 cm interval (representing modern conditions) and a bottom 0.5 cm interval (representing pre-industrial conditions) using the methods described in Korosi & Smol (2012a, 2012b). Bottom intervals chosen for analysis ranged from 17.25 to 29.25 cm. Approximately 1 g of wet sediment was

deflocculated in 100 mL of a 10% potassium hydroxide (KOH) solution and heated to ~70°C for 30 minutes. The KOH-sediment mixture was then filtered through a 36 µm sieve to remove excess organic matter and rinsed with distilled water. The residue was transferred to a vial, with a few drops of 80% ethanol added as a preservative, and two drops safranin-glycerol solution added to colour the remains. Next, 50 µL aliquots were transferred to microscope slides and allowed to dry until the water had evaporated. Slide covers were mounted using glycerin jelly and examined under an Amscope B690C-PL microscope to identify and count the cladoceran remains at 200-400X magnification. Cladocera remains were identified to the species level using Korosi & Smol (2012a, 2012b) as taxonomic guides. The most frequent body part identified for each species was used as a measure of the number of individuals (Korhola & Rautio, 2001). A minimum count of 50 individuals was used for lakes with low cladoceran abundances, and a minimum of 70 individuals was used for lakes where Cladocera diversity and abundances were higher (Kurek et al. 2010).

2.3.5 Statistical Analyses

Variation in Cladocera assemblages across the 23 study lakes, and changes in Cladocera assemblages between the modern and pre-industrial samples, were summarized using a principal component analysis (PCA) in RStudio Version 1.2.5033 using the vegan package (Oksanen et al., 2019). Prior to undertaking the ordination, taxa that did not meet the criteria of ≥2% relative abundance in at least five samples were excluded to reduce the influence of rare species on the PCA. The relative abundance data were also square root transformed to stabilize variance. Modern samples were plotted actively, and pre-industrial samples were plotted passively in the ordination. A PCA was also used to visualize variation in measured environmental variables

across the study lakes. Prior to analysis, in order to meet the assumptions of normality, all environmental variables with the exception of pH were log (base 10)-transformed. A Pearson correlation matrix was also produced using the Hmisc package (Harrell, 2020) to assess correlations among the measured environmental variables. The variable antimony was excluded from the subsequent redundancy analysis (RDA) only because of its high positive correlation with arsenic.

The RDA with forward selection was performed using the rioja (Juggins 2017) and vegan packages (Oksanen et al., 2012) to examine the relationships between modern cladoceran assemblages and the measured environmental variables. Data transformation for the RDA followed the same methods used for the PCAs. Significance of the RDA was tested using the vegan package's permutation test for redundancy analysis, 'anova.cca' (Oksanen et al., 2012). Forward selection was done using the 'adespatial package' (Dray et al., 2012). Rarefied species richness and Hill's N2 diversity, were calculated using the rioja and vegan packages in RStudio (Oksanen et al., 2012). Statistical differences in species diversity (N2) and rarefied species richness between modern and pre-industrial samples among all lakes was tested using the Wilcoxon signed-rank test (also known as paired samples Wilcoxon test).

The relationship between rarefied species richness and arsenic, and Hill's N2 species diversity and arsenic, were also explored using linear regression. The regression models were run both with and without the major outliers (four most highly arsenic-contaminated lakes, BC 17, BC 20, Frame and David; [As] > 100 µg/L). To compare all Cladocera assemblages across four *a priori* defined arsenic classes, the ANOSIM test based on Bray-Curtis dissimilarity was performed using the vegan package (Oksanen et al., 2019).

An analysis of similarity (ANOSIM) test, based on Bray-Curtis dissimilarity, was used to test for significant differences in the Cladocera assemblages between modern and pre-industrial samples across all 23 lakes, using the vegan package for RStudio (Oksanen et al., 2012). The exclusion criteria and square-root-transformation used for the PCA were also used for this test. Differences in relative abundance of individual abundant taxa since pre-industrial times were also examined in the context of the four *a priori* defined arsenic classes. The Kruskal-Wallis nonparametric analysis of variance, using R Studio version 1.2.5033, was used to test whether differences in cladoceran taxa among arsenic categories were statistically significant.

2.4 Results

2.4.1 Differences in water chemistry variables amongst Yellowknife study lakes

PCA axis 1 of the environmental variables was primarily influenced by water quality variables indicative of impact from mining emissions, including arsenic and antimony, and to a lesser extent sulfate. PCA axis 2 represented a gradient of lake area, aluminum, pH, and sulfates (Figure 2.2a). Cumulatively, these two axes explained 74% of the variation in the limnological variables of the 23 lakes. Arsenic and antimony plotted in the opposite direction to lake depth and distance from Giant Mine (Figure 2.2a). Not surprisingly, these variables were also negatively correlated (Pearson correlation coefficients <-0.52).

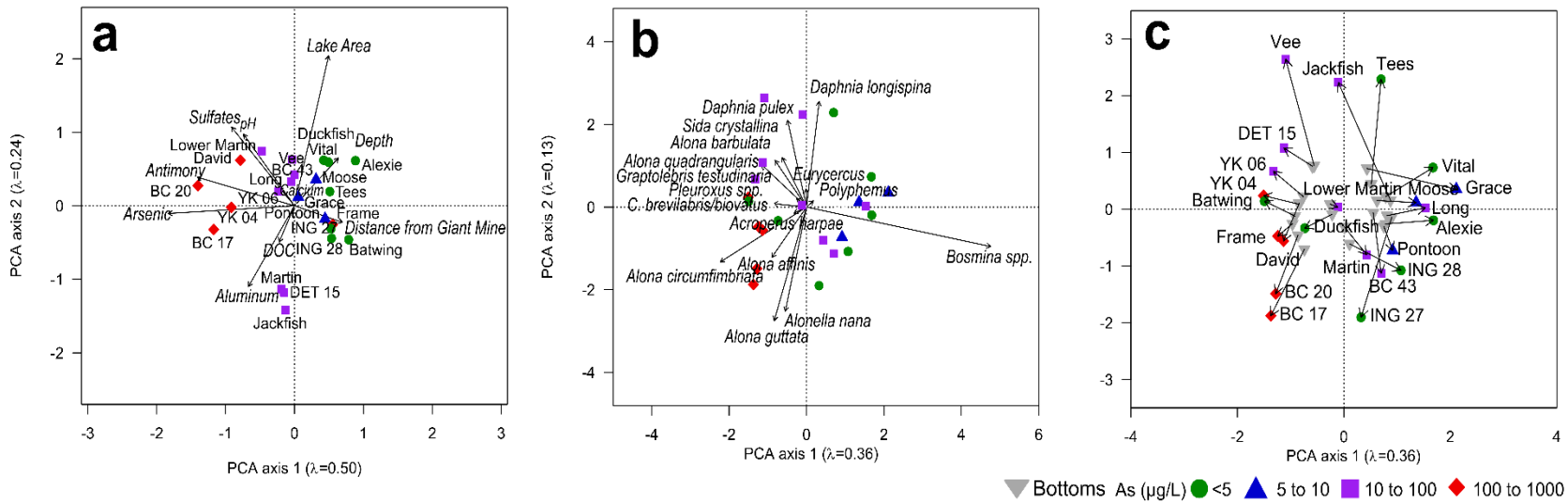

Figure 2.2. Principal component analysis (PCA) showing: a) the distribution of the study lakes along the gradients of the measured environmental variables; b) variation in cladoceran species assemblages in the surface sediments of Yellowknife lakes; c) direction of change in cladoceran species assemblages in Yellowknife lakes between present-day (surface sediments) and the pre-industrial (bottom sediments) periods. Cladocera assemblages in the bottom sediment interval are plotted passively. The shapes of the symbols represent the present-day surface water arsenic concentrations

2.4.2 Differences in Cladocera assemblages from the surface sediments of the Yellowknife study lakes

We identified 28 Cladocera species, both littoral and pelagic, across our 23-lake dataset. The dominant taxa were *Bosmina* spp., *Chydorus brevilabris/biovatus*, and several taxa belonging to the genus *Alona* (*A. circumfimbriata*, *A. guttata*, *A. affinis*, *A. quadrangularis* and *A. barbulata*). *Bosmina* were present in 15 of the 23 study lakes, ranging in relative abundance from ~2 to 91% in the lakes where it was present, with an average relative abundance of ~27% across all lakes (Figure 2.3, Table 2.2). *C. brevilabris/biovatus* was found in 22 of the 23 lakes at ~2 to 49 %, and an average relative abundance of 12.3% (Figure 2.3, Table 2.2). At comparatively lower relative abundances were the littoral taxa *Acroperus harpae* (present in 18 lakes at an average of ~ 3%), *Euycercus* (present in 10 lakes at an average of ~1.5%), *Pleuroxus* (present in 11 lakes at an average of ~1.6%), and the predatory cladoceran taxon *Polyphemus* (present in 9 lakes at an average of ~1%). *Daphnia pulex* and *Daphnia longispina* occurred sporadically across the lake set but were often the dominant taxa when present (Figure 2.3).

Table 2.2. List of the most common cladoceran taxa encountered in the 23 study lakes, a summary of their distribution (abundance and number of lakes) and basic ecology. Information on basic ecology was adapted from Korosi & Smol, 2012a, 2012b

Cladocera taxa	**Number of Lakes**	**Average relative abundance**	**Basic ecology**
Acroperus harpae	18	3.07	Commonly found in littoral zones of lakes and exhibits high habitat heterogeneity. Species is indifferent to pH (Walseng et al., 2001, 2003).
Alona affinis	16	6.43	Epiphytic taxon that shows habitat specificity, being associated with vegetation beds (Tremel et al., 2000). Taxon is indifferent to pH (Walseng et al., 2008).
Alona barbulata	15	6.82	Associated with soft water lakes in northeastern North America (Megard, 1967).
Alona circumfimbriata	16	9.50	Eurybiontic species (Megard, 1967) not frequently found in soft water lakes.
Alona guttata	7	4.67	Occurring more in lakes with a circumneutral pH (Walseng et al., 2001, 2008). Adapted to both mud-vegetation and sandy-rocky habitats (Chengalath, 1982).
Alona quadrangularis	9	4.07	Large mud dwelling taxon abundant in muddy areas with vegetation (Evans, 1984; Tremel et al., 2000). Observed over a broad pH range (Walseng et al., 2008).

Cladocera taxa	Number of Lakes	Average relative abundance	Basic ecology
Alonella nana		8.01	Small taxon commonly found in the littoral zones of lakes in Canada (Chengalath, 1982). Found to be uncommon at pH <5.0 (Walseng et al., 2008).
Bosmina	15	27.19	A planktonic taxon often found in the epilimnion of lakes. It is ubiquitous, with its subfossil remains being the most commonly recovered among all cladocerans.
Chydorus brevilabris/biovatus	22	12.12	*C. brevilabris* and *C. biovatus* are the two branches of the *C. sphaericus* complex. The former dominates in southeastern Canada and northeastern USA (Chengalath, 1982) and the latter is more abundant in northern North America (Frey, 1980).
Daphnia longispina	4	4.69	Planktonic, filter feeding taxa observed in both ephemeral rock pools and lakes (Pajunen & Pajunen, 2007).
Daphnia pulex	10	3.68	Relatively large, planktonic filter feeding taxa. High variability between populations in response to toxins from cyanobacteria (Laurén-Määttä et al., 1997)

Cladocera taxa	Number of Lakes	Average relative abundance	Basic ecology
Eurycercus	10	1.62	Largest of the chydorid family (Frey, 1971). Widely found in northeastern North America (Hann, 1982; Bekker et al., 2012).
Graptolebris testudinaria	8	0.78	Epiphytic taxon commonly observed in water bodies with dense vegetation (Chengalath, 1982; Walseng et al., 2008).
Pleuroxus spp.	11	1.51	Consistent but not abundant species comprising the chydorid assemblage of soft water lakes.
Polyphemus	9	0.87	Commonly littoral, raptorial, omnivorous taxon that feeds on *Daphnia* and *Bosmina*. One species, Polyphemus pediculus exhibits high ecological plasticity (Xu et al., 2009).
Sida crystallina	7	1.63	Filter feeder commonly found in the littoral zones of lakes attached to plant surfaces. Found to be common at pH >5.0 (Walseng et al., 2003).

