Development, Assessment and Application of Benthic Algal Biomonitoring Protocols for Canadian Waters

by

Kathryn Elizabeth Thomas

A thesis
presented to the University of Waterloo
in fulfilment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Biology

Waterloo, Ontario, Canada, 2014

Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Stressors such as residential and industrial development and climate warming are escalating in North America, which increases stress to aquatic ecosystems. In the face of this, monitoring biologists must continually improve protocols for long-term monitoring programs in order to adequately characterize changes in biological communities. To address this need, this thesis has developed, applied, and assessed benthic algal biomonitoring protocols in lakes and rivers. In the Muskoka-Haliburton area of Ontario, benthic algal protocols were developed to assess effects of differences in shoreline development. In the South Nahanni River watershed, Northwest Territories, benthic algal biomonitoring protocols were developed to assess effects of two mining companies on rivers in an otherwise pristine ecosystem.

In the Muskoka-Haliburton area I developed and evaluated bioassessment protocols based on benthic algae growing in the littoral zone of lakes to track effects of shoreline development. To do this, I sampled a suite of study sites (n = 28 in 2006, n = 29 in 2007) spanning a gradient of shoreline development (e.g., intact forests, cottages, marinas). The protocols were modified from protocols developed for rivers (Biggs and Kilroy, 2000), and five levels of assessment were completed for each site that differed in the amount of time, resources and expertise required. Level 1 comprised visual assessments of benthic algal cover. Level 2 involved biomass estimates (ash-free dry mass and chlorophyll-*a*). Level 3 included coarse-level taxonomic enumeration of benthic algal community composition (i.e., to major algal classes). Level 4 included quantification of pigment concentrations using High-Performance Liquid Chromatography (HPLC). Level 5 involved high-taxonomic resolution enumeration of diatom community composition (to species and sub-species levels). Uni- and multivariate analyses were used to assess relations between shoreline development, water chemistry and benthic algal metrics. Results of this study showed that Level 5 (diatom community composition) best

discriminated among shoreline development categories and, despite the higher technical skill and time required, was recommended for use as the most promising metric for Precambrian Shield lake nearshore biomonitoring with benthic algae. Photosynthetic pigment concentration (Level 4) showed modest potential as a biomonitoring tool, but further development is required for their use in monitoring protocols.

The South Nahanni River watershed is remote with good water quality. However, activities conducted by two mining operations within the watershed potentially threaten the water quality and ecological integrity of downstream sites. Here, I conducted three studies. The first study examined physical and chemical conditions at river sites unaffected by human activities and how the conditions related to three algal metrics (benthic algal community composition, diatom community composition, and photosynthetic pigment concentration). To do this, I sampled 44 reference sites (i.e., unaltered by human activities such as mining or other infrastructure) from across the South Nahanni River watershed in 2008 and 18 sites in 2009 (12 repeated from 2008, 6 new). Multivariate analyses were utilized to assess patterns of variation in physical and chemical data and their relation to benthic algal community composition. Results showed that physical and chemical conditions differed distinctly between two ecoregions within the Nahanni Watershed (Selwyn Mountain and Nahanni-Hyland ecoregions). Patterns of variation in the benthic algal metrics corresponded well with gradients of physical and chemical variables. Diatom community composition discriminated best between the two ecoregions. Photosynthetic pigment concentration only discriminated between the ecoregions in 2009, showing some promise as a biological monitoring tool.

The second study examined the extent that algal pigment versus taxonomic descriptors of algal community structure varied due to the Cantung mine along the Flat River in the South

Nahanni River watershed in order to evaluate the use of photosynthetic pigment concentration as a biomonitoring approach. To do this, I sampled 4 sites upstream and 6 sites adjacent to and downstream of the Cantung mine site and compared relations of water physico-chemical conditions with photosynthetic pigment concentration and taxonomic-based benthic algal community composition at the study sites. Patterns evident in ordinations by PCA and RDA identified that photosynthetic pigment concentrations varied along Flat River and were related to variance in physical and chemical variables. My analyses showed that there were substantial and often statistically significant differences in photosynthetic pigment concentration at non-exposed sites located upstream of the mine versus exposed sites located adjacent to and downstream of the mine. Photosynthetic pigment concentrations were more strongly and consistently associated with physical and chemical conditions than the taxonomy-based data, suggesting pigment analysis is effective for detecting environmental degradation. Additionally, cost comparisons showed that the base analytical cost for in-house analysis of pigment was low ($66.48/sample) and generally lower than traditional taxonomy-based assessments, making it a cost-effective alternative for biomonitoring protocols.

In the third study, I developed Reference Condition Approach (RCA) models based on benthic algae for the South Nahanni River watershed. To do this, I sampled a suite of reference sites across the watershed in 2008 (n = 44) and 2009 (n = 18; 12 resampled from 2008 and n = 6 new) and test sites (potentially affected) downstream of two mining companies (n=20 in 2008 and n = 17 in 2009). The BEAST (BEnthic Assessment of SedimenT) model was used to develop the benthic algal RCA models for each of the three benthic algal metrics. All reference sites (unaffected by mining activities) from 2008 and 2009 were grouped into biologically similar assemblages. Only physical and chemical variables unaffected by mining activities

were used in developing the RCA model. The biological assemblages at test sites were compared to their predicted reference assemblage using non-metric Multimetric Dimensional Scaling Analysis (MDS) and assessed for impairment. Three probability ellipses were used to create four categories of impairment: Category 1: ≤ 90% (reference condition), Category 2: 90 – 99% (possibly stressed), Category 3: 99 – 99.9% (stressed), Category 4: ≥ 99.9% (severely stressed). Patterns of downstream impairment were assessed and zones of influence were identified for each algal metric in each year. Assessments downstream showed that the RCA models identified reasonably consistent 'zones' of stress downstream of Cantung mine along Flat River. However, changes in photosynthetic pigment concentrations were more prominent compared to the other two metrics. Along Prairie Creek, only photosynthetic pigment concentrations identified sites outside of the reference condition directly downstream of the Prairie Creek mine. My results show that benthic algal RCA models (specifically photosynthetic pigment concentration models) show promise as biological monitoring tools, but should be tested in other ecosystems to assess the widespread utility of the method.

I developed, applied and assessed benthic algal community compositions for Canadian lakes and rivers. I assessed a variety of algal metrics in different ecosystems and associated with differing stressors, and found that photosynthetic pigments were the most sensitive metric to differences in physical and chemical conditions downstream of the two mines. Conversely, diatoms were the most responsive metric to differences between ecoregions, and similarly to differences in shoreline development categories in Muskoka lakes. Photosynthetic pigment concentrations can be influenced differently by stressors such as light compared to other metrics or biological traits. Indeed, I found that differences in pigment concentrations were often associated with differences in turbidity and thus, light may play an important role in

pigment concentration in biological assessments. Despite this, pigments and the RCA approach show promise as a biomonitoring tool for detection of impairment, and should be further tested and refined based on studies in other watersheds.

Acknowledgements

I would like to take this opportunity to thank Roland Hall, Andrew Paterson, and Garry Scrimgeour for all the support throughout the stages of this thesis, from helping to come up with the conceptual ideas and research questions, field logistics and statistical analyses to the seemingly endless edits. This thesis wouldn't be the same without your input, knowledge and expertise.

Roland, you have always given careful thought to the ideas, questions and road blocks that have come up with this thesis. Your advice and mentoring always helped to point me in the right direction. Thank you for sending me on all those 'work-cations', or conferences; they always helped to inspire me. Thank you for giving me the opportunity to teach your course; it was an eye-opening introduction into the world of lecturing and was a very rewarding experience.

Thanks to all the people who helped me in field (Dana Haggarty, Mike Sokal, Stephanie Lyons) for teaching me to take deep breaths when things didn't go my way and providing comical relief while filtering water, catching mice in our cabin, and living out princess dreams.

Garry, you ran the Nahanni show like a pro and were a great mentor to look up to. I have learned a great deal from you, including, but not limited to, how far to stand away from you when you teach me how to cast off while fishing. Someday I too hope to call myself a 'science geek'. Dana and Garry, I will always remember those 'grueling' field days in Nahanni soaring over mountain peaks, eating fresh pastries, relaxing in the hot springs, and watching two seasons of Flight of the Conchords; we truly had it rough!

I would like to recognize all the people in the labs, you guys were always willing to help keep things fun and provide advice, instruction and constructive feedback. Special thanks to Lauren MacDonald for being my personal cheering squad!

Above all to my parents; mom and dad you have been there for me from beginning to end of this thesis and I truly appreciate all the love and support you have given me. Thank you to the rest of my family and friends (Brad, Robyn, Alina, Elise, Kevin, and Emma), I couldn't have done it without everyone's support!

The first part of this project was funded by the Ontario Ministry of Environment's Best in Science program, NSERC Discovery grant (to R.I. Hall), and the University of Waterloo (Graduate student assistance). Thank you to Kevin Turner for assistance in developing the site map, and staff at the Ontario Ministry of Environment's Dorset Environmental Science Centre for logistical and analytical support.

The second part of this project was funded by Parks Canada Agency, NSERC Strategic Partnership Projects Grant (to R.I. Hall), Aboriginal Affairs and Northern Development Canada, Environment Canada, North American Tungsten, Canadian Zinc Corporation, Northern Scientific Training Program (student travel funding), and the University of Waterloo (graduate student assistance). Shape files for maps of Nahanni, copyright: Department of Natural Resources Canada and Parks Canada Agency, all rights reserved.

Table of Contents

List of Figures

List of Tables

Chapter 1

General Introduction

1.1 Freshwater biomonitoring potential

Freshwater ecosystems provide a number of important services to humans, but are under increasing pressure due to human activities and climate warming. As the climate warms and urban, rural, and industrial development increases, aquatic ecosystems become increasingly affected by both natural processes and human influences. The intensity of the cumulative effects of both natural and anthropogenic stressors threatens to degrade water quality and ecological integrity of aquatic ecosystems (Chambers et al., 2001; Schindler & Smol, 2006). However, disentangling the effects of human activities and climate warming from natural variability is difficult (Clements et al., 1992, 2010). In the face of these pressures, improved long-term monitoring protocols are required to inform policies and practices that can safeguard against deterioration of water-quality and ecological integrity.

My study was conducted to develop, assess, and apply methods for monitoring changes in water quality and ecological condition based on benthic algae in the nearshore zone of temperate-zone lakes and northern rivers. This study was located in two areas of Canada under intensifying, yet different, development pressures. The two study areas were used to assess the effects of residential development on water quality and ecological integrity of lakes in the Muskoka-Haliburton area (Ontario), and mining in rivers of the South Nahanni River watershed (NWT). The research in this thesis was performed in partnership with the Ontario Ministry of Environment and Parks Canada and so has great potential to be incorporated into policies and procedures for groups ranging from local communities to First Nations and governmental organizations.

1.2 Biomonitoring of temperate lakes

One of the most widespread and major causes of water-quality and ecological impairment in lakes is cultural eutrophication, due to elevated nutrient loading. To date, monitoring programs in lakes have commonly focused on characterizing the chemical and biological conditions (e.g., water chemistry, chlorophyll-*a* concentration, phytoplankton and zooplankton metrics) at a central, open-water (or pelagic) location (King et al., 2006). For example, phosphorus concentrations are extensively used as indicators of lake trophic status and are highly correlated with phytoplankton biomass (Dillon & Rigler, 1974). Paleoecological studies have tracked shifts in diatom community composition, taken at central, open-water locations within lakes, to infer TP concentrations down-core using transfer functions (Hall & Smol, 1996). Human activities have increasingly encroached on the shorelines of lakes and so are likely to exert the greatest effects on the nearshore zone. However, there has been relatively little emphasis on monitoring conditions in the littoral zone of lakes, despite that the littoral zone is often the first to be influenced by human activities, and given that benthic algae often contribute importantly to primary production in lakes (Vadeboncoeur et al., 2001, 2003). Only in the last 10 years has the focus of lake monitoring begun to shift toward nearshore monitoring with the recognition that monitoring of periphyton could provide a more sensitive assessment and early warning of impending changes compared to phytoplankton and water chemistry from the pelagic zone of lakes (e.g., Lambert & Catto, 2008; Rosenberger et al., 2008; Lambert et al., 2008).

Periphyton communities, unlike phytoplankton, are not always correlated with open water TP concentration and often indicate a higher trophic status of the lake. Previous studies have suggested that periphyton receive pulses of nutrients from the surrounding land and assimilate

it before dilution via mixing with offshore waters (Cattaneo, 1987; Poulíčková et al., 2004). Changes in periphyton biomass and community composition associated with different gradients of shoreline development have been observed in lakes (Lambert & Catto, 2008; Lambert et al., 2008). For example, Rosenberger et al., (2008), studied algal communities along a gradient of shoreline development in oligotrophic lakes in the USA and found increased algal biomass and shifts in algal community composition to more nuisance forms of algae at more developed sites. Shifts observed in algal communities may be associated with changes in aesthetic appeal for recreational purposes, and also with the alteration of foodwebs and water quality (Rosenberger et al., 2008). Thus, nearshore monitoring of periphyton not only gives insights into the trophic status of the lake as a whole but provides a 'report card' of sorts for different levels of development and the degree of changes they cause.

1.3 The Muskoka-Haliburton area of Ontario

The Muskoka-Haliburton area is located in South-Central Ontario (Figure 1.1). It is an ecologically and economically important area. This area is located on the Canadian Shield, and many of the lakes are acid-sensitive and oligo- to mesotrophic. The lakes are subjected to multiple stressors including: climate variability, climate change, acidic deposition, pollution (e.g., Hg contamination), invasive species (e.g., *Bythotrephes*), and development (Dillon & Molot, 1996; Schindler et al., 1996; Schindler, 2001; Yan et al., 2001). The area is only 4% developed with settlements and agriculture/open fields, but with varying degrees of natural state and development around lakes (Tran, 2007). Therefore, increased development around these lakes may make effects of increasing contaminants more noticeable, especially when in conjunction with climate change.

1.4 Biomonitoring of northern rivers

Freshwaters in northern Canada are increasingly subjected to human-caused changes, including industrial development, encroachment of expanding human populations and long-distance transport of contaminants (Schindler & Smol, 2006). Mining activities are expected to nearly double between 2011 and 2020 (The Conference Board of Canada, 2013). Some areas in northern Canada are naturally rich in metals, however, mining can mobilize metals in aquatic environments causing degradation of water quality and ecological integrity (Wrona et al., 2006). The detection of effects of metal mining can be difficult, because studies are often initiated after pollution has occurred [Clements et al., 2000; Hill et al., 2000a; Clark & Clements, 2005; Rhea et al., 2006; Hall et al., 2012; Thomas et al., 2013 (Chapter 4 of this thesis)] and lack of knowledge of natural loading and variability impair determination of the effects of mining. Thus, it is important to adequately define reference conditions in order to understand the natural variability among sites and to effectively assess the effects of mining contamination at potentially affected test sites (Hawkins et al., 2010).

Methods for river assessment are well established. Examples include CABIN protocols (Environment Canada [EC], 2011), NIWA benthic algal protocols (Biggs & Kilroy, 2000), and European river monitoring protocols (CEN, 2003, 2004); however, they are still evolving. Many study designs can be utilized to identify environmental impacts of industrial activities on surface waters, including control-impact (CI), before-after-control impact (BACI), gradient and reference condition approaches (RCA; Green, 1979; Underwood, 1994; Bailey et al., 2004). Each method takes a different approach to defining the conditions at reference sites (sites least disturbed by stressor of interest) ranging from using one or a few reference sites upstream of the stressor of interest (e.g., CI, BACI, gradient) to using many reference sites from adjacent

streams not exposed to stressor of interest (RCA). Despite the differences in each method, all methods compare reference sites to test sites (possibly exposed to the stressor of interest) typically using water chemistry or measures of biological communities or ecosystem function [e.g., Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2013 (Chapter 4)]. The benefit of the RCA model is that it uses many regional reference sites (typically from across a watershed) to adequately characterize the reference condition of an area (Reece & Richardson, 1999; Rosenberg et al., 1999; Bowman et al., 2010). The RCA assumes that biological communities are primarily influenced by the surrounding physical and chemical conditions at each site. Thus, each reference site is also selected to be similar in physical and chemical conditions to the potentially affected 'test' sites (Hulbert, 1984; Reynoldson et al., 1997; Bailey et al., 2004). RCA models have been successfully developed for invertebrate communities across Canada (e.g., Reynoldson et al., 1997; Reynoldson et al., 2001; Bowman et al., 2010). However, few studies have developed models for benthic algal communities, and only one study (Bowman et al., 2010) developed a preliminary model for algal communities within the South Nahanni River watershed.

1.5 The South Nahanni River watershed

The South Nahanni River watershed (35 000 km^2) is located in the southwest of the Northwest Territories (61°39', 125°34') and is a pristine, remote wilderness with high preservation value and cultural significance. A portion of the watershed was designated a National Park (Nahanni National Park Reserve; 30 050 km^2) in 1976 and a UNESCO world heritage site in 1978 (Figure 1.2). In 2012, a portion of the most northern extent of the watershed was designated as a National Park Reserve (Nááts'ihch'oh National Park Reserve; 4 850 km^2). The South Nahanni River (540 km long) runs the length of the watershed (through the two national park

reserves) and was named a Canadian Heritage River in 1987. The South Nahanni River watershed is a headwater tributary for the Mackenzie River. It is underlain by Proterozoic formations of glaciomarine conglomerates and carbonates with major veins of heavy metals including lead, zinc and silver sulphides, and tungsten ($CaWO_4$) (EC, 1991). Airborne contaminants are considered to be less influential within the watershed than those derived from land and water (EC, 1991).

The watershed is located within the Taiga Cordillera, Taiga Plains, and Boreal Cordillera ecozones and includes diverse topography (e.g., canyons, karst features, mountains, plains, and plateaus) and vegetation (e.g., boreal forest and alpine tundra; EC, 1991). The western portion of the watershed is underlain primarily by shale and has the Logan Mountains and the Ragged Ranges, while the eastern portion is underlain primarily by carbonates and incorporates the Nahanni Karst and Ram plateaus (Caron et al., 2008). The hydrology within the watershed is influenced by many factors including snowmelt in late winter, and glacier melt, and precipitation in summer. Flows range from 55 – 1500 m^3/s at Virginia Falls (EC, 1991; Halliwell & Catto, 2003). Peak flow occurs during spring snowmelt, and summer flows are heavily influenced by rain; thus rivers within the South Nahanni River watershed are prone to flash flooding. Although the South Nahanni River watershed is considered to be pristine, it is also considered to have great potential for mineral extraction (Falck & Wright, 2007; Caron et al., 2008). With industrial development projected to nearly double between 2011 and 2020 (The Conference Board of Canada, 2013), there is concern about the continued 'good ecological status' of this watershed through time. Along with the potential for increased development within the borders of the South Nahanni River watershed, there are two established mining operations located within the watershed. There are concerns about effects of

these mines on the downstream water quality within the Nahanni National Park Reserve; thus studies have been conducted to assess water quality and biological integrity downstream of the two mines [e.g., Halliwell & Catto, 2003; Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2013 (Chapter 4)].

North American Tungsten's Cantung mine is a fully operating tungsten mine located along the Flat River (~200 km upstream of the confluence with the South Nahanni River) in the western portion of the South Nahanni River watershed (61°87', 128°13'; Figure 1.2). The Flat River is underlain primarily by shale (Caron et al., 2008). Tungsten is mined from a schelite ($CaWO_4$) deposit. The Flat River is a 4th order stream at the mine site, with flow from hot springs and mineral springs along with fluctuating sediment loads resulting in high natural variability along the river (Halliwell & Catto, 2003). Flow at the mouth of the Flat River ranges from 247 – 900 m^3/s (Halliwell & Catto, 2003). North American Tungsten began operations in the 1950s and remained fully operating until 1986, then reopened in 2001. Metal-rich mine tailings and nutrient-rich sewage are pumped into a series of 3 tailings ponds. Most of the effluent is pumped into tailings pond 3 (Figure 1.3A). Leachate enters the Flat River immediately adjacent to, and up to several hundred meters downstream of, the tailings ponds. However, there is limited information on how leachate enters the river which complicates assessment of effects on water quality and biota (Spencer et al., 2008). Mining activities at North American Tungsten are associated with elevated concentrations of Al, As, Cr, Cu, Fe, Pb, Mn and W and shifts in biological communities downstream of the tailings ponds [Spencer et al., 2008; Bowman et al., 2010; Scrimgeour, 2013; Thomas et al., 2013 (Chapter 4)].

Canadian Zinc Corporation has an advanced exploration mine (Prairie Creek mine) located along Prairie Creek (~45 km upstream of the confluence with the South Nahanni River) in the

eastern portion of the South Nahanni River watershed (61°33', 124°47'; Figure 1.2). Prairie Creek is underlain primarily by limestone and dolostone with mineralized veins containing zinc, lead, copper, and silver sulfides (Halliwell & Catto, 2003). Prairie Creek flows through canyons in the upper and lower reaches, which results in lower suspended sediments compared to other rivers in the South Nahanni River watershed (Halliwell & Catto, 2003). Measurements of flow taken from upstream of Virginia Falls ranges from 0.5 m^3/s in the winter to 30 m^3/s in the summer (EC, 1991). The Prairie Creek mine was started in the 1950's with a mill complex and tailings ponds constructed in the 1980s. Due to financial difficulties, the mine shut down in 1982 and was not re-started until 1991 when operation was taken over by Canadian Zinc Corporation which later began advanced exploration of Ag, Cu, Pb and Zn. All exfiltrate from exploration is pumped into a polishing pond; the overlying water from the polishing pond is drained into a catchment pond and then diverted directly into Prairie Creek via Harrison Creek (Figure 1.3B). Mining activities at Prairie Creek mine are associated with elevated concentrations of Al and Zn and shifts in biological communities such as macroinvertebrates and benthic algae adjacent to and downstream of the mine (Spencer et al., 2008; Bowman et al., 2010; Scrimgeour, 2013).

1.6 The use of algal communities for biomonitoring

Current monitoring programs for rivers and lakes typically rely on assessment of chemical and/or biological conditions. Measurements of water chemistry are easy to obtain; however, ability to detect long-term trends is limited by the spatial resolution and temporal frequency at which samples are collected. Water chemistry measurements do not provide direct information about changes occurring within the biotic communities. They also capture information over much shorter timescales compared to biota and thus may be less able to capture signals of

pulses of nutrients or contaminants that occur between sampling episodes. (Reavie et al., 2006; Lambert et al., 2008). Macroinvertebrates and fish communities are often used in biomonitoring programs, nevertheless they too have limitations. Fish move throughout their environment and thus may not reflect changes in physical and chemical conditions at the sampling site. They may not be plentiful in all ecosystems and thus sampling may be deleterious to the overall populations in an area. Fish also do not provide an early warning of possible impairment (Kilgour et al., 2007). Macroinvertebrates are more sedentary than fish; however, they do not necessarily track changes in all contaminants (e.g., nutrients; Resh, 2008).

Among the numerous aquatic ecosystem components that can be monitored (e.g., water chemistry, algae, macroinvertebrates, fish), benthic algae possess many features that predispose them to be effective sentinels of changes in water quality and ecological status of lakes and rivers caused by anthropogenic disturbances [(Reavie & Smol, 1998; Rott et al., 1998; Hill et al., 2000b; Leland & Porter 2000; Thomas et al., 2011 (Chapter 2 of this thesis), 2013 (Chapter 4)]. As primary producers, benthic algae play important roles in the structure and function of aquatic foodwebs (Sabater & Admiraal, 2005; Resh, 2008). Benthic algal communities are abundant, widespread, and diverse, and so require a relatively low amount of sampling effort during field collection to obtain useful ecological information (Biggs & Kilroy, 2000; Resh, 2008). They can rapidly assimilate pulses of nutrients due to their rapid potential growth rate, and, due to their short generation time, can respond quickly to changes in climate and anthropogenic disturbances. For these reasons, benthic algae are considered to be early warning indicators of environmental and ecological change (Sabater & Admiraal, 2005). Also, benthic algal communities accrue in aquatic systems over time periods spanning several weeks

to months, and so can store information about influential events and changes for substantial periods of time. To better understand the cumulative effects of climate change and anthropogenic alteration on aquatic ecosystems, it is essential that monitoring programs measure biotic metrics that track changes in the structure and functioning of the biological communities in relation to shifting physical and chemical conditions. Despite these features that suggest benthic algal communities possess distinct advantages compared to other biota, they are not widely used in bioassessments of lake and river conditions. Monitoring lower trophic levels can be more cost-effective and can serve as surrogates for ecological status of higher trophic levels (Kilgour et al., 2005; Rhea et al., 2006).

1.7 Objectives of study

The overall objectives of this study were to develop and assess the use of benthic algae for biomonitoring of water quality and ecological integrity in selected Canadian lakes and rivers. To do this, several different levels of analyses (or metrics) of benthic algal communities (e.g., visual assessments, biomass assessments, taxonomic assessments, and the novel approach of pigment quantification) were assessed for effectiveness as biomonitoring tools. The research in this thesis was conducted in two different areas of Canada. In the Muskoka-Haliburton area of Ontario, effects of shoreline development were assessed on water quality and benthic algal communities in the nearshore zones of lakes. In the South Nahanni River watershed, NWT, effects of industrial development were assessed on the water quality and benthic algal communities in rivers downstream of two mines.

In the Muskoka-Haliburton area of Ontario, we adapted and applied benthic algal biomonitoring protocols, developed originally in rivers, for use in the nearshore zone of lakes. The lakes possess generally good water quality (oligo- to meso-trophic), but recreational uses

and increasing shoreline development threaten to degrade environmental quality. Thus, development of effective nearshore biomonitoring protocols was considered a priority by the Ontario Ministry Of Environment (OMOE) for surveillance and protection of lakes with good water quality and recreational potential. The overall goal was to assess if benthic algal communities were affected by differences in shoreline development and if a range of benthic algal metrics could discriminate differences in sites located along a gradient of shoreline development in oligo- to meso-trophic Precambrian Shield lakes. Specifically, we examined and compared five levels of benthic algal bioassessment. Four of the levels were adapted from the National Institute of Water and Atmospheric research (NIWA) stream monitoring program (Biggs & Kilroy, 2000), and one, based on quantification of photosynthetic pigments by High-Performance Liquid Chromatography (HPLC), is new to agency-based biomonitoring. The five levels differed in the amount of time, effort, and expertise required for field and laboratory procedures.

In the South Nahanni River watershed, we used established benthic algal biomonitoring protocols for taxonomic assessments and HPLC analysis of algal pigments. The South Nahanni River watershed is considered to be pristine. However, two mining operations within the watershed pose a potential threat to downstream water quality within the Nahanni National Park Reserve. The goals of this study were to: 1) characterize the benthic algal communities at undisturbed reference sites across the watershed and their relationship with physical and chemical conditions of their surrounding environment; 2) assess the potential for the use of photosynthetic pigment concentration as a bioassessment tool compared to traditional taxonomic assessments; 3) develop a RCA model for benthic algal communities and apply it to assess if communities are altered, downstream of two mining companies.

1.8 Outline of the thesis

This thesis began as a study in partnership with the OMOE to assess biomonitoring tools for detecting effects of nearshore development on benthic algal communities. From there, it expanded into a larger project in partnership with Parks Canada Agency to assess biomonitoring tools for detecting effects of mining on benthic algal communities using the RCA. The overall objectives of this thesis are to develop, apply, and assess benthic algal biomonitoring protocols for use in the nearshore zone of lakes and rivers in Canada. This was done by assessing different levels of analysis (e.g., visual assessments, taxonomic assessments, and the novel approach of pigment quantification) of benthic algal communities for effectiveness as biomonitoring tools.

This thesis is divided into four 'data chapters' (chapter 2 – 5) which were prepared as independent articles for publication in scientific journals. Chapter 2 was published in the Journal of Lake and Reservoir Management (Thomas et al., 2011). Chapter 3 will be submitted to Hydrobiologia, after receiving feedback from the examination committee. Chapter 4 was published in Environmental Monitoring and Assessment in 2013 (Thomas et al., 2013), and Chapter 5 will be submitted to Freshwater Biology or Freshwater Science, after receiving feedback from the examination committee. The citation for each chapter is listed below:

Chapter 2: Thomas KE, Kluke A, Hall RI, Paterson AM, Winter JG. 2011. Assessment of benthic algal biomonitoring protocols to evaluate effects of shoreline development on the nearshore zone of Precambrian Shield lakes in Ontario. *Lake and Reservoir Management,* 27, 398-413. DOI 10.1080/07438141.211.633307.

Chapter 3: Thomas KE, Hall RI, Scrimgeour GJ. Relations between limnological conditions and composition of benthic algal communities in the South Nahanni River watershed, NWT (Canada): defining the reference condition. *Hydrobiologia*. In preparation.

Chapter 4: Thomas KE, Hall RI, Scrimgeour GJ. 2013. Evaluating the use of algal pigments to assess the biological condition of streams. *Environmental Assessment and Monitoring*, 185(9), 7895-7913. DOI 10.1007/s10661-013-3143-1.

Chapter 5: Thomas KE, Hall RI, Scrimgeour GJ. Development of a benthic algal reference condition model to assess ecological integrity within the South Nahanni River watershed. *Freshwater Biology* or *Freshwater Science*. In preparation.

In Chapter 2, a spatial survey of 28 sites in 2006 and 29 sites in 2007 within five lakes in the Muskoka-Haliburton area of Ontario was employed to develop and assess the ability of benthic algal bioassessment protocols to detect differences in shoreline development at sites within Precambrian-Shield lakes. In this chapter, I assessed 4 levels of benthic algal bioassessment. Level 1 involved visual descriptions of algal cover. Level 2 entailed biomass measurements (Chl-*a*, ash-free dry mass). Level 3 involved enumeration of algae to a coarse taxonomic level (i.e., the major algal classes). Level 4 involved quantification of photosynthetic pigments by High-Performance Liquid Chromatography (HPLC). Level 5 involved high taxonomic resolution enumeration of diatom communities. This chapter established a 'best practice' for benthic algal biomonitoring in Precambrian-Shield lakes. As well, the knowledge gained from this study is being used in an ongoing project on the bioassessment of cumulative effects on nearshore periphyton communities within the Muskoka River watershed. Chapter 2 was based

on the published paper; however minor changes were made to the terminology to be consistent throughout the thesis. Original water chemistry data is located in Appendix A.

In Chapter 3 a double stratified random sampling design was used to select 44 reference sites in 2008 and 18 reference sites in 2009 (12 repeated sampling from 2008, 6 newly sampled in 2009) from across the South Nahanni River watershed, NWT that lack proximate human activities. In this chapter, the physical and chemical conditions across the watershed were assessed and related to the benthic algal communities. The merits of 3 levels of benthic algal communities (benthic algal community composition, diatom community composition, and photosynthetic pigment concentration) for bioassessment, specifically their relative sensitivity to the physical and chemical variables was also assessed. This study provided important baseline information about the benthic algal communities in light of increasing industrial development within the South Nahanni River watershed. Original water chemistry data is located in Appendix B.

Chapter 4 used a gradient design to assess how benthic algal communities along a 10.5 km stretch of the Flat River, were affected by a fully-operating Tungsten mine (Cantung mine, NWT). This study compared the sensitivity of the more traditional benthic algal taxonomic assessments (to Class or Family level) with quantification of photosynthetic pigment concentration as a biomonitoring approach. The relative costs of each method were assessed and recommendations were made based on the relative merits of the methods. The results of this study will help establish quantification of photosynthetic pigment concentration as a viable biomonitoring tool for river monitoring downstream of metal mines in Canada. This chapter was based on the published paper; however, minor changes have been made to the terminology to be consistent throughout the thesis.

In Chapter 5, a double-stratified random sampling design, applied to the South Nahanni River watershed, was used to select 44 reference sites in 2008 and 18 reference sites in 2009 (12 repeated sampling from 2008, 6 newly sampled in 2009). Reference sites were located upstream of two mining companies and in other streams located throughout the watershed. Reference sites were comparable to test sites downstream of 2 mining companies in the South Nahanni River watershed sites (n = 13 and n = 8 along Flat River and Prairie Creek respectively). Reference condition approach models were created for each of three algal metrics (benthic algal community composition, diatom community composition and photosynthetic pigment concentration) and used to evaluate the ecological health of streams downstream of the two mining companies. The three RCA models and resulting test site assessments were used to make comparisons of the ability of the three algal metrics to reflect changes in water chemistry.

1.9 Major contributions of contributing authors

Chapters	Contributing Authors
Chapter 2	
Idea and planning:	KE Thomas, A Kluke, RI Hall, AM Paterson, JG Winter
Field work:	KE Thomas, A Kluke, RI Hall
Laboratory analyses:	KE Thomas, A Kluke, except water chemistry samples which were analyzed at the OMOE's Dorset Environmental Science Center
Data analysis and Figures:	KE Thomas
Writing:	KE Thomas (body of the text), RI Hall, AM Paterson, JG Winter (comments and assistance with text)
Chapter 3	
Idea and planning:	KE Thomas, RI Hall, GJ Scrimgeour
Field work:	KE Thomas, GJ Scrimgeour

Chapters	Contributing Authors
Laboratory analyses:	KE Thomas, except water chemistry samples which were analyzed at Environment Canada's National Laboratory for Environmental Testing, Burlington, Ontario and physical data which was provided by Parks Canada Agency.
Data analysis and Figures:	KE Thomas
Writing:	KE Thomas (body of the text), RI Hall, GJ Scrimgeour (comments and assistance with text)
<u>Chapter 4</u>	
Idea and planning:	KE Thomas, RI Hall, GJ Scrimgeour
Field work:	KE Thomas, GJ Scrimgeour
Laboratory analyses:	KE Thomas, except water chemistry samples which were analyzed at Environment Canada's National Laboratory for Environmental Testing, Burlington, Ontario and physical data which was provided by Parks Canada Agency.
Data analysis and Figures:	KE Thomas
Writing:	KE Thomas (body of the text), RI Hall, GJ Scrimgeour (comments and assistance with text)
<u>Chapter 5</u>	
Idea and planning:	KE Thomas, RI Hall, GJ Scrimgeour
Field work:	KE Thomas, GJ Scrimgeour
Laboratory analyses:	KE Thomas, except water chemistry samples which were analyzed at Environment Canada's National Laboratory for Environmental Testing, Burlington, Ontario and physical data which was provided by Parks Canada Agency.
Data analysis and Figures:	KE Thomas
Writing:	KE Thomas (body of the text), RI Hall, GJ Scrimgeour (comments and assistance with text)

1.10 Figures

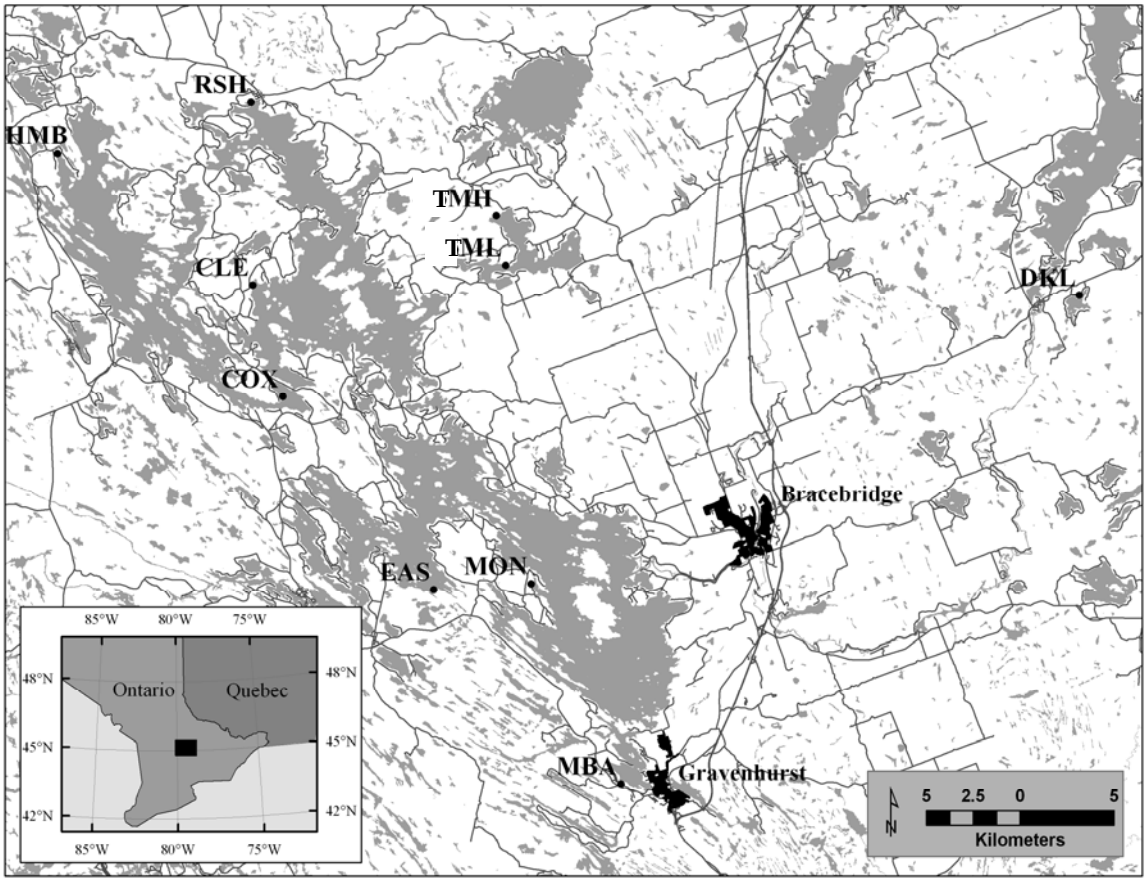


Figure 1.1 Map showing the location of the study area in south-central Ontario and the approximate locations of the study sites (n = 29) in the 5 study lakes.

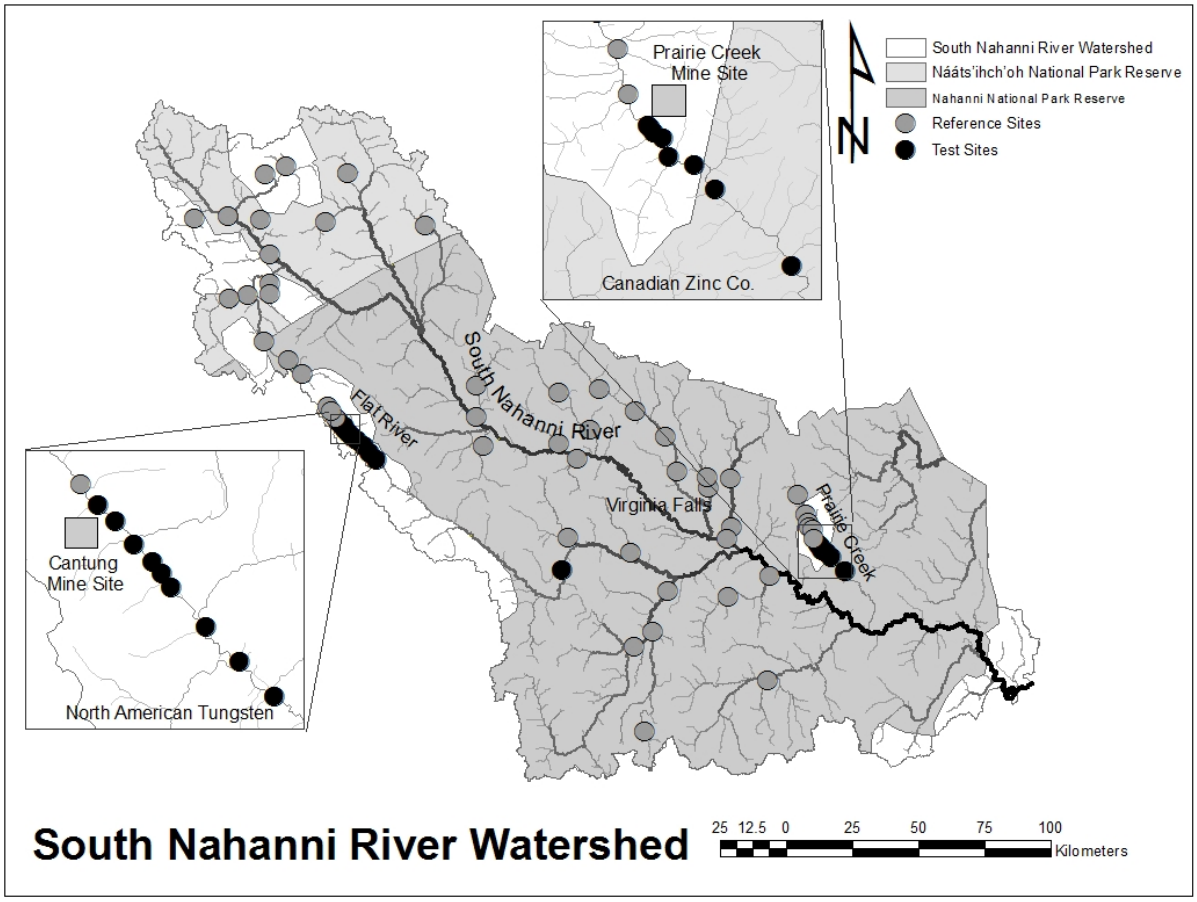


Figure 1.2 Location of study sites within the South Nahanni River watershed, Northwest Territories, Canada. A total of 44 reference sites (grey) were selected in 2008 and 18 reference sites (grey) in 2009 (12 repeated sampling from 2008; 6 newly sampled in 2009) and 20 test sites (black) were sampled between 2008 and 2009. Inserts show sites downstream of two mining companies, North American Tungsten (Cantung mine) and Canadian Zinc Corporation (Prairie Creek mine).

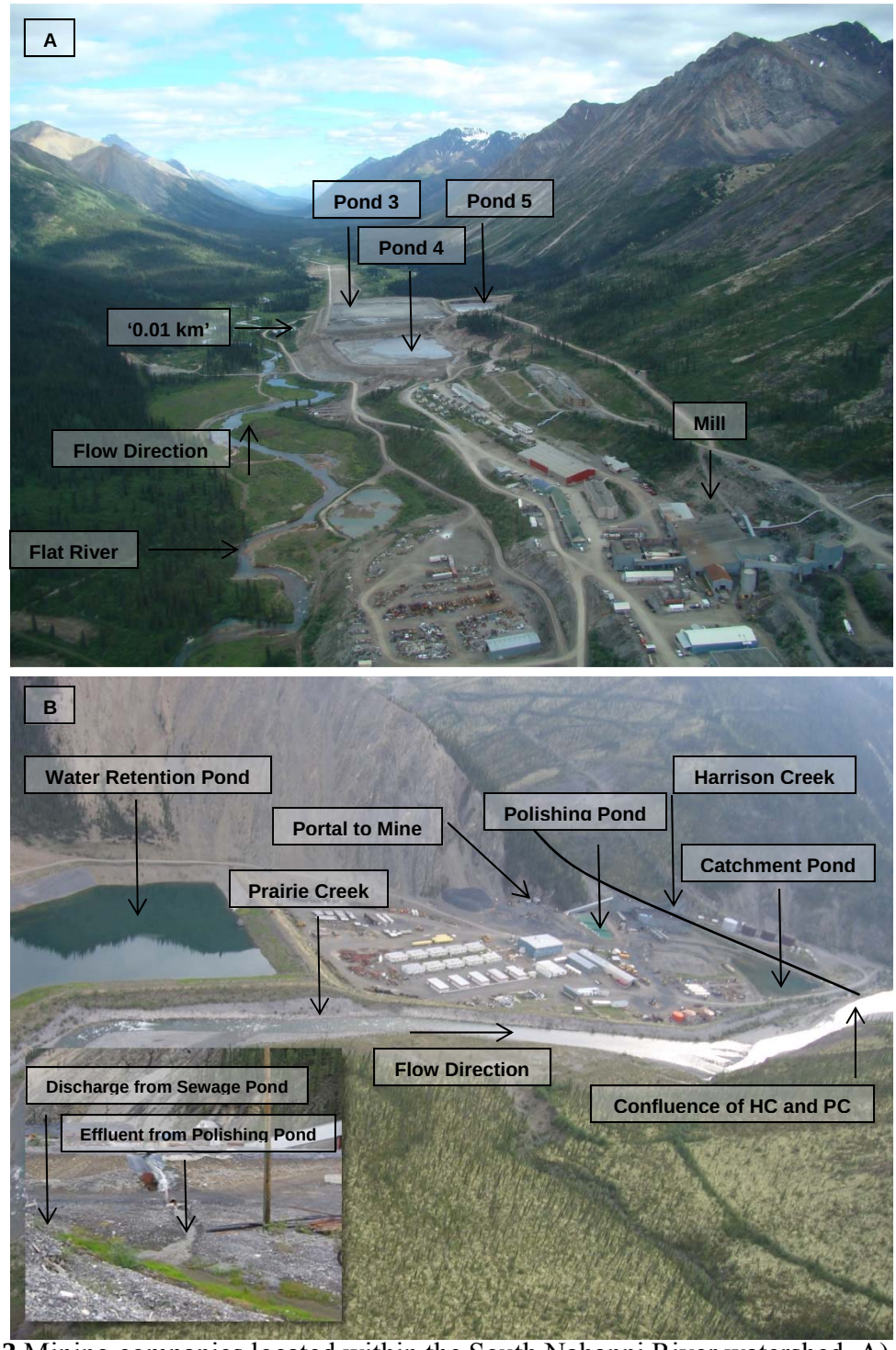


Figure 1.3 Mining companies located within the South Nahanni River watershed. A) North American Tungsten, Cantung mine located along Flat River showing location of site 0.01 km along Flat River, tailings ponds, mill and Flat River (Photo Dana Haggarty). B) Canadian Zinc Corporation, Prairie Creek mine located along Prairie Creek, showing location of confluence of Harrison Creek (HC) and Prairie Creek (PC), polishing and catchment ponds and mill.

Chapter 2

Assessment of benthic algal biomonitoring protocols to evaluate effects of shoreline development on the nearshore zone of Precambrian Shield lakes in Ontario

2.1 Overview

We assessed the ability of benthic algal biomonitoring protocols, for use in the littoral zone of oligo- to meso-trophic Precambrian Shield lakes in south-central Ontario, to detect effects of differences in shoreline development. The study sites (n = 28 in Aug. 2006, n = 29 in Aug. 2007) spanned a broad gradient of shoreline development (e.g., intact forest, cottages, marinas) but a modest gradient of nutrient concentration (3-22 µg/L TP). Each site was sampled for water chemistry (nutrients, ions, metals, pH) and 5 levels of benthic algal bioassessment, differing in the amount of time, resources and expertise required. Level 1 involved visual descriptions of algal cover. Level 2 involved biomass measurements (Chl-*a*, ash-free dry mass). Level 3 involved enumeration of algae to a coarse taxonomic level (i.e., to class or order level). Level 4 involved quantification of photosynthetic pigments by High-Performance Liquid Chromatography. Level 5 involved high taxonomic resolution enumeration of diatom communities. Multi- and uni-variate numerical analyses (e.g., PCA, ANOSIM, ANOVA) were used to assess relationships between measurements of shoreline development, water chemistry, and benthic algal metrics. Results identified that Level 5 was the most sensitive to track differences in the shoreline development among sites. For lakes on the Precambrian Shield, we suggest that benthic algal biomonitoring programs focus on Level 5, despite the higher requirements of time and technical skill/training. We further recommend that the other levels of bioassessment be explored further in other regions where broader gradients of shoreline development and lake trophic status exist.

2.2 Introduction

As human development increases, so too does the need to improve scientific methods to detect and quantify degradation of water quality and ecological integrity. This study was designed in partnership with the Ontario Ministry of Environment (OMOE) to provide a scientific foundation for a long-term, nearshore biomonitoring program based on benthic algae. To date, monitoring programs in lakes have commonly focused on characterizing the chemical and biological conditions (e.g., water chemistry, chlorophyll-*a* concentration, phytoplankton and zooplankton metrics) at a central, open-water (or, pelagic) location (King et al., 2006). There has been relatively little emphasis on monitoring conditions in the littoral zone of lakes based on benthic algae, despite the fact that the littoral zone is often the first to receive influences of human activities and that benthic algae often contribute importantly to primary production in lakes (Vadeboncoeur et al., 2001, 2003).

The ability to detect long-term trends in chemical water quality is often limited by the spatial resolution and temporal frequency of sample collection. For example, water samples are commonly collected at widely spaced intervals in time, such as monthly, annually, or even less frequently (Reavie et al., 2006). Furthermore, water chemistry samples do not provide direct information about changes occurring in biotic communities, though laws and policies that protect water quality are commonly based on the underlying principle that they will minimize undesirable biological changes (Loeb, 1994; Reavie et al., 2006; Lambert et al., 2008).

Benthic algae (or, periphyton) are potentially useful biomonitors. They live attached to substrata in the littoral zone of water bodies (Graham & Wilcox, 2000), and they are abundant in most aquatic systems (Vadeboncoeur et al., 2001, 2003), a feature that ensures sufficient sample size can be obtained efficiently (Biggs & Kilroy, 2000; Resh, 2008). Benthic algae play

important roles in the structure and function of aquatic foodwebs (Resh, 2008), and they respond rapidly to environmental changes. Their taxa possess well-defined growth optima along environmental gradients, and they integrate water-quality information over useful time scales (weeks to months; Biggs & Kilroy, 2000; Lavoie et al., 2008a; Resh, 2008). Moreover, benthic algae are often the first biological group to intercept nutrients delivered from adjacent lands (Hansson, 1988) and, therefore, may respond quickly and sensitively to shoreline development. Importantly, biota can rapidly assimilate pulses of nutrients (e.g., after precipitation events) and maintain a "record" of their effects. In contrast, spot water chemistry measurements may be less likely to capture these signals, because waves and currents dilute pulsed nutrients with offshore waters. Thus, assessment of benthic algae in the littoral zone may provide an early warning of degradation due to human activities (Jacoby et al., 1991; Poulíčková et al., 2004). As presented below, long-term monitoring programs that collect and analyze benthic algae have the potential to complement many of the shortcomings of programs based on water sampling alone.

The past 20 years have seen pronounced development of benthic algal biomonitoring protocols for use in rivers [e.g., Europe (Comité Européen de Normalisation - CEN, 2003, 2004), New Zealand (National Institute of Water and Atmospheric Research - NIWA; Biggs & Kilroy, 2000) and the USA (Environmental Protection Agency - EPA; Stephenson & Bahls, 1999). These protocols provide useful and sensitive measures of changes due to human activities in watersheds of rivers (Biggs & Kilroy, 2000). However, benthic algal biomonitoring protocols have not yet been widely adapted for use in lakes. A few studies have employed relatively crude levels of assessment, such as estimates of biomass [e.g., chlorophyll-*a* concentration (chl-*a*) and ash-free dry mass (AFDM)], but standardized

protocols have not yet been developed for lakes in Canada (King et al., 2006; Lambert & Cattaneo, 2008; Lambert et al., 2008; Rosenberger et al., 2008).

Here, we adapted and applied benthic algal biomonitoring protocols, developed originally in rivers, for use in the nearshore zone of lakes located on the Precambrian Shield in south-central Ontario. The lakes possess generally good water quality (oligo- to meso-trophic), but recreational uses and increasing shoreline development threaten to degrade environmental quality. Thus, development of effective nearshore biomonitoring protocols was considered a priority by the OMOE for surveillance and protection of lakes with good water quality and recreational potential. Our overall goal was to assess if benthic algal communities were affected by differences in shoreline development and if a range of benthic algal metrics could discriminate differences in the algal communities along a gradient of shoreline development in oligo- to meso-trophic Precambrian Shield lakes. Specifically, we examined and compared 5 different levels of benthic algal bioassessment, which are described in detail in the Methods section. Four of the levels were adapted from the NIWA stream monitoring program (Biggs & Kilroy, 2000), and one based on quantification of photosynthetic pigments by High-Performance Liquid Chromatography (HPLC; Level 4, below) is new to agency-based biomonitoring. The 5 levels differed in the amount of time, effort and expertise required for field and laboratory procedures. The specific questions addressed were: do water chemistry variables measured in the nearshore region of lakes differ among sites due to differences in the amount of shoreline development; do nearshore benthic algal metrics differ due to differences in the amount of shoreline development; and which levels of bioassessment are most effective at discriminating these differences.

2.3Methods

2.3.1 The five levels of benthic algal bioassessment

Level 1 bioassessment ("rapid visual assessment") characterized visual attributes (e.g., colour, texture, length of filaments, and thickness of mats) of the benthic algal mats, recorded as percent cover. Level 2 ("biomass assessment") estimated biomass using rapid and routine methods to quantify Chl-*a* concentration and AFDM. Level 3 ("benthic algal community composition") assessed benthic algal community composition to a coarse taxonomic level (e.g., class or order level, such as Bacillariophyta, Chroococcales, Nostocales). Level 4 ("photosynthetic pigment concentration") quantified concentrations and composition of photosynthetic pigments, and represents a relatively new, rapid, and time- and cost-effective method for benthic algal bioassessment. Level 5 ("diatom community composition") determined community composition of diatom algae to the finest taxonomic level possible (e.g., species, sub-species or variety).

2.3.2 Study sites

Samples were collected from lakes located in the Muskoka-Haliburton region of Ontario, a region which has received seasonal recreational use for many decades (Figure 2.1). The lakes are situated on the Precambrian Shield and are acid-sensitive, soft water and oligo- to meso-trophic in nature (Chapman & Putnam, 1984; Girard et al., 2006). Human development along the shorelines varied from minimal (forested and protected areas) to extensive (high-use resorts, golf courses and marinas; Table 2.1).

A total of 28 sites were sampled along shorelines of 5 lakes (Dickie Lake, Lake Joseph, Lake Muskoka, Lake Rosseau, and Three Mile Lake; Figure 2.1) in August 2006 and 2007 (with an additional site in 2007, n = 29). The sites were selected to span a range of type and

intensity of shoreline development. The type and amount of shoreline development, characterized along a 100-m long, 50-m wide riparian strip directly adjacent to the site, was recorded to assess the influence of terrestrial development on water chemistry and benthic algal metrics at each site. Each site was placed into 1 of 3 shoreline development categories: low (forested shoreline in pristine to sparsely-cottaged areas); medium (high density of cottages with developed shoreline; i.e., lawns, constructed beach or shoreline); or high (resorts, marinas, golf courses, and trailer parks; Table 2.1). Similar shoreline development categories have been applied successfully in biomonitoring studies (Lambert & Cattaneo, 2008; Lambert et al., 2008; Rosenberger et al., 2008). These categories were used in multi- and uni-variate analyses of the data to assess if water chemistry variables and benthic algal metrics differed among the shoreline development categories.

To assess possible differences in meteorological conditions between the sampling years (2006, 2007), seasonal and inter-annual variation in precipitation, temperature, and wind speed were compared relative to average climatic conditions (precipitation and temperature: 1939-2006; wind speed: 1996-2005), using data from Muskoka Airport (Bracebridge, ON). The Muskoka-Haliburton area of Ontario experienced average to slightly above average temperatures during 2006 and 2007. Monthly precipitation was above average in July of 2006 and 2007, while precipitation in August was half the normal amount in 2006 and average in 2007 (Figure 2.2). Daytime (5:00 am to 9:00 pm) hourly wind speed data were compiled during June to August for the years 1996-2005, and the 90^{th} (19 km/hr) and 95^{th} (22 km/hr) percentiles were calculated. The total number of hours that wind speeds exceeded the 90^{th} and 95^{th} percentiles was higher during the sampling period in 2007 (29 hrs, 11 hrs, respectively) than in 2006 (3 hrs, 0 hrs, respectively). However, the number of times wind speeds exceeded

the 90th and 95th percentiles did not differ markedly between the study years during the two-week period prior to field sampling (14 hrs, 4 hrs in 2006 versus 12 hrs, 1 hr in 2007, respectively).

2.3.3 Sample collection

At each site, samples were collected for analysis of water chemistry and benthic algae along a 9-m long transect positioned parallel to the shoreline at 40-60-cm water depth. Each transect was divided into 9 contiguous 1-m diameter circular plots in order to capture the variation within a site (or, transect; Figure 2.3).

2.3.3.1 Water chemistry and environmental variables

At each site, water samples (1 L) were collected from all 9 of the plots along a transect and pooled into a 9 L "site composite" sample. Water from the composite samples was filtered through an 80-µm Nitex mesh to remove zooplankton and other large particles, and then analyzed for a suite of chemical variables by the OMOE's Dorset Environmental Science Center following standard methods (OMOE, 1983; Janhurst, 1994). Water chemistry variables included concentrations of ions (Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, SO_4^{2-}), nutrients (ammonium/ammonia, nitrate/nitrite, TKN, and TP), reactive silicate (SiO_3^{2-}), dissolved organic carbon (DOC), metals (Al, Ba, Fe, Pb, Mn, Sr, and Zn), and colour, Gran alkalinity, pH, and conductivity. To assess if nearshore water chemistry conditions tracked differences in shoreline development or were strongly influenced by offshore waters, selected variables were compared between nearshore and offshore sites of each lake (data from pelagic sites was provided by OMOE). The light extinction coefficient of photosynthetically active radiation (Kd_{par}) was estimated in 2007 only using an Apogee Instruments Quantum meter (Model

QMSS-SUN). Three measurements of PAR were recorded at approximately 5-depth intervals per site and were used to calculate Kd_{par} following methods outlined in Wiklund et al., (2010).

2.3.3.2 Benthic algal sampling

Level 1 bioassessment (rapid visual assessment) was conducted at all 9 contiguous 1-m diameter plots along each transect. In contrast, samples for Levels 2-5 were collected at every second plot along the 9-m transect (plots 1, 3, 5, 7 and 9; Figure 2.3). We sampled benthic algae from cobbles or boulders wherever possible to reduce noise due to confounding influence of different substrate types. Sand substrates were sampled for analysis at 9 sites where cobbles and boulders were not present (2 sites at Three Mile Lake, 3 sites at Lake Rosseau, 2 sites at Lake Muskoka and 2 sites at Lake Joseph). Benthic algal samples were removed and collected from rock and cobble substrates using a 2.6-cm internal diameter syringe sampler fitted with a toothbrush head (used to dislodge the algae from the substrate) and an attached second syringe was used to obtain the dislodged algal slurry (Lobe, 1981). For the 9 sites that lacked cobbles or boulders, benthic algae were collected from sand using an inverted Petri dish (surface area = 25-cm^2) and spatula to obtain the top sediments (< 1-cm). At each plot within a transect (i.e., plots 1, 3, 5, 7, and 9; Figure 2.3), three "replicate samples" were obtained from distinct areas of the plot (i.e., from either three cobbles or three different sand samples) and were pooled into one 500 mL bottle. This comprised one "plot sample." At each site, these plot samples (five plots per transect) were diluted to a volume of 350 mL with distilled water. An aliquot of 20 mL was removed from each plot sample and pooled to create one site composite sample for each site (100 mL/site). Collection of algal samples for levels 2-5 bioassessment required approximately 1 h in the field.

2.3.3.3 Level 1: Rapid visual assessment

Rapid visual assessments were carried out by modifying protocols developed by NIWA for monitoring streams in New Zealand (Biggs & Kilroy, 2000). Laminated charts of benthic algal growth forms and colour (from Biggs & Kilroy, 2000) were taken into the field and used for rapid identification of benthic algal growth types based on visual appearance (colour, texture, filament length, mat thickness). Data for Level 1 were recorded as percent cover. Collection of the rapid visual assessment data required approximately 15 min at each site.

2.3.3.4 Level 2: Biomass assessment

Samples for determination of Chl-*a* concentration and AFDM of the benthic mats were obtained in 2007 by combining sub-samples (50 mL) from each of the five plot samples at a site into one composite sample (250 mL). These samples were then filtered onto GF/F glass fiber filters for Chl-*a* determination and onto pre-ashed and pre-weighed GF/C glass fiber filters for determination of AFDM. The filters were wrapped individually in aluminum foil and stored at -20 °C until analyzed. Analysis of Chl-*a* was performed by the OMOE, Dorset Environmental Science Center, while the AFDM samples were analyzed at the University of Waterloo, following standard protocols (Stainton et al., 1977; Biggs & Kilroy 2000).

2.3.3.5 Level 3: Benthic algal community composition

The samples for benthic algal community composition were prepared by sub-sampling 1.5 mL of well-mixed composite sample from each site into an Utermöhl chamber and diluting to 3 mL with deionized water. The benthic algae were allowed to settle for 24 h and were enumerated using an inverted microscope at 400x magnification. Approximately 300 organisms per sample were counted to a coarse taxonomic level (e.g., class and order such as, Bacillariophyta,

Chroococcales, Nostocales, Oedogoniales, Desmidiales) following nomenclature of Prescott (1951) and Wehr & Sheath (2003). Single-celled algae and cells in colonies were tallied as individual cells, while filaments consisting of multiple cells were tallied as one organism or "count," following methods of Biggs & Kilroy (2000). Counts were completed rapidly (within 1-2 h) with a relatively low level of initial training (e.g., 1-3 d).

2.3.3.6 Level 4: Photosynthetic pigment concentration

Samples for the analysis of photosynthetic pigment concentration were obtained by combining sub-samples (50 mL) from each of the five plot samples at a site into one composite sample (250 mL). The samples were then filtered onto GF/F glass fiber filters, wrapped in aluminum foil, and stored at -20°C until analysis at the University of Waterloo. Filtered samples for HPLC analysis were extracted in a mixture of acetone:methanol:water (80:15:5 by volume) for 24 h in the dark at -20°C. Samples were then filtered (0.22 µm PTFE syringe filter) to remove the filter paper and other impurities, dried under inert gas (N_2) and re-eluted in injection solution consisting of an acetone:ion pairing reagent:methanol solution (70:25:5 by volume). The Ion-Pairing Reagent (IPR) solution consisted of 0.75 g tetrabutylammonium acetate and 7.7 g ammonium acetate in 100 mL of water. The re-eluted samples (500 µL) were injected into a Waters HPLC, which ran on a reverse-phase procedure following the methods of Leavitt et al., (1989) as modified from Mantoura & Lleywellyn (1983), with a Waters 2998 PhotoDiod Array (PDA) detector, Waters 2475 Multi λ fluorescence detector, and a Symmetry C18 column (3.5 µm, 4.6 x 75 mm). The separation of the pigments was through a gradient delivery of mobile phase A and B [mobile phase A: methanol and IPR (90:10 by volume); mobile phase B: methanol and acetone (73:27 by volume)]. The column was equilibrated and calibrated for 10 minutes prior to the first sample injection of each run. Sudan II, calibration solution and a

geranium sample standard were analyzed at the beginning and end of each run to account for any noise or shift in the pigment peaks during the run. Sudan II was also run as an internal standard in each sample of algal pigments to account for any dilution and injection errors (Leavitt & Findlay, 1994). Pigments were identified by comparing the spectral characteristics of each pigment to standards from Jeffery et al., (1997), as well as by the chromatographic mobility of the pigments (i.e., elution time; Leavitt et al., 1989). Data were expressed as nMoles of pigment per cm^2 of substrate.

2.3.3.7 Level 5: Diatom community composition

The diatom community composition consisted of counting benthic diatom algae to the finest possible level (e.g., species, subspecies or variety). Diatoms were chosen in accordance with established NIWA protocols (Biggs & Kilroy, 2000). Also, prepared microscope slides for diatom enumeration preserve for many decades or longer, and so provide a useful archive for long-term monitoring programs (Smol & Stoermer, 2010). Samples for high-resolution diatom counts were prepared by measuring 15 mL of each sample preserved with Lugol's solution into individual test tubes, allowing the algae and other materials to settle to the bottom for 24 h before removing two-thirds of the supernatant and replacing it with de-ionized water. This sequence of settling for 24 h, followed by removal of supernatant and replacement with de-ionized water, was repeated until all the Lugol's preservative was removed. The samples were then oxidized using 30% hydrogen peroxide at room temperature for 1 week. Acid residues were removed by rinsing repeatedly with deionized water after diatom valves were allowed to settle for 24 h, until a neutral pH was reached. The resulting cleaned slurries of diatoms in water were dried onto circular coverslips and mounted onto microscope slides with Naphrax mounting medium. Approximately 300-500 diatom valves were counted per sample using a

compound light microscope at 1000x magnification (Zeiss Axioskop 2Plus, numerical aperture = 1.30). Taxonomic identifications relied upon Krammer & Lange-Bertalot (1986-1991) and Lavoie et al., (2008b). Data were expressed as taxon relative (%) abundances of the total diatom sum. Diatom analyses required 2-5 h per sample, and a relatively high level of initial training (~1 mo).

2.3.4 Numerical analyses

Principal Components Analysis (PCA) of the water chemistry data was used to explore the main patterns of differences in chemical conditions among the study sites. Analysis of Similarities (ANOSIM) tests were used to: 1) determine if water chemistry conditions differed significantly among the shoreline development categories, and 2) determine if water chemistry conditions differed among the study lakes where each site was located (i.e., Dickie Lake, Lakes Rosseau, Joseph and Muskoka, and Three Mile Lake). All ANOSIM tests were performed using the software PRIMER version 6. Prior to analysis, all non-normal water chemistry data were ln(x+1) transformed. As well, prior to ANOSIM tests, water chemistry variables were standardized and then matrices were calculated based on Euclidean distances. Separate numerical and statistical analyses were carried out for the 2006 and 2007 field seasons. PCA ordinations were performed using CANOCO version 4.5 software.

Paired t-tests were performed on selected water chemistry variables to assess if nearshore and offshore conditions differed in the lakes. For the paired t-tests, nearshore values were obtained by averaging the values for all nearshore sites within each lake (i.e., Dickie Lake, Lakes Joseph, Rosseau and Muskoka, and Three Mile Lake). Three Mile Lake consists of 2 distinct embayments (Main basin and Hammell's Bay) that differ in offshore water chemistry conditions, and consequently the embayments were considered as distinct sites in the paired t-

tests. To compare with the nearshore data collected in August of 2006 and 2007, available data from the end of July to the end of August were averaged from the offshore sites in both study years. However, water chemistry data were collected only in the spring of 2006 or 2007 at offshore sites of Lakes Joseph, Rosseau, and Muskoka (Lake Muskoka = 2006 and Lakes Joseph and Rosseau = 2007), and we used these data for those sites.

Benthic algal bioassessment levels 1, 3, 4 and 5 were analyzed using PCA ordinations to explore the main patterns of difference in community composition among sites. PCA ordinations were performed using CANOCO 4.5. One-way ANOSIM tests of significance were used to determine if community composition differed significantly among shoreline development categories. The one-way ANOSIM analyses were based on Bray-Curtis similarity matrices calculated using PRIMER version 6. Prior to all analyses, the biological data were square-root (Levels 1, 3, and 5) or ln(x+1) (Level 4) transformed to down-weight the influence of the most abundant taxa and to equalize variances. Analyses of the high taxonomic resolution algal counts used only taxa with ≥ 1% relative abundance in at least 1 sample. For all lakes, separate analyses were performed using data from 2006 and 2007.

In addition to the multivariate analyses described above, mat thickness, a metric arising from the rapid visual benthic algal assessment (Level 1), was analyzed independently across all plot samples using a Chi-squared Goodness of Fit test. The purpose was to assess if the frequency of visually determined benthic algal mat thickness categories (Thick: > 3 mm, Medium: 0.5-3 mm, Thin: < 0.5 mm) differed among shoreline development categories. Mat thickness was used because a previous study had indicated that it was affected by differences in shoreline development (Lambert & Cattaneo, 2008). The Chi-squared test was performed using the software SPSS version 16.0.

Level 2 data (Chl-*a* and AFDM) were analyzed to test if biomass of benthic algal mats differed among the shoreline development categories. Chl-*a* and AFDM datasets were analyzed using one-way ANOVA tests on log(x+1) transformed data. Bonferroni post-hoc tests were used to determine which of the shoreline development categories differed significantly in mean Chl-*a* content. For all statistical tests, significance was assessed at alpha = 0.1.

2.4 Results

2.4.1 Water chemistry

Ordination by PCA showed that water chemistry conditions did not differ markedly among the three different shoreline development categories in either of the study years (2006, 2007; Figure 2.4). Axis 1 of the PCA ordination for 2006 captured 96.4% of the total variation and separated study sites mainly due to differences in concentrations of ions, alkalinity, and pH. The second axis captured 1.4% of the variation and separated sites along a gradient of water colour and concentrations of DOC, nutrients (i.e., nitrate/nitrite, TKN, TP), Fe, and Mn. Despite the high proportion of variance explained by the first 2 PCA axes (97.8%), sample scores were not well separated according to the different shoreline development categories. Similar patterns were apparent in a comparable analysis of water chemistry data from 2007, though axes 1 and 2 explained different amounts of variation compared to 2006 (Figure 2.4). In 2007, measurements of Kd_{par} were made, and the PCA ordination identified strong correlation of Kd_{par} with DOC concentration.

Interestingly, one-way ANOSIM tests identified that water chemistry conditions differed significantly among the five study lakes (Global R = 0.57, p-value < 0.01), but did not differ significantly among the three categories of shoreline development (Global R = -0.02, p-value = 0.57; Table 2.2). These findings were consistent in both study years (2006, 2007). Paired t-tests

of select water chemistry variables from nearshore and offshore sites confirmed these findings (Table 2.3). While water chemistry variables such as alkalinity, conductivity, DOC, and TKN did not differ significantly between nearshore and offshore sites, they were consistently lower or higher between the locations (i.e., −t value or +t value respectively) in both study years. Measures of pH were significantly different between nearshore and offshore sites in both study years, however, in 2006 the nearshore sites had lower pH and in 2007 they had higher pH than offshore sites. Concentrations of TP and SiO_3 were significantly different between nearshore and offshore sites in one of the two study years, but not consistently between years.

2.4.2 Level 1: Rapid visual assessment

In both study years, percent cover estimates from rapid visual assessment of benthic algal cover did not discriminate effectively among the 3 categories of shoreline development, as assessed by PCA ordination (Figure 2.5). Axis 1 of the PCA ordination from 2006 captured 41.0% of the total variation and separated study sites with relatively high cover of medium algal mats (positioned to the left) from those with relatively high cover of thin mats, colonies, and filaments (positioned to the right). The second axis captured 18.3% of the variation and separated sites with predominantly thick algal mats from all other sites. Despite the high amount of variation explained (59.3%), sample scores were not well separated according to the different shoreline development categories. Similar patterns recurred in the PCA ordination plot based on visual assessment in 2007. One-way ANOSIM tests of the rapid visual assessment data confirmed these results by identifying that differences among the shoreline development categories were not statistically significant during 2006 or 2007, except for the comparison of high and low shoreline development categories in 2006 only ($p < 0.1$; Table

2.4). Thus, the distribution of algal growth forms among plot samples at the study sites does not form a coherent, interpretable pattern along the gradient of shoreline development.

