Development and Evaluation of a Periphytic Diatom Biomonitoring Platform for the Assessment of Cumulative Effects in Lakes of the Muskoka River Watershed, Ontario, Canada

by

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Authors 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.

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Abstract

Anthropogenic stressors such as urban development, agricultural practices and industrial activities are a growing concern throughout the Muskoka River watershed of Ontario, Canada, because they can alter physical, chemical and biological conditions of aquatic ecosystems. Cumulative effects of multiple stressors are often difficult to anticipate based on what is known about the individual effects of each stressor acting in isolation; thus, it is important to track cumulative effects on ecological integrity of our aquatic resources. Periphytic diatoms have potential to serve as an early indicator of ecological degradation in aquatic systems. Within the Muskoka River watershed there is a need to maintain the perceived naturalness of the region as it is a key driver of the tourism based economy. This thesis examines relations between diatom community composition, water chemistry and the abundance of anthropogenic stressors within the Muskoka River watershed and uses these relations to develop a bioassessment framework for tracking changes in biological integrity within lakes of the South Muskoka River Watershed. To do this, 86 lakes were examined to assess relations between diatom community composition, water chemistry and measures of nine anthropogenic stressors based on analyses by GIS. These relations were assessed using a combination of univariate and mulitvariate statistical methods. Composition of periphytic diatom communities was found to be associated with the concentration of ions from anthropogenic sources (i..e, application of road salt and dust suppressants), but relations became weaker at sites with a low abundance of anthropogenic stressors due to confounding influence of lake-water pH.

Relations between periphytic diatom community composition and anthropogenic stressors were sufficiently strong to permit development of bioassessment indices for the evaluation of ecological degradation at a watershed-scale. Multiple variations of two primary indices (Eastern Canadian Diatom Index (IDEC), Index of Biological Integrity (IBI)) were developed and assessed to identify the best index

for evaluating biological integrity of lakes. To reduce the cofounding influence of pH at low levels of stressors, the indices were developed separately for acidified lakes (pH 5.23-6.45) and circumneutral lakes (pH 6.52-7.47). IBI-3-Acidified and IBI-3-Circumneutral, which are based on a continuous IBI method, were found to be the best indices to assess lakes across the watershed. However, IBIs make use of only a small number of 'indicator' taxa, and as a result, may encounter no-analogue situations when applied to some lakes. Consequently, use of IBI-3-Acidified and IBI-3-Circumneutral is recommended for assessment of lakes within the Muskoka River watershed. But we suggest that the IDEC also be included as a backup in lakes where the IBI-3 index encounters no-analogue situations. Advantages of the use of periphytic diatom-based bioassessment protocols are presented in the thesis. By increasing ecological monitoring capacity, management agencies will be better prepared to avoid or mitigate cumulative effects of ever-evolving stressors.

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"*Many men go fishing all of their lives without knowing that it is not fish they are after*."

-Henry David Thoreau

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List of Abbreviations

Abbreviation	Meaning
ALK	Gran alkalinity
BI	Biotic Index
CA	Correspondence Analysis
CCA	Canonical Correspondence Analysis
COND	Conductivity
DCA	Detrended Correspondence Analysis
DIC	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
IBI	Index of Biological Integrity
IDEC	Eastern Canadian Diatom Index
mIDEC	(modified) Eastern Canadian Diatom Index
MRW	Muskoka River Watershed
PCA	Principal Component Analysis
RDA	Redundancy Analysis
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphorus

-Chapter 1-

General Introduction

1.1 Introduction

Human activities often exert profound influence on physical, chemical and biological conditions of aquatic ecosystems (Schindler, 1998; Smucker et al., 2013). Anthropogenic stressors can take on a wide range of forms, such as urban development, agricultural practices and industrial activities, which directly and indirectly alter habitat, hydrological processes and the supply of nutrients and other materials to aquatic ecosystems (Paul and Meyer, 2001; Walsh et al. 2005; Quinlan et al., 2008; Smucker et al., 2013). The resulting cumulative effects from the recurrence of stressors over time, or from multiple stressors acting in concert, are often difficult to anticipate based on what is known about the individual effects of each stressor acting in isolation (Folt et al. 1999; Christensen et al. 2006; Paterson et al., 2008; Smol, 2010). Thus, it is important that the impacts of cumulative effects are monitored to track and understand how anthropogenic stressors affect ecological integrity of our aquatic resources.