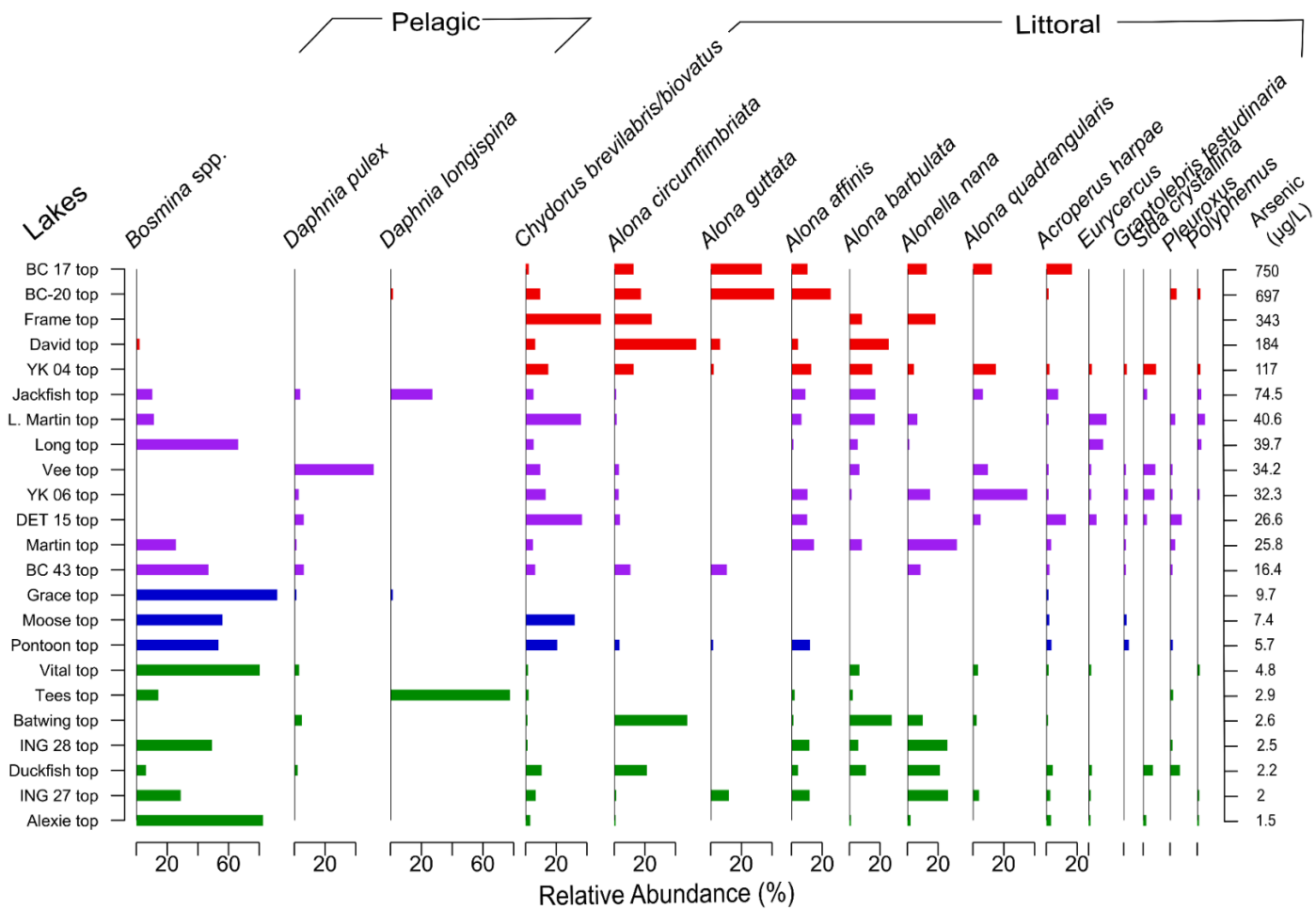

Figure 2.3. Simplified Cladocera relative frequency diagram of the most common cladoceran taxa in the surface sediments for our twenty-three lakes in Yellowknife, Northwest Territories. The sites are arranged in order of increasing arsenic (As) concentrations, as shown on the right. The colours of the individual bars represent the varying arsenic concentrations at the site (red 100 - 1000 µg/l, purple 10–100 µg/l, blue 5–10 µg/l, and green < 5 µg/l)

PCA of the cladoceran data showed that the variation in the assemblages across the study lakes was driven mainly by *Bosmina*, *Daphnia* (both the *D. pulex* and *D. longispina* complex), *Sida crystallina*, *Alonella nana*, and *Alona* species (Figure 2.2b, Figure 2.2c). Cumulatively, PCA axis 1 and PCA axis 2 explained 49% of the variance in the modern Cladocera assemblages ($\lambda_{axis1} = 0.36$, $\lambda_{axis2} = 0.13$; Figure 2.2b). The lakes with the highest arsenic concentrations (100 – 1000 µg/L) plotted in the lower left quadrant of the ordination (Figure 2.2b), which was primarily driven by the littoral species *Alona circumfimbriata*, *A. affinis*, and *A. guttata*. The exception to this trend was YK-04, for which the modern sample scores plotted in the top left quadrant of the ordination. YK-04 had the lowest surface water arsenic concentration of the five lakes in the 100 to 1000 µg/L arsenic group with a concentration of 117 µg/L. This value is likely an overestimation of open-water arsenic concentrations since it is measured on a water sample collected in winter under the ice, and arsenic and other solutes are excluded during ice formation (Palmer et al., 2019). *Bosmina* were absent or rare in both the pre-industrial and modern time periods in the five lakes with the highest arsenic concentrations (Figure 2.2b). The RDA with forward selection only identified one variable, namely arsenic, as explaining the variation in assemblages, and given the lack of dimensionality in the cladoceran assemblages, the RDA was not pursued further.

Cladoceran species diversity (Hill's N2) and rarefied species richness in the surface sediments showed a significant positive relationship with lake water arsenic concentration (Hill's N2: $p = 0.002$, $R^2 = 0.46$, F-statistic: 14.31 on 1 and 17 *df*; richness: $p = 0.004$, $R^2 = 0.39$, F-statistic: 11.04 on 1 and 17 *df*) when the four lakes with the highest arsenic concentrations (BC 20, BC 17, Frame and David) were removed (Figure 2.4 a, c). However, the inclusion of BC 20, BC 17, Frame and David Lake resulted in trends not being statistically significant (Figure 2.4 b,

d). For lakes with [As] <5 µg/L, Hill's N2 and rarefied species richness ranged from 1.3 to 7.1, and 4.0 to 11.0, respectively. Lakes within the [As] 10 to 100 µg/L category had values that ranged from 1.7 to 6.0 (N2) and 7.9 to 12.0 (richness). Lake YK 04 ([As] > 100 µg/L) had the highest Hill's N2 and richness values at 8.8 and 12.8, respectively. David, BC 20, and BC 17 have Hill's N2 values ranging from 2.6 to 4.9, and similar species richness values of 6.9. Frame Lake has a species richness value of ~4 and a Hill's N2 value of 3.1.

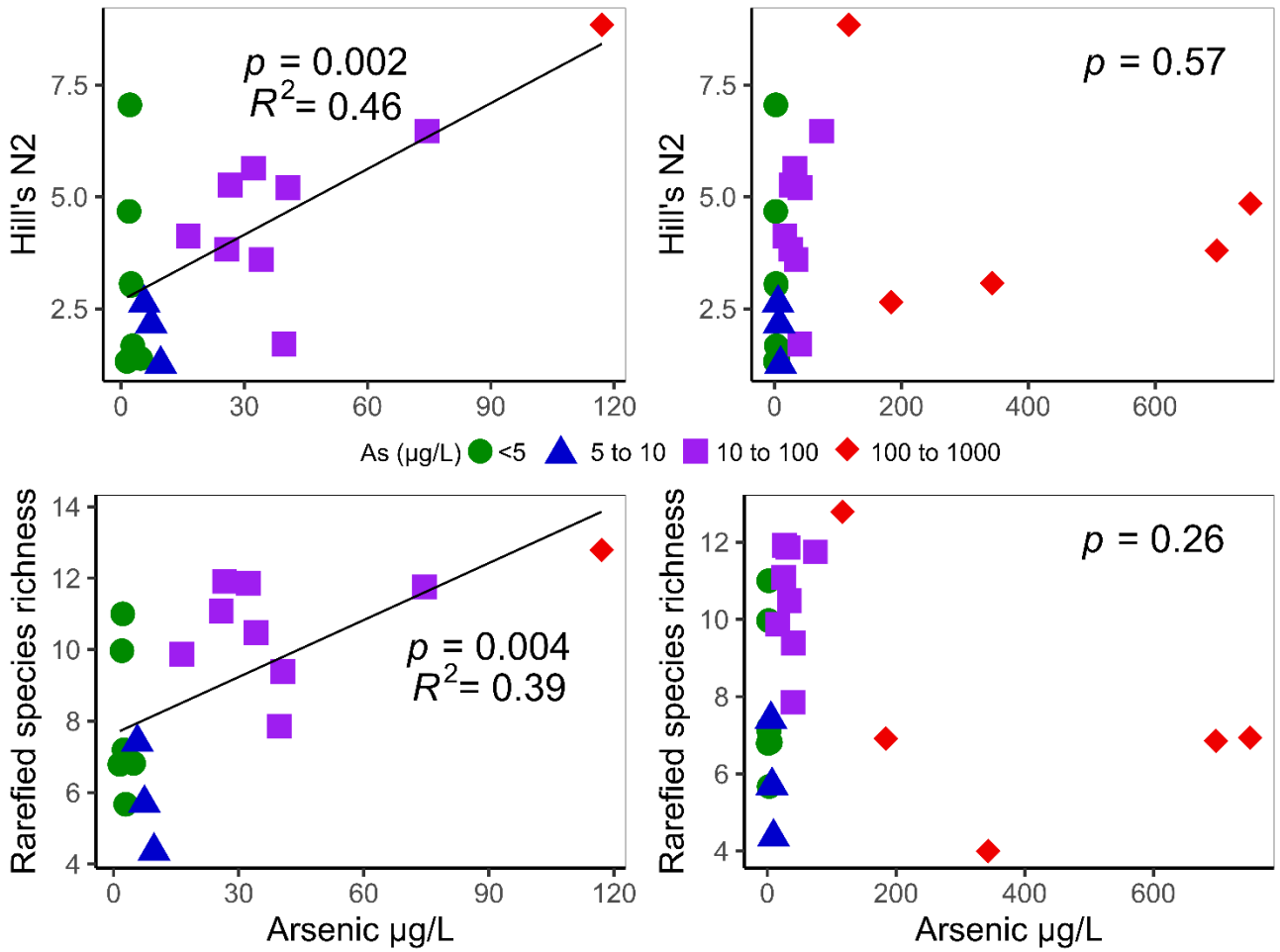

Figure 2.4. Regression plots showing the relationship between: a) cladoceran species diversity (Hill's N2, surface sediments) and lake water arsenic concentrations for 20 of the 23 study lakes (3 lakes with the highest arsenic concentrations removed); b) species diversity (Hill's N2) regression plot with all 23 study lakes included; c) rarefied species richness (surface sediments) and lake water

2.4.3 Regional changes in Cladocera assemblages since pre-industrial times

There were no significant differences in regional cladoceran assemblage between the present-day and pre-industrial sediment intervals (one-way ANOSIM, R = -0.047, p = 0.982, n = 999 permutations). Cladoceran species richness and Hill's N2 diversity were similarly not significantly different (paired Wilcoxon signed-rank, species richness – p = 0.67; Hill's N2 – p = 0.92) between the top and bottom intervals (Figure 2.5). Although no significant regional differences in cladoceran assemblages and diversity indices were observed, many individual lakes exhibited notable changes in cladoceran assemblages since pre-industrial times (Figure 2.6). The lakes that had the greatest dissimilarity between top and bottom assemblages (based on Bray-Curtis Dissimilarity) were Grace Lake (As = 9.7 µg/L), ING-27 (As = 2.0 µg/L) and Frame Lake (As = 343 µg/L).