Chi-square goodness of fit tests identified that the frequency distribution of thickness categories of algal mats, based on rapid visual assessment, differed significantly among the shoreline development categories (2006: Chi-square = 36.29, $p = 2.53 \times 10^{-7}$, d.f. = 4; 2007: Chi-square = 67.70 , $p = 6.93 \times 10^{-14}$, d.f. = 4). However, the patterns of difference did not appear to provide a useful basis for discriminating the shoreline development categories in both study years (Figure 2.6). In 2006, 'thin' mats (< 0.5 mm) were more frequent at sites in the low shoreline development category than at sites in the medium and high shoreline development categories, 'medium' mats (0.5-3 mm) were most frequent at sites in the medium shoreline development category, and 'thick' mats (> 3 mm) were most common at sites with high shoreline development. In 2007, the 'thin' and 'medium' mats followed a similar trend as in 2006, however, the 'thick' mats were absent from the high shoreline development category. Only thin algal mats exhibited a consistent frequency distribution among the shoreline development categories in both study years, and they were most abundant at sites with low shoreline development but also moderately abundant at sites with high shoreline development. Thus, the distribution of mat thickness types did not form a coherent, interpretable pattern along the gradient of shoreline development in either study year.

2.4.3 Level 2: Biomass assessment

Estimates of benthic algal biomass were obtained in August of 2007 as AFDM and Chl-*a*. AFDM did not differ significantly among the shoreline development categories (AFDM; one-way ANOVA: F = 0.45, p = 0.64, d.f. = 2, 26). Chl-*a* did differ significantly among the shoreline development categories (Chl-*a*: one-way ANOVA: F = 6.38, $p < 0.01$, d.f. = 2, 26),

but did not show an interpretable pattern along a gradient of shoreline development. Specifically, Chl-*a* concentrations differed significantly between sites in low- versus medium-shoreline development categories and between sites in medium- versus high-shoreline development categories, based on Bonferroni post-hoc tests ($p < 0.05$), but not between sites in low- versus high- shoreline development categories ($p = 1.0$).

2.4.4 Level 3: Benthic algal community composition

Sample scores were not well separated according to the 3 shoreline development categories in a PCA ordination of the benthic algal community composition data from 2006 (Figure 2.7). Axis 1 captured 71.0% of the total variation and separated sites with high relative abundance of diatoms (positioned to the right) from those dominated by cyanobacteria (Chroococcales; positioned to the left). The second axis captured 14.1% of the total variation and identified sites with high relative abundance of cyanobacteria (Nostocales, Oscillatoriales; positioned high on axis 2) and green algae (Oedogoniales and Zygnematales). Similar patterns were evident in the PCA ordination plot based on the data obtained in 2007, but sample scores based on the 2007 data were able to discriminate to some extent among the shoreline development categories (Figure 2.7). Specifically, sample scores from sites with high shoreline development were positioned low on axis 2, and generally were separated from sites in the other shoreline development categories and associated with lower abundance of Nostocales and higher abundance of Zygnematales. Accordingly, one-way ANOSIM tests identified that benthic algal community composition did not differ significantly among the shoreline development categories in 2006, but did differ significantly in 2007 (Table 2.4). In 2007, community composition differed significantly between the high- and low-shoreline development categories

and between the medium- and low-shoreline development categories, but not between the medium- and high-shoreline development categories.

2.4.5 Level 4: Photosynthetic pigment concentration

Samples collected in 2007 were analyzed for concentration and composition of photosynthetic pigments. Ordination of the data by PCA showed considerable overlap of sample scores from sites in the different shoreline development categories, mainly because sample scores from sites with low shoreline development were dispersed throughout the ordination space (Figure 2.8). Sites with high- and medium-shoreline development were generally well separated. Axis 1 of the PCA ordination explained 48.3% of the total variance and captured mainly a gradient of concentrations of all pigments except those from cyanobacteria. Sites with low pigment concentrations (many of the sites with high shoreline development and some of the low shoreline development sites) were positioned to the left along axis 1, whereas sites with high pigment concentrations (many of the sites with medium shoreline development and some of the low shoreline development sites) were positioned to the right. Axis 2 captured 21.1% of the variance, and sites with high concentrations of cyanobacterial pigments (i.e., myxoxanthophyll and aphanizophyll) were positioned high on axis 2. A one-way ANOSIM test identified that composition and concentration of pigments differed significantly between the sites in the high shoreline development category and those in the low- and medium-shoreline development categories ($p < 0.1$; Table 2.4).

2.4.6 Level 5: Diatom community composition

Compared to the other levels of benthic algal bioassessment, PCA ordination of the diatom community composition data resulted in better separation of site scores according to their

shoreline development categories, both for 2006 and 2007 (Figure 2.9). Despite a moderate amount of overlap, sites with high shoreline development were generally positioned to the right along PCA axis 1 in 2006, whereas sites with medium- to low-shoreline development were positioned to the left. Sites with high shoreline development tended to possess high relative abundance of *Achnanthes hungarica*, *Aulacoseira ambigua*, *Navicula notha*, *Nitzschia palea*, and *Staurosirella pinnata*, among others. Sites with low shoreline development tended to possess high relative abundance of *Anomoeoneis vitrea*, *Cymbella descripta*, *Cymbella laevis*, and *Fragilaria crotonensis*, among others. A PCA ordination of the 2007 data showed similar patterns. Diatom community composition was distinctive in Three Mile Lake (positioned to the right along PCA axis 1) due to high relative abundance of taxa belonging to *Achnanthes*, *Fragilaria*, *Staurosira*, and *Staurosirella*, and in Dickie Lake (positioned high on axis 2 in 2006 and low on axis 2 in 2007) due to high relative abundance of taxa belonging to *Eunotia* and *Nitzschia*. One-way ANOSIM tests identified that community composition, based on high taxonomic resolution diatom counts, differed significantly between sites with low shoreline development and those with medium- and high-shoreline development, for both study years ($p < 0.1$; Table 2.4).

2.5 Discussion

Nearshore water chemistry conditions did not differ among the shoreline development categories. Instead, our results show that nearshore water chemistry conditions closely matched those at offshore locations and consistently distinguished differences among lakes. In contrast, benthic algal assessment at Level 5 (high taxonomic resolution diatom counts) could detect differences in the benthic algal communities among shoreline development categories in these oligo- to meso-trophic Precambrian Shield lakes, especially between highly developed (resorts,

golf courses, marinas) and relatively undeveloped shorelines (forested, sparse seasonal cottages). This finding is consistent with other studies which have shown that the abundance and community composition of benthic algae reflect shoreline development, whereas snapshot samples of nearshore water quality are strongly influenced by exchange with open water conditions (Lambert et al., 2008; Rosenberger et al., 2008).

It is well known that increases in nutrient supplies can result in shifts in algal community composition from diatoms to those dominated by green algae and cyanobacteria, via degradation of water quality and changes to food webs (Rosenberger et al., 2008). Consequently, we anticipated that all five levels of benthic algal bioassessment would be able to track, to varying degrees, differences in the benthic algal communities due to differences in shoreline development among sites in this study, which included lakes that range from oligo- to meso-trophic. Interestingly, biomass assessment and coarse taxonomic level assessment (rapid algal counts) did not provide data of sufficient sensitivity to discriminate among the shoreline development categories, and results were inconsistent between the study years.

Lambert & Cattaneo (2008) suggested that the thickness of algal mats and biomass measurements (i.e., Chl-*a* content) can be used to discriminate between developed and undeveloped shorelines, but our results suggest that these metrics are not sufficiently sensitive for use in oligo- to meso-trophic lakes, at least not based on the gradient of shoreline development typical of Precambrian Shield lakes in South-Central Ontario. The thickness of algal mat cover showed modest potential. For example, in 2006, thin mats were most frequent at sites with low shoreline development, medium mats were most common at sites in the low- and medium- shoreline development categories, and thick mats were most common at sites in the high shoreline development category and absent from the low shoreline development

category - a pattern consistent with the gradient of nutrient availability. However, this pattern was not repeated in 2007, and the lack of a consistent inter-annual trend in mat thickness along the gradient of shoreline development categories makes it a problematic metric to rely on for a provincial biomonitoring program. The sampling season of 2007 was characterized by a higher frequency of strong winds compared to 2006, with 29 hours exceeding the 90^{th} percentile of daytime wind speeds for the 1996-2005 decadal dataset and 11 hours exceeding the 95^{th} percentile in 2007 compared to 3 hours and 0 hours in 2006. Also, more rain fell in 2007 (comparable to the long-term average) than in 2006 (half the long-term average). The higher amount of wind and rain in 2007 suggest more storms and associated wave action in that year. Stronger wave action in 2007 could have resulted in the preferential removal of the thickest algal mats, but this factor alone likely cannot account for differences observed between 2006 and 2007 in frequency distributions of the algal mat thickness types, because the frequency of thick mats increased in 2007 (relative to 2006) at sites in the low shoreline development category but decreased at sites in the high shoreline development category.

Based on findings by Rosenberger et al., (2008), we anticipated that benthic algal community composition data (Level 3) and photosynthetic pigment concentration (Level 4) would be able to provide complementary, cost-effective and informative bioassessment of effects of shoreline development. However, we show here that rapid benthic algal counts could only discriminate between sites in high- and low-shoreline development categories in one of the two study years. The use of rapid algal counts, therefore, did not appear to provide a practical approach for a regional-scale biomonitoring program to identify environmental and ecological differences due to human activities along the nearshore zone of lakes. However, this finding may be influenced by the relatively modest range of lake trophic status and shoreline

development across the study sites. Thus, while rapid benthic algal counts did not appear to work well along a gradient of shoreline development in the oligo- to meso-trophic, P-limited lakes of our study (Molot & Dillon, 1991) that are affected mainly by summer recreational activities, it may prove useful in lakes that span a broader gradient of shoreline development, trophic status and water quality. Consequently, we suggest that similar studies should be undertaken in other regions to explore this level of bioassessment.

Photosynthetic pigment concentrations (Level 4) were able to discriminate between sites in the high shoreline development category from those in medium- and low-shoreline development categories, despite that sites with moderate shoreline development had the highest concentrations of pigments and sites with high shoreline development had comparably lower pigment concentrations. Generally, human recreational activities are associated with increasing nutrient supply and elevated algal production. In our study, however, many of the sites with high shoreline development were located near marinas and resorts (highly exposed areas), where wave action from the strong winds of 2007, boat traffic and contact by swimmers with benthic surfaces may elevate benthic algal loss rates (similar to the decrease in algal mat thickness observed in 2007). Regardless of the mechanisms accounting for the observed patterns, pigment analysis appears to have limited ability to discriminate among shoreline development categories in these oligo- to meso-trophic lakes. As with Level 3 assessment, we suggest further studies explore the potential of pigment analyses in meso- to eu-trophic lakes.

In our study, the level of bioassessment that employed the highest degree of taxonomic resolution (i.e., Level 5) was able to discriminate, for the most part, differences in benthic algal communities among shoreline development categories. Many studies have utilized diatoms to track limnological changes due to eutrophication in lakes (i.e., Hall & Smol, 1996; Quinlan et

al., 2008) and for biomonitoring of river water quality (i.e., Reavie & Smol 1998; Winter & Duthie 2000; Winter et al., 2003; Lavoie et al., 2008a), but diatoms appear to be rarely used for biomonitoring of shoreline conditions in lakes. Our results promote the use diatom community composition as a tool for littoral-zone biomonitoring in Precambrian Shield lakes with high water quality. Collection of diatom samples and preparation for analysis using a light microscope is routine, and high taxonomic resolution can be achieved (species or sub-species level) and appears to provide the most sensitive and robust ability to discriminate among the shoreline development categories. Moreover, microscope slides with diatom samples preserve a permanent archive that can be used by long-term monitoring programs for many decades to centuries for environmental-change detection. The clear distinction between diatom community composition at sites in the high- and low-shoreline development categories within a lake system demonstrates that they can provide a useful biomonitoring tool, despite the greater training and analytical effort required. Diatom community composition was the only level of bioassessment that provided consistent and statistically significant discrimination among the shoreline development categories in both study years, despite marked differences in the amount of wind and, presumably, wave action in August of 2006 and 2007 - a feature which allows us to recommend it for long-term monitoring programs.

A larger study with more sites and lakes situated along a broader gradient of lake trophic status and shoreline development is warranted to confirm the usefulness of benthic algal assessments in lakes, in particular to assess the ability of the easier and cheaper rapid assessment techniques (Levels 1-4) to discriminate eutrophic conditions. Increased sample size would also allow for the potential identification of indicator taxa of the different shoreline development categories as well as the use of a reference condition approach (RCA) which

could provide a more practical approach to studying development along shorelines (Bailey et al., 2004).

Overall, benthic algae have promising attributes to contribute to long-term lake monitoring programs. We recommend that future provincial biomonitoring protocols assessing effects of shoreline development on Precambrian Shield lakes include diatom community composition analysis (Level 5). For lakes with high water quality and recreational value, we recommend Level 5 despite the higher amount of technical skill and time required compared to the other levels, because it provides the strongest ability to detect differences in the benthic algal communities among shoreline development categories, and it is the only level of bioassessment that can discriminate between sites in medium- and low-shoreline development categories as well as those with high- and low-shoreline development.

2.6 Figures

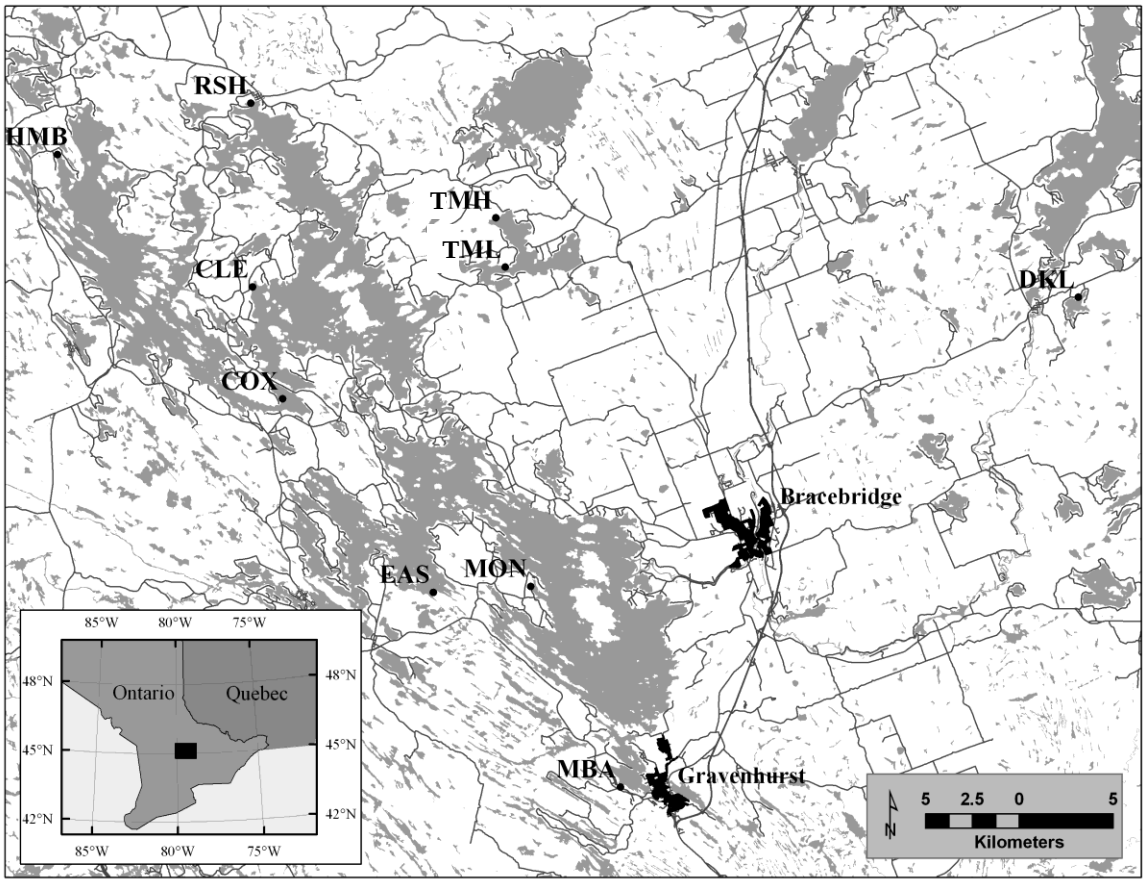


Figure 2.1 Map showing the location of the study area in south-central Ontario and the approximate locations of the study sites (n = 29) in the 5 study lakes.

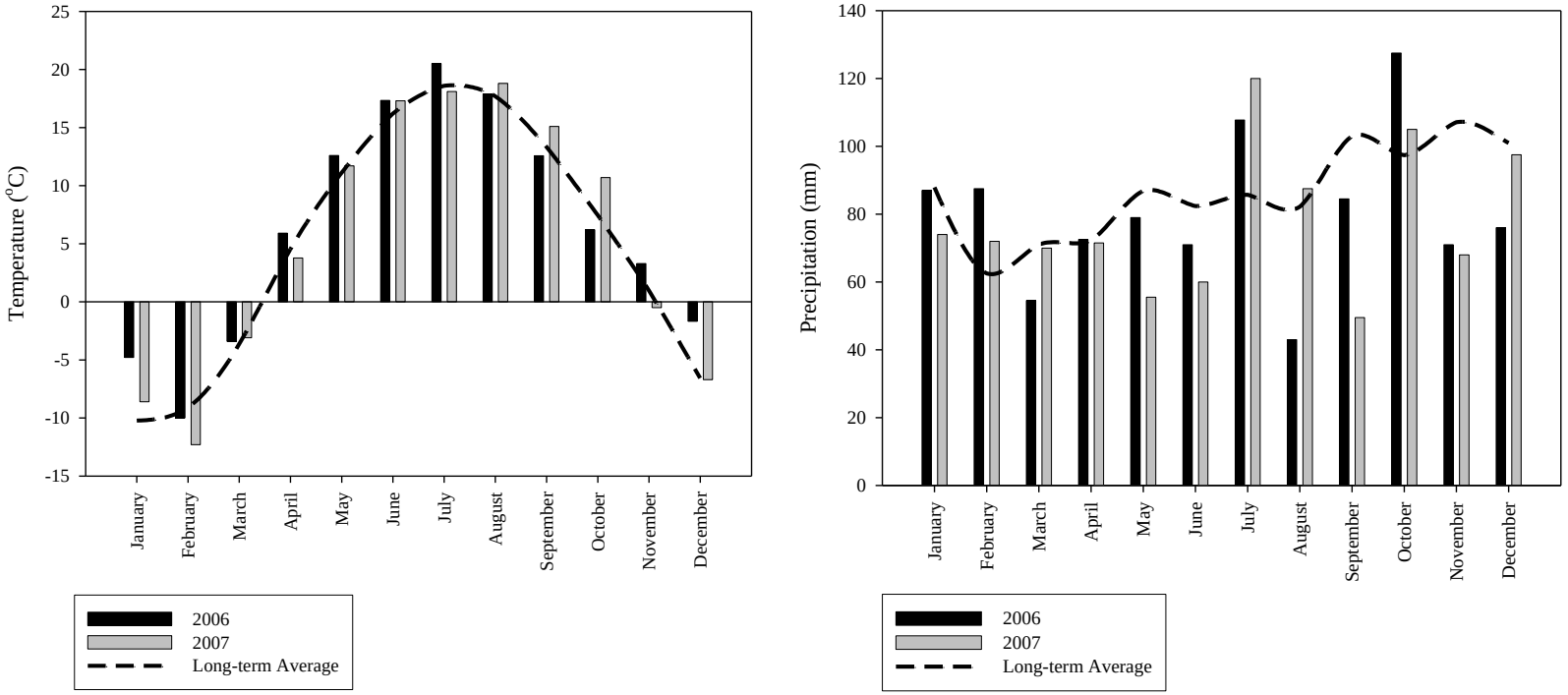


Figure 2.2 Bar graph of temperature and precipitation monthly average values for long term averages: 1939-2006 (dotted line), and 2006 (black) and 2007 (grey) averages from the Muskoka airport (Bracebridge, ON), provided by the Ontario Ministry of Environment, 2006 (black) and 2007 (grey).

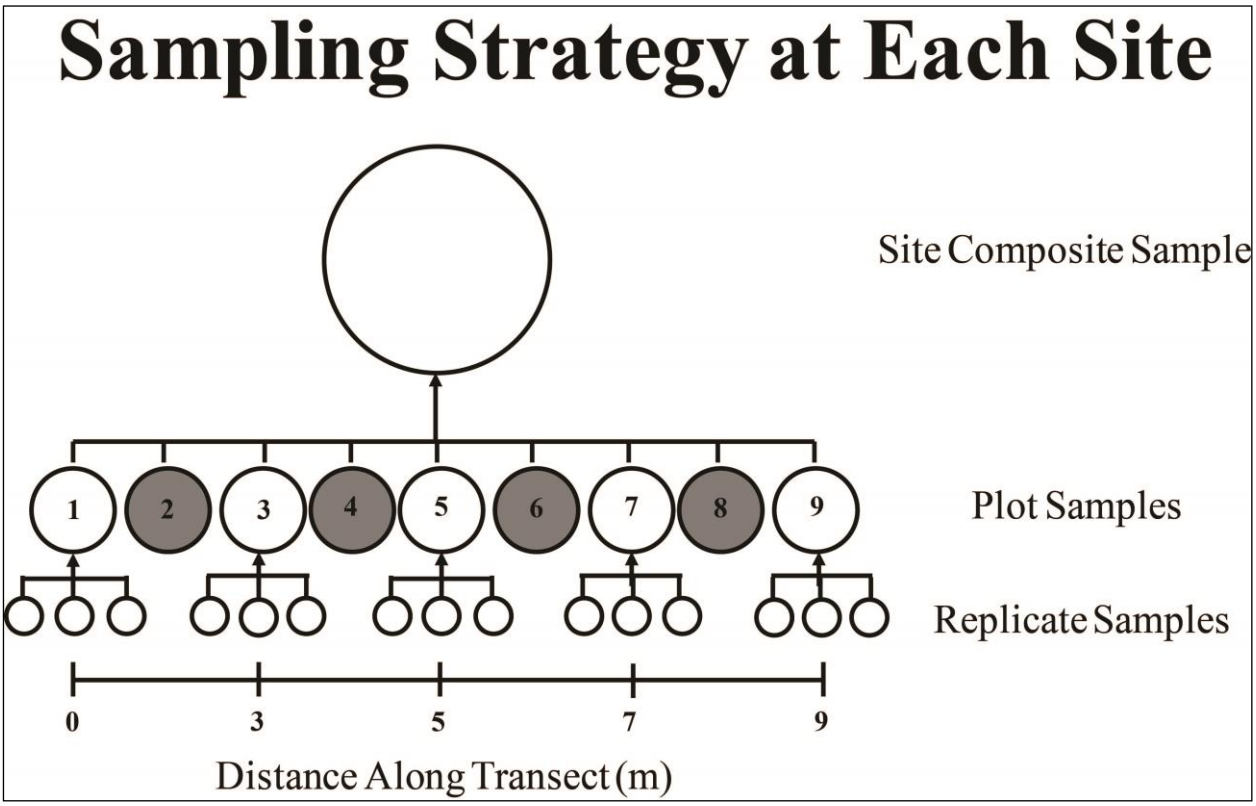


Figure 2.3 Schematic diagram to illustrate the sampling design at each study site, including the different types of benthic algal samples that were collected and the position of the nine 1 m diameter sampling plots along a 9 m long transect at each sample site. Rapid visual assessment (Level 1) and samples (1 L) for water chemistry were collected from all of the 9 plots. The water samples were pooled to make a site composite sample. Samples for biomass, pigment and taxonomic analyses (Level 2-5 bioassessments) were collected at 5 of the plots (indicated as open circles; plots numbered 1, 3, 5, 7, 9). These samples were obtained from 3 replicate samples (individual cobbles or sediment samples) that were pooled from each of the 5 plots. Subsamples (20 mL) from each of the 5 plot samples were then pooled to make a site composite sample (100 mL), as described in the Methods.

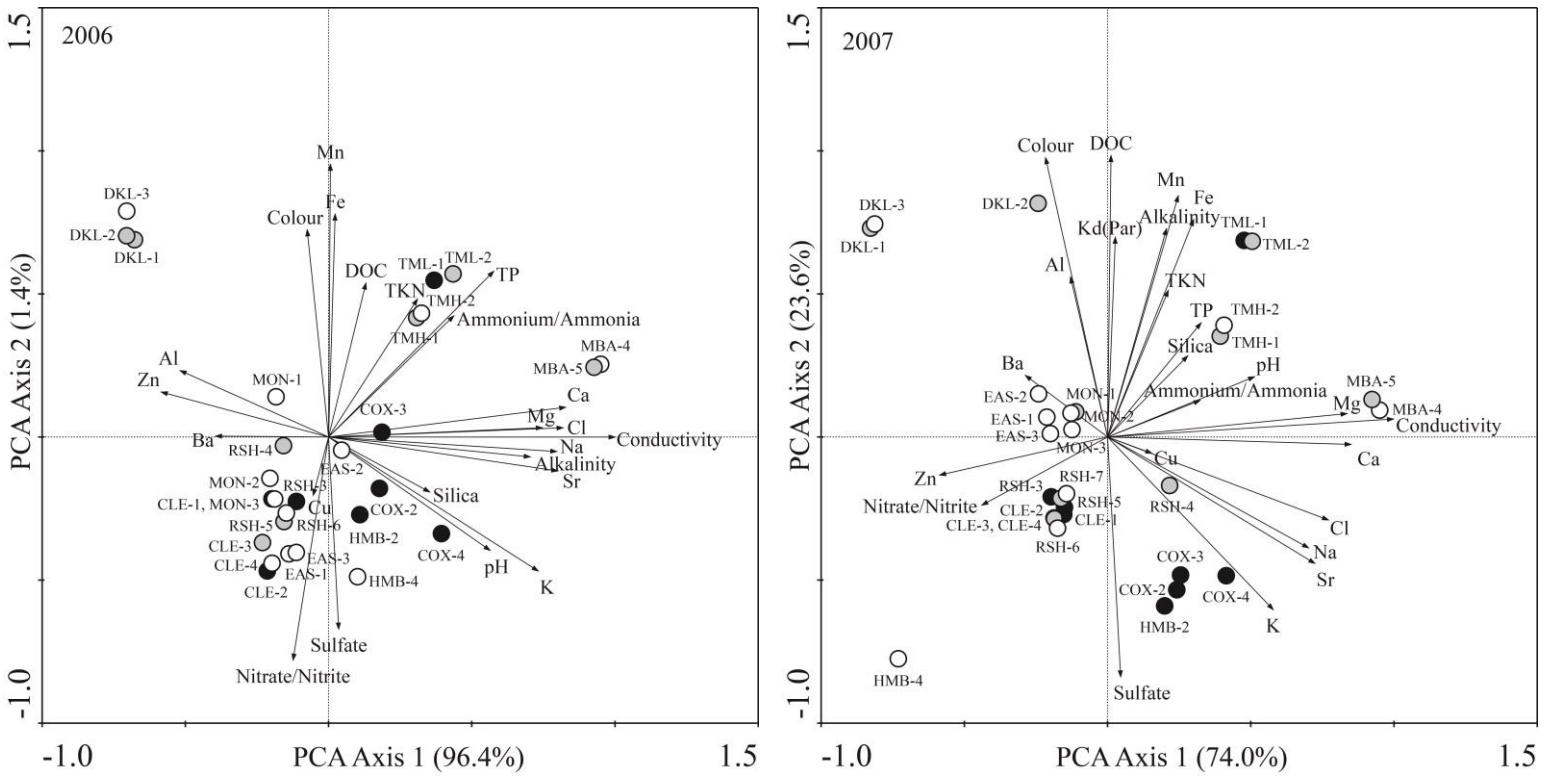


Figure 2.4 Principal Components Analysis (PCA) plots of the limnological data from each of the study sites in 2006 (left, n = 28) and 2007 (right, n = 29). Site scores are coded according to 1 of the 3 shoreline development categories (High = black, Medium = grey, Low = white). Vectors represent direction of variation in the chemical variables. Values of water chemistry variables are provided in Appendix A, Tables 2.1 and 2.2.

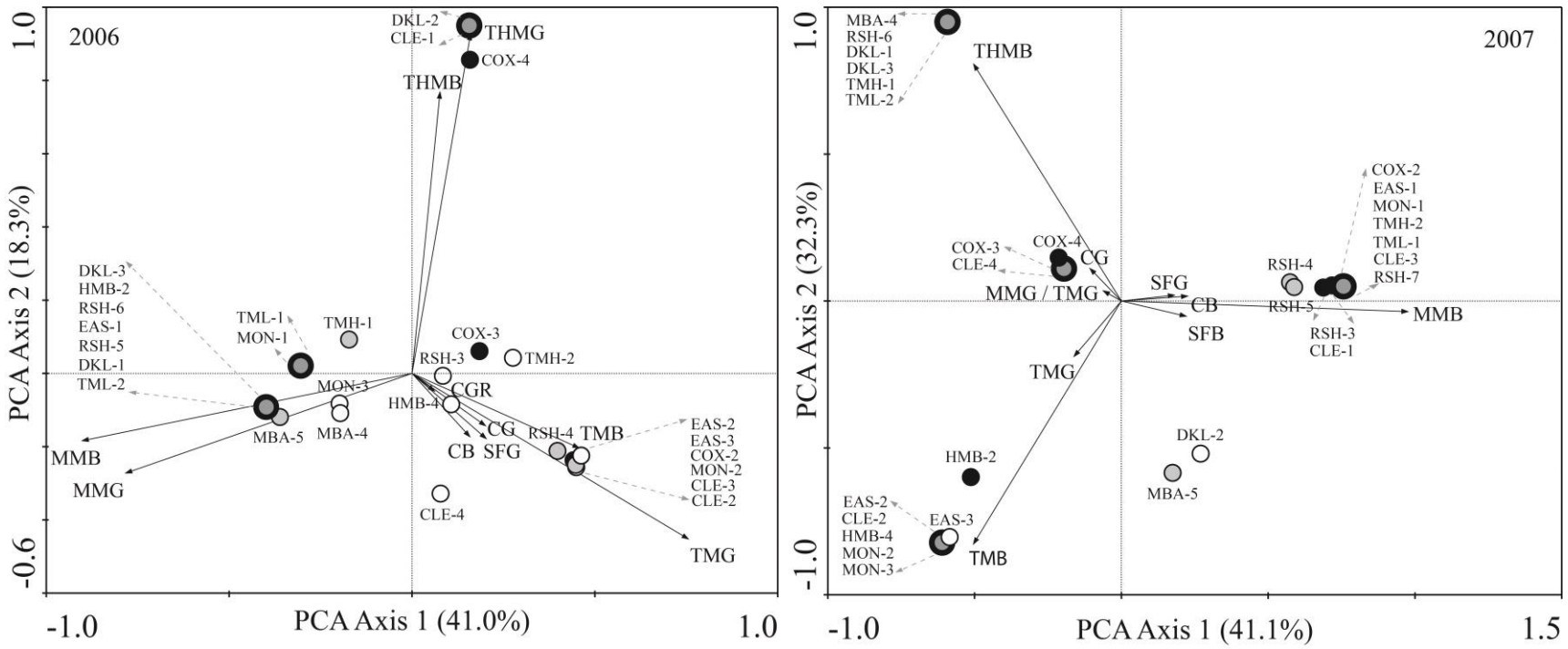


Figure 2.5 Principal Components Analysis (PCA) plots of the rapid visual assessment (Level 1) data from each of the study sites in 2006 (left, n = 28) and 2007 (right, n = 29). Site scores are coded according to 1 of the 3 shoreline development categories (High = black, Medium = grey, Low = white). A double circle (large black circle with smaller grey circle inset) represents a group of sites belonging to multiple shoreline development categories but plotting in the same place in ordination space. Vectors display directions of variation in the percent cover of the rapid visual assessment categories of benthic algal growth (TMB = thin mat brown; TMG = thin mat green; MMB = medium mat brown; MMG = medium mat green; THMB = thick mat brown; THMG = thick mat green)

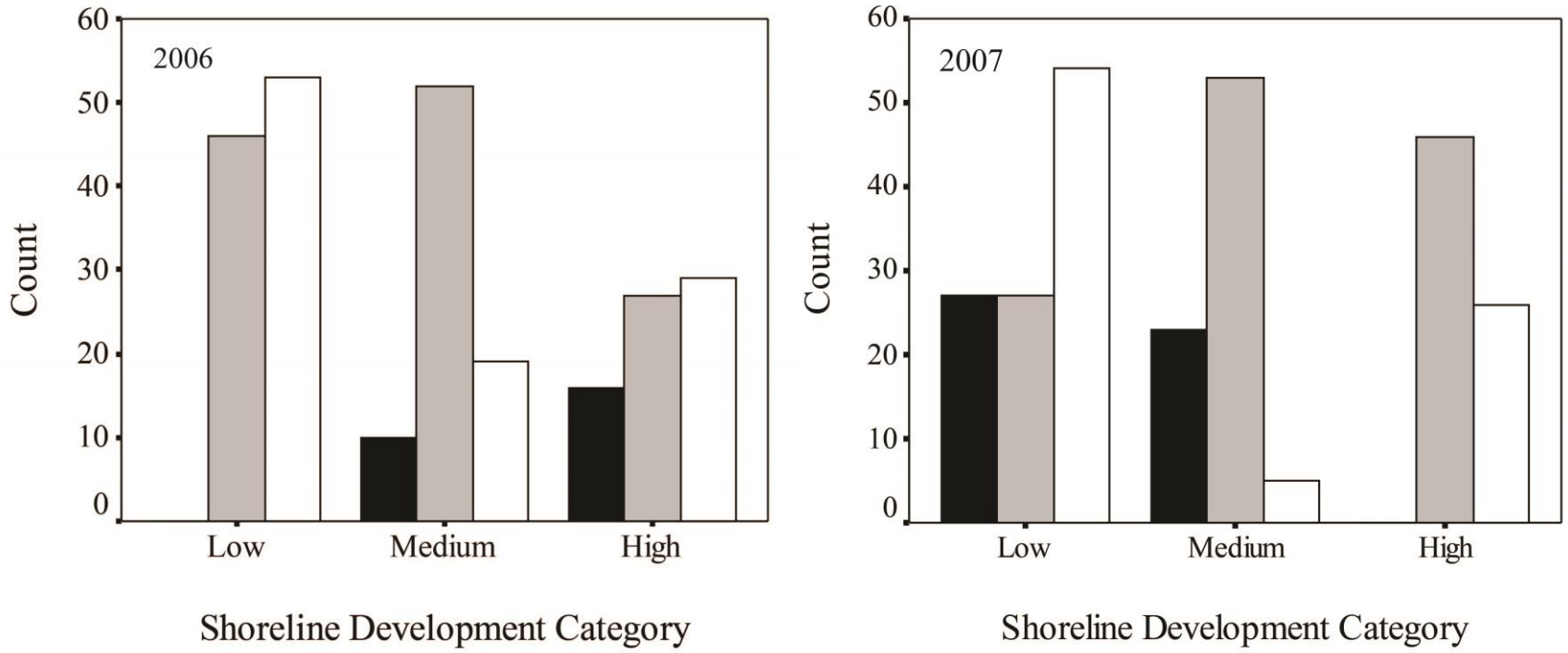


Figure 2.6 Bar charts showing frequency distributions of the type of benthic algal mats [Thick mat (> 3 mm) = black, Medium mat (0.5-3 mm) = grey, Thin mat (< 0.5 mm) = white] in each of the plots in 2006 (left, n = 252) and 2007 (right, n = 261) along transects across the 3 shoreline development categories.

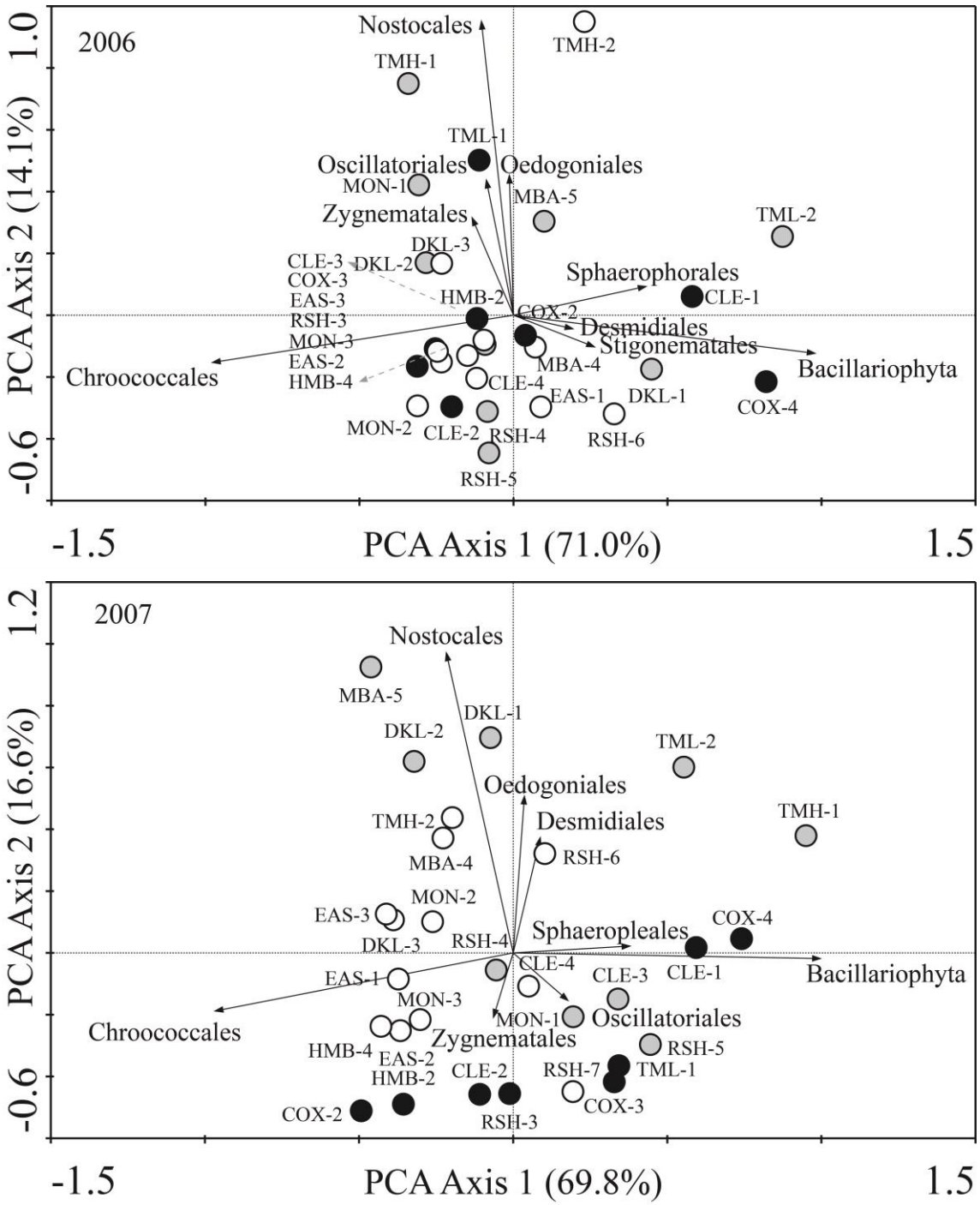


Figure 2.7 Principal Components Analysis (PCA) plots of the benthic algal community composition (Level 3) from each of the study sites in 2006 (top, n = 28) and 2007 (bottom, n = 29). Each site is coded according to 1 of the 3 shoreline development categories (High = black, Medium = grey, Low = white). Vectors display directions of variation in the relative abundance of algal taxa enumerated at each site.

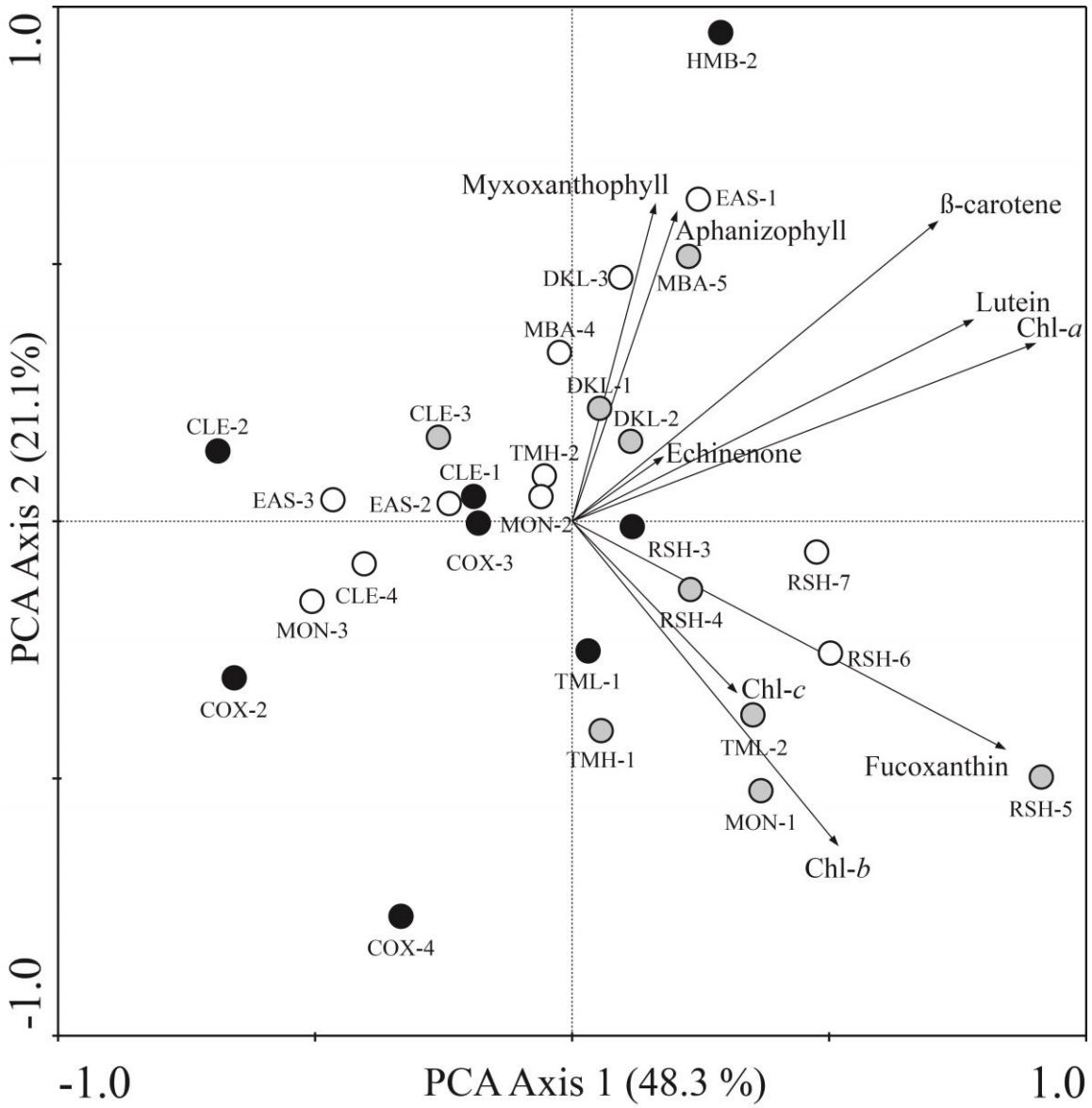


Figure 2.8 Principal Components Analysis (PCA) plots of the photosynthetic pigment concentrations (Level 4) obtained in 2007 only (n = 28). Each site is coded according to 1 of the 3 shoreline development categories (High = black, Medium = grey, Low = white). Vectors display directions of variation in the concentrations of the different photosynthetic pigments.

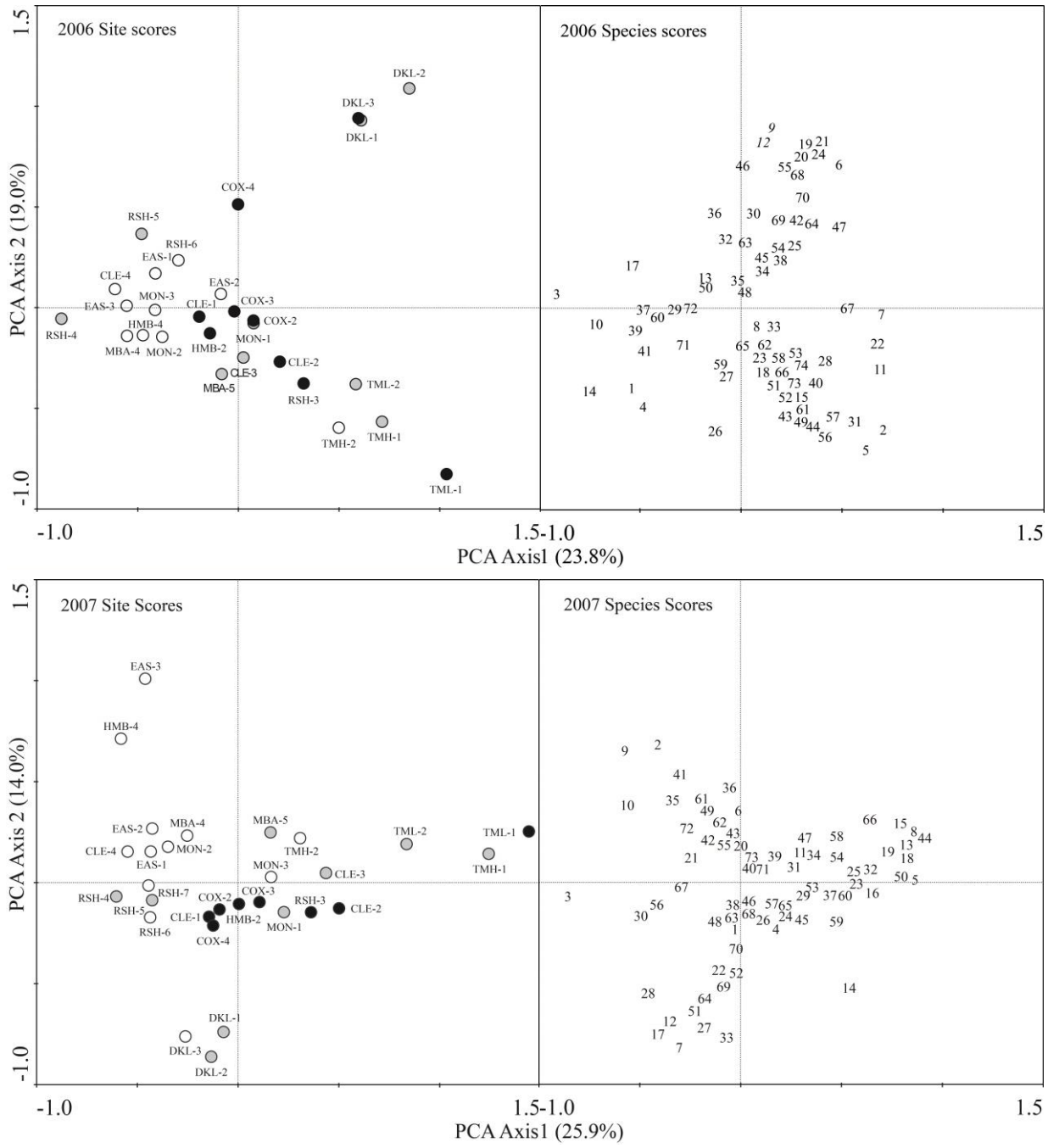


Figure 2.9 Principal Component Analysis (PCA) plots of sample and species scores in 2006 (upper graphs, n = 28) and 2007 (lower graphs, n = 29) based on diatom community composition. Each site is coded according to 1 of the 3 shoreline development categories (High = black, Medium = grey, Low = white). Taxa are presented as numbers and their corresponding names are presented in Appendix A, Table 2.3.

2.7 Tables

Table 2.1: Selected shoreline development characteristics of the 29 study sites in the Muskoka-Halliburton district of south-central Ontario. Descriptions of shoreline development categories are provided in the Methods.

Site Name	Development Category	Shoreline Description	Lake
HMB-2	High	Marina & Resort	Joseph
TML-1	High	Trailer Park within 50m	Three Mile
CLE-1	High	Resort	Rosseau
CLE-2	High	Resort & Boat House	Rosseau
RSH-3	High	Marina & Cottages	Rosseau
COX-2	High	Golf course & resort	Joseph
COX-3	High	Marina & road	Joseph
COX-4	High	Resort & Construction	Joseph
TMH-1	Medium	Cottages	Three Mile
TML-2	Medium	Cottages	Three Mile
MON-1	Medium	Cottages	Muskoka
MBA-5	Medium	Marina & Cottages	Muskoka
CLE-3	Medium	Cottages	Rosseau
RSH-4	Medium	Cottage / Construction	Rosseau
RSH-5	Medium	Cottages	Rosseau
DKL-1	Medium	Cottages	Dickie
DKL-2	Medium	Cottages	Dickie
HMB-4	Low	Forest & Exposed Rock	Joseph
TMH-2	Low	Forested / Cottages	Three Mile
MON-2	Low	Shrubs	Muskoka
MON-3	Low	Forested	Muskoka
MBA-4	Low	Forested	Muskoka
CLE-4	Low	Forested / Cottages	Rosseau
RSH-6	Low	Forested / Cottages	Rosseau
RSH-7	Low	Forested / Cottages	Rosseau
DKL-3	Low	Forested / Cottages	Dickie
EAS-1	Low	Conservation Area	Muskoka
EAS-2	Low	Conservation Area	Muskoka
EAS-3	Low	Conservation Area	Muskoka

Table 2.2 Results (R statistic and p-values) obtained from one-way ANOSIM tests of water chemistry data obtained from the nearshore sites in 2006 (n = 28) and 2007 (n = 29) from the study lakes (n = 5) in south-central Ontario. The ANOSIM tests were used to assess if water chemistry conditions differed among the 3 shoreline development categories and among the 5 different lakes. For all comparisons, 9999 permutations were performed for Monte-Carlo tests of significance. Values in bold text indicate comparisons that resulted in significant differences at alpha = 0.1. Values of water chemistry variables are provided in Appendix A, Tables 2.1 and 2.2.

	2006		2007	
Groups	**R statistic**	***p*-value**	**R statistic**	***p*-value**
Pairwise tests: Shoreline development categories				
High, Medium	−0.02	0.47	0.07	0.17
High, Low	−0.04	0.59	0.08	0.14
Medium, Low	−0.02	0.48	0.02	0.30
Pairwise tests: Lakes				
Three Mile, Rosseau	**1.00**	**< 0.01**	**0.98**	**< 0.01**
Three Mile, Joseph	**0.71**	**0.02**	**0.60**	**< 0.01**
Three Mile, Dickie	**1.00**	**0.03**	**1.00**	**0.03**
Three Mile, Muskoka	**0.29**	**0.08**	**0.29**	**0.05**
Rosseau, Joseph	**0.98**	**< 0.01**	**0.79**	**< 0.01**
Rosseau, Dickie	**1.00**	**< 0.01**	**0.10**	**< 0.01**
Rosseau, Muskoka	**0.12**	**0.06**	**0.49**	**< 0.01**
Joseph, Dickie	**1.00**	**0.02**	**0.76**	**0.04**
Joseph, Muskoka	**0.31**	**0.03**	**0.20**	**0.08**
Dickie, Muskoka	**0.42**	**0.06**	**0.39**	**0.07**

Table 2.3 Results of paired-samples t-tests to assess if water chemistry conditions differ between nearshore and offshore sites in the study lakes (n = 4 in 2006, n = 5 in 2007). Results are presented for select variables obtained in 2006 and 2007. Values in bold text indicate comparisons that resulted in significant (alpha = 0.1) differences.

	2006		2007	
	t	**p-value**	**t**	**p-value**
Alkalinity	1.28	0.29	0.91	0.42
Conductivity	1.16	0.33	0.85	0.45
pH	−3.64	**0.04**	2.30	**0.08**
DOC	−0.15	0.89	−0.49	0.65
TKN	0.68	0.54	1.58	0.19
TP	−2.36	**0.10**	−0.85	0.44
SiO_3	−2.26	0.11	−3.53	**0.02**

Table 2.4 Results (R statistic and p-values) obtained from one-way ANOSIM tests of levels 1, 3, 4 and 5 benthic algal bioassessment. The overall test assessed if benthic algal metrics differed among the 3 shoreline development categories (n = 28 sites in 2006, n = 29 sites in 2007). Results are shown for all pairwise comparisons between the shoreline development categories. For all comparisons, 9999 permutations were performed for Monte-Carlo tests of significance. Values in bold text indicate comparisons that resulted in significant difference at alpha = 0.1.

		Levels						
		Level 1: Rapid Visual Assessment		Level 3: Benthic Algal Community Composition		Level 4: Photosynthetic Pigment Concentration	Level 5:Diatom Community Composition	
	Year	2006	2007	2006	2007	2007	2006	2007
Global	R statistic	0.06	0.01	0.01	**0.26**	**0.01**	**0.11**	**0.16**
	p-value	0.14	0.34	0.40	**< 0.01**	**0.06**	**0.04**	**< 0.01**
High, Low	R statistic	**0.12**	0.02	0.06	**0.36**	**0.12**	**0.20**	**0.28**
	p-value	**0.07**	0.30	0.19	**< 0.01**	**0.09**	**0.02**	**< 0.01**
High, Medium	R statistic	-0.01	0.02	-0.09	0.05	**0.15**	0.03	-0.02
	p-value	0.49	0.33	0.92	0.22	**0.04**	0.33	0.50
Medium, Low	R statistic	0.04	0.01	0.02	**0.30**	0.02	**0.09**	**0.17**
	p-value	0.21	0.38	0.29	**< 0.01**	0.33	**0.10**	**0.02**

Chapter 3

Relations between limnological conditions and composition of benthic algal communities in the South Nahanni River watershed, NWT (Canada): defining the reference condition.

3.1 Overview

Aquatic ecosystems in northern Canada are threatened by rapid climate warming and increased industrial activities such as mining. Monitoring studies are needed to assess the natural variability of baseline conditions in order to effectively assess effects of human activities. We examined physical and chemical conditions and their relations to benthic algal communities in rivers across the South Nahanni River watershed, NWT, in 2008 and 2009. We also assessed the ability of different benthic algal metrics (benthic algal community composition, diatom community composition and pigment concentrations) to track differences in physical and chemical conditions among ecoregions. Multivariate analyses (principal components analysis, ANOSIM tests) identified that chemical variables differed significantly ($P < 0.05$) between the ecoregions in the South Nahanni River watershed and highlighted the importance of bedrock geology in influencing chemical variables in different ecoregions (Selwyn Mountain ecoregion, Nahanni-Hyland ecoregions). Multivariate analyses (ANOSIM tests, correspondence analysis and canonical correspondence analysis) of benthic algal metrics (benthic algal community composition, diatom community composition, and photosynthetic pigment concentrations) showed that these metrics also differed significantly ($p < 0.05$) and were associated with differences in physical and chemical variables between ecoregions. These relations were consistent in both 2008 and 2009 data. The results of this study can improve future monitoring programs by effective selection of reference sites in the diverse landscape of the South Nahanni River watershed.

3.2 Introduction

Defining reference conditions is a central precursor to identifying environmental effects of anthropogenic activities. Aquatic ecosystems in northern Canada are often remote from human activities, but rapid climate warming and northward expansion of industrial activities threaten to degrade water quality and ecological integrity (Schindler & Smol, 2006). Climate warming is projected to be greater in the north than the global average (Kattsov et al., 2005). Warming is expected to lengthen growing seasons, increase evaporative water losses, alter river hydrographs towards more pluvial regimes (from more nival regimes), increase water temperatures, alter water chemistry, and increase productivity (Prowse et al., 2006, 2011; Schindler & Smol, 2006; Wrona et al., 2006). In aquatic ecosystems, these changes are expected to alter composition, richness, and diversity of biotic communities and trophic interactions (Secretariat of the Convention on Biological Diversity, 2003).

In addition to climate warming, freshwaters in northern Canada are increasingly subjected to other human-caused changes including industrial development, encroachment of expanding human populations, and long-distance transport of contaminants (Schindler & Smol, 2006). Increases in mining activities are of particular concern, as outputs from mining activities in northern Canada are expected to almost double in the next decade (The Conference Board of Canada, 2013). Although some regions are naturally rich in minerals, increased mobilization of metals and nutrients by industrial activities can impair downstream aquatic ecosystems (Wrona et al., 2006). Pollution from metal mining can degrade water quality and modify biological communities, but detection of the effects has proven difficult because studies are often initiated only after industrial development has begun [Clements et al., 2000; Hill et al., 2000a; Clark & Clements, 2005; Rhea et al., 2006; Hall et al., 2012; Thomas et al., 2013 (Chapter 4)]. Also,

natural loads of contaminants can be high and vary both spatially and temporally, a feature that challenges the ability of monitoring programs to determine the relative roles of natural and human processes (Clements et al., 1992, 2010). In particular, the lack of knowledge of baseline (or, pre-impact) conditions and natural variability reduces our ability to quantify impairment due to industrial activities (e.g., Hall et al., 2012). Consequently, there is an increasing need to generate knowledge of baseline physical and chemical conditions, and biological communities in remote northern ecosystems before the magnitude of climate warming and industrial development escalate further. Indeed, many studies recognize the need to obtain information for many regional reference sites (currently unaffected by anthropogenic activities) for use in assessing potentially effected sites (Hughes et al., 1986; Reynoldson et al., 1997; Bowman & Somers, 2005). This need is driven, at least in part, by recognition that the structure and function of biological communities in pristine northern ecosystems are sensitive to changes in physical and chemical conditions (Wrona et al., 2006).

Among the numerous components that can be monitored (e.g., water chemistry, algae, macroinvertebrates, fish), benthic algae possess many features that predispose them to provide effective monitoring of changes in water quality and ecological status of lakes and rivers caused by anthropogenic disturbances [Reavie & Smol, 1998; Rott et al., 1998; Hill et al., 2000b; Leland & Porter 2000; Thomas et al., 2011 (Chapter 2), 2013 (Chapter 4)]. As primary producers, benthic algae play influential roles in the structure and function of aquatic foodwebs (Sabater & Admiraal, 2005; Resh 2008). Benthic algal communities are abundant, widespread, and diverse, and so require a relatively low sampling effort during field collection to obtain high content of ecological information (Biggs & Kilroy, 2000; Resh, 2008). They can rapidly assimilate pulses of nutrients due to their rapid potential growth rate and short generation time,

and thus can respond quickly to changes in climate and anthropogenic disturbances. For these reasons, benthic algae are considered to be early warning indicators of environmental and ecological change (Sabater & Admiraal, 2005). Also, benthic algal communities accrue in aquatic ecosystems over time periods spanning several weeks to months, and so can store information about influential events and changes during these timescales. Spot water-chemistry samples, in contrast, capture information over much shorter timescales, and so may be less able to capture signals of pulses of nutrients or contaminants that occur between sampling episodes. To better understand the cumulative effects of climate change and anthropogenic alteration on aquatic ecosystems, it is imperative that monitoring programs measure biotic metrics that track changes in the structure and functioning of the biological communities in relation to shifting physical and chemical conditions (Dubé et al., 2013). Despite these features, which suggest that benthic algal communities possess distinct advantages compared to other biota, they have not been as widely used in bioassessments of stream conditions (e.g., Reynoldson et al., 1997; Rosenberg et al., 1999; Clements et al., 2000; Sylvestre et al., 2005; Clements et al., 2010). In fact, use of fish and benthic macroinvertebratres is more common than algae, likely due, at least in part, to the cultural significance of fish and interest in their main food source.

The South Nahanni River watershed (37 000 km^2) in southwestern NWT ($61^o39'N$, $125^o34'W$) is a remote wilderness area with high preservation value and cultural significance. Despite its protection within two National Park Reserves, mining development within the watershed and climate change are causing concerns about degradation of river water quality and ecological integrity. The Nahanni National Park Reserve was established in 1976 and became a UNESCO World Heritage Site in 1978. The adjacent Nááts'ihch'oh National Park Reserve was established in 2012 (Figure 3.1). The South Nahanni River watershed is located

within the Taiga Cordillera and Taiga Plains and Boreal Cordillera Ecozones with diverse topography (e.g., canyons, karst features, mineral springs, mountains, plains, and plateaus) and vegetation (e.g., boreal forests, alpine tundra; Environment Canada [EC], 1991). The watershed is known for the abundance of pristine or nearly pristine rivers within its boundaries (EC, 1991; Halliwell & Catto, 2003). The South Nahanni River (540 km long) transects this watershed and was established as a Canadian Heritage River in 1987. Because of the presence of deposits of metals, current mining and future mining within the watershed pose potential threats to downstream water quality within the two National Park Reserves and are causing concerns about the effect of mining activities within the watershed [e.g., Spencer et al., 2008; Bowman et al., 2010; Scrimgeour 2013; Thomas et al., 2013 (Chapter 4)]. Although this watershed is remote, the projected global increase in temperature, and associated hydrological shifts, raise further concerns (Schindler & Smol, 2006; Wrona et al., 2006).

The overall objective of this study is to assess the patterns of variation in physical and chemical conditions across the South Nahanni River watershed at spatially dispersed sampling sites unaffected by direct human activities and their relations with benthic algal community composition (44 and 18 sites in 2008 and 2009 respectively; Figure 3.1). Addressing this objective required several steps. To assess variation in benthic algal community composition, we analyzed three benthic algal metrics (benthic algal community composition, diatom community composition, and photosynthetic pigment concentrations). These metrics have been shown to provide useful information to track changes in community composition within river and lake ecosystems [Rott et al., 1998; Hill et al., 2000b; Hirst et al., 2002; Rosenberger et al., 2008; Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2011 (Chapter 2), 2013 (Chapter 4)], and they were selected to assess the level of effort and expertise needed to track

biotic differences among sites. Finally, we explored relations between the benthic algal metrics and physical and chemical variables to improve understanding of controls on benthic algal communities in streams of the South Nahanni River watershed unaffected by direct human activities. It is intended that this information will be utilized by ongoing and future biomonitoring programs to quantify changes caused by human activities and climatic changes. Two previous studies reported differences in chemical conditions and community composition of benthic algae, macroinvertebrates and fish downstream of two mining developments, but they did not undertake a comprehensive examination of reference communities across the entire watershed (Spencer et al., 2008; Bowman et al., 2010). Natural variability in physical and chemical variables can introduce unwanted noise in biological monitoring data used to assess changes due to anthropogenic activities. Understanding what physical and chemical processes are associated with shifts in biological communities can help to disentangle natural variability from the effects of anthropogenic influences.

3.3 Methods

3.3.1 Study area

The South Nahanni River watershed forms part of the headwaters of the Mackenzie River, connecting via the Liard River. The watershed is underlain by Proterozoic glaciomarine conglomerates, early Paleozoic formations, late Devonian to Jurassic formations, and Cretaceous granitic rock formations. The watershed contains economic deposits of lead, zinc, silver, and tungsten (EC, 1991). Due to the location and nature of the bedrock and landscape, airborne contaminants are considered to be less influential than those derived from the land and water within the watershed (EC, 1991).

The landscape across the South Nahanni River watershed is diverse with the rugged snow-capped, glaciated terrain of the Selwyn Mountain ecoregion in the northwest and the lower summits of the Nahanni Plateau, Sibbeston Lake Plain and Hyland Highland ecoregions (referred to hereafter as the Nahanni-Hyland ecoregions) to the south and east (Figure 3.1). The Selwyn Mountain ecoregion is part of the Selwyn and Mackenzie mountain ranges and is characterized by mean summer temperatures of 9.5 °C. It is primarily underlain by a mixture of shale and plutonic suites. The Nahanni-Hyland ecoregions are characterized by mean summer temperatures of 9 °C – 10 °C. They are underlain primarily by shale and limestone/dolostone (Ecological Stratification Working Group, 1996; Caron et al., 2008). Due to the diversity in terrain, the hydrology within the South Nahanni River watershed is influenced by multiple factors (e.g., snowmelt during late-winter and spring, glacier melt and precipitation during the summer). Flow rates along the South Nahanni River at Virginia Falls range from 55 to 150 m^3/s (EC, 1991; Halliwell & Catto 2003). Peak flows along the rivers occur during snowmelt in early to mid-June. During summer, flows are influenced strongly by precipitation events.

For this study, 44 sites were selected in 2008 and 18 sites in 2009 (12 repeated sampling from 2008; 6 newly sampled in 2009) based on a double stratified random sampling design (Figure 3.1). The two strata used were stream order (3^{rd} to 6^{th} order streams) and % ice cover (or % glacier cover in the sub-watershed; removing those sites with >40% ice cover) because these strata are well known to influence physical and chemical stream conditions. By stratifying samples according to stream order and % ice cover, we aimed to detect variability in chemical characteristics and benthic algal communities due to other factors operating across the watershed. Firstly, potential sampling sites were randomly identified that fell into the 3^{rd} to 6^{th} order stream-categories. Then, those sites with >40% ice cover within the catchment were

removed. This led to the identification of 140 potential sampling sites. Not all sites were sampled due to a lack of safe helicopter landing sites, lower flow than expected along Flat River and Prairie Creek, or wildfires that did not allow safe passage to the stream site. All sites were selected from undisturbed areas of the South Nahanni River watershed to determine benthic algal community structure and physical and chemical conditions at stream and river sites with no direct human influence (e.g., no source of contaminants adjacent to or upstream). The sites were sampled early to mid-August in 2008 and 2009. No sites downstream of mining operations were used in this analysis (Figure 3.1). Multiple sites were sampled along Caribou River, Cathedral Creek, Clearwater Creek, Flat River, Flood Creek, Prairie Creek, Little Nahanni River, and Mary River. Sites along the same stream were located greater than 2 km apart with multiple 1st to 3rd order streams joining between sampling locations, and were, therefore, treated as independent from each other.

To assess meteorological conditions during this study and inter-annual differences between the two study years, we compared monthly (May - September) precipitation and mean air temperature for the two study years (2008, 2009) with corresponding long-term monthly averages (May - September: 1997 & 2001 - 2007) based on Environment Canada's meteorological data collected at the Rabbit Kettle station (Climate ID: 2203342) within the Nahanni National Park Reserve.

3.3.2 Field and laboratory methods

3.3.2.1 Physical and chemical analysis

Stream sites were remote and therefore accessed by helicopter. At each stream site, water samples (2 L) for chemical analysis were collected at a midstream location and at approximately 30-cm depth. Samples were stored in the dark and kept cool until transported to

a temporary field laboratory for processing at the end of each sampling day. At the temporary field laboratory, all water samples were filtered through an 80-µm mesh to remove large particles before being processed for analysis of total concentrations of 34 metals (Ag, Al, As, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, La, Li, Mn, Mo, Nb, Ni, Pb, Pt, Rb, Sb, Se, Sn, Sr, Tl, U, V, W, Y, Zn) and nutrients [dissolved inorganic carbon (DIC; filtered through a 0.45-µm cellulose acetate filter), dissolved organic carbon (DOC; filtered through a 0.45-µm cellulose acetate filter), nitrate + nitrite (NO_2+NO_3), total nitrogen (TN), and total phosphorus (TP; preserved with 30% H_2SO_4)]. Metals were analyzed using inductively coupled plasma mass spectrometry. Concentrations of DOC and DIC were analyzed using an UV-persulfate TOC analyzer, and samples for NO_2+NO_3, NH_3, TN, and TP were analyzed using an automated continuous-segmented-flow analyzer at Environment Canada's National Laboratory for Environmental Testing, Burlington, Ontario (EC, 1994). At each site, we also measured conductivity and pH (using a YSI model 650 meter), turbidity (using a LaMotte model 2020e turbidity meter), and water velocity and depth (mean of 5 - 10 measures; using a Marsh McBirney flow mate).

A suite of physical variables were available for each site, which included quantitative, categorical, and binary data (Appendix B, Table 3.1). Variables such as canopy cover, habitat type, macrophyte coverage (visual estimation of abundance), riparian vegetation, and percent gravel and cobble within the river bed were visually estimated following protocols described by EC (2011). Wetted widths were measured with a Bushnell range finder (± 0.5 m). Elevation was measured using a Garmin GPS. Variables such as stream order and bedrock were derived from GIS layers. The full list of physical variables used in this is located in Appendix B, Table 3.1.

Metals concentrations were compared to the Canadian Council of Ministers of the Environment's Canadian Environmental Quality Guidelines for the Protection of Aquatic Life (CCME Guidelines; CCME, 2003). Guidelines for concentrations of metals such as Cd, Cu, Ni, and Pb were calculated using an average water hardness value derived from reference site measurements taken in 2006 across the South Nahanni River watershed (172.3 mg/L $CaCO_3$; Monique Dubé, EP Total, Calgary, Alberta, unpublished data). These measurements were used because we did not record water hardness during our 2008 to 2009 field surveys.

3.3.2.2 Benthic algae

Benthic algal samples were collected from the upper surface of cobbles collected from the stream bed. Sampling was restricted to one type of substrate (cobbles) in order to reduce variation due to different substrate types (Biggs & Kilroy, 2000). Separate benthic algal samples were collected for each type of metric (e.g., benthic algal community composition, diatom community composition, and pigment concentrations). Each sample was collected by removing benthic algae from 5 to 10 cobbles using a syringe sampler [as described in Thomas et al., 2013 (Chapter 4)] and combining the material to make one sample with a known surface area of 26.5 to 53.1 cm^2. Samples were stored in the dark and kept cool until further processing at the end of each day at the field base. Benthic algal samples for quantification of photosynthetic pigments by HPLC were filtered onto Whatman GFF filters (0.7 µm), wrapped in aluminum foil and frozen until analysis at the University of Waterloo. Benthic algal samples for taxonomic analyses were preserved using Lugol's iodine and transported to the University of Waterloo for further analysis.

3.3.2.3 Benthic algal community composition

Sub-samples of 2 mL of well-mixed sample were placed into Utermöhl chambers and allowed to settle for 24 h. An inverted microscope (1000 x magnification) was used to identify a total of 300 units of algae to class (e.g., Bacillariophyceae) or family (e.g., Chrococcaceae, Oscillatoriaceae) level following the nomenclature of Prescott (1951) and Wehr & Sheath (2003). Relative abundances were calculated by dividing the number of units enumerated per taxonomic group by the total number of units enumerated in the sample and multiplying by 100.

3.3.2.4 Diatom community composition

Sub-samples of 15 mL of well-mixed samples were placed into individual test tubes and allowed to settle for 24 h. The supernatant was then removed and replaced with deionized water and allowed to settle for 24 h. This was repeated until most of the Lugol's solution was removed. The samples were then oxidized by addition of 30% hydrogen peroxide to remove organic material. Samples were allowed to react with the hydrogen peroxide for one week at room temperature. Acid residues were removed by repeatedly siphoning off two-thirds of the supernatant, diluting the remaining supernatant with deionized water and allowing the mixture to settle for 24 hours. These steps were repeated until the solution reached a pH comparable to that of the deionized water. The resulting cleaned diatom slurries were then dried onto glass coverslips and mounted onto microscope slides using Naphrax mounting medium. A total of 300 to 500 diatom valves were identified and counted using a compound light microscope at 1000x magnification (Zeiss Axioskop 2Plus, numerical aperture = 1.30). Taxonomic identifications followed Krammer & Lange-Bertalot (1986-1991) and Lavoie et al., (2008).