1.2 Stewardship Challenges for the Muskoka River Watershed

The Muskoka River watershed (MRW) occupies more than 5,200 km^2 of land in south-central Ontario and includes more than 2,000 lakes that support a diversity of ecosystems (Fig. 1.1; OMNR, 2003; O'Connor et al., 2009). The beauty of the forested landscape, with abundant lakes and natural areas, makes tourism a major driver of the regional economy (Winter et al., 2002; DMM, 2005; Dillon et al., 2007). However, rising concerns over growth of anthropogenic activities have stimulated a need to quantify biological responses to the cumulative effects of multiple stressors on lake ecosystems. Anthropogenic stressors such as residential development, road construction and the introduction of invasive species can all be attributed to increases in human activity within the watershed (Tran &

Brouse, 2009; Weisz & Yan, 2010; Palmer et al., 2011). In 2011, the Muskoka River Watershed Monitoring and Management Consortium was formed with support from the Canadian Water Network's 'Canadian Watershed Research Consortium' Program in response to growing public concerns. The consortium's primary goal is to work in partnership with researchers, local and regional agencies, and members of the public to develop robust approaches for assessing and managing cumulative effects at a watershed scale. Research conducted as part of the consortium is designed to further our understanding of the underlying processes driving the hydrology, chemistry and ecology of streams and lakes of the MRW. A fundamental principle of this program is that the research findings will be more readily translated into beneficial actions if the research is conducted collaboratively, via active participation and dialogue between researchers, local stakeholders and end-users. As part of this consortium, the research reported in this thesis contributes to the development of a novel biomonitoring approach that will inform future environmental and planning policies, and initiatives that better protect lakes in the MRW against ecological degradation. Many natural and anthropogenic processes are important in defining aquatic ecosystems of the MRW. This thesis will focus on addressing the responses observed to contemporary, local-scale anthropogenic stressors affecting the ecological function of lakes.

1.3 Periphytic Diatoms as Bioindicators of Anthropogenic Stress

Environmental monitoring programs have focused primarily on measurements of physical and chemical variables to assess degradation of lakes, as they are relatively easy to collect and are perceived to require fewer resources than programs based on biological measurements. However, this approach is limited because it provides only point-in-time measurements of variables that potentially drive biological responses, rather than directly measuring the biotic changes that are of main interest (Fausch et al., 1990; Karr, 1993; Knopman and Smith, 1993; Cassidy and Jordon, 2011). In contrast, biomonitoring, the process of investigating trends and changes in environmental conditions over time via systematic, repeated measurements of biota, can be used to track cumulative effects by relating the magnitude of multiple stressors to ecological responses (Rosenberg & Resh, 1993). Biota integrate information about fluctuations in environmental conditions during the weeks to months prior to sample collection (Schindler et al., 1987; Reavie et al., 2006). This includes the influence of important pulse-type events, such as storm events, snowmelt and episodic releases of nutrients and other materials due to human activities, all of which may be missed by periodic water chemistry monitoring (Reavie et al., 2006; Lambert et al. 2008). Biomonitoring can also be used to detect changes in habitat structure that may be missed by solely measuring physical and chemical variables in the lake water (Kilgour et al. 2007; Spencer et al. 2008). Commonly employed biotic groups include benthic invertebrates and fish, but can span a wide range of organisms including macrophytes (Rooney and Bayley 2011; 2012), crayfish (Schilderman et al., 1999), algae (Paterson et al., 2008; Thomas et al., 2011) and zooplankton (Yan et al., 2008b) to assess aquatic ecosystem status and trends.