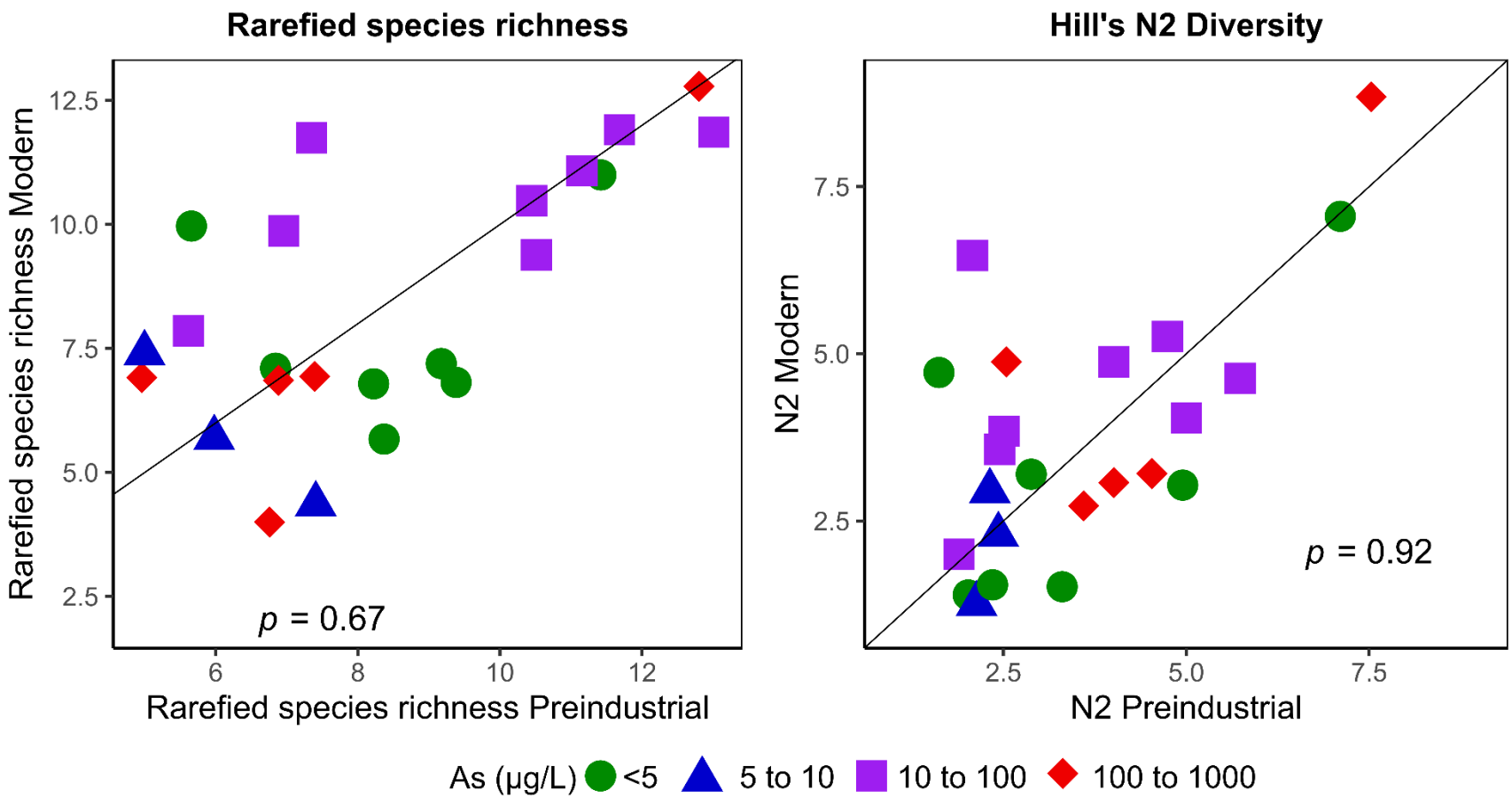

Figure 2.5. Scatterplots showing cladoceran rarefied species richness and species diversity (Hill's N2) in present-day and pre-industrial sediment intervals. Samples that plot above the 1:1 line have higher rarefied species richness and Hill's N2 diversity in the surface interval compared to the bottom interval. Significance values are based on the Wilcoxon tests. The shapes of the symbols represent the surface water arsenic concentrations.

In Frame Lake, *Chydorus brevilabris/biovatus* increased from 12.1% relative abundance in the bottom sediments to 49.0% in the surface sediments while many *Alona* guttata, *A. affinis* and *A. quadrangularis*, which were present in the bottom sediments, were absent in the surface sediments abundance. In Grace Lake, *Daphnia longispina* decreased by 59.6% relative abundance from the bottom sediments to the surface sediments, with a corresponding 68.2% increase in *Bosmina* by (Figure 2.6). In contrast, *Bosmina* decreased by 41.4% relative abundance in Lake ING 27, while *Alonella nana* increased by 16.4% relative abundance (Figure 2.6). Jackfish Lake (As = 74.5 µg/L) and Tees Lake (As = 2.9 µg/L) also exhibited notable declines in *Bosmina* relative abundance, with corresponding increases in *D. longispina* (Figure 2.6, Figure 2.2b). The lakes with the least dissimilarity between top and bottom cladoceran assemblages were BC-17 (the lake with the highest surface water arsenic concentration) and Lower Martin Lake.

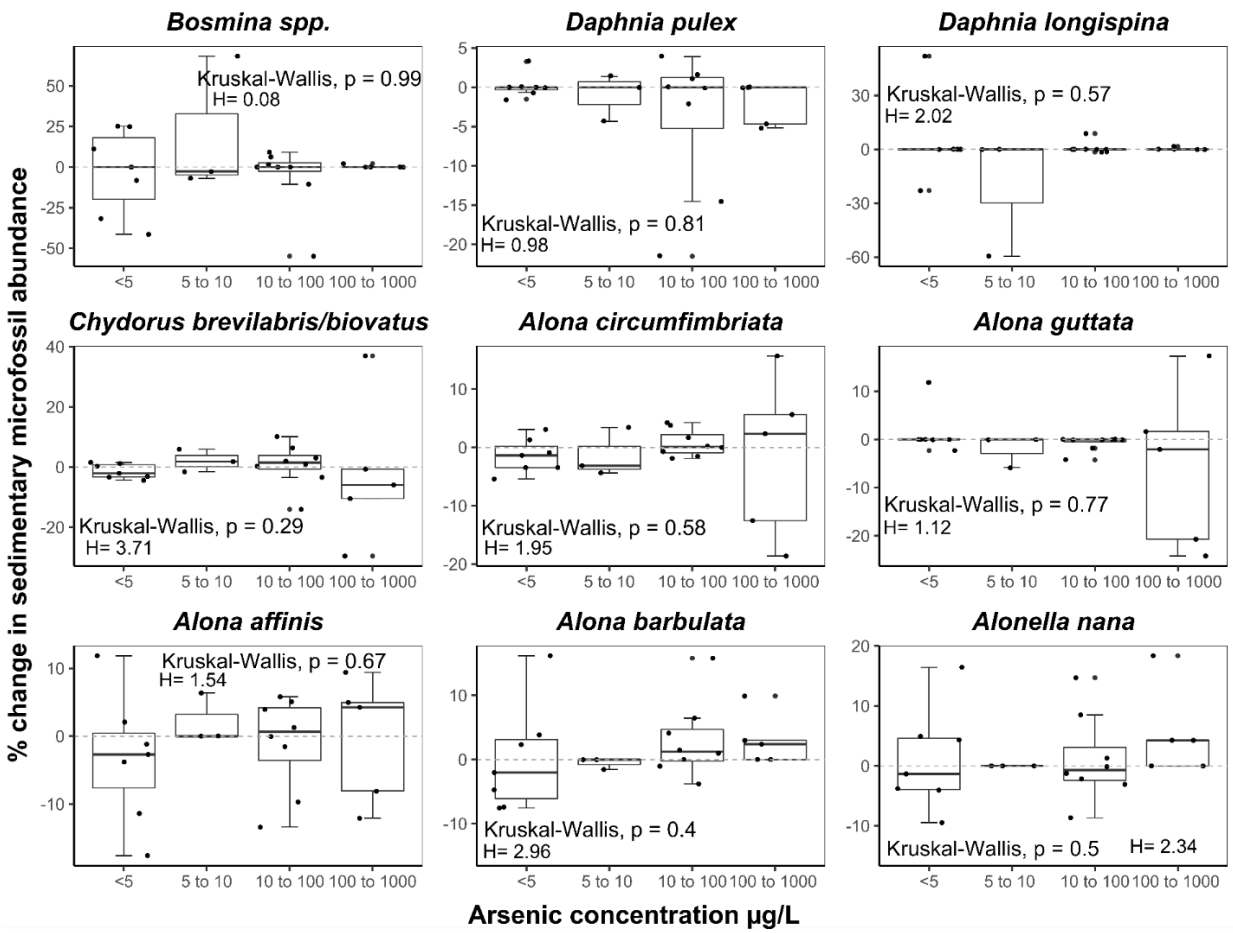

Figure 2.6. Changes in the relative abundance of the most abundant cladoceran taxonomic between present-day and pre-industrial times for the 23 study lakes arranged by measured surface water arsenic concentrations. Kruskal-Wallis nonparametric analysis of variance for all arsenic classes: <5 µg/L (n = 7); 5 to 10 µg/L (n = 3); 10 to 100 µg/L (n = 8); and 100 to 1000 µg/L (n = 5) are provided in each plot.