Only species contributing greater than 1% abundance in at least one sample were included in numerical analyses.

3.3.2.5 Photosynthetic pigment concentration

For quantification of photosynthetic pigments, samples (Whatman GFF filters with the algae) were extracted in a mixture of acetone:methanol:water (80:15:5 by volume) for 24 h at -20 ºC. Once extracted, the samples were filtered through a 0.22-µm polytetrafluoroethylene (PTFE) syringe-filter to remove large particles and other impurities. The filtrate was then dried in the dark under inert gas (N_2). Once dried, the pigments were re-eluted in 500 µL of injection solution [acetone:ion-pairing reagent:methanol; 70:25:5 by volume (ion-pairing reagent = 0.75g tetrabutylammonium acetate and 7.7g ammonium acetate)] and analyzed using a Waters HPLC in reverse-phase with a Symmetry C18 column (3.5-µm bead diameter, column dimensions = 4.6 x 75 mm), following the methods of Leavitt et al., (1989) as modified from Mantoura & Lleywellyn (1983).

Each sample was analyzed individually using a gradient delivery of two mobile phases to separate the pigments within the sample. Mobile phase A consisted of methanol:IPR (90:10 by volume), and mobile phase B consisted of methanol:acetone (73:27 by volume). Sudan II was used as an external standard (at the beginning and end of a group of samples) and as an internal standard added to each sample to account for dilution and injection errors. Pigment samples from Geranium plants were placed at the start and end of each batch of samples to account for shifts in chromatographic mobility of individual pigments. Pigment signatures were measured by a Waters 2998 PDA detector and a Waters 2475 Multi λ Fluorescence detector. Pigments were subsequently identified using chromatographic mobility (Leavitt et al., 1989) and spectral

characteristics (Jeffrey et al., 1997). Concentrations of all pigments were expressed as $\mu g/cm^2$ (mass per unit area of cobble surface).

3.3.3 Numerical Analyses

3.3.3.1 Physical and chemical data

Box plots were created to assess patterns of variation between ecoregions. Principal Components Analysis (PCA) was used to assess patterns of spatial variability in physical and chemical variables among sites. We completed the analyses on the data from 2008 and 2009 separately. The chemical variables were assumed to be the primary influences of biological communities, driven by variations in physical variables. We reduced the number of physical variables to include only those variables that explained important amounts of variation in water chemistry variables. To do this, individual Redundancy Analyses (RDA's) were run with a single physical variable at a time, and Monte Carlo permutation tests (with 999 random permutations) were used to identify physical variables that accounted for significant amounts of variance in water chemistry samples ($\alpha = 0.05$). Only those variables that were significant along the first axis were retained for further analysis. All statistically significant variables were then used in a series of RDA's and the variables with highest Variance Inflation Factors (VIF's) were sequentially removed until all VIF's were ≤ 20. Using this method, subsets of physical variables were selected for further use in PCAs as supplemental data. For the PCA ordinations, the scaling focused on inter-sample distances and the variables were centered and standardized.

Exploratory ordinations by PCA demonstrated striking differences in chemical and physical characteristics between the main ecoregions of the watershed (Selwyn Mountain ecoregion and the Nahanni-Hyland ecoregions). To describe patterns in these relations, we subsequently

coded sites according to their ecoregion to assess how conditions varied. One-way analysis of similarities (ANOSIM) tests (2008, 2009) were run on all the chemical data to determine if chemical conditions differed significantly between sites in the Selwyn Mountain ecoregion versus those in the Nahanni-Hyland ecoregions. All the physical and chemical variables were normalized (variables had their mean subtracted then divided by the standard deviation) prior to one-way ANOSIM tests in order to equalize variances before calculation of Euclidean distances. For each one-way ANOSIM test, 9999 computations were completed (Clark and Warwick 2001). One-way analysis of variance (ANOVA) tests were used to determine if concentrations of individual physical and chemical variables differed significantly between the two ecoregions. Separate tests were performed on data obtained in 2008 and 2009.

Prior to numerical analyses, all quantitative physical and chemical variables were assessed for normality using Kolmogorov-Smirnov tests of normality ($\alpha = 0.05$). All non-normal variables were transformed. Variables measured as percent were square-root transformed. Most other variables with non-normal distributions were ln(x+b)-transformed, where b = half the minimum non-zero value among sites. Binary and categorical variables were not transformed, nor were air and water temperature, measures of precipitation (total, snowfall, rainfall), latitude, longitude, pH, and Julian day. Some water chemistry variables (e.g., Ag, Bi, Cs, NH_3) had values below detection limits. For water chemistry variables that had values missing at < 15% of the sites, values were randomly generated between 0 and the detection limit and these variables were kept for further analysis (i.e., Cs, Tl, Pb). Variables with values below detection limits at > 15% of the sites were removed from further analysis (i.e., NH_3, Ag, Bi, and Nb).

3.3.3.2 Benthic algal metrics

Detrended Correspondence Analysis (DCA) was used to determine if unimodal or linear models were best suited for the data obtained from the three different benthic algal metrics (benthic algal community composition, diatom community composition and pigment concentrations). The DCA was run with detrending by linear segments. Gradient lengths of 1.5 to 2.2 standard deviation units were found for benthic algal metrics in both 2008 and 2009. Since unimodal methods (Correspondence Analysis (CA), Canonical Correspondence Analysis (CCA)) are considered to be a robust method for percentage data with gradient lengths ≥ 1.5 SD (Birks, 2010), we used CA to assess patterns of variation in community structure and CCA to assess their associations with physical and chemical variables. As with PCA, the physical variables were included in the CCA ordinations as supplementary data. Because the number of physical and chemical variables exceeded the number of sites, we needed to reduce the number of variables used in the CCA ordinations. To do this, individual CCAs were run with a single variable at a time, and Monte Carlo permutation tests (with 999 random permutations) were run to identify the variables that accounted for significant directions of variance in the benthic algal metrics (at $\alpha = 0.05$). Only those variables which were significant along the first axis were included in further CCAs. Then, a series of CCA ordinations were run starting with one that included all the significant chemical variables. The variable with the highest Variance Inflation Factor (VIF) above 20 was eliminated. This was repeated until all remaining variables had VIFs ≤ 20. Using this method, subsets of chemical and supplemental physical variables were selected for CCA ordinations of benthic algal community composition, diatom community composition, and pigment concentrations, separately for both study years (Appendix B, Table 3.1). Prior to all ordinations that involved the physical variables (PCAs

and CCAs), categorical environmental variables (e.g., stream order, sediment type and ecoregions) were converted to dummy variables. Sites 40, 48, 53 and 73 were outliers based on analyses of the pigment data and were removed from all analyses (CA, CCA, ANOSIM and SIMPER) in order to readily observe trends in the data.

One-way ANOSIM tests were performed on data from each of the three benthic algal metrics (benthic algal community composition, diatom community composition, and pigment concentrations) to determine if they identify differences in benthic algal communities between sites in the Selwyn Mountain ecoregion versus the Nahanni-Hyland ecoregions. For the ANOSIM tests that resulted in significant differences, Similarities of Percentages (SIMPER) analyses were used to identify the main taxa (benthic algal families or classes, diatom taxa or pigments) that contributed to the differences between the Selwyn Mountain and Nahanni-Hyland ecoregions. The main taxa accounting for differences between the ecoregions were identified as those that contributed > 2% to the average within-group similarity and >2% to the average between-group dissimilarity, following the criteria of Sokal et al., (2008). One-way ANOSIM tests and SIMPER analyses involving the benthic algal metrics were based on Bray-Curtis similarity coefficients. P-values were computed for each test by comparing the distribution of within- and across-group rank Bray-Curtis similarities (9999 computations) to the initial rank similarity, as reported by the global R value (Clarke & Gorley; 2006, Clarke & Warwick, 2001).

Two analyses were performed to determine the strength of the three metrics in differentiating between the two ecoregions. Mahalanobis or generalized distances were calculated between the two sets of reference sites for each algal metric to determine the dissimilarity between the two ecoregions (Legendre & Legendre, 1998). RELATE analyses

were used to determine if sample scores for all combinations of algal metrics (i.e., benthic algal community composition and diatom community composition; benthic algal community composition and photosynthetic pigment concentrations; diatom community composition and photosynthetic pigment concentration) shared similar structure (i.e., were correlated). All analyses (Mahalanobis distances and RELATE analyses) were performed separately for 2008 and 2009. RELATE analyses used spearman ranked-correlation tests with 999 random permutations to determine correlations between sites using data matrices (Bray-Curtis matrices for biological data, Euclidean distances for environmental data; Clark and Warwick 2001).

For the above numerical analyses (CA, CCA, ANOSIM, SIMPER, Mahalanobis distances, and RELATE), benthic algal community composition data and diatom community composition data (percent abundances) were square-root transformed to down-weight the influence of the most abundant taxa, whereas pigment concentrations were log(x+1) transformed to equalize variances. The ANOSIM tests and SIMPER analyses were performed using the software PRIMER version 6 (Clark & Gorley, 2006). Ordinations by PCA, CA and CCA were performed using CANOCO version 4.5 software (ter Braak & Šmilauer, 2002). Kolmogorov-Smirnov tests, Mahalanobis distances, and one-way ANOVA tests were performed using the software SPSS version 11. Statistical tests were considered significant if $p \leq 0.05$ and marginally significant if $0.1 \leq p > 0.05$.

3.4 Results

3.4.1 Meteorological conditions

In 2008, mean monthly air temperature was at or just below the long-term average from June through September and monthly precipitation was below the long-term average during July, August, and September (Figure 3.2). Precipitation in July 2008 was nearly double that of July

2009. In 2009, mean monthly air temperature was at or above the long-term average from May to September, while precipitation was approximately equal to the long-term average for every month, except for July when precipitation was about 25 mm below the long-term average. The warmer and dryer conditions in July 2009 coincided with higher incidence of fires within the watershed compared to 2008.

3.4.2 Physical and chemical analyses

Sites within the South Nahanni River watershed were characterized by broad ranges of nutrient concentrations, conductivity and turbidity, and by relatively high concentrations of metals. Ranges of TP (0.5 - 13.5 µg/L), TN (43 - 349 µg/L), NO_2+NO_3 (12 - 217 µg/L), DIC (1.1 - 56 mg/L) and DOC (0.5 - 6 mg/L) were considerable over the course of the two study years. The upper ranges of some metal concentrations exceeded the CCME guidelines for aquatic life in both years (e.g., Al, Cd, Cu, Fe, Se, and Zn). The metals Ni and W exceeded the guideline in 2008 only. The pH ranged from 4.9 to 9.1 across the watershed with an average value of 8.4. Conductivity ranged from 45 to 411 µS/cm, and turbidity ranged from 0.9 to 21 NTU.

Sites within the Selwyn Mountain ecoregion had median concentrations of some metals that were 1.3- to 9.6-fold higher than sites within the Nahanni-Hyland ecoregions (e.g., Al, As, Be, Cd, Co, Fe, Mn, Ni, W, Zn; Table 3.1). Box plots of metals showed that for most metals there was overlap in the interquartile ranges (middle 50% of the data) between ecoregions, however that concentrations were often higher and had greater range in the Selwyn Mountain ecoregion compared to the Nahanni-Hyland ecoregion, while concentrations of TP did not differ between ecoregions (Figure 3.3 & 3.4). The Selwyn Mountain ecoregion was also characterized by 1.7-fold greater median slope and altitude, and 168- fold higher median percent ice coverage (Table 3.2). Box plots of physical variables showed that although the interquartile ranges for

percent intrusive and sedimentary bedrocks overlapped between ecoregions, the Selwyn Mountain ecoregion typically had higher amounts of intrusive bedrock and less sedimentary bedrock compared to Nahanni-Hyland ecoregions (Figure 3.5). The Nahanni-Hyland ecoregions had 1.3- to 3.9-fold higher median concentrations of dissolved carbon (DIC, DOC), NO_2NO_3 and TN, and 1.4- to 3.6-fold higher median concentrations of some metals (e.g., Ba, Cr, Mo, Pb, Sr, U, V) (Table 3.1). Box plots of nutrients such as TN, NO_2NO_3 and DIC, and pH, conductivity and turbidity show that the interquartile ranges between the ecoregions only overlap for turbidity, but that values were consistently higher in the Nahanni-Hyland ecoregions than the Selwyn Mountain ecoregion (Figure 3.4). The Nahanni-Hyland ecoregions was also characterized by 3.0-fold larger median drainage area, 1.9-fold larger median perimeter of upstream drainage area, 1.6-fold higher median percent forest cover, and 1.4 to 1.7-fold larger median bankfull and wetted stream widths than the Selwyn Mountain ecoregion (Table 3.2). Boxplots of physical chemical variables showed that although interquartile ranges overlap for drainage areas, perimeters of upstream drainage areas, bankfull widths and maximum velocities, values were consistently higher in the Nahanni-Hyland ecoregions compared to Selwyn Mountain ecoregion (Figure 3.5). Several physical and chemical variables differed significantly (One-way ANOSIM: $p \leq 0.05$) between ecoregions in both years (2008: DIC, TN, NO_2NO_3, Ba, Mo, Rb, Se, Sr, U, V, Zn, pH, conductivity, altitude, percent gravel, percent forest cover, percent ice cover, perimeter, slope, stream density, stream order, pools; 2009: DIC, TN, NO_2NO_3, As, B, Ba, Cd, Ce, Co, Cs, La, Li, Mn, Mo, Ni, Se, Sr, U, W, Zn, pH, conductivity, altitude, bankfull minus wetted width, drainage area, percent boulder, percent forest cover, percent ice cover, vegetation, slope, wetted width, stream order, macrophytes, embededness). Some other physical and chemical variables differed marginally significantly

(One-way ANOVA tests: $0.1 \leq p > 0.05$) between ecoregions in both years (2008: Sb, deciduous trees, grasses/ferns; 2009: max depth, perimeter, stream density).

Chemical conditions of the streams differ significantly between the two ecoregions in both study years (One-way ANOSIM: 2008: Global R = 0.167, $p < 0.01$; 2009: Global R = 0.533, $p < 0.01$). Ordination by PCA revealed that sample scores based on physical and chemical conditions differed between river sites of the Selwyn Mountain and Nahanni-Hyland ecoregions (Figure 3.6). Eigenvalues for the 1st and 2nd axes were 0.419 and 0.197 in 2008 and 0.472 and 0.151 in 2009, explaining 61.6% and 62.3% of the total variation among sites, respectively. In 2008 and 2009, sites within the Selwyn Mountain ecoregion were characterized by relatively higher concentrations of several metals including, Al, As, Ce, Co, Cs, Cu, Li, Ni, Mn, and W, and TP. These chemical variables were associated with relatively higher slopes, stream densities, percent ice, latitudes, percent intrusive bedrock, June mean temperature, and percent pools and straight runs. In 2008 and 2009, the Nahanni-Hyland ecoregions were characterized by higher concentrations of some metals including B, Ba, Mo, Sb, Se, Sr, U, and V and nutrients including DIC, NO_2NO_3, TN and conductivity and pH. These chemical variables were associated with higher drainage areas, bankfull and wetted widths, percent sedimentary bedrock, percent forested land cover, and total rain, precipitation and snow. Although there were many similarities in chemical variables between years, there were also some differences. For example, in both 2008 and 2009 sites with high turbidity (primarily within the Selwyn Mountain ecoregion) had relatively high TP concentration but relatively low TN, NO_2NO_3 and pH. In 2008, Cu and Pb were positively correlated with metals such as Cd, Li, Ni, and Zn, but in 2009 they were not. In 2008, percent intrusive bedrock and percent sedimentary bedrock were the most closely correlated with PCA axis 1. In 2009,

percent intrusive and sedimentary bedrock did not account for a significant amount of variation along PCA axis 1, however the patterns in other physical and chemical variables remained similar to 2008.

3.4.3 Benthic algal metrics

3.4.3.1 Benthic algal community composition

Based on taxonomic analysis of benthic algae, communities at stream sites within the South Nahanni River watershed consisted mainly of diatoms (2008: 5 - 99%, 2009: 27 - 99%), cyanobacteria (2008: 0 - 71%, 2009: 0 - 23%) and green algae and charophytes (2008: 0 - 12%, 2009: 0 - 56%). Ordination by CA showed differences in composition of benthic algal communities between the Selwyn Mountain and Nahanni-Hyland ecoregions (Figure 3.7). There was overlap between sites in each ecoregion in 2008, however, sites within the Selwyn Mountain ecoregion generally plotted to the right along axis 1, associated with higher relative abundance of Oedogoniaceae, Oscillatoriaceae, Phormidiaceae, Scenedemaceae, and Zygnemataceae. Sites within the Nahanni-Hyland ecoregions generally were positioned further to the left along axis 1, associated with higher relative abundance of Bacillariophyceae, Desmidiaceae, and Euglena species. Despite fewer sites sampled in 2009, generally similar patterns of benthic algal community composition were observed in both years but with greater separation between the ecoregions. Consistent with results of ordination by CA, ANOSIM tests identified that benthic algal communities differed significantly between the two ecoregions (2008: Global $R = 0.272$, $p < 0.01$; 2009: Global $R = 0.382$, $p = < 0.01$). Based on results of a SIMPER analysis, Oscillatoriaceae and Phormidiaceae were identified as indicator families of the Selwyn Mountain ecoregion in 2008, and Merismopediaceae and Oscillatoriaceae were identified as indicator families of the Selwyn Mountain ecoregion in 2009. However, none of

the algal families met criteria as indicator taxa for the Nahanni-Hyland ecoregions in either year (Table 3.3).

3.4.3.2 Diatom community composition

Diatom communities within the South Nahanni River watershed were dominated by *Achnanthidium minutissimum* (2008: 0.4 - 88%, 2009: 11 - 94%), *Hannaea arcus* (2008: 0 - 94%, 2009: 0 - 54%), *Gomphonema micropus* (2008: 0 - 83%, 2009: 0.6 - 25%), *Fragilaria capucina* var. *gracilis* (2008: 0 - 65%, 2009: 0 - 9%), and *Diatoma tenuis* (2008: 0 - 23%, 2009: 0.4 - 30 %). Based in ordinations by CA of the 2008 data, sample scores from several sites in the Nahanni-Hyland ecoregions were positioned low along axes 1 and 2 relative to sites from the Selwyn Mountain ecoregion, although there was considerable overlap among sample scores from the two ecoregions (Figure 3.8). Separation of sample scores was greater between the ecoregions in 2009, when fewer sites were sampled. In 2008 and 2009, sites from Selwyn Mountain ecoregion had higher relative abundance of taxa belonging to *Encyonema*, *Eunotia*, *Fragilaria, Nitzschia*, *Planothidium, Staurosirella,* and *Sellaphora*, whereas sites in the Nahanni-Hyland ecoregions had higher relative abundance of *Gomphonema* taxa. One-way ANOSIM tests demonstrated that diatom community composition differs significantly between the two ecoregions (2008: Global $R = 0.194$, $p < 0.01$; 2009: Global $R = 0.551$ $p < 0.01$). Based on SIMPER analysis, *F. capucina* var. *gracilis* was identified as an indicator for the Selwyn Mountain ecoregion in 2008, and *E. minutum, E. silesiacum, F. capucina* var. *gracilis, N. palea* and *S. parvus* were indicator taxa in 2009. For the Nahanni-Hyland ecoregions, *G. species 1* (Appendix B, Figure 3.1) and *S. ulna* were identified as indicator taxa in 2008, and *Cymbella affinis* and *G. species 1* were indicator taxa in 2009 (Table 3.3).

3.4.3.3 Photosynthetic pigment concentration

Photosynthetic pigment concentrations in the samples from the South Nahanni River watershed were dominated by chlorophyll-*a* (2008: 0.002 - 0.6 µg pigment/cm^2, 2009: 0 - 0.3 µg pigment/cm^2), fucoxanthin (2008: 0 - 0.3 µg pigment/cm^2, 2009: 0 - 0.2 µg pigment/cm^2), chlorophyll-*b* (2008 & 2009: 0 - 0.2 µg pigment/cm^2), chlorophyll-*a*' (2008: 0 - 0.1 µg pigment/cm^2, 2009: 0 - 0.2 µg pigment/cm^2), aphanizophyll (2008 & 2009: 0 - 0.1 µg pigment/cm^2), and lutein-zeaxanthin (2008 & 2009: 0 - 0.1 µg pigment/cm^2). In 2008, there was extensive overlap between the ecoregions, and differences could not be discerned (Figure 3.9). Overlap of sample scores remained considerable in 2009 for the two ecoregions. However, sites from the Selwyn Mountain ecoregion tended to cluster towards the right along axis 1, associated with relatively higher concentrations of aphanizophyll, chlorophyll-*a*, chlorophyll-*c*2, fucoxanthin, and myxoxanthophyll. Sites from the Nahanni-Hyland ecoregions were characterized by higher concentrations of alloxanthin, β-carotene, and phaeophytin-*a* than most sites from the Selwyn Mountain ecoregion. In 2008, abundance and composition of pigments did not differ significantly between the ecoregions (one-way ANOSIM test, Global R = 0.048, p = 0.111). However, in 2009 abundance and composition of pigments differed significantly between the ecoregions (one-way ANOSIM test, R = 0.295, $p < 0.01$). Results of the SIMPER analysis showed that in 2008, chlorophyll-*a*' was an indicator pigment for stream sites with the Selwyn Mountain ecoregion, and fucoxanthin was an indicator pigment for stream sites within the Nahanni-Hyland ecoregions. In 2009, aphanizophyll, chlorophyll-*a*' and phaeophytin-*b* were indicator pigments for stream sites in the Selwyn Mountain ecoregion, and β-carotene was the only indicator pigment of the Nahanni-Hyland ecoregions.

3.4.4 Relations between benthic algal metrics and physical and chemical conditions

3.4.4.1 Benthic algal community composition

Ordination by CCA was used to assess relations between benthic algal community composition and variations in physical and chemical conditions among sites. Eigenvalues along the first and second axes were 0.183 and 0.107 in 2008, respectively, and 0.118 and 0.075 in 2009 (Figure 3.10). They explained 29.0% and 19.3% of the total variation among sites in 2008 and 2009, respectively.

The relative positions of the sample scores remained similar in the CA ordination of the benthic algal community composition data (Figure 3.7) and the corresponding CCA of the benthic algal community composition data and water chemistry variables (Figure 3.10), indicating that the measured chemical variables captured important gradients of variation in the algal communities among sites. Consistent with this, species-environment correlations were high for CCA axes 1 and 2 (0.94 and 0.0.89, 0.82 and 0.79, for axis 1 and 2 in 2008 and 2009 respectively). Sample scores for sites in the Selwyn Mountain ecoregion were located primarily in the right half of the ordination, characterized by higher percent abundance of filamentous and colonial cyanobacteria (e.g., Oscillatoriaceae, Phormidiaceae, Merismopodiaceae and Microcystaceae), and green algae and charophytes (Zygnematales, Chaetophoraceae, Odedogonales, Ulotrichales) and higher concentrations of TP and metals (e.g., As, Cu, Fe, Ni, W, Zn), steeper slope, higher proportions of intrusive bedrock and ice, and higher mean June temperature. They were generally well separated from the sample scores for sites in the Nahanni-Hyland ecoregions, which were positioned mainly in the left half of the CCA ordinations. Sites in the Nahanni-Hyland ecoregions were characterized by higher percent abundance of Bacillariophyta, Desmidaceae, Euglenoids, and Nostocaceae (2009 only) and

higher concentrations of TN, NO_2+NO_3, DIC, higher conductivity, and pH, higher concentrations of metals such as Sr, Mo, and U, higher percentage of sedimentary bedrock and forest cover, and larger bank-full width, and maximum flow velocity.

3.4.4.2 Diatom community composition

Ordination by CCA of diatom taxon relative abundances and the physical and chemical data captured 37.6% (λ_1 = 0.243, λ_2 = 0.133) and 35.2% (λ_1 = 0.22, λ_2 = 0.131) of the total among-site variation in 2008 and 2009, respectively (Figure 3.11). The relationships between the distribution of diatom taxa and physical and chemical variables were consistent between years. Indeed, the relative positions of sample scores remained fairly consistent between the CCA and corresponding CA, suggesting the supplied environmental variables explain variations in diatom community composition among the sites (species-environmental correlations: 0.97 and 89, 0.99 and 0.95 for axis 1 and 2 in 2008 and 2009 respectively) (Figures 3.8, 3.10). The majority of the sites within the Selwyn Mountain ecoregion were positioned toward the upper right portion of the CCA diagrams in 2008 & 2009 and were characterized by relatively higher abundance of several taxa belonging to the genera *Eunotia*, *Encyonema*, *Fragilaria*, *Nitzschia*, *Planothidium*, and *Sellaphora*, among others. These taxa were typically Species in the Selwyn Mountain ecoregion (located toward the upper right portion of the ordination) were associated with higher concentrations of TP, higher turbidity, percentages of ice, latitude, depth, stream density and macrophyte presence, and steeper slope. A few sites within the Selwyn mountain ecoregion were located in the upper left portion of the CCA ordination in 2008. These sites possessed higher relative abundance of *Cyclotella comensis* than all other sites, and were associated with lower concentrations of TP, turbidity, and metals. In contrast, species in the Nahanni-Hyland ecoregions were positioned toward the lower left portion of the ordinations in

2008 & 2009, and were characterized primarily by higher relative abundance of taxa in the genus *Gomphonema*. Communities at these sites were typically associated with higher concentrations of NO_2+NO_3, DIC, and higher conductivity, pH, flow velocity, bank-full and wetted widths, percent boulder, larger drainage areas, and higher percentage of forest cover.

3.4.4.3 Photosynthetic pigment concentration

Ordination by CCA of pigment and physical and chemical data captured 22.7% ($\lambda_1 = 0.165$, $\lambda_2 = 0.062$) and 25.3% ($\lambda_1 = 0.177$, $\lambda_2 = 0.076$) of the total variation in 2008 and 2009, respectively (Figure 3.12). In contrast to the CA ordinations, there were distinct differences between ecoregions when pigment data were constrained by chemical variables. Species-environmental correlations were lowest in each respective year compared to benthic algal and diatom community composition (species environmental correlations: 0.92 and 0.94, 0.76 and 0.63 for axis 1 and 2 in 2008 and 2009 respectively). Based on the data from 2008, sites located within the Selwyn Mountain ecoregion had higher relative abundances of pigments such as β-carotene, chlorophyll-*a*, echinenone, and myxoxanthophyll associated with higher concentrations of metals such as W and Zn, higher amounts of macrophytes, pools, average velocities, and percent intrusive bedrock. Sites located within the Nahanni-Hyland ecoregions had higher relative abundances of diatoxanthin, diadinoxanthin, fucoxanthin, and phaeophytin-*a*, associated with higher concentrations of metals (e.g., Be and Sr), turbidity, TN, NO_2NO_3, higher amounts of percent cobble, conifers, and grasses/ferns. In 2009, sites along the left side of axis 1 were mainly located within the Nahanni-Hyland ecoregions and had higher relative concentrations of pigments such as alloxanthin, okenone, β-carotene, and phaeophytin-*a*, associated with lower concentrations of metals Li and Zn. Sites to the right along axis 1 tended to occur within the Selwyn Mountains ecoregion and had higher relative concentrations of

aphanizophyll, chlorophyll-*c*2, chlorophyll-*a*, chlorophyll-*a*', and myxoxanthophyll, associated with higher concentrations of Li and Zn, and higher % gravel.

3.4.5 Comparison of algal metrics

Diatom community composition was greatest with Mahalanobis distances of 2204.2 and 20843.0 for 2008 and 2009, respectively. Mahalanobis distances for benthic algal community composition (6.3 and 160.1 for 2008 and 2009 respectively) and photosynthetic pigment concentrations (3.9 and 301.2 for 2008 and 2009 respectively) were both lower than diatom community composition distances. RELATE analyses were used to identify which groups shared more similar structures (or were correlated). Results of RELATE analyses showed that benthic algal community composition was significantly correlated to diatom community composition in 2008 (Rho = 0.269, $p < 0.01$), whereas photosynthetic pigment concentrations structure were not (Rho = 0.07, p = 0.13). In 2009 the opposite was true, where photosynthetic pigment concentration was significantly correlated to diatom community composition (Rho = 0.253, p = 0.05) and benthic algal community composition was not (Rho = 0.024, p = 0.41). This was consistent with the Mahalanobis distance results in that benthic algal community composition had a larger Mahalanobis distance in 2008 compared to photosynthetic pigment concentration and *vise-versa* in 2009. RELATE analyses of benthic algal community composition and photosynthetic pigment concentration showed that they did not share similar structure in either 2008 or 2009 (Rho = 0.05, p = 0.18 in 2008; Rho = 0.15, p = 0.14 in 2009).

3.5 Discussion

How we define reference conditions is central to identifying human impacts in aquatic ecosystems and to understand the natural variability among sites (Hawkins et al., 2010). Our

study sampled sites that were relatively unaffected by human influences (no adjacent or upstream activity) and thus allowed us to explore natural factors regulating physical and chemical conditions and benthic algal communities. This study shows that even in the absence of upstream human disturbances, the physical and chemical conditions vary widely among the reference stream sites within the South Nahanni River watershed. Ecoregions have been proposed as an *a priori* way to group study sites; a concept that assumes that catchment characteristics strongly influence aquatic ecology (Hynes, 1975; Johnson, 1999; Hawkins et al., 2000; Johnson, 2000). Physical and chemical conditions differ distinctly between the ecoregions in our study sites. Many physical variables appear to influence the differences between chemical conditions and biological communities in different ecoregions including: bedrock geology (% sedimentary, % intrusive), landcover (% ice, % forest), slope, and latitude. Previous studies have found that differences in biological groups can be associated with physical and chemical differences among sites such as geology, vegetation, landforms, and nutrients (e.g., Leland, 1995; Johnson, 1999; Johnson 2000; Leland & Porter, 2000, Neff & Jackson, 2011). In the absence of anthropogenic land use, bedrock geology can be a significant contributor to differences in water chemistry and biological communities (Leland, 1995; Leland & Porter, 2000). In our study, differences in geology (percent intrusive and percent sedimentary bedrock) were highly correlated with PCA axis 1. The bedrock found within the southeast of the watershed (Nahanni-Hyland ecoregions) is comprised primarily of limestone/dolostone and shale, while the bedrock found within the northwest of the watershed (Selwyn Mountain ecoregion) is comprised primarily of shale and cretaceous plutons (Caron et al., 2008). These underlying differences in bedrock are associated with differences in concentrations of nutrients and metals among sites. For example, the Selwyn Mountain

ecoregion is characterized by higher concentrations of metals such as Al, As, Be, Co, Mn, Ni, W, and Zn and TP and the Nahanni-Hyland ecoregions are characterized by higher concentrations of nutrients such as TN, NO_2NO_3, and DIC and pH. Typically, TN and TP are correlated; however in our study TN and TP were uncorrelated in both 2008 and 2009. Previous studies in the South Nahanni River watershed had found that TP appeared to be correlated with turbidity suggesting that it was associated with suspended sediments in the water column and possibly unavailable for biological regulation [Scrimgeour, 2013; Thomas et al., 2013 (Chapter 4)].

Algal metrics differ significantly between the two ecoregions and are closely associated with gradients of nutrients, metals, and physical conditions in the adjacent watershed. Other studies have assessed relationships of benthic algae to chemical and physical variables at varying scales and found that natural differences in watershed characteristics (e.g., ecoregion, geology, conductivity, and vegetation) explain much of the variability in these communities (Briggs, 1990; Leland, 1995; Leland & Porter, 2000; Antoniades et al., 2009; Tornés et al., 2012). Algae are also known to be sensitive to changes in nutrient concentrations (Resh, 2008). In fact, differences in nutrients across the South Nahanni River watershed (i.e., TN, NO_2NO_3, and DIC/DOC in Nahanni-Hyland ecoregions and TP in Selwyn Mountain ecoregion) are associated with differences in benthic algal communities. The goal of every basic monitoring program is to select sites representative of the possible 'reference' biological communities present for best comparison with possibly affected test sites. Moreover, the RCA assumes that biological communities are influenced by the physical and chemical conditions of the surrounding environment (Reynoldson et al., 1997; Bailey et al., 2004). This study shows that the benthic algal communities in the South Nahanni River watershed are influenced by the

physical and chemical conditions of their surrounding environment and thus the algal metrics show promise for use in an RCA monitoring design in this area. These important differences in biological communities and their associations with physical and chemical variables can also be used to help guide how reference sites should be selected in future studies within the South Nahanni River watershed.

The main characteristics of lasting monitoring programs include: 1) inexpensive programs that will survive budget cuts; 2) simple programs that can be carried out by multiple personnel without compromising quality of data, and 3) measurements taken must be sensitive to changes in the surrounding environment (Schindler, 1987). Benthic algal community composition and diatom community composition metrics have both been used in algal river monitoring studies [Winter & Duthie, 2000; Lavoie et al., 2006; Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2013 (Chapter 4)]. However, they do require a large investment in time and money for taxonomic identification of the algal taxa. Photosynthetic pigment concentrations on the other hand is a relatively new metric for algal river monitoring, but is more time- and cost-effective [Thomas et al., 2013 (Chapter 4)]. Schindler (1987) pointed out that measures of ecosystem function were often not sufficiently sensitive to detect the early signs of perturbation by anthropogenic stressors, whereas measures of community composition were often the most sensitive indicators of ecosystem change. In our study, we show that diatom community composition is the most sensitive metric to differences in water physical and chemical conditions between the two ecoregions, and photosynthetic pigment concentrations (measure of ecosystem function) shows promise as an algal biomonitoring metric as it is comparable with benthic algal community composition (a commonly used algal metric), similar to previous studies conducted within the South Nahanni River watershed [Thomas et al., 2013 (Chapter 4)]

Many studies have employed the use of coarse taxonomic biological assessments (e.g., Spencer et al., 2008; Bowman et al., 2010), diatom assessments (e.g., Reavie & Smol, 1997; Winter et al., 2003), and photosynthetic pigments [(e.g., Dorigo et al., 2004, 2007; Thomas et al., 2013 (Chapter 4)] in river monitoring studies. While all of these types of assessment require a high amount of technical training [Resh, 2008; Thomas et al., 2013 (Chapter 4)], either in taxonomic identifications (coarse taxonomic assessments, diatom assessments) or in analytical techniques for a HPLC (photosynthetic pigments), they are all routine methods and high quality data (i.e., resulting in reproducible results) can be achieved. Additionally, the metrics used in this study (benthic algal community composition, diatom community composition, and photosynthetic pigment concentration) have unique merits that make them viable options for river biomonitoring. Benthic algal community composition to a coarse level taxonomy is comparably rapid [Thomas et al., 2011 (Chapter 2)]. For diatom community composition, diatom samples are preserved on permanent microscope slides for future use in biological monitoring studies. Photosynthetic pigment concentration provides a relatively course level of taxonomic resolution (e.g., division) compared to taxonomic assessments of benthic algal communities (e.g., division to species). They can also be influenced by factors such as light supply, nutrients storage, and different algal taxa producing different quantities of pigments per cell. Thus, pigment data do not necessarily equate to relative abundances (Hill, 1996). However, despite the weaker ability of pigments to discriminate between ecoregions, Thomas et al., (2013; Chapter 4) have shown that they are a cost-effective method that provides comparable data to algal community composition. In our study, we have shown that all three of these metrics are sensitive to differences in physical and chemical conditions across the South Nahanni River watershed in one or both of our study years. However, further studies

need to assess if all these metrics are sensitive to even small differences in physical and chemical conditions downstream of mining operations when compared to reference sites (e.g., regional reference sites as part of a RCA study, or upstream sites as part of a gradient type design).

Benthic algal community structure can vary among years due to factors such as weather, grazing pressure, competition, and succession patterns (Hill, 1996). Our study was conducted over two years which had different meteorological conditions. In spring 2009, precipitation was approximately half the amount in the previous year. Lower precipitation combined with higher temperatures in 2009 could have resulted in lower discharge within the river system and thus a higher concentration of solutes in the water. As we show in Thomas et al., (2013; Chapter 4), many water chemistry variables were higher in concentration in 2009 compared to 2008 and benthic algal communities showed more distinct alterations in response to possible stressors in 2009 compared to 2008. Despite the temporal variation, our study found that relationships between algal metrics (with the exception of photosynthetic pigments) and physical and chemical variables were consistent between years. The close correspondence between algal metrics and physical and chemical conditions means that benthic algae have the potential to be able to infer water chemistry conditions, and thus can be used to track ecological changes. The data provided in this study are valuable at the present, and in the future, with other stressors such as climate change (Schindler and Smol, 2006) and long-range N-deposition (Chambers et al., 2001) becoming more influential in these remote locations.

Bioassessments require adequate characterization of natural variability to effectively assess the degree of alteration by human activities at test sites (Hawkins et al., 2010). Study designs need to describe natural variability to adequately describe community composition at

unaffected reference sites in order to be able to detect subtle changes in biological communities at test sites due to human activities and other stressors. A variety of methods have emerged from stream assessments [e.g., Control-Impact (CI), Before-After-Control-Impact (BACI), Gradient and Reference Condition Approach (RCA)] which characterize the variability in the reference condition in different ways. Control-Impact and BACI designs often use only one control site to compare to potentially impacted sites (e.g., Spencer et al., 2008). Gradient designs compare multiple upstream sites to multiple downstream sites, where each site is considered as a replicate [e.g., Rott et al., 1998; Thomas et al., 2013 (Chapter 4)]. Monitoring methods such as BACI, CI and gradient designs have been criticized for their inability to adequately characterize the reference condition against which degradation is quantified, as well as their inability to avoid pseudoreplication within rivers (Hurlbert, 1984; Cooper & Barmuta, 1993; Downes et al., 2002). Many alternative methods have been proposed including the RCA. Reference condition approach designs compare many reference sites that are similar in physiographic features (e.g., catchment size, stream order, surrounding vegetation, bedrock, particle size) to test sites. Each of the reference sites are replicates and typically collected across an entire watershed (e.g., Reece & Richardson, 1999; Rosenberg et al., 1999; Bowman et al., 2010). Regardless of the type of assessment conducted, reference sites need to share similar physical and chemical characteristics with test sites in order for researchers to assume the biological communities would be the same in reference condition (Hulbert, 1984; Reynoldson et al., 1997; Bailey et al., 2004). Our study provides the reference sites for an RCA approach and highlights the importance of differences between ecoregions on physical and chemical conditions and benthic algal communities, and the influence this can have on site selection within this watershed. Previous studies conducted within the South Nahanni River

watershed utilized CI, gradient, and RCA designs to identify environmental impacts of mining developments on biological communities [Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2013 (Chapter 4)]. Of these studies, only Bowman et al., (2010) used regional reference sites from across the watershed. They used an RCA design to assess stream condition downstream of the two mining companies within the watershed.

Despite the promising attributes of the RCA model, there are some problems with the RCA that need to be overcome. The RCA is designed to identify physical attributes that best discriminate between groups of biological communities (or reference sites) and then use these physical variables to identify group affiliation of potential test sites. Given the stark contrasts in benthic algal communities between ecoregions, the RCA should be able to effectively group reference sites based on benthic algal data appropriately within the South Nahanni River watershed and consequently assign test sites to appropriate reference groupings. However, since the RCA typically uses *a posteriori* methods of grouping reference sites (e.g., hierarchal clustering of sites based on biological community composition) the RCA has the potential to group reference sites differently. Our study can help disentangle reference groupings within the South Nahanni River watershed. The natural, inherent differences in limnological conditions and benthic algal communities between the ecoregions in the South Nahanni River watershed can potentially cause problems for the design and application of an environmental monitoring program. Selection of reference sites from a different ecoregion than where the test sites are located for example can lead to misinterpretation of results and incorrect assessments. Assessment of spatial variation among reference sites in algal communities and their relations with physical and chemical conditions could strengthen the studies employed by others in the past and studies that may occur in the future by providing confirmation of suitable reference

site selection. Using an RCA study design in northern systems could be problematic as northern ecosystems are often remote, requiring helicopter assistance to access study sites. This study could aid in reducing costs associated with RCA designs because it shows that the selection of representative reference sites need only occur within each ecoregion and not from across the entire watershed.

3.6 Figures

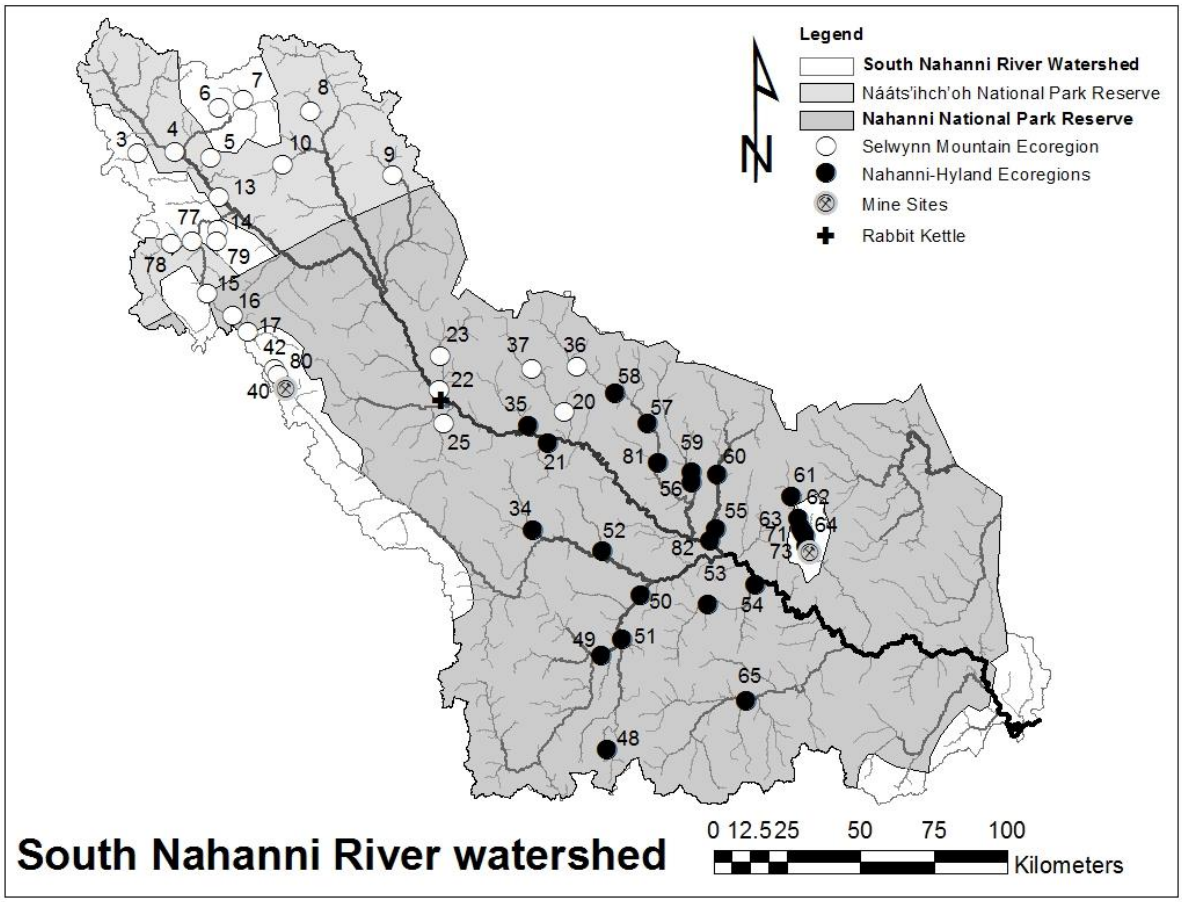


Figure 3.1 Map showing the South Nahanni River watershed and the locations of sites sampled during August of two years (2008, 2009). Sites are coded according to ecoregion: Selwyn Mountain ecoregion sites are white circles, Nahanni-Hyland ecoregions sites are solid black circles. Grey shaded areas of the map indicate the area protected by the Náátsá'ihch'oh and Nahanni National Park Reserves. Mine symbols represent the two mining companies within the South Nahanni River watershed.

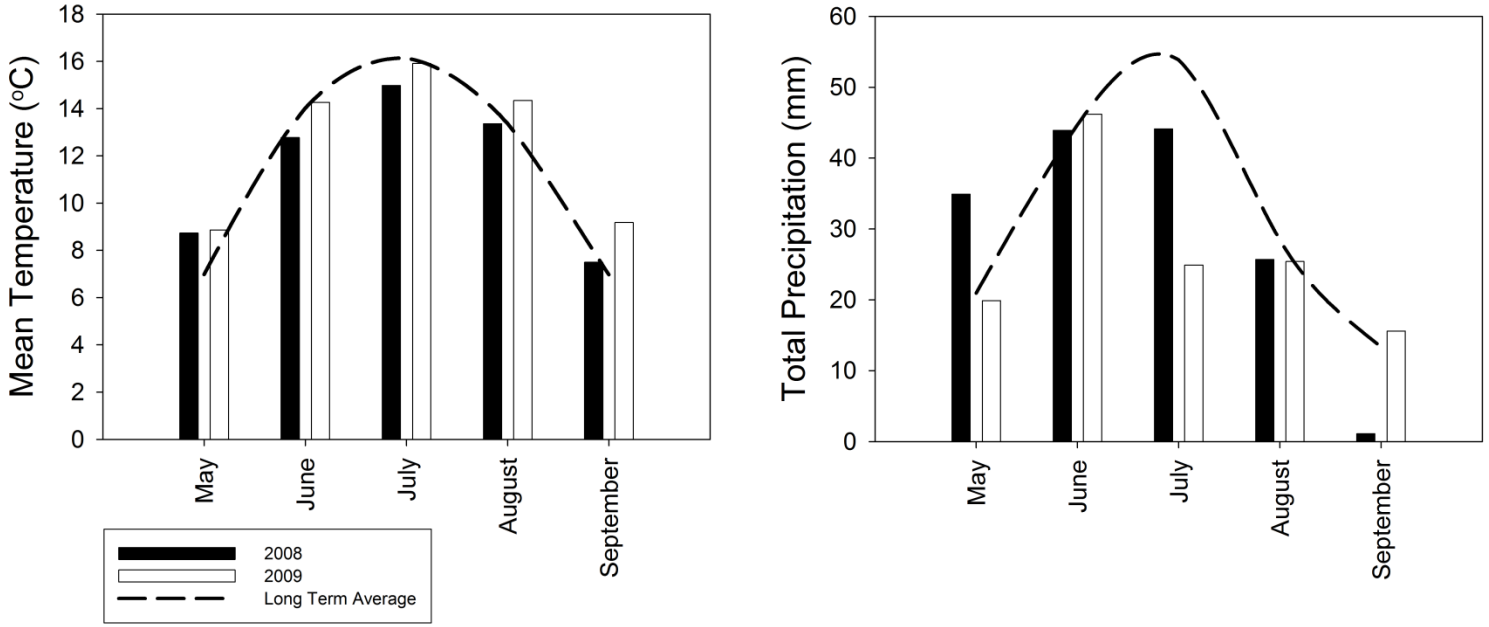


Figure 3.2 Long-term averages (May - September: 1997 & 2001 – 2007; dashed line) overlaying monthly averages (May - September) for each year (2008: black bars, 2009: white bars) of mean temperature and total precipitation data calculated from Environment Canada meteorological data collected from Rabbit Kettle, NWT station within the Nahanni National Park Reserve.

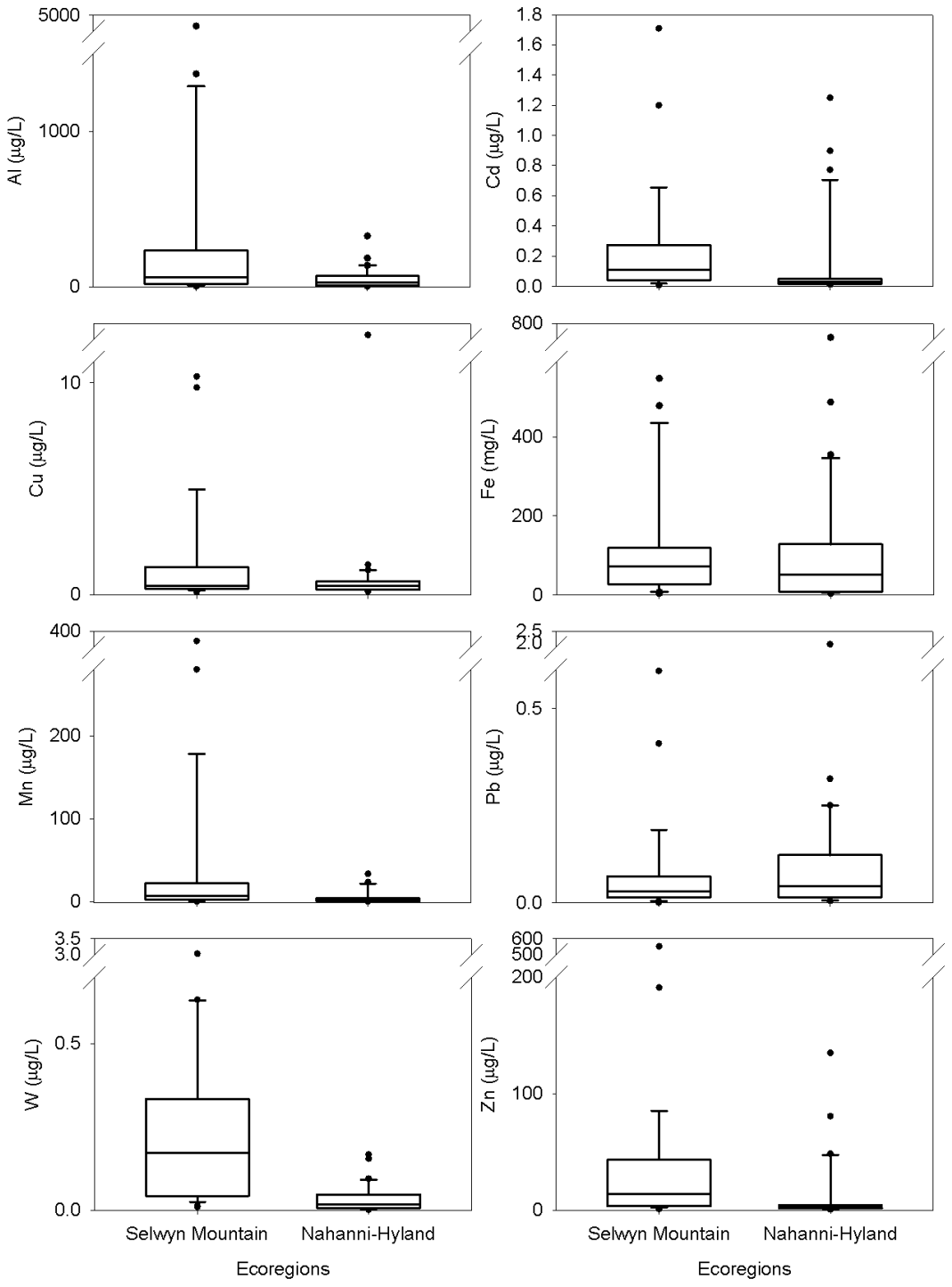


Figure 3.3 Box plots of selected metal concentrations in the Selwyn and Nahanni-Hyland ecoregions.

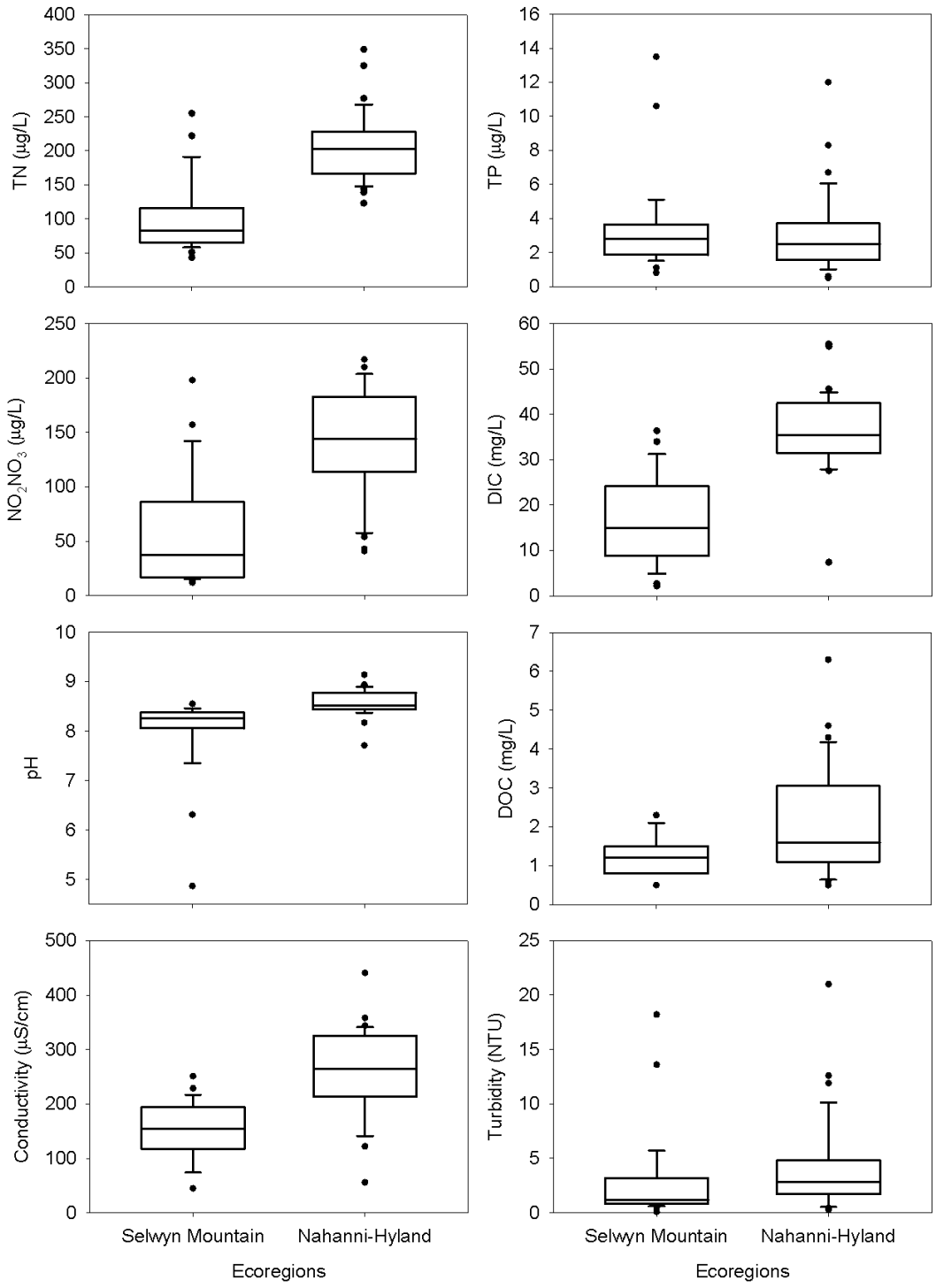


Figure 3.4 Box plots of selected nutrient concentrations and ions in the Selwyn and Nahanni-Hyland ecoregions.

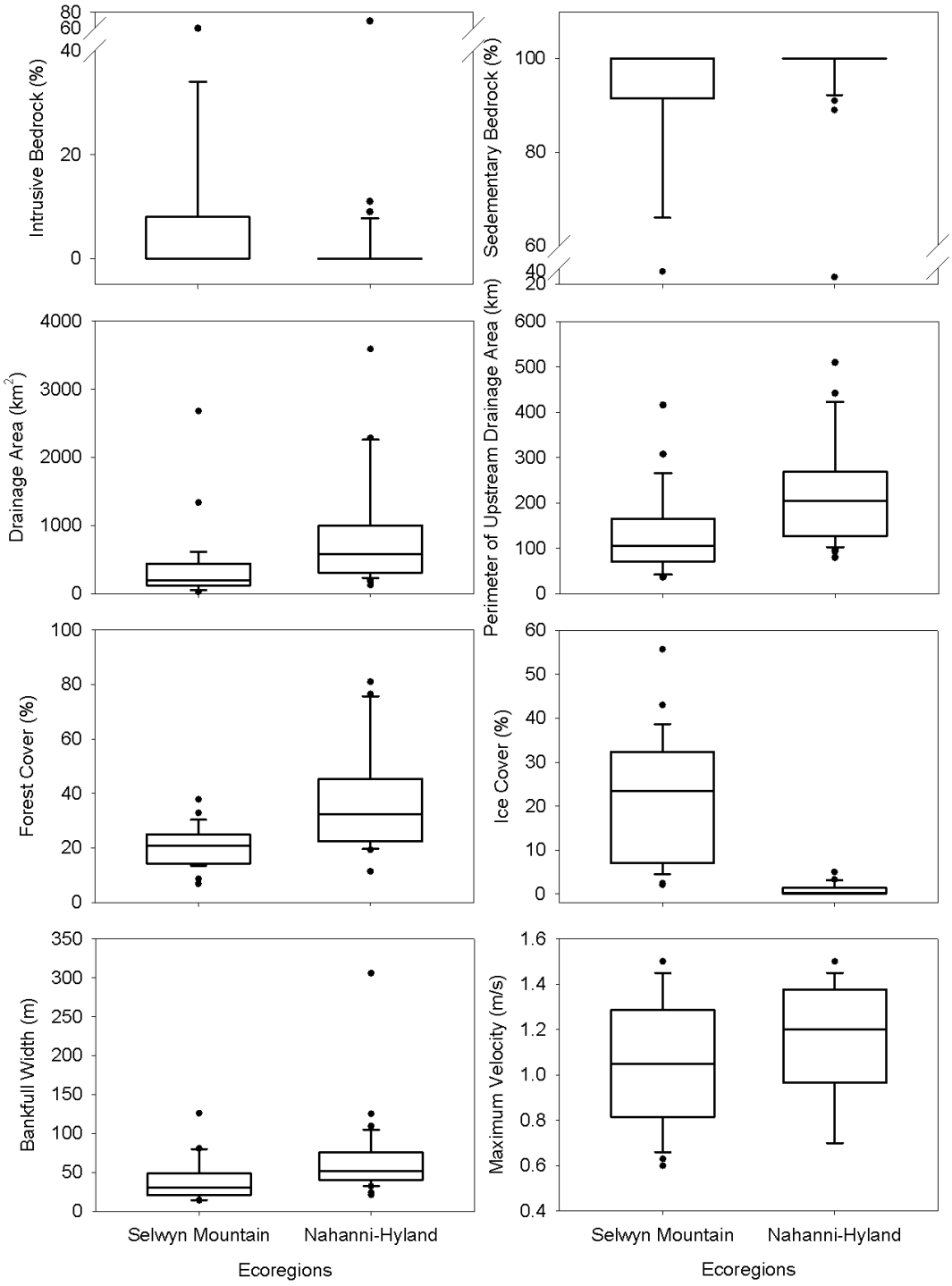


Figure 3.5 Box plots of selected physical variables in the Selwyn and Nahanni-Hyland ecoregions.

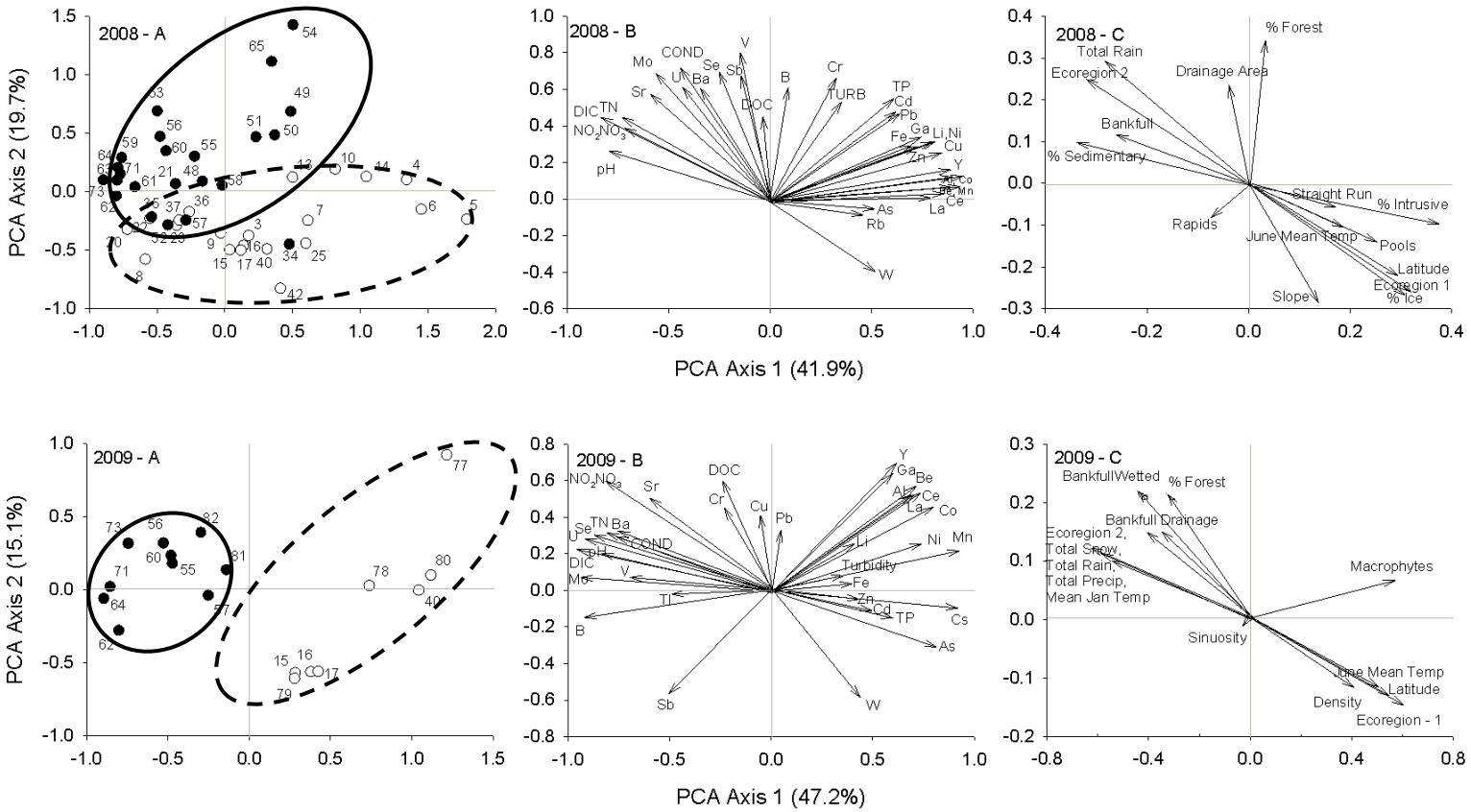


Figure 3.6 Principal components analysis (PCA) ordination plots based on water chemistry data obtained in 2008 (top panel) and 2009 (bottom panel). Panel A) displays the site scores, B) vectors for the water chemistry variables (active variables), and C) vectors for the physical variables (supplementary variables). Black circles with solid ellipses encircling them are study sites within the Nahanni-Hyland ecoregions, white circles with dashed ellipses encircling them are study sites within the Selwyn Mountain ecoregion.

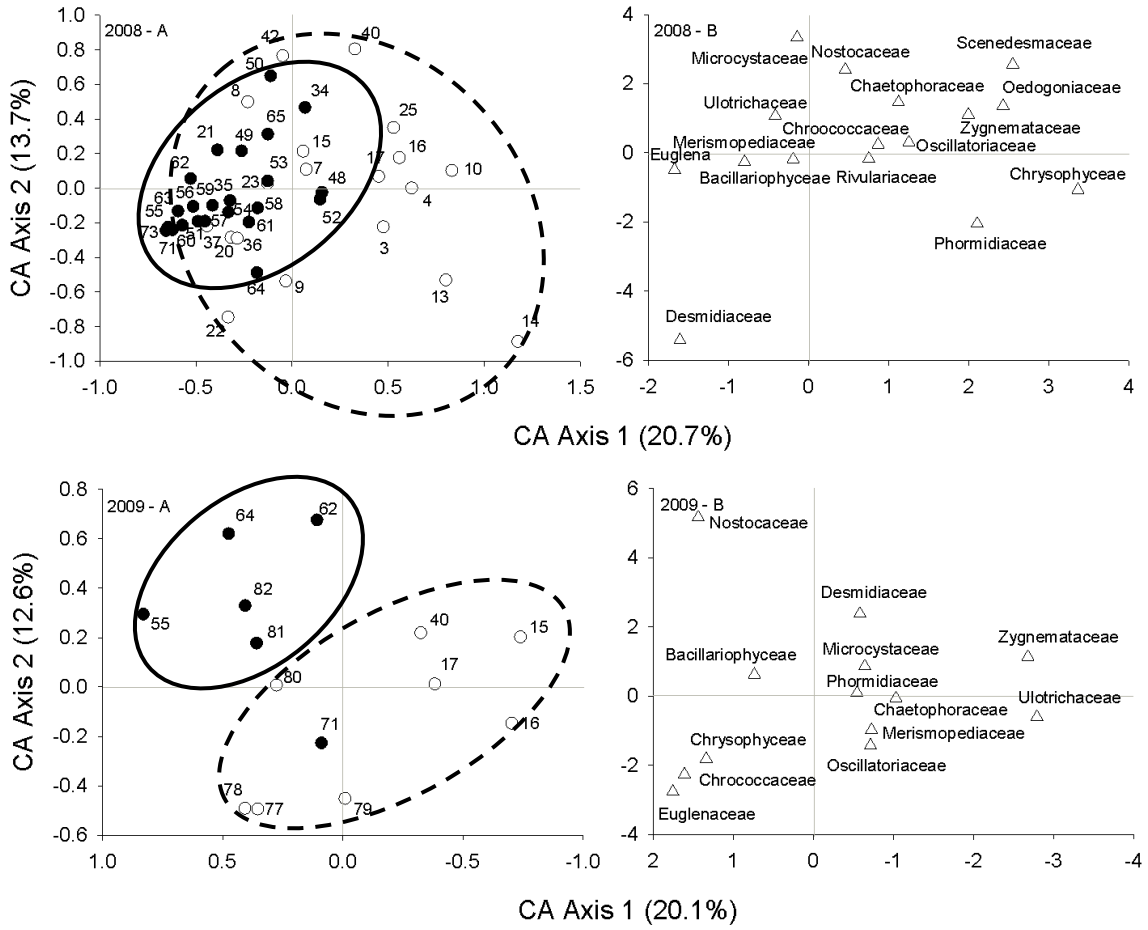


Figure 3.7 Correspondence analysis (CA) of the benthic algal community composition data obtained from the study sites in 2008 (top panel) and 2009 (bottom panel). Black circles with solid ellipses encircling them are study sites within the Nahanni-Hyland ecoregions, white circles with dashed ellipses encircling them are study sites within the Selwyn Mountain ecoregion. Open triangles in the right-hand graphs (top and bottom panels) represent taxon scores.

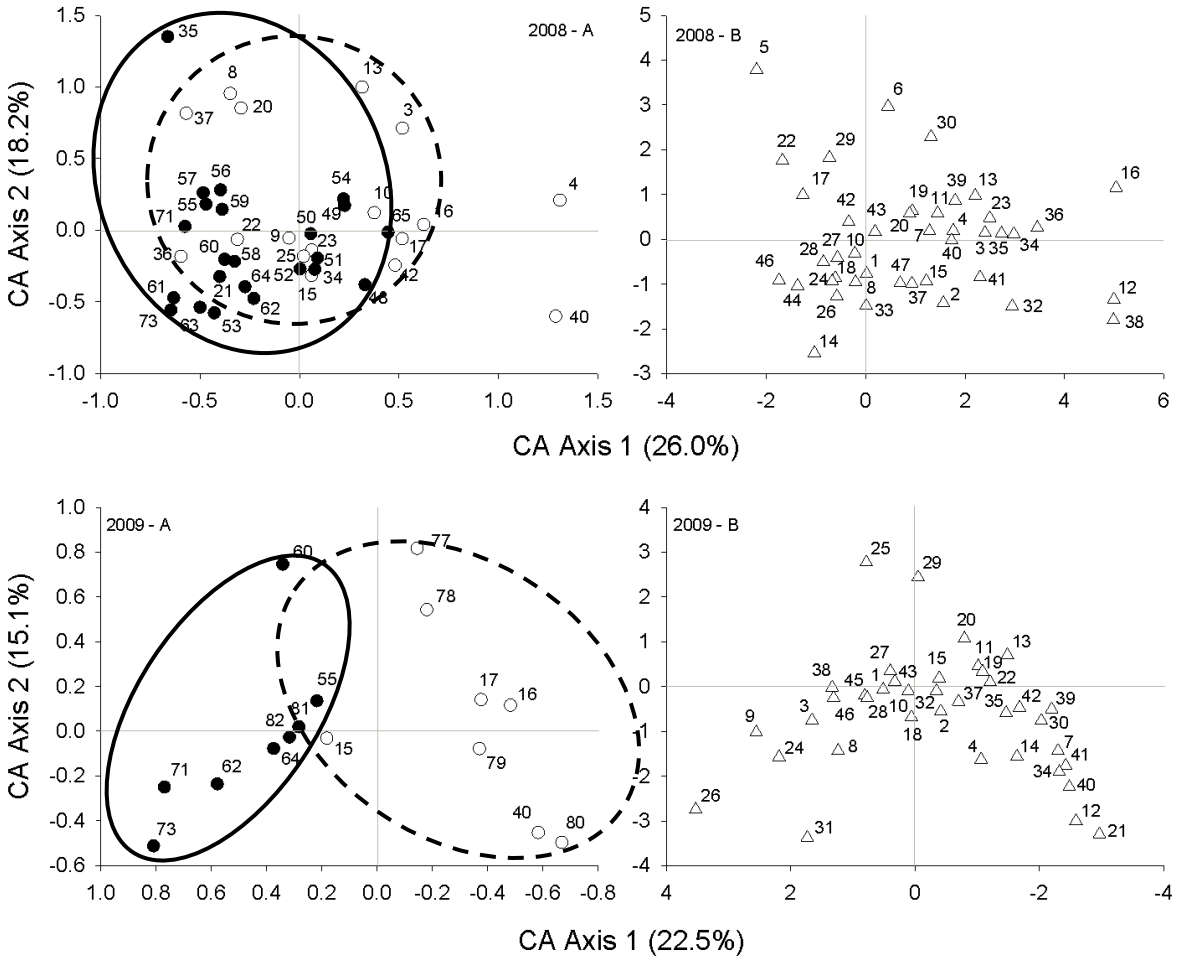


Figure 3.8 Correspondence analysis (CA) of the benthic diatom community composition data obtained from the study sites in 2008 (top panel) and 2009 (bottom panel). Black circles with solid ellipses encircling them are study sites within the Nahanni-Hyland ecoregions, white circles with dashed ellipses encircling them are study sites within the Selwyn Mountain ecoregion. Open triangles in the right-hand graphs (top and bottom panels) represent taxon scores. Corresponding diatom taxon names for the number codes presented in panel b (2008 & 2009) are located in Appendix B, Table 3.2.