For decades, biomonitoring programs in North America have systematically employed algal biomass and composition as indicators of aquatic ecosystem health (Patrick, 1949; Schindler, 1987). Algae function as primary producers at the base of aquatic food-webs, and thus have the ability to exert a bottom-up influence on food-web structure and function (Sabater & Admiraal, 2005; Resh, 2008). Furthermore, algae are ubiquitous, highly abundant and diverse, features which make it relatively easy to obtain samples that yield high information content. Due to their rapid growth rates and few barriers to dispersal, they are amongst the first organisms to respond to environmental changes (Sabater & Admiraal, 2005; Lavoie et al., 2008; Resh, 2008). Diatoms (Division: Bacillariophyceae), in particular, are widely recognized for their responsiveness to environmental fluctuations and ability to assess changes in pH and concentrations of ions, nutrients and metals in surface waters (Hall and Smol, 1996; Dixit et al., 1999; O'Connor et al., 2000, Potapova & Charles, 2003). The diatom cell wall is composed of silica and preserves well within aquatic sediments, a feature that enables their use in paleolimnological studies to assess past environmental changes (Smol & Stoermer, 2010). This allows biomonitoring programs to couple information regarding current conditions and trends (obtained from contemporary monitoring) with longer time-series of information about past environmental changes (obtained from sediment cores). The combination of these methods can be extremely useful in disentangling the influence of human activities from that due to natural processes (e.g., Wolfe et al., 2012).

Periphytic diatoms grow attached to submerged surfaces; including, rocks (epilithic), sediment (epipelic) and macrophytes (epiphytic). Consequently, periphytic diatoms are relatively sessile organisms, and so are able to provide information about the locations from which they were sampled. This is an advantage compared to more mobile biota, such as fish or aquatic invertebrates, which may migrate into and out of the areas sampled. For the above reasons, periphytic diatom community composition has been suggested as a promising method to detect impairment from multiple stressors in the nearshore zone of lakes (Prygiel and Coste, 1993; Fore and Grafe, 2002; Thomas et al., 2011; Gottschalk & Kahlert, 2012). The nearshore zone is of specific interest to lake managers, because it is the first zone to receive materials provided by anthropogenic stressors acting within the catchments of lakes, and it is often where habitats are altered or impaired by human activities (Vadeboncoeur et al. 2001; 2003). The nearshore zone is also the part of a lake where most human activities occur, and thus ecological impairment of this zone often results in highest levels of societal concern. Thus, sensitive biota sampled in nearshore regions of lakes, such as periphytic diatoms, may serve as early indicators of ecosystem-level responses to anthropogenic stressors (Jacoby et al. 1991; Poulíčková et al. 2004). Interestingly, biomonitoring programs have incorporated periphytic diatoms in stream assessments (e.g., NIWA (New Zealand) Stream Periphyton Monitoring Program (Biggs & Kilroy, 2000), EPA (United States) Periphyton Protocols as part of the Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al. 1999)), but they have not been widely developed and applied for lakes.

Instead, phytoplankton communities in the central, deep-basin of lakes have traditionally been the main source of algal biomonitoring data.

In a pilot project, Thomas et al. (2011) demonstrated that periphyton sampling protocols developed for use in streams (Biggs & Kilroy, 2000) can be effectively adapted for use in the nearshore reaches of lakes within the MRW. Using diatoms as bioindicators, relations between community composition, water chemistry and varying levels of shoreline development were examined. In their study, water chemistry did not differ significantly across the categories of increasing shoreline development, and Thomas et al. (2011) concluded this was due to dilution of nearshore waters by offshore waters. This feature, thus, may limit effectiveness of water chemistry monitoring to track anthropogenically-driven increases in the flux of materials from the catchment to lakes. However, Thomas et al. (2011) demonstrated that the differences in periphytic diatom community composition were significantly associated with differences in shoreline development, suggesting that effective biomonitoring programs can be developed based on analysis of periphytic diatoms. The study by Thomas et al. (2011) provides an important foundation for use of periphytic diatoms in the bioassessment of lakes, and Chapter 2 of this thesis expands upon that study by examining 86 lakes across the MRW, including use of better quantitative estimates of anthropogenic stress acting within the lakes and their local catchments that are based on analyses by GIS. Examination of the relations between diatom community composition and anthropogenic stressors among lakes, provides the basis for the development of watershed-scale bioassessment.