The Kruskal-Wallis nonparametric analysis of variance for the four *a priori* defined arsenic lake categories showed no significant regional differences for the common cladoceran taxonomic groups between present-day and pre-industrial sediments in any of the categories (Figure 2.6). Additionally, while the ANOSIM indicated differences between the present-day Cladocera assemblages across the four arsenic groups (one-way ANOSIM, $p = 0.003$, n = 999 permutations), a low R statistic value of 0.28 suggests that these differences were not pronounced. Of the 6 lakes with [As] <5 µg/L in which *Bosmina* was present, the taxa increased in 3 lakes since pre-industrial times, but also decreased in 3 lakes. Alona *circumfimbriata* and *Alona affinis* increased in 3 of the 4 lakes with [As] >100 µg/L (Figure 2.6).

2.5 Discussion

Our study aimed to provide a regional snapshot of Cladocera assemblage changes from the pre-industrial period to the modern day and identify possible bioindicators for arsenic contamination in Yellowknife lakes. Our findings showed that cladoceran species richness and diversity increased along an arsenic gradient until a critical threshold (>100 µg/L) was reached, after which both diversity and richness decreased. Littoral/benthic taxa dominated the assemblages in the highly contaminated lakes, while the pelagic *Bosmina* dominated in lakes with lower arsenic. Overall, no consistent regional trends in cladoceran assemblage shifts were detected between present-day and pre-1850 sediments, and instead cladoceran changes were lake-specific.

2.5.1 Differences in Cladocera species richness and diversity along a gradient of arsenic

We found that arsenic was positively correlated with rarefied species richness and alpha diversity across our study lakes that spanned an arsenic gradient of 1.5 µg/L to 117 µg/L, when

the four most heavily contaminated lakes were removed from the analysis. This differs from the findings of Winegardner et al. (2017) for mining-impacted lakes in Québec (Canada) and Leppänen et al. (2018b) for mining-impacted lakes in Finland, where Cladocera species richness and diversity decreased with metal pollution. In their study, the primary metals of concern were copper, nickel, and zinc, which are well known to have deleterious impacts on cladoceran communities (Yan et al., 2004; Labaj et al., 2015). In Yellowknife, the main metal(loid) pollutants released from mine emissions were arsenic and antimony, while copper, nickel, and zinc are not a concern in this region.

Our findings indicate that lake ecosystem responses to arsenic pollution may actually result in increases to cladoceran species richness and diversity below a certain threshold, in contrast to other metals more commonly studied in the aquatic environment. The four most contaminated lakes in our dataset (BC 17, BC 20, Frame and David; [As] > 180 µg/L) clearly deviated from the trend of increasing richness and diversity with arsenic concentrations, and may indicate a negative influence of arsenic on Cladocera once a threshold in arsenic is reached. However, the positive relationship between cladoceran richness and diversity with arsenic concentrations may also be a spurious correlation, instead tracking the influence of lake depth on Cladocera. A clear relationship was evident between lake depth and arsenic concentrations in our dataset, where shallower lakes were more likely to have elevated surface arsenic concentrations. Lakes with low water levels have been shown to have higher Cladocera richness and diversity relative to lakes with higher levels (Ghidini & Santos-Silva, 2018), and shallow lakes are often dominated by diverse littoral/benthic assemblages (Nevalainen, 2011; Gałka et al., 2014).

If the positive relationship between species richness/diversity and arsenic is direct (i.e. driven by arsenic and not an indirect association with depth or other related environmental

variable), we might expect increases in species richness and diversity since pre-industrial times for lakes that have received arsenic deposition, with the exception of the most heavily contaminated lakes where arsenic concentrations exceed a threshold where toxic effects become evident (somewhere between 115 µg/L to 180 µg/L based on our data). There were no significant differences in species richness and alpha diversity (Hill's N2) between the pre-industrial and modern time periods across all lakes, although many lakes with [As] between 10 and 100 µg/L did exhibit increases in species diversity and richness since the pre-industrial time period. Of the five lakes with [As] > 100 µg/L, David Lake and Lake YK-04 exhibited increases in Hill's N2 diversity and rarefied species richness, while BC 17 and BC 20 decreased. Arsenic concentrations in YK-04 are based on measurements in water samples taken under the ice, and solute exclusion during ice formation likely resulted in higher arsenic than would be measured if the water sample had been collected in the open-water season.

2.5.2 Subfossil Cladocera species assemblages as paleoecological indicators of arsenic contamination

In a detailed paleolimnological analysis of Pocket Lake (Thienpont et al., 2016), *Daphnia* appeared and then increased in relative abundance at the onset of arsenic contamination, but were then extirpated at the height of arsenic emissions, indicating that they are relatively tolerant of arsenic until a threshold is reached. Lab bioassays support this, with EC_{50} values of 49.6 ± 9.0 mg arsenate/L documented for *Daphnia pulex* (Passino & Novak, 1984), and 0.54 mg arsenate/L for *Daphnia similis* (de Sales et al., 2016). The one lab bioassay study available for *Bosmina* recorded a 96 h EC_{50} of 0.85 ± 0.12 mg arsenate/L for *Bosmina longisrostris*, lower than what was documented for *Daphnia* (Passino & Novak, 1984). These effects concentrations are much

higher than the values we report for the most contaminated lakes in our study; however, EC_{50} and other similar metrics are functions of time, and may not necessarily capture the responses of Cladocera to long-term exposure (years), as is the case for Yellowknife lakes.

Although we did not document similarly strong associations between cladoceran assemblages and arsenic in our study, some patterns were evident in the presence and absence of certain taxa in lakes with high surface water arsenic concentrations. The most heavily arsenic-contaminated lakes were dominated by *Alona* taxa, particularly *A. circumfimbriata* and *A. guttata*, while *C. brevilabris/bioavatus* were also common. *C. brevilabris* has been observed at a surface water arsenic concentration of 1113 µg/L in Cart Lake, in northeastern Ontario (Little et al., 2020), and has also been shown to increase with copper and nickel contamination in lakes near Wawa and Sudbury in Ontario, Canada (Jeziorski et al., 2013; Labaj et al., 2015).

Arsenic contamination appears to have a disproportionate effect on pelagic organisms relative to littoral/benthic ones, and benthic algal/microbial mats may be protecting benthic taxa by binding or detoxifying arsenic and other contaminants (Bender et al., 1994; Roeselers et al., 2008; Thienpont et al., 2016). In Yellowknife lakes, pelagic feeding fish tended to have higher arsenic concentrations relative to littoral feeding taxa (Chételat et al. 2019). Yellowknife lakes with [As] >5 µg/L also had higher abundances of opportunistic benthic diatoms (small fragilarioid taxa and *Achnanthidium minutissimum*) (Sivarajah et al., 2019). *Daphnia* species, which increased with the onset of arsenic contamination in Pocket Lake (Thienpont et al. 2016), were not an important part of the assemblage in our high-arsenic lakes.

Bosmina were dominant across many of the lakes in our dataset, including shallow lakes (e.g. Moose Lake, depth = 1 m, *Bosmina* relative abundance ~60%), but were absent in the highly contaminated lakes ([As] <100 µg/L) in both the surface and pre-industrial sediments.

Several lakes within 40 km of Giant Mine's roaster stack had naturally high geogenic arsenic (Cheney et al., 2020), which may explain the absence of *Bosmina* in both surface and pre-industrial sediments. A paleolimnological study documented decreases in the abundance of bosminids in Yangzong Lake and Datun Lake, China, coincident with high arsenic enrichment (Chen et al. 2015). Toxicity bioassays have shown that *Bosmina longirostris* are more sensitive to arsenic than *Daphnia magna* and *Daphnia pulex* (Passino & Novak, 1984); however, in Cobalt (Ontario), *Bosmina* were reported in lakes with surface water arsenic concentrations as high as 1113 μg/L (Little et al., 2020). Geographic variation in genetic background (Burge et al., 2018; Radzikowski et al., 2018), the bioavailability and form of arsenic to which the taxa is exposed, and the influence of other water quality variables such as calcium and phosphorus on arsenic toxicity (Awoyemi et al., 2020) can act collectively to influence species ecotoxicological responses to arsenic concentration across different regions.

Notably, highly contaminated lakes in our dataset showed only muted changes in cladoceran assemblages since pre-industrial time, indicating that Giant Mine emissions were not a significant driver of regional change in Cladocera. Most of our study lakes are situated in organic-rich catchments, in contrast to Pocket Lake which is situated in a granite basin and experienced an apparent extirpation of all Cladocera as a result of arsenic pollution (Thienpont et al., 2016). Organic matter facilitates the microbially-mediated conversion of highly toxic arsenic trioxide released from Giant Mine into less toxic arsenic sulphides, as it acts as a substrate for microbial growth (Galloway et al., 2017). In contrast, arsenic trioxide particulate matter persists in granitic outcrops (Palmer et al., 2015). This suggests that lakes with high organic matter may be more protected from arsenic toxicity than those in predominantly granitic basins (such as Pocket Lake, Thienpont et al. 2016).

2.5.3 Subfossil Cladocera assemblage and diversity changes in Yellowknife lakes since pre-industrial times

Several of our study lakes showed notable changes in cladoceran assemblages between present-day and pre-industrial times, but we did not detect any regional patterns of directional changes in Cladocera assemblages and diversity indices. From this, we infer that site-specific limnological characteristics are more significant drivers of Cladocera assemblage changes in Yellowknife lakes, rather than regional stressors. Grace Lake, Frame Lake, and Jackfish Lake, all located within the City of Yellowknife, exhibited the greatest dissimilarity in cladoceran assemblages between present-day and pre-industrial times, although species-specific changes differed between the three lakes. For example, Grace Lake experienced a decline in *Daphnia longispina* coincident with increases in *Bosmina*, while Jackfish Lake showed the opposite trend.

In Grace Lake, nutrient enrichment from increased urbanization has been inferred from subfossil diatom assemblages analyzed in a sediment core (Sivarajah et al., 2020). Gianuca et al. (2018) examined taxonomic, functional and phylogenetic metacommunity ecology of Cladocera across an urbanization gradient and found that species of the Daphniidae family are negatively associated with urbanization, but positively associated with phosphorus. Jackfish Lake has experienced water quality problems associated with algal blooms in recent years possibly linked to high phosphorus concentrations (Mackenzie Valley Land and Water Board, 2015). An ecological shift from *Daphnia* to *Bosmina* has been linked to the combined stressors of eutrophication, climate warming, and increases in cyanobacteria blooms that can negatively impact *Daphnia* (Li et al. 2016); however, despite documented algal blooms, *Daphnia* have increased in Jackfish Lake while *Bosmina* have decreased. This shift from *Bosmina* to *Daphnia*

occurred at the onset of mining, and it is therefore possible that it was driven by metal(loid) pollution (Sivarajah, unpublished data).

Frame Lake has also been subjected to multiple stressors associated with urbanization, including nutrient enrichment from urban runoff and sewage seepage, compounded by high arsenic concentrations in the sediments linked to Giant Mine emissions (Dirszowsky & Wilson, 2016; Gavel et al., 2018). Winter fish kills in Frame Lake are becoming more common as a result of a high biological oxygen demand in the sediments, which contributes to dysoxic conditions under the ice when the lake is sealed off from atmospheric oxygen inputs (Gavel et al., 2018). In our study, we observed an increase in *C. brevilabris/biovatus* in Frame Lake since pre-industrial times, with corresponding decreases in *A. circumfimbriata, A. guttata and A. affinis. Bosmina* were absent in both the surface and pre-industrial sediments. *Chydorus* are known to be tolerant of eutrophication, as well as urbanization and pollution more generally (Bartoli et al., 2018; Gianuca et al., 2018).