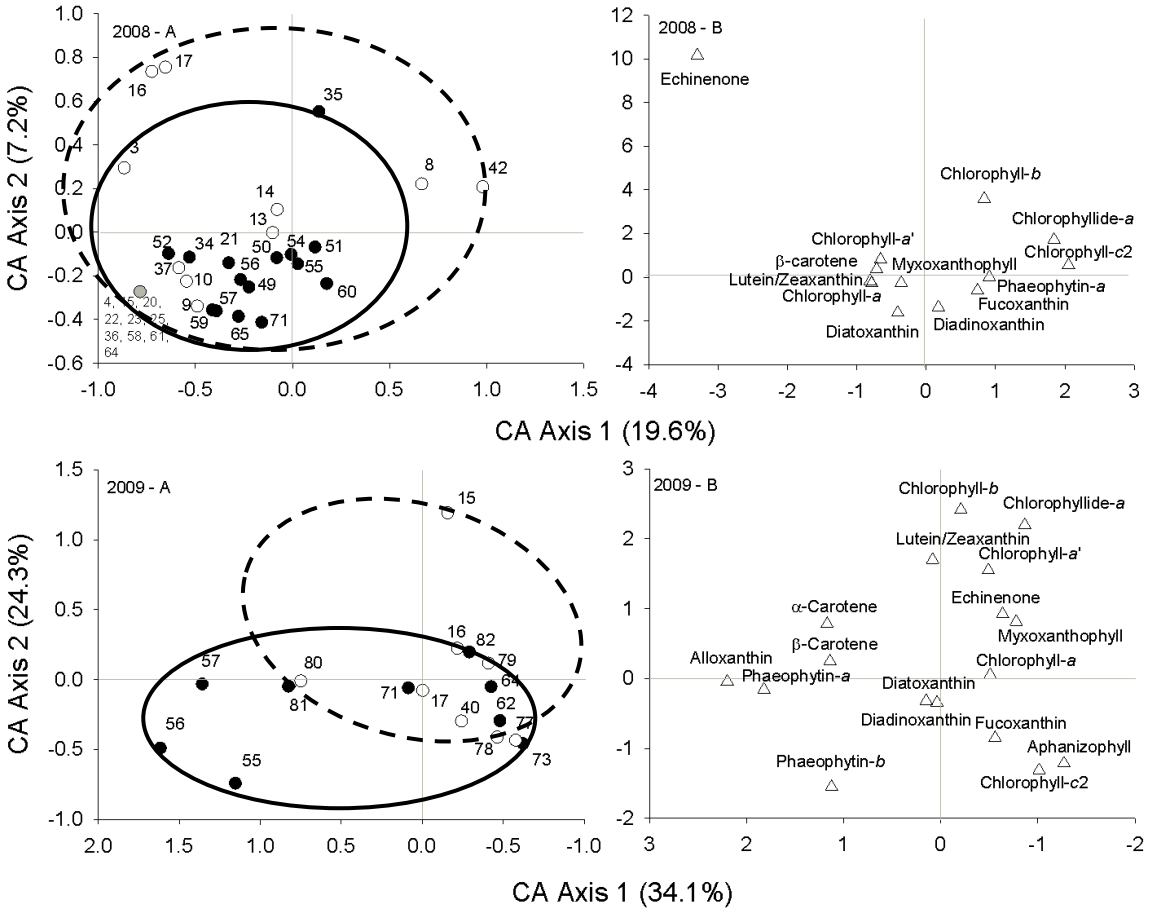


Figure 3.9 Correspondence analysis (CA) of the pigment concentration data obtained from the study sites in 2008 (top panel) and 2009 (bottom panel). Black circles with solid ellipses encircling them are study sites within the Nahanni-Hyland ecoregions, white circles with dashed ellipses encircling them are study sites within the Selwyn Mountain ecoregion. Open triangles in the right-hand graphs (top and bottom panels) represent taxon scores.

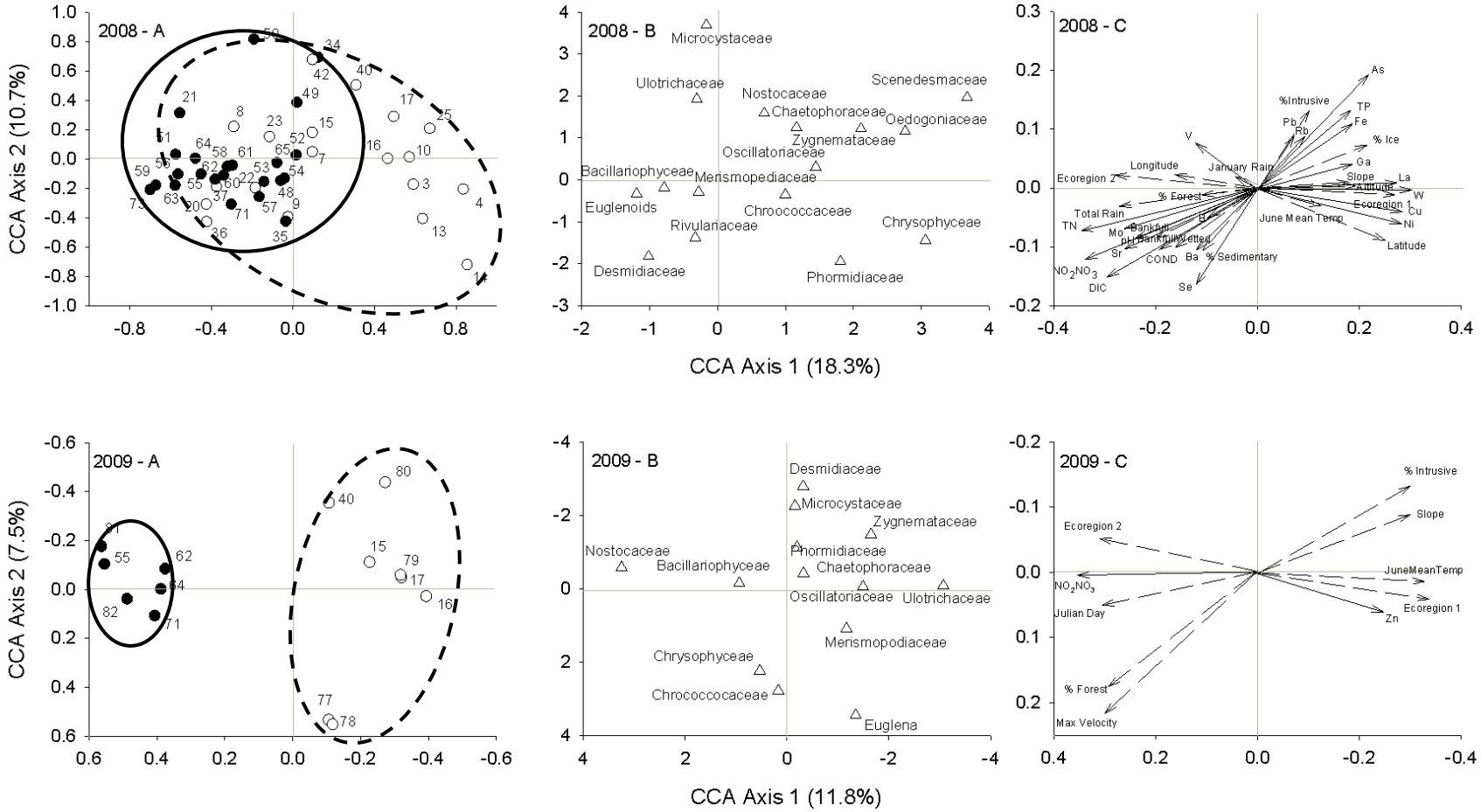


Figure 3.10 Canonical correspondence analysis (CCA) of the benthic algal community composition data (relative abundances) obtained from the study sites in 2008 (top panel) and 2009 (bottom panel) constrained to chemical variables (active environmental variables; solid vectors) and physical variables (supplemental environmental variables; dashed vectors). Panel A) displays the site scores; Black circles with solid ellipses encircling them are study sites within the Nahanni-Hyland ecoregions, white circles with

dashed ellipses encircling them are study sites within the Selwyn Mountain ecoregion. Panel B) displays the taxon scores. Panel C) displays the vectors for the chemical variables (active environmental variables; solid vectors) and physical variables (supplemental environmental variables; dashed vectors).

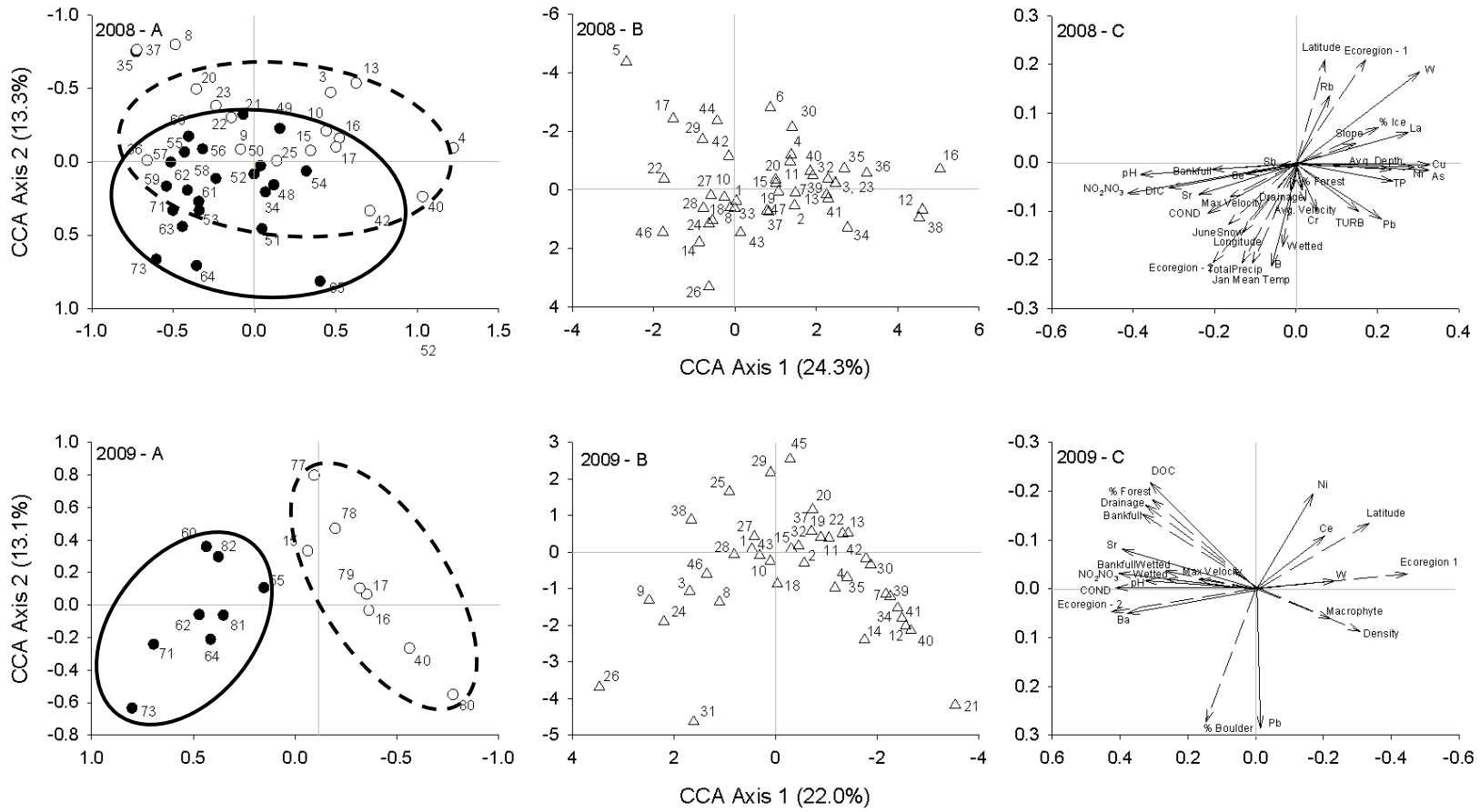


Figure 3.11 Canonical correspondence analysis (CCA) of the benthic diatom community composition data (relative abundances) obtained from the study sites in 2008 (top panel) and 2009 (bottom panel) constrained to chemical variables (active environmental variables; solid vectors) and physical variables (supplemental environmental variables; dashed vectors). Panel A) displays the site scores; Black circles with solid ellipses encircling them are study sites within the Nahanni-Hyland ecoregions, white circles with dashed ellipses encircling them are study sites within the Selwyn Mountain ecoregion. Panel B) displays the taxon scores;

corresponding diatom taxon names for the number codes presented in panel b (2008 & 2009) are located in Appendix B, Table 3.2. Panel C) displays the vectors for the chemical variables (active environmental variables; solid vectors) and physical variables (supplemental environmental variables; dashed vectors).

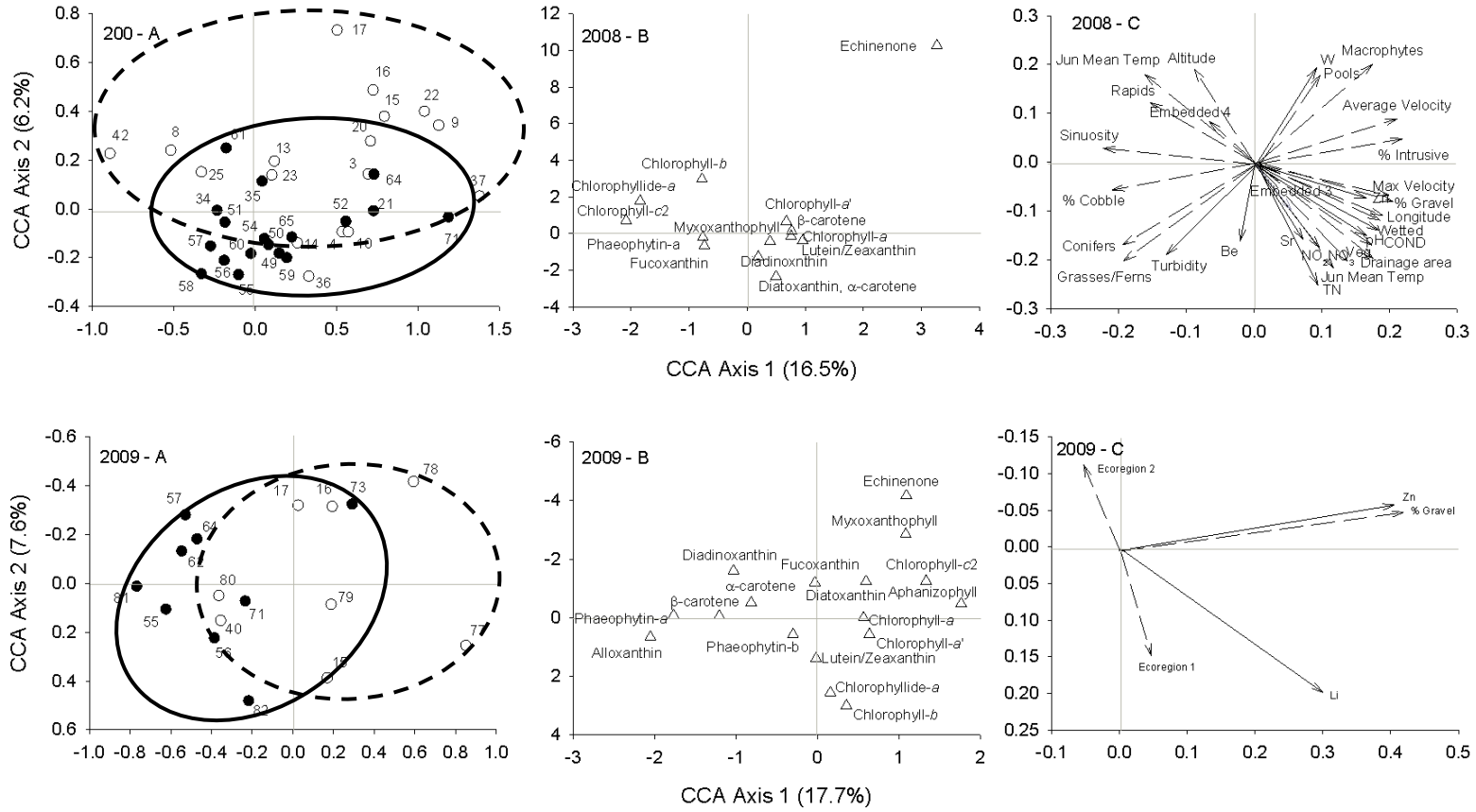


Figure 3.12 Canonical correspondence analysis (CCA) of the pigment concentration data (relative abundances) obtained from the study sites in 2008 (top panel) and 2009 (bottom panel) constrained to chemical variables (active environmental variables; solid vectors) and physical variables (supplemental environmental variables; dashed vectors). Panel A) displays the site scores; Black circles with solid ellipses encircling them are study sites within the Nahanni-Hyland ecoregions, white circles with dashed ellipses encircling them are study sites within the Selwyn Mountain ecoregion. Panel B) displays the taxon scores. Panel C) displays the

vectors for the chemical variables (active environmental variables; solid vectors) and physical variables (supplemental environmental variables; dashed vectors).

3.7 Tables

Table 3.1 Values of selected chemical variables for each of the study sites. For river sites sampled in both 2008 and 2009 values for each year are reported respectively. The ecoregion for each site is provided because physical and chemical conditions differ between Selwyn Mountain Ecoregion (SM) and the Nahanni-Hyland ecoregions (NH).

Site	Ecoregion	TN (µg/L)	TP (µg/L)	NO_2NO_3 (µg/L)	DIC (mg/L)	Cu (µg/L)	Fe (µg/L)	Pb (µg/L)	W (µg/L)	Zn (µg/L)
	CCME Guideline					3.8	300	NA	1.0	30
	SM - mean	99.6	3.3	54.1	16.4	1.5	121.4	0.08	0.3	45.1
	SM - standard deviation	50.7	2.7	49.5	9.9	2.6	155.6	0.1	0.6	104.9
3	SM	64	2.8	12	16.1	1.6	82	0.001	0.04	25.6
4	SM	89	2.8	22	4.9	10.3	479	0.07	0.3	191
5	SM	64	2.0	37	1.1	9.8	324	0.2	0.01	552
6	SM	64	3.4	37	1.7	5.0	111	0.06	3.0	65.7
7	SM	89	2.8	56	5.1	0.8	41.3	0.05	0.2	18.5
8	SM	121	1.8	102	34.0	0.3	3.7	<0.005	0.03	1.0
9	SM	113	1.5	96	25.7	0.3	49	0.01	0.6	26
10	SM	82	5.1	48	17.6	1.6	435	0.1	0.1	72.9
13	SM	83	4.2	27	22.7	1.5	124	0.03	0.6	85.2
14	SM	116	3.1	76	16.4	2.9	26.2	0.03	0.1	42.1
15	SM	66, 110	2.3, 3.6	20, 22	16.4, 18.2	0.4, 0.4	48.3, 30.4	0.02, 0.03	0.05, 0.5	12.1, 8.1
16	SM	65, 116	2.8, 4.4	15, 14	13.6, 13.7	0.4, 0.4	72.1, 72.4	0.03, 0.07	0.4, 0.4	18.4, 17.4
17	SM	43, 61	2.9, 3.4	17, 17	11.7, 12	0.4, 0.5	89.8, 88.9	0.03, 0.05	0.2, 0.5	13.9, 11.9
20	SM	191	0.8	157	36.4	0.1	7.5	<0.005	0.1	1.8
22	SM	144	1.1	115	30.8	0.3	7.3	0.005	0.1	5.1
23	SM	146	1.9	108	29.9	0.3	8.3	<0.005	0.03	2.3
25	SM	93	3.2	38	4.9	0.9	28.7	0.01	0.3	15.7
36	SM	222	1.6	198	31.3	0.2	103	0.008	0.01	2.6
37	SM	255	1.7	142	29.8	0.1	36.4	0.02	0.1	4.4
40	SM	65, 72	4, 10.6	26, 27	11.2, 10	0.3, 0.6	123, 413	0.09, 0.4	0.3, 0.2	3.6, 2.9
42	SM	74	3.7	15	4.9	0.3	114	0.1	0.03	2.1
77	SM	73	1.9	47	14.9	1.2	11.6	0.05	0.04	44.2
78	SM	98	1.8	46	14	0.7	9.5	0.07	0.2	47.7
79	SM	58	2.1	17	16.6	0.4	34.4	0.03	0.3	11.5
80	SM	51	13.5	15	7.7	0.7	548	0.6	0.1	3.2

Site	Ecoregion	TN (µg/L)	TP (µg/L)	NO_2NO_3 (µg/L)	DIC (mg/L)	Cu (µg/L)	Fe (µg/L)	Pb (µg/L)	W (µg/L)	Zn (µg/L)
	NH - mean	205.5	3.0	142.9	36.1	1.5	108.3	0.1	0.03	13.2
	NH - standard deviation	49.1	2.3	49.9	9.9	5.6	165.5	0.3	0.04	28.1
21	NH	349	1.5	113	34.1	0.3	12.2	0.01	0.1	2.6
34	NH	139	3.8	43	7.4	0.6	170	0.05	0.1	1.6
35	NH	198	1.0	166	35.1	0.2	12.5	<0.005	0.02	1.6
48	NH	157	3.8	41	27.6	0.5	104	0.1	0.02	2.7
49	NH	123	6.7	54	35.4	1.2	488	0.1	0.02	48.4
50	NH	190	4.6	63	7.4	0.9	334	0.1	0.02	31.6
51	NH	144	3.6	77	39.3	0.8	261	0.07	0.01	12.9
52	NH	254	2.5	217	39.2	0.2	24.5	0.02	0.01	1.9
53	NH	210	2.6	114	55.5	0.7	9.3	0.05	0.01	135
54	NH	188	12.0	117	55.0	1.4	756	0.3	0.03	80.6
55	NH	227, 240	3.4, 2.2	191, 179	36, 33.2	0.3, 0.3	96.9, 59.3	0.07, 0.06	0.004, 0.2	2.7, 1.5
56	NH	325, 277	3.4, 3.4	210, 192	34.7, 31.2	0.5, 0.7	50.4, 84.9	0.04, 0.1	0.01, 0.2	1.1, 2.5
57	NH	219, 236	3.2, 2.5	194, 183	30.4, 30.2	0.2, 0.3	44, 33.4	0.03, 0.3	0.02, 0.08	1.3, 3.0
58	NH	213	2.3	192	28.4	0.3	204	0.1	0.01	3.1
59	NH	247	1.7	217	35.1	0.4	19.6	0.02	0.002	0.7
60	NH	207, 229	2.6, 1.9	182, 165	33.5, 31.8	0.05, 0.05	55.2, 68.8	0.04, 0.04	0.007, 0.07	1.5, 0.8
61	NH	179	2.3	110	38.4	0.3	6.8	0.07	0.01	0.6
62	NH	162, 170	1.1, 1.4	133, 116	42.4, 40.8	0.3, 0.3	4.8, 5	0.006, 0.03	0.04, 0.05	2.7, 2.4
63	NH	163	1.0	141	43.5	0.2	8.1	0.01	0.01	4.7
64	NH	162, 181	2.2, 0.6	144, 125	43.7, 42.8	0.2, 0.2	6.7, 5	0.01, 0.04	0.002, 0.03	3.9, 3.1
65	NH	153	8.3	88	45.6	1.2	355	0.3	0.01	45.6
71	NH	224, 195	1.6, 1.9	162, 139	43.1, 41.4	0.5, 0.5	3.8, 9.5	0.02, 0.1	0.004, 0.02	3.4, 4.3
73	NH	203, 193	1, 0.5	166, 142	42.6, 41.4	0.2, 32.8	3.6, 50.5	0.01, 1.9	0.002, 0.09	3.0, 4.2
81	NH	215	5.1	176	31.0	0.2	74.2	0.2	0.03	1.1
82	NH	211	4.6	164	33.2	0.5	152	0.1	0.1	2.9

Table 3.2 Values of selected physical variables for each of the study sites. For river sites sampled in both 2008 and 2009 values for each year are reported respectively. The ecoregion for each site is provided because physical and chemical conditions differ between Selwyn Mountain Ecoregion (SM) and the Nahanni-Hyland ecoregions (NH).

Site	Ecoregion	Altitude (m above Sea Level)	Drainage Area (km^2)	Forest (%)	Ice (%)	Intrusive Bedrock (%)	Sedimentary Bedrock (%)
	SM - mean	3329.8	370.2	20.6	21.7	7.6	92.0
	SM - standard deviation	468.6	518.9	7.1	14.1	16.1	16.1
3	SM	3648	140.5	25.2	5.9	0	100
4	SM	3648	1336.5	29.7	2.1	7	93
5	SM	3648	25.3	19.3	10.9	10	90
6	SM	3609	53.5	21.3	23.6	60	40
7	SM	3484	107.2	21.9	23.2	60	40
8	SM	3464	153.6	15.9	15.2	0	100
9	SM	3740	257.7	8.6	33.5	0	100
10	SM	3349	254.9	20.8	19.6	1	99
13	SM	2559	2682.1	37.8	7.1	0	91
14	SM	2920	121.3	25.7	21.8	0	100
15	SM	3310	612.3	19.1	23.8	4	96
16	SM	3625	301.4	14.0	31.2	8	92
17	SM	3714	192.8	14.3	37.2	13	87
20	SM	2214	405.4	32.8	2.5	0	100
22	SM	2086	580.7	26.3	8.7	0	100
23	SM	3805	27.8	13.5	5.7	0	100
25	SM	2719	305.2	21.2	4.5	34	66
36	SM	3474	457.0	24.8	6.9	0	100
37	SM	3120	189.0	30.4	5.3	0	100
40	SM	3655	120.9	16.6	38.6	0	100
42	SM	3773	60.1	6.8	55.7	0	100
77	SM	2916	502.3	24.7	23.5	0	100
78	SM	3041	422.3	24.5	25.0	0	100
79	SM	3051	111.7	24.3	23.8	0	100
80	SM	3687	87.1	14.1	43.0	0	100
	NH - mean	2273.8	812.2	37.6	0.8	2.9	97.1
	NH - standard deviation	694.5	762.1	0.8	1.3	12.1	12.2
21	NH	2017	198.8	34.3	0.3	11	89
34	NH	1876	579.8	26.6	0.6	69	31
35	NH	2076	304.8	28.1	1.8	0	100
48	NH	2946	457.0	73.2	0	0	100
49	NH	1975	2220.0	74.6	0	9	91
50	NH	1509	3591.7	70.3	2	6	94
51	NH	1866	765.4	64.4	0	0	100
52	NH	1866	276.9	52.2	1.3	0	100

Site	Ecoregion	Altitude (m above Sea Level)	Drainage Area (km^2)	Forest (%)	Ice (%)	Intrusive Bedrock (%)	Sedimentary Bedrock (%)
53	NH	1965	408.5	76.5	0	0	100
54	NH	1204	810.4	76.5	0	0	100
55	NH	1446	2286.7	35.6	1.5	0	100
56	NH	1981	615.7	38.6	0.01	0	100
57	NH	2601	999.0	22.8	3.3	0	100
58	NH	3011	652.8	23.8	5.0	0	100
59	NH	2040	580.9	36.8	0	0	100
60	NH	600	827.1	32.5	0.1	0	100
61	NH	3431	173.4	11.4	0.2	0	100
62	NH	3182	274.7	19.3	0.2	0	100
63	NH	3080	297.8	20.2	0.2	0	100
64	NH	3038	307.2	20.8	0.1	0	100
65	NH	1939	1282.6	81.0	3	0	100
71	NH	3002	414.8	21.9	0.1	0	100
73	NH	2900	460.0	23.2	0.1	0	100
81	NH	2221	140.5	27.2	2.7	0	100
82	NH	1400	1109.9	33.5	0.06	0	100

Table 3.3 List of the 'indicator' taxa, as determined from use of Similarities percentage (SIMPER) analysis, that best accounted for differences in the three algal metrics between the two ecoregions (Selwyn Mountain, Nahanni-Hyland). See Methods for further details.

Benthic algal metric	Year	Ecoregion	
		Selwyn Mountain ecoregion	Nahanni-Hyland ecoregions
Benthic algal community composition	2008	Oscillatoriaceae, Phormidiaceae	None
	2009	Merismopediaceae, Oscillatoriaceae	None
Diatom community composition	2008	*E. minutum , F. c. gracilis, , F. c. rumpens*	*G. species 1, S. ulna*
	2009	*E. silesiacum, F. c. gracilis*	*C. affinis, G. species 1*
Photosynthetic pigment concentration	2008	Chlorophyll-*a*'	Fucoxanthin
	2009	Aphanizophyll, Chlorophyll-*a*', Phaeophytin-*b*	β-carotene

Chapter 4

Evaluating the use of algal pigments to assess the biological condition of streams

4.1 Overview

Assessments of stream condition using benthic algal communities have traditionally relied on taxonomy-based approaches to compare community structure at sites exposed to a stressor versus reference sites. Taxonomy-based methods require high levels of training and are relatively time-consuming and expensive. We examined the utility of assessing stream biological condition using algal pigments. We used gradient and control-impact study designs in 2008 and 2009 to compare the extent that algal pigments versus taxonomic descriptors of algal community structure varied along a 10.5-km stretch of the Flat River (South Nahanni River watershed, NWT, Canada) encompassing a gradient of nutrients and metals at sites upstream, adjacent to and downstream of a northern metals mine. We also calculated costs to quantify algal pigments relative to taxonomy-based methods. Multivariate analyses (ANOSIM tests, redundancy analysis) identified that pigment concentrations from benthic algal samples differed significantly ($P < 0.05$) between non-exposed and exposed river sites and were related to variations in water physical and chemical conditions. By contrast, community composition determined from taxonomy-based enumeration to the Class and Family levels did not differ significantly between non-exposed and exposed sites, and relations with water physical and chemical conditions were weaker and inconsistent between the study years. In-house costs to quantify algal pigments were lower than commercial rates to describe community structure using taxonomy. Thus, our data suggests that analysis of benthic algal pigments represents a viable and cost-effective bio-monitoring method for assessing anthropogenic effects on stream condition that merits further evaluation.

4.2. Introduction

Developing cost-effective tools to quantify degradation of aquatic ecosystems by environmental stressors is a central challenge to monitoring biologists (Walker et al., 2003; King et al., 2006). In lotic systems, monitoring programs typically rely on taxonomy-based assessments that describe the community structure of benthic algae, benthic macroinvertebrates, and/or fish (e.g., Stevenson & Bahls, 1999; Biggs & Kilroy, 2000; Walker et al., 2003; Kilgour et al., 2007). Algae have been acknowledged as promising indicators for detection of metal and nutrient enrichment, but they remain infrequently adopted within monitoring programs compared to fish and macroinvertebrates (Dubé et al., 1997; Chambers et al., 2001; Kilgour et al., 2005, 2007). In Canada, for example, monitoring of environmental effects is mandatory for the mining sector and typically includes monitoring of benthic macroinvertebrate and fish communities (e.g., Kilgour et al., 2007; Spencer et al., 2008). These biota vary in their responsiveness to differences in physical and chemical conditions of the surrounding aquatic habitat. Both fish and macroinvertebrates are thought to integrate the conditions of lower trophic levels and, therefore, be representative of the overall ecological integrity of stream ecosystems. However, there are limitations to monitoring fish and macroinvertebrate communities (e.g., due to their mobility, monitoring data may not reflect conditions of the site where they were collected; Resh, 2008). In addition, monitoring lower trophic levels can be more cost-effective and can serve as surrogates for ecological status of higher trophic levels (Kilgour et al., 2005; Rhea et al., 2006).

Assessments of river health based on benthic algae typically involve comparisons of community structure among sites based on taxonomic identifications at the family, genus or

species levels (Reavie & Smol, 1998; Rott et al., 1998; Winter & Duthie, 1998; Hill et al., 2000a). Despite the use of sub-sampling procedures that reduce the number of cells that are used to describe the community, taxonomic descriptions of community structure require high levels of training, and are time-consuming and moderately expensive. Alternatively, variation in the structure of algal communities can be assessed using algal pigments since variation in the abundance and composition of algal groups coincides with changes in concentrations of algal pigments (e.g., Leavitt & Hodgson, 2001; Lauridsen et al., 2011). Algal pigments provide a potentially more cost- and time-effective method of assessing algal community structure and function to monitoring biologists and have been shown to respond to a diversity of anthropogenic stressors. In fact, algal pigments have been used as an indication of shifts in algal communities in paleolimnological studies, nearshore benthic algal biomonitoring studies in lakes, studies of river phytoplankton, and studies of diffuse-source agricultural pollution in European rivers. Only a few studies have used pigments to assess shifts in the structure of algal communities in response to development of lake shorelines (Thomas et al., 2011; Chapter 2), eutrophication (Hall et al., 1997), mining (Sabater et al., 2003), or responses to herbicides (Guasch & Sabater, 1998; Dorigo et al., 2004, 2007). To our knowledge, no study has evaluated the use of a suite of algal pigments to assess environmental impacts in Canadian rivers, including those associated with hard-rock mining operations. Despite recognition of high-throughput potential of pigment analysis by HPLC and well characterized taxon-pigment associations, the extent to which algal pigments represent a potentially effective and cost-saving alternative to taxonomy-based river monitoring approaches remains poorly known.

The primary objective of our study was to evaluate the use of algal pigments as a bio-assessment and bio-monitoring approach. We addressed this goal using a gradient and control-

impact study design in a river that received metal-rich discharge (Spencer et al., 2008) from a tungsten mine in Northwest Territories, Canada. We compared relations of water physical and chemical conditions with pigment and taxonomy-based (to Class/Family level) descriptors of benthic algal communities at 10 sites located upstream and downstream of the mine in 2008 and 2009. Our intent was to evaluate the use of algal pigments as a bio-monitoring approach rather than completing a broad-scale application of the method to assess site condition. Two previous studies reported taxonomy-based assessments that detected differences in the benthic algal communities (composition and biomass) corresponding to differences in chemical stressors (i.e., metals and nutrients) adjacent to the tungsten mine (Spencer et al., 2008; Bowman et al., 2010). Thus, we predicted that algal community structure and pigments would vary among sites located upstream and downstream of the main tailings pond, because there is a sufficiently large range in potential chemical stressors associated with the mine effluent to alter the composition of benthic algal communities and potentially their pigment signatures. Algal pigments could be an alternative to traditional taxonomy-based assessment and monitoring methods if: i) variance in pigments is strongly related to chemical stressors (e.g., nutrients and metals) and ii) these relations are concordant with variance and relations between taxonomy-based descriptions of algal community structure and chemical stressors. Finally, we calculated in-house and commercial costs to quantify algal pigments using high performance liquid chromatography (HPLC) and compared these with costs to assess site condition using traditional algal taxonomy approaches. Low algal pigment analytical costs and positive relations between variance in concentrations of algal pigments and water physical and chemical variables would further suggest that algal pigments provide a viable cost-effective alternative to taxonomy-based assessment and monitoring approaches.

4.3 Methods

4.3.1 Study area and study sites

This study centered on the Flat River, which is located within the South Nahanni River watershed. Portions of both rivers lie within the Nahanni National Park Reserve in western Northwest Territories, Canada. The Flat River is underlain predominantly by shale, limestone, dolostone and sandstone bedrock, which contains commercially viable metals including tungsten in the form of a schelite ($CaWO_4$) deposit. The Flat River watershed contains boreal forests, alpine tundra, shrubland and bog (Halliwell & Catto, 2003).

The North American Tungsten Cantung mine (61^0 57', 128^0 13') is located within the upper portion of the Flat River and is a fourth-order river at this site (Figure 4.1A). Metal-rich mine tailings and nutrient-rich sewage from the mine site are pumped into a series of three tailings ponds (Figure 4.1B). The majority of tailings are currently deposited into Tailings Pond 3. A small portion of leachate from this tailings pond enters the Flat River immediately adjacent to the mine site, and mining activities are associated with elevated concentrations of aluminum, arsenic, chromium, copper, iron, lead, manganese, and tungsten downstream of the mine site (e.g., Spencer et al., 2008). Although leachate from the tailings pond likely enters the Flat River along a distance of several hundred meters, we defined the 10 study sites based on their proximity to Tailings Pond 3. Sites located 2 to 7 km upstream of Tailings Pond 3 were defined as non-exposed sites and sites located 1 km upstream to 3.5 km downstream of Tailings Pond 3 were defined as exposed sites (Figure 4.1A). The site located 1 km upstream of Tailings Pond 3 was included in the exposed-site category, because it was located adjacent to an old, reclaimed tailings pond and a floodplain where mine tailings had previously been deposited. The 10.5-km study reach does not receive appreciable inputs from tributaries and a

few small 1st and 3rd order streams enter the study reach. Average water velocities along the Flat River ranged from 0.44 to 1.08 (cm/s) at the time of sampling and were not considerably different between years (Table 4.1).

Meteorological data collected by Environment Canada at the Rabbit Kettle, NWT station within the Nahanni National Park Reserve (Climate ID: 2203342), approximately 50 km west of the mine, were used to assess differences in meterological conditions between the study years. Comparisons were based on monthly mean temperature and monthly total precipitation during the summer (May to August).

4.3.2 Water physical and chemical analysis

At each site, conductivity, dissolved oxygen concentration, and pH were measured using a YSI model 650 meter, and turbidity was measured using a LaMotte model 2020e turbidity meter. Water velocity and depth were measured using a Marsh McBirney flow mate. Wetted widths were measured with a Bushnell range finder (± 0.5 m). We visually estimated percent gravel and cobble within the river bed, following protocols described by Environment Canada (2011).

Water samples for chemical analyses were collected from the midstream of flow at approximately 30-cm depth. Samples were stored in the dark in a cooler during transport to the field-base for processing. At the field-base, water samples were sub-sampled and analyzed for concentrations of 34 metals (Ag, Al, As, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, La, Li, Mn, Mo, Nb, Ni, Pb, Pt, Rb, Sb, Se, Sn, Sr, Tl, U, V, W, Y, and Zn), and nutrients [ammonia + ammonium (NH_3+NH_4), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), nitrate+nitrite (NO_2+NO_3), total phosphorus (TP), and total nitrogen (TN)]. Concentrations of metals were determined using inductively coupled plasma mass spectrometry. Samples for DIC/DOC were analyzed using an UV-persulfate TOC analyzer, and samples for NH_3+NH_4,

NO_2+NO_3, TN, and TP measurements were analyzed using an automated continuous segmented flow analyzer. All samples were analyzed for nutrients and metals at Environment Canada's National Laboratory for Environmental Testing, Burlington, Ontario, following standard methods (Environment Canada [EC], 1994).

The Canadian Council of Ministers of the Environment's Canadian Environmental Quality Guidelines (CCME Guidelines; CCME, 2003) were used to assess if the metal concentrations along Flat River exceeded thresholds for the protection of aquatic life. The CCME guidelines for metals such as Cd and Pb were calculated using an average hardness value collected from Flat River in fall 2006 (Hardness = 112.93 mg/L $CaCO_3$; Monique Dubé, EP Total, Calgary, Alberta, unpublished data).

4.3.3 Collection of benthic algal samples

Benthic algae were collected from the upper surfaces of cobbles (maximum width and length = 10 to 15 cm) from one large riffle area (25 to 75 m long) at each site where mean water depths and velocities ranged from 0.14 to 0.45 m and from 0.44 to 1.08 m/s respectively. Benthic algal samples were collected from August 2nd to 13th in 2008 and from August 7th to 14th in 2009, using a modified syringe sampler (Lobe, 1981; Biggs & Kilroy, 2000). Cobbles were removed from the riverbed and placed on the river bank for 5 to 10 minutes to allow partial drying of the algal mat. The modified syringe was then placed on the cobble, and the plunger fitted with a toothbrush head, was placed into the syringe and was rigorously rotated against the cobble surface to dislodge the biofilm. The brush plunger was then removed from the cobble and the material on the brush was rinsed into a 100 mL plastic container.

At each site, separate algal samples were collected for photosynthetic pigment concentration and benthic algal community composition analysis. Each sample consisted of

scrapings from five to ten cobbles using the syringe sampler, which were combined into one bottle each for photosynthetic pigment concentration and benthic algal community composition analysis. Thus, each bottle contained 5 to 10 scrapings (1 from each of the 5 to 10 rocks) that represented a total surface area of 26.5 to 53.1 cm^2. Samples were stored in the dark in a cooler in the field until transported to the field-base for processing. For photosynthetic pigment concentration, samples were filtered onto Whatman GFF filters (0.7 μm) and frozen until analyzed by HPLC at the University of Waterloo. Samples for benthic algal community composition were preserved with Lugol's solution until enumerated at the University of Waterloo.

4.3.4 Photosynthetic pigment concentration

Pigments were extracted from each sample for 24 hours at -20^0C in a solution of acetone:methanol:water (80:15:5, by volume). Once extracted, the solution was filtered through a 0.22-μm polytetrafluoroethylene (PTFE) syringe filter to remove large particles and other impurities. The filtrate was then dried under inert gas (N_2) and re-eluted in 500 μL of injection solution (acetone:ion pairing reagent:methanol; 70:25:5, by volume) prior to analysis using a Waters HPLC reverse-phase system with a Symmetry C18 column (3.5 μm) following the methods of Leavitt et al., (1989) as modified from Mantoura & Lleywellyn (1983). A gradient delivery of 2 mobile phases was used to separate the pigment compounds. Mobile phase A consisted of methanol:ion pairing reagent (90:10, by volume) and mobile phase B consisted of methanol:acetone (73:27, by volume). Ion pairing reagent solution consisted of 0.75g tetrabutylammonium acetate and 7.7g ammonium acetate. Sudan II was used as an external standard, positioned as the first and last sample for each batch of samples processed through the HPLC. Since Sudan II has carotenoid-like absorption characteristics and

consistently elutes positioned between aphanizophyll and myxoxanthophyll in the chromatograph, it was also used as an internal standard added to each sample to account for dilution and injection errors (Leavitt & Findlay, 1994). Geranium samples were also positioned near the beginning and end of each batch to account for shifts in retention time of pigments during the run time. Pigments were measured using a Waters 2998 PDA detector and a Waters 2475 Multi λ Fluorescence detector. Pigments were identified using the chromatographic mobility (Leavitt et al., 1989) and spectral characteristics, following information provided by Jeffrey et al., (1997). Standards for algal pigments (including: fucoxanthin, lutein, zeaxanthin, diadinoxanthin, echinenone, chlorophyll-*c*3, myxoxanthophyll, phaeophytin-*a*, chlorophyll-*a*, chlorophyll-*b*, β-carotene, and a mixed pigment standard) were used to calibrate the HPLC machine prior to analysis. These standards were ordered from DHI Lab Products, Horsholm, Denmark. Concentrations of pigments were expressed as μg pigment/cm^2.

Photosynthetic pigments are produced by all algae and some pigments are taxonomically diagnostic. Chlorophyll-*a* (chl-*a*) and β-carotene are produced by all algae and are used to estimate total algal biomass (e.g., Clausen & Biggs, 1997). Preliminary analyses showed that benthic algal communities from the Flat River contained detectable levels of a suite of pigments including: alloxanthin (cryptophytes – 2009 only), aphanizophyll (N-fixing cyanobacteria), β-carotene (all algae), chl-*a* (all algae), chlorophyll-*b* (chl-*b*; green algae), chlorophyll-*c*2 (chl-*c*2; diatoms and chrysophytes), diadinoxanthin (diatoms and chrysophytes - 2009 only), echinenone (cyanobacteria), fucoxanthin (diatoms and chrysophytes), myxoxanthophyll (colonial cyanobacteria), and okenone (purple sulfur bacteria – 2009 only). Lutein/zeaxanthin (green algae and cyanobacteria, respectively) were expressed as one pigment as our system was unable to separate them. Many degradation products of chl-*a*

(chlorophyllide-*a* – 2008 only, phaeophytin-*a* – 2009 only) and chl-*b* (phaeophytin-*b*) were also detected including isomers of chl-*a* (chlorophyll-*a´*).

4.3.5 Benthic algal community composition

Samples for benthic algal community composition were prepared by subsampling 2 mL of well-mixed sample into an Utermöhl chamber. The samples were diluted with deionized water to 3 mL and allowed to settle for 24 hr. Approximately 300 cells of algae per sample were enumerated to Class (i.e., Bacillariophyceae, Chrysophyceae) or Family (i.e., Chaetophoraceae, Chroococcaceae, Closteriaceae, Desmidiaceae, Hydrodictyaceae, Oedogoniaceae, Oscillatoriaceae, Merismopediaceae, Microcystaceae, Nostocaceae, Rivulariaceae, and Zygnemataceae), following the nomenclature of Prescott (1951) and Wehr and Sheath (2003). Single-celled algae and each cell within a colony were counted as individual units, while entire filaments consisting of multiple cells were counted as one unit. We performed analyses on the benthic algal community composition data expressed both as taxon relative abundances (expressed as percentages), cell density (number of algal units/cm^2), and as biovolume ($\mu m^3/cm^2$). Preliminary analyses showed that comparable results were obtained for all three types of data, but patterns were less apparent and statistical test results were weaker when based on the data expressed as cell density and biovolume. Thus, to reduce redundancy in this paper, we present analyses based on relative abundances of algal cells only.

4.3.6 Numerical analyses

We described spatial variation in concentrations of metals, nutrients, photosynthetic pigment concentration and benthic algal community composition data by plotting their concentrations versus distance upstream or downstream of the main tailings pond (Tailings Pond 3). Because

rainfall in 2008 exceeded that of 2009, we explored spatial patterns in water physical and chemical conditions, photosynthetic pigment concentration and benthic algal community composition separately for each year.

Detrended correspondence analysis (DCA) was used to calculate gradient lengths for each algal metric separately. Since gradient lengths were < 1.5 SD units, linear ordination methods were used to determine general trends in the data (Birks, 2010). Principal components analyses (PCA) were used to explore the differences among sites in water chemistry, photosynthetic pigment concentration and benthic algal community composition data for both 2008 and 2009. Ordination by redundancy analysis (RDA) was subsequently used to explore relations between photosynthetic pigment concentrations and water physical and chemical conditions, and between benthic algal community composition and water physical and chemical conditions. Our primary interest was to define the relations between biota and water physical and chemical conditions; however, these relations may not necessarily reflect direct causal effects of the water physical and chemical conditions.

Gradient and upstream-downstream designs can be problematic if physical and chemical characteristics at one site influence that at other sites (i.e., when there is spatial dependence). We used Pearson correlations to test for spatial autocorrelations in physical and chemical characteristics of sites. For these analyses, we systematically ordered data from Site 1 to Site 10 and then created a separate column of data by spatially lagging data at Sites 1 to 10 by one site. This creates a data matrix where values at sites 1 to 9 are compared to data at sites 2 to 10 (i.e., spatial lag of one site). We tested for spatial autocorrelations in select physical and chemical variables that we had identified as being important in explaining variance in algal community structure (i.e., DIC, NO_2+NO_3, TN, TP, pH, DO, turbidity, Al, Cd, Cu, Fe, Mn, W,

average and maximum depth, average and maximum velocity, wetted widths, and percent gravel and cobble).

Because the number of environmental variables exceeded the number of sites, we reduced the number of environmental variables (metals and nutrients) used in the RDA analyses. This was achieved by completing a series of preliminary RDA ordinations with a single environmental variable at a time and retaining the top nine variables that accounted for the greatest amount of variation along the first ordination axis. We further reduced the number of variables by selectively eliminating those that were the most highly correlated ($r > 0.80$) until the variance inflation factors (VIF's) were below 20 for all variables. These methods were performed separately for each biological metric and each study year. Using this approach, subsets of water physical and chemical variables were selected for RDAs based on pigment concentrations and taxonomy-based data separately, for both 2008 and 2009. The variables DOC, NO_2+NO_3, turbidity, Cd, Pb, W, and Zn were retained for the RDA of pigment concentrations in 2008, whereas analyses of pigment concentrations collected in 2009 were based on NO_2+NO_3, TP, pH, Cu, Mn, and W. For RDAs with the taxonomy-based data, DIC, DOC, TN, TP, conductivity, turbidity, Mn, and Zn were retained for 2008, and DIC, NO_2+NO_3, TN, TP, pH, Cu, and W were retained for 2009.

One-way Analysis of Similarities (ANOSIM) tests were used to determine if physical and chemical water conditions differed significantly between non-exposed and exposed sites. ANOSIM tests were also used to determine if composition of benthic algae (based on pigment concentrations or taxonomy-based data) differed significantly between non-exposed and exposed sites. We also used one-way Analysis of Variances (ANOVA) tests to determine if concentrations of individual water physical and chemical variables and individual pigments

differed significantly between non-exposed and exposed sites. Statistical tests were considered significant if $p < 0.05$, and marginally significant for $0.10 \geq p < 0.05$.

RELATE analyses were used to determine if sample scores for photosynthetic pigment concentration and sample scores for water physical and chemical variables at each site shared similar structure (i.e., were correlated). Similarly, RELATE analyses were performed to determine if sample scores for benthic algal community composition data and sample scores for water physical and chemical variables at each site shared similar structure. Also, RELATE analyses were performed to assess if sample scores for photosynthetic pigment concentration and sample scores for benthic algal community composition data at each site shared similar structure. RELATE analyses were performed separately for 2008 and 2009. RELATE analyses used Spearman ranked correlation tests with 999 random permutations to determine correlations between sites using data matrices (Bray-Curtis matrices for biological data, Euclidean distances for environmental data; Clark & Warwick, 2001).

Prior to ordinations and one-way ANOVA tests, water physical and chemical data were tested for normality using Shapiro-Wilks tests. All non-normal variables were ln(x+b)-transformed, where b = 0.5 x the minimum non-zero value. Prior to one-way ANOSIM tests and RELATE analyses, all water physical and chemical data were normalized (variables had their mean subtracted and then were divided by the standard deviation) in order to equalize variances for calculation of Euclidean distances (Clark & Warwick, 2001). Photosynthetic pigment concentration data were transformed using a log(x+1)-transformation, and benthic algal community composition data were square-root transformed prior to running PCA and RDA ordinations, one-way ANOSIM tests, one-way ANOVA tests and RELATE analyses to down-weight the most abundant species and equalize variances. The one-way ANOSIM tests

and RELATE analyses of photosynthetic pigment concentration and benthic algal community composition data were based on Bray-Curtis similarity matrices. All one-way ANOSIM tests and RELATE analyses were performed using the software PRIMER version 6 (Clark & Gorley, 2006). PCA and RDA ordinations were performed using CANOCO version 4.5 software (ter Braak & Šmilauer, 2002). Shapiro-Wilks and one-way ANOVA tests were performed using the software IBM SPSS statistics 20.

4.4 Results

4.4.1 Water physical and chemical conditions

On average, the total metal concentration was 1.8-fold higher at the exposed sites compared to the non-exposed sites. Of the metal and nutrient variables that were elevated at exposed sites (e.g., Al, Cd, Fe, Mn, W, TN, and NO_2+NO_3), average concentrations (2008 to 2009) were 1.4 – 2.1-fold higher than at the non-exposed sites. For most sites, concentrations of several metals (e.g., Al, Cu, Fe, Mn, W), nutrients (e.g., TP), turbidity, and flow velocity were higher in 2009 than 2008, while water levels were lower in 2009 compared to 2008 (Table 4.1, Figure 4.2). These differences coincided with a warmer drier summer in 2009 compared to 2008. One-way ANOSIM tests showed that water physical and chemical conditions differed significantly between non-exposed and exposed sites in 2008 (global $R = 0.39$, $p = 0.03$) and with marginal significance in 2009 (global $R = 0.24$, $p = 0.08$).

One-way ANOVA tests showed that concentrations of Cu, Fe, Zn, and DIC were marginally significant to significantly higher at exposed sites compared to non-exposed sites in 2009 only (Cu: $F = 7.5$, $p = 0.03$; Fe: $F = 7.0$, $p = 0.03$; Zn: $F = 3.4$, $p = 0.10$; DIC: $F = 4.9$, $p = 0.06$; Figure 4.2). Concentrations of Mn, W and NO_2+NO_3 were marginally significant to significantly higher at exposed compared to non-exposed sites in both study years (Mn – 2008:

F = 14.7, p = 0.01; Mn – 2009: F = 9.3, p = 0.02; W – 2008: F = 28.7, p < 0.01; W – 2009: F = 5.4, p = 0.05; NO_2+NO_3 – 2008, F = 7.5, p = 0.03, NO_2+NO_3 – 2009: F = 3.8, p = 0.09). All other water physical and chemical variables did not differ significantly between exposed and non-exposed sites.

Some metals exceeded CCME guidelines at both non-exposed and exposed sites. Concentrations of Cd (2009) and W (2008 and 2009) exceeded CCME guidelines at the exposed sites, but not at the non-exposed sites (CCME guidelines: Cd = 0.037 µg/L; W = 1.0 µg/L). Concentrations of iron exceeded guidelines at all non-exposed and exposed sites in 2009, but not at any sites in 2008 (CCME guideline: Fe = 300.0 µg/L).

Sites along Flat River were located in riffle-run habitats that were similar in terms of water depths and velocities (Table 4.1). Wetted widths ranged between 9.6 to 29.6 m (2008) and 9.8 to 31.2 m (2009). Average depths ranged from 14.4 to 44.6 cm in 2008, and 17.1 to 30.0 cm in 2009. Average velocity ranged from 0.47 to 0.82 cm/s (2008) and 0.44 to 1.08 cm/s (2009), and % cobble and gravel ranged from 29 to 64 % (2008) and 20 to 66 % (2009).

Pearson correlation analyses showed statistically significant (p < 0.05) positive serial correlations in only 7 of 20 variables (DIC, Cu, Fe, Mn, W, Wetted Widths, % cobble). Of these, only two showed evidence of positive correlation in both 2008 and 2009 (Mn & W). These analyses revealed, at most, only modest evidence of spatial autocorrelation among sites in 2008 and 2009, with high levels of inter-annual variability.

4.4.2 Comparison of taxonomy- and pigment-based analysis of benthic algal communities

Based on benthic algal community composition analyses, benthic algal communities in the Flat River were dominated by diatoms and chrysophytes (14 – 92%), cyanobacteria (2 – 76%), and, to a lesser extent, by green algae and charophytes (0 – 20%; Figure 4.3). Non-exposed sites

were dominated by diatoms and chrysophytes (20 – 92%) or colonial cyanobacteria (2 – 59%). Abundance of filamentous cyanobacteria increased markedly (30 - 76%) adjacent to the mine (-1 km to 0.01 km from Tailings Pond 3). Exposed sites further downstream (1 – 3.5 km downstream of Tailings Pond 3) were dominated by diatoms and chrysophytes (42 –76%; Figure 4.3).

Analysis of photosynthetic pigment concentration showed communities at non-exposed sites along the Flat River to have variable concentrations of fucoxanthin, chl-c2, and diadinoxanthin (diatoms and chrysophytes), and chl-*a* (all algae; Figure 4.4). However, concentrations of aphanizophyll (N-fixing cyanobacteria), echinenone (cyanobacteria), myxoxanthophyll (colonial cyanobacteria), and lutein/zeaxanthin (green algae and cyanobacteria) increased at the sites adjacent to and downstream of the mine in both years. Concentrations of Chl-*c*2, diadinoxanthin and fucoxanthin increased further downstream (1 to 3 km downstream of Tailings Pond 3).

4.4.2.1 Photosynthetic pigment concentration

Concentrations of algal pigments varied appreciably among sites and between years, and were generally 1.5 to 17-fold higher in 2009 than in 2008 (Figure 4.4). These patterns coincided with 2- to 5-fold increases in concentrations of Al, Cu, Fe and total phosphorus in 2009 compared to 2008 (Figure 4.2). Indeed, one-way ANOSIM tests showed that the composition and abundance of algal pigments differed significantly between non-exposed sites and exposed sites in 2009 (global $R = 0.54$, $p = 0.01$), but not in 2008 (global $R = 0.04$, $p = 0.31$). In 2009, concentrations of chl-*a* and β-carotene (all algae), aphanizophyll (N-fixing cyanobacteria), myxoxanthophyll (colonial cyanobacteria) and lutein/zeaxanthin (green algae and cyanobacteria) were 1.3 to 11.9-fold higher at exposed sites compared to non-exposed sites

(Figure 4.4). Based on one-way ANOVA tests, concentrations of aphanizophyll and phaeophytin-*b* (chl-*b* derivative) at the six exposed sites exceeded values at the four non-exposed sites in 2008 (aphanizophyll: $F = 4.3$, $p = 0.07$; phaeophytin-*b*: $F = 4.7$, $p = 0.06$). In 2009, concentrations of aphanizophyll, β-carotene, lutein/zeaxanthin, and phaeophytin-*a* (chl-*a* derivative) were higher at the exposed sites than the non-exposed sites (aphanizophyll: $F = 11.9$, $p = 0.01$; β-carotene: $F = 7.8$, $p = 0.02$; lutein/zeaxanthin: $F = 3.5$, $p = 0.10$; phaeophytin-*a*: $F = 3.8$, $p = 0.09$). All other pigments did not differ significantly between non-exposed and exposed sites in 2008 or 2009.

Ordination by PCA was used to characterize variation in photosynthetic pigment concentrations among sites. For the 2008 data, eigenvalues for the first and second PCA axes were 0.77 and 0.10 respectively, explaining 87% of the total variation in pigment abundances among sites. In 2009, they were 0.65 and 0.21 respectively, explaining 86% of the total variation among sites (Figure 4.5). Sample scores for non-exposed and exposed sites occupied distinct positions in PCA space in 2009. In 2008, there was some overlap among the sample scores for the non-exposed sites and those for the exposed sites furthest downstream. These findings were consistent with the results of the multivariate ANOSIM tests, which showed significant difference in photosynthetic pigment composition between exposed and non-exposed sites in 2009 but not in 2008. In 2008, sites closest to the mine (-1 km to 1 km) were characterized by higher concentrations of β-carotene, echinenone and myxoxanthophyll relative to sites upstream and further downstream (2 to 3.5 km). Sites 2 to 3.5 km downstream plotted alongside the upstream sites (-3 & -7 km) and were characterized by lower concentrations of all pigments. Upstream sites (e.g., -2 km) were characterized by higher concentrations of chlorophyll-*b,* fucoxanthan and phaeophytin-*b* relative to all other sites. In

2009, sites adjacent to the mine (-1 km and 0.01 km) were characterized by relatively higher concentrations of chlorophyll-*a*, echinenone and myxoxanthophyll compared to all other sites. Sites further downstream (1 to 3.5 km) were characterized by relatively higher concentrations of chlorophyll-*c*2, diadinoxanthin, fucoxanthin, and phaeophytin-a, and upstream (non-exposed) sites were characterized by relatively lower concentrations of all pigments (with the exception of okenone which was present at low concentration at site -4.5 km) compared to other sites.

We used RDA to characterize relationships between pigment concentrations and physical and chemical variables (Figure 4.6). For the 2008 dataset, eigenvalues for the first and second axes were 0.54 and 0.15 respectively, accounting for 69% of the total variation in photosynthetic pigment concentration among sites. In 2009, eigenvalues for the first and second axes were 0.36 and 0.22 respectively, accounting for 58% of the total among-site variation. There was clear separation of non-exposed and exposed sites in 2009 (similar to PCA ordinations). However in 2008, separation of non-exposed and exposed sites was less clear with little to no separation of sites immediately adjacent to the mine from sites upstream and further downstream. Apparent in the RDA ordinations is that relations between photosynthetic pigment concentration and water physical and chemical data differed between exposed and non-exposed sites in the Flat River. Concentrations of pigments and water physical and chemical variables at non-exposed sites were lower than that at downstream sites in both years, with the exception of the site 2 km upstream from Tailings Pond 3 in 2008, which was associated with high concentrations of several pigments [e.g., aphanizophyll (N-fixing cyanobacteria), chl-*b* (green algae) and fucoxanthin (chrysophytes and diatoms)] and Zn (Figure 4.6). Additionally, in 2009 concentrations of okenone (purple sulfur bacteria) were

highest at the non-exposed sites compared to exposed sites. At exposed sites closest to the mine (-1 to 1 km), increased concentrations of β-carotene and chl-*a* (all algae), echinenone (cyanobacteria), and myxoxanthophyll (colonial cyanobacteria) were positively related to increased concentrations of NO_2+NO_3 and DIC. However, photosynthetic pigment concentration and associated water physical and chemical variables at exposed sites located 1 to 3.5 km downstream varied between years. In 2008, these exposed sites were associated with relatively low concentrations of pigments and water chemistry variables (with the exception of DOC in 2008). In 2009, the exposed sites were associated with relatively high concentrations of pigments [e.g., chl-*c*2, chl-*b*, diadinoxanthin (diatoms and chrysophytes) and fucoxanthin], TP, several metals (e.g., Al, Cu, Fe, Mn, W), and pH.

Although there were some differences in photosynthetic pigment concentration between years, the same general trend of increased concentrations of β-carotene and chl-*a* (all algae), echinenone (cyanobacteria) and myxoxanthophyll (colonial cyanobacteria) along with increased concentrations of DIC and NO_2+NO_3 at sites closest to the mine was apparent in both years (Figure 4.6). RELATE analysis was used to determine the correlation of sample scores between photosynthetic pigment concentration and water chemistry data. In both years, these two data sets were marginally significantly correlated (2008: Rho = 0.25, p = 0.08; 2009: Rho = 0.20, p = 0.10).

4.4.2.2 Benthic algal community composition

The PCA ordination of benthic algal community composition data from 2009 captured 73% of the total variation (λ_1 = 0.39, λ_2 = 0.34), but showed very little separation of sample scores from the non-exposed and exposed sites (Figure 4.7). Similarly, the RDA ordination of the 2008 data captured 71% of the total variation (λ_1 = 0.59, λ_2 = 0.12), with considerable overlap

of the sample scores from non-exposed and exposed sites (Figure 4.8). Consistent with these patterns, one-way ANOSIM tests revealed that non-exposed and exposed sites did not differ significantly using the benthic algal community composition data from 2008 and 2009 (2008: R = -0.01, p = 0.42; 2009: 0.10, p = 0.23). In 2009 only, there was minimal separation of sites adjacent to the mine (-1 to 0.01 km) from all other sites. Exposed sites were characterized by relatively high abundances of Oscillatoriaceae, Nostocaceae, and Ulotrichaceae, which corresponded with HPLC analysis showing relatively high concentrations of cyanobacterial pigments at these sites (Figures 4.5 & 4.6).

The RDA ordinations of benthic algal community composition data from 2009 captured 51% of the total among-site variation ($\lambda_1 = 0.29$, $\lambda_2 = 0.22$), and showed even less separation of sample scores from non-exposed and exposed sites than the 2009 PCA ordination (Figure 4.8). The 2008 RDA ordination captured 54% of the total among site variation ($\lambda_1 = 0.33$, $\lambda_2 = 0.21$), and showed a greater separation of sites at -1 km and 2 km along the Flat River from all other sites. Relations between benthic algal communities and environmental conditions were variable between study years when using the taxonomy-based data (Figure 4.8). In 2008, for example, high relative abundances of Merismodpediaceae, Microcystaceae, and Chrococcaceae were associated with relatively high concentrations of TN and turbidity. However, in 2009 these taxa were associated with relatively low concentrations of nutrients, low pH and high concentrations of Cu and W. Abundance of Bacillariophyceae and Zygnemataceae was associated with higher concentrations of TP in both years. Also, Site 5, which is located 1 km upstream of the mine, was characterized by relatively high abundance of Hydordictyaceae in 2008, and high relative abundance of Oscillatoriaceae, Nostocaceae, and Ulotrichaceae in 2009. Although there were different types of algae present at this site between years, the algae

were consistently associated with DIC and NO_2+NO_3 in both years. Thus, the photosynthetic pigment concentration data appeared to capture more consistent biota-environmental relations during the two years of the study compared to the benthic algal community composition assessment. Consistent with this finding, results of RELATE analysis showed no statistically significant correlation between sample scores from benthic algal community composition data and water physical and chemical concentrations in either study year (2008 Rho = -0.23, p = 0.96; 2009 Rho = -0.01, p = 0.51). In addition, results of RELATE analysis showed a marginally statistically significant correlation between sample scores from benthic algal community composition data and photosynthetic pigment concentration in 2009 (Rho = 0.24, p = 0.07), but not in 2008 (Rho = -0.03, p = 0.54). Also, the direction of the correlation differed between the two study years.

4.4.3 Costs to quantify photosynthetic pigment concentrations

We estimated the cost per sample for our laboratory at University of Waterloo to quantify algal pigments in a benthic algal sample from a river site using a high performance liquid chromatography (HPLC) system by including costs of supplies, labour and the purchase, maintenance, servicing, and replacement of the HPLC (Table 2). Supplies included all consumable solvents, filters, and other materials. Labour costs were estimated based on a full-time dedicated HPLC technician assuming an annual salary (including benefits) of $70 000 per year and a throughput of 1500 samples per year. Maintenance and servicing costs assumed expenses of $15 000 per year to replace lamps, columns and an annual service visit. Replacement costs were estimated based on a purchase-price of $80 000 and a 20-year lifespan for the HPLC system. Using these major cost components, the base cost of analyzing an individual sample for algal pigments was calculated to be $66.84. We then compared this base

cost for pigment analysis by HPLC to commercial rates to quantify algal community structure using taxonomy-based approaches ($180.00 – 250.00/sample) and pigment analysis ($125.00 – 175.00/sample). Overall, the base costs for pigment analysis using in-house HPLC is approximately half that of a commercial rate and nearly one-third the cost for the commercial rate of taxonomy-based assessment.

4.5 Discussion

We used gradient and control-impact (upstream-downstream) study designs to evaluate whether algal pigments could be used as an alternative to traditional taxonomy-based approaches for assessing stream condition. We quantified algal community structure using both algal pigments and traditional taxonomic descriptors of taxon relative abundance at the Class and Family level, and then tested for relationships between both measures of community structure with longitudinal variance in water physical and chemical concentrations created by the release of metal- and nutrient-rich discharge to the Flat River. A prerequisite to the use of algal pigments as a potential bio-indicator is that they are time- and cost-effective for sampling and analysis, and responsive to gradients in water physical and chemical concentrations, including potential chemical stressors (e.g., metals and nutrients). Patterns evident in ordinations by PCA and RDA identified that concentrations of algal pigments varied across the longitudinal gradient and were related to variance in water physical and chemical concentrations. Additionally, our control-impact comparisons showed substantial, and often statistically significant, differences in concentrations of algal pigments at non-exposed sites located upstream of the mine versus exposed sites located adjacent to and downstream of the mine.

Our analyses, based on RDA ordinations, showed that algal pigments were sensitive to differences in concentrations of metals and nutrients among sites. Previous studies have shown that metals such as cadmium and zinc can result in differences in algal communities including decreased biomass, decreased density of diatoms, community shifts from dominance by diatoms to green algae and cyanobacteria, and shifts from sensitive to tolerant species (Genter et al., 1987; Gold et al., 2003; Morin et al., 2008). Increased concentrations of nutrients such as dissolved inorganic nitrogen can result in increased biomass (Chambers et al., 2006). In our study, based on pigment analysis we found increased biomass of all algae (as β-carotene and chl-*a*) and cyanobacteria (echinenone and myxoxanthophyll) associated with elevated concentrations of NO_2+NO_3 and DIC at the sites closest to the mine site (-1 km to 1 km). Biomass was also lowest at the non-exposed sites. These observed patterns are concurrent with results of two other studies that used benthic algae and benthic macroinvertebrates to assess impacts of the North American Tungsten Cantung mine on the Flat River (Spencer et al., 2008; Bowman et al., 2010). Of note, these studies identified increased biomass (in the form of chlorophyll-*a*) of the overall algal mat, and abundance of cyanobacteria in benthic algal communities near the mine, similar to our findings based on pigment analysis. In our study, the pigment data were more strongly and consistently associated with water physical and chemical conditions than the taxonomy-based data, suggesting pigment analysis is more informative of environmental conditions. For example, RELATE analyses showed consistent and marginally significant associations between photosynthetic pigment concentration and water physical and chemical conditions in both years. In contrast, benthic algal community composition data showed positive associations between some algal taxa and nutrient and metal concentrations in one year but negative associations in the other year. Also, patterns of photosynthetic pigment

concentration and benthic algal community data were correlated in only one of the two study years (2009). Consequently, benthic algal community composition assessment does not appear to provide consistent assessment of environmental conditions among years.

Analysis of algal pigments provides a coarser level of taxonomic resolution (typically division to subdivision level, but can be coarser than division level) than can be achieved by taxonomic analyses (typically division to species). Thus, a potential concern for the use of algal pigments as a biomonitor is that photosynthetic pigment concentrations may be capable of detecting only relatively coarse levels of changes in community composition. Contrary to this expectation, our results suggest that analysis of photosynthetic pigment concentration outperforms benthic algal community composition. For example, the PCA and RDA based on the photosynthetic pigment concentration explained 87% (2008 - PCA) and 86% (2009 - PCA), and 69% (2008 - RDA) and 58% (2009 - RDA) of the variation among sites, which was higher than variation explained by taxonomy-based assessment (71% in 2008, 73% in 2009 - PCA; 54% in 2008, 51% in 2009 - RDA). Importantly, the PCA and RDA based on the algal pigments showed greater separation of non-exposed and exposed sites compared to the PCA and RDA using taxonomy-based data. Consequently, our results suggest that algal pigments track variations in environmental conditions of rivers at least as effectively, if not more so, than traditional taxonomy-based assessments.

Monitoring biologists apply a suite of study designs to identify environmental impacts of industrial activities on surface waters, including control-impact (CI), before-after-control impact (BACI), gradient, and reference condition approaches (RCA; Green, 1979; Underwood, 1994; Bailey et al., 2004). We used a gradient design to assess the use of photosynthetic pigments as a biomonitoring tool. Since rivers are continua of river water and biological

communities, this type of study design has raised concerns about pseudoreplication (Cooper & Barmuta, 1993). We tested for spatial dependence by completing correlation analyses of spatially lagged data. These analyses showed that sites along the Flat River were, at worse, only moderately autocorrelated (7 of 20 variables tested over 2 years showed evidence of first order spatial correlation). Moreover, only 2 of these variables showed evidence of autocorrelation in both study years, highlighting the interannual variability in autocorrelation results. Despite challenges related to spatial autocorrelation, gradient and control impact designs are pervasive and are approved approaches within the environmental-effects monitoring guidelines in Canada (EC, 2012). While reference condition approaches are clearly valuable, they may not be economically feasible in some locations, especially in many northern settings where the remote nature of the sites requires helicopter access, often at costs of $10,000 to $15,000 per day. Results from upstream-downstream designs can be comparable to that using reference condition approaches based on sampling of sites. Bowman et al., (2010) used the reference condition approach, sampling reference sites from independent streams rather than an upstream-downstream design to assess impairment. Interestingly, conclusions related to impairment at the North American Tungsten Cantung mine site presented by Bowman et al., (2010) were similar to those of Spencer et al., (2008) despite differences in study designs (i.e., gradient, control-impact and RCA). For example, both studies found increased algal biomass and cyanobacteria at the sites closest to the mine, an observation that we also documented. Moreover, Bowman et al., (2010) did not detect statistically significant differences in community composition at exposed and non-exposed sites in multivariate ordination space, similar to our results using benthic algal community composition data.