1.4 Biological Indices as a Method of Assessing Ecological Status of Lakes

Analysis of biomonitoring data can be complex because programs frequently measure dozens of taxa and environmental variables (Cao & Hawkins, 2011). To help facilitate use of information by decision makers, investigators often seek to condense these large multivariate data sets down to a single value, typically an index or a grade, which represents the overall ecological status of a site (Resh, 1995; Uys et al. 1996; Stark 1998). The development of bioassessment indices allows for complex ecological data to be condensed into an intuitive value that can be used to communicate the ecological condition of a site to decision-makers and the general public.

Bioassessment indices assess the ecological status of sites by comparing the relative abundance of taxa observed to the relative abundance of taxa expected at reference locations (Cao & Hawkins, 2011). There are two main categories of indices that are widely used in bioassessment, which are known as biotic indices (BI) and indices of biological integrity (IBI). Both BIs and IBIs, in the context for which they will be used in this thesis, rely on assessments of biotic communities using taxonomic identification of species and quantification of their relative abundance. The influence of anthropogenic stressors can lead to degradation of environmental conditions, and commonly results in the shifting of resources available for aquatic organisms (Karr, 1981). Resulting changes in community composition can then be measured and quantified through the use of a bioassessment index (Karr, 1981; Abbasi & Abbasi, 2012). Sites are then assessed relative to each other on the basis of the community composition observed. A key difference between IBIs and BIs is that IBIs utilize data from a subset of selected taxa that are identified as possessing strong bioindicator value for the stressors of interest, whereas BIs use information from all observed taxa.

Other types of indices examining community diversity, richness and evenness have been used extensively to describe both terrestrial and aquatic communities. These measures, however, do not include information on the taxonomic identification of the observed taxa. As a result, they have been criticized because they lose important information conveyed by the identity of the taxa (Peet, 1975; Karr, 1981; Abbasi & Abbasi, 2012). For example, in a diversity index (e.g., Shannon-Weaver index or Simpson index), if both pristine and degraded sites have comparable species richness and abundances, diversity indices may assess them to be of similar quality, even if the community composition is very different (i.e., comprise mainly of taxa that are sensitive versus tolerant to stressors). Comparatively, BIs and IBIs, incorporate variation in community structure, which allows greater ability to assess ecological degradation (Washington, 1984; Karr, 1991). BIs and IBIs do have limitations in that they can be computationally challenging to develop. However, this is balanced by the amount of biological information they incorporate.

In targeted studies, periphytic diatoms have become well established for use in the regulatory assessment of stream environments (e.g., Medley & Clements, 1998; Thomas et al. 2013; Pool et al. 2013). While periphytic diatoms remain largely underutilized for bioassessment of lake environments (Thomas et al. 2011, Gottschalk & Kahlert, 2012), investigation of stream periphyton is most successful when analysis is paired with a bioassessment index. Biotic indices (BIs), such as the Polluo-sensitivity Index (IPS; Coste 1982), the Diatom Biological Index (IBD; Lenoir and Coste, 1996), the Trophic Diatom Index (TDI; Kelly and Whitton, 1995), the Sladecek Index (SLA; Sladecek, 1973) and the Eastern Canadian Diatom Index (IDEC; Lavoie et al. 2006, 2013b), have all been designed to assess ecological response to the anthropogenic stressors, where formulae calculate an index value based on the relative abundance of all taxa present. However, with the exception of the IDEC, each of these indices requires substantial *a priori* information. For example, environmental optima for each taxon must be quantified, requiring a suite of physical, chemical and biological information from a large number of reference sites prior to assessment. The IDEC avoids this limitation, as site assessments are based solely on the orientation of sample scores along the gradient of maximum variance (first axis) of a multivariate ordination analysis (correspondence analysis; CA) performed on taxon relative abundance data (Lavoie et al. 2006; 2013b). For each site, sample scores are calculated by summing weighted taxon abundance values (ter Braak & Smilauer, 2002). Sample sites can then be evaluated on the basis of relative abundances of the taxa observed, and ranked along the gradient of stress being assessed. Because the IDEC is an ordination-based index, the process of developing the index is somewhat involved. However, once the index has been 'trained' for a specific region, calculating test-site scores is relatively simple. Provincial monitoring agencies are interested in the potential use of the IDEC in lake environments, as it has become established as the primary method of periphyton assessment in Ontario streams (Lavoie et al. 2013a).