Subarctic freshwater ecosystems, such as those in Yellowknife, are particularly susceptible to limnological changes associated with climate warming (Vincent et al., 2012). In the northern hemisphere, widespread shifts in diatom assemblages towards assemblages dominated by the planktonic, cylotelloid taxa of the *Discostella stelligera/pseudostelligera* complex have been attributed to stronger lake thermal stratification and a longer ice-free season (Rühland et al., 2003, 2008; Rühland & Smol, 2005; Rühland et al. 2015). However, the responses of Cladocera to climate warming are less understood and characterized. Increases in *Bosmina* in subarctic lakes of northwest Finland have been attributed to regional climate warming (Leppänen et al., 2017), while Thienpont et al., (2015) suggested that climate warming in western Arctic lakes triggered shifts from generalist taxa (e.g., *C. brevilabris, A.*

circumfimbriata) to specialized planktonic (*Bosmina*) and littoral/benthic taxa (e.g. mud-dwelling *Leydigia leydigi*). In our Yellowknife study lakes, we did not observe significant regional, directional changes in Cladocera assemblages that would suggest that climate warming on its own is a strong driver of Cladocera changes since pre-industrial times. These relatively muted changes in Cladoceran assemblages compared to diatom changes are consistent with findings from other Arctic and subarctic regions within Canada (Sweetman et al., 2008; Rühland et al., 2014; Jeziorski et al., 2015).

2.5.4 Conclusions and Future Directions

Our top-bottom paleolimnological analyses provided evidence of Cladocera assemblage changes in several lakes, but we observed no regional, directional changes. As such, assemblages in Yellowknife did not appear to be significantly influenced by regional external forcing factors of climate change, urbanization and mining, despite significant changes noted for diatom taxa in similar regions. The most notable trend in Cladocera changes was a positive correlation between species richness and diversity along an arsenic gradient of 1.5 µg/L to 117 µg/L. This increase was driven mainly by littoral/benthic taxa, potentially attributed to conditions in the benthic/littoral zone that buffer the effects of arsenic contamination on these taxa, and the fact that shallow lakes typically have higher arsenic concentrations.

Bosmina was absent in highly contaminated lakes, but dominant in many others with lower arsenic concentrations. Our findings highlight the complex relationships between Cladocera and metal(loid) contamination in subarctic lakes. The apparent sensitivity of *Bosmina* to arsenic has been noted in one traditional lab bioassay but the long-term toxicological responses and sensitivity of *Bosmina* to arsenic are still poorly understood. The application of

modern ecological techniques, such as resurrection ecology studies (Jeppesen et al., 2001), may serve to provide important insights. Additionally, a potential confounding factor to the changes in Yellowknife could be antimony, which increased during the period of mining (Palmer et al., 2015; Cheney et al., 2020) and was highly correlated with arsenic in our PCA. There is a general knowledge gap on the ecotoxicological effects of antimony on Cladocera assemblages, apart from toxicity assays involving *Daphnia magna* (Kimball, 1978; Waaijers et al., 2013). As such, the extent to which antimony is influencing the assemblages in Yellowknife remains unclear. The relationship between the diversity of lake taxa and arsenic should also be explored in future studies to determine if the positive relationship is limited to Cladocera or extends to other plankton groups.

Traditional laboratory bioassays focus on arsenic effects to one or a few model organisms, and littoral and benthic taxa are underrepresented. Furthermore, littoral and benthic taxa are also underrepresented in contemporary surveys of zooplankton assemblages. As such, subfossil remains in sediments, which integrate bioindicators from all lake habitats, are highly effective for investigations on littoral and benthic cladoceran communities because key taxonomic diagnostic features (e.g., headpores) are readily visible on subfossil remains (Frey, 1960), and sediments collected from the centre of a lake integrate the remains of organisms that live throughout the lake in different habitats through sediment focusing (Korhola & Rautio, 2001). Sediment focusing is a process in which lake sediments are moved from shallower zones of a lake to deeper zones by water turbulence (Blais & Kalff, 1995). Thus, paleo-ecotoxicological approaches (Korosi et al. 2017a, 2017b) are well designed to identify patterns of potential ecotoxicological significance in these underrepresented groups that can be examined in subsequent studies using diverse methodologies.

2.6 Acknowledgments

We thank David Eickmeyer, Joshua Thienpont, Mija Azdajic, Claudia Tanamal, and Martin Pothier for field work assistance. Logistical support and advice by Mike Palmer (Aurora Research Institute) are also greatly appreciated. This work was funded by a Natural Science and Engineering Research Council of Canada (NSERC) Strategic Grant to JMB and JPS (Grant # STPGP 462955-14), the Banting Postdoctoral Fellowship program (JBK) as well as Northern Scientific Training Program grants to CLC, and BS. Helicopter support for lake sediment coring was provided by the Polar Continental Shelf Program (JMB).

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Chapter 3 : Assessing the viability of *Daphnia pulex* complex resting eggs in a subarctic lake (Yellowknife, Northwest Territories, Canada) impacted by historic gold mining

3.1 Abstract

All species of the Cladocera (Branchiopoda, Crustacea) taxa *Daphnia* produce diapausing eggs that are critical for autogenous population restoration following pollution. Their long-term preservation and viability within sediments have made them excellent ecotoxicological indicators of evolutionary responses to metal contamination. The focus of this study was to determine whether *Daphnia pulex* resting eggs in a sediment core from Pocket Lake (Yellowknife, Northwest Territories) were viable for use in future resurrection ecology studies, and whether the resting egg bank was potentially compromised by sediment arsenic toxicity. Pocket Lake is a small, shallow subarctic lake that was historically contaminated by arsenic, which resulted in dramatic ecological impacts. I found *Daphnia* ephippia throughout the entire 25 cm sediment core except for the surface 2 cm, and there was a notable peak at 12.0-13.5 cm core depth likely indicating the early mining period. All ephippia were empty, and I was unable to extract viable resting eggs from any sediment intervals. The absence of any viable resting eggs preserved in the sediments is notable but inconclusive, as it could indicate that a) the eggs successfully hatched; b) resting egg production was aborted, or c) original viable resting eggs degraded over time. This study shows that while past *Daphnia pulex* populations in Pocket Lake were possibly tolerant of extreme arsenic contamination, the direct examination of these past populations through resurrection ecology is not feasible.

3.2 Introduction

The adoption of dormancy (suspended or reduced development and activity) strategies is one of the conspicuous features of zooplankton (Araújo et al. 2013). One example of a dormancy

strategy is the production of resting stages, which is triggered during periods of adverse environmental disturbances (Dahms 1995; Radzikowski et al. 2018). Resting stages are characterized by low physiological demands and reduced functionality, allowing the organism to maintain structural integrity compared to active populations (Kawasaki et al. 2004; Ślusarczyk et al. 2019). The production of resting eggs is a fundamental ecological process for maintaining genetic diversity within populations, and colonizing new lake habitats as resting eggs are dispersed by birds, macrophytes, aquatic insects and currents (Bilton et al. 2001; De Meester et al. 2006; Van De Meutter et al. 2008; Santangelo et al. 2011; Araújo et al. 2013; Battauz et al. 2017; Hessen et al. 2019). The resting egg bank is also critical to ecological recovery in lakes adversely impacted by pollution and other stressors (Dahms 1995; Santangelo et al. 2011; Radzikowski et al. 2018). For this reason, many toxicological studies examine diapausing egg production and viability in response to exposure to metals and other pollutants (e.g., Conde-Porcuna et al. 2014).

Daphnia, which are large crustacean zooplankton, are one of the most commonly used model organisms in toxicology. Several species of *Daphnia* reproduce via cyclical parthenogenesis, a reproduction mode that involves both sexual recombination and amictic parthenogenesis (Decaestecker et al. 2009). Under normal environmental conditions, female *Daphnia* undergo amictic parthenogenesis, a form of asexual reproduction where genetically identical clones of females are produced (Innes and Hebert 1988; Decaestecker et al. 2009). When environmental conditions deteriorate, males are parthenogenetically (asexually) produced and females undergo sexual reproduction, producing haploid (single set of chromosomes) resting eggs (Innes and Hebert 1988; Decaestecker et al. 2009). The parthenogenetically produced males fertilize these eggs, called ephippia, which then function as resting stages that subsequently hatch

under the right environmental conditions (Innes and Hebert 1988). Other species of *Daphnia*, including *Daphnia pulex,* are obligate parthenogens, meaning they reproduce exclusively by asexual parthenogenesis, producing diploid (paired chromosomes) resting eggs (Hebert and Crease 1980, 1983; Innes and Hebert 1988; Subramoniam 2017). Obligate parthenogens can parthenogenetically produce males; however, they are not involved in the production of offspring (Innes and Hebert 1988).

Reactivation of resting eggs is influenced by the biochemistry of the eggs and ephippia (Ślusarczyk et al. 2019), but external hatching stimuli also play a major role. The main hatching stimuli for *Daphnia* resting eggs include optimal photoperiod light and temperature regimes (Cuenca Cambronero & Orsini 2018; Radzikowski et al. 2018). All resting eggs produced may not be exposed to the optimal hatching stimuli, and therefore, instead of hatching, viable eggs can accumulate within the sediment matrix of a lake basin (Radzikowski 2013; Burge et al. 2018), with multiple generations overlapping (García-Roger et al. 2006; Araújo et al. 2013). *Daphnia* resting eggs within sediments are resilient, and can remain viable for decades to centuries (Kawasaki et al. 2004; Radzikowski 2013; Frisch et al. 2014). *Daphnia* can be successfully hatched from these eggs by stimulating spring-time conditions, allowing for the direct examination of historical populations (Burge et al. 2018; Cuenca Cambronero and Orsini 2018). This novel approach to studying evolution and adaption is known as ‘resurrection ecology’ and has been successfully applied to study the responses of *Daphnia* (and other planktonic groups that produce resting stages) to metals (e.g., Kerfoot et al. 1999; Rogalski 2017) and other contaminants such as pesticides (e.g., Buser et al. 2012; Simpson et al. 2015).

The abundance, distribution and composition of the resting egg bank are affected by natural and anthropogenic factors (Santangelo et al. 2011; Araújo et al. 2013). These

characteristics have been used to infer the responses of resting egg producing lake biota to changing environmental conditions, including changes in food availability, photoperiod light, and contamination. For example, the production of ephippia by the obligate parthenogenetic species *Daphnia pulicaria* was negatively correlated with the photoperiod light phase (i.e., when *D. pulicaria* ephippia received illumination) in a high alpine lake in Spain (Conde-Porcuna et al. 2014). On the other hand, the number of empty ephippia produced (no resting eggs in the chitin envelope) was negatively correlated with the epibiont burden of *Korshikoviella gracilipes*, a chlorophyte (Conde-Porcuna et al. 2014). High epibiont burden is indicative of high food availability, therefore, the negative correlation indicates that the number of empty ephippia increased as food availability decreased (Conde-Porcuna et al. 2014). Additionally, acidification of lakes in the High Tatra Mountains, Poland, was inferred to have caused decreases in the abundance of *Daphnia* ephippia (Marková et al. 2006). Significant increases in ephippia abundance also occurred coincident with periods of warm temperatures, pointing to a possible influence of climate on resting egg production (Marková et al. 2006).