The reference condition approach (Bailey et al., 2004) is becoming increasingly used to detect ecological impairment in Canada, and when combined with process studies, to provide a mechanistic understanding of plausible cause-effect relationships (Reynoldson et al., 1997; Bowman et al., 2005; Scrimgeour et al., 2008; Bowman et al., 2010; White et al., 2011). RCA uses clustering analysis, discriminate function analysis, and ordination to compare potentially exposed test sites to an appropriate subset of reference sites using physical, chemical, and biological data. The ecological condition of test sites is assessed by evaluating the extent to which their community composition differs from that at sites identified as un-impacted and in the reference condition (Reynoldson et al., 1997; Bailey et al., 2004; Bowman et al., 2005). The RCA is thought to offer a powerful alternative to other study designs as it captures the variation present within the reference condition using each reference site as a replicate, compared to designs such as CI and BACI whose replicates typically consist of multiple measurements at each site (Reynoldson et al., 1997; Bailey et al., 2012).

A potential cause of concern in use of algal pigments within reference condition designs is that factors other than those that are influenced by the stressors of concerns may alter algal pigment fingerprints. For example, algal pigments can be influenced by factors such as light, temperature, and grazing pressure. Thus, interpretations based on pigment data should be made with caution and attention to possible confounding factors. For example, phytoplankton will produce higher concentrations of carotenoid accessory pigments in high light environments in order to reduce photoinhibition. Additional research is required to better understand how variance in concentrations of algal pigments might be influenced by chemical stressors due to anthropogenic activities versus those that reflect natural variance in water physical and chemical concentrations (Hill, 1996). However, in our study the discharge from the mine did

not greatly alter water clarity, and hence light attenuation. Thus, results from our study suggest algal pigments may serve as a good surrogate and alternative endpoints for monitoring and assessment.

We evaluated the use of algal pigments to serve as a cost-effective bio-indicator through multiple yet indirect lines of evidence. This approach included: i) establishing relations between photosynthetic pigment concentration and water physical and chemical conditions, ii) comparing these relationships to those between relative abundances of algal cells at the Class and Family level and water physical and chemical conditions, and iii) quantifying costs to identify algal pigment signatures. Our cost comparisons showed that the base analytical costs for in-house analysis of pigments were low ($66.48/sample). Additionally, the commercial cost of analyzing of a pigment sample is generally lower than the commercial cost of taxonomy-based assessments, making them a reliable, cost-effective alternative to traditional taxonomic-based assessments. However, our study centered on one type of human activity, the effects of mining at one river, and the broad application of pigment analysis for biomonitoring of river condition in response to other types of human activities remains untested. Our study supports a growing body of evidence that algal communities are highly sensitive to metal enrichment (e.g., Peterson et al., 1984; Macfie et al., 1994; Gensemer et al., 1999; Cervantes et al., 2001; Pinto et al., 2003; Küpper et al., 2002). Because industrial effluents are a mixture of many potential chemical stressors, identifying causal relations between shifts in biological communities and industrial effluents will require experimentation. This is required to better establish causal linkages between shifts in algal pigment signatures and chemical stressors present in metal-mine effluents. Further studies are also needed to compare environmental assessments using algal pigments with those using other biological endpoints, such as

taxonomic counts of benthic algae or macroinvertebrates, to better determine the relative sensitivity of algal pigments versus other biological endpoints for detection of ecological impairment.

4.6 Figures

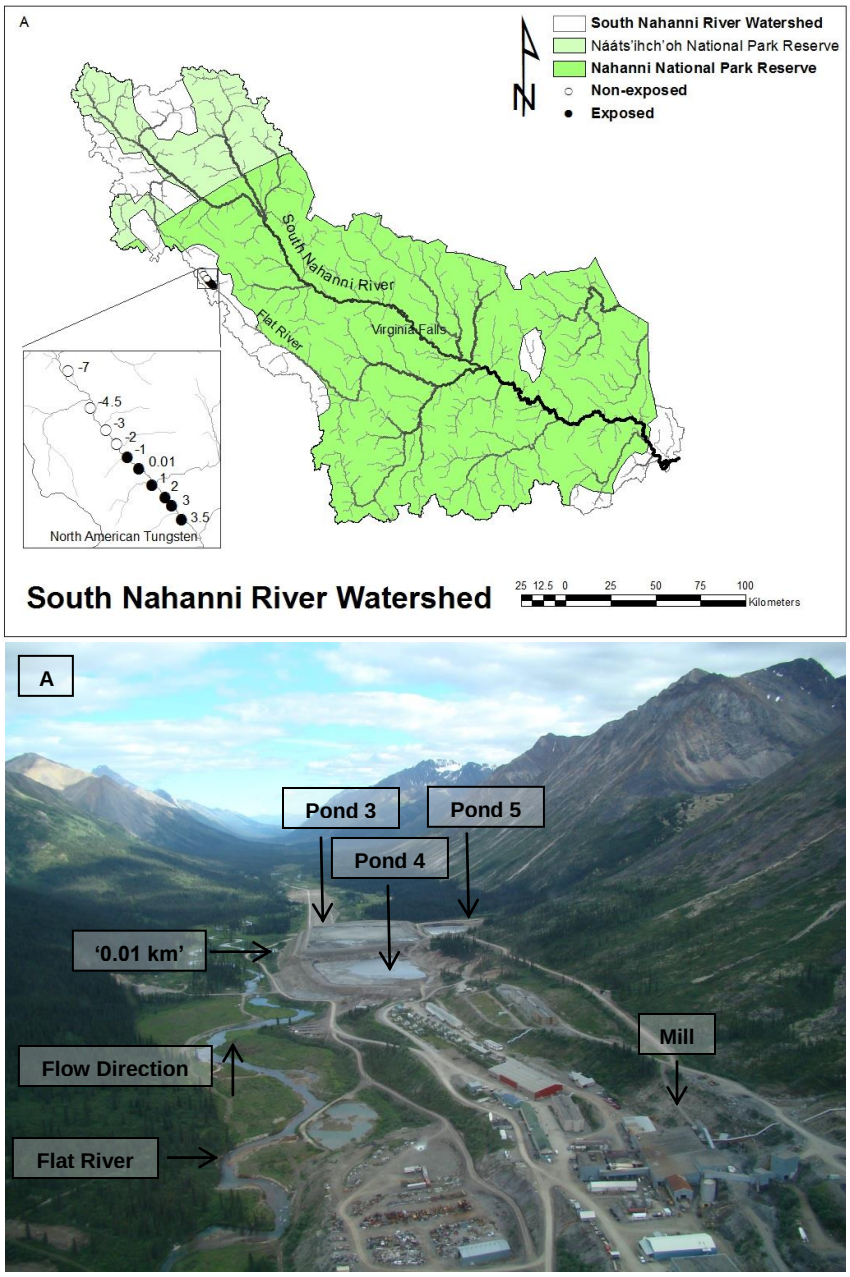


Figure 4.1 Location of study sites along the Flat River in the South Nahanni Watershed, Northwest Territories, Canada (A) and an aerial view showing the location of the tailings pond at the North American Tungsten Cantung Mine adjacent to the Flat River (B) (Photo Dana Haggarty). Each of the 10 study sites shown in plot A are defined based on its proximity to Tailings Pond 3 which receives metal- and nutrient-rich effluent from the mine site. The green shaded regions in the upper plot are the boundaries of the National Parks (Nahanni and Nááts'ihch'oh National Park Reserves).

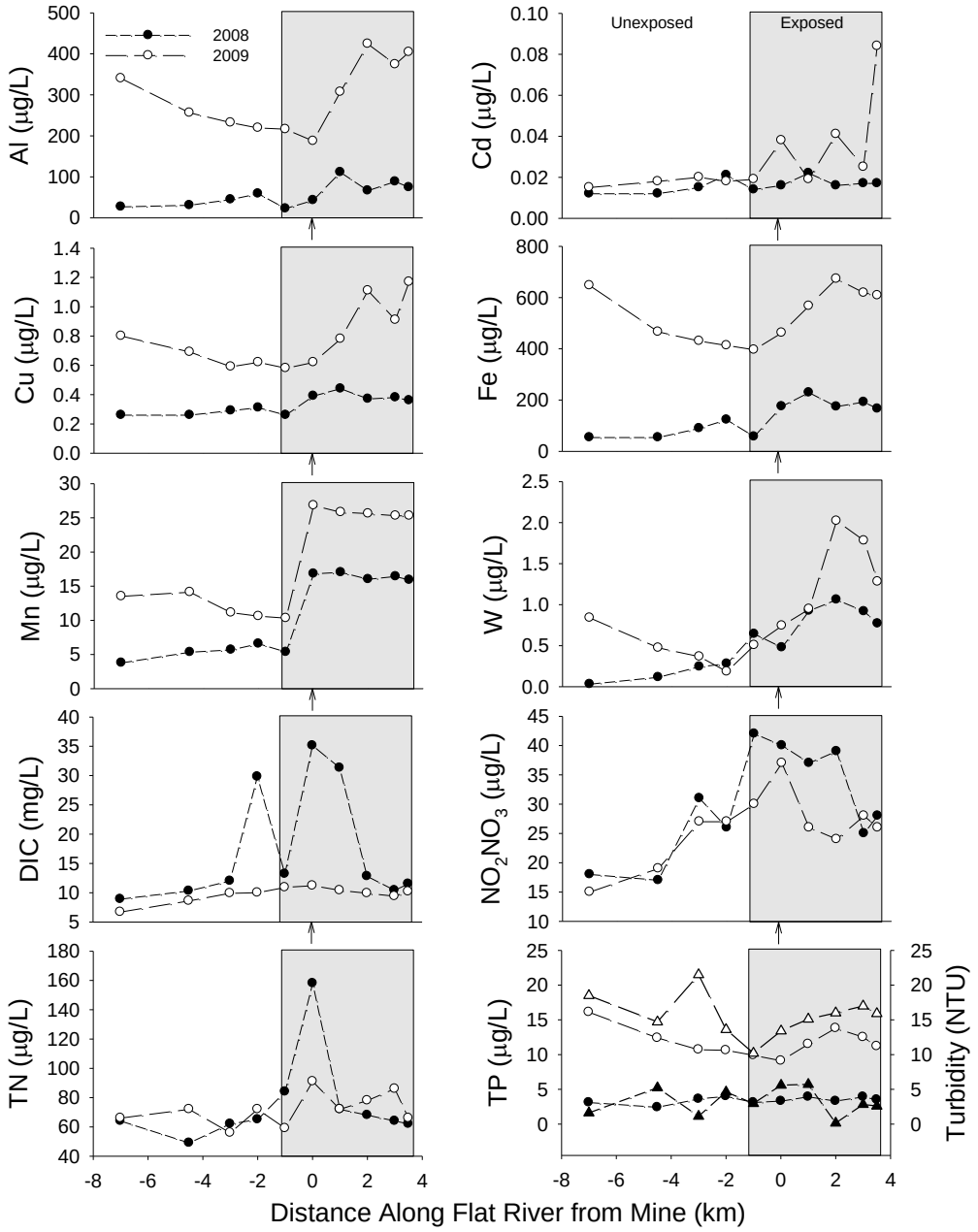


Figure 4.2 Longitudinal variation in concentrations of metals and nutrients in the Flat River in 2008 (closed circle) and 2009 (open circle). Turbidity is represented by closed and open triangles for 2008 and 2009, respectively. All sites are expressed as a distance upstream (negative) and downstream (positive) of Tailings Pond 3 (closest site = 0.01 km; identified as vertical arrows along the x-axes). Sites defined as exposed are enclosed in a grey box, while those defined as non-exposed are not.

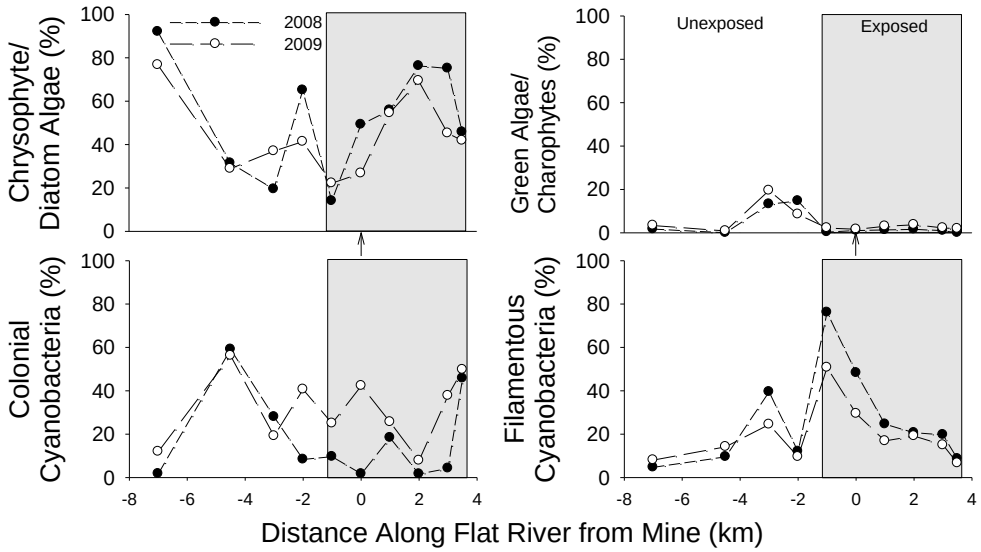


Figure 4.3 Longitudinal variation in percent abundance of selected benthic algal taxa based on analyses at the Class and Family level in the Flat River in 2008 (closed circle) and 2009 (open circle). All sites are expressed as distances upstream (negative) and downstream (positive) of Tailings Pond 3 (closest site = 0.01 km; identified as vertical arrows along the x-axes). Sites defined as exposed are enclosed in a grey box, while those defined as non-exposed are not.

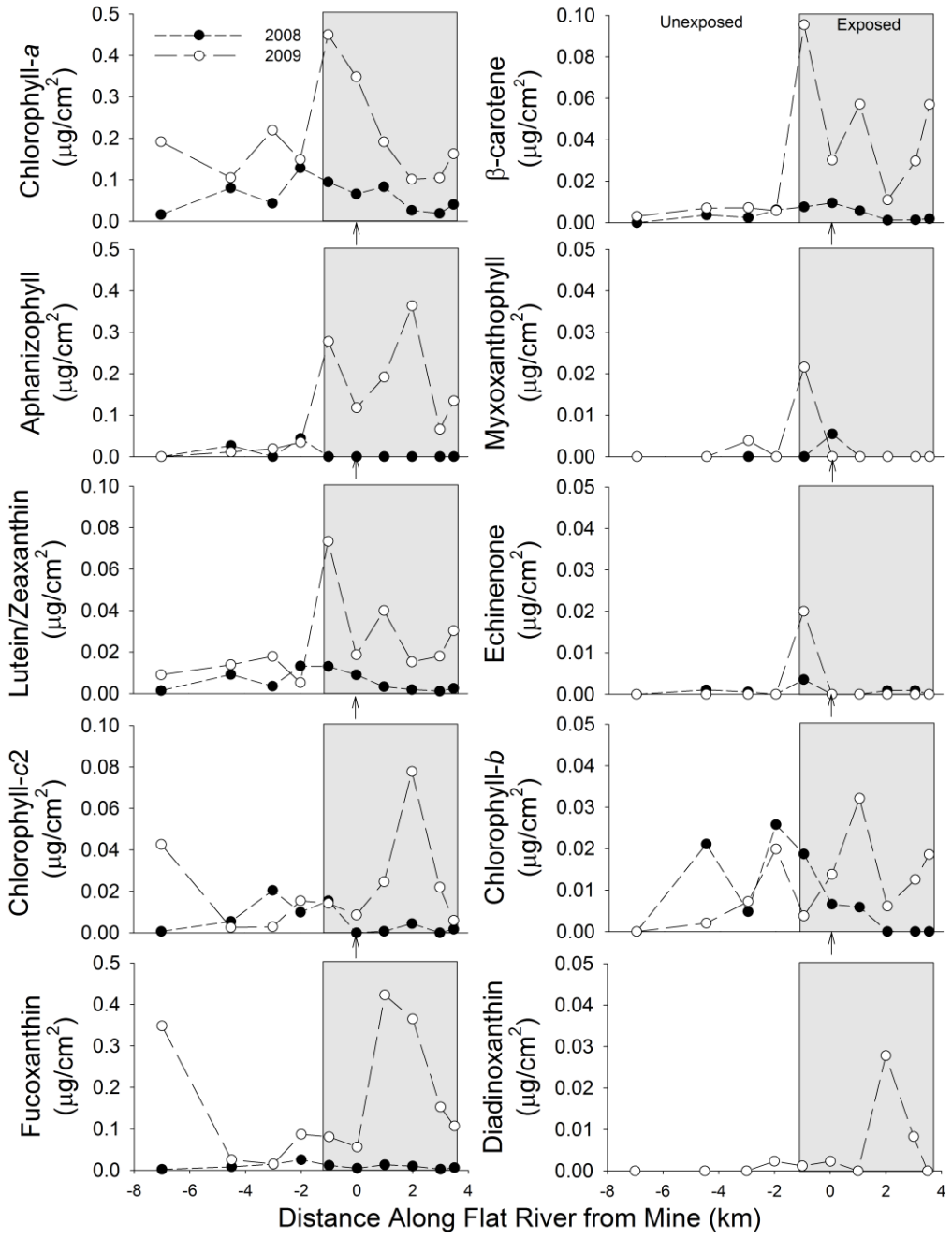


Figure 4.4 Longitudinal variation in concentrations of selected algal pigments in the Flat River in 2008 (closed circle) and 2009 (open circle). All sites are expressed as distances upstream (negative) and downstream (positive) of Tailings Pond 3 (closest site = 0.01 km; identified as vertical arrows along the x-axes). Sites defined as exposed are enclosed in a grey box, while those defined as non-exposed are not.

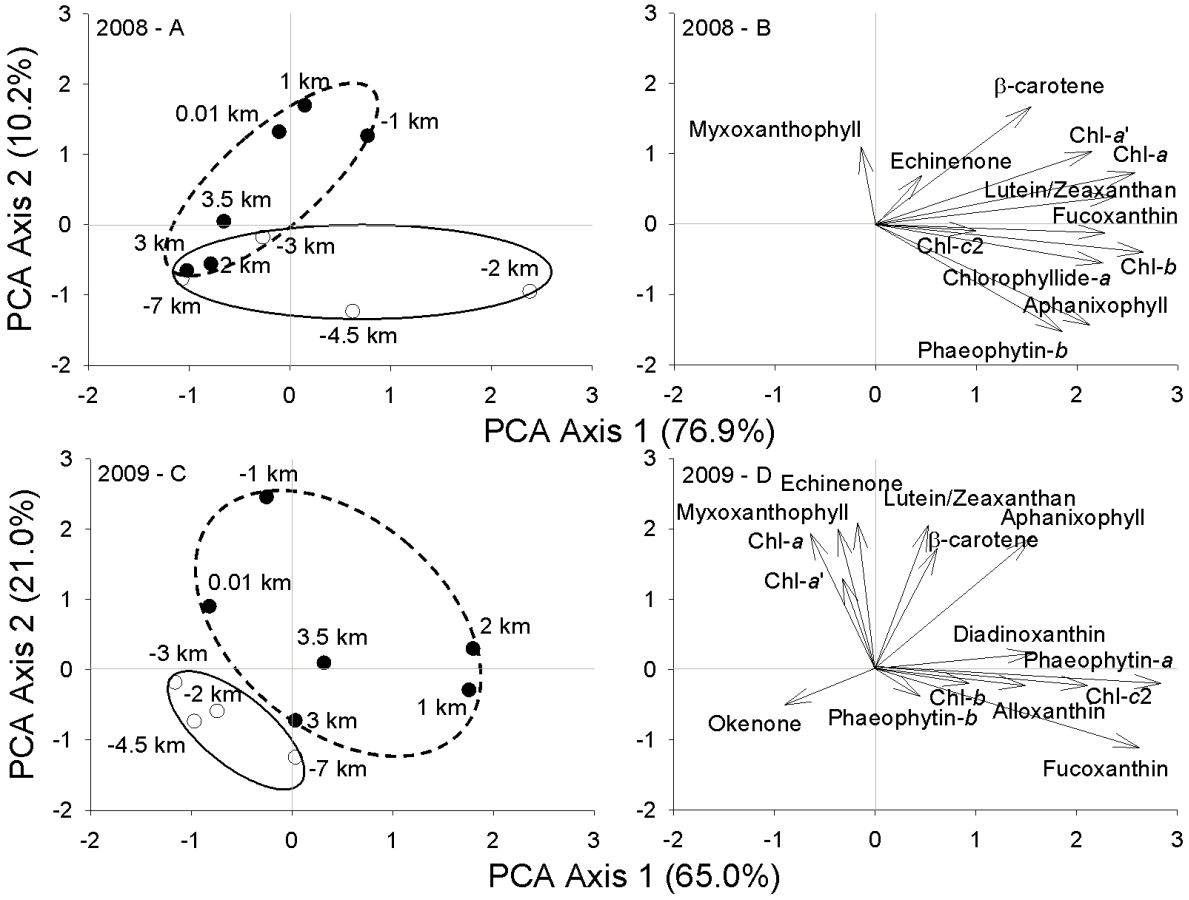


Figure 4.5 Principal components analysis ordination biplots showing distributions of photosynthetic pigment concentrations in the Flat River in 2008 (A – B) and 2009 (C – D). Each site is labeled according to their distance along Flat River and coded according to their category (i.e., non-exposed sites = white, exposed sites = black). Ellipses were drawn around sample scores for the non-exposed (solid line) and exposed (dashed line) sites. Arrows indicate the eigenvectors for the corresponding concentrations of pigments.

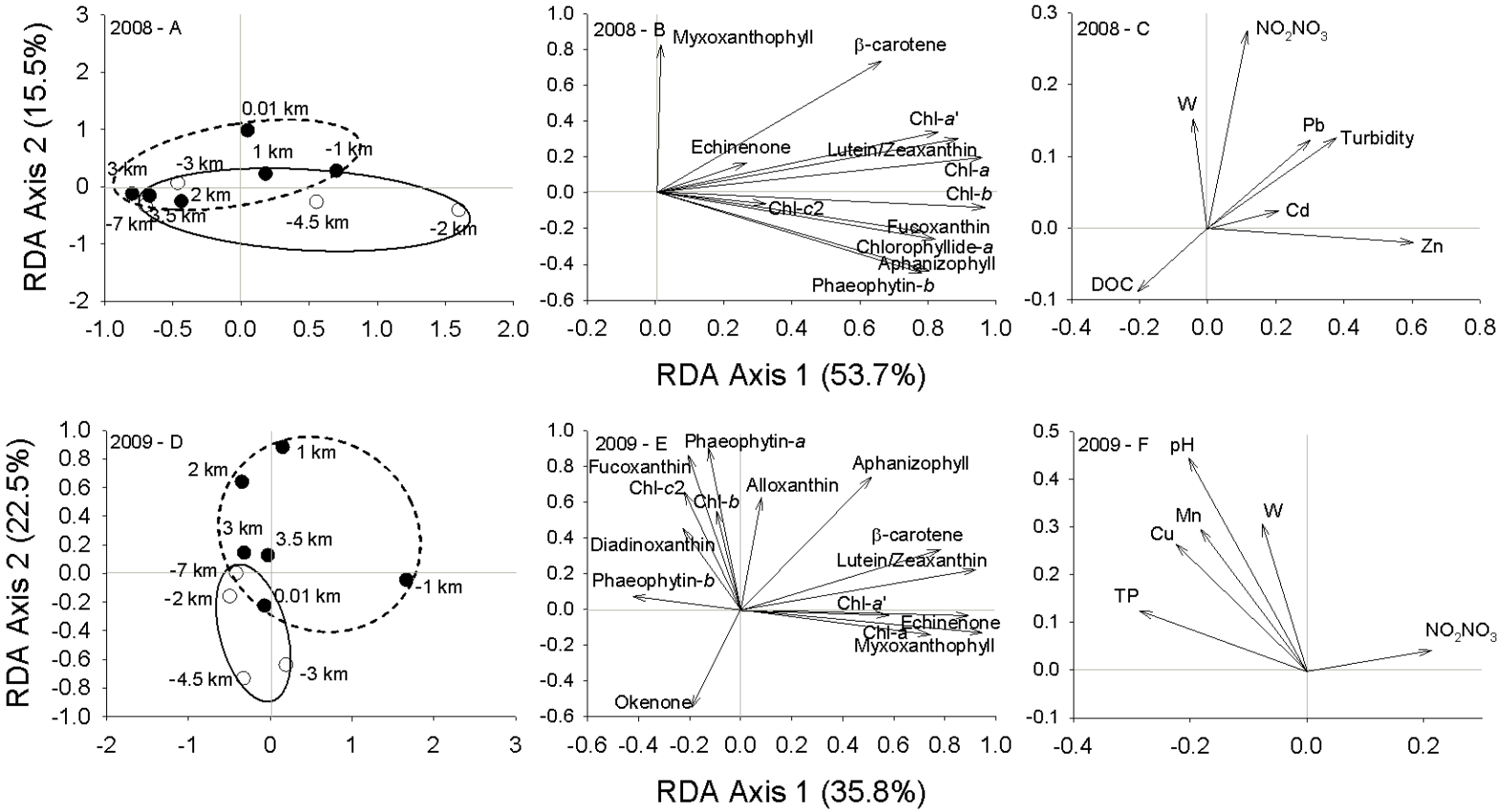


Figure 4.6 Redundancy analysis ordination biplots showing distributions of photosynthetic pigment concentrations and environmental variables in the Flat River in 2008 (A – C) and 2009 (D – F). Each site is labeled according to their distance along Flat River and coded according to their category (i.e., non-exposed sites = white, exposed sites = black). Ellipses were drawn around sample scores for the non-exposed (solid line) and exposed (dashed line) sites. Arrows indicate the eigenvectors for the corresponding concentrations of environmental variables and pigments.

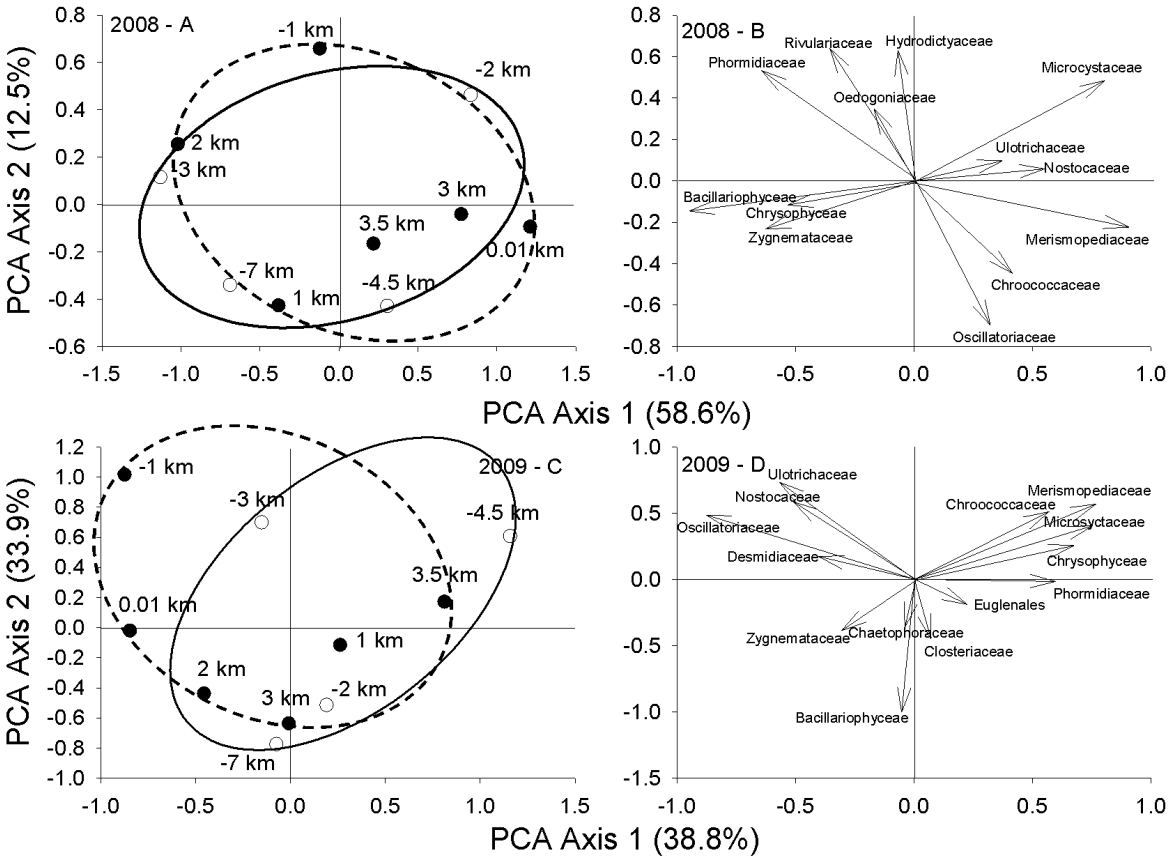


Figure 4.7 Principal components analysis ordination biplots showing distribution of benthic algal community composition (Class and Family level) based on relative abundance benthic algal counts from the Flat River in 2008 (A – B) and 2009 (C – D). Each site is labeled according to their distance along Flat River and coded according to their category (i.e., non-exposed sites = white, exposed sites = black). Ellipses were drawn around sample scores for the non-exposed (solid line) and exposed (dashed line) sites. Arrows indicate the eigenvectors for the corresponding benthic algal taxa.

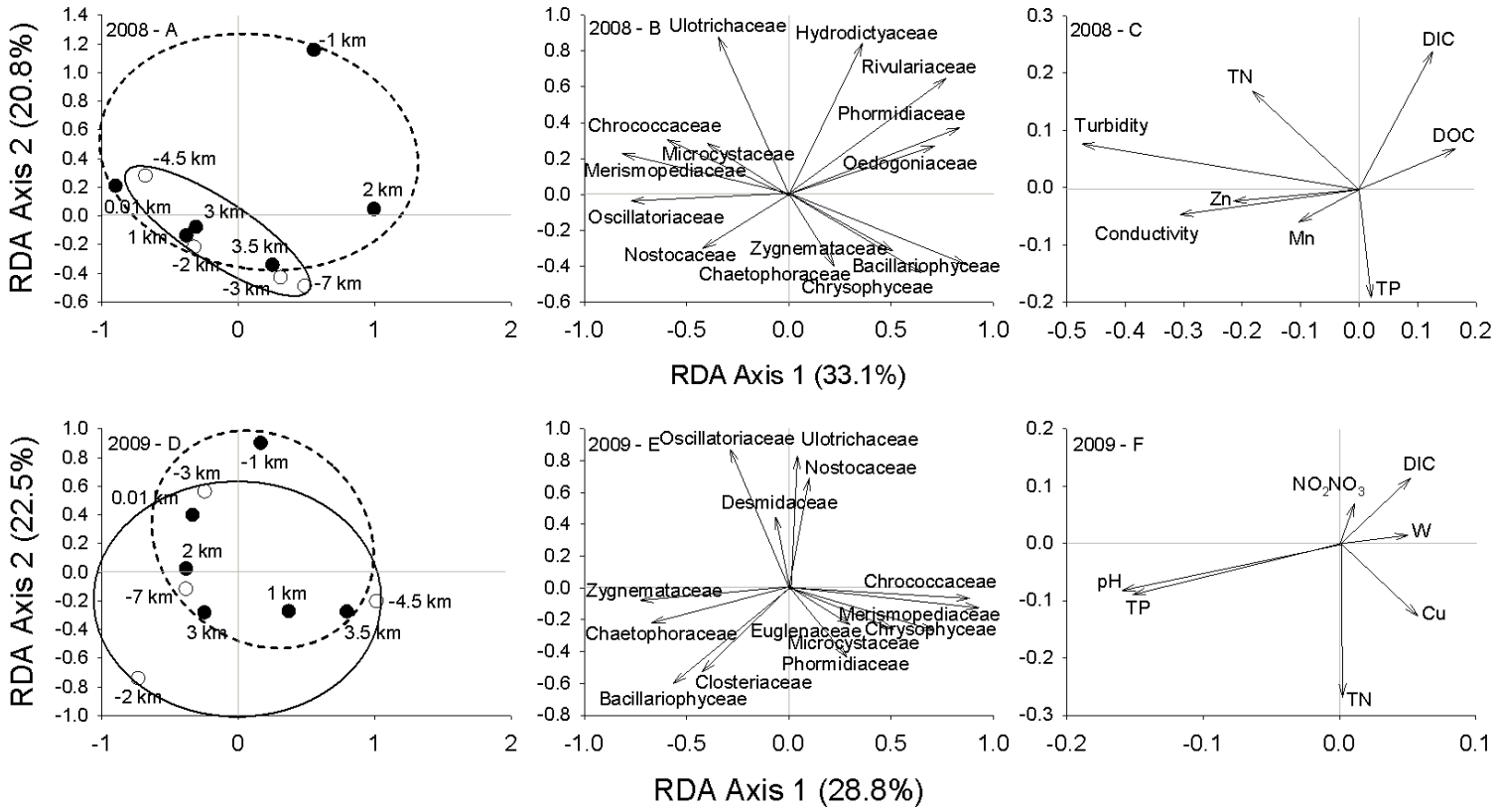


Figure 4.8 Redundancy analysis ordination biplots showing distribution of benthic algal community compositions (Class and Family level), based on relative abundance benthic algal counts, and environmental variables from the Flat River in 2008 (A – C) and 2009 (D – F). Each site is labeled according to their distance along Flat River and coded according to their category (i.e., non-exposed sites = white, exposed sites = black). Ellipses were drawn around sample scores for the non-exposed (solid line) and exposed (dashed line) sites. Arrows indicate the eigenvectors for the corresponding concentrations of environmental variables and benthic algal taxa.

4.7 Tables

Table 4.1 Locations and physical attributes of study sites in the Flat River, Northwest Territories, Canada. Reference sites are located upstream of the mine site whereas exposed sites are located adjacent to the mine site, or downstream of the main tailings pond. All physical descriptors are based on mean values measured at each site in each year. NTU = nephelometric turbidity units. See Methods for descriptions of study sites. Site distances with negative values are located upstream of the tailings pond whereas positive values are located adjacent to and downstream of the tailings pond.

Site number	Site location (km upstream or downstream of tailings pond)	Site type	Physical descriptors													
			Wetted width (m)		Average Water depth (cm)		Average Water velocity (cm/s)		Percent cobble and gravel		Dissolved oxygen (mg/L)		pH		Turbidity (NTU)	
			2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
1	-7	Ref.	19.4	16.0	19.1	30.0	0.53	0.67	29	32	10.9	12.0	8.2	8.2	1.6	18.5
2	-4.5	Ref.	9.6	13.2	22.7	17.6	0.54	1.08	53	34	11.5	9.6	8.1	8.0	5.2	14.7
3	-3	Ref.	17.0	13.4	19.1	23.0	0.47	0.64	37	20	11.1	9.8	8.1	7.9	1.1	21.5
4	-2	Ref.	17.6	14.6	44.6	27.5	0.82	0.67	43	20	11.9	9.6	8.0	8.1	4.6	13.6
5	-1	Exp.	14.4	9.8	26.6	20.2	0.52	0.77	43	63	11.7	10.8	7.6	8.0	3.0	10.2
6	0.01	Exp.	17.6	12.8	31.7	17.1	0.57	0.54	64	83	11.9	10.9	8.2	8.1	5.6	13.4
7	1	Exp.	21.4	14.6	25.9	29.3	0.54	0.44	47	33	12.8	9.6	8.1	8.5	5.7	15.1
8	2	Exp.	26.2	26.2	14.4	26.3	0.51	0.51	55	30	11.4	9.7	8.0	8.4	0.1	16.0
9	3	Exp.	27.0	23.6	29.7	25.4	0.65	0.69	45	66	11.7	12.7	7.7	8.2	2.8	17.0
10	3.5	Exp.	29.6	31.2	25.9	24.7	0.52	0.47	56	50	11.9	9.9	7.9	8.2	2.6	15.9

Table 4.2 Total sample cost and itemized cost components to quantify algal pigment concentrations using a high performance liquid chromatographer (HPLC). Costs are in Canadian dollars. Labour costs are based on a full time technician at an annual salary of $70,000, including benefits, to complete sample preparation, and process and coordinate all activities related to running, maintaining and servicing the HPLC.

Cost item	Activity description and major costs components	Cost ($)
1	Sample collection, extraction and preparation (GF/F glass fiber, extraction solution, filtration/purification equipment, N_2 gas and injection solution).	6.00
2	Characterizing the algal pigment with the HPLC (Mobile phase A & B, inserts for vials)	1.50
3	Per-sample costs associated with annual maintenance and servicing of the HPLC. Annual costs = $15,000 year / 1500 samples analyzed each year.	10.00
4	Per-sample costs associated with the purchase of the HPLC (Purchase price = $80,000 / 20 years = $4000 year. Per-sample cost = $4000 year / 1500 samples analyzed each year.	2.67
5	Technician to run and maintain the HPLC. Annual cost = $70,000 / 1500 samples analyzed each year.	46.67
	Total costs per sample	66.84

Chapter 5

Development of a benthic algal reference condition model to assess ecological integrity within the South Nahanni River watershed

5.1 Overview

Monitoring biologists are continually striving to improve monitoring protocols in order to effectively assess alteration of water quality and biological communities due to contaminants. We developed benthic algal RCA models for the South Nahanni River watershed, NWT. For this, we sampled a suite of least disturbed sites across the watershed in 2008 (n = 44) and 2009 (n = 18; 12 resampled from 2008 and 6 new) and test sites (potentially affected) downstream of two mining companies (n = 20 in 2008 and n = 18 in 2009). Benthic algal communities were assessed using three metrics: 1) benthic algal community composition (coarse taxonomic resolution counts), 2) diatom community composition (high-taxonomic resolution counts) and 3) photosynthetic pigment concentrations. The BEAST (BEnthic Assessment of SedimenT) model was used to develop the benthic algal RCA models. Patterns of impairment downstream of the two mines (Cantung mine along Flat River and Prairie Creek mine along Prairie Creek) were assessed and zones of influence were identified for each algal metric in each year. Results showed that the three models identified reasonably consistent 'zones' of stress downstream of Cantung mine along Flat River. However, changes in photosynthetic pigment concentration were more sensitive compared to the other two metrics. Along Prairie Creek, only photosynthetic pigment concentration identified sites outside of the reference condition directly downstream of the Prairie Creek mine. Our results show that benthic algal RCA models (specifically photosynthetic pigment concentration models) show promise as biological monitoring tools.

5.2 Introduction

Rivers of northern Canada are increasingly threatened by industrial development and climate change (Prowse et al., 2006; Schindler & Smol, 2006; Wrona, 2006; Prowse et al., 2011). Industrial development such as mining in Canada's north is anticipated to nearly double between 2011 and 2020 (The Conference Board of Canada, 2013). In the face of these pressures, improved long-term monitoring protocols are required to inform policies and practices that can safeguard against deterioration of water-quality and ecological integrity. Monitoring of streams and rivers in northern Canada consists primarily of the collection of water chemistry variables, macroinvertebrates and fish. Among the numerous biota that can be monitored, benthic algae possess many features that predispose them to provide effective monitoring of mining activities in rivers [Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2013 (Chapter 4)]. It has also been recognized that monitoring lower trophic levels could reflect changes at higher levels (e.g., macroinvertebrates and fish communities; Kilgour et al., 2005, 2007). Monitoring protocols for sampling benthic algae in rivers have been developed in Europe (CEN; CEN, 2003, 2004), New Zealand (NIWA; Biggs & Kilroy, 2000), and the USA (US EPA; Stevenson & Bahls, 1999). In Canada, benthic algae have been utilized in studies of streams and rivers in southern, temperate regions (Reavie & Smol, 1998, Winter & Duthie, 2000; Lavoie et al., 2006), but standardized benthic algal monitoring protocols have not yet been evaluated for northern Canadian rivers.

Environmental effects monitoring (EEM) is mandatory for metal mines in Canada under the metal mining effluent regulations (Environment Canada [EC], 2012). Study designs recommended for EEM include control-impact (CI) and reference condition approach (RCA). The benefit of the RCA model is that it uses many regional reference sites to characterize the

reference condition. Each reference site is an individual site selected from across a large area (typically a watershed) in order to adequately characterize the reference conditions in the area (Reece & Richardson, 1999; Rosenberg et al., 1999; Bowman et al., 2010). The RCA assumes that the biological communities at sites are primarily influenced by the physical and chemical conditions at each site. Thus, each reference site is also selected to be similar in physical and chemical conditions to the potentially affected 'test' sites (Hulbert, 1984; Reynoldson et al., 1997; Bailey et al., 2004). RCA models have been successfully developed for invertebrate communities across Canada (e.g., Reynoldson et al., 1997; Reynoldson et al., 2001; Bowman et al., 2010). However, few studies have developed models for benthic algal communities, and only one study (Bowman et al., 2010) developed a preliminary model for algal communities within the South Nahanni River watershed.

The South Nahanni River watershed has a high preservation value and cultural significance. However, deposits of metals within the watershed have led to current and future mining operations that may threaten downstream water quality and ecological integrity. There are currently two mine operations within the watershed. Previous studies have assessed the effects of these mines on biological communities (i.e., fish, macroinvertebrates, and benthic algae) and water quality downstream of the mines [Spencer et al., 2008; Bowman et al., 2010; Scrimgeour, 2013; Thomas et al., 2013 (Chapter 4)]. Two of these studies found alteration of benthic algal community composition downstream of the two mining sites [Spencer et al., 2008; Thomas et al., 2013 (Chapter 4)]. Only one study developed a preliminary RCA model based on fish, macroinvertebrate, and benthic algae (Bowman et al., 2010). This study did not find significant differences in benthic algal community composition downstream of the mine sites. However, the study sampled a smaller number of reference sites than our study. Here, we

develop a more comprehensive RCA model based on composition of benthic algal communities within the South Nahanni River watershed. With the larger number of reference sites in our study, the resulting RCA models are predicted to have better ability to detect impairment. We collected water chemistry and benthic algal samples from 44 reference sites in 2008 and 18 reference sites in 2009 from across the South Nahanni River watershed. We analyzed three benthic algal metrics (benthic algal community composition, diatom community composition and photosynthetic pigment concentrations) which have been shown to provide useful information to track changes in river and lake conditions in this and other regions [Rott et al., 1998; Hill et al., 2000b; Hirst et al., 2002; Rosenberger et al., 2008; Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2011 (Chapter 2), 2013 (Chapter 4)]. The objectives of this study were three-fold: 1) using the data collected, we developed RCA models for each algal metric (benthic algal community composition, diatom community composition and photosynthetic pigment concentrations) to evaluate the ecological health of stream sites downstream of two mining companies, 2) we used the models to determine potential zones of influence of each mine, 3) the three RCA models and resulting test-site assessments were used to make comparisons of the ability of the three algal metrics to reflect changes in water chemistry.

5.3 Methods

5.3.1 Study sites

The South Nahanni River watershed is located in southwestern Northwest Territories. The watershed is characterized by subarctic climate including mean air temperatures of 9°C during summer and -19.5°C during winter (EC, 1991). As part of the Mackenzie Mountains Ragged Ranges, the elevation in South Nahanni River watershed ranges from less than 1372 m above

sea level to approximately 2770 m above sea level. The watershed is underlain by Proterozoic glaciomarine conglomerates, early Paleozoic formations, late Devonian to Jurassic formations, and Cretaceous granitic rock formations (Halliwell & Catto, 2003). The west portion of the watershed is underlain primarily by shale and has the ragged, snow-capped mountains of the Logan Mountains and the Ragged Ranges, while the east is underlain primarily by carbonates and incorporates the Nahanni Karst and Ram plateaus (Caron et al., 2008). The geological formations are naturally abundant in deposits of tungsten, lead, zinc, silver, and gold, which has resulted in mining claims throughout the watershed including an operational tungsten mine on Flat River (North American Tungsten, Cantung Mine) and an advanced lead, silver, and zinc exploration mine on Prairie Creek (Canadian Zinc Corporation, Prairie Creek Mine) (Scrimgeour, 2013).

For this study, a total of 44 sites were selected in 2008 and 18 sites in 2009 (12 repeated sampling from 2008; 6 newly sampled in 2009) based on a double-stratified random-sampling design (Figure 5.1). The two strata used were stream order and percent ice cover, because they are well known to influence physical and chemical stream conditions. By stratifying samples according to stream order and % ice cover, we aimed to minimize effects of stream order and percent ice cover in order to better detect variability in chemical characteristics and benthic algal communities due to other factors operating across the watershed. Firstly, potential sampling sites were randomly identified that fell into the 3rd to 6th order stream category. Then, those sites with >40% ice cover were removed. This led to the identification of 140 potential sampling sites. Not all sites were sampled due to unsafe helicopter landing sites, lower flow than expected along Flat River and Prairie Creek, or wildfires that did not allow safe passage to the stream site. All reference sites were selected from undisturbed areas of the South Nahanni

River watershed to determine benthic algal community structure and physical and chemical conditions at stream and river sites having no direct human influence (e.g., no source of contaminants adjacent to or upstream). Multiple reference sites were sampled along Caribou River, Cathedral Creek, Clearwater Creek, Flat River, Flood Creek, Prairie Creek, Little Nahanni River, and Mary River. However, these sites were located greater than 2 km apart with multiple confluences of 1st to 3rd order streams between sampling locations, and were, therefore, considered independent from each other.

A total of 39 test sites (21 in 2008 and 18 in 2009) were sampled between August 2nd and 13th in 2008 and between August 7th and 14th in 2009 (Figure 5.1).The test sites were located downstream of two mines within the watershed (North American Tungsten – Cantung Mine and Canadian Zinc Corporation – Prairie Creek Mine). At the time of sampling, the Cantung Mine was an operational tungsten mine located along the Flat River in the west of the watershed at the Northwest Territories – Yukon border (61° 57', 128° 13'; Figure 5.1). Flat River is located in the Selwyn Mountain ecoregion and is underlain primarily by shale (Caron et al., 2008). Tailings rich in heavy metals from mine processing and nutrients from sewage are pumped into a series of three tailings ponds (Figure 5.2A). The majority of tailings were deposited into Tailings pond 3 at the time of sampling. A small amount of leachate from the tailings ponds enters the Flat River immediately adjacent to the mine site and likely enters the Flat River along a distance of several hundred meters. Thus, we defined the 12 test sites along Flat River based on their proximity to Tailings Pond 3. Mining activities along Flat River have been associated with elevated concentrations of Al, As, Cr, Cu, Fe, Pb, Mn, and W downstream of the mine site (e.g., Spencer et al., 2008).

The Prairie Creek Mine is an advanced lead, silver and zinc exploration mine located along Prairie Creek in the east portion of the watershed (61° 33', 124° 47'; Figure 5.1). Prairie Creek is located in the Nahanni Plateau ecoregion and is underlain primarily by limestone and dolostone bedrock with veins of zinc, lead, copper, and silver mineralization (Caron et al., 2008). Metal-enriched water is pumped into a series of settling ponds which eventually drain into Prairie Creek via Harrison Creek at the time of sampling (Figure 5.2B). Similarly, nutrient-rich sewage effluent drains from a settling tank directly into Prairie Creek via Harrison Creek. Unlike the Cantung Mine, leachate from the settling ponds at the Prairie Creek Mine enter Prairie Creek via Harrison Creek with minimal amounts entering downstream of the confluence of the two creeks. Thus, we defined the 8 test sites along Prairie Creek based on their proximity to the confluence of Prairie Creek and Harrison Creek. Mining activities along Prairie Creek have been associated with elevated concentrations of heavy metals such as Al and Zn downstream of the mine site (e.g., Spencer et al., 2008).

5.3.2 Field and laboratory methods

5.3.2.1 Physical and chemical data

At each site, water samples (2L) for chemical analyses were collected from the midstream of flow at approximately 30-cm depth. Samples were stored in the dark and kept cool until transported to a temporary field laboratory for processing. At the temporary field laboratory, all water samples were first filtered through an 80-µm mesh to remove large debris, and then sub-sampled and processed for analysis of concentrations of total metals (Ag, Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, La, Li, Mn, Mo, Nb, Ni, Pb, Pt, Rb, Sb, Se, Sn, Sr, Tl, U, V, W, Y, Zn), nutrients [dissolved inorganic carbon (DIC; filtered through a 0.45-µm cellulose acetate filter), dissolved organic carbon (DOC; filtered through a 0.45-µm cellulose acetate

filter), nitrite + nitrate (NO_2+NO_3), total nitrogen (TN), and total phosphorus (TP; preserved with 30% H_2SO_4)]. Metals were analyzed using inductively coupled plasma mass spectrometry. Concentrations of DOC and DIC were analyzed using an UV-persulfate TOC analyzer, and samples for NO_2+NO_3, TN, and TP measurements were analyzed using an automated continuous segmented flow analyzer at Environment Canada's National Laboratory for Environmental Testing, Burlington, Ontario (EC, 1994). Measurements of conductivity and pH were taken at each site using a YSI model 650 meter, while measurements of turbidity were taken at each site using a LaMotte model 2020e turbidity meter. Water velocity and depth were measured using a Marsh McBirney flow mate.

Physical variables included quantitative, categorical and binary data encompassing the physical characteristics of the streams and riparian habitats at the sites (Appendix C, Table 5.1). Variables such as canopy cover, habitat types, macrophyte coverage, riparian vegetation, substrate size, and percent gravel and cobble within the river bed were determined using protocols described by Environment Canada (2011). Bankfull and wetted widths were measured with a Bushnell range finder (± 0.5 m) or with a tape measure. Variables describing basin morphology, climate, land cover, and bedrock geology were derived from landscape-scale GIS data (geo-spatial databases including: digital elevation model [www.geobase.ca], stream network [www.geobase.ca], climate [http://sis.agr.gc.ca/cansis/nsdb/ecostrat/district/climate.html], and bedrock geology [http://www.lib/uwo.ca/madgic/geospatial/can_geo1860_1997_data.htm]. For full description of variables and how they were measured see Appendix C, Table 5.1.

5.3.2.2 Benthic algal sampling

Benthic algal samples were collected from the upper surface of 5 to 10 cobbles at each site. Sampling was restricted to cobbles in order to reduce within-site and among-site variation due to the confounding influence of different substrate types (Biggs & Kilroy, 2000). Separate benthic algal samples were collected for each type of metric including taxonomic metrics (benthic algal community composition and diatom community composition) and quantification of photosynthetic pigment concentration. Each sample was collected by removing benthic algae from cobbles using a syringe sampler (as described in Thomas et al., 2013; Chapter 4) and combining the material from the 5 to 10 cobbles to make one composite sample representing a measured surface area (range = 26.5 to 53.1 cm^2). Samples were stored in the dark and kept cool until further processing. Benthic algal samples for quantification of photosynthetic pigments by HPLC were filtered onto Whatman GFF filters (0.7 µm), wrapped in aluminum foil and frozen until analysis at the University of Waterloo. Benthic algal samples for taxonomic analyses were preserved using Lugol's preservative and transported to the University of Waterloo for further analysis.

5.3.2.3 Benthic algal community composition

The samples for determination of benthic algal community composition were processed by sub-sampling 2 mL of well mixed sample and placing it into Utermöhl chambers. These samples were allowed to settle for 24 hours before algal cells were identified and enumerated using an inverted microscope (at 1000 x magnification). Approximately 300 units of algae were identified to Class (e.g., Bacillariophyceae) or Family (e.g., Chrococcaceae, Oscillatoriaceae) level following the nomenclature of Prescott (1951) and Wher & Sheath (2003). The relative abundances of each type of algae were calculated by dividing the number

of units enumerated per taxonomic group by the total number of units enumerated in the sample and multiplying by 100.

5.3.2.4 Diatom community composition

The samples for determination of diatom community composition (percent composition) were assessed by sub-sampling 15 mL of well-mixed sample into individual test tubes which were allowed to settle for 24 hours. The supernatant was removed from the sample and replaced with deionized water and allowed to settle for another 24 hours. This method for rinsing samples was repeated until most of the Lugol's solution was removed from the samples. Upon removal of Lugol's solution the supernatant was again removed and the samples were oxidized by the addition of 30% hydrogen peroxide to remove organic material. Samples were left at room temperature for one week to allow sufficient time for reaction of hydrogen peroxide and organic matter. Then, acid residues were removed by repeated siphoning of the supernatant, dilution with deionized water and settling for 24 hours until the solution reached a pH comparable to the deionized water. This resulted in cleaned slurries of diatom cells, which were dried onto glass coverslips and mounted onto microscope slides using Naphrax mounting medium. At least 300 diatom values per sample were identified and enumerated using a compound light microscope at 1000x magnification (Zeiss Axioskop 2Plus, numerical aperture = 1.30). Taxonomic identifications followed the nomenclature of Krammer & Lange-Bertalot (1986 – 1991) and Lavoie et al., (2008b). Only species contributing greater than 1% abundance in at least one sample were included in the numerical analyses.

5.3.2.5 Photosynthetic pigment concentration

We extracted photosynthetic pigments from samples in a mixture of acetone:methanol:water (80:15:5 by volume) for 24 hours at -20°C. Once extracted, the supernatant was filtered through a 0.22 µm polytetrafluoroethylene (PTFE) syringe filter to remove large particles and other impurities. The filtrate was then dried under inert (N_2) gas to remove all water and extraction solution. The dried pigments were re-eluted in 500 µL of injection solution consisting of acetone:ion-pairing reagent: methanol (70:25:5 by volume). Ion-pairing reagent (IPR) consisted of 0.75 g tetrabutylammonium acetate and 7.7 g ammonium acetate. A Waters HPLC, reverse-phase system with a symmetry C18 column (3.5 µm, 4.6 x 75mm) was used to separate pigments following the methods of Leavitt et al., (1989), modified from Mantoura & Lleywellyn (1983). Pigments were separated using a gradient delivery of Mobile Phase A, consisting of methanol:IPR (90:10 by volume) and Mobile Phase B, consisting of methanol:acetone (73:27 by volume). Sudan II was used as an external standard at the beginning and end of each run to account for changes in chromatographic mobility and as an internal standard to account for dilution and injection errors. Pigments were extracted from a sample of geranium leaves and were placed at the beginning and end of each run to account for the chromatographic mobility of individual pigments during the run. Pigment signatures were measured by a Waters 2998 PDA detector and a Waters 2475 multi λ fluorescence detector. Identification of pigment signatures was based on chromatographic mobility (Leavitt et al., 1989) and spectral characteristics (Jeffrey et al., 1997). Pigments were expressed as $\mu g/cm^2$ (i.e., mass per unit area of cobble surface).

5.3.3 Numerical analyses

5.3.3.1 RCA model development

The RCA has been developed as an assessment tool based on the assumption that biological communities can be predicted from the physical chemical characteristics of their surrounding environment. To this effect, the RCA involves characterization of the physical attributes and biological communities at a number of relatively undisturbed reference sites to characterize the range of variation in reference conditions. These references sites are assumed to be influenced by their surrounding physical and chemical characteristics. The first step in the RCA is to use hierarchical agglomerative cluster analysis (along with non-metric Multi-Dimensional Scaling Analysis [MDS] ordination and statistical tests) to group reference sites that possess similar biological assemblages. The second step is to identify the physical attributes (not affected by stressor of interest) that best discriminate between the sites with similar biological assemblages. This is accomplished using Discriminant Function Analysis (DFA). The resultant model then uses the selected environmental variables to predict which reference assemblages are most appropriate for each test site to be compared with, again using DFA. These reference assemblages represent the range of expected natural variability for the test site, assuming it was not affected by anthropogenic stress (Sylvestre et al., 2005). The third step in the RCA is to compare biological communities at a potentially affected 'test' site to their predicted reference assemblages using ordination techniques (MDS ordination). In the MDS ordination, combinations of three axes are used to assess the position of the test site relative to the expected reference communities. The closer the sites are in ordination space, the more similar they are (and *vice-versa*). The difference between the expected reference communities and the community at each test site indicates the degree of anthropogenic stress (Sylvestre et al., 2005).

The status of each test site was assessed using confidence ellipses. If a test site falls within the 90% confidence ellipse for the reference sites it is considered to be in "reference condition". If it falls between the 90% and 99% confidence ellipses it is considered to be "possibly stressed". If it falls between the 99% and 99.9% confidence ellipse it is considered to be "stressed", and if it falls outside the 99.9% confidence ellipse it is considered to be "severely stressed" (Reynoldson et al., 1997; Bailey et al., 2004; Sylvestre et al., 2005). Additionally, we added two extra ellipses (80% and 85%) to identify sites which were close to possibly stressed in order to aid in interpretation of sites that were identified as in reference condition but were bounded by otherwise stressed sites. Sites between 80 and 85% ellipses were recorded as 'reference condition*' and sites between 85 and 90% ellipses were recorded as 'reference condition**'. If a site was predicted to two assemblages with roughly equal probability, it was tested with both assemblages and results were recorded using both assessments.

For the first step of the RCA design (grouping of reference sites into biologically similar assemblages), hierarchical agglomerative clustering and MDS ordinations were used to discriminate reference site assemblages that had similar composition of benthic algal communities, and to identify outliers. One-way ANOSIM tests (with 9999 permutations) were used to determine if community composition differed significantly among the assemblages identified by hierarchical agglomerative cluster analysis and MDS ordinations. The second step of the RCA design involved identifying the physical variables that best discriminated between the groups of reference assemblages, followed by predicting test site assemblage affiliation. This step was performed using DFA to produce predictive models. In a first step, backward selection was used to identify a subset of environmental variables that discriminated among reference site assemblages. Then, environmental variables were added and removed until the

best combination of environmental variables was found (i.e., the combination that produced the strongest model). The performance of the models (how often a site would be reclassified into the proper assemblage) was determined by a jackknifed (cross-validated) classification matrix in which the site to be tested was removed prior to model development and then tested for correct classification (Sylvestre et al., 2005). Once the model that best discriminated between reference assemblages was created, it was used to predict assemblage membership of each test site (i.e., the probability of belonging to each assemblage). The assemblage with the highest probability of membership for each test site was used to assess the test site for impairment. Test sites that were predicted equally to more than one reference assemblage were compared to both assemblages and both findings were incorporated into the results.

Test sites were assessed for impairment by plotting each individual test site with their associated reference assemblages using MDS ordinations. Biplot ordinations were subsequently created using the first three axes of the MDS ordinations (i.e., axis 1 & 2, axis 1 & 3 and axis 2 & 3). Individual assessments were carried out for each test site and the associated reference assemblages. Probability ellipses of site scores were created in each biplot for test site assessment. Test sites were assessed based on where they plotted relative to the confidence ellipses. If a test site fell on top of an ellipse, it was recorded as both categories (e.g., possibly stressed – stressed).

The above methods were carried out individually for each biological metric (benthic algal community composition, diatom community composition and pigment concentration). Metrics based on relative abundance data (benthic algal community composition and diatom community composition) were square-root transformed prior to all analyses to down-weight the influence of the most abundant taxa. Pigment concentrations were log(x+1) transformed to

equalize variances. Cluster analyses, MDS ordinations and ANOSIM tests were performed using the software PRIMER version 6 (Clark & Gorley, 2006). Discriminant function analyses were performed using the software SYSTAT version 11 (SYSTAT Software Inc. 2004).

In order to delineate zones of influence of each mine site on nutrient and metal concentrations of the river water and subsequently to use those zones to assess against the benthic algal-derived assessment of impairment, PCA ordinations of nutrient and metal concentrations at sites along Flat Rivera and Prairie Creek were run separately and for each year. PCA Axis 1 scores were extracted and plotted against distance along each creek. From this, zones of changes in Axis 1 scores were used to determine zones of influence of the mine. All water chemistry values (except pH) were tested for normality prior to analysis and transformed accordingly using ln(x+b) transformations, where b was half the smallest non-zero value. The PCA ordinations were performed using CANOCO 4.5.

5.4 Results

5.4.1 Development of RCA model

5.4.1.1 Cluster analysis and ordination

The cluster analysis of benthic algal community composition showed that reference sites formed three distinct assemblage types consisting of between 11 and 32 sites (Figure 5.3). Similarly, ordination by MDS of the benthic algal communities showed that the three clusters of sites occupied distinct areas along the first two axes (Figure 5.4A). Along the 1st axis, sites differed according to differing relative abundances of diatoms and filamentous algae. Along the 2nd axis, sites differed in relative abundance of colonial cyanobacteria and desmid/chrysophyte assemblages (Figure 5.4A; Table 5.1). Sites characterized by assemblage 1 were positioned to the right along axis one, and possessed higher relative abundance of

filamentous algae (e.g., Zygnemataceae, Oscillatoriaceae and Phormidiaceae), colonial cyanobacteria (e.g., Merismopedia) and lower relative abundance of diatoms (Bacillariophyceae). Sites characterized by Assemblage 1 were also associated with the highest altitude, longitude, percent cobble substrate and lowest bankfull width (Table 5.1). Sites within assemblages 2 and 3 were positioned to the left along axis 1 and had higher relative abundance of diatoms (Bacillariophyceae) compared to assemblage 1. Sites within assemblage 2 differed from those within assemblage 3 due to higher relative abundance of colonial cyanobacteria such as Chroococcus and Microcystaceae than sites within assemblage 2 (but roughly the same amount as assemblage 1). Sites within assemblage 2 also had higher relative abundance of filamentous algae such as Oscillatoriaceae and Phormidiaceae than those within assemblage 3 (but less than assemblage 1). Sites within assemblage 2 were associated with the highest amount of percent ice cover and bankfull width compared with sites with the other assemblages. Assemblage 3 differed from assemblage 2 in that it had a higher relative abundance of Merismopedia compared to assemblage 2 (but less than assemblage 1; Table 5.1). Assemblage 3 had the lowest altitude, longitude and percent ice cover and highest stream order and percent forest cover. Based on results of an ANOSIM test, composition of all 3 assemblage types differed significantly ($p < 0.01$).

Diatom community composition differed distinctly among the reference sites based on ordination and cluster analysis and formed three distinct assemblages consisting of between 13 and 25 sites (Figure 5.3B; Figure 5.4B). Assemblages appeared to differentiate along two main gradients. Along the 1st axis, assemblage 1 was separated from assemblages 2 and 3 and were characterized by higher relative abundances of *Cyclotella*, *Fragilaria*, *Hannaea* and *Navicula*, whereas assemblages 2 and 3 were characterized by higher relative abundances of

Achnanthidium, *Brachysira*, *Encyonopsis* and *Navicula*. Along the second axis, assemblage 2 was separated from assemblage 3 associated with higher relative abundances of *Encyonema*, *Eucocconeis* and *Fragilaria* and lower relative abundances of *Gomphonema* (Figure 5.4B; Table 5.1). More specifically, sites with assemblage 1 were characterized by higher relative abundances of *Hannaea arcus* (56%) compared to assemblages 2 and 3. Sites with assemblage 1 were associated with the lowest altitude of all three assemblages and highest percent forest cover (Table 5.1). Sites with assemblage 2 and 3 were characterized by higher relative abundances of *Achnanthidium minutissimum* compared to sites with assemblage 1. Sites with assemblage 3 were also characterized by the highest relative abundances of *Gomphonema parvulum* var. *micropus* (12.5%), *Gomphonema olivaceum* (3.4%) and *Gomphonema* unknown (3.0%) compared to those with assemblages 1 and 2 (Table 5.1). Sites with assemblage 2 were associated with the highest altitude, longitude and percent ice, while sites with assemblage 3 had the lowest longitude and percent ice cover and highest bankfull width. Assemblage 1 had the highest relative abundance of *Hannaea arcus* (56.4%) and *Fragilaria capucina* var. gracilis (6.6%), while assemblage 2 had the highest relative abundances of *Fragilaria capucina* var. *vaucheria* (9.1%), *Diatom tenuis* (8.2%) and *Encyonema minutum* (1.9%; Table 5.1). Community compositions differed significantly among all three assemblages ($p < 0.01$), based on an ANOSIM test.

For the pigment concentration data, sites were clustered into 4 assemblages based on cluster analysis and confirmed by MDS ordination which showed sites grouping into four distinct assemblages consisting of between 4 and 24 sites (Figure 5.3C; Figure 5.4C). Three of the 4 assemblage types separated along axis 1, along a single gradient from lowest pigment concentrations in assemblage 3 to highest pigment concentrations in assemblage 2 with

intermediate pigment concentrations in assemblage 1 (Figure 5.4C, Table 5.1). Sites in Assemblages 1 through 3 differed in their environmental variables with assemblage 1 having the lowest altitude, longitude and bankfull width and having the highest percent forest, percent cobble and stream order. Sites in Assemblage 2 had the highest altitude, longitude, percent ice and bankfull width, while assemblage 3 had the lowest stream order, percent forest and percent ice cover (Table 5.1). Sites in assemblage 4 were located along a second gradient and were distinctly different from all other sites. These sites were characterized by high concentrations of phaeophytin-*a* and phaeophytin-*b* (Table 5.1). Sites with assemblage 4 were characterized by the lowest altitude, longitude and percent ice and the highest stream order and bankfull width among all four assemblages. An ANOSIM test identified that all assemblages differ significantly from each other ($p < 0.01$). Because assemblage 4 only consisted of 4 sites, it was eliminated from further analyses.

The assemblages created for the benthic algal community composition and diatom community composition data roughly coincided with the two major ecoregions (Selwyn Mountain ecoregion and Nahanni-Hyland ecoregions) across the watershed (Appendix C, Figure 5.1A-G). Assemblages 1 and 2 included mostly sites located within the Selwyn Mountain ecoregion (61 – 78%). Assemblage 3 for the benthic algal community composition data included sites primarily located within the Nahanni-Hyland ecoregion (70%), however about half of the reference sites with assemblage 3 of the diatom community composition were located in each ecoregion. The assemblages for pigment concentration data were located approximately equally in each ecoregion (Appendix C, Figure 5.1H-J). For all algal metrics, assemblage 2 contained the highest amount of reference sites along Flat River (3, 3, 2; benthic algal community composition, diatom community composition and pigment concentrations,

respectively), while assemblage 3 contained the highest amount of reference sites along Prairie Creek (5, 5, 3 benthic algal community composition, diatom community composition and pigment concentrations, respectively).

5.4.1.2 Discriminant function analysis

Discriminant function analysis found that 20 variables successfully discriminated among the three benthic algal community composition assemblages (Appendix C, Table 5.2). This model had an overall classification success of 75% using cross-validation methods. Classification success was best for assemblage 3 (78% of the sites correctly classified). Classification success for assemblages 1 was only slightly lower than for assemblage 2 (i.e., 69% of the sites for assemblage 1 and 73% of the sites for assemblage 2 were correctly classified). This model primarily predicted Flat River test sites to assemblage 1 & 2 and Prairie Creek test sites to assemblage 3 (Appendix C, Table 5.3).

Twenty-one physical variables were found to successfully discriminate among the 3 reference site assemblages for diatom community composition using DFA (Appendix C, Table 5.2). Overall, this model predicted 70% of the sites correctly using cross-validation methods. Classification success for assemblages 1 and 2 were 62% and 67%, respectively. This model primarily predicted Flat River test sites to assemblages 1 & 2 and Prairie Creek test sites to assemblage 3 (Appendix C, Table 5.3).

Twenty-five variables successfully discriminated among the 3 assemblages of reference sites for pigment concentrations using DFA (Appendix C, Table 5.2). This model predicted 73% of the sites correctly. Classification success varied slightly across all assemblages (i.e., assemblages 1, 2 and 3 correctly classified 79%, 64% and 75% of the sites). This model primarily predicted Flat River and Prairie Creek test sites to assemblage 3 in 2008, Flat River

test sites to assemblage 2 in 2009 and Prairie Creek test sites to all three assemblages in 2009 (Appendix C, Table 5.3).

5.4.2 Assessment of test sites

5.4.2.1 Water physical and chemical data

Concentrations of various metals along Flat River consistently increased adjacent to and downstream of Tailings Pond 3 (Figure 5.5). Concentrations of metals were elevated at downstream sites compared to upstream sites until 2 to 4 km downstream, where they began to decline back toward reference condition. Around 9 km along Flat River, concentrations of metals began to increase again and were often elevated as much or more than at sites located directly adjacent to the mine. Concentrations of TP along Flat River varied between 0.003 – 0.004 mg/L at sites -1 to 4 km along Flat River in 2008 (Figure 5.5). In 2009, there was a slight increase from 2 to 4 km along Flat River, however concentrations were still within the range of upstream reference sites. In both study years, TP increased from 9 to 90 km along Flat River, until approximately double the concentration of upstream sites. Turbidity and TP followed the same trend. Nitrogen and carbon (TN, NO_2NO_3 and DIC) concentrations, on the other hand, had an increasing trend along the entire length of Flat River. Despite this, they did increase above reference concentrations directly adjacent to the mine site. The PCA axis 1 scores identified three zones along Flat River. Zone 1 consisted of the reference sites from -8 to -2 km along Flat River. Zone 2 consisted of sites directly adjacent to the mine from -1 to 6 km along Flat River where the direct influence of the mine was observed. Zone 3 included downstream sites from 8 to 90 km along Flat River where water chemistry concentrations appeared to increase above concentrations at all other sites (Figure 5.5).

Elevated concentrations of metals along Prairie Creek were localized around the confluence of Harrison Creek and Prairie Creek (within 2 km of the confluence; Figure 5.6). Concentrations of metals returned to reference concentrations a few kilometers below the confluence of the two rivers. Concentrations of phosphorus did not appear to be influenced by the mine in 2008, but in 2009 there was an increase in concentrations of TP directly at the confluence of Harrison Creek and Prairie Creek (0.31 km), after which it declined back to reference concentrations (Figure 5.6). Both TN and NO_2NO_3 showed a general increasing trend along the entirety of Prairie Creek in both 2008 and 2009. PCA Axis 1 scores identified three zones along Prairie Creek. Zone 1 included all the reference sites from -23 to -0.5 km along Prairie Creek. Zone 2 included sites directly adjacent to the confluence of Prairie Creek and Harrison Creek from 0.2 to 1.5 km along Prairie Creek where the primary influence of the mine is observed. Zone 3 consisted of sites from 4 to 10 km along Prairie Creek where sites returned to reference concentrations (Figure 5.6).

5.4.2.2 Benthic algal community composition

Based on the RCA, benthic algal community composition identified that sites along Flat River downstream of the Cantung mine were possibly stressed to severely stressed within zone 1 from -1 to 0.01 km (directly adjacent to the mining site). Sites in zone 1 from 1 to 2 km downstream were in reference condition (Table 5.2; Appendix C, Figure 5.2A and B). From 3 to 6 km downstream, sites were assessed to be in reference**/possibly stressed to stressed condition. Then, in zone 2, from 8 km to 90 km (downstream of where two 3rd order stream enters the Flat River) sites ranged from reference condition to severely stressed and stressed conditions for the remainder of the stretch of river. Only in 2008 did sites return toward reference/possibly stressed conditions at 90 km downstream.