Indices of Biological Integrity (IBIs) are widely used in the assessment of aquatic ecosystems throughout North America (Rooney & Bayley, 2012). IBIs differentiate themselves from other indices by employing information from only a select number of indicator taxa (Karr, 1981). These taxa are common throughout the ecosystem and illustrate either a strong positive or negative association with anthropogenic stressor abundance (Karr, 1981). These indicator taxa can act as surrogates of ecological integrity for the entire ecosystem (Beck & Hatch, 2009). Collectively, the indicator taxa can be summarized as an index value that represents the level of degradation observed at a given sample location (Karr et al., 1986; McKenzie et al., 1992; Carignan & Villard, 2002). IBIs have become a common method for analyzing biomonitoring data in stream environments in North America (Karr, 1981; Abbasi & Abbasi, 2012; Rooney & Bayley, 2012). However, IBIs are designed to be robust and therefore easily adapted for use in lakes and wetlands (Beck & Hatch 2009). By focusing on only a limited number of taxa, IBIs avoid noise introduced by taxa that do not respond strongly to changes in the stressors (Karr , 1991; Ruaro & Gubiani, 2013). This conceptually simple approach allows users to easily adapt the index to track biological responses observed from a wide variety of individual and multiple stressors (Dyer et al., 1998; Karr, 1999; Merovich et al., 2007).

1.5 Thesis Overview

The overall research goal of this project is to develop and evaluate a novel biomonitoring framework to improve bioassessment of changes in ecological integrity in response to the cumulative effects of anthropogenic activities on lakes of the MRW. Specifically, the ability of periphytic diatom communities and water chemistry will be evaluated to detect the influence of multiple anthropogenic stressors in the nearshore zone of lakes for the purposes of bioassessment. This thesis is comprised of four chapters, of which Chapters 2 and 3 will address two key objectives that define the project. The objectives of this thesis are outlined as follows:

Objective A: *Explore the distributions of water chemistry and periphytic diatom community composition in lakes of the MRW, and evaluate relations along gradients of multiple anthropogenic stressors.*

In Chapter 2, I examine the distribution of water chemistry variables and periphytic diatom community composition among 86 lakes of the MRW, and evaluate relations among water chemistry, periphytic diatom community composition and quantitative estimates of total anthropogenic stress acting within their catchments. This goal is achieved by employing three steps. First, I explore the distribution of multiple anthropogenic stressors within the catchments of study lakes using indirect gradient ordination, and quantify the magnitude of stress using the sample scores along the first ordination axis. Second, I use indirect gradient ordinations to explore the distributions of water chemistry variables and periphytic diatom community composition among the study lakes. Finally, direct gradient ordination methods and correlation analyses are employed to assess relations among anthropogenic stressors acting in the catchment, water chemistry variables and periphytic diatom community composition. These exploratory analyses provide a foundation for developing biomonitoring protocols and indices for assessing cumulative effects of anthropogenic stressors on ecological integrity of lakes using periphytic diatom communities, which is the topic of the next chapter.