During periods of intense contamination, resting stages play a critical role in ecosystem recovery, as they provide a temporal refuge for biota (Santangelo et al. 2011; Araújo et al. 2013; Piscia et al. 2016). In ponds located in Paris, France, along a gradient of metal pollution, Ringot et al. (2018) observed that *Daphnia* ephippia production increased with increasing metal contamination. In examining the viability of resting eggs impacted by pollution, Rogalski (2015) found that the hatching success of *Daphnia ambigua*, an indication of the viability of resting eggs, was negatively correlated with metal pollution. Moreover, in instances where eggs successfully hatched, juvenile mortality had a strong positive relationship with sediment metal concentrations (Rogalski 2015). In addition, Winegardner et al. (2017) found that metal

contamination likely influenced the ratio of unhatched resting eggs to empty cladoceran ephippia. An analysis of *Daphnia* ephippia in Lake Knob and Lake Dauriat (near Schefferville, Quebec, Canada) observed a higher proportion of unhatched resting eggs to empty Cladocera ephippia in Lake Dauriat, the more contaminated of the two (Winegardner et al. 2017). The authors posited that the empty ephippia may be indicative of successful hatching; however, the absence of eggs could also be attributed to other mechanisms, such as poorer preservation of resting eggs or failed resting egg production (Winegardner et al. 2017).

While there is much more to be understood about cladoceran resting egg banks, their usefulness in interpreting the responses of Cladocera to ecosystem changes, including metal(loid) contamination cannot be understated. In this study, I examine the impacts of arsenic pollution on *Daphnia* resting egg production in Pocket Lake (Yellowknife, Northwest Territories, Canada), where a history of gold mining has directly resulted in the legacy contamination by arsenic and other metal(loid)s (Palmer et al. 2015; Thienpont et al. 2016). A multi-proxy paleolimnological study of Pocket Lake documented striking ecological impacts at multiple trophic levels coincident with the onset of arsenic pollution in the ~1950's, when the sediments consisted of more than 3% arsenic by dry weight. Notably, the subfossil record of Pocket Lake revealed the appearance of *Daphnia pulex* at the time of arsenic enrichment, which was previously absent from the sediments of Pocket Lake, suggesting a high tolerance of the species to arsenic (Thienpont et al. 2016). As arsenic levels peaked, *Daphnia* was inferred to have been functionally extirpated (Thienpont et al. 2016). Based on these findings, Thienpont et al. (2016) hypothesized that (1) *Daphnia* was initially favoured by arsenic contamination due to a competitive advantage over other zooplankton species that may be more sensitive to arsenic, and

(2) that the eventual extirpation of *Daphnia* was due to its critical threshold for arsenic exposure being exceeded (i.e., the lake became too toxic for *Daphnia*).

The purpose of this study is to examine trends in *Daphnia* ephippia production during the pre-mining period, the mining period, and the post-mining period, to provide new insights into the mechanisms underlying *Daphnia* responses to arsenic pollution in Pocket Lake as inferred in Thienpont et al. (2016). Specifically, the objectives of my study are to 1) investigate if sediment arsenic toxicity compromised the integrity of the *Daphnia* resting egg bank in Pocket Lake; and 2) assess the viability of resting eggs in Pocket Lake sediments for use in future resurrection ecology studies.

3.3 Methods

3.3.1 Study Site Description

Pocket Lake is located within the southern portion of the Baker Creek watershed, on the Giant Mine lease territory (Figure 1). The nearest urban centre is the City of Yellowknife, located approximately 4 km to the south of lake. Pocket Lake is a small headwater lake with a relatively small surface area (4.8 ha) and shallow mean depth (2.0 m based on sampling depth from Thienpont et al. (2016)). The landscape is dominated by bedrock uplands, which constitutes approximately 78% of the lake's basin (Spence 2006; Ecosystem Classification Group 2008; Thienpont et al. 2016)). Vegetation is characterized by black spruce (*Picea mariana*), lichen on the bedrock outcrop, mosses, dwarf willow (*Salix spp.)* and grasses (Spence 2006; Thienpont et al. 2016). The climate for the region of Yellowknife is subarctic continental, and permafrost is discontinuous (Wolfe 1998). Mean annual temperature is -4.38°C and mean annual precipitation is 289 mm, based on meteorological data recorded by the nearest weather station at Yellowknife Airport averaged over the period of 1981 to 2010 (Environment and Climate Change Canada,

2019). The north Great Slave Lake region, in which Pocket Lake is situated, has a long history of habitation by the Yellowknives Dene (Weledeh) First Nation, who have engaged in subsistence use of the land and surface water resources (Sandlos & Keeling 2016).

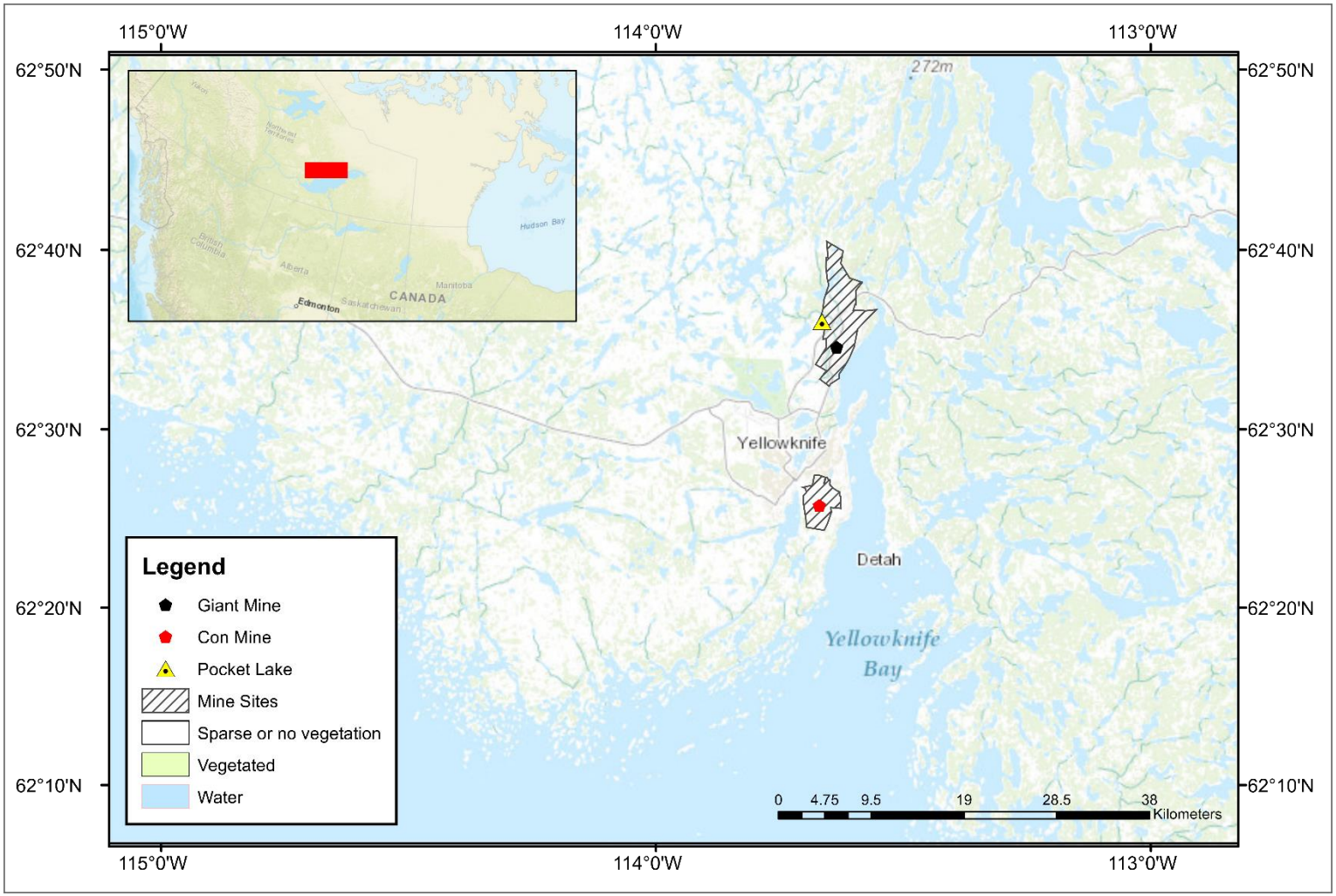

Figure 3.1. Map of the Yellowknife area (Northwest Territories, Canada) showing the location of Pocket Lake (yellow triangle), Giant Mine (north of Yellowknife; black pentagon), and Con Mine (south of Yellowknife; red pentagon). The respective footprints of the two mines are shown. Inset map shows the location of the study region (red rectangle) within Canada.

The sediments of Pocket Lake had a median arsenic concentration of 1750 µg/g and ranged from 640 µg/g at 24.25 cm to 33,000 µg/g at 9.25 cm, the latter of which occurred at the peak of mining. Surface sediments, 0-0.5 cm, had an arsenic concentration of 730 µg/g. Lake water arsenic concentration is ~2000 µg/L, based on point measurements taken between March 2014 and July 2016 (Sivarajah et al. 2019).

3.3.2 Field Methods

A sediment core was collected from Pocket Lake using a Uwitec gravity corer (Mondsee, Austria) in March 2016. The 25 cm long core was sectioned into 0.5 cm intervals for the first 15 cm intervals and then at 1 cm intervals for the remainder of the core, using a modified Glew (1988) vertical extruder. Sediments were stored cold (4°C) or frozen until analysis at York University.

3.3.4 Laboratory methods

Daphnia resting eggs were isolated from the sediment matrix using a modified Onbé-Marcus sugar flotation method (Vandekerkhove et al. 2004; Marcus, 1990; Onbé, 1978). Every other 0.5 cm interval was analyzed fully from 0 to 14.5 cm, an average sediment volume of 22 mL per interval; however, sediments were extracted from five intervals at the 8.0-11.5 cm section of the core (median core depths of 8.25, 8.75, 9.75, 10.25 and 11.25 cm) to intensify counting efforts at sediment depths estimated to represent the period in which *Daphnia* dominated the zooplankton assemblage of Pocket Lake (Thienpont et al. 2016). For core depths below 14.5 cm, which were divided into 1 cm intervals, sediments were extracted from every other interval, and had an average volume of ~46.4 mL per interval.

Sediment intervals were placed into separate centrifuge tubes. Sugar and water were mixed in a 1:1 ratio and added to the tubes with sediments. Sediments mixed with the sugar solution were centrifuged at 3000 rpm for 3 minutes to homogenize the mixture. The supernatant from the centrifugation was then then rinsed with Milli-Q water through a 45 µm sieve to remove the sugar. The filtrate was checked regularly to ensure that no ephippium was lost at this filter size. Following filtration, small aliquots of the wet sediment were transferred to petri dishes and resuspended with the addition of Milli-Q water. Sediments were then analyzed under a Motic SMZ-161 R2LED Stereo Zoom Microscope at X10 magnification to identify ephippia. Ephippia were separated into separate petri dishes and were decapsulated based on methods in Cuenca Cambronero & Orsini (2018) to determine the physical condition of the ephippia and viability of the resting eggs based on the categories established in Marková et al. (2006). Ephippia were also identified to the species complex level using Mergeay et al. (2005) as a guide. There was no predetermined maximum count for ephippia; all ephippia from each selected interval were analyzed and corrected for volume of sediment used. As no viable resting eggs were identified, resurrection ecology methods could not be used.