Benthic algal community composition along Prairie Creek was in reference condition the entire length downstream of the confluence of Harrison Creek and Prairie Creek (Table 5.2; Appendix C, Figure 5.3A and B). Only 3 sites were the exception to this (5 to 7 km downstream), however, these sites were bounded by sites in reference status and were predicted to different reference assemblages than all other sites along Prairie Creek (Appendix C, Table 5.3).

5.4.2.3 Diatom community composition

Diatom community composition showed similar trends to benthic algal community composition in the lower reaches of Flat River in both 2008 and 2009. Diatom communities in 2008 were assessed to be in reference condition from -1 to 2 km along Flat River (zone 2). In 2009, they were stressed in zone 1 between -1 to 0.01 km along Flat River, then in reference condition at 1 km and back to stressed at 2 km along Flat River. Although the communities appeared to be in reference condition at 1 km, that site was bounded by stressed communities at 0.01 and 2 km along Flat River. Sites located in zone 1 (3 to 6 km downstream) were possibly stressed to stressed and sites within zone 2 (8 to 20 km downstream) ranged from being in reference condition to stressed returning to a possibly-stressed state at 20 km downstream (Table 5.2; Appendix C, Figure 5.2C and D).

Diatom community composition along Prairie Creek was in reference condition the entire length downstream of the confluence of Harrison Creek and Prairie Creek (Table 5.2; Appendix C, Figure 5.3C and D).

5.4.2.4 Photosynthetic pigment concentration

Photosynthetic pigment concentration identified the largest area of influence within zone 1 along Flat River, extending from -1 km to 2 km downstream of the Cantung mine. From 3 to 4 km downstream, sites were primarily in reference condition with the exception of 4 km downstream in 2009, which was stressed to severely stressed (Table 5.2; Appendix C, Figure 5.2E and F). From 6 km (zone 1) to 20 km (zone 2), the majority of sites were in varying states of stress (possibly stressed, stressed and severely stressed).

Along Prairie Creek, photosynthetic pigment analysis identified a zone of influence from 0 km to 1.5 km downstream of the confluence of Harrison Creek and Prairie Creek corresponding to zone 1 defined by the site scores of PCA Axis 1 for water chemistry variables (Table 5.2; Appendix C, Figure 5.3E and F). From 4 km to 10 km downstream, sites were mostly in reference condition to possibly-stressed conditions.

5.4.2.5 Overall trends

Flat River

Algal metrics identified that sites were variably stressed along Flat River, but showed similar patterns of stress adjacent to and downstream of the tailings ponds (Table 5.2; Appendix C, Figure 5.2). All algal metrics (with the exception of the diatom community compositions in 2008) showed possibly stressed to stressed communities from -1 km to 0.01 km along Flat River in both study years. From 2 km downstream to 20 km downstream, algal metrics varied in their assessment of stress. Communities generally appeared to return to a state of reference condition or possibly-stressed state in a stretch ranging from 3 km to 6 km downstream of the mine. Then, from 8 to 20 km downstream of the mine the communities deviated from reference

again, coinciding with the entrance of two 3rd order streams and with increases in concentrations of metals and nutrients in the lower part of Flat River (Figures 5.5).

Prairie Creek

Benthic algal community composition and diatom community composition showed sites throughout Prairie Creek to be primarily within reference condition (Table 5.2; Appendix C, Figure 5.3). Photosynthetic pigment concentration identified the zone of influence to be within 1.5 km of the mine in 2008 and within 0.5 km in 2009. The rest of the way downstream appeared to be mostly in reference condition or possibly-stressed conditions in both years.

5.5 Discussion

For effective monitoring studies, it is important to select appropriate biota. Biological communities typically used for river monitoring include fish, macroinvertebrates, and algae. Fish are the most widely used biological group as they are considered to have recreational and economic value and to be important for their ecosystem and they have long generation times. However, fish are mobile in their environments and, thus, may not accurately reflect the conditions at the site where they were sampled (Resh, 2008). Fish also may not be sufficiently abundant or present in some northern ecosystems. For example, in the South Nahanni River watershed, Spencer et al., (2008) recommended that fish not be used for monitoring due to their low populations. Macroinvertebrates have many positive attributes that make them attractive endpoints, including their widespread distribution, diverse communities, limited mobility and long generation times. However, macroinvertebrates are considered difficult to identify and can have poor response levels to contaminants such as nutrients compared to algal communities (Resh, 2008). Algae are the least used endpoint of the three, despite their positive attributes such as being widespread and diverse, their low mobility and short generation times

that allow for the integration of changes in pollution over shorter periods of time compared to macroinvertebrates and fish. However, algae have their own challenges including their heterogeneous nature (i.e., microhabitat differences in algal community structure), and the expertise needed to identify them (Resh, 2008). Many studies use multiple biotic groups and some have recommended the use of macroinvertebrates and algae together, because macroinvertebrates typically respond strongly to metal contamination and algae to nutrients (e.g., Spencer et al., 2008). Benthic algae are also generally thought to be early-warning indicator organisms, because they are so intricately linked with their surrounding environments (Resh, 2008).

Developing cost-effective monitoring protocols are essential for northern latitudes where costs to assess impairment are high. We developed and compared a RCA model using three measures of algal community structure (benthic algal community composition, diatom community composition and photosynthetic pigment concentrations) to assess alternative endpoints. Results showed that algal metrics identified zones of stress downstream of the two mines, coinciding with zones defined using water chemistry. However, results varied among metrics. Along Flat River there were three zones of change in algal communities. All three zones were very similar among algal metrics, however changes in photosynthetic pigment concentration were more pronounced compared to the other metrics and better reflected changes in water chemistry. Photosynthetic pigment concentration identified two zones of change along Prairie Creek (0 to 1.5 km along Prairie Creek was Stressed; 1.5 to 10 km along Prairie Creek was mostly possibly stressed), while the other two metrics indicated that sites were within reference condition along Prairie Creek. A RCA model based on benthic macroinvertebrates within the South Nahanni River watershed showed that macroinvertebrate

communities were possibly stressed from 0.01 to 3 km along Flat River and possibly stressed from 0 to 2 km along Prairie Creek in 2009 only (Scrimgeour, 2013). Despite the identification of similar zones directly adjacent to and downstream of the mines along Flat River and Prairie Creek, the results contrast with our study where we identified influences of the mines further downstream along Flat River using all three algal metrics and along Prairie Creek using photosynthetic pigment concentration in both 2008 and 2009. This would suggest that the algal metrics are more sensitive than macroinvertebrate communities, possibly reflecting changes in nutrient concentrations as identified in Thomas et al., (2013; Chapter 4).

In chapter 3 of this thesis, we showed that benthic algal communities varied between ecoregions. Diatom community composition data appeared to reflect differences in physical and chemical variables between ecoregions best, and photosynthetic pigment concentrations appeared to be the least sensitive of the benthic algal metrics. Consequently, we expected the diatom community composition metric to respond most sensitively to fluctuations in nutrient and metal concentrations downstream of the two mines, and that photosynthetic pigment concentration would be less sensitive. Three previous studies have been conducted in the South Nahanni River watershed to assess river health using benthic algae. Two of the studies found changes in benthic algal communities near the Cantung mine along Flat River and the Prairie Creek Mine along Prairie Creek. The study conducted by Thomas et al., (2013; Chapter 4) used similar sites to this study, except that instead of using a RCA model approach they used upstream-downstream comparisons of benthic algal community composition and pigment concentration to assess impairment of downstream communities and also to assess relative performance of the two metrics as monitoring tools. They found that photosynthetic pigment concentration showed changes in algal communities along Flat River. Spencer et al., (2008)

assessed benthic algal communities (chlorophyll-*a*, diatoms, diversity and richness of algae and number of cells/sample) and found changes in algal communities downstream of both mines. They attributed decreases in specific species of diatoms (e.g., *Achnanthes minutissimum*) at the near-field site with increased metal concentrations (Spencer et al., 2008). Consequently, we expected to see changes in all our metrics outside of the reference condition adjacent to and downstream of both metal mines. While all our metrics reflected changes in water chemistry adjacent to and downstream of the mine, there was some variability among and between metrics. Diatom community composition appeared to be the least sensitive metric as it was inconsistently sensitive to differences in water chemistry directly adjacent to and downstream of tailings pond 3. My results showed that algal pigment concentrations mirrored the benthic algal community composition results along Flat River, and in fact were more pronounced, but were the only metric reflecting fluctuations in contaminants along Prairie Creek. These results were consistent with a previous study along Flat River which showed photosynthetic pigment concentration to mirror fluctuations in contaminants more strongly than benthic algal community composition (Thomas et al., 2013; Chapter 4). Although photosynthetic pigment concentration was the least responsive to differences in limnological conditions among reference sites, they may be the most sensitive to effects of mining activities. In Thomas et al., (2013; Chapter 4), algal pigment concentrations differed along with differences in nutrients and turbidity adjacent to and downstream of the mine sites. Algal pigments are sensitive to differences in light and thus, light (represented here by turbidity) may play an important role in the use of algal pigments as a biomonitoring and bioassessment tool. Prior to implementation in monitoring programs, mesocosm experiments should be conducted to better understand the influence of metal and nutrient concentrations on pigment concentration.

Bioassessments require adequate characterization of natural variability and grouping of reference sites to effectively assess the degree of alteration by human activities at test sites (Hawkins et al., 2010). There are two main factors to consider in selecting reference sites: 1) the adequate representation of geographically distinct areas and 2) the minimum number of reference sites to adequately make comparisons. Reference sites should represent all possible reference biological communities for reliable comparison with possibly affected biological communities at test sites. In this study, we sampled a broad range of sites from across the watershed. When we separated these sites into biologically similar assemblages for each metric, we identified 3 to 4 assemblages for each biological metric measured. Each of these assemblages was biologically distinct with minimal overlap between assemblages based on hierarchical cluster analysis and MDS ordination. In Chapter 2 of this thesis significant differences in all algal metrics were found between two major ecoregions. The RCA should be able to effectively group reference sites based on benthic algal data appropriately within the South Nahanni River watershed given the stark contrasts in benthic algal communities between the ecoregions. However, the RCA has the potential to 1) group reference sites inappropriately and 2) assign group membership of test sites incorrectly. Following the methods of the RCA, sites along the Flat River were typically grouped in the same assemblages (1 & 2 for benthic algal community composition and diatom community composition) and Prairie Creek sites were typically grouped into assemblage 3. However, the natural, inherent differences in limnological conditions between ecoregions in the South Nahanni River watershed were not observed as distinctly in the benthic algal communities, thus the natural inherent differences in biological communities appear to have led to differences in the design and application of a RCA in the South Nahanni River watershed where sites are grouped *a posteriori* and perhaps

future application of a RCA approach could incorporate *a priori* grouping of reference sites. For the development of a preliminary RCA model, it is recommended that no less than 25 reference sites be used (Bailey et al., 2004, Sylvestre et al., 2005). A previous study (Bowman et al., (2010) used a RCA design, based on biovolume of benthic algae, to assess stream health. Contrary to our study, they found sites to be within reference condition at all their sites along Flat River. Differences in results between our study and the study by Bowman et al., (2010) could be attributed to differences in the models developed. While Bowman et al., (2010) developed a RCA model based on biovolume of benthic algae, it was a preliminary model and the test sites along Flat River were only compared to 4 to 7 reference sites. Using so few reference sites can result in inaccurate test-site assessments. Other studies have recommended 10 to 15 reference sites per assemblage as a minimum for comparison with test sites (Bailey et al., 2004, Sylvestre et al., 2005). Thus, if there are sites which are clustered into assemblages of less than 10 to 15 sites, they are typically not used in the RCA model. In our study, we found one residual assemblage (assemblage 4 of photosynthetic pigment concentration metric) which was biologically distinct from all other assemblages (as determined by cluster analysis and MDS ordination) and was thus removed from further analyses. Interestingly, Bowman et al., (2010) compared test sites along Prairie Creek to > 10 reference sites and found similar results to our study in that test sites were within reference condition downstream of the Prairie Creek mine sites based on benthic algal community compositions. Thus, when an appropriate amount of reference sites are used, assessments appear to be consistent. Therefore, results should be interpreted with caution when less than 10 reference sites are used to compare to a test community.

Conclusions about assessment of downstream sites along Flat River are potentially confounded by the influence of inflowing lower-order streams along its path. All the algal metrics used in this study consistently showed a potential zone of influence from inflowing streams at approximately 8 to 90 km along Flat River where sites were variably stressed. The assessment of 'stressed' conditions among algal metrics in this stretch of the Flat River coincided with increases in concentrations of contaminants (Figure 5.5). This suggests that the influence of external factors on river algal communities along this stretch of Flat River were important. There are two 3rd order rivers which enter Flat River 6 - 9 km downstream of Tailings Pond 3. There are also springs (warm and hot, alkaline mineral springs) located along Flat River just downstream of the mine which could influence water chemistry (Caron et al., 2008). Concordantly, assessment of select metal and nutrient variables along Flat River showed elevated concentrations in many variables 8 - 10 km downstream of Tailings Pond 3 (Figure 5.5). No previous studies conducted along Flat River have assessed natural variability along Flat River in relation to benthic algal assessments of river health [e.g., Spencer et al., 2008; Bowman et al., 2010; Thomas et al., 2013 (Chapter 4)]. In fact, apart from Thomas et al., (2013; Chapter 4), these studies have not assessed the gradient of contaminants downstream of the mine. One study (Scrimgeour, 2013) assessed the gradient downstream of Cantung mine using benthic macroinvertebrate communities to assess river health. However, the benthic macroinvertebrates did not appear to be influenced by fluctuations in nutrient and metal concentrations along this portion of Flat River. These results highlight the possible implications of not considering natural variability along Flat River when assessing algal communities. Consequently, when using benthic algal communities to assess river health

downstream of the Cantung mine, it is possible that reliable results can only be obtained within 8 km of the mine site.

The RCA design often incorporates study sites sampled over multiple years into the creation of the reference model (e.g., Reynoldson et al., 2001; Sylvestre et al., 2005). However, there is concern about the effect of inter-annual variability and the long term effects of climate change on reference communities. One study assessed the effects of inter-annual variability on both reference and test sites and found that reference sites were predicted to the same assemblages and had the same assessment (in reference condition) in both years studied (Sylvestre et al., 2005). They found that some of their test sites were grouped into the same reference assemblages while some were not. They attributed these differences to slight difference in habitat characteristics measured each year (e.g., channel width, depth etc.). However, despite these differences they found that the majority of their test sites (5/7) resulted in the same assessment between years (Sylvestre et al., 2005). In our study, we sampled a large set of sites (44 = reference, 20 = test) in 2008 and a smaller subset of sites (18 = reference, 18 = test) in 2009. We found that benthic algal community composition and diatom community composition models predicted test sites relatively similarly to assemblages in both years. However, prediction of test site assemblages for the photosynthetic pigment concentration metric was variable and test sites along Flat River and Prairie Creek were not assigned to assemblages in a predictable way. Although there were some differences between predictions of test sites to different reference assemblages in 2008 and 2009, all algal metrics had similar patterns (i.e., zones of change) along Flat River in both study years. Patterns of sensitivity appeared to be relatively similar (benthic algal community composition) or slightly greater (diatom community composition and photosynthetic pigment concentration) in 2009 than

2008. This finding is similar to a finding by Thomas et al., (2013; Chapter 4) which attributed the differences between years to variation in meteorological conditions. Bailey et al., (2012) found that the starting community plays an important role in long-term assessment of a site. They suggest that it is important to characterize the reference condition in each assessment year in order to re-calibrate the RCA model each year to reduce any associate error rates. However, they did not indicate how many reference sites should be re-sampled (i.e., a subsample or all of the reference sites). When working in remote northern landscapes it may not be economically feasible to re-sample the number of reference sites required for an RCA model each year and so the cost of running an effective RCA may be a limiting factor to its implementation. Further, more comprehensive, long-term studies need to be conducted to assess multi-year change and the effects of climate change on RCA development and its subsequent suitability as a monitoring tool in remote northern ecosystems in Canada.

5.6 Figures

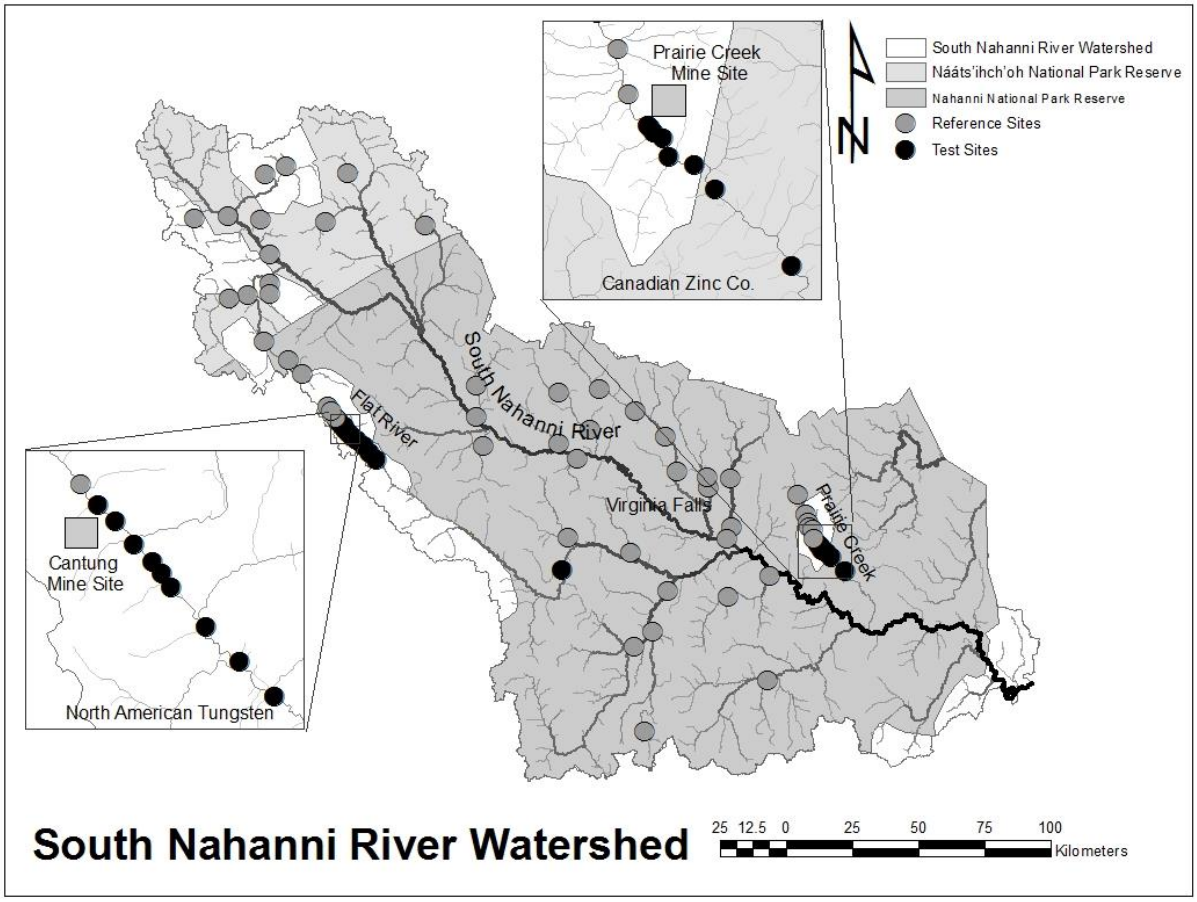


Figure 5.1 Location of study sites within the South Nahanni River watershed, Northwest Territories, Canada. A total of 44 reference sites (grey) were selected in 2008 and 18 reference sites (grey) in 2009 (12 repeated sampling from 2008; 6 newly sampled in 2009) and 20 test sites (black) were sampled between 2008 and 2009. Inserts show sites downstream of two mining companies, North American Tungsten (Cantung mine) and Canadian Zinc Corporation (Prairie Creek mine).

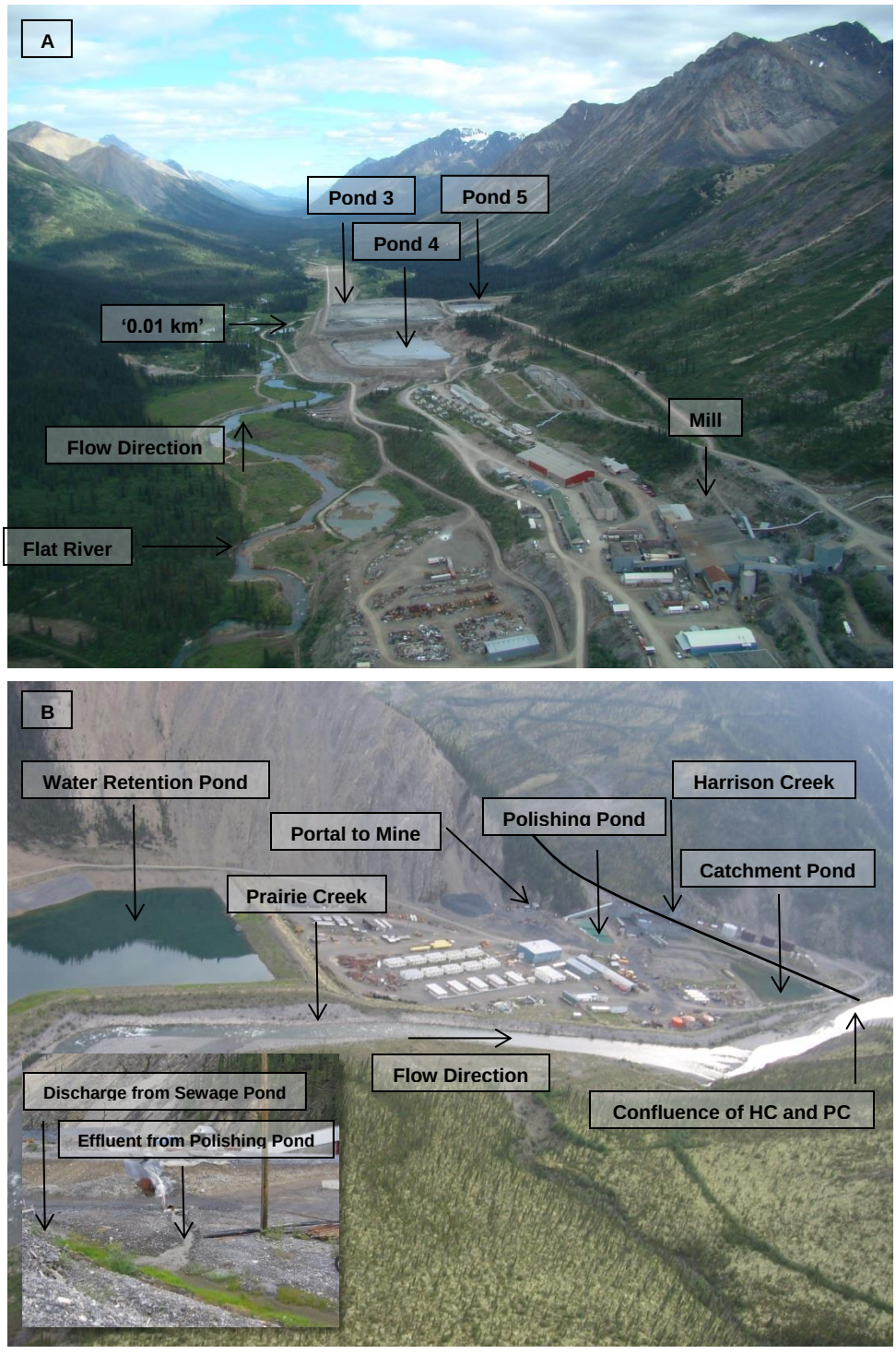


Figure 5.2 Mining companies located within the South Nahanni River watershed. A) North American Tungsten, Cantung mine located along Flat River showing location of site 0.01 km along Flat River, tailings ponds, mill and Flat River (Photo Dana Haggarty). B) Canadian Zinc Corporation, Prairie Creek mine located along Prairie Creek, showing location of confluence of Harrison Creek (HC) and Prairie Creek (PC), polishing and catchment ponds and mill.

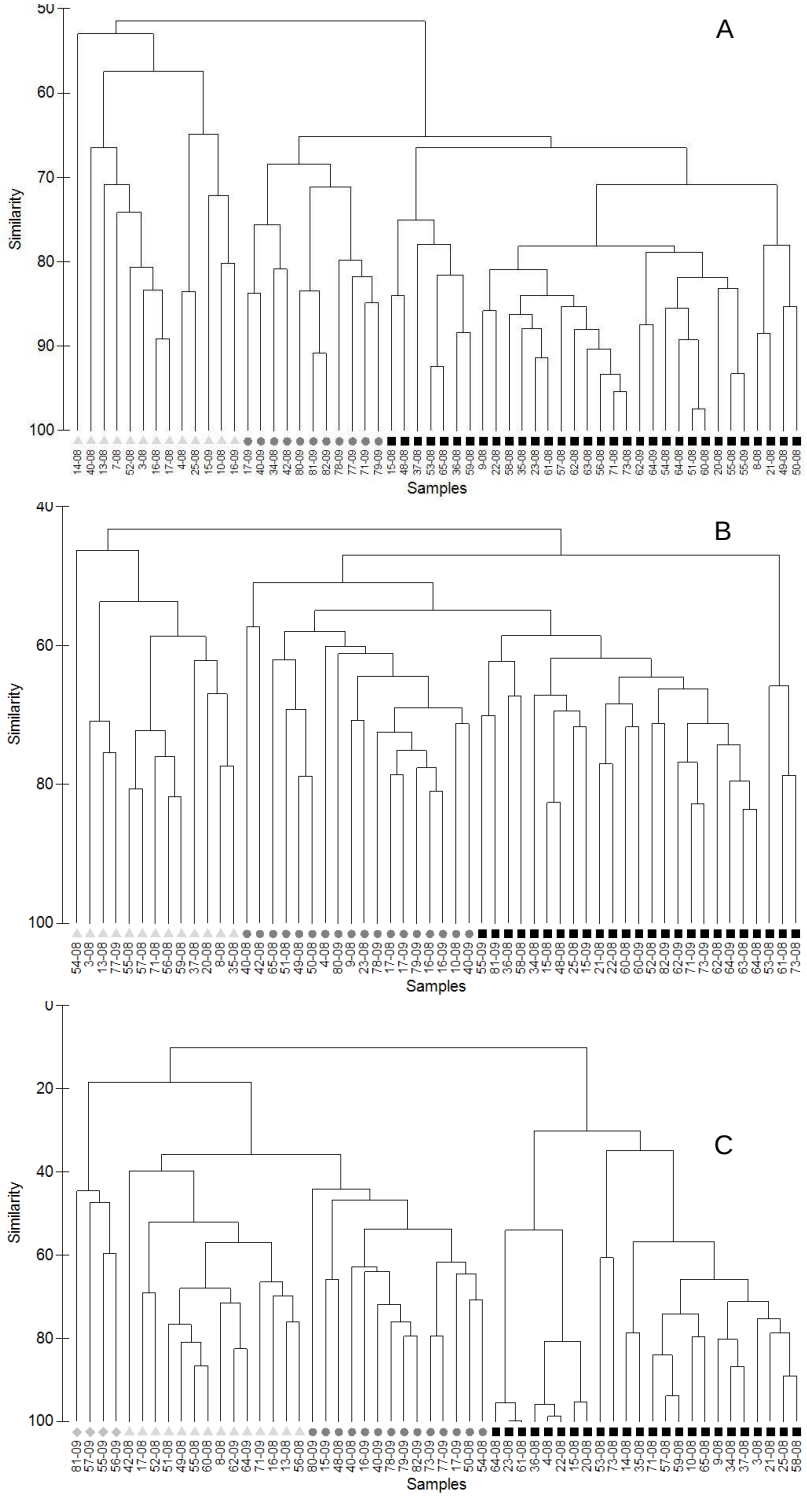


Figure 5.3 Cluster dendrograms of biological assemblages for each algal metric among the reference sites. Panels A is benthic algal community composition, B is diatom community composition and C is photosynthetic pigment concentration. Assemblage 1 = light grey triangles, Assemblage 2 = dark grey circles, Assemblage 3 = black squares.

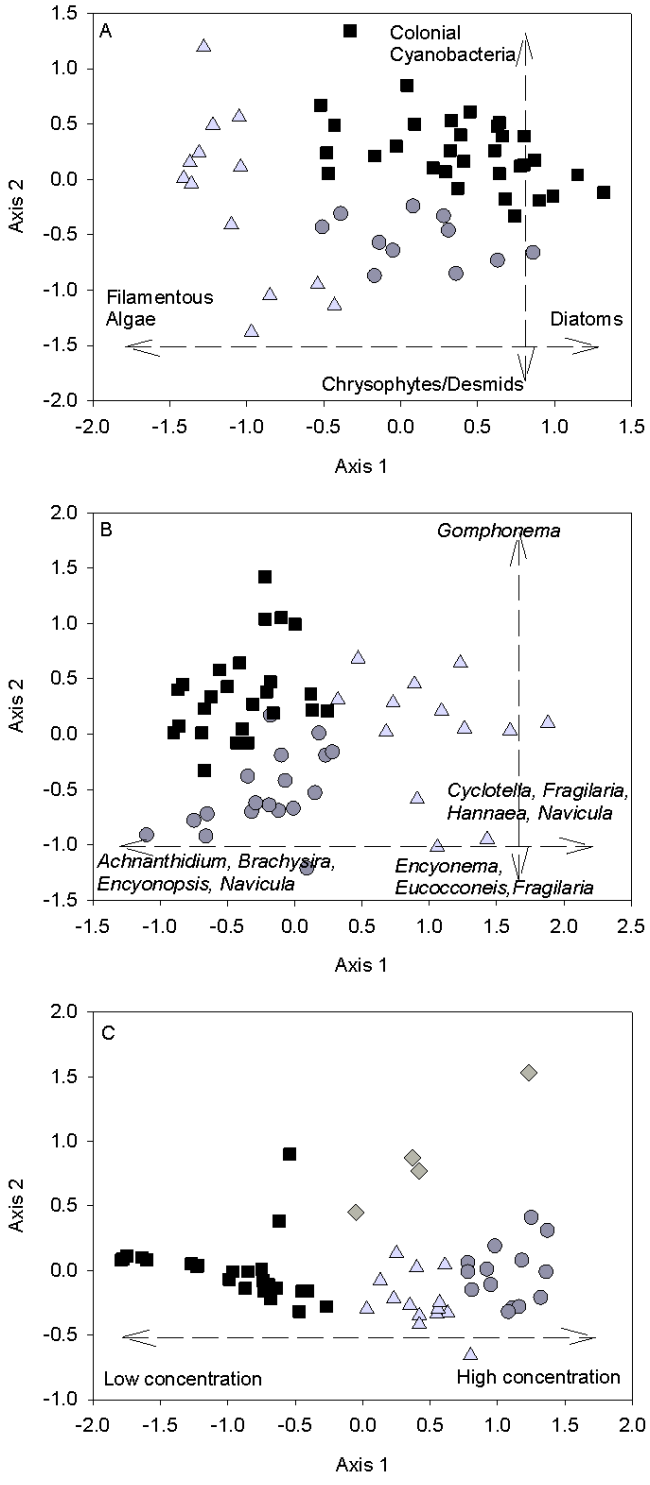


Figure 5.4 MDS ordinations of biological assemblages for each algal metric among the reference sites. Panel A is benthic algal community composition, B is diatom community composition and C is photosynthetic pigment concentration. Assemblage 1 = light grey triangles, Assemblage 2 = dark grey circles, Assemblage 3 = black squares.

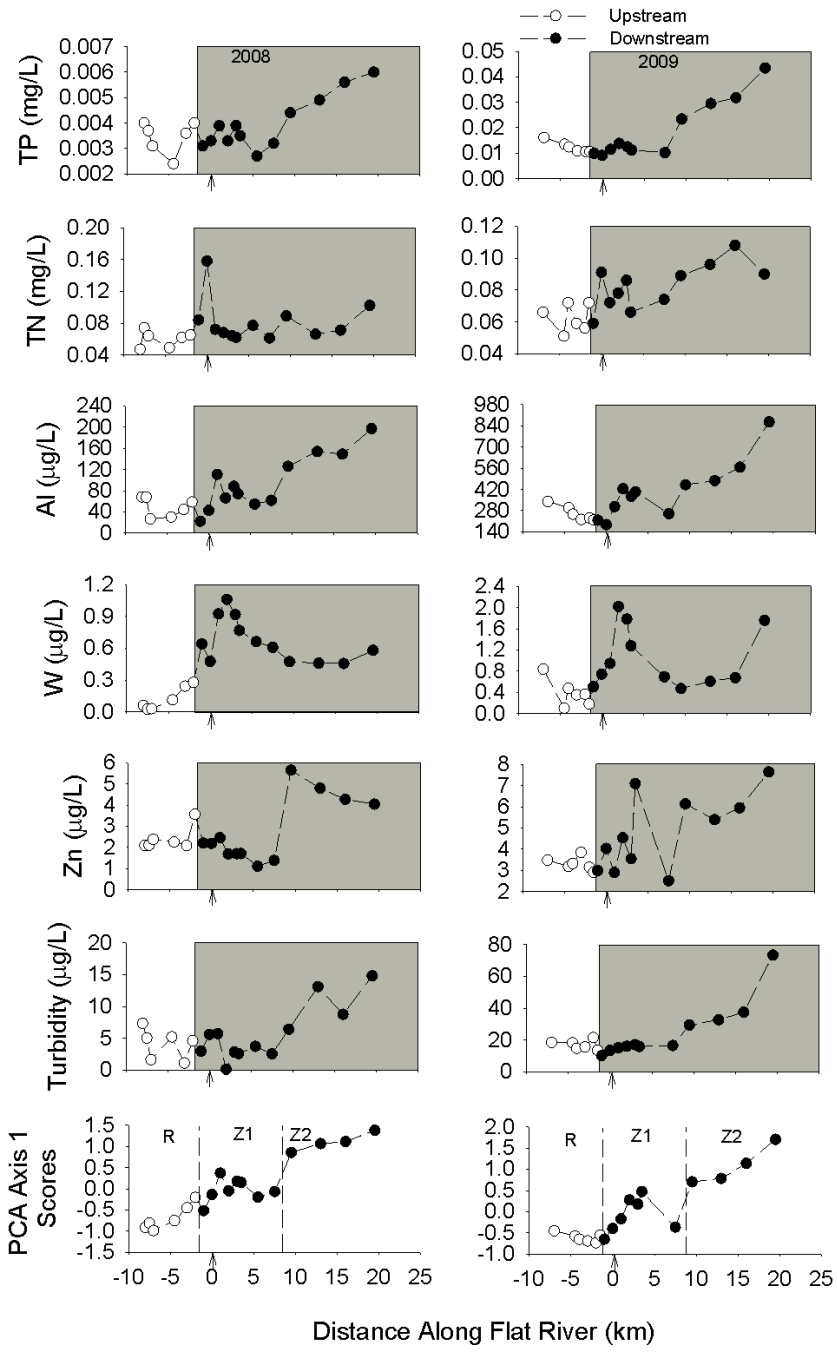


Figure 5.5 Concentrations of selected nutrients, metals and physical variables along Flat River both upstream (white circles) and adjacent to/downstream (black circles) of the mining site in 2008 and 2009. The shaded box indicates the all sites downstream of the mine. The arrow indicates the position of Tailings Pond 3. PCA axis 1 scores for 2008 and 2009 are also displayed. Vertical lines delineate boundaries of each zone of mining influence (R = reference, Z1 = zone 1, and Z2 = zone 2).

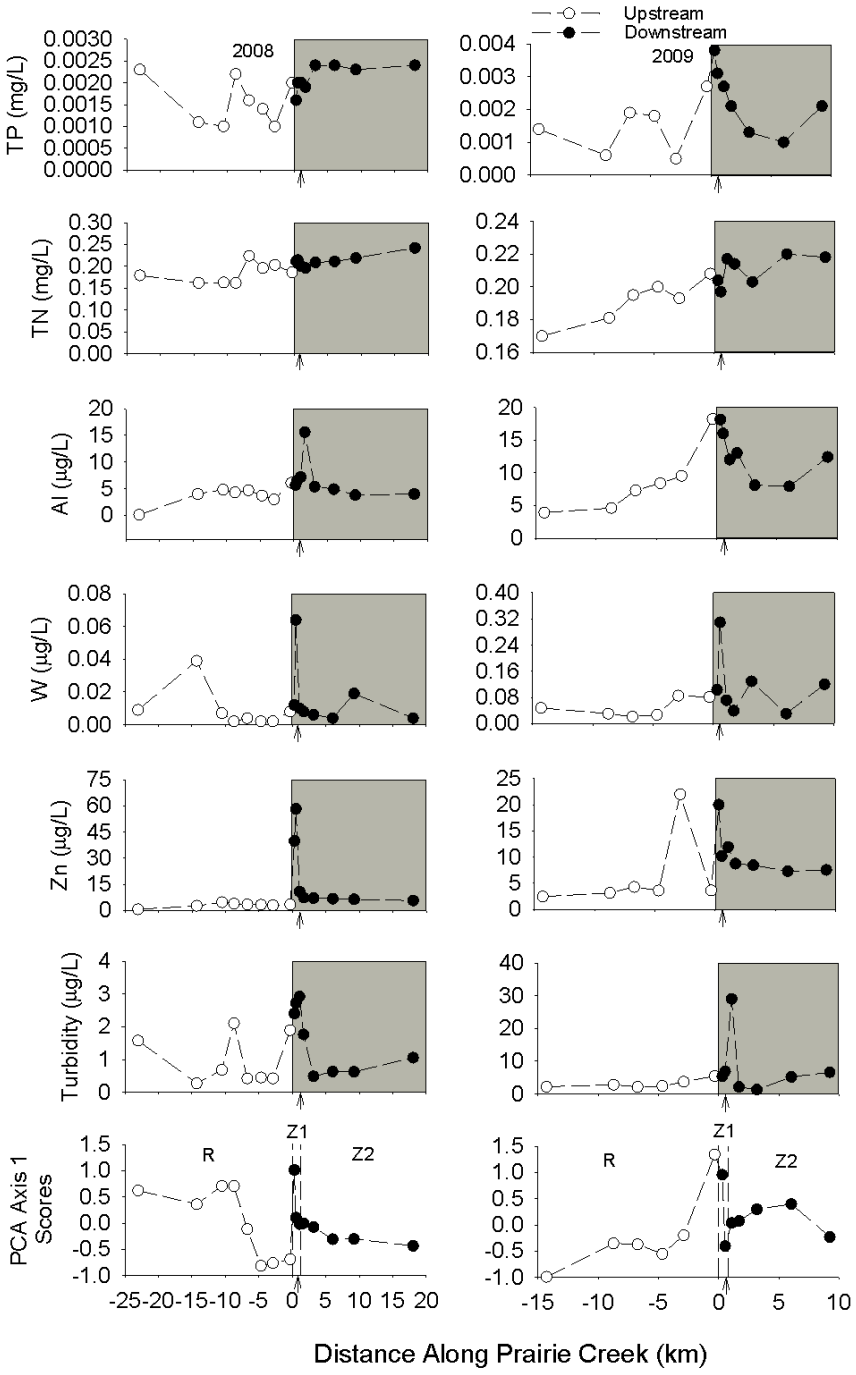


Figure 5.6 Concentrations of selected nutrients, metals and physical variables along Prairie Creek both upstream (white circles) and adjacent to/downstream (black circles) of the mining site in 2008 and 2009. The shaded box indicates the all sites downstream of the mine. The arrow indicates the position of Tailings Pond 3. PCA axis 1 scores for 2008 and 2009 are also displayed. Vertical lines delineate boundaries of each zone of mining influence (R = reference, Z1 = zone 1, and Z2 = zone 2).

5.8 Tables

Table 5.1 Average values of physical variables and biological communities among all assemblages for each benthic algal metric (Benthic algal community composition, diatom community composition, and photosynthetic pigment concentration).

Benthic Algal Community Composition			
		Assemblage	
Environmental Variables	1	2	3
Altitude (m above sea level)	3240.2	2916.6	2540.3
Longitude (decimal)	-127.8	-127.5	-126.0
Stream Order	4.2	4.4	4.5
% Cobble	43.9	40.6	42.3
% Forest	24.0	21.3	33.9
% Ice	19.0	22.8	3.8
Bankfull Width (m)	22.3	66.5	56.0
Biological Communities			
Bacillariophyceae	27.8	74.9	78.7
Chaetophoraceae	7.7	6.0	0.6
Chroococcus	1.2	1.6	0.6
Chrysophyceae	0.6	2.0	0.0
Desmidiaceae	0.02	0.0	0.0
Euglena	0.0	0.1	0.1
Hydrodictyaceae	0.0	0.0	0.0
Merismopediaceae	30.7	4.5	16.0
Microcystaceae	2.4	2.3	1.4
Nostocaceae	0.4	0.1	0.1
Oedogoniaceae	0.1	0.1	0.0
Oscillatoriaceae	17.1	4.3	1.5
Phormidiaceae	11.8	4.1	1.0
Rivulariaceae	0.0	0.0	0.0
Scenedesmaceae	0.04	0.0	0.0
Ulotrichaceae	0.2	0.2	0.0
Zygnemataceae	3.0	0.6	0.1
Diatom Community Composition			
		Assemblage	
Environmental Variables	1	2	3
Altitude (m above sea level)	2482.4	3187.3	2548.6
Longitude (decimal)	-126.8	-127.3	-125.9
Stream Order	4.5	4.4	4.5
% Cobble	48.6	38.5	40.8
% Forest	32.9	29.0	29.5
% Ice	5.1	23.7	3.2
Bankfull Width (m)	50.3	44.3	64.7

Biological Communities				
Achnanthidium minutissimum	9.4	46.4	60.8	
Encyonema minutum	0.3	1.9	0.1	
Encyonema silesiacum	0.4	1.4	0.0	
Diatoma tenuis	4.3	8.2	5.2	
Hannaea arcus	56.4	6.2	4.7	
Fragilaria capucina var. gracilis	6.6	4.9	0.8	
Fragilaria capucina subsp. rumpens	1.0	2.2	2.7	
Fragilaria capucina var. vaucheria	6.6	9.1	1.4	
Staurosirella pinnata	0.0	1.3	0.0	
Gomphonema parvulum var. micropus	7.0	4.1	12.5	
Gomphonema olivaceum	1.6	2.4	3.4	
Gomphonema unknown	3.0	0.2	3.7	
Synedra ulna	1.2	3.7	0.8	

Photosynthetic Pigment Composition

	Assemblage			
Environmental Variables	1	2	3	4
Altitude (m above sea level)	2559.9	2900.9	2830.4	2062.3
Longitude (decimal)	-125.9	-127.5	-126.8	-125.7
Stream Order	4.6	4.4	4.3	5.3
% Cobble	48.6	38.4	40.8	47.5
% Forest	32.0	31.8	28.5	31.0
% Ice	10.7	20.5	6.4	1.9
Bankfull Width (m)	43.7	58.6	54.2	74.3
Biological Communities				
α-carotene	0	0.000758	0	0.000393
β-carotene	0.003243005	0.015057	0.000968	0.006012
Alloxanthin	0	0.000238	0	0
Aphanizophyll	0.00097256	0.027213	0	0
Chlorophyll_*a*	0.065046722	0.246556	0.009537	0.015959
Chlorophyll_*a*'	0.01464848	0.050377	0.000686	0.000482
Chlorophyll_*b*	0.001052396	0.027741	0	0
Chlorophyll_*c*2	0.010251231	0.014251	0.000154	0.000147
Chlorophyllide_*a*	0.009254477	0.014223	0.00039	0
Diadinoxanthin	0.00064143	0.00164	0.0000247	0
Diatoxanthin	0	0.00071	0	0
Echinenone	0.000857286	0.00031	0.0000357	0
Fucoxanthin	0.040371952	0.097011	0.001362	0.004282
Lutein	0.002746729	0.02088	0.0000941	0.001615

Myxoxanthin	0.002278047	0.004727	0	0
Okenone	0.0000198	0.001322	0	0.002355
Phaeophytin_*a*	0.004507753	0.083258	0	0.081214
Phaeophytin_*b*	0	0.016725	0	0.027323

Table 5.2 Results of RCA assessment of 2008 and 2009 test sites along Flat River and Prairie Creek for each algal metric (Benthic algal community composition, diatom community composition, and pigment concentration).

	Distance From Tailings Pond	2008			2009		
Site	(km)	Benthic Algae	Diatoms	Pigments	Benthic Algae	Diatoms	Pigments
Flat River							
NNP-1	-1	Possibly Stressed	Reference	Possibly Stressed - Stressed	Stressed	Stressed	Reference ** - Severely Stressed
NNP-38	0.01	Severely Stressed	Reference	Reference	Possibly Stressed	Stressed	Stressed
NNP-39	1	Reference **	Reference	Possibly Stressed	Reference *	Reference	Possibly Stressed – Severely Stressed
NNP-2	2	Reference **	Stressed	Severely Stressed	Reference	Reference *	Severely Stressed
NNP-32	3	Stressed	Possibly Stressed	Reference	Reference ** – Possibly Stressed	Possibly Stressed	Reference * - Reference **
NNP-31	4	Possibly Stressed – Stressed	Stressed	Reference * - Reference **	Possibly Stressed	Reference **	Stressed – Severely Stressed
NNP-19	6	Reference	Possibly Stressed	Stressed	NA	NA	NA
NNP-30	8	Possibly Stressed	Reference *	Severely Stressed	Possibly Stressed	Stressed	Severely Stressed
NNP-29	10	Possibly Stressed	Reference *	Possibly Stressed	Severely Stressed	Possibly Stressed	Possibly Stressed
NNP-28	13	Severely Stressed	Stressed	Possibly Stressed	Stressed	Reference *	Reference
NNP-27	16	Possibly Stressed	Reference - Possibly Stressed	Stressed	Stressed	Reference	Possibly Stressed - Severely Stressed
NNP-	20	Reference	Possibly	Severely	Stressed	Possibly	Stressed

	Distance From Tailings Pond	2008			2009		
Site	(km)	Benthic Algae	Diatoms	Pigments	Benthic Algae	Diatoms	Pigments
26			Stressed	Stressed		Stressed	
NNP-33	90	Possibly Stressed	Reference * - Possibly Stressed	Reference **	NA	NA	NA
Prairie Creek							
NNP-43	0	Reference	Reference	NA	Reference	Reference	Stressed
NNP-46	0.5	Reference	Reference	Severely Stressed	Reference	Reference	Stressed
NNP-44	1	Reference	Reference	NA	Reference	Reference	Reference * - Stressed
NNP-45	1.5	Reference	Reference	Stressed	Reference **	Reference	Reference *
NNP-70	4	Reference	Reference	Reference – Severely Stressed	Reference	Reference	Possibly Stressed
NNP-69	5	Possibly Stressed	Reference	Reference	Possibly Stressed	Reference	Possibly Stressed
NNP-68	7	Stressed	Reference	Possibly Stressed – Stressed	Reference **	Reference *	Possibly Stressed
NNP-67	10	Reference	Reference	Severely Stressed	NA	NA	NA

Chapter 6

Synthesis and Recommendations

Freshwaters in North America are increasingly subjected to a variety of stressors from human development (including industrial and residential) and climate change (Schindler & Smol, 2006). In the face of these stressors, it is imperative that studies improve protocols for implementation by monitoring biologists for long-term monitoring programs in order to effectively track alteration of biological communities (Walker et al., 2003; King et al., 2006). For rivers, many studies utilize fish and macroinvertebrate communities as sources of biomonitoring data to assess water quality and ecological integrity. For lakes, concentrations of TP are also used due to their relationship with phytoplankton in the open water (pelagic) zone (Dillon & Rigler, 1974). Benthic algae are less-utilized, but possess numerous features that predispose them to provide effective monitoring of changes in water quality and ecological status of lakes and rivers caused by anthropogenic disturbances [Reavie & Smol, 1998; Rott et al., 1998; Hill et al., 2000b; Leland & Porter, 2000; Thomas et al., 2011 (Chapter 2), 2013 (Chapter 4)]. As demonstrated in this thesis, based on development and assessment of multiple algal metrics (different levels of taxonomic resolution and quantification of photosynthetic pigments) in lakes within the Muskoka-Haliburton area of south-central Ontario and in stream and river sites within the South Nahanni River watershed, benthic algae are able to effectively indicate changes in community composition and water quality. Below, I provide a synthesis of the key findings as a basis for presenting recommendations for application of benthic algal biomonitoring protocols in long-term monitoring programs and for future research priorities.

In the Muskoka-Haliburton area, where there is generally good water quality and low to moderate levels of shoreline development, we found that the highest taxonomic resolution

benthic algal metric (diatom community composition) discriminated best among shoreline development categories. Photosynthetic pigment concentration showed modest potential and should be explored further over larger trophic gradients. All other metrics (visual assessments, biomass assessments and benthic algal community composition) did not sufficiently discriminate among categories for widespread application as provincial biomonitoring metrics. Despite the higher cost in time and training required for diatom community composition analysis, we recommend this metric for future monitoring purposes as it provides a sufficient amount of detail to track changes in shoreline development in lakes of this region.

Estimates of diatom community composition, based on percent abundance data, were able to discriminate between nearshore lake sites categorized as having low and high shoreline development. But, they could not discriminate sites in the medium category from those in the low and high categories. It could be that larger sample sizes may provide the added power required to discriminate between categories of shoreline development. However, the diatom community composition metric appears to have detection limits and can only discriminate the highest from the lowest shoreline development categories in Precambrian Shield lakes. Nevertheless, this may still be a useful level of discrimination for management of shoreline development as managers are able to effectively identify sites where human activities are altering biological communities and can target them for further study or for implementation of remediation efforts along the nearshore (e.g., best management practices). Alternatively, human disturbances are relatively low in the Muskoka-Haliburton region and so only two categories (lowest and highest development) can be discriminated effectively. In other regions where human activities span broader gradients, three or more shoreline development categories may be discriminated, but more research is needed to discover the limits of detection. Starker contrasts between, and better definition of shoreline-development categories could aid in the discrimination of

categories among metrics and may result in the identification of other useful metrics (e.g., benthic algal community composition, biomass). In areas where there is a larger gradient of shoreline development, it would be advisable to test cruder, more cost-effective measures of benthic algal communities (e.g., benthic algal community composition and biomass) to determine the ideal metric to incorporate into monitoring protocols. Thus, a broader gradient of trophic status of lakes is needed to get a sense of the wider applicability of these methods to lakes other than those in the Precambrian Shield. Studies could also be conducted over longer time-series to assess if the metrics can detect changes over time.

In the South Nahanni River watershed, three metrics were used to assess benthic algal communities. Taxonomic metrics included a low taxonomy approach (benthic algal community composition) and a high taxonomy approach (diatom community composition). Quantification of photosynthetic pigment concentration was the third metric used. Comparisons were made among the different algal metrics between ecoregions to assess shifts of algae relative to physical and chemical variables. The algal metrics were also assessed for their ability to measure changes at sites downstream of two metal mining companies using two methods (upstream-downstream and RCA models).

The spatial survey of reference sites showed that diatom community composition was the most effective metric at tracking major differences in physical and chemical conditions between the two ecoregions. Benthic algal community composition and photosynthetic pigment concentration metrics did not achieve this very well. Diatom community composition was the highest resolution taxonomy used in the study and thus may be more sensitive to changes in physical and chemical variables. Benthic algal community composition and photosynthetic pigment concentration metrics tracked differences in physical and chemical

conditions between ecoregions in 2009 corresponding with higher concentrations of nutrients and metals and thus a more stark contrast between ecoregions compared to 2008. Thus, they did show promise in tracking differences in physical and chemical variables across the watershed, however, when differences are not as large they may lose the ability to discriminate as effectively between ecoregions. These metrics are a lower taxonomic resolution compared to diatom community composition and so may not be as sensitive to changes across the watershed. They also could be influenced by other factors such as grazing and light.

Despite the fact that analysis of photosynthetic pigment concentrations provides the lowest level of taxonomic analysis and was the worst at discriminating between ecoregions, it was the most sensitive metric to changes in physical and chemical conditions downstream of the two mining companies. Conversely, diatom community composition was the best metric at discriminating between ecoregions and the least sensitive metric for assessing changes in physical and chemical conditions downstream of the two mines. Photosynthetic pigment concentrations were comparable (or more sensitive) than taxonomic-based assessments in assessing test sites using both an upstream-downstream and an RCA model approach. Using both methods (upstream-downstream and RCA models), photosynthetic pigment concentration defined a zone of influence that corresponded with elevations of nutrient and metal concentrations in the river waters. Diatom communities have been shown to be sensitive to metal contamination, whereas overall benthic algal community composition reflects major changes in the communities and could reflect shifts from diatom-dominated communities to cyanobacterial-dominated communities due to increases in nutrient concentrations. Also, photosynthetic pigments are sensitive to changes in light, and in this study, changes at sites close to the mine site were found to be correlated with turbidity. Differences observed in

assessments between photosynthetic pigment concentrations and the other metrics (benthic algal community composition and diatom community composition) could reflect the higher sensitivity of photosynthetic pigment concentrations to variations in light environment (measured here as turbidity). Thus, differences in the relative abilities of algal metrics to assess test sites could be influenced differently by concentrations of metals and nutrients or differences in light. Since the RCA assumes that biological communities are influenced by surrounding physical and chemical conditions, the weaker ability of photosynthetic pigment concentration to detect differences among reference sites may affect the application of the RCA. Further understanding of the influences on photosynthetic pigment concentration and the effect on biological assessments of contaminants are needed. Mesocosm experiments should be conducted to help in understand how physical and chemical variables affect pigment concentrations in relation to mining activities.

In the face of increasing anthropogenic influences and climate change, monitoring biologists are challenged to develop cost-effective tools to quantify degradation of aquatic ecosystems in monitoring programs (Walker et al., 2003; King et al., 2006). Many methods have been developed to assess changes in water quality and ecological integrity using biological communities including control-impact (CI), before-after-control impact (BACI), gradient, and RCA (Green, 1979; Underwood, 1994; Bailey et al., 2004). However, some of these methods (e.g., CI and gradient) have come under scrutiny due to concerns of pseudoreplication (Cooper & Barmuta, 1993). Despite these differences and concerns, we found agreement of results from our upstream-downstream and RCA study designs. Both study designs detected changes in algal communities downstream of the Cantung mine along Flat River. We found that photosynthetic pigment concentration was more sensitive than benthic algal community

composition using both study methods. Despite the concordance between methods in test site assessments, we did find that the results from the RCA approach were more pronounced than the upstream-downstream approach using both metrics. Spencer et al., (2008) used a CI study design to assess conditions at a near-field and far-field site; using this method they also found changes in the benthic algal communities downstream of the Cantung mine. Thus, it appears that these methods produce similar results. The best method to use for assessing downstream impairment may depend on the question being asked by the monitoring biologist. For example, RCA models are informative when we want to assess if the test site is impaired; however, other study designs need to be implemented in order to determine how a test site is impaired. It may also depend on budgetary constraints. For example, RCA model development requires more reference sites from a wider area compared to a gradient of CI design and thus could be more expensive to conduct on a regular basis, specifically in remote northern areas.

RCA study designs have been used extensively to create benthic macroinvertebrate models and have been integrated into the environmental effects monitoring for metal mining effluent regulations (Environment Canada 2012). However, only one other study has developed a preliminary RCA model using benthic algae and thus, the use of an algal-based RCA model is in its infancy (Bowman et al., 2010). Our results indicate that the RCA models appear to be more sensitive (indicating larger differences in biological communities) compared to our upstream-downstream study. For example, in 2008, benthic algal community composition using the upstream-downstream approach appears to not deviate significantly from reference sites. But when using the RCA model, test sites adjacent to and downstream of the mine are not within reference condition. RCA models use many regional reference sites to assess one test site and, thus, may have more power to detect differences compared to upstream-

downstream approaches. However, the RCA may also introduce more variability among reference sites. The RCA model is an initial assessment tool for managers to determine if more in-depth investigations are needed at sites possibly affected by mining activities.

There are many methods used for biomonitoring in river ecosystems. Predictive models include both multimetric (e.g., IBI, Karr 1981; RBP, Plafkin et al., 1989) and multivariate (e.g., RIVPACS, Wright et al., 1993; AUSRIVAS, Simpson and Norris, 2000; ANNA, Linke et al., 2005; BEAST, Reynoldson et al., 1997) approaches. Predictive models are used to determine if a site is affected by potential stressors, but they are not able to diagnose how a biological community is impaired (how it is different from reference), or the potential cause of impairment. Metric-based assessments such as traits assessments are able to provide the link between biological response and environmental variables (Culp et al., 2010). However, traits or metrics in multimetric models can have correlation among metrics. There are positive and negative attributes to each type of bioassessment methods. Some studies have suggested concurrent use of different method types. For example, Bowman and Somers (2006) created the Test Site Approach (TSA) using a combination of multimetric and multivariate methods in a new statistical method. Alternatively, a predictive method may be used initially to determine if a test site is impaired, and is then followed up with a metric approach to identify causative agents.

This thesis has developed, applied and assessed benthic algal metrics for river biomonitoring in the South Nahanni River watershed. We have shown that metrics were sensitive, to varying degrees, to changes in water physical and chemical conditions across the watershed (between ecoregions) and downstream of two mining companies. We have shown that there is great potential in the use of algal metrics using an RCA approach. We have also

shown that there is great potential in the use of photosynthetic pigment concentration as a bioassessment tool. However, there was variability among metrics using the RCA approach and within each metric that needs to be better understood prior to widespread implementation of the methods. For example, although general patterns were discernible along Flat River, assessments were variable downstream and were influenced by incoming streams limiting the ability of the RCA to detect impairment beyond 8 km downstream of Cantung mine. Conversely, patterns along Prairie Creek were less variable and easier to interpret. Thus, prior to further implementation of algal based RCA models, development of models in other ecosystems should be conducted to assess the relative ability to assess stream condition. Metal mining in the South Nahanni River watershed is expected to increase in the coming years. RCA models could be developed for sites along rivers where mining companies will develop in the future, incorporating data from this study into the model development. As well, continued assessments downstream of Prairie Creek mine could be conducted to assess if test sites become more stressed when the mine becomes a fully operating lead-zinc-silver mine.

References

Antoniades, D., Douglas, M.S.V., & Smol, J.P. (2009). Biogeographic distributions and environmental controls of stream diatoms in the Canadian Arctic Archipelago. *Botany*, 87, 443-454.

Bailey, R.C., Norris, R.H., & Reynoldson, T.B. (2004). *Bioassessment of freshwater ecosystems using the reference condition approach*. Norwell, MA: Kluwer Academic Publishers.

Bailey, R.C., Scrimgeour, G., Coté, D., Kehler, D., Linke, S., & Cao, Y. (2012). Bioassessment of stream ecosystems enduring a decade of simulated degradation: lessons for the real world. *Canadian Journal of Fisheries and Aquatic Science*, 69, 784-796.

Biggs, B.J.F. (1990). Periphyton communities and their environments in New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research,* 24, 367-386.

Biggs, B.J.F., & Kilroy, C. (2000). *Stream periphyton monitoring manual*. Christchurch, New Zealand: National Institute for Water and Atmospheric Research.

Birks, H.J.B. (2010). Numerical methods for the analysis of diatom assemblage data. In Smol, J.P., Stoermer, E.F. (Ed.). *The Diatoms: Applications for the environmental and earth sciences*. Cambridge, UK: Cambridge University Press, 29 pp.

Bowman, M.F., & Somers, K.M. (2005). Considerations when using the reference condition approach for bioassessment of freshwater ecosystems. *Water Quality Research Journal of Canada*, 40(3), 347-360.

Bowman, M.F., & Somers, K.M. (2006). Evaluating a novel Test Site Analysis (TSA) bioassessment approach. *Journal of the North American Benthological Society*, 25(3), 712-727.

Bowman, M., Spencer, P., Dubé, M., & West, D. (2010). Regional reference variation provides ecologically meaningful protection criteria for northern World Heritage Site. *Integrated Environmental Assessment and Management*, 6, 12-27.

Caron, M., Grasby, S.E., & Ryan, M.C. (2008). Spring water trace element geochemistry: A tool for resource assessment and reconnaissance mineral exploration. *Applied Geochemistry,* 23, 3561-3578.

Cattaneo, A. (1987). Periphyton in lakes of different trophy. *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 296-303.

CCME [Canadian Council of Ministers of the Environment]. (2003). *Canadian water quality guidelines for the protection of aquatic life*. Hull, QC: Environment Canada.

[CEN] Comité Européen de Normalisation. (2003). *Water quality – Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers*. Geneva: EN 13946 rev.

[CEN] Comité Européen de Normalisation. (2004). *Water quality – Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from rivers*. Geneva: prEN 14407 rev.

Cervantes, C., Campos-Gracía, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J. C., & Moreno-Sánchez, R. (2001). Interactions of chromium with microorganisms and plants. *FEMS Microbial Reviews*, 25, 335-347.

Chambers, P.A., Culp, J.M., Glozier, N.E., Cash, K.J., Wrona, F.J., & Noton, L. (2006). Northern rivers ecosystem initiative: Nutrients and dissolved oxygen – issues and impacts. *Environmental Monitoring and Assessment*, 113, 117-141.

Chambers, P.A., Guy, M., Roberts, E.S., Charlton, M.N., Kent, R., Gagnon, C., Grove, G., & Foster. N. (2001). *Nutrients and their impact on the Canadian environment*. Agriculture and Agri-Food Canada, Environment Canada, Fisheries and Oceans Canada, Health Canada and Natural Resources Canada, pp. 241.

Chapman, L.J., & Putnam, D.F. (1984). *The physiography of southern Ontario*. Sudbury, ON: Ontario Geological Survey Special vol 2.

Clark, J.L., & Clements, W.H. (2005). The use of in situ and stream microcosm experiments to assess population- and community-level responses to metals. *Environmental Toxicology and Chemistry,* 25(9), 2306-2312.

Clark, K.R., & Gorley, R.N. (2006). *PRIMER v6: user manual/tutorial*. Plymouth: PRIMER-E.

Clark, K.R., & Warwick, R.M. (2001). *Change in marine communities: an approach to statistical analysis and interpretation*. 2nd edition. Plymouth; PRIMER-E.

Clausen, B., & Biggs, B.J.G. (1997). Relationships between benthic biota and hydrological indices in New Zealand streams. *Freshwater Biology*, 38, 327-342.

Clements, W.H., Carlisle, D.M., Lazorchak, J.M., & Johnson, P.C. (2000). Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications*, 10(2), 626-638.

Clements, W.H., Cherry, D.S., & Hassel, J.H.V. (1992). Assessment of the impact of heavy metals on benthic communities at the Clinch River (Virginia): Evaluation of an index of community sensitivity. *Canadian Journal of Fisheries and Aquatic Sciences*, 49, 1686-1694.

Clements, W.H., Vieira, N.K.M., & Church, S.E. (2010). Quantifying restoration success and recovery in a metal-polluted stream: a 17-year assessment of physicochemical and biological responses. *Journal of Applied Ecology*, 47, 899-910.

Cooper, S.D., & Barmuta, L.A. (1993). Field experiments in biomonitoring. In Rosenberg, D.M., & Resh, V.H. (Eds). *Freshwater biomonitoring and benthic macroinvertebrates*. New York: Chapman and Hall, pp. 399-441.

Culp, J.M., Armanini, D.G., Dunbar, M.J., Orlofske, J.M., Poff, N.L., Pollard, A.I., Yates, A.G., & Grant, C. (2010). Incorporating traits in aquatic biomonitoring to enhance casual diagnosis and prediction. *Integrated Environmental Assessment and Management*, 7(2), 187-197.

Dillon, P.J., & Molot, L.A. (1996). Long-term phosphorus budgets and an examination of a steady-state mass balance model for central Ontario lakes. *Water Resources*, 30(10), 2273-2280.

Dillon, P.J., & Rigler, F.H. (1974). The phosphorus-chlorophyll relationship in lakes. *Limnology and Oceanography,* 19(5), 767-773.

Dorigo, U., Bourrain, X., Bérard, A., & Leboulanger, C. (2004). Seasonal changes in the sensitivity of river microalgae to atrazine and isoproturon along a contamination gradient. *The Science of the Total Environment*, 318, 101-114.

Dorigo, U., Leboulanger, C., Bérard, A., Bouchez, A., Humbert, J.F., Montuelle, B. (2007). *Lotic biofilm community structure and pesticide tolerance along a contamination gradient in a vineyard area*. Aquatic Microbial Ecology, 50, 91-102.

Downes, B.J., Barmuta, L.A., Fairweather, P.G., Faith, D.P., Keough, M.J., Lake, P.S., Mapstone, B.D., & Quinn, G.P. (2002). *Monitoring ecological impacts: Concepts and Practice in Flowing Waters*. Cambridge, UK: Cambridge University Press, pp. 446.

Dubé, M., Culp, J., & Scrimgeour, G. (1997). Nutrient limitation and herbivory: processes influenced by bleached kraft pulp mill effluent. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, 2584-2595.

Dubé, M., Duinker, P., Greig, L., Carver, M., Servos, M., McMaster, M., Nobel, B., Schreier, H., Jackson, L., & Munkittrick, K.R. (2013). A framework for assessing cumulative effects in watersheds: An introduction to Canadian case studies. *Integrated Environmental Assessment and Management*, 9(3), 363-369.

Ecological Stratification Working Group. (1996). *A national ecological framework for Canada*. Ministry of Supply and Service. Cat. No. A42-65/1996E.

Environment Canada. (1991). *Protecting the waters of the Nahanni National Park Reserve, Northwest Territories*. Yellowknife, NT: Inland Waters Directorate and Canadian Parks Service Report TR91-1/NAH.

Environment Canada. (1994). *Manual of analytical methods: major ions and nutrients, Volume 1*. Burlington, ON: National Laboratory for Environmental Testing, Canadian Center for Inland Waters.

Environment Canada. (2011). *Canadian aquatic biomonitoring network, field manual, wadeable streams*. Dartmouth, NS: Ministry of the Environment, Catalog # En84-87/2012E.

Environment Canada. (2012). *Metal Mining Technical Guidance for Environmental Effects Monitoring*. Gatineau, QC: Catalog # En14-61/2012E.

Falck, H., & Wright, D.F. (Eds.). (2007). *Mineral and Energy Resource Potential of the Proposed Expansion to the Nahanni National Park Reserve, Northern Cordillera*, Northwest Territories. Geological Survey of Canada, Open File, 5344.

Gensemer, R.W., & Playle, R.C. (1999). The bioavailability and toxicity of aluminum in aquatic environments. *Critical Reviews in Environmental Science and Technology*, 29(4), 315-450.

Genter, R.B., Cherry, D.S., Smith, E.P., & Cairns, J. (1987). Algal-periphyton population and community changes from zinc stress in stream mesocosms. *Hydrobiologia*, 153, 261-275.

Girard, R.E., Clark, B.J., Yan, N.D., Reid, R.A., David, S.M., Ingram, R., & Findeis, J.G. (2006). *A history of chemical, physical and biological sampling methods, sample locations and lake morphometry for the Dorset Environmental Science Center (1973-2006)*. Dorset, ON: Ontario Ministry of Environment.

Gold, C., Feurtet-Mazel, A., Coste, M., & Boudou, A. (2003). Impacts of Cd and Zn on the development of periphytic diatom communities in artificial streams located along a river pollution gradient. *Archives of Environmental Contamination and Toxicology*, 44, 189-197.

Graham, L.E., & Wilcox, L.W. (2000). *Algae*. Upper Saddle River, NJ: Prentice Hall.

Green, R.H. (1979). *Sampling design and statistical methods for environmental biologists*. United States: John Wiley and Sons.

Guasch, H., & Sabater, S. (1998). Light history influences the sensitivity to atrazine in periphytic algae. *Journal of Phycology*, 34, 233-241.

Hall, R.I., Leavitt, P.R., Smol, J.P., & Zirnhelt, N. (1997). Comparison of diatoms, fossil pigments and historic records as measures of lake eutrophication. *Freshwater Biology*, 38, 401-417.

Hall, R.I., & Smol, J.P. (1996). Paleolimnological assessment of long-term water-quality changes in south-central Ontario lakes affected by cottage development and acidification. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 1-17.

Hall, R.I., Wolfe, B.B., Wiklund, J.A., Edwards, T.W.D., Farwell, A.J., & Dixon, D.G. (2012). Has Alberta oil sands development altered delivery of polycyclic aromatic compounds to the Peace-Athabasca Delta? *PLOS ONE,* 7(9), e46089. DOI:10.1371/journal.pone.0046089

Halliwell, D., & Catto, S. (2003). How and why is aquatic quality changing at Nahanni National Park Reserve, NWT, Canada? *Environmental Monitoring and Assessment*, 88, 243-281.

Hansson, L. (1988). Effects of competitive interactions on the biomass development of planktonic and periphytic algae in lakes. *Limnology and Oceanography*, 33(1), 121-128.

Hawkins, C.P., Norris, R.H., Gerritsen, J., Hughes, R.M., Jackson, S.K., Johnson, R.K., & Stevenson, R.J. (2000). Evaluation of the use of landscape classifications for the prediction of freshwater biota: synthesis and recommendations. *Journal of North American Benthological Society*, 19(3), 541-556.

Hawkins, C.P., Olson, J.R., & Hill, R.A. (2010). The reference condition: predicting benchmarks for ecological and water-quality assessments. *Journal of North American Benthological Society*, 29(1), 312-333.

Hill, W.R., (1996). Factors Affecting Benthic Algae. In Stevenson, R.J., Bothwell, M.L., & Lowe, R.L. (Ed.). *Algal ecology freshwater benthic ecosystems*. San Diego, CA: Academic Press, Inc., pp. 121-144.

Hill, B.H., Herlihy, A.T., Kaufmann, P.R., Stebenson, R.J., McCormick, F.H., & Burch Johnson, C. (2000a). Use of periphyton assemblage data as an index of biotic integrity. *Journal of North American Benthological Society*, 19, 50-67.

Hill, B.H., Willingham, W.T., Parrish, L.P., & McFarland, B.H. (2000b). Periphyton community responses to elevated metal concentrations in a Rocky Mountain stream. *Hydrobiologia*, 428, 161-169.

Hirst, H., Jüttner, I., & Ormerod, S.J. (2002). Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology,* 47, 1752-1765.

Hughes, R.M., Larsen, D.P., & Omernik, J.M. (1986). Regional reference sites: a method for assessing stream potentials. *Environmental Management*, 10(5), 629-635.

Hulbert, S.H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54, 187-211.

Hynes, H.B.N. (1975). The stream and its valley. *Verhandlungen der international Vereinigung für Theoretische und Angewandte Limnologie,* 19, 1-15.

Jacoby, J.M., Bouchard, D.D., & Patmont, C.R. (1991). Response of periphyton to nutrient enrichment in Lake Chelan, WA. *Lake and Reservoir Management,* 7, 33-43.

Janhurst, S. (Ed.). (1994). *Water quality analyses section: 1992 performance report*. Sudbury, ON: Laboratory Services Branch, Ontario Ministry of Environment and Energy.