Objective B: *Using the relations observed between stressor abundance and biological responses (i.e., Objective A), develop an index for assessing changes in ecological integrity of lakes in the MRW in response to cumulative effects of multiple stressors.*

The primary objective of Chapter 3 is to develop and evaluate the ability of bioassessment indices to measure ecological responses to stressors influencing lakes of the MRW. Specifically, the performance is assessed for two unique indices based on periphytic diatom community composition. The two indices are the IDEC, following the methods outlined by Lavoie et al. (2013b; IDEC 3.0), and the IBI (Karr et al. 1981). The IDEC was developed and tested in streams throughout Ontario, Quebec and the Maritimes, including a number of sample locations from within lakes of the MRW (Lavoie et al. 2013b). Here, we assess the performance of the current IDEC, as well as a version modified to be specific to the range of conditions observed within the MRW. The IBI will be evaluated using three distinct variations of index calculation: a continuous scoring method, a binned scoring method, and a continuous scoring method whereby each taxon is scored proportionally to the maximum abundance of that taxon observed across the watershed. Taking into consideration the performance of the indices, the ease of implementation and resource requirements, the end goal is to recommend the best index for use by natural resource managers and other end-users to evaluate impacts of anthropogenic stressors for lakes within the MRW.

Chapter 4 provides a summary of the key findings generated by this study, and it offers recommendations for future development of periphytic diatom biomonitoring practices. This chapter will highlight how the findings of the study can be incorporated into advancing aquatic monitoring programs and environmental protection within the MRW. Supplementary information cited in this thesis is included as a series of Appendices located at the end.