3.4 Results and discussion

All ephippia observed in Pocket Lake were produced by individuals in the *Daphnia pulex* complex based on ephippia morphology (Mergeay et al. 2005). All ephippia were empty, with no viable or degraded resting eggs observed in the ephippia casings. *Daphnia* ephippia abundance was relatively low in sediment intervals collected from 14-24 cm core depths, ranging from 0.063 ephippia/mL (19.5 cm core depth, 3 ephippia) to 0.222 ephippia/mL (21.5 cm core depth, 10 ephippia) (Table 3.1, Figure 3.1). Ephippia abundance increased above 15 cm, particularly in the 12 -13.25 cm (0.962 – 1.0 ephippia/mL; 23-25 ephippia), and 9.5 cm (1.083 ephippia/mL; 26

ephippia) intervals. Ephippia abundance sharply declined above 8.5 cm core depth to an abundance of 0.208 ephippia/mL, with only 5 ephippia found (Table 3.1, Figure 3.1). Empty egg membranes were found in comparatively low numbers (<6), at core depths of 4.5-8.5 cm, and 13-13.5 cm, indicating at least some of the ephippia likely hatched successfully (Table 3.1, Figure 3.1). Ephippia and egg membranes were completely absent in the upper 2 cm of the core (Table 3.1, Figure 3.1).

Table 3.1. Mass and volume of sediment from each interval of Pocket Lake and the number of empty ephippia and empty egg membranes identified

Depth (cm)	**Mass of Sediment (g)**	**Volume of Sediment (mL)**	**Empty ephippia**	**Empty egg membrane**
0-0.5	14.50	13	0	0
1.0-1.5	22.86	21	0	0
1.5-2.0	23.94	22	0	0
2.5-3.0	22.55	21	6	0
3.5-4.0	20.45	19	3	0
4.5-5.0	23.15	21	13	1
6.0-6.5	22.11	20	9	2
7.0-7.5	23.64	22	8	6
8.0-8.5	26.23	24	5	3
8.5-9.0	27.19	25	5	0
9.5-10.0	25.41	24	26	0
10.0-10.5	26.58	25	10	0
11.0-11.5	20.16	19	9	0
12.0-12.5	24.61	23	23	0
13.0-13.5	28.50	26	25	1
14.0-14.5	23.10	21	2	0
15-16	49.12	45	7	0
17-18	47.95	44	3	0
19-20	51.82	48	3	0
21-22	48.29	45	10	0
23-24	53.92	50	10	0

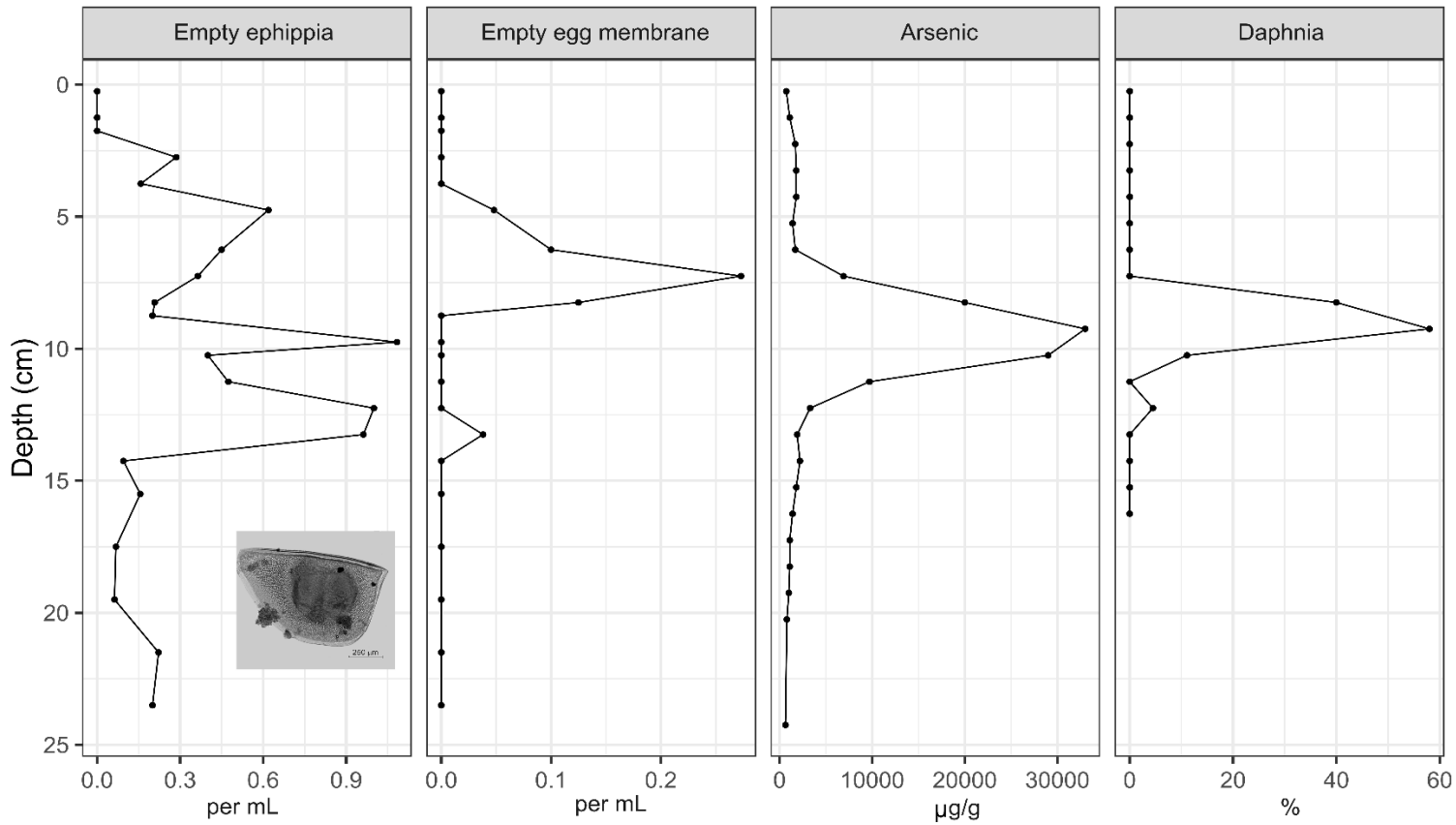

Figure 3.2 Number of empty ephippia and egg membranes corrected for volume of sediment used found in a sediment core from Pocket Lake (Yellowknife, Northwest Territories, Canada). Trends in arsenic concentrations and % *Daphnia* are recreated from data presented in Thienpont et al. (2016). Our analysis was conducted on a different sediment core to what was analyzed in Thienpont et al. (2016), and so trends are not directly comparable. The image presented is that of a typical *Daphnia* ephippia (image taken by J. Korosi)

3.4.1 Temporal trends in Daphnia ephippia production

Trends in *Daphnia* ephippia abundance are consistent with the previous findings of *Daphnia* population trends inferred from *Daphnia* post-abdominal claws (Thienpont et al. 2016), where *Daphnia* increase in abundance during the early mining period and subsequently declined. Based on *Daphnia* trends reconstructed in Thienpont et al. (2016), we infer that the increase in *D. pulex* ephippia abundance is an approximate marker of the onset of mining activities and arsenic enrichment in Pocket Lake, and that the decline in *Daphnia* ephippia reflects the decline in *Daphnia* populations after arsenic concentrations peaked. Similarly, I recorded no *Daphnia* in the surface 2.0 cm intervals, further supporting the conclusion that *Daphnia* have been extirpated from Pocket Lake. Present-day lake-water arsenic concentration is 2.07 mg/L in Pocket Lake (Sivarajah et al. 2019). *Daphnia pulex* and *D. magna* have LC50 values (concentration at which 50% of the population perishes) of 2.6 mg arsenite/L (Shaw et al. 2007) and 0.51 mg arsenic trioxide/L (Fikirdeşici et al. 2012), respectively.

In contrast to Thienpont et al. (2016), I found *Daphnia* ephippia in the pre-mining period, showing that *Daphnia* were present prior to the onset of arsenic pollution. The discrepancy in the results is likely attributed to the amount of sediments analyzed. Using a reasonable counting effort for Cladocera subfossils (where *Daphnia* are typically recovered as postabdominal claws), in which approximately 1-2 g of sediments are analyzed (Korosi & Smol 2012), it is unlikely that *Daphnia* subfossils could have been found given the relatively low abundance of ephippia (5-13) I encountered in the upper and lower sediments using ~22 mL of sediments per 0.5 cm interval. It is likely that an analysis of more sediments by Thienpont et al. (2016) would have led to the identification of a small number postabdominal claws. However, subfossil analysis of such a large amount of sediment would be both difficult and inefficient. This illustrates the benefit of

analyzing the resting egg bank, it allows for a comparatively more efficient method of analyzing a large volume of sediment.

3.4.2 Viability of resting eggs in Pocket Lake for future resurrection ecology studies

There were no degraded eggs or resting eggs outside of ephippial cases (177) and empty egg membranes (13), all of which were empty (Figure 1, Table 1). The basis of resurrection ecology studies is the hatching of resting eggs and maintenance of clonal lineages (Rogalski 2015; Burge et al. 2018; Radzikowski et al. 2018; Cuenca Cambronero and Orsini 2018). Moreover, the number of *Daphnia* resting eggs impacted by metal contamination used in resting studies is relatively large, for example, >273 (Rogalski 2015). "Empty ephippial covers" was the most common category of the 4,948 ephippia found in the cores of six High Tatra lakes (Marková et al. 2006). While intact resting eggs were found by Marková et al. (2006), these numbered only 94, representing a very low 1.9% of the total 4,948 ephippia. It should be noted that the ephippia counting strategy, analyzing all sediments in 0.5 and 1 cm intervals, used in the analysis of the High Tatra lakes was also applied in my study (Marková et al. 2006). The possibility exists that viable resting eggs may be present in Pocket Lake but at extremely low abundances. While degraded eggs were also common in the High Tatra lakes (Marková et al. 2006), this category was completely absent in Pocket Lake. As no viable resting eggs appear to be present in Pocket Lake, or are present only at very low abundances, future resurrection ecology studies to generate historic clonal lineages for use in arsenic toxicity studies are improbable.

3.4.3 Impacts of contamination on the Daphnia resting egg bank of Pocket Lake

The presence of only empty ephippial covers and egg membranes in our study could be mean either 1) resting eggs were present and successfully hatched, leaving only empty ephippia covers; 2) *Daphnia* failed to deposit resting eggs in the ephippia, i.e. resting egg abortion (failed development of eggs) occurred (Marková et al. 2006; Conde-Porcuna et al. 2014); or 3) resting eggs degraded over time.

While metal contamination triggers the shift to the production of diapausing eggs, hatching has been inferred as a potential mechanism explaining the presence of empty ephippia in contaminated lakes. For example, Winegardner et al. (2017) found that in Lake Knob, which had metal concentrations below the probable effect level, had a higher proportion of empty ephippia compared to unhatched eggs, suggesting that empty ephippia may be indicative of successful hatching. Within a second lake in the region, Lake Dauriat, which had levels above the probable effect level, while the proportion of unhatched eggs was greater, empty ephippia were found pointing to possible hatching even in highly contaminated lakes (Winegardner et al. 2017). Empty ephippia as representative of successful hatching were also noted in the acidified High Tatra lakes, supporting the notion that termination of diapause can occur within perturbed ecosystems (Marková et al. 2006). As such, successful hatching of resting eggs could have occurred in Pocket Lake despite it being highly contaminated. The fact that empty ephippia were noted prior to peak arsenic contamination, together with the presence of empty egg membranes, supports this possibility. Successful termination of diapause would suggest that the resting egg bank supported the maintenance of *Daphnia* populations (autogenous restoration), although high juvenile mortality following hatching, a consequence of metal contamination (Rogalski 2015) may still have occurred.