Jeffrey, S.W., Mantoura, R.F.C., & Wright, S.W. (1997). *Phytoplankton pigments in oceanography*. Paris, France: UNSECO Publishing.

Johnson, C. (2000). Use of periphyton assemblage data as an index of biotic integrity. *Journal of North American Benthological Society*, 19, 50-67.

Johnson, R.K. (1999). Regional representativeness of Swedish reference lakes. *Environmental Management,* 23(1), 115-124.

Karr, J.R. (1981). Assessment of biotic integrity using fish communities. *Fisheries*, 6(6), 21-27.

Kattsov, V.M., Källén, E., Cattle, H., Christensen, J., Drange, H., Hanssen-Bauer, I., Jóhannesen, T., Karol, I., Räisänen, J., & Svensson, G. (2005). Future climate change: modeling and scenarios for the Arctic. ACIA. In *Arctic Climate Impact Assessment*. Cambridge, UK: Cambridge University Press, pp. 99-150

Kilgour, B.W., Dubé, M.G., Hedley, K., Portt, C.B., & Munkittrick, K.R. (2007). Aquatic environmental effects monitoring guidance for environmental assessment practitioners. *Environmental Monitoring and Assessment*, 130, 423-436.

Kilgour, B.W., Munkittrick, K.R., Portt, C.B., Hedley, K., Culp, J., Dixit, S., & Pastershank, G. (2005). Biological criteria for municipal wastewater effluent monitoring programs. *Water Quality Research Journal of Canada*, 40, 374-387.

King, L., Clarke, G., Bennion, H., Kelly, M., & Yallop, M. (2006). Recommendations for sampling littoral diatoms in lakes for ecological status assessments. *Journal of Applied Phycology*, 18, 15-25.

Krammer, K., & Lange-Bertalot, H. (1986-1991). Bacillariophyceae. In Ettl, H., Gerloff, J., Heynig, H., & Mollenhauer, D. (Eds). *Stubwasserflora von mitteleuropa band 2/1-4*. Gustav Fischer Verlag, Stuttgart.

Küpper, H., Šetlík, I., Spiller, M., Küpper, F.C., & Prášil, O. (2002). Heavy metal-induced inhibition of photosynthesis: Targets of in vivo heavy metal chlorophyll formation. *Journal of Phycology*, 38, 429-441.

Lambert, D., & Cattaneo, A. (2008). Monitoring periphyton in lakes experiencing shoreline development. *Lake and Reservoir Management*, 24, 190-195.

Lambert, D., Cattaneo, A., & Carignan, R. (2008). Periphyton as an early indicator of perturbation in recreational lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 65, 258-265.

Lauridsen, T.L., Schluter, L., & Johansson, L.S. (2011). Determining algal assemblages in oligotrophic lakes and streams: comparing information from newly developed pigment/chlorophyll a ratios with direct microscopy. *Freshwater Biology*, 56, 1638-1651.

Lavoie, I., Campeau, S., Darchambeau, F., Cabana, G., & Dillon, P.J. (2008a). Are diatoms good integrators of temporal variability in stream water quality? Freshwater Biology, 53, 827-841.

Lavoie, I., Campeau, S., Grenier, M., & Dillon, P. (2006). A diatom-based index for the biological assessment of eastern Canadian rivers : an application of correspondence analysis (CA). *Canadian Journal of Fisheries and Aquatic Sciences*, 8, 1793-1811.

Lavoie, I., Hamilton, P.B., Campeau, S., Grenier, M., & Dillon, P.J. (2008b). *Guide d'identification des Diatomées des rivières de I'Est du Canada*. Québec, QC: Presses de I'Université du Québec.

Leavitt, P.R., Carpenter, S.R., & Kitchell, J.F. (1989). Whole-lake experiments: the annual record of fossil pigments and zooplankton. *American Society of Limnology and Oceanography*, 34(4), 700-717.

Leavitt, P.R., & Findlay, D.L. (1994). Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic lake 227, Experimental Lakes Area, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 51, 2286-2299.

Leavitt, P.R., & Hodgson, D.A. (2001). Sedimentary Pigments. In Smol, J.P., Birks, H.J.B., Last, W.M. (Ed.). *Tracking environmental change using lake sediments. Volume 3: terrestrial, algal, and siliceous indicators* (pp. 295-325). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Legendre, P., & Legendre, L. (1998). *Numerical ecology*. (2nd Ed.). New York: Elsevier.

Leland, H.V. (1995). Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use, and other environmental factors. *Canadian Journal of Fisheries and Aquatic Sciences*, 52, 1108-1129.

Leland, H.V., & Porter, S.D. (2000). Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. *Freshwater Biology,* 44, 279-301.

Linke, S., Norris, R.H., Faith, D.P., & Stockwell, D. (2005). ANNA: A new prediction method for bioassessment programs. *Freshwater Biology*, 50, 147-158.

Lobe, S.L. (1981). An in situ method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. *Limnology and Oceanography*, 26(2), 394-399.

Loeb, S.L. (1994). An ecological context for biological monitoring. In Loeb, S.L., & Spacie, A. (Eds). *Biological monitoring of aquatic systems*. Boca Raton, FL: CRC Press, Inc., pp. 3-7.

Macfie, S.M., Tarmohamed, Y., & Welbourn, P.M. (1994). Effects of cadmium, cobalt, copper, and nickel on growth of the green alga *Chlamydomonas reinhardtii*: The influences of the cell wall and pH. *Archives of Environmental Contamination and Toxicology*, 27, 454-458.

Mantoura, R.F.C., & Llewellyn, C.A. (1983). The rapid determination fo algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Analytica Chimica Acta,* 151, 297-314.

Molot, L.A., & Dillon, P.J. (1991). Nitrogen/phosphorus ratios and the prediction of chlorophyll in phosphorus-limited lakes in central Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 48, 140-145.

Morin, S., Thuy Duong, T., Herlory, O., Feurtet-Mazel, A., & Coste, M. (2008). Cadmium toxicity and bioaccumulation in freshwater biofilms. *Archives of Environmental Contamination and Toxicology*, 54, 173-186.

Neff, M.R., & Jackson, D.A. (2011). Effects of broad-scale geological changes on patterns in macroinvertebrate assemblages. *Journal of North American Benthological Society,* 30(2), 459-473.

[OMOE] Ontario Ministry of Environment. (1983). *Handbook of analytical methods for environmental samples*. Sudbury, ON. Laboratory Services Branch, Ontario Ministry of Environment and Energy. Volumes 1 and 2.

Plafkin, J.L., Barbour, M.T., Porter, K.D., Gross, S.K., & Hughes, R.M. (1989). *Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish*. Washington, DC. U.S. Environmental Protection Agency. EPA 444/4- 89-001.

Peterson, H.G., Healey, F.P., & Wagemann, R. (1984). Metal toxicity to algae: A highly pH dependent phenomenon. *Canadian Journal of Fisheries and Aquatic Science*, 41, 974-979.

Pinto, E., Sigaud-Kutner, T.C.S., Leitão, M.A.S., Okamoto, O.K., Morse, D., & Colepicolo, P. (2003). Heavy metal-induced oxidative stress in algae. *Journal of Phycology*, 39, 1008-1018.

Poulíčková, A., Duchoslav, M., & Dokulil, M. (2004). Littoral diatom assemblages as bioindicators of lake trophic status: a case study from perialpine lakes in Austria. *European Journal of Phycology*, 39, 143-152.

Prescott, G.W. (1951). *Algae of the western Great Lakes area*. Bloomfield Hills, MI: The Cranbrook Press.

Prowse, T., Alfredsen, K., Beltaos, S., Bonsal, B.R., Bowde,, W.B., Duguay, C., Korhola, A., McNamara, J., Vincent, W.F., Vuglinsky, V., Walter Anthony, K.M., & Weyhenmeyer, G.A. (2011). Effects of changes in arctic lake and river ice. *Ambio,* 40, 63-74.

Prowse, T.D., Wrona, F.J., Reist, J.D., Gibson, J.J., Hobbie, J.E., Lévesque, L.M.J., & Vincent, W.F. (2006). Climate change effects on hydroecology of Arctic freshwater ecosystems. *Ambio,* 35(7), 347-358.

Quinlan, R., Hall, R.I., Paterson, A.M., Cumming, B.F., & Smol, J.P. (2008). Long-term assessments of ecological effects of anthropogenic stressors on aquatic ecosystems from

paleoecological analyses: challenges to perspectives of lake management. *Canadian Journal of Fisheries and Aquatic Sciences*, 65(5), 933-944.

Reavie, E.D., Axler, R.P., Sgro, G.V., Danz, N.P., Kingston, J.C., Kireta, A.R., Brown, T.N., Hollenhorst, T.P., & Ferguson, M.J. (2006). Diatom–based weighted–averaging transfer functions for great lakes coastal water quality: relationships to watershed characteristics. *Journal of Great Lakes Research*, 32, 321-347.

Reavie, E.D., & Smol, J.P. (1998). Epilithic diatoms of the St. Laurence River and their relationships to water quality. *Canadian Journal of Botany*, 76, 251-257.

Reece, P.A., & Richardson, J.S. (1999). *Biomonitoring with the reference condition approach for the detection of aquatic ecosystem risk*. Victoria, BC: Proceedings of a Conference on the Biology and Management of Species and Habitats at Risk, Kamloopps. BC. Volume 2: British Columbia Ministry of Environment, Lands and Parks, pp. 520.

Resh, V.H. (2008). Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. *Environmental Monitoring and Assessment*, 138, 131-138.

Reynoldson, T.B., Norris, R.H., Resh, V.H., Day, K.E., & Rosenberg, D.M. (1997). The reference condition: A comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. *Journal of the North American Benthological Society*, 16(4), 833-852.

Reynoldson, T.B., Rosenberg, D.M., & Resh, V.H. (2001). Comparison of models predicting invertebrate assemblages for biomonitoring in the Fraser River catchment, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*. 58, 1395-1410.

Rhea, D.T., Harper, D.D., Farag, A.M., & Brumbaugh, W.G. (2006). Biomonitoring in the Boulder River watershed, Montana, USA: Metal concentrations in biofilm and macroinvertebrates, and relations with macroinvertebrate assemblage. *Environmental Monitoring and Assessment*, 115, 381-393.

Rosenberg, D.M., Reynoldson, T.B., & Resh, V.H. (1999). *Establishing reference conditions for benthic invertebrate monitoring in the Fraser River catchment, British Columbia, Canada*. Winnipeg, MB: Department of Fisheries and Oceans, Freshwater Institute.

Rosenberger, E.E., Hampton, S.E., Fradkin, S.C., & Kennedy, B.P. (2008). Effects of shoreline development on the nearshore environment in large deep oligotrophic lakes. *Freshwater Biology*, 53, 1673-1691.

Rott, E., Duthie, H.C., & Pipp, E. (1998). Monitoring organic pollution and eutrophication in the Grand River, Ontario, by means of diatoms. *Canadian Journal of Fisheries and Aquatic Sciences,* 55, 1443-1453.

Sabater, S., & Admiraal, W. (2005). Periphyton as biological indicators in managed aquatic ecosystems. In Azim, M.E., Verdegem, M.C.J., Van Dam, A.A., & Beveridge, M.C.M. (Eds.). *Periphyton: ecology, exploitation and management*. Wallingford, Oxfordshire, UK: CAB International, pp. 159 – 178.

Sabater, S., Buchaca, T., Cambra, J., Catalan, J., Guasch, H., Ivorra, N., Muñoz, I., Navarro, E., Real, M., & Romaní, A. (2003). Structure and function of benthic algal communities in an extremely acid river. *Journal of Phycology*, 39, 481-489.

Schindler, D.W. (1987). Detecting ecosystem responses to anthropogenic stress. *Canadian Journal of Fisheries and Aquatic Sciences*, 44(Suppl. 1), 6-25.

Schindler, D.W. (2001). The cumulative effects of climate warming and other human stresses on Canadian freshwaters in the new millennium. *Canadian Journal of Fisheries and Aquatic Sciences*. 58, 18-29.

Schindler, D.W., Bayley, S.E., Parker, B.R., Beaty, K.G., Cruikshank, D.R., Fee, E.J., Schindler, E.U., & Stainton, M.P. (1996). The effects of climate warming on the properties of boreal lakes and streams at the experimental lakes area, Northwestern Ontario. *Limnology and Oceanography*, 41(5), 1004-1017.

Schindler, D.W., & Smol, J.P. (2006). Cumulative effects of climate warming and other human activities on freshwaters of Arctic and Subarctic North America. *Ambio*, 35(4), 160-168.

Scrimgeour, G.J. (2013). *An assessment of the ecological integrity of streams in the South Nahanni Watershed: development and application of a reference condition model*. Parks Canada Agency. Interm Report.

Scrimgeour, G.J., Hvenegaard, P.H., & Tchir, J. (2008). Cumulative effects of industrial activity on the occurrence and composition of stream fish communities in two boreal forest watersheds. *Environmental Management*, 42, 957-970.

Secretariat of the Convention on Biological Diversity. (2003). *Interlinkages between biological diversity and climate change. Advice on the integration of biodiversity considerations into the implementation of the United Nations Framework Convention on Climate Change and its Kyoto protocol*, Montreal, SCBD. CBD Technical Series no. 10, pp. 154.

Simpson, J.C., & Norris, R.H. (2000). Biological assessment of river quality: Development of AUSRIVAS models and outputs. In Wright, J.F., Sutcliffe, D.W., & Furse, M.T. (Eds.). *Assessing the biological quality of fresh waters: RIVPACS and other techniques*. Cumbria, UK: Freshwater Biological Association, pp 143–163.

Smol, J.P., & Stoermer, E.F., (Eds.). (2010). *The diatoms: applications for the environmental and earth sciences* (2nd Ed.). Cambridge, UK: Cambridge University Press.

Sokal, M.A., Hall, R.I., & Wolfe, B.B. (2008). Relationships between hydrological and limnological conditions in lakes of the Slave River Delts (NWT, Canada) and quantification of their roles on sedimentary diatom assemblages. *Journal of Paleolimnology,* 39, 533-550.

Spencer, P., Bowman, M.F., & Dubé, M.G. (2008). A multitrophic approach to monitoring the effects of metal mining in otherwise pristine and ecologically sensitive rivers in Northern Canada. *Integrated Environmental Assessment and Management*, 4, 327-343.

Stainton, M.P., Capel, M.J., & Armstrong, F.A.J. (1977). *The chemical analysis of freshwater*. Fisheries and Oceans Canada Special Publication No 25.

Stephenson, R.J., & Bahls, L.L. (1999). Periphyton protocols. In Barbour, M.T., Gerritsen, J., Synder, B.D., & Stribling, J.B. (2nd Ed.). *Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish*. Washington, DC: US Environmental Protection Agency.

Sylvestre, S., Fluegel, M., & Tuominen T. (2005). *Benthic invertebrate assessment of streams in the Georgia basin using the reference condition approach: Expansion of the Fraser River invertebrate monitoring program 1998-2002*. Vancouver BC: Environment Canada, Environmental Conservation Branch, Aquatic and Atmospheric Sciences Division. EC/GB/01/81.

ter Braak, C.J.F., & Šmilauer, P. (2002). C*anoco reference manual and canodraw for windows user's guide. Software for canonical community ordination (ver 4.5)*. Ithaca, NY: Microcomputer Power.

The Conference Board of Canada. (2013). *The future of mining in Canada's North*. Canada: AERIC Inc. Agreement No. 40063028.

Thomas, K.E., Hall, R.I., & Scrimgeour, G.J. (2013). Evaluating the use of algal pigments to assess the biological condition of streams. *Environmental Assessment and Monitoring*, 185, 7895-7913.

Thomas, K.E., Kluke, A., Hall, R.I., Paterson, A.M., & Winter, J.G. (2011). Assessment of benthic algal biomintoring protocols to evaluate effects of shoreline development on the nearshore zone of Precambrian Shield lakes in Ontario. *Lake and Reservoir Management*, 27, 398-413.

Tornés, E., Leira, M., & Sabater, S. (2012). Is the biological classification of benthic diatom communities concordant with ecotypes? *Hydrobiologia,* 695, 43-55.

Tran, P. (2007). *Muskoka river watershed inventory project, Final Report*. The Muskoka Heritage Foundation, The District Municipality of Muskoka, The Ontario Ministry of Natural Resources, The Muskoka Watershed Council.

Underwood, A.J. (1994). On beyond BACI – sampling designs that might reliably detect environmental disturbances. *Ecological Applications*, 4, 3-15.

Vadeboncoeur, Y., Lodge, D.M., & Carpenter, S.R. (2001). Whole–lake fertilization effects on distribution of primary production between benthic and pelagic habitats. *Ecology*, 82(4), 1065-1077.

Vadeboncoeur, Y., Jeppesen, E., Vander Zanden, M.J., Schierup, H., Christoffersen, K., & Lodge, D.M. (2003). From Greenland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography*, 18(1), 1108-1118.

Walker, S., Ribey, S., Trudel, L., & Porter, E. (2003). Canadian environmental effects monitoring: Experiences with pulp and paper and metal mining regulatory programs. *Environmental monitoring and assessment*, 88, 311-326.

Wehr, J.D., & Sheath, R.G., (Eds.). 2003. *Freshwater Algae of North America: Ecology and Classification*. San Diego, CA: Academic Press.

White, M.S., Xenopoulos, M.A., Metcalfe, R.A., & Somers, K.M. (2011). Water level fluctuation thresholds of benthic macroinvertebrate richness, structure, and function in boreal stony littoral habitats. *Canadian Journal of Fisheries and Aquatic Sciences*, 68, 1695-1704.

Wiklund, J.A., Bozinovski, N., Hall, R.I., & Wolfe, B.B. (2010). Epiphytic diatoms as flood indicators. *Journal of Paleolimnology,* 44, 25-42.

Winter, J.G., Dillon, P.J., Paterson, C., Reid, R.A., & Somers, K.M. (2003). Impacts of golf course construction and operation on headwater streams: bioassessment using benthic algae. *Canadian Journal of Botany*, 81, 848-858.

Winter, J.G., & Duthie, H.C. (1998). Effects of urbanization on water quality, periphyton and invertebrate communities in a southern Ontario stream. *Canadian Water Resources Journal*, 23(3), 245-257.

Winter, J.G., & Duthie, H.C. (2000). Stream biomonitoring at an agricultural test site using benthic algae. *Canadian Journal of Botany*, 78, 1319-1325.

Wright, J.F., Furse, M.T., & Armitage, P.D. (1993). RIVPACS: A technique for evaluating the biological quality of rivers in the UK. *European Water Pollution Control*, 3, 15-25.

Wrona, F.J., Prowse, T.D., Reist, J.D., Hobbie, J.E., Lévesque, L.M.J., & Vincent, W.F. (2006). Climate change effects on aquatic biota, ecosystem structure and function. *Ambio,* 35(7), 359-369.

Yan, N.D., Blukacz, A., Sprules, W.G., Kindy, P.K., Hackett, D., Girard, R.E., & Clark, B.J. (2001). Changes in zooplankton and the phenology of the spiny water flea, *Bythotrephes*, following its invasion of Harp Lake, Ontario, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*. 58, 2341-2350.

Appendix A - Chapter 2

Assessment of benthic algal biomonitoring protocols to evaluate effects of shoreline development on the nearshore zone of Precambrian Shield lakes in Ontario

Appendix A, Table 2.1 Selected nutrient and ion characteristics obtained at each of the 29 nearshore study sites in the Muskoka-Haliburton district of south-central Ontario. The Site Names are presented in Figure 2.1 and Table 2.1

Site Name	TP µg/L		TKN µg /L		NO_3^- µg /L		NH_4^+ µg /L		SO_4^{2-} mg/L		DOC mg/L		COND µS/cm		pH	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
HMB-2	3.4	4.5	252.0	205.0	6.0	8.0	30.0	12.0	6.8	6.7	3.1	2.6	61.2	62.8	6.0	6.4
TML-1	18.2	25.8	484.0	493.0	4.0	2.0	108.0	78.0	4.7	4.6	6.9	5.9	67.2	71.4	6.6	7.3
CLE-1	6.9	5.0	322.0	287.0	16.0	16.0	32.0	28.0	5.9	5.7	4.1	3.6	53.8	54.6	6.5	7.0
CLE-2	4.6	7.7	260.0	283.0	16.0	14.0	30.0	28.0	5.9	6.0	4.0	3.5	53.4	54.6	6.5	7.1
RSH-3	5.9	7.5	248.0	258.0	18.0	24.0	32.0	10.0	6.1	6.1	3.5	3.3	55.8	53.6	6.6	7.1
COX-2	3.1	4.1	197.0	251.0	2.0	2.0	18.0	16.0	6.8	6.6	3.0	3.1	62.8	63.8	6.5	7.0
COX-3	12.6	4.8	205.0	250.0	2.0	2.0	20.0	12.0	6.8	6.3	2.9	3.1	63.0	64.2	6.5	7.1
COX-4	3.5	4.7	207.0	208.0	16.0	10.0	22.0	14.0	6.7	7.2	2.9	3.0	68.0	68.2	6.5	7.0
TMH-1	11.6	14.0	382.0	347.0	2.0	2.0	74.0	44.0	5.3	4.8	4.9	5.2	65.8	68.8	6.2	7.1
TML-2	18.4	24.4	475.0	495.0	4.0	12.0	102.0	68.0	4.8	4.7	6.5	5.8	68.8	72.2	6.6	7.3
MON-1	5.5	5.9	299.0	269.0	18.0	30.0	32.0	26.0	5.7	5.7	4.4	4.2	54.0	56.2	6.5	6.9
MBA-5	7.1	7.3	301.0	279.0	4.0	2.0	28.0	18.0	6.2	6.3	4.3	4.6	80.6	81.6	6.9	7.1
CLE-3	4.8	5.3	292.0	267.0	12.0	16.0	26.0	24.0	6.0	5.7	3.5	3.4	53.0	53.8	6.6	7.0
RSH-4	4.2	5.2	240.0	244.0	16.0	32.0	30.0	10.0	6.2	6.1	2.9	3.5	54.8	64.0	6.6	6.4
RSH-5	4.1	4.9	233.0	533.0	16.0	28.0	30.0	162.0	6.1	6.1	2.8	3.6	54.8	54.4	6.6	7.2
DKL-1	4.9	5.2	258.0	296.0	2.0	4.0	30.0	8.0	5.4	5.2	4.3	6.1	42.4	39.6	5.8	6.7
DKL-2	5.0	6.2	249.0	283.0	2.0	6.0	22.0	8.0	5.5	5.4	4.0	6.2	41.8	54.2	5.8	7.0
HMB-4	4.3	3.9	273.0	249.0	8.0	8.0	30.0	14.0	6.7	6.8	2.6	2.8	61.0	39.6	6.0	6.3
TMH-2	11.9	12.2	374.0	333.0	2.0	2.0	66.0	44.0	5.1	4.8	4.9	5.1	66.2	69.2	6.2	7.2
MON-2	4.6	5.6	328.0	287.0	18.0	30.0	28.0	32.0	5.9	5.5	4.4	4.1	53.6	55.8	6.5	6.9
MON-3	4.8	5.7	287.0	286.0	18.0	30.0	30.0	24.0	5.8	5.7	5.0	3.9	54.0	55.8	6.1	7.0
MBA-4	22.1	7.7	316.0	295.0	4.0	2.0	38.0	18.0	6.1	6.3	4.3	4.5	81.2	82.2	6.9	7.2
CLE-4	4.4	5.0	247.0	208.0	14.0	16.0	24.0	18.0	5.9	5.9	4.3	3.5	53.8	53.8	6.6	7.1
RSH-6	5.0	4.5	231.0	263.0	18.0	24.0	28.0	24.0	6.2	5.9	2.5	3.6	55.0	54.0	6.7	7.0

Site Name	TP µg/L		TKN µg /L		NO_3^- µg /L		NH_4^+ µg /L		SO_4^{2-} mg/L		DOC mg/L		COND µS/cm		pH	
	2006	2007	2006	2007	2006	2007	2006	2007		2006	2007	2006	2007	2006	2007	2006
RSH-7	N/A	4.5	N/A	255.0	N/A	28.0	N/A	8.0	N/A	6.1	N/A	3.4	N/A	55.0	N/A	7.1
DKL-3	5.6	4.7	273.0	272.0	2.0	4.0	24.0	8.0	5.5	5.2	4.0	6.1	41.8	40.0	5.9	6.4
EAS-1	4.4	6.6	247.0	239.0	18.0	46.0	38.0	18.0	6.1	5.5	3.8	4.4	55.2	53.8	6.5	6.9
EAS-2	6.6	6.5	261.0	258.0	12.0	52.0	30.0	18.0	5.9	5.7	4.2	4.6	59.6	53.2	6.5	7.0
EAS-3	4.9	5.9	245.0	235.0	18.0	48.0	34.0	16.0	5.9	5.7	4.2	4.2	55.8	54.0	6.5	6.9

Appendix A, Table 2.2 Selected metal values obtained at each of the 29 nearshore study sites in the Muskoka-Haliburton district of south-central Ontario. The Site Names are presented in Figure 2.1 and Table 2.1. Metal concentrations that were below detection limits were represented as ND.

Site Name	Al µg/L		Ba µg /L		Cd µg /L		Cu µg /L		Fe µg /L		Mn µg /L		Sr µg /L		Zn µg /L	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
HMB-2	10.0	6.4	8.7	9.0	0.1	1.0	1.5	0.5	19.0	13.2	2.3	2.3	40.2	43.4	1.7	1.9
TML-1	19.2	83.4	4.7	6.9	0.2	1.0	0.5	0.8	84.4	179.0	16.2	22.5	35.5	37.1	0.5	0.9
CLE-1	26.4	26.3	10.7	10.7	0.04	0.9	0.6	0.4	35.2	28.6	4.1	2.3	31.3	31.3	2.0	2.1
CLE-2	13.7	47.4	10.0	11.3	0.2	1.3	0.2	0.7	13.2	67.1	2.9	10.5	30.3	31.5	1.2	2.6
RSH-3	23.9	31.4	11.0	10.9	ND	0.7	0.4	1.0	75.5	63.6	6.5	5.7	31.8	1.5	11.0	2.5
COX-2	7.3	11.7	9.2	9.3	0.0	1.2	0.7	0.6	13.3	16.6	4.0	4.0	40.1	41.4	1.2	1.6
COX-3	16.2	16.1	9.2	9.3	ND	1.0	0.8	0.4	19.5	26.8	4.3	4.7	40.2	41.3	1.3	1.8
COX-4	23.7	11.5	14.5	9.7	ND	1.5	0.5	0.6	26.3	20.0	5.1	6.4	41.9	43.7	1.0	2.0
TMH-1	14.4	43.7	9.7	11.5	ND	1.1	0.7	0.7	41.6	107.0	12.9	27.3	32.6	34.4	1.0	0.7
TML-2	19.5	52.5	7.2	6.2	0.1	0.6	0.7	0.3	82.3	147.0	19.2	23.1	35.5	35.8	0.5	1.0
MON-1	15.1	22.8	14.7	14.0	ND	1.3	1.0	0.9	45.4	43.1	6.9	5.5	30.6	29.8	1.6	1.2
MBA-5	3.9	5.8	14.0	13.9	ND	0.6	0.7	1.1	33.8	38.9	13.3	12.5	44.9	45.2	0.4	1.3
CLE-3	16.1	17.7	10.0	10.4	ND	1.2	0.8	0.4	18.5	14.4	3.1	1.9	30.6	31.1	1.4	1.7
RSH-4	17.2	15.0	10.5	10.8	ND	0.7	0.7	0.9	30.7	15.2	4.5	3.0	30.4	30.5	1.2	2.7
RSH-5	16.5	15.3	10.6	10.6	0.2	0.9	0.6	0.8	38.9	13.8	4.5	3.2	30.3	30.0	1.5	3.9
DKL-1	23.0	27.3	14.7	14.2	ND	0.7	0.4	1.0	55.5	57.0	25.1	29.3	26.0	25.8	4.3	4.1
DKL-2	23.7	30.5	0.01	14.5	ND	0.8	0.7	0.9	62.5	60.0	25.9	27.6	25.6	25.6	4.3	4.4
HMB-4	4.1	6.0	8.6	9.0	0.2	0.7	1.2	1.1	8.7	4.4	1.8	1.3	39.9	40.7	1.1	2.1
TMH-2	15.3	20.5	9.9	11.0	0.2	1.1	0.9	0.3	46.9	65.9	13.6	22.4	33.0	34.7	0.6	0.3

MON-2	14.8	23.0	14.4	14.2	ND	0.9	0.8	0.7	34.9	36.9	6.4	5.9	30.4	30.3	1.4	1.9
MON-3	12.0	21.1	14.3	14.2	0.3	1.1	1.1	0.4	38.6	35.1	6.1	5.0	30.4	30.2	0.7	1.9

Site Name	**Al µg/L**		**Ba µg /L**		**Cd µg /L**		**Cu µg /L**		**Fe µg /L**		**Mn µg /L**		**Sr µg /L**		**Zn µg /L**	
	2006	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**
MBA-4	3.0	6.6	14.1	13.6	ND	0.7	0.4	1.2	36.6	37.4	15.3	12.0	45.3	45.2	1.2	1.5
CLE-4	14.0	19.2	0.0	10.6	ND	1.0	0.7	0.6	14.5	19.7	3.1	2.4	30.9	31.3	1.2	7.2
RSH-6	16.5	20.4	10.6	10.5	0.2	1.0	0.6	0.5	38.9	30.0	4.5	2.6	30.3	30.8	1.5	1.7
RSH-7	NA	16.0	NA	10.7	NA	0.5	NA	0.5	NA	23.7	NA	4.6	NA	30.0	NA	2.0
DKL-3	23.9	22.5	14.7	13.4	0.02	0.5	0.7	0.3	65.8	45.1	36.3	22.6	26.0	25.4	4.0	3.9
EAS-1	13.3	17.4	14.0	14.3	ND	1.2	1.0	0.2	16.8	20.0	3.6	3.4	29.7	28.9	1.9	3.3
EAS-2	20.5	14.2	14.3	14.2	0.1	1.0	0.3	0.2	44.7	25.5	7.0	3.8	30.8	29.1	1.3	1.6
EAS-3	13.8	14.3	14.1	14.3	ND	1.3	0.8	0.4	16.8	18.6	4.0	3.0	30.3	29.3	1.1	2.0

Appendix A, Table 2.3 List of diatom taxon names for Level 5: High taxonomic resolution benthic algal counts. Taxon numbers are presented in Figure 2.9.

Taxon Number		Taxon Name	Number of Occurrences		Mean Relative Abundance		Maximum Relative Abundance	
2006	2007		2006	2007	2006	2007	2006	2007
1	1	***Achnanthes bioretii*** Germain	10	1	0.27	0.06	1.76	1.78
2	NA	***Achnanthes curtissima*** Carter	14	NA	0.25	NA	1.23	NA
3	2	***Achnanthes didyma*** Hustedt	7	11	0.15	0.36	1.33	3.17
4	3	***Achnanthes exigua*** Grunow	5	8	0.23	0.15	3.54	1.13
5	4	***Achnanthes hungarica*** (Grunow) Grunow	16	10	1.66	0.49	19.07	5.91
NA	5	***Achnanthes levanderi*** Hustedt	2	NA	0.09	NA	2.27	NA
6	NA	***Achnanthes marginulata*** Grunow	8	NA	0.16	NA	1.08	NA
7	6	***Achnanthes pusilla***(Grunow) De Toni	15	19	0.49	0.52	2.06	2.84
8	NA	***Achnanthes saccula*** Carter	16	NA	0.37	NA	1.15	NA
9	7	***Achnanthidium minutissimum*** Czarnecki [*Achnanthes minutissima* Kützing 1833]	28	29	33.85	43.14	53.52	66.14
10	8	***Adlafina sp. [A. cf. bryophila*** (Petersen) Moser et al.] (*Navicula bryophila* Petersen)	27	23	5.29	3.04	36.79	36.13

Taxon Number		Taxon Name	Number of Occurrences		Mean Relative Abundance		Maximum Relative Abundance	
NA	9	***Amphora inariensis*** Krammer	NA	8	NA	0.14	NA	1.19
NA	10	***Amphora veneta*** Kützing	NA	3	NA	0.07	NA	1.14
11	NA	***Asterionella formosa*** Hassall	10	NA	0.22	NA	1.18	NA
12	11	***Aulacoseira ambigua***(Grunow) Simonsen	14	8	0.63	0.51	5.71	4.66
13	12	***Brachysira brebissonii*** Ross [*Anomoeoneis brachysira* (Bréisson) Grunow]	22	23	1.79	1.24	11.01	5.84
14	13	***Brachysira styriaca*** (Grunow) Ross [*Anomoeoneis styriaca* (Grunow) Hustedt]	7	22	0.38	0.61	3.58	3.09
15	14	***Brachysira vitrea*** (Grunow) Ross [*Anomoeoneis vitrea* (Grunow) Ross]	27	29	9.23	11.72	26.49	33.60
16	NA	***Calonies bacillum*** (Grunow) Cleve	3	NA	0.12	NA	2.36	NA
NA	15	***Cocconeis placentula*** *var*. ***placentula*** Ehrenberg	NA	5	NA	0.18	NA	3.56
17	NA	***Cocconeis placentula var. raphid*** Ehrenberg	10	NA	0.19	NA	1.41	NA
NA	16	***Cyclostephanos dubius*** (Fricke) Round	NA	1	NA	0.09	NA	2.63
18	NA	***Cyclotella bodanica*** *var*. ***lemanica*** Grunow in Schneider (O. Müller ex Schröter) Bachmann	20	NA	0.63	NA	4.44	NA
19	NA	***Cyclotella distinguenda*** Hustedt	17	NA	0.39	NA	2.27	NA
20	17	***Cyclotella ocellata*** Pantocsek	5	9	0.16	0.13	1.65	1.01
21	18	***Cyclotella pseudostelligera*** Hustedt	27	13	1.21	0.27	2.56	1.98
22	19	***Cyclotella stelligera*** Cleve & Grunow	7	17	0.27	0.34	5.56	1.86
23	NA	***Cymbella cistula*** (Ehrenberg) Kirchner	10	NA	0.16	NA	1.06	NA
24	20	***Cymbella descripta***(Hustedt) Krammer & Lange-Bertalot	26	29	3.70	4.81	10.00	12.50
25	21	***Cymbella hillardii*** (Grunow) Cleve	8	8	0.18	0.15	1.10	1.16
26	22	***Cymbella laevis*** Naegeli	18	14	2.05	1.56	11.70	13.71
27	23	***Encyonema minutum*** (Hilse ex Rabenhorst) Mann (*Cymbella minuta* Hilse ex Rabenhorst)	10	12	0.23	0.32	1.58	2.38
28	24	***Encyonema neogracile*** Krammer [*Cymbella gracilis* ((Ehrenberg) Kützing)]	28	27	2.61	1.87	15.98	10.02
29	25	***Encyonema silesiacum*** (Bleisch) Mann (*Cymbella silesiaca* Bleisch)	25	27	1.27	1.38	3.91	18.25
NA	26	***Encyonopsis cesatii*** (Rabenhorst) Krammer [*Cymbella cesatii* (Rabenhorst) Grunow]	NA	9	NA	0.32	NA	2.94
NA	27	***Eolimna subminuscula***(Mangium) Moser, Lange-Bertalot and Metzeltin (*Navicula subminuscula* Manguin)	NA	17	NA	0.82	NA	7.56
30	NA	***Epithemia adnata***(Kützing) Brébisson	3	NA	0.10	NA	1.62	NA
31	28	***Eucocconeis flexella*** (Kützing) Cleve	15	8	0.86	0.17	4.08	1.52
32	29	***Eunotia bilunaris***(Ehrenberg) Mills	7	1	0.16	0.06	1.48	1.64
33	NA	***Eunotia minor*** (Kützing) Grunow	14	NA	0.63	NA	4.94	NA

Taxon Number		Taxon Name	Number of Occurrences		Mean Relative Abundance		Maximum Relative Abundance	
34	30	***Eunotia pectinalis*** *var.* ***pectinalis*** Rabenhorst	12	6	0.74	0.19	6.45	2.65
NA	31	***Eunotia praerupta*** Ehrenberg	NA	1	NA	0.04	NA	1.17
35	32	***Eunotia subarcuatoides*** Alles, Nörpel & Lange-Bertalot	9	10	0.60	0.17	5.08	1.03
NA	33	***Fragilaria capucina*** Desmazières	NA	8	NA	0.26	NA	2.17
36	34	***Fragilaria capucina*** *var.* ***capucina*** Desmazières	15	6	0.30	0.41	2.11	5.12
37	NA	***Fragilaria capucina*** *var.* ***mesolepta***(Rabenhorst) Rabenhorst	10	NA	0.20	NA	2.11	NA
38	35	***Fragilaria capucina*** *var.* ***vaucheriae*** (Kützing) Lange-Bertalot	9	21	0.17	0.60	1.06	3.12
39	36	***Fragilaria crotonensis*** Kitton	25	9	1.66	0.34	5.86	2.46
NA	37	***Fragilaria tenera*** (W. Smith) Lange-Bertalot	NA	1	NA	0.04	NA	1.29
40	38	***Frustulia amphipleuroides*** **(**Grunow) Cleve-Euler [*Frustulia rhomboids var. amphipleuroides* (Grunow) De Toni]	14	18	0.78	0.41	5.79	1.55
41	39	***Gomphonema acuminatum*** Ehrenberg	13	4	0.38	0.10	2.42	1.50
42	40	***Gomphonema angustum*** Agardh	7	16	0.21	0.48	3.59	2.20
NA	41	***Gomphonema anoenum*** Lange-Bertalot	NA	2	NA	0.14	NA	3.61
43	NA	***Gomphonema clavatum*** Ehrenberg	27	NA	1.75	NA	13.54	NA
44	42	***Gomphonema clevei*** Fricke	1	17	0.04	0.34	1.06	2.40
NA	43	***Gomphonema*** girdle view	NA	2	NA	0.07	NA	1.17
45	44	***Gomphonema gracile*** Ehrenberg	18	9	0.53	0.21	2.63	1.55
NA	45	***Gomphonema parvulum*** (Krützing) Krützing	NA	4	NA	0.07	NA	1.03
NA	46	***Gomphonema truncatum*** Ehrenberg	NA	5	NA	0.08	NA	1.00
46	NA	***Gyrosigma acuminatum*** (Kützing) Rabenhorst	1	NA	0.06	NA	1.57	NA
NA	47	***Karayevia clevei*** (Grunow) Round and Bukhtiyarova(*Achnanthes clevei* Grunow)	NA	2	NA	0.12	NA	2.97
47	NA	***Karayevia laterostrata*** (Hustedt) Kingston (*Achnanthes laterostrata* Hustedt)	1	NA	0.04	NA	1.23	NA
48	NA	***Navicula angusta*** Grunow	8	NA	0.25	NA	1.52	NA
NA	48	***Navicula cincta*** (Ehrenberg) Ralfs	NA	3	NA	0.07	NA	1.12
49	NA	***Navicula concentrica*** Carter	4	NA	0.12	NA	1.17	NA
50	49	***Navicula cryptocephala*** Kützing	7	10	0.51	0.30	7.81	2.83
51	50	***Navicula cryptotenella*** Lange-Bertalot	18	20	0.76	0.84	3.31	3.88
52	51	***Navicula halophila***(Grunow) Cleve	13	2	0.20	0.14	1.23	3.56
53	52	***Navicula notha*** Wallace	27	29	6.14	5.50	15.14	20.79
54	53	***Navicula radiosa*** Kützing	25	15	1.02	0.34	3.05	1.70
NA	54	***Navicula rhynchocephala*** Kützing	NA	3	NA	0.07	NA	1.51

Taxon Number		Taxon Name	Number of Occurrences		Mean Relative Abundance		Maximum Relative Abundance	
NA	55	***Navicula submuralis*** Hustedt	NA	1	NA	0.08	NA	2.34
55	NA	***Neidium ampliatum*** (Ehrenberg) Kirchner	6	NA	0.12	NA	1.10	NA
56	56	***Nitzschia amphibia*** Grunow	13	3	0.35	0.06	1.69	1.01
57	57	***Nitzschia palea***(Kützing) W. Smith	28	26	3.26	1.79	11.61	7.06
58	58	***Nitzschia radicula*** Hustedt	9	25	0.17	1.54	1.80	11.49
NA	59	***Nitzschia vitrea*** Norman	NA	2	NA	0.04	NA	1.08
59	NA	***Pinnularia subrostrata***(A. Cleve) Cleve-Euler	2	NA	0.11	NA	2.73	NA
60	NA	***Placoneis placentula***(Ehrenberg) Heinzerling [*Navicula placentula* (Ehrenberg) Kützing]	8	NA	0.19	NA	1.33	NA
61	NA	***Planothidium biporomum*** (Hohn et Hellerman) Lange-Bertalot [*Achnanthes lanceolata ssp. biporoma* (Hohn et Hellerman) Lange-Bertalot]	1	NA	0.04	NA	1.15	NA
NA	60	***Planothidium lanceolatum*** (Brébisson ex Kützing) Round and Bukhtiyarova [*Achnanthes lanceolata* (Brébisson) Grunow]	NA	0.16	NA	0.16	NA	1.97
62	NA	***Psammothidium bioretii*** (Germain) Bukhtiyarova et Round (*Achnanthes bioretii* Germain)	12	NA	0.42	NA	3.09	NA
NA	61	***Psammothidium subatomoides*** (Hustedt) Bukhtiyarova and Round [*Achnanthes subatomoides* (Hustedt) Lange-Bertalot et Archibald]	NA	4	NA	0.13	NA	2.27
NA	62	***Pseudostaurosira parasitica*** (W. Smith) Morales [*Fragilaria parasitica* (Smith) Grunow]	NA	9	NA	0.17	NA	1.57
63	63	***Rhopalodia gibba***(Ehrenberg) O. Müller	4	3	0.11	0.07	1.41	1.24
NA	64	***Sellaphora pupula*** (Kützing) Mereschkowksy (*Navicula pupula* Kützing)	NA	10	NA	0.18	NA	1.21
NA	65	***Sellaphora stroemii*** (Hustedt) H. Kobayasi (*Navicula stroemii* Hustedt)	NA	4	NA	0.12	NA	1.46
64	66	***Staurosira construens*** Ehrenberg [*Fragilaria construens* (Ehrenberg) Grunow]	2	7	0.05	0.29	1.06	5.86
NA	67	***Staurosira construens var. venter*** (Ehrenberg) Hamilton [*Fragilaria construens* var. *venter* (Ehrenberg) Grunow et van Heurck]	NA	13	NA	1.37	NA	16.14
65	68	***Staurosirella pinnata*** (Ehrenberg) Williams and Round (*Fragilaria pinnata* Ehrenberg)	14	18	2.67	1.65	30.54	18.50
66	69	***Stenopterobia curvula***(W. Smith) Krammer	16	12	0.81	0.40	6.72	3.10
NA	70	***Stephanodiscus minutulus*** (Krützing) Cleve & Möller	NA	4	NA	0.08	NA	1.19
67	NA	***Synedra ulna*** Ehrenberg	1	NA	0.05	NA	1.32	NA
68	71	***Tabellaria flocculosa***(Roth) Kützing	28	28	3.13	3.04	12.58	16.23
69	NA	***Tabellaria quadriseptata*** Knudson	12	NA	0.33	NA	2.04	NA
70	72	***Tryblionella angustata*** W. Smith [*Nitzschia angustata* (W. Smith) Grunow]	17	6	0.34	0.10	2.20	1.61

Appendix B - Chapter 3

Relations between limnological conditions and composition of benthic algal communities in the South Nahanni River watershed, NWT (Canada): defining the reference condition

Appendix B, Table 3.1 Physical and chemical variables measured at each site in 2008 and 2009. Black circles indicate variables selected for use in ordinations.

Variable	Units	Overall Benthic Algal Taxonomy		Diatom Taxonomy		Pigment Concentrations	
		2008	2009	2008	2009	2008	2009
Physical Variables							
Julian Day	Quantitative		●				
Latitude	Quantitative (Hours, Minutes, Seconds)	●		●	●		
Longitude	Quantitative (Hours, Minutes, Seconds)	●		●		●	
Altitude	Quantitative (m)	●				●	
Stream Order	Categorical (Strahler)						
Ecoregion	Categorical (1 – 2; 1-Selwyn mountain ecoregion, 2-Nahanni-Hyland ecoregions)	●	●	●	●		●
Bedrock	Quantitative (Percentage)						
Boulders	Quantitative (Percentage)				●		
Cobbles	Quantitative (Percentage)					●	
Gravel	Quantitative (Percentage)					●	●
Pebbles	Quantitative (Percentage)						
Sand	Quantitative (Percentage)						
Silt & Clay	Quantitative (Percentage)						
Bankfull – Wetted	Quantitative (cm)	●			●		
Bankfull Width	Quantitative (m)	●		●	●		
Wetted Width	Quantitative (m)			●	●	●	
Intrusive Bedrock	Quantitative (Percentage)	●	●			●	
Sedimentary Bedrock	Quantitative (Percentage)	●					

Variable	Units	Overall Benthic Algal Taxonomy		Diatom Taxonomy		Pigment Concentrations	
		2008	2009	2008	2009	2008	2009
Average Depth	Quantitative (cm)			●			
Maximum Depth	Quantitative (cm)						
Streamside Vegetation	Categorical (1 – 4; 1-ferns/grasses, 2-shrubs, 3- deciduous trees, 4-coniferous trees)					●	
Drainage Area	Quantitative (km^2)			●	●	●	
Presence of Pools, Rapids, Riffles, Runs	Binary (presence – absence)					●	
Forest Cover	Quantitative (Percentage)	●	●	●	●		
Ice Cover	Quantitative (Percentage)	●		●			
Macrophyte coverage	Quantitative (Percentage)				●	●	
Perimeter of Upstream Drainage Area	Quantitative (km)						
Presence of Coniferous Trees, Deciduous Trees, Grasses & Ferns, Shrubs	Binary (presence – absence)					●	
Sinuosity (The ratio of distance measured along a watercourse between two points, divided by the straight line distance between the same two points.)	m of stream within a 2 km linear distance of stream					●	
Slope	Quantitative (m/m)	●	●	●			
Stream Density	Quantitative (m stream/km^2 drainage area)				●		
Secondarily Dominant Sediment Size	Categorical (0 – 9)						
Dominant Sediment	Categorical (0 – 9)						

Variable	Units	Overall Benthic Algal Taxonomy		Diatom Taxonomy		Pigment Concentrations	
		2008	2009	2008	2009	2008	2009
Size							
Sediment Embeddedness [a measure of how entrenched coarse substrate (e.g., gravel, cobbles and boulders) are in finer substrates (e.g., silt and clay)].	Categorical (1 – 5; 1 = completely embedded, 5 = unembedded)					●	
Sediment Surrounding Material	Categorical (0 – 9)						
Average Velocity	Quantitative (m/s)			●		●	
Maximum Velocity	Quantitative (m/s)		●	●	●	●	
Median Particle Size (Wolman)	Quantitative (cm)						
Geometric Mean Particle Size (Wolman)	Quantitative (cm)						
Canopy Cover	Quantitative (percentage)						
June Min Temperature	Quantitative (°C)						
June Max Temperature	Quantitative (°C)						
June Mean Temperature	Quantitative (°C)	●	●			●	
Jan Min Temperature	Quantitative (°C)						
Jan Max Temperature	Quantitative (°C)						
Jan Mean Temperature	Quantitative (°C)			●			
June Rain	Quantitative (mm)						
June Snow	Quantitative (mm)			●			
June Precipitation	Quantitative (mm)						
Jan Rain	Quantitative (mm)	●					

Variable	Units	Overall Benthic Algal Taxonomy		Diatom Taxonomy		Pigment Concentrations	
		2008	2009	2008	2009	2008	2009
Jan Snow	Quantitative (mm)						
Jan Precipitation	Quantitative (mm)						
Total Snow	Quantitative (mm)						
Total Rain	Quantitative (mm)	●					
Total Precipitation	Quantitative (mm)			●			
Chemical Variables							
NO2NO3		●	●	●	●	●	
DOC					●	●	
DIC		●		●			
TN		●				●	
TP		●		●			
pH		●		●	●		
Conductivity		●		●	●	●	
Turbidity				●			
Al						●	
As		●		●			
B		●		●			
Ba		●			●	●	
Be							
Cd							
Ce					●		
Co						●	
Cr				●			
Cs							
Cu		●		●			
Fe		●					
Ga		●					
La		●		●			
Li							●

Variable	Units	Overall Benthic Algal Taxonomy		Diatom Taxonomy		Pigment Concentrations	
		2008	2009	2008	2009	2008	2009
Mn							
Mo		●					
Ni		●		●	●		
Pb		●		●	●		
Rb		●		●		●	
Sb				●			
Se		●		●		●	
Sr		●		●	●	●	
Tl							
U							
V		●				●	
W		●		●	●	●	
Y							
Zn			●			●	●

Appendix B, Table 3.2 List of diatom taxon names for each sample in 2008 and 2009.

Taxon Number 2008	Taxon Number 2009	Taxon Name
1	1	***Achnanthidium minutissimum*** (Kützing) Czarnecki [*Achnanthes minutissima* Kützing 1833]
2	2	***Achnanthidium pyrenaicum*** (Hustedt) H. Kobayasi (*Achnanthes biasolettiana* Grunow)
3	3	***Brachysira vitrea*** (Grunow) Ross [*Anomoeoneis vitrea* (Grunow) Ross]
4	4	***Cocconeis placentula*** *var*. ***placentula*** Ehrenberg
5	NA	***Cyclotella comensis*** Grunow
6	NA	***Cyclotella ocellata*** Pantocsek
7	7	***Cyclotella rossii*** Håkansson
8	8	***Cymbella affinis*** Kützing
NA	9	***Denticula kuetzingii*** Grunow
10	10	***Diatoma tenuis*** Agardh
11	11	***Encyonema minutum*** (Hilse ex Rabenhorst) Mann (*Cymbella minuta* Hilse ex Rabenhorst)
12	12	***Encyonema reichardtii*** Krammer
13	13	***Encyonema silesiacum*** (Bleisch) Mann (*Cymbella silesiaca* Bleisch)
14	14	***Encyonopsis microcephala*** (Grunow) Krammer (*Cymbella microcephala* Grunow)
15	15	***Eucocconeis flexella*** (Kützing) Meister [*Achnanthes flexella* (Krützing) Brun
16	NA	***Eunotia subarcuatoides*** Alles, Nörpel & Lange-Bertalot
17	NA	***Fragilaria capucina*** Desmazières
18	18	***Fragilaria capucina subsp. rumpens*** (Kützinng) Lange-Bertalot [*Fragilaria capucina var. rumpens* (Kützing) Lange-Bertalot ex Bukhtiyarova]
19	19	***Fragilaria capucina var. gracilis*** (Oestrup) Hustedt
20	20	***Fragilaria capucina var. vaucheriae*** (Kützing) Lange-Bertalot
NA	21	***Fragilaria construens*** (Ehrenberg) Grunow
22	22	***Fragilaria crotonensis*** Kitton
23	23	***Gomphoneis clevei*** Fricke (***Gomphonema clevei*** Fricke)
24	24	***Gomphonema affine*** Krützing
NA	25	***Gomphonema angustatum*** (Krützing) Rabenhorst
26	26	***Gomphonema minutum*** (Agardh) Agardh
27	27	***Gomphonema olivaceum*** (Hornemann) Brébisson
28	28	***Gomphonema parvulum var. micropus*** (Kützing) Cleve (*Gomphonema micropus* Krützing)
29	29	***Hannaea arcus*** (Ehrenerg) Patrick [*Fragilaria arcus* (Ehrenberg) Cleve]
30	30	***Meridion circulare*** (Greville) Agardh
NA	31	***Navicula cryptocephala*** Kützing
32	32	***Navicula cryptotenella*** Lange-Bertalot
33	NA	***Navicula notha*** Wallace
34	34	***Nitzschia capitellata*** Hustedt
35	35	***Nitzschia palea*** (Kützing) W. Smith
36	NA	***Planothidium lanceolatum*** (Brébisson ex Kützing) Lange-Bertalot [*Achnanthes lanceolata* (Brébisson) Grunow]
37	37	***Reimeria sinuata*** (Gregory) Kociolek and Stoermer (*Cymbella sinuata* Gregory)
38	38	***Sellaphora stroemii*** (Hustedt) H. Kobayasi (*Navicula stroemii* Hustedt)
39	39	***Staurosira venter*** (Ehrenberg) Kobayasi [*Fragilaria construens* var. *venter*

Taxon Number		Taxon Name
		(Ehrenberg) Grunow et van Heurck]
40	40	***Staurosirella leptostauron var. dubia*** (Grunow) [*Fragilaria leptostauron var. dubia* (Grunow) Hustedt]
41	41	***Staurosirella pinnata*** (Ehrenberg) Williams and Round (*Fragilaria pinnata* Ehrenberg)
42	42	***Stephanodiscus parvus*** Stoermer and Håkansson
43	43	***Synedra ulna*** Ehrenberg
44	NA	***Unknown Achnanthes in girdleband view***
NA	45	***Unknown Cymbella***
46	46	***Unknown Gomphonema***
47	NA	***Unknown Navicula***

Appendix B, Table 3.3 Water chemistry data for the South Nahanni River water sites sampled in 2008

Site	NO3NO2	NH3	DOC	DIC	Ag	Al	As	B	Ba	Be	Bi	Cd
	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
NNP-1-08	0.042	0.013	1.3	13.2	0.001	22.3	0.53	1.3	29.7	0.003	0.008	0.014
NNP-2-08	0.039	0.013	1.4	12.8	< 0.001	66.3	1.22	6.4	29.8	0.007	0.037	0.016
NNP-3-08	0.012	0.008	2.1	16.1	0.002	95.8	0.18	0.9	106	0.012	< 0.001	0.327
NNP-4-08	0.022	< 0.005	2.1	4.9	0.007	1370	0.39	6.9	36.4	0.15	0.001	1.71
NNP-5-08	0.037	0.005	1.5	1.1	0.003	4660	0.09	1.8	26.6	0.711	< 0.001	1.2
NNP-6-08	0.037	< 0.005	0.9	1.7	0.002	1290	3.07	29.9	10.8	0.251	< 0.001	0.276
NNP-7-08	0.056	< 0.005	1.2	5.1	0.001	194	1.83	19.4	8.42	0.04	0.004	0.176
NNP-8-08	0.102	< 0.005	1.2	34	0.001	22.3	0.07	2.8	37.7	0.003	< 0.001	0.009
NNP-9-08	0.096	< 0.005	1.2	25.7	< 0.001	37.6	0.09	2.9	28.8	0.003	< 0.001	0.172
NNP-10-08	0.048	0.005	1.3	17.6	0.001	670	0.51	2.2	46.6	0.043	0.008	0.273
NNP-11-08	0.018	0.009	1.1	8.9	< 0.001	26.7	0.54	1.1	23.4	0.004	0.004	0.012
NNP-12-08	0.031	0.007	1.1	12	< 0.001	44.4	0.61	1.2	28.2	0.004	0.004	0.015
NNP-13-08	0.027	< 0.005	1.8	22.7	0.002	244	0.19	2.6	83.2	0.037	0.003	0.655
NNP-14-08	0.076	< 0.005	1.1	16.4	< 0.001	533	0.46	7.4	30.5	0.107	0.001	0.108
NNP-15-08	0.02	0.006	1.3	16.4	< 0.001	19.9	0.57	1.8	17	0.003	0.001	0.095
NNP-16-08	0.015	0.005	1.2	13.6	< 0.001	37.3	0.72	1.7	20.2	0.003	0.007	0.161
NNP-17-08	0.017	0.006	1.1	11.7	< 0.001	15	0.82	1.7	21.5	0.002	0.003	0.151
NNP-18-08	0.017	0.009	0.9	10.3	< 0.001	30.5	0.55	1.2	23.9	0.004	0.002	0.012
NNP-19-08	0.03	0.007	1	11.9	< 0.001	54.8	1.95	7	23	0.006	0.042	0.011
NNP-20-08	0.157	< 0.005	1.5	36.4	< 0.001	4.1	0.1	1.9	42.5	0.001	0.004	0.04
NNP-21-08	0.113	< 0.005	1.9	34.1	< 0.001	9.1	0.37	4.2	55.3	0.003	0.014	0.088
NNP-22-08	0.115	< 0.005	1.3	30.8	< 0.001	10	0.28	2.6	78.7	0.001	0.002	0.085
NNP-23-08	0.108	< 0.005	1.1	29.9	< 0.001	13.3	0.32	1.7	73.7	0.005	0.002	0.046
NNP-24-08	0.094	< 0.005	0.8	14.7	0.005	969	2.61	3.5	48.3	0.069	0.122	0.159
NNP-25-08	0.038	0.007	1.8	4.9	0.001	53.8	0.8	4.6	9.87	0.03	0.004	0.042
NNP-26-08	0.055	< 0.005	0.8	12.6	0.002	197	1.47	7.8	29.8	0.028	0.06	0.033

Site	NO3NO2	NH3	DOC	DIC	Ag	Al	As	B	Ba	Be	Bi	Cd
	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
NNP-27-08	0.052	< 0.005	0.9	12.3	0.001	149	1.36	6.7	27.6	0.028	0.029	0.028
NNP-28-08	0.048	< 0.005	0.8	11.5	0.001	154	1.42	6.1	26.6	0.03	0.033	0.03
NNP-29-08	0.04	< 0.005	1	10.4	0.001	126	1.46	6.3	25.1	0.031	0.02	0.027
NNP-30-08	0.029	0.007	0.8	12.1	< 0.001	61.7	1.75	7.7	26.8	0.006	0.031	0.014
NNP-31-08	0.028	0.008	0.9	11.6	< 0.001	74.3	1.41	9.6	30.6	0.007	0.024	0.017
NNP-32-08	0.025	0.009	2.1	11.5	0.001	88.3	1.57	9.1	29.1	0.009	0.034	0.017
NNP-33-08	0.046	0.005	1.4	20.2	0.001	118	0.76	5.3	42.2	0.018	0.016	0.062
NNP-34-08	0.043	0.009	3.1	7.4	0.001	76	1.13	4.4	7.74	0.012	0.003	0.011
NNP-35-08	0.166	0.005	1.1	35.1	< 0.001	9.1	0.28	1.6	70	0.004	< 0.001	0.018
NNP-36-08	0.198	0.008	0.8	31.3	< 0.001	64.8	0.09	2.5	17	0.016	< 0.001	0.023
NNP-37-08	0.142	0.005	0.8	29.8	< 0.001	26	0.15	1.3	26.3	0.004	0.007	0.047
NNP-38-08	0.04	0.009	0.9	12.4	0.002	42.7	0.63	1.7	29.8	0.005	0.033	0.016
NNP-39-08	0.037	0.009	1	12	0.002	111	1.22	3.3	28.8	0.01	0.101	0.022
NNP-40-08	0.026	0.01	1.1	11.2	0.001	58.8	0.66	1.3	28.6	0.005	0.009	0.021
NNP-41-08	0.01	0.012	0.8	4.8	0.001	68.1	0.73	0.7	8.69	0.008	0.005	0.007
NNP-42-08	0.015	0.013	1.7	4.9	< 0.001	67.9	0.7	0.7	8.82	0.007	0.008	0.007
NNP-43-08	0.171	0.005	1.3	42.2	0.002	5.7	0.28	9.4	77.8	0.001	< 0.001	0.072
NNP-44-08	0.161	0.005	1.3	41.5	0.001	7.2	0.24	9.4	79.5	0.002	< 0.001	0.029
NNP-45-08	0.161	0.005	2.7	41.3	0.001	15.6	0.27	9.4	80.2	0.003	< 0.001	0.031
NNP-46-08	0.172	0.005	2.9	42.9	< 0.001	6.5	0.28	9.3	77.5	0.002	0.002	0.084
NNP-47-08	0.158	0.005	1.8	41	< 0.001	6.1	0.24	8.9	80.2	0.002	< 0.001	0.023
NNP-48-08	0.041	0.01	6.3	27.6	0.001	19.3	0.25	5.2	174	0.004	0.002	0.057
NNP-49-08	0.054	0.007	2.1	35.4	0.007	184	0.61	4.7	82	0.021	0.001	0.898
NNP-50-08	0.063	0.007	0.5	7.4	0.003	137	0.48	5.3	83.4	0.016	0.001	0.607
NNP-51-08	0.077	0.007	4.3	39.3	0.002	135	0.25	11.5	103	0.016	0.002	0.169
NNP-52-08	0.217	< 0.005	4	39.2	< 0.001	16.9	0.68	1	28.7	0.002	< 0.001	0.037
NNP-53-08	0.114	0.005	3.6	55.5	0.001	5.5	0.25	6.2	93.8	0.002	< 0.001	1.25
NNP-54-08	0.117	0.006	4.6	55	0.006	326	0.6	13.5	99.3	0.021	0.006	0.772

Site	NO3NO2 mg/L	NH3 mg/L	DOC mg/L	DIC mg/L	Ag µg/L	Al µg/L	As µg/L	B µg/L	Ba µg/L	Be µg/L	Bi µg/L	Cd µg/L
NNP-55-08	0.191	0.006	2.9	36	0.001	61.1	0.15	4.3	42.1	0.005	0.007	0.04
NNP-56-08	0.21	0.005	3	34.7	0.002	30	0.19	8	78.4	0.004	0.001	0.017
NNP-57-08	0.194	0.005	0.5	30.9	< 0.001	38.8	0.07	2.3	17.4	0.007	0.001	0.014
NNP-58-08	0.192	0.005	2.3	28.4	< 0.001	78.8	0.07	1.9	12.2	0.024	< 0.001	0.025
NNP-59-08	0.217	0.005	1.4	35.1	0.002	13.9	0.16	7.3	77.8	0.002	< 0.001	0.012
NNP-60-08	0.182	0.007	3.8	33.5	0.001	34.1	0.16	5.7	68.1	0.004	< 0.001	0.012
NNP-61-08	0.11	0.006	2.7	38.4	< 0.001	6.5	0.1	7.2	73.7	0.001	< 0.001	0.012
NNP-62-08	0.133	0.005	0.9	42.4	< 0.001	4	0.11	7.5	76.9	0.001	< 0.001	0.023
NNP-63-08	0.141	< 0.005	1.6	43.5	< 0.001	4.8	0.1	7.6	79.2	0.001	< 0.001	0.034
NNP-64-08	0.144	0.005	2.7	43.7	< 0.001	4.3	0.12	7.4	78.9	0.001	< 0.001	0.033
NNP-65-08	0.088	0.006	3.9	45.6	0.009	121	0.73	16.6	140	0.016	0.002	0.473
NNP-66-08	0.195	< 0.005	2.6	38.1	< 0.001	17.2	0.23	6.1	77.3	0.002	< 0.001	0.021
NNP-67-08	0.184	< 0.005	1.1	39.9	< 0.001	4	0.23	7.3	77.1	0.001	< 0.001	0.024
NNP-68-08	0.183	< 0.005	1.1	43.4	0.001	3.8	0.25	8.4	79.2	0.001	< 0.001	0.026
NNP-69-08	0.171	0.005	1.7	42.6	< 0.001	4.9	0.24	9	80.1	0.002	< 0.001	0.033
NNP-70-08	0.175	< 0.005	1.4	43.8	< 0.001	5.4	0.24	9.1	78.6	0.001	0.001	0.031
NNP-71-08	0.162	< 0.005	1.1	43.1	< 0.001	4.7	0.14	8.9	81.2	0.002	< 0.001	0.03
NNP-72-08	0.164	< 0.005	2.9	43.2	< 0.001	3.7	0.14	8.8	80.9	0.002	< 0.001	0.026
NNP-73-08	0.166	0.005	1.7	42.6	< 0.001	3	0.14	8.7	79.6	0.001	< 0.001	0.023

Appendix B, Table 3.3, continued

Site	Ce µg/L	Co µg/L	Cr µg/L	Cs µg/L	Cu µg/L	Fe µg/L	Ga µg/L	La µg/L	Li µg/L	Mn µg/L	Mo µg/L	Nb µg/L
NNP-1-08	0.072	0.126	0.054	0.049	0.26	57.1	0.007	0.064	2.3	5.34	0.556	0.002
NNP-2-08	0.089	0.137	0.071	0.116	0.37	174	0.023	0.062	5.5	16	0.63	0.015
NNP-3-08	0.044	0.376	0.022	0.012	1.55	82	0.002	0.033	1.3	18.4	0.691	< 0.001

Site	Ce µg/L	Co µg/L	Cr µg/L	Cs µg/L	Cu µg/L	Fe µg/L	Ga µg/L	La µg/L	Li µg/L	Mn µg/L	Mo µg/L	Nb µg/L
NNP-4-08	2.11	12.1	0.177	0.126	10.3	479	0.037	1.21	10	280	0.39	0.001
NNP-5-08	5.17	59	0.144	0.224	9.78	324	0.058	2.74	36.2	381	0.029	< 0.001
NNP-6-08	3.14	7.07	0.148	2.68	4.96	111	0.057	2.12	12.3	71.9	1	0.002
NNP-7-08	0.304	0.977	0.06	0.514	0.78	41.3	0.013	0.214	2.7	15.8	1.23	0.002
NNP-8-08	0.014	0.023	0.076	0.024	0.25	3.7	0.002	0.007	1.7	0.15	0.991	< 0.001
NNP-9-08	0.016	0.653	0.085	0.009	0.27	49	0.007	0.008	2.8	13.2	0.628	< 0.001
NNP-10-08	0.496	6.22	0.185	0.043	1.6	435	0.027	0.249	6.2	25.6	1.02	0.004
NNP-11-08	0.035	0.112	0.055	0.078	0.26	53.7	0.008	0.02	2	3.76	0.437	0.002
NNP-12-08	0.113	0.159	0.081	0.06	0.29	89.4	0.012	0.093	2.4	5.67	0.552	0.002
NNP-13-08	0.159	2.01	0.059	0.017	1.45	124	0.007	0.067	6	47.9	0.994	0.001
NNP-14-08	13.5	12.4	0.075	0.117	2.87	26.2	0.16	9.35	15	178	1.14	< 0.001
NNP-15-08	0.054	0.07	0.032	0.047	0.41	48.3	0.005	0.046	3.3	2.9	0.854	< 0.001
NNP-16-08	0.056	0.09	0.04	0.042	0.37	72.1	0.006	0.031	2.4	5.79	1.2	0.001
NNP-17-08	0.078	0.082	0.047	0.064	0.35	89.8	0.005	0.043	2.3	4.85	0.934	0.001
NNP-18-08	0.095	0.158	0.052	0.062	0.26	53.7	0.007	0.09	2.3	5.33	0.488	0.002
NNP-19-08	0.071	0.089	0.066	0.181	0.29	122	0.023	0.049	5.9	9.8	0.555	0.014
NNP-20-08	0.007	0.012	0.049	0.007	0.13	7.5	0.004	0.006	1.2	0.26	2.22	< 0.001
NNP-21-08	0.082	0.027	0.089	0.016	0.3	12.2	0.007	0.095	3	0.59	3.78	0.002
NNP-22-08	0.01	0.025	0.041	0.009	0.25	7.3	0.004	0.009	1.9	0.37	2.05	< 0.001
NNP-23-08	0.135	0.023	0.033	0.009	0.25	8.3	0.005	0.169	1.8	0.39	1.84	< 0.001
NNP-24-08	1.04	0.674	0.706	0.425	0.96	855	0.326	0.553	6.4	20.5	2.79	0.341
NNP-25-08	0.317	0.752	0.041	0.068	0.91	28.7	0.032	0.427	8.6	5.42	0.825	0.003
NNP-26-08	0.962	0.735	0.218	0.156	0.62	274	0.059	0.848	6.5	13.3	0.813	0.026
NNP-27-08	0.998	0.78	0.163	0.142	0.53	198	0.05	0.954	6.1	13.4	0.755	0.021
NNP-28-08	1.13	0.886	0.153	0.15	0.58	205	0.05	1.09	6.3	15.3	0.497	0.02
NNP-29-08	1.14	0.924	0.112	0.171	0.47	156	0.04	1.15	6.6	14.9	0.491	0.018
NNP-30-08	0.072	0.105	0.072	0.203	0.3	124	0.025	0.051	6.5	9.52	0.547	0.019
NNP-31-08	0.101	0.136	0.06	0.275	0.36	166	0.03	0.078	8.2	15.9	0.639	0.023

Site	Ce µg/L	Co µg/L	Cr µg/L	Cs µg/L	Cu µg/L	Fe µg/L	Ga µg/L	La µg/L	Li µg/L	Mn µg/L	Mo µg/L	Nb µg/L
NNP-32-08	0.105	0.131	0.077	0.248	0.38	192	0.035	0.072	7.7	16.4	0.615	0.025
NNP-33-08	0.312	0.23	0.079	0.051	0.62	117	0.017	0.258	7.8	6.58	1.43	0.006
NNP-34-08	0.632	0.092	0.076	0.028	0.55	170	0.026	0.623	3.5	6.43	2.08	0.02
NNP-35-08	0.144	0.035	0.051	0.008	0.16	12.5	0.005	0.164	1.2	0.26	1.98	< 0.001
NNP-36-08	0.036	0.706	0.038	0.006	0.21	103	0.004	0.017	3.1	11.4	3.37	< 0.001
NNP-37-08	0.131	0.034	0.078	0.014	0.14	36.4	0.012	0.126	1	0.51	1.56	0.003
NNP-38-08	0.077	0.154	0.079	0.062	0.39	175	0.013	0.061	2.6	16.8	0.503	0.003
NNP-39-08	0.135	0.159	0.103	0.112	0.44	229	0.038	0.083	3.5	17	0.519	0.031
NNP-40-08	0.108	0.16	0.095	0.069	0.31	123	0.016	0.078	2.3	6.56	0.489	0.004
NNP-41-08	0.137	0.202	0.103	0.11	0.36	113	0.017	0.1	1.8	5.62	0.071	0.006
NNP-42-08	0.103	0.196	0.102	0.115	0.34	114	0.017	0.053	1.8	5.55	0.076	0.006
NNP-43-08	0.009	0.021	0.092	< 0.005	0.39	9.9	0.004	0.006	2.9	0.46	3.31	< 0.001
NNP-44-08	0.115	0.015	0.088	0.005	0.33	9.2	0.005	0.081	2.9	0.22	3.37	< 0.001
NNP-45-08	0.065	0.027	0.099	0.008	0.31	29	0.007	0.04	3	0.67	3.38	< 0.001
NNP-46-08	0.01	0.018	0.083	0.006	0.35	11.2	0.003	0.006	3	0.31	3.29	< 0.001
NNP-47-08	0.018	0.015	0.106	< 0.005	0.28	8.6	0.004	0.016	2.8	0.17	3.29	< 0.001
NNP-48-08	0.078	0.043	0.07	0.005	0.53	104	0.004	0.055	1.5	3.15	2.01	0.001
NNP-49-08	0.144	1.54	0.21	0.046	1.17	488	0.025	0.066	4.9	23.6	3.65	0.002
NNP-50-08	0.108	0.929	0.156	0.03	0.93	334	0.017	0.05	4.6	13.6	3.29	0.002
NNP-51-08	0.167	1.28	0.099	0.018	0.82	261	0.016	0.109	3.8	33.6	1.93	0.004
NNP-52-08	0.037	0.034	0.081	0.006	0.24	24.5	0.006	0.019	1.6	0.65	0.364	0.002
NNP-53-08	0.017	0.054	0.054	< 0.005	0.7	9.3	0.002	0.014	2.7	1.13	13.7	< 0.001
NNP-54-08	0.51	0.595	0.502	0.111	1.41	756	0.093	0.253	9.4	15.2	13	0.052
NNP-55-08	0.092	0.109	0.134	0.017	0.32	96.9	0.02	0.057	2.4	2.1	2.27	0.002
NNP-56-08	0.043	0.037	0.134	0.014	0.54	50.4	0.017	0.037	3.4	0.82	4.62	< 0.001
NNP-57-08	0.05	0.216	0.057	0.005	0.15	44	0.006	0.029	2.1	3.59	1.53	< 0.001
NNP-58-08	0.092	1.37	0.057	0.006	0.29	204	0.007	0.044	3.4	23.6	2.08	< 0.001
NNP-59-08	0.006	0.022	0.098	0.008	0.41	19.6	0.014	0.005	2.8	0.35	4.62	< 0.001