1.6 Figures

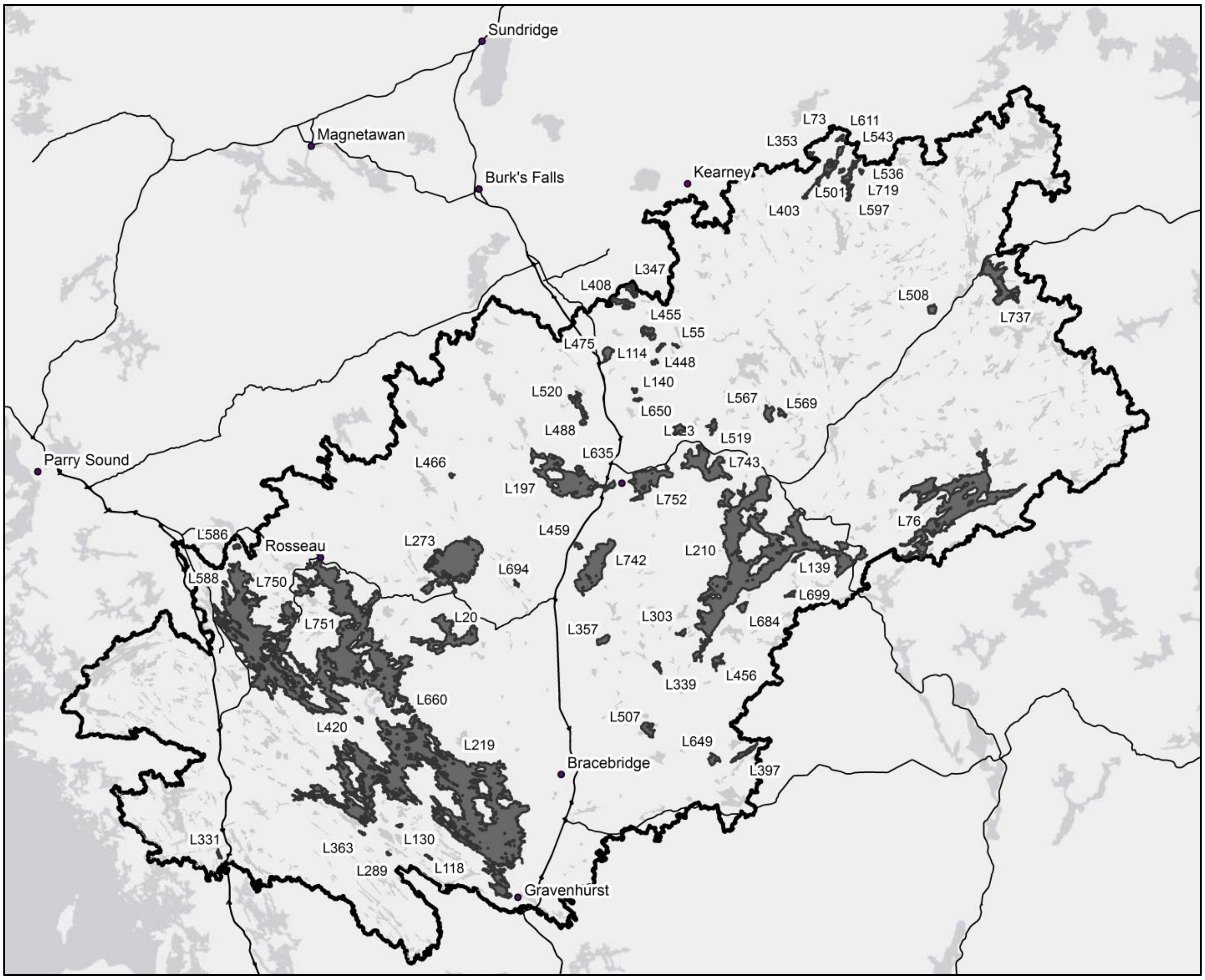

Figure 1.1. Map of the Muskoka River Watershed, in south-central Ontario (outlined in thick black boarder). Lake basins shaded in dark grey were sampled for water chemistry and periphytic diatom community composition. Lake codes correspond with information provided in Appendix A.

-Chapter 2 -

Relations among Multiple Anthropogenic Stressors, Water Chemistry and Composition of Periphytic Diatom Communities in the Muskoka River Watershed, Ontario, Canada

2.1. Introduction

The goal of this chapter is to evaluate the responses of water chemistry and periphytic diatom community composition to the influence of multiple anthropogenic stressors among 86 lakes within the Muskoka River watershed (MRW). This goal is achieved by using multivariate statistical analyses in a sequence of three steps. First, I explored the distribution of multiple anthropogenic stressors within the catchments of study lakes using indirect gradient ordination, including the use of axis one sample scores as a means to quantify and summarize the magnitude of stressors acting on each lake. Second, I used indirect gradient ordinations to explore the distributions of water chemistry variables and periphytic diatom community composition among the study lakes. Finally, I quantified relations among the anthropogenic stressors, water chemistry variables and periphytic diatom community composition using direct gradient ordination methods and correlation analysis. These analyses provide a foundation for developing biomonitoring protocols and indices able to assess cumulative effects using periphytic diatom communities, which is the topic of the next chapter.

2.2. Methods

2.2.1 Study Location and Site Selection

Located on the southern edge of the Precambrian Shield, in south-central Ontario, the MRW contains over 2,000 lakes, with more than 800 having surface areas ≥ 30 ha. The watershed is comprised primarily of impermeable granite bedrock (O'Connor et al., 2009). Where soils accumulate, they are typically shallow and sandy with a high permeability and low moisture-retention capacity (Chapman and Putnam, 1984; Eimers et al., 2009). These characteristics lead to the predominance of soft-water, acid-sensitive, oligo- to meso-trophic lakes (Girard et al. 2007).

The climate of the MRW is characterized by cool to moderate temperatures. Maximum monthly mean air temperature is 18.5^{o}C (based on the July mean, 1971-2000 climate normal from Muskoka airport, Station CYQA) and minimum monthly mean air temperature is -10.4^{o}C (January mean, 1971-2000; Environment Canada, 2012). The MRW is one of the wettest regions in the province, receiving approximately 1000 mm of precipitation annually, of which approximately one-third falls as snow (Environment Canada, 2012).

The MRW has experienced more than a hundred years of substantial anthropogenic influence, dating back to the early European colonization of the region. This includes extensive forest harvesting, which left the landscape of the watershed largely barren by the 1920s (Michalski et al., 1973). Since then, forests have re-generated and the MRW retains a high percentage of closed-canopy mixed-forest, wetlands and barrens. Many of the human activities within the MRW are localized, with varying amounts of residential development along the shorelines of lakes and concentrations of activity at the towns of Gravenhurst, Huntsville and Bracebridge. These three towns all have populations greater than 10,000 (Statistics Canada, 2012).