Alternatively, the large number of empty ephippia in Pocket Lake may be linked to resting egg abortion due to decreased food availability or metal contamination. Conde-Porcuna et al. (2014) determined that empty ephippial covers were negatively correlated with food availability (i.e. decreased food availability results in empty ephippia). Diatom analysis of Pocket Lake showed that coincident with the peak of sediment arsenic concentrations was a loss in planktonic diatoms, most notably *Discostella stelligera/ pseudostelligera* (Thienpont et al. 2016). As an herbivorous, planktonic cladoceran, a loss in planktonic diatoms would represent a decrease in food availability for *Daphnia*, which in turn would increase the production in empty ephippia.

With respect to metal contamination, Ponti et al. (2010) observed that exposure of *Daphnia galeata Sars* to ionic copper (39-87 µg Cu^{2+}/ L) led to increases in the number of eggs produced, but also an increased rate of egg abortion. While the effect of arsenic toxicity on subitaneous and diapausing egg abortion has not been well studied, Quach et al. (2019) found abnormal occurrences in the production of subitaneous eggs in *D. magna* exposed to 50 µg As/L. Within the brood chamber of the adult female *Daphnia*, broken and adhesive eggs were found. Therefore, it could be possible that the high concentrations of arsenic in Pocket Lake may have compromised the development of resting eggs. Moreover, while the increase of arsenic was one of the most striking changes in Pocket Lake, coincident with this period was a substantial increase in lead (Pb) within the lake's sediments. Araujo et al. (2020) found that egg abortion of haploid resting eggs occurred when *D. magna* are chronically exposed to lead as an environmental pollutant, and therefore the presence of empty ephippia may have been influenced by lead. For this reason, future bioassays should investigate the impacts of food availability, and arsenic toxicity on resting egg production of *Daphnia pulex.*

Although ephippia can remain viable for decades, over time, degradation and senescence of resting eggs contribute to a reduced abundance in the egg bank (DeStasio 1989; Santangelo et al. 2011), which was observed for *Diaptamus sanguineus*, a freshwater copepod (DeStasio 1989). Marková et al.(2006) also found that some resting eggs of *Daphnia pulicaria* stored in the sediments from lakes in the High Tatra Mountains were degraded. However, notable traces of the resting eggs were found in the lakes of the Tara Mountains (Marková et al. 2006), but were not found by Winegardner et al. (2017) in their study of the lakes near Schefferville, Quebec, Canada. Similarly, there were no degraded eggs observed in my study, but the possibility cannot be ruled out, especially for the lower core depths in which eggs are considerably older.

This study provided evidence of *Daphnia*'s presence both after the peak in arsenic concentrations and during the pre-mining period. The peak in ephippia abundance was consistent with the inferred peak in *Daphnia pulex* complex abundance from the subfossil analysis conducted by Thienpont et al (2016). However, the absence of viable resting eggs in the sediments of Pocket Lake, possibly due to either successful hatching, resting egg abortion, or degradation of eggs, indicates that future resurrection ecology studies are infeasible.

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Chapter 4 : General conclusions and Future Directions

4.1 Thesis Summary and Conclusions

Historical arsenic trioxide emissions in Yellowknife (Northwest Territories, Canada), particularly from Giant Mine, has resulted in the contamination of many freshwater lakes in the region. One paleolimnological study on Pocket Lake, which has experienced extreme arsenic contamination, noted dramatic ecological impacts to the Cladocera (Class Branchiopoda), a group of crustacean zooplankton; however, little is known about the long-term impacts to Cladocera in other contaminated lakes in the region.

The primary goals of my thesis were to investigate the long-term impacts of arsenic on Cladocera in Yellowknife lakes along a broad arsenic gradient, and to evaluate the usefulness of Cladocera-based paleo-ecotoxicological techniques in studying ecological impacts of arsenic in Yellowknife lakes. To accomplish these goals, I aimed to assess the regional changes in Cladocera assemblages in lakes along an arsenic gradient, as well as the changes in the resting egg bank, a source of autogenous restoration, of *Daphnia* (cladoceran taxa commonly used in resting egg studies) in the heavily contaminated Pocket Lake. The research documented in this thesis aimed to achieve the primary goal by answering four main research questions regarding the regional changes in Cladocera assemblages and the trends in the *Daphnia* resting egg bank of Pocket Lake. These questions were explored in two separate studies presented in Chapters 2 and 3.

4.1.1 How have regional Cladocera assemblages in Yellowknife lakes changed since pre-1850?

Sediment cores from 23 lakes in Yellowknife along a lake-water arsenic gradient (1.5 to 750 µg/L) within a 40 km radius of Giant Mine were analyzed, with the goal of assessing

changes in regional Cladocera assemblages since pre-1850 (pre-industrial time period). This goal was achieved by using a "top-bottom" paleolimnological approach, where the Cladocera subfossils in the top sediments (representative of recent times) and bottom sediments (pre-1850) from each lake were analyzed.

Overall, there were no significant regional, directional changes in Cladocera assemblages since pre-1850 and therefore Cladocera changes were not driven by regional forcing factors. Instead, changes were based on local, lake-specific limnological conditions. The greatest changes in Cladocera assemblages occurred in lakes impacted by urban development in Yellowknife, which were characterized by a complete shift in the dominant taxa. Interestingly, while arsenic contamination was not identified as a regional forcing factor, species richness and diversity of modern assemblages increased along the lake-water arsenic gradient, when the four most contaminated lakes were excluded. This particular finding contrasts with other studies on metal contamination that showed that other metals, especially copper, decrease species diversity (Winegardner et al. 2017; Leppänen 2018), highlighting the need for greater research regarding arsenic's effect on Cladocera.

4.1.2 Which cladoceran taxa are sensitive or tolerant of arsenic, and therefore candidate ecological indicators for future arsenic contamination studies?

Changes in individual Cladocera taxa along the arsenic gradient were analyzed to determine taxa that are sensitive or tolerant of arsenic, which can be explored in future studies as candidate ecological indicators of arsenic contamination. Overall, lakes highly contaminated by arsenic (>100 µg/L) were dominated by littoral/benthic taxa, primarily species within the genus *Alona*, and *Chydorus brevilabris/biovatus*. The presence of these taxa at relatively high arsenic concentrations has been reported in previous studies (Thienpont et al. 2016; Little et al. 2020),

indicating a potential high tolerance. On the other hand, the pelagic taxa, *Bosmina* dominated in most lakes with comparatively lower arsenic concentrations, indicating a relatively low tolerance to arsenic. The long-term sensitivity of *Bosmina* to arsenic was inferred from paleolimnological analysis (Chen et al. 2015), and more directly in a toxicological study which proposed its usage as an ecological indicator (Passino and Novak 1984).

4.1.3 Did arsenic contamination compromise the Daphnia resting egg bank in Pocket Lake?

Analysis of *Daphnia pulex* complex ephippia was conducted on a single core collected from Pocket Lake. Coincident with the period of arsenic enrichment was an increase in the abundance of ephippia, which is in keeping with the inferred dominance of *Daphnia* based on its subfossil record (Thienpont et al. 2016). Ephippia were also present following the peak in arsenic contamination and prior to the onset of mining in the region, periods in which its smaller postabdominal claw remains were absent (Thienpont et al. 2016). These findings indicate a relatively high tolerance of the *Daphnia pulex* complex to arsenic contamination.

An assessment of the physical condition of the ephippia showed that they were all empty, indicating that either a) the eggs successfully hatched; b) resting egg production was aborted, or c) original viable resting eggs degraded over time. The possibility that eggs successfully hatched suggests that the *Daphnia* resting egg bank in Pocket contributed to the maintenance of its population through autogenous restoration during arsenic contamination. The other two possibilities suggest that the resting egg bank was compromised. The impacts of arsenic on the production of resting stages need to be studied in controlled toxicity studies to explain these observations.

4.1.4 Are the Daphnia pulex complex resting eggs in Pocket Lake still viable to be used in future resurrection ecology studies?

All ephippia identified from the Pocket Lake sediment core were decapsulated to determine the number of viable resting eggs contained. No resting eggs were identified in the ephippia nor were there traces of degraded resting eggs in the sediments. Resurrection ecology studies hatch resting eggs from different time periods to analyze the responses of historical populations to stimuli. Unfortunately, the consequence of there being no viable resting eggs in Pocket Lake is that future resurrection ecology studies are not feasible.

4.2 Future Directions

The major findings presented in this thesis highlight the complex responses of Cladocera to multiple stressors in subarctic lakes, particularly arsenic. Moreover, this research demonstrates that paleo-ecotoxicology can provide new insights regarding the responses of Cladocera not inferred from traditional toxicity studies or traditional subfossil analysis, such as a high tolerance of some taxa to arsenic. Much more remains to be studied about the responses of Cladocera to arsenic contamination, including a much more direct analysis of changes in species richness and diversity with arsenic. Furthermore, arsenic toxicity studies should also dedicate focus to the critical role ephippia production plays in the responses of zooplankton during periods of contamination.

While viable resting eggs could not be found in Pocket Lake, resting eggs may exist in other arsenic contaminated lakes, which can be used to determine evolutionary responses, potentially addressing the lack of eggs in Pocket Lake. This information can be particularly useful not only for future research in different regions, but also for environmental management, and remediation projects, where a lack of knowledge about arsenic contamination may compromise the efficacy of corrective actions. Arsenic contamination is a major stressor to freshwater ecosystems in many regions where it is a contaminant, and the use of Cladocera as

biological indicators, together with the application of paleo-ecotoxicological techniques can help us in determining and potentially ameliorating its impacts.

4.3 References

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Glossary

Term	Definition
Alpha diversity	A local measure that refers to the average species diversity of a given area.
Diapausing eggs	Eggs have a period of dormancy and do not hatch until environmental conditions are optimal.
Diploid	Quality of an organism or cell having double the haploid number of chromosomes, i.e. paired chromosomes
Dysoxic	Low oxygen concentration; between hypoxia and anoxia.
EC_{50}	The concentration at which a drug or substance gives a half maximal response, i.e. estimate of toxicant concentration that produces an effect in 50% of an exposed population.
Genotoxic agent	A substance that damages cellular DNA, which can potentially result in genetic mutations and/or cancer.
Haploid	Quality of an organism or cell having a single set of chromosomes.
LC_{50}	The concentration at which a substance of drug will result in the deaths of 50% of the exposed population.
Paleo-ecotoxicology	Paleo-ecotoxicology is an applied paleolimnological approach that combines traditional approaches in paleolimnology with ecotoxicology
Paleolimnology	Paleolimnology is a field in which the chemical, physical and/or biological markers preserved within lake sediments are analyzed to reconstruct the past conditions of the lake.
Parthenogenesis	Reproduction via embryo that does not require fertilization by sperm. Typically results in a female gamete, or very rarely, a male gamete.
Photoperiod	The period in which an organism receives illumination
Species richness	Number of different species within an ecosystem or ecological community
Subitaneous eggs	Eggs that immediately hatch or hatch within a short period of time. There is no period of dormancy.
Teratogen	Teratogens are substances that cause human congenital abnormalities.