Site	Ce µg/L	Co µg/L	Cr µg/L	Cs µg/L	Cu µg/L	Fe µg/L	Ga µg/L	La µg/L	Li µg/L	Mn µg/L	Mo µg/L	Nb µg/L
NNP-60-08	0.03	0.049	0.148	0.014	0.5	55.2	0.019	0.024	3.4	1.07	3.5	0.001
NNP-61-08	0.079	0.013	0.093	< 0.005	0.25	6.8	0.005	0.052	1.7	0.3	3.1	< 0.001
NNP-62-08	0.009	0.018	0.089	< 0.005	0.25	4.8	0.003	0.008	2	0.19	3.93	< 0.001
NNP-63-08	0.007	0.014	0.094	< 0.005	0.23	8.1	0.004	0.005	2.1	0.22	3.9	< 0.001
NNP-64-08	0.006	0.013	0.09	< 0.005	0.23	6.7	0.003	0.005	2	0.18	3.94	< 0.001
NNP-65-08	0.289	0.673	0.214	0.046	1.15	355	0.029	0.171	6.5	18.6	12	0.005
NNP-66-08	0.029	0.031	0.1	0.008	0.29	34	0.008	0.015	2.1	0.62	2.83	< 0.001
NNP-67-08	0.007	0.014	0.085	< 0.005	0.27	6.1	0.004	0.005	2.4	0.14	3.11	< 0.001
NNP-68-08	0.006	0.014	0.083	< 0.005	0.29	5.1	0.004	0.004	2.7	0.15	3.46	< 0.001
NNP-69-08	0.006	0.017	0.087	< 0.005	0.29	5.8	0.003	0.005	2.9	0.17	3.69	< 0.001
NNP-70-08	0.007	0.014	0.087	< 0.005	0.28	7.6	0.004	0.006	2.9	0.18	3.44	< 0.001
NNP-71-08	0.009	0.013	0.092	< 0.005	0.53	3.8	0.003	0.009	2.5	0.11	3.52	< 0.001
NNP-72-08	0.031	0.013	0.098	< 0.005	0.26	3.9	0.004	0.032	2.6	0.11	3.46	< 0.001
NNP-73-08	0.004	0.011	0.086	< 0.005	0.24	3.6	0.003	0.003	2.6	0.09	3.4	< 0.001

Appendix B, Table 3.3, continued

Site	Ni µg/L	Pb µg/L	Pt µg/L	Rb µg/L	Sb µg/L	Se µg/L	Sn µg/L	Sr µg/L	Tl µg/L	U µg/L	V µg/L	W µg/L
NNP-1-08	2.25	0.11	< 0.001	0.57	0.05	0.16	< 0.005	69.3	0.001	0.668	0.078	0.641
NNP-2-08	1.87	0.058	< 0.001	1.01	0.048	0.09	0.005	64.9	0.002	0.701	0.13	1.06
NNP-3-08	12.7	0.01	< 0.001	0.29	0.154	0.87	< 0.005	111	0.003	0.569	0.047	0.035
NNP-4-08	67.7	0.069	< 0.001	0.65	0.07	0.63	< 0.005	87.4	0.007	0.587	0.152	0.284
NNP-5-08	153	0.188	< 0.001	0.61	0.029	0.39	< 0.005	78.5	0.004	0.329	0.043	0.013
NNP-6-08	35	0.057	< 0.001	1.39	0.106	0.24	< 0.005	35.7	0.008	4.18	0.102	3.03
NNP-7-08	6.38	0.053	< 0.001	0.78	0.153	0.39	< 0.005	58.7	0.005	2.11	0.167	0.173
NNP-8-08	0.19	< 0.005	< 0.001	0.8	0.035	0.11	< 0.005	202	< 0.001	0.745	0.08	0.025
NNP-9-08	7.04	0.014	< 0.001	0.18	0.055	0.38	< 0.005	190	0.002	1.13	0.087	0.632

Site	Ni µg/L	Pb µg/L	Pt µg/L	Rb µg/L	Sb µg/L	Se µg/L	Sn µg/L	Sr µg/L	Tl µg/L	U µg/L	V µg/L	W µg/L
NNP-10-08	25	0.106	< 0.001	0.79	0.099	0.67	< 0.005	127	0.006	1.31	0.211	0.066
NNP-11-08	2.2	0.041	0.001	0.42	0.052	0.18	< 0.005	61.7	0.001	0.44	0.087	0.03
NNP-12-08	2.25	0.059	< 0.001	0.51	0.051	0.18	< 0.005	72.2	0.001	0.684	0.121	0.242
NNP-13-08	20.9	0.028	< 0.001	0.48	0.163	1.53	< 0.005	132	0.006	2.16	0.126	0.63
NNP-14-08	50.6	0.029	< 0.001	1.04	0.037	0.49	< 0.005	101	0.002	1.77	0.267	0.135
NNP-15-08	2.06	0.02	< 0.001	0.66	0.125	0.28	< 0.005	101	0.003	0.782	0.214	0.053
NNP-16-08	3.14	0.029	< 0.001	0.39	0.174	0.33	< 0.005	71.9	0.004	0.91	0.29	0.377
NNP-17-08	2.54	0.025	< 0.001	0.42	0.159	0.31	< 0.005	68.4	0.004	0.689	0.214	0.174
NNP-18-08	2.47	0.036	< 0.001	0.44	0.049	0.18	< 0.005	72	0.001	0.617	0.083	0.113
NNP-19-08	1.14	0.051	< 0.001	0.94	0.05	0.11	< 0.005	57.4	0.003	0.706	0.133	0.661
NNP-20-08	1.11	< 0.005	0.001	0.9	0.186	0.93	< 0.005	137	0.002	5.37	0.116	0.093
NNP-21-08	0.68	0.013	< 0.001	1.12	0.229	0.77	< 0.005	104	0.003	3.09	1.5	0.058
NNP-22-08	1.79	0.005	< 0.001	0.9	0.24	0.85	< 0.005	156	0.004	3.53	0.119	0.085
NNP-23-08	1.24	< 0.005	< 0.001	1.1	0.128	0.54	< 0.005	155	0.004	3.34	0.12	0.031
NNP-24-08	4.44	0.485	< 0.001	4.98	0.265	0.99	0.072	96.3	0.03	2.31	2.69	0.323
NNP-25-08	8.79	0.014	< 0.001	1.2	0.07	< 0.05	< 0.005	40.9	0.002	0.731	0.184	0.292
NNP-26-08	5.38	0.185	< 0.001	1.36	0.076	0.25	0.006	73.8	0.004	1.02	0.515	0.58
NNP-27-08	5.73	0.131	< 0.001	1.23	0.065	0.24	0.007	72.9	0.003	0.775	0.362	0.456
NNP-28-08	6.19	0.128	< 0.001	1.11	0.057	0.16	< 0.005	70.4	0.003	0.669	0.332	0.458
NNP-29-08	6.49	0.096	< 0.001	1.07	0.052	0.14	< 0.005	71.3	0.003	0.678	0.242	0.473
NNP-30-08	1.3	0.061	< 0.001	1.03	0.052	0.13	0.005	60.2	0.003	0.745	0.14	0.609
NNP-31-08	1.64	0.066	< 0.001	1.15	0.048	0.15	0.006	62.1	0.004	0.742	0.186	0.768
NNP-32-08	1.61	0.079	< 0.001	1.17	0.052	0.14	0.008	60.9	0.004	0.696	0.183	0.917
NNP-33-08	4.15	0.07	< 0.001	1.49	0.136	0.37	< 0.005	130	0.006	1.93	0.298	0.425
NNP-34-08	1.25	0.049	< 0.001	0.97	0.066	< 0.05	< 0.005	33.8	0.003	1.12	0.273	0.046
NNP-35-08	0.95	< 0.005	< 0.001	0.52	0.216	0.62	< 0.005	112	0.002	3.78	0.106	0.018
NNP-36-08	1.92	0.008	< 0.001	0.68	0.18	1.01	< 0.005	138	0.003	4.82	0.107	0.01
NNP-37-08	1.27	0.023	< 0.001	1.05	0.209	0.67	< 0.005	99.6	0.003	4.68	0.13	0.05

Site	Ni µg/L	Pb µg/L	Pt µg/L	Rb µg/L	Sb µg/L	Se µg/L	Sn µg/L	Sr µg/L	Tl µg/L	U µg/L	V µg/L	W µg/L
NNP-38-08	2.16	0.067	< 0.001	0.63	0.048	0.15	< 0.005	67.2	0.002	0.589	0.118	0.476
NNP-39-08	1.84	0.116	< 0.001	1.07	0.048	0.15	0.007	62.1	0.005	0.635	0.207	0.924
NNP-40-08	2.23	0.092	< 0.001	0.57	0.049	0.16	< 0.005	67.9	0.002	0.591	0.132	0.278
NNP-41-08	2.56	0.1	< 0.001	0.53	0.025	< 0.05	< 0.005	48.5	0.002	0.102	0.079	0.06
NNP-42-08	2.48	0.104	< 0.001	0.52	0.026	0.05	< 0.005	48.9	0.001	0.105	0.074	0.025
NNP-43-08	1.42	0.535	< 0.001	0.31	1.02	1.3	< 0.005	314	0.011	5.3	0.444	0.012
NNP-44-08	1.32	0.12	< 0.001	0.3	0.281	1.2	< 0.005	314	0.011	4.79	0.471	0.01
NNP-45-08	1.27	0.099	< 0.001	0.31	0.221	1.2	< 0.005	319	0.01	4.67	0.49	0.008
NNP-46-08	1.45	0.331	< 0.001	0.34	0.543	1.32	< 0.005	315	0.012	5.87	0.428	0.064
NNP-47-08	1.2	0.024	< 0.001	0.28	0.152	1.19	< 0.005	311	0.008	4.42	0.487	0.008
NNP-48-08	1.63	0.133	< 0.001	0.35	0.167	0.81	< 0.005	80.1	0.005	0.901	0.212	0.018
NNP-49-08	15.3	0.146	< 0.001	0.91	0.355	0.93	< 0.005	183	0.032	2.2	1.85	0.015
NNP-50-08	11.1	0.123	< 0.001	0.78	0.293	0.78	0.007	191	0.018	2.09	0.949	0.018
NNP-51-08	7.85	0.067	< 0.001	0.56	0.193	0.43	< 0.005	205	0.014	1.76	0.299	0.008
NNP-52-08	0.28	0.017	< 0.001	0.34	0.052	0.13	< 0.005	134	< 0.001	0.831	0.174	0.005
NNP-53-08	40.5	0.005	< 0.001	0.24	0.64	2.72	< 0.005	345	0.038	13.5	1.2	0.012
NNP-54-08	31.1	0.319	< 0.001	1.05	0.713	2.66	0.005	361	0.052	14.1	2.12	0.026
NNP-55-08	0.97	0.067	< 0.001	0.37	0.149	0.74	< 0.005	417	0.037	2.78	0.365	0.004
NNP-56-08	0.65	0.041	< 0.001	0.27	0.131	1.25	< 0.005	1610	0.003	3.44	0.696	0.014
NNP-57-08	0.98	0.031	< 0.001	0.39	0.11	0.48	< 0.005	110	0.002	2.25	0.102	0.022
NNP-58-08	2.59	0.102	< 0.001	0.52	0.143	0.6	< 0.005	105	0.003	3.17	0.139	0.006
NNP-59-08	0.55	0.015	< 0.001	0.22	0.126	1.17	< 0.005	1620	0.002	3.46	0.671	0.002
NNP-60-08	0.67	0.036	< 0.001	0.24	0.112	1.06	< 0.005	974	0.002	2.7	0.641	0.007
NNP-61-08	0.54	0.007	< 0.001	0.2	0.072	0.79	< 0.005	223	0.005	2.71	0.546	0.009
NNP-62-08	1.31	0.006	< 0.001	0.22	0.116	1.1	< 0.005	265	0.007	4.66	0.505	0.039
NNP-63-08	1.44	0.01	< 0.001	0.24	0.126	1.21	< 0.005	259	0.007	5.12	0.479	0.007
NNP-64-08	1.36	0.011	< 0.001	0.25	0.131	1.22	< 0.005	254	0.007	5.1	0.464	0.002
NNP-65-08	18.5	0.25	< 0.001	0.66	0.486	2.23	< 0.005	213	0.05	6.68	1.62	0.01

Site	Ni µg/L	Pb µg/L	Pt µg/L	Rb µg/L	Sb µg/L	Se µg/L	Sn µg/L	Sr µg/L	Tl µg/L	U µg/L	V µg/L	W µg/L
NNP-66-08	0.92	0.045	< 0.001	0.28	0.166	0.92	< 0.005	268	0.01	4.25	0.39	0.005
NNP-67-08	0.97	0.055	< 0.001	0.25	0.196	1.05	< 0.005	261	0.008	4.71	0.36	0.004
NNP-68-08	1.12	0.052	< 0.001	0.26	0.209	1.18	< 0.005	296	0.009	5.03	0.397	0.019
NNP-69-08	1.25	0.03	< 0.001	0.28	0.219	1.25	< 0.005	319	0.009	5.24	0.424	0.004
NNP-70-08	1.19	0.045	< 0.001	0.28	0.221	1.25	< 0.005	317	0.009	4.9	0.442	0.006
NNP-71-08	1.22	0.02	< 0.001	0.26	0.124	1.23	< 0.005	308	0.009	4.53	0.499	0.004
NNP-72-08	1.19	0.009	< 0.001	0.26	0.128	1.23	< 0.005	313	0.008	4.52	0.507	0.002
NNP-73-08	1.12	0.014	< 0.001	0.26	0.125	1.18	< 0.005	306	0.008	4.47	0.46	0.002

Appendix B, Table 3.3, continued

Site	Y µg/L	Zn µg/L	TN mg/L	TP mg/L	Temp (°C)	pH	Specific Conductance µS/cm	DO mg/L	Turbidity NTU
NNP-1-08	0.05	2.2	0.084	0.0031	7.32	7.60	118.00	11.70	2.96
NNP-2-08	0.048	1.69	0.068	0.0033	7.75	8.00	109.00	11.42	0.14
NNP-3-08	0.185	25.6	0.064	0.0028	8.34	8.04	135.00	12.20	0.79
NNP-4-08	3.3	191	0.089	0.0028	10.31	7.61	141.00	11.30	3.91
NNP-5-08	13.1	552	0.064	0.002	10.86	4.87	196.00	10.64	0.99
NNP-6-08	2.13	65.7	0.064	0.0034	5.11	6.31	45.00	11.40	1.10
NNP-7-08	0.308	18.5	0.089	0.0028	6.51	7.35	45.00	11.90	1.80
NNP-8-08	0.033	0.97	0.121	0.0018	6.97	8.55	200.00	12.20	1.86
NNP-9-08	0.16	26	0.113	0.0015	11.12	8.36	163.00	11.07	1.17
NNP-10-08	1.98	72.9	0.082	0.0051	8.63	8.26	165.00	12.80	2.48
NNP-11-08	0.022	2.38	0.064	0.0031	10.20	8.17	107.00	10.90	1.62
NNP-12-08	0.073	2.09	0.062	0.0036	10.21	8.11	137.00	11.10	1.12
NNP-13-08	0.621	85.2	0.083	0.0042	5.15	8.39	217.00	13.20	1.70
NNP-14-08	5.94	42.1	0.116	0.0031	4.93	8.12	199.00	13.00	1.67

Site	Y µg/L	Zn µg/L	TN mg/L	TP mg/L	Temp (°C)	pH	Specific Conductance µS/cm	DO mg/L	Turbidity NTU
NNP-15-08	0.055	12.1	0.066	0.0023	10.10	8.20	147.00	12.10	0.59
NNP-16-08	0.043	18.4	0.065	0.0028	9.73	8.10	101.00	11.60	0.77
NNP-17-08	0.049	13.9	0.043	0.0029	11.55	8.26	190.00	10.90	0.68
NNP-18-08	0.065	2.25	0.049	0.0024	10.10	8.05	133.00	11.50	5.22
NNP-19-08	0.041	1.11	0.077	0.0027	8.40	7.95	114.00	11.80	3.71
NNP-20-08	0.018	1.82	0.191	0.0008	8.90	8.29	155.00	12.30	0.89
NNP-21-08	0.116	2.61	0.349	0.0015	8.90	8.17	169.00	12.10	0.73
NNP-22-08	0.023	5.08	0.144	0.0011	9.40	8.35	229.00	12.20	0.64
NNP-23-08	0.142	2.33	0.146	0.0019	9.80	8.46	212.00	12.30	0.88
NNP-24-08	0.383	13.6	0.117	0.0149	8.18	8.10	114.00	13.50	46.00
NNP-25-08	0.211	15.7	0.093	0.0032	8.40	8.20	119.00	13.20	2.30
NNP-26-08	0.46	4.04	0.102	0.006	6.91	7.86	125.00	12.80	148.00
NNP-27-08	0.52	4.26	0.071	0.0056	7.56	7.84	114.00	12.90	8.77
NNP-28-08	0.564	4.79	0.066	0.0049	8.05	7.98	124.00	12.70	13.10
NNP-29-08	0.542	5.64	0.089	0.0044	9.10	7.97	117.00	12.30	6.40
NNP-30-08	0.04	1.38	0.061	0.0032	9.51	8.22	121.00	12.40	2.55
NNP-31-08	0.052	1.71	0.062	0.0035	10.50	7.94	122.00	11.90	2.60
NNP-32-08	0.053	1.7	0.064	0.0039	11.70	7.65	132.00	11.70	2.83
NNP-33-08	0.234	5.93	0.098	0.0032	11.70	8.65	181.00	12.10	4.59
NNP-34-08	0.425	1.59	0.139	0.0038	13.20	7.71	56.00	11.60	2.19
NNP-35-08	0.142	1.58	0.198	0.001	11.40	8.70	285.00	12.30	0.73
NNP-36-08	0.138	2.59	0.222	0.0016	12.20	8.42	192.00	11.50	0.82
NNP-37-08	0.108	4.36	0.255	0.0017	11.00	8.45	251.00	11.90	0.09
NNP-38-08	0.047	2.19	0.158	0.0033	8.24	8.23	126.00	11.90	5.60
NNP-39-08	0.056	2.46	0.072	0.0039	8.70	8.10	124.00	12.80	5.72
NNP-40-08	0.066	3.56	0.065	0.004	9.81	8.03	123.00	11.86	4.61
NNP-41-08	0.057	2.09	0.047	0.004	10.70	7.91	74.00	12.50	7.34

Site	Y µg/L	Zn µg/L	TN mg/L	TP mg/L	Temp (°C)	pH	Specific Conductance µS/cm	DO mg/L	Turbidity NTU
NNP-42-08	0.038	2.1	0.074	0.0037	10.80	7.93	74.00	11.80	5.06
NNP-43-08	0.022	39.8	0.211	0.0016	10.60	8.48	137.00	12.30	2.40
NNP-44-08	0.076	10.8	0.2	0.002	10.60	8.46	308.00	12.40	2.92
NNP-45-08	0.063	7.37	0.196	0.0019	10.60	8.54	306.00	12.30	1.76
NNP-46-08	0.023	58.2	0.214	0.002	10.80	8.50	191.00	12.60	2.72
NNP-47-08	0.03	3.43	0.186	0.002	11.60	8.43	312.00	12.40	1.89
NNP-48-08	0.076	2.66	0.157	0.0038	12.40	8.51	189.00	12.20	1.64
NNP-49-08	0.52	48.4	0.123	0.0067	14.60	8.50	217.00	12.10	7.48
NNP-50-08	0.367	31.6	0.19	0.0046	13.90	8.46	287.00	12.40	6.43
NNP-51-08	0.317	12.9	0.144	0.0036	12.50	8.41	325.00	12.40	5.31
NNP-52-08	0.036	1.87	0.254	0.0025	8.21	8.40	211.00	13.00	2.14
NNP-53-08	0.097	135	0.21	0.0026	8.20	8.37	358.00	13.00	1.83
NNP-54-08	0.35	80.6	0.188	0.012	12.30	8.50	441.00	12.30	21.00
NNP-55-08	0.077	2.74	0.227	0.0034	14.80	8.51	194.00	12.10	7.40
NNP-56-08	0.056	1.14	0.325	0.0034	16.80	8.55	309.00	11.40	2.44
NNP-57-08	0.059	1.32	0.219	0.0032	8.70	8.43	122.00	13.40	3.20
NNP-58-08	0.232	3.09	0.213	0.0023	7.35	8.37	221.00	13.70	3.69
NNP-59-08	0.031	0.68	0.247	0.0017	13.00	8.49	284.00	12.20	1.94
NNP-60-08	0.045	1.52	0.207	0.0026	13.90	8.51	229.00	12.10	2.81
NNP-61-08	0.062	0.63	0.179	0.0023	11.23	8.52	265.00	12.50	1.57
NNP-62-08	0.026	2.68	0.162	0.0011	13.70	8.44	335.00	11.60	0.27
NNP-63-08	0.021	4.72	0.163	0.001	13.10	8.47	337.00	11.80	0.68
NNP-64-08	0.022	3.9	0.162	0.0022	13.80	8.54	344.00	11.80	2.11
NNP-65-08	0.327	45.6	0.153	0.0083	9.87	8.40	326.00	13.40	11.90
NNP-66-08	0.03	3.08	0.246	0.0029	11.50	8.57	284.00	13.50	2.99
NNP-67-08	0.019	5.66	0.242	0.0024	10.60	8.53	297.00	13.20	1.05
NNP-68-08	0.019	6.39	0.219	0.0023	10.60	8.54	319.00	13.10	0.62

Site	Y µg/L	Zn µg/L	TN mg/L	TP mg/L	Temp (°C)	pH	Specific Conductance µS/cm	DO mg/L	Turbidity NTU
NNP-69-08	0.028	6.7	0.211	0.0024	11.10	8.52	330.00	13.00	0.63
NNP-70-08	0.021	7.13	0.209	0.0024	11.78	8.50	334.00	12.63	0.48
NNP-71-08	0.028	3.43	0.224	0.0016	12.24	8.54	330.00	12.38	0.42
NNP-72-08	0.037	3.25	0.196	0.0014	12.29	8.55	330.00	12.33	0.45
NNP-73-08	0.019	2.95	0.203	0.001	12.20	8.51	327.00	12.40	0.42

Appendix B, Table 3.4 Water chemistry variables for South Nahanni River water sites sampled in 2009

Sample Ids	NO3NO2 mg/L	NH3NH4 mg/L	DOC mg/L	DIC mg/L	Ag µg/L	Al µg/L	As µg/L	B µg/L	Ba µg/L	Be µg/L	Bi µg/L	Cd µg/L
NNP1-09	0.03	0.008	0.5	10.9	0.004	216	1.58	1.8	27.9	0.017	0.039	0.019
NNP2-09	0.024	0.011	0.5	9.9	0.007	424	2.94	5.8	27.9	0.029	0.131	0.041
NNP11-09	0.015	0.008	0.5	6.7	0.004	340	2.13	1.4	19.7	0.026	0.049	0.015
NNP12-09	0.027	0.006	0.5	9.9	0.003	232	1.64	1.8	24.8	0.018	0.031	0.02
NNP15-09	0.022	0.01	0.9	18.2	< 0.001	9.5	0.62	3.1	18.9	0.002	0.004	0.082
NNP16-09	0.014	0.019	0.8	13.7	0.001	17.3	0.8	2.7	22.9	0.003	0.003	0.155
NNP17-09	0.017	0.007	0.8	12	0.001	19.2	0.93	2	24.2	0.002	0.007	0.139
NNP18-09	0.019	0.009	0.5	8.6	0.003	256	1.72	1.5	21.5	0.018	0.036	0.018
NNP26-09	0.052	0.005	0.5	11.6	0.015	865	2.52	7.4	31.2	0.066	0.507	0.052
NNP27-09	0.055	0.006	0.4	10.8	0.008	567	2.49	6.6	26.1	0.049	0.127	0.041
NNP28-09	0.048	< 0.005	0.4	10.3	0.006	478	2.38	6.4	24.5	0.044	0.084	0.032
NNP29-09	0.044	0.007	0.5	9.3	0.006	451	2.45	5.7	23.1	0.047	0.07	0.038
NNP30-09	0.036	0.005	0.9	10.1	0.003	259	2.87	5.9	23.8	0.02	0.091	0.018
NNP31-09	0.026	< 0.005	0.5	10.2	0.007	404	2.88	10.4	29.6	0.032	0.102	0.084
NNP32-09	0.028	0.005	0.5	9.4	0.005	374	2.93	7.7	26.5	0.028	0.239	0.025
NNP38-09	0.037	0.006	0.6	11.2	0.003	187	1.46	2.1	28.7	0.016	0.034	0.038
NNP39-09	0.026	0.007	0.5	10.4	0.005	307	2.31	3.1	27.8	0.023	0.084	0.019

Sample Ids	NO3NO2 mg/L	NH3NH4 mg/L	DOC mg/L	DIC mg/L	Ag µg/L	Al µg/L	As µg/L	B µg/L	Ba µg/L	Be µg/L	Bi µg/L	Cd µg/L
NNP40-09	0.027	0.008	0.5	10	0.003	219	1.6	1.1	25.2	0.018	0.029	0.018
NNP43-09	0.141	0.005	1.2	41.8	< 0.001	18.1	0.25	10	76.1	0.003	0.006	0.046
NNP44-09	0.146	< 0.005	1.2	43.4	< 0.001	12	0.25	10.1	74.4	0.003	0.001	0.041
NNP45-09	0.149	< 0.005	1.4	40.2	0.001	13	0.26	9.7	74	0.003	< 0.001	0.034
NNP46-09	0.138	< 0.005	1.2	39.8	0.001	16	0.26	10	76.5	0.003	0.002	0.034
NNP47-09	0.135	< 0.005	1.2	41	0.001	18.2	0.22	10.2	76.7	0.002	0.002	0.024
NNP55-09	0.179	< 0.005	1	33.2	0.001	39.4	0.14	4.5	39.8	0.003	0.001	0.016
NNP56-09	0.192	< 0.005	1.5	31.2	0.003	52.5	0.19	7.8	66	0.004	0.005	0.026
NNP57-09	0.183	< 0.005	0.6	30.2	< 0.001	24.7	0.08	2.9	18.9	0.006	0.002	0.032
NNP60-09	0.165	< 0.005	1.2	31.8	< 0.001	45.1	0.17	5.9	64.8	0.004	0.001	0.012
NNP62-09	0.116	< 0.005	1	40.8	< 0.001	3.9	0.12	7.9	74.3	0.001	< 0.001	0.023
NNP64-09	0.125	< 0.005	1.1	42.8	< 0.001	4.6	0.13	8.4	77.3	0.002	< 0.001	0.031
NNP66-09	0.16	< 0.005	1	35.8	0.001	53.3	0.23	6.8	70.1	0.005	0.001	0.022
NNP68-09	0.152	< 0.005	1.3	42.2	< 0.001	12.4	0.23	9.2	75.5	0.003	< 0.001	0.028
NNP69-09	0.15	< 0.005	1.2	43	0.001	7.9	0.25	9.8	74.1	0.002	< 0.001	0.031
NNP70-09	0.151	< 0.005	1.2	43.2	< 0.001	8.1	0.24	8.8	72.5	0.002	0.001	0.031
NNP71-09	0.139	< 0.005	1.2	41.4	< 0.001	7.3	0.15	10.3	73.5	0.002	< 0.001	0.027
NNP72-09	0.138	0.006	1.2	42.2	< 0.001	8.4	0.16	8.9	75.8	0.003	< 0.001	0.023
NNP73-09	0.142	< 0.005	1.2	41.4	0.003	9.5	0.17	10.1	74.9	0.002	0.001	0.03
NNP76-09	0.021	0.007	0.6	9.3	0.003	221	1.62	1.2	23.7	0.019	0.039	0.029
NNP77-09	0.047	< 0.005	2.3	14.9	< 0.001	198	0.6	0.3	25.2	0.038	< 0.001	0.5
NNP78-09	0.046	< 0.005	0.8	14	< 0.001	94.5	0.27	0.5	19.1	0.008	0.001	0.579
NNP79-09	0.017	< 0.005	0.8	16.6	< 0.001	8.8	0.61	2.4	19.9	0.002	0.002	0.1
NNP80-09	0.015	0.005	0.5	7.7	0.003	298	1.94	1.2	23.7	0.022	0.035	0.02
NNP81-09	0.176	< 0.005	0.7	31	0.001	43.1	0.11	2.5	21.1	0.006	0.003	0.014
NNP82-09	0.164	< 0.005	1.2	33.2	0.002	98.1	0.23	6.8	63.5	0.008	0.001	0.032

Appendix B, Table 3.4, continued

Sample	Ce µg/L	Co µg/L	Cr µg/L	Cs µg/L	Cu µg/L	Fe µg/L	Ga µg/L	La µg/L	Li µg/L	Mn µg/L	Mo µg/L	Nb µg/L
NNP1-09	0.326	0.305	0.285	0.144	0.58	396	0.056	0.179	2.5	10.3	0.426	0.027
NNP2-09	0.453	0.368	0.409	0.319	1.11	673	0.152	0.24	5.8	25.6	0.482	0.138
NNP11-09	0.527	0.496	0.47	0.222	0.8	647	0.092	0.251	2.4	13.5	0.241	0.039
NNP12-09	0.365	0.345	0.31	0.163	0.59	430	0.061	0.194	2.5	11.1	0.372	0.03
NNP15-09	0.027	0.029	0.033	0.06	0.43	30.4	0.005	0.027	3.9	2.47	0.904	0.001
NNP16-09	0.054	0.06	0.041	0.043	0.39	72.4	0.007	0.032	2.4	4.51	1.32	0.001
NNP17-09	0.069	0.081	0.048	0.064	0.51	88.9	0.007	0.038	2.2	5.93	0.98	0.002
NNP18-09	0.427	0.464	0.336	0.169	0.69	466	0.068	0.239	2.5	14.1	0.34	0.032
NNP26-09	2.34	1.03	1.13	0.381	2.11	1380	0.264	1.5	7.2	23.9	0.589	0.104
NNP27-09	1.86	0.852	0.671	0.262	1.17	875	0.179	1.36	6.5	20.2	0.526	0.091
NNP28-09	1.38	0.862	0.541	0.235	0.97	723	0.153	0.982	6.5	20.3	0.405	0.085
NNP29-09	1.37	0.908	0.505	0.248	0.91	682	0.148	1.03	6.4	20	0.396	0.085
NNP30-09	0.328	0.218	0.243	0.282	0.61	406	0.094	0.174	6.1	15	0.448	0.087
NNP31-09	0.412	0.365	0.431	0.43	1.17	608	0.142	0.216	8.4	25.3	0.547	0.137
NNP32-09	0.449	0.316	0.318	0.384	0.91	618	0.14	0.233	7.2	25.3	0.472	0.131
NNP38-09	0.305	0.327	0.256	0.132	0.62	462	0.054	0.164	2.8	26.8	0.409	0.023
NNP39-09	0.402	0.329	0.314	0.23	0.78	567	0.104	0.213	3.9	25.8	0.444	0.091
NNP40-09	0.342	0.34	0.297	0.15	0.62	413	0.059	0.182	2.6	10.6	0.376	0.025
NNP43-09	0.438	0.039	0.085	0.016	0.38	27.2	0.011	0.376	2.7	0.85	2.88	< 0.001
NNP44-09	0.029	0.018	0.125	0.007	0.37	23.9	0.005	0.017	2.7	0.68	3.07	< 0.001
NNP45-09	0.029	0.024	0.169	0.009	0.87	25.6	0.006	0.015	2.7	0.77	3.06	< 0.001
NNP46-09	0.05	0.027	0.095	0.015	0.34	29.4	0.007	0.029	2.7	0.81	2.91	< 0.001
NNP47-09	0.023	0.023	0.093	0.012	0.31	26.9	0.007	0.016	2.7	0.65	2.9	< 0.001
NNP55-09	0.216	0.043	0.104	0.017	0.32	59.3	0.017	0.186	2.2	0.97	2.35	< 0.001
NNP56-09	0.047	0.054	0.186	0.017	0.73	84.9	0.022	0.032	2.7	1.75	4.13	< 0.001
NNP57-09	0.035	0.228	0.068	0.005	0.28	33.4	0.006	0.018	1.7	4.2	1.76	< 0.001

Sample	Ce µg/L	Co µg/L	Cr µg/L	Cs µg/L	Cu µg/L	Fe µg/L	Ga µg/L	La µg/L	Li µg/L	Mn µg/L	Mo µg/L	Nb µg/L
NNP60-09	0.046	0.052	0.159	0.019	0.49	68.8	0.021	0.04	3.1	1.32	3.38	0.001
NNP62-09	0.007	0.01	0.092	< 0.005	0.25	5	0.003	0.006	1.9	0.17	3.9	< 0.001
NNP64-09	0.023	0.011	0.082	0.006	0.24	5	0.004	0.016	1.9	0.13	3.83	< 0.001
NNP66-09	0.103	0.058	0.277	0.025	1.01	102	0.021	0.055	1.9	1.93	2.33	0.002
NNP68-09	0.133	0.023	0.179	0.01	1.11	21.7	0.007	0.099	2.7	0.53	3.2	< 0.001
NNP69-09	0.017	0.018	0.124	0.009	0.5	10.7	0.004	0.014	2.8	0.27	3.53	< 0.001
NNP70-09	0.013	0.016	0.129	0.005	0.9	12.9	0.004	0.008	2.8	0.36	3.13	< 0.001
NNP71-09	0.009	0.014	0.094	0.006	0.57	9.5	0.004	0.006	2.6	0.28	2.75	< 0.001
NNP72-09	0.019	0.015	0.091	0.005	0.81	7.4	0.004	0.017	2.5	0.27	3.09	< 0.001
NNP73-09	0.036	0.036	3.68	0.008	32.8	50.5	0.005	0.022	2.5	0.38	3.46	< 0.001
NNP76-09	0.361	0.422	0.32	0.151	0.66	412	0.059	0.201	2.5	12.7	0.363	0.026
NNP77-09	13.7	3.33	0.041	0.067	1.16	11.6	0.134	10.7	4.8	45	0.405	< 0.001
NNP78-09	0.18	0.575	0.021	0.034	0.68	9.5	0.006	0.136	2.7	14	0.346	< 0.001
NNP79-09	0.039	0.027	0.025	0.045	0.36	34.4	0.004	0.036	3.2	2.31	0.985	< 0.001
NNP80-09	0.5	0.487	0.38	0.202	0.74	548	0.08	0.245	2.4	16.4	0.323	0.034
NNP81-09	0.107	0.151	0.094	0.013	0.23	74.2	0.012	0.073	1.9	3.3	1.69	< 0.001
NNP82-09	0.083	0.095	0.227	0.032	0.53	152	0.035	0.045	3.5	2.93	3.51	0.006

Appendix B, Table 3.4, continued

Sample	Ni µg/L	Pb µg/L	Pt µg/L	Rb µg/L	Sb µg/L	Se µg/L	Sn µg/L	Sr µg/L	Tl µg/L	U µg/L	V µg/L	W µg/L
NNP1-09	2.3	0.406	< 0.001	0.98	0.049	0.14	0.015	65.1	0.005	0.534	0.253	0.506
NNP2-09	1.83	1.07	< 0.001	2.33	0.059	0.1	0.044	54.7	0.014	0.572	0.615	2.02
NNP11-09	2.48	0.638	< 0.001	1.1	0.047	0.1	0.007	50.8	0.008	0.299	0.335	0.839
NNP12-09	2.57	0.966	< 0.001	0.96	0.048	0.13	0.006	64.2	0.005	0.464	0.259	0.365
NNP15-09	1.55	0.031	< 0.001	0.92	0.135	0.28	0.009	116	0.004	0.788	0.168	0.463

Sample	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Tl	U	V	W
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
NNP16-09	2.92	0.066	< 0.001	0.49	0.202	0.36	0.016	76.6	0.006	0.839	0.302	0.398
NNP17-09	2.34	0.05	< 0.001	0.47	0.182	0.34	< 0.005	75.5	0.004	0.722	0.18	0.479
NNP18-09	2.52	0.566	< 0.001	0.94	0.047	0.12	0.016	64.2	0.006	0.454	0.28	0.473
NNP26-09	5.29	1.15	< 0.001	2.47	0.095	0.21	0.018	66.5	0.015	0.895	1.79	1.76
NNP27-09	5.19	0.64	< 0.001	2.03	0.073	0.21	0.016	67	0.011	0.686	0.997	0.676
NNP28-09	5.42	0.493	< 0.001	1.79	0.061	0.14	0.016	65.5	0.01	0.579	0.791	0.607
NNP29-09	5.83	0.473	< 0.001	1.76	0.057	0.13	0.014	66.1	0.01	0.575	0.732	0.471
NNP30-09	1.34	0.327	< 0.001	1.7	0.052	0.09	0.018	52.9	0.009	0.636	0.379	0.692
NNP31-09	2.06	1.16	< 0.001	2.42	0.074	0.11	0.053	56	0.012	0.642	0.612	1.28
NNP32-09	1.71	0.474	< 0.001	2.27	0.047	0.09	0.028	51.5	0.013	0.579	0.529	1.78
NNP38-09	2.29	3.52	< 0.001	0.95	0.054	0.13	0.05	64.6	0.005	0.551	0.221	0.743
NNP39-09	1.78	0.487	< 0.001	1.75	0.05	0.11	0.023	58.1	0.009	0.627	0.416	0.945
NNP40-09	2.3	0.41	< 0.001	0.93	0.051	0.12	0.005	63.5	0.004	0.495	0.228	0.182
NNP43-09	1.33	0.156	< 0.001	0.33	0.696	1.02	< 0.005	342	0.011	4.29	0.449	0.103
NNP44-09	1.3	0.114	< 0.001	0.27	0.335	1.08	< 0.005	337	0.011	4.34	0.416	0.071
NNP45-09	1.2	0.161	< 0.001	0.28	0.259	1.04	< 0.005	334	0.009	4.14	0.418	0.039
NNP46-09	1.18	0.104	< 0.001	0.33	0.368	1.01	< 0.005	340	0.01	3.99	0.469	0.309
NNP47-09	1.16	0.036	< 0.001	0.32	0.132	0.99	< 0.005	344	0.01	3.72	0.474	0.081
NNP55-09	0.73	0.06	< 0.001	0.33	0.136	0.73	< 0.005	512	0.029	2.59	0.317	0.167
NNP56-09	0.79	0.122	< 0.001	0.26	0.121	1	0.127	1520	0.003	2.91	0.7	0.155
NNP57-09	1	0.251	< 0.001	0.37	0.116	0.56	0.017	123	0.004	2.41	0.098	0.082
NNP60-09	0.63	0.042	< 0.001	0.25	0.111	0.97	< 0.005	984	0.002	2.54	0.632	0.075
NNP62-09	1.23	0.028	0.001	0.21	0.112	1.01	< 0.005	256	0.007	4.34	0.453	0.048
NNP64-09	1.24	0.038	< 0.001	0.23	0.126	1.14	< 0.005	248	0.007	4.67	0.413	0.03
NNP66-09	0.91	0.155	< 0.001	0.38	0.156	0.73	< 0.005	249	0.011	3.52	0.439	0.034
NNP68-09	1.18	0.131	< 0.001	0.28	0.238	1.04	< 0.005	317	0.011	4.58	0.417	0.12
NNP69-09	1.23	0.055	< 0.001	0.27	0.244	1.07	< 0.005	337	0.009	4.66	0.398	0.03
NNP70-09	1.22	0.138	< 0.001	0.27	0.248	1.04	0.005	339	0.01	4.26	0.41	0.129

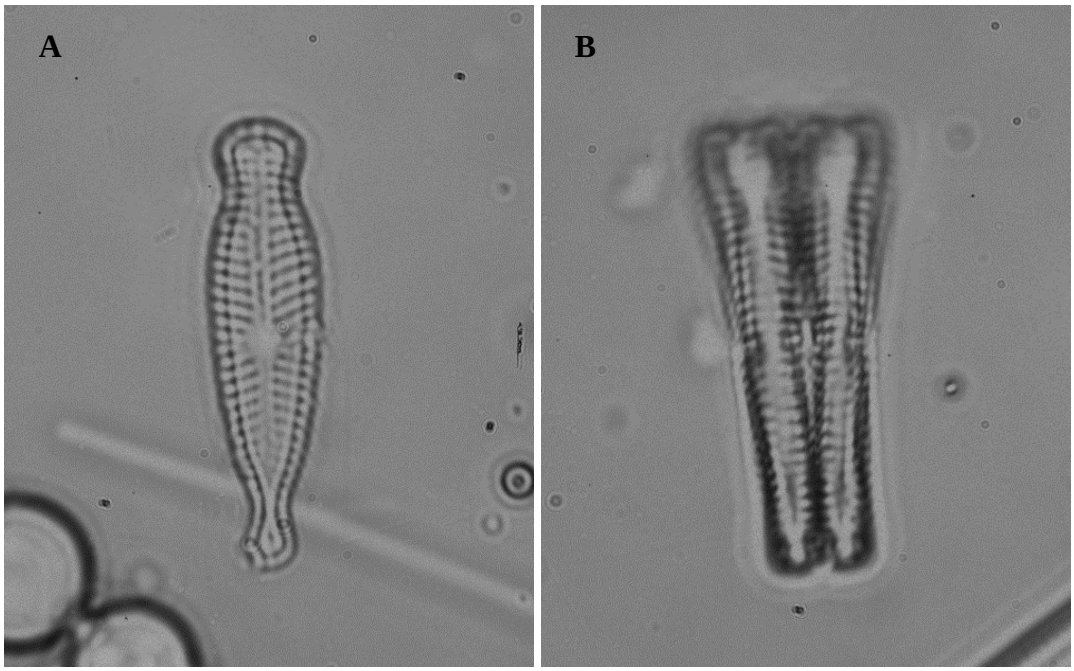
Sample	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Tl	U	V	W
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
NNP71-09	1.1	0.096	< 0.001	0.27	0.105	0.95	< 0.005	370	0.011	3.38	0.521	0.021
NNP72-09	1.12	0.045	< 0.001	0.25	0.117	1	0.035	328	0.008	3.84	0.446	0.027
NNP73-09	1.33	1.93	< 0.001	0.26	0.124	0.98	0.13	331	0.009	3.85	0.458	0.085
NNP76-09	2.44	0.436	< 0.001	0.9	0.053	0.13	0.023	62.5	0.005	0.47	0.241	0.353
NNP77-09	22.5	0.047	< 0.001	0.63	0.052	0.44	< 0.005	170	0.003	1.02	0.07	0.036
NNP78-09	9.32	0.066	< 0.001	0.65	0.048	0.38	0.015	167	0.002	0.963	0.032	0.234
NNP79-09	1.86	0.029	< 0.001	0.72	0.151	0.32	0.008	103	0.003	0.795	0.173	0.245
NNP80-09	2.25	0.597	0.001	1.04	0.058	0.13	0.005	52.3	0.005	0.379	0.299	0.101
NNP81-09	0.87	0.153	< 0.001	0.39	0.118	0.54	< 0.005	132	0.002	2.37	0.156	0.03
NNP82-09	2	0.089	< 0.001	0.38	0.118	1.04	< 0.005	972	0.005	2.74	0.721	0.095

Appendix B, Table 3.4, continued

Sample	Y	Zn	TN	TP	Temp	pH	Specific Conductance	DO	Turbidity
	µg/L	µg/L	mg/L	mg/L	°C		µS/cm	mg/L	NTU
NNP1-09	0.109	2.98	0.059	0.0099	9.10	7.98	103.00	10.80	10.20
NNP2-09	0.142	4.53	0.078	0.0138	10.20	8.39	109.00	9.70	16.00
NNP11-09	0.135	3.48	0.066	0.0161	10.10	8.24	77.00	12.00	18.50
NNP12-09	0.115	3.14	0.056	0.0107	9.10	7.92	114.00	9.80	21.50
NNP15-09	0.025	8.12	0.11	0.0036	10.90	8.31	163.00	10.90	5.70
NNP16-09	0.046	17.4	0.116	0.0044	11.00	8.36	77.00	10.90	4.92
NNP17-09	0.038	11.9	0.061	0.0034	10.20	8.29	142.00	11.80	0.50
NNP18-09	0.14	3.32	0.072	0.0124	9.30	8.03	100.00	9.60	14.70
NNP26-09	0.776	7.64	0.09	0.0434	7.10	8.27	110.00	12.60	73.30
NNP27-09	0.67	5.94	0.108	0.0318	6.90	8.26	110.00	12.40	37.40
NNP28-09	0.555	5.39	0.096	0.0295	7.00	8.31	107.00	12.20	32.70
NNP29-09	0.585	6.13	0.089	0.0234	7.20	8.34	110.00	12.20	29.40

Sample	Y	Zn	TN	TP	Temp	pH	Specific Conductance	DO	Turbidity
	µg/L	µg/L	mg/L	mg/L	°C		µS/cm	mg/L	NTU
NNP30-09	0.11	2.5	0.074	0.0102	7.70	8.38	147.00	12.80	16.50
NNP31-09	0.138	7.09	0.066	0.0112	9.60	8.24	100.00	9.90	15.90
NNP32-09	0.138	3.54	0.086	0.0125	8.60	8.23	100.00	12.70	17.20
NNP38-09	0.102	4.03	0.091	0.0091	8.80	8.11	116.00	10.90	13.40
NNP39-09	0.125	2.89	0.072	0.0115	10.60	8.46	120.00	9.60	15.10
NNP40-09	0.112	2.91	0.072	0.0106	9.60	8.07	116.00	10.10	13.60
NNP43-09	0.198	20	0.204	0.0038	11.60	8.85	325.00	12.10	5.38
NNP44-09	0.043	11.9	0.217	0.0027	8.60	8.92	232.00	14.10	29.00
NNP45-09	0.036	8.72	0.214	0.0021	7.50	8.89	280.00	14.10	2.10
NNP46-09	0.049	10.2	0.197	0.0031	11.40	8.89	306.00	12.10	6.90
NNP47-09	0.031	3.64	0.208	0.0027	12.00	8.83	300.00	12.00	5.44
NNP55-09	0.111	1.49	0.24	0.0022	13.10	9.14	258.00	12.80	3.83
NNP56-09	0.063	2.48	0.277	0.0034	9.80	8.78	230.00	13.60	4.60
NNP57-09	0.044	3.02	0.236	0.0025	6.80	8.86	211.00	13.10	5.01
NNP60-09	0.054	0.81	0.229	0.0019	13.50	8.77	226.00	12.30	3.61
NNP62-09	0.018	2.44	0.17	0.0014	12.40	8.78	122.00	12.00	2.20
NNP64-09	0.032	3.13	0.181	0.0006	11.20	8.92	307.00	12.70	2.80
NNP66-09	0.082	4.56	0.225	0.0037	10.90	8.82	256.00	15.08	5.36
NNP68-09	0.073	7.53	0.218	0.0021	6.70	8.79	272.00	13.40	6.60
NNP69-09	0.027	7.3	0.22	0.001	6.60	8.90	276.00	14.10	5.20
NNP70-09	0.023	8.42	0.203	0.0013	4.80	8.92	337.00	14.70	1.30
NNP71-09	0.025	4.29	0.195	0.0019	9.90	8.94	286.00	13.60	2.10
NNP72-09	0.039	3.6	0.2	0.0018	10.30	8.87	200.00	13.30	2.40
NNP73-09	0.043	22	0.193	0.0005	9.10	8.70	283.00	13.80	3.70
NNP76-09	0.118	3.85	0.059	0.0109	10.10	8.14	109.00	10.40	15.60
NNP77-09	4.27	44.2	0.073	0.0019	8.50	8.27	123.00	11.50	1.20
NNP78-09	0.214	47.7	0.098	0.0018	9.00	8.46	155.00	11.60	0.90

Sample	Y µg/L	Zn µg/L	TN mg/L	TP mg/L	Temp °C	pH	Specific Conductance µS/cm	DO mg/L	Turbidity NTU
NNP79-09	0.027	11.5	0.058	0.0021	11.20	8.46	161.00	10.70	0.90
NNP80-09	0.122	3.18	0.051	0.0135	10.60	8.13	88.00	12.30	18.20
NNP81-09	0.08	1.1	0.215	0.0051	8.10	8.85	221.00	13.10	12.60
NNP82-09	0.073	2.87	0.211	0.0046	11.80	8.84	235.00	12.50	3.81



Appendix B, Figure 3.1 Images of Gomphonema Species 1 in valve view (A) and girdleband view (B).

Appendix C - Chapter 5

Development of a benthic algal reference condition model to assess ecological integrity within the South Nahanni River watershed

Appendix D, Table 5.1 Physical variables measured at each site in 2008 and 2009.

Variable type	Description and Units
	Julian Day
	Ecoregion (1 – 2; 1-Selwyn mountain ecoregion, 2-Nahanni-Hyland ecoregion)
Drainage area	Latitude (Hours, Minutes, Seconds)
	Longitude (Hours, Minutes, Seconds)
	Altitude (m)
	Stream Order (Strahler)
	Drainage Area (km^2)
	Perimeter of Upstream Drainage Area (km)
Land cover and bedrock geology	Intrusive Bedrock (Percentage)
	Sedimentary Bedrock (Percentage)
	Forest Cover (Percentage)
	Ice Cover (Percentage)
Channel and site	Boulders (Percentage)
	Cobbles (Percentage)
	Gravel (Percentage)
	Pebbles (Percentage)
	Sand (Percentage)
	Silt & Clay (Percentage)
	Bankfull – Wetted (cm)
	Bankfull Width (m)
	Wetted Width (m)
	Average Depth (cm)
	Maximum Depth (cm)
	Streamside Vegetation (1 – 4; 1-ferns/grasses, 2-shrubs, 3- deciduous trees, 4-coniferous trees)
	Presence of Pools, Rapids, Riffles, Runs (presence – absence)
	Macrophyte coverage (Percentage)
	Presence of Coniferous Trees, Deciduous Trees, Grasses & Ferns, Shrubs (presence – absence)
	Sinuosity (The ratio of distance measured along a watercourse between two points, divided by the straight line distance between the same two points.) (m of stream within a 2 km linear distance of stream)
	Slope (m/m)

	Stream Density (m stream/km^2 drainage area) Dominant Sediment Size (0 – 9) Secondarily Dominant Sediment Size (0 – 9) Sediment Embeddedness [a measure of how entrenched coarse substrate (e.g., gravel, cobbles and boulders) are in finer substrates (e.g., silt and clay)]. (1 – 5; 1 = completely embedded, 5 = unembedded) Sediment Surrounding Material (0 – 9) Average Velocity (m/s) Maximum Velocity (m/s) Median Particle Size (Wolman) (cm) Geometric Mean Particle Size (Wolman) (cm) Canopy Cover (percentage)
Climate	June Min Temperature (°C) June Max Temperature (°C) June Mean Temperature (°C) Jan Min Temperature (°C) Jan Max Temperature (°C) Jan Mean Temperature (°C) June Rain (mm) June Snow (mm) June Precipitation (mm) Jan Rain (mm) Jan Snow (mm) Jan Precipitation (mm) Total Snow (mm) Total Rain (mm) Total Precipitation (mm)

Appendix C, Table 5.2 List of physical variables included in the discriminant model for each biological metric for the 2008-2009 models.

	Physical Variables Included in Discriminant Model
Benthic Algal Community Composition	Stream Order, % Boulder, % Cobble, % Gravel, % Pebble, % Sand, Bankfull-Wetted, % Intrusive, % Sedimentary, Average Depth, Drainage Area, Pools, Rapids, Straight Run, % Forest, % Ice, Perimeter, January Snow, Deciduous, Sinuosity
Diatom Community Composition	Latitude, Longitude, Altitude, Stream Order, % Cobble, % Gravel, % Pebble, % Sand, Average Depth, Maximum Depth, Drainage Area, Rapids, Straight Run, % Forest, % Ice, Macrophyte, Perimeter, Density, 2nd Dominant Substrate, Bankfull, D50Wolman
Photosynthetic Pigment Concentration	Julian Day, Longitude, Altitude, % Cobble, % Gravel, % Pebble, Average Depth, Vegetation, Drainage Area, Pools, Rapids, % Forest, Macrophyte, Perimeter, January Rain, June Rain, Total Rain, Conifers, Deciduous, Shrubs, Sinuosity, Slope, Substrate, Average Velocity, Bankfull

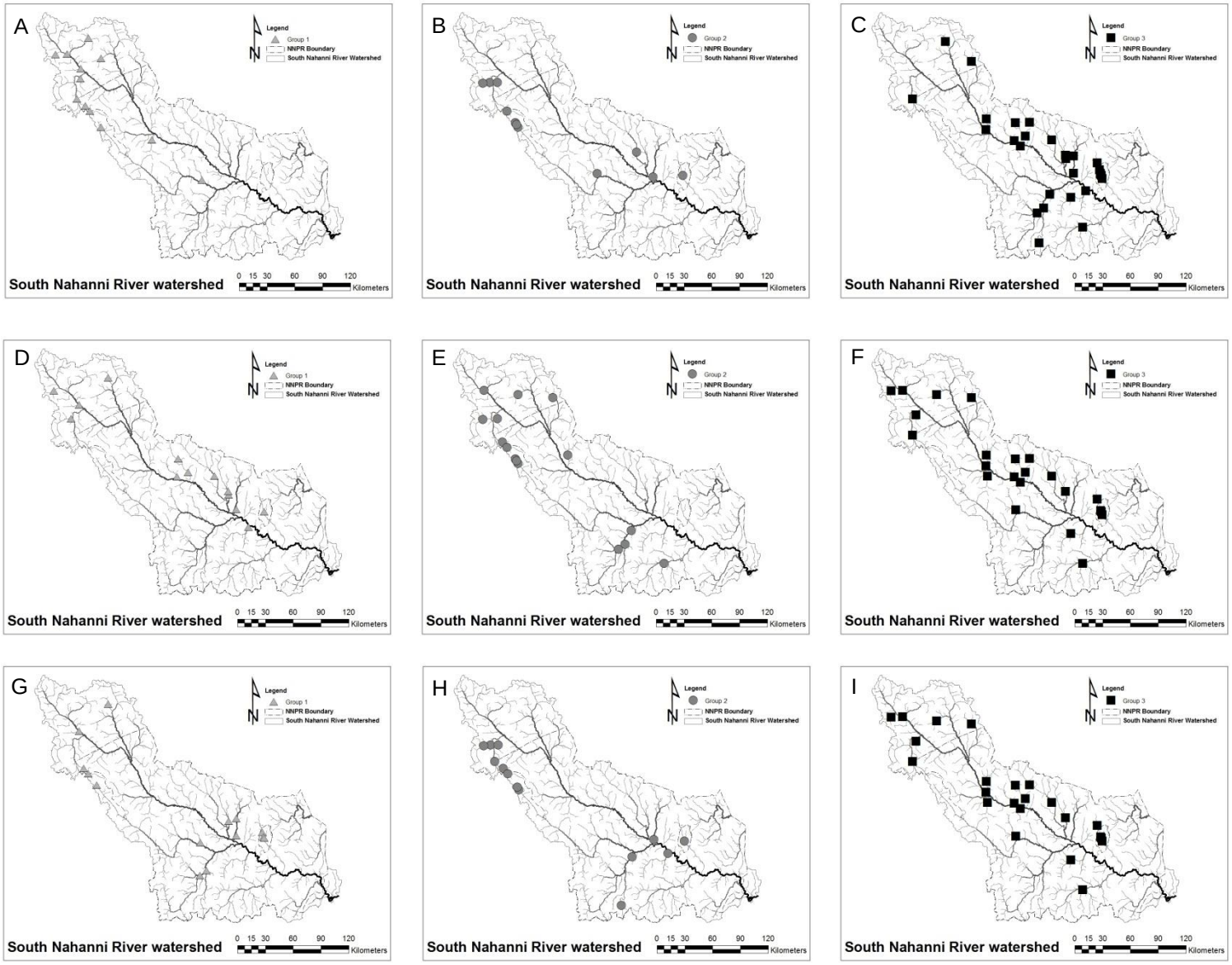
Appendix C, Table 5.3 Probability of assemblage membership of test sites downstream of Cantung mine and Prairie Creek mine sites in 2008 and 2009 for each algal metric.

Probability of Assemblage Membership – benthic algal community compositions							
	2008				2009		
Sites	Assemblage 1	Assemblage 2	Assemblage 3	sites	Assemblage 1	Assemblage 2	Assemblage 3
1	0.004	0.991	0.004	1	0.0	0.999	0.001
2	0.0	1.0	0.0	2	0.0	0.748	0.252
19	0.774	0.184	0.041	NA	NA	NA	NA
26	0.840	0.159	0.0	26	0.0	1.0	0.0
27	0.0	0.919	0.081	27	0.027	0.951	0.022
28	0.0	0.875	0.125	28	0.0	0.994	0.006
29	0.0	0.998	0.002	29	0.0	0.998	0.002
30	0.163	0.803	0.034	30	0.0	0.978	0.022
31	0.0	0.975	0.024	31	0.043	0.024	0.933
32	0.001	0.789	0.210	32	0.045	0.953	0.001
33	0.992	0.008	0.0	NA	NA	NA	NA
38	0.0	0.991	0.009	38	0.0	0.644	0.356
39	0.001	0.882	0.117	39	0.0	0.704	0.296
43	0.0	0.003	0.997	43	0.001	0.004	0.959
44	0.004	0.008	0.989	44	0.0	0.003	0.997
45	0.0	0.001	0.999	45	0.023	0.01	0.967
46	0.001	0.124	0.875	46	0.002	0.002	0.996
66	0.715	0.001	0.285	66	0.052	0.022	0.926
67	0.003	0.010	0.987	NA	NA	NA	NA
68	0.022	0.907	0.070	68	0.0	0.032	0.968
69	0.933	0.001	0.066	69	0.0	0.0	1.0
70	0.018	0.159	0.823	70	0.0	0.032	0.968
Probability of Assemblage Membership – diatom community compositions							
	2008				2009		
Sites	Assemblage 1	Assemblage 2	Assemblage 3	sites	Assemblage 1	Assemblage 2	Assemblage 3
1	0.140	0.764	0.097	1	0.828	0.172	0.0
2	1.0	0.0	0.0	2	0.006	0.991	0.003
19	0.097	0.793	0.111	NA	NA	NA	NA
26	0.999	0.001	0.0	26	0.428	0.231	0.341
27	0.553	0.003	0.443	27	0.025	0.847	0.127
28	0.002	0.005	0.993	28	0.019	0.032	0.949
29	0.081	0.581	0.339	29	0.0	0.026	0.974
30	0.039	0.941	0.021	30	0.992	0.0	0.008
31	0.021	0.903	0.077	31	0.031	0.969	0.0
32	0.077	0.966	0.026	32	0.689	0.311	0.0
33	0.0	0.335	0.665	NA	NA	NA	NA
38	0.006	0.994	0.0	38	1.0	0.0	0.0
39	0.044	0.526	0.430	39	0.001	0.997	0.001

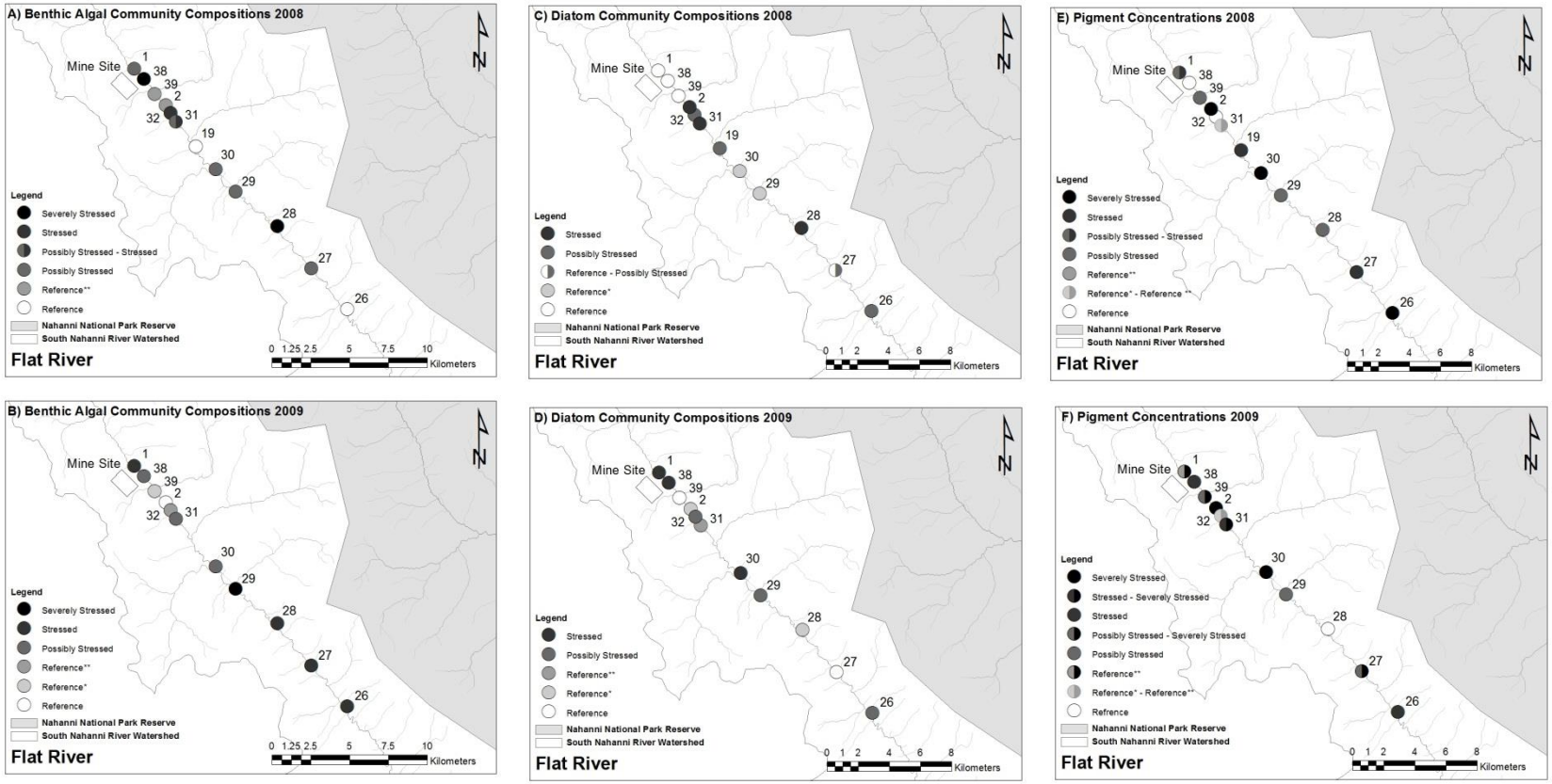
43	0.016	0.003	0.981	43	0.120	0.011	0.869
44	0.001	0.001	0.998	44	0.007	0.0	0.993
45	0.011	0.0	0.989	45	0.0	0.0	1.0
46	0.001	0.001	0.999	46	0.217	0.0	0.783
66	0.0	0.0	1.0	66	0.0	0.0	1.0
67	0.074	0.0	0.926	NA	NA	NA	NA
68	0.0	0.0	1.0	68	0.0	0.0	1.0
69	0.0	0.002	0.998	69	0.006	0.0	0.994
70	0.001	0.001	0.998	70	0.0	0.0	1.0

Probability of Assemblage Membership – photosynthetic pigment concentration

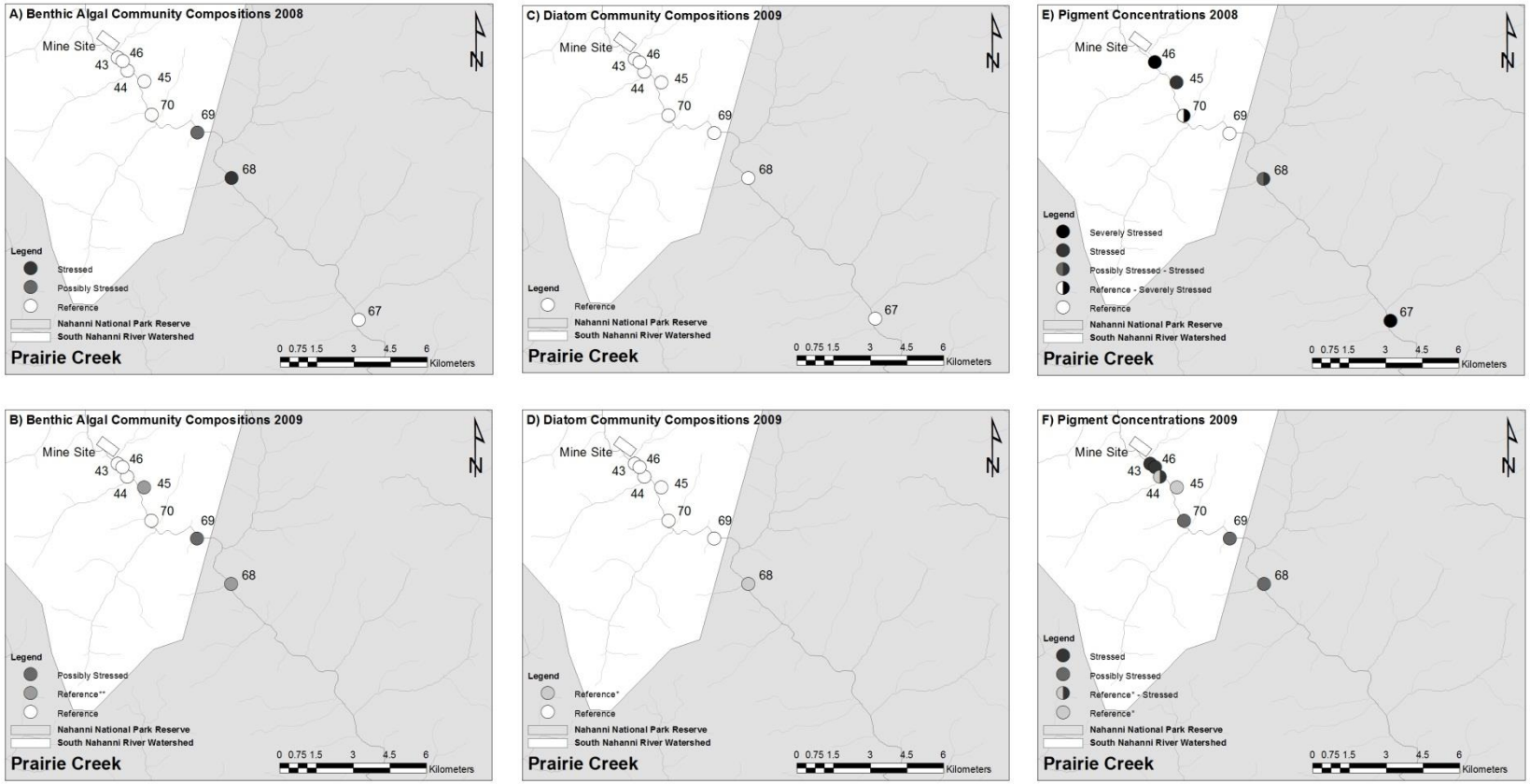
	2008				2009		
Sites	Assemblage 1	Assemblage 2	Assemblage 3	sites	Assemblage 1	Assemblage 2	Assemblage 3
1	0.002	0.470	0.528	1	0.444	0.556	0.0
2	0.002	0.790	0.208	2	0.0	0.003	0.997
19	0.0	0.0	1.0	NA	NA	NA	NA
26	0.0	0.0	1.0	26	0.001	0.993	0.006
27	0.0	0.0	1.0	27	0.002	0.409	0.589
28	0.0	0.0	1.0	28	0.0	0.985	0.015
29	0.0	0.0	1.0	29	0.001	0.983	0.016
30	0.004	0.0	0.996	30	0.0	0.0	1.0
31	0.0	0.0	1.0	31	1.0	0.0	0.0
32	0.0	0.0	1.0	32	0.007	0.992	0.0
33	1.0	0.0	0.0	NA	NA	NA	NA
38	1.0	0.0	0.0	38	0.762	0.216	0.022
39	0.0	0.002	0.998	39	0.0	0.425	0.575
43	NA	NA	NA	43	0.932	0.05	0.018
44	NA	NA	NA	44	0.001	0.507	0.492
45	0.0	0.001	0.999	45	0.06	0.544	0.397
46	0.053	0.032	0.915	46	0.027	0.006	0.967
66	1.0	0.0	0.0	66	1.0	0.0	0.0
67	0.061	0.889	0.05	NA	NA	NA	NA
68	0.0	0.0	1.0	68	1.0	0.0	0.0
69	0.004	0.003	0.993	69	0.973	0.014	0.013
70	0.0	0.487	0.513	70	1.0	0.0	0.0


A
B
C
D
E
F
G
H
I
Legend
Group 1
Group 2
Group 3
NNPR Boundary
South Nahanni River Watershed
South Nahanni River watershed
0 15 30 60 90 120
Kilometers

Appendix C, Figure 5.1 Locations of reference sites in each assemblage (A, D, G = Assemblage 1 – light grey triangles; B, E, H = Assemblage 2 – dark grey circles; C, F, I = Assemblage 3 – black squares) within the South Nahanni River watershed.



Appendix C, Figure 5.2 Test site assessments downstream of North American Tungsten along Flat River. Each test site is coded for their level of stress (white = reference condition; varying shades of grey = reference *, reference **, possibly stressed and stressed; black = severely stressed; sites in between levels of stress (e.g., possibly stressed – stressed) re represented by combinations of the two (e.g., light grey and dark grey).



Appendix C, Figure 5.3 Test site assessments downstream of Canadian Zinc Corporation along Prairie Creek. Each test site is coded for their level of stress (white = reference condition; varying shades of grey = reference *, reference **, possibly stressed and stressed; black = severely stressed; sites in between levels of stress (e.g., possibly stressed – stressed) re represented by combinations of the two (e.g., light grey and dark grey).