Using GIS-based land-use data, as outlined in Murison (2012), nine anthropogenic stressors were identified and quantified for each lake catchment within the MRW from a list of land-use and land-cover attributes (OMNR, 2000; OMNR, 2010, Weisz & Yan, 2010). A stressor is defined herein as an attribute that is potentially detrimental to the ecological status of lakes within the MRW. The measured stressors include: 1) proximity to roadways; 2) density of paved roadways; 3) density of unpaved roadways; 4) percentage of catchment containing golf courses; 5) percentage agricultural lands; 6) percentage of urban/rural development; 7) percentage of other major land uses (e.g., utilities, railway corridors, unknown land features); 8) the presence of *Bythotrephes* (i.e., the spiny water flea – a pervasive influential invasive zooplankton species); and 9) the presence of hydraulic control structures (i.e., dams). For land-use stressors (#2-7), values represent activities within 300 m of the lake basin perimeter. The 300 m buffer region was employed as it corresponds with the stressor radius used in the lake-shore capacity model (Paterson et al., 2006). Roadway proximity (#1) is an absolute measure from the lake basin perimeter to the closest section of roadway. In total, stressors were quantified for more than 800 lake basins within the MRW (including both entire lakes and embayments within large lakes) possessing surface area ≥ 30 ha. From this dataset, 86 study lake basins were selected for field sampling that span the full gradient of anthropogenic stressors within the MRW. To achieve this, I included 19 least-disturbed lake basins with minimal contemporary sources of human activities in their watersheds, the 10 lake basins with the greatest abundance of human disturbance, 13 lake basins for which long-term monitoring records exist, 26 basins of strategic importance to the District of Muskoka, and 18 lake basins which were randomly selected (See Appendix A). Lake selection was conducted in collaboration with other researchers and stakeholders within the Muskoka River Watershed Monitoring and Management Consortium to ensure that scientific objectives as well as the needs of end-users were met. All 86 unique lake basins were sampled across the MRW within an eight-week period during July and August 2012.

2.2.2 *Sample Collection*

Prior to sample collection, each lake basin was divided into four approximately equal quadrants, in which compass direction defined the quadrant boundaries. Three of these quadrants were selected for the collection of water chemistry and periphyton samples through a single-blind selection method. Within each quadrant, a sampling station was selected with the precise location chosen as the first available section of shoreline containing an adequate amount of cobble, or other suitable substrate (bedrock or boulder) if sufficient cobble was not available (occurred at approximately 10% of sample sites). A maximum of one sampling station per quadrant was sampled. However, if no adequate sampling substrate was available within a quadrant (i.e., cobble, bedrock or boulders), the unselected quadrant was substituted as a replacement. At six lakes where adequate sampling substrate was absent from two quadrants, chemical and biological samples were collected from the remaining two quadrants. In three cases where adequate sampling substrate was absent from more than two quadrants, the lakes were abandoned and a different lake basin was randomly selected. At each sampling station, samples for chemistry and periphyton analysis were collected along a 6-m transect positioned parallel to the shoreline at water depth between 0.4 - 0.6 m, as described below.

2.2.2.1 Water Sampling and Chemical Analysis

A 1 L sample of water was collected from the centre of each transect and combined with equal volumes of water from the other two quadrants within the basin to form a single 'basin composite' sample. The water samples were collected from a boat before periphyton samples were obtained in order to minimize the influence of substrate disturbance during periphyton sampling on water chemistry measurements. All bottles were triple-rinsed with filtered lake water (80-µm mesh) prior to sample collection. The 1-L water sample was then collected from a depth of 0.25-m below the surface, filtered through an 80-µm mesh and placed into a composite bottle. Composite water samples from each basin were submitted for chemical analysis following methods outlined in Ingram et al. (2006). Lake-water pH, Gran alkalinity, conductivity, concentrations of nutrients (total phosphorus (TP), nitrogen species (NO_2/NO_3, NH_3/NH_4, TKN)), DOC, DIC, and major ions (Cl, Ca, Mg, Na, K, SiO_3, SO_4) were analyzed at the Dorset Environmental Science Centre using standard MOE methods (OMOE 1983, Janhurst 1994).

2.2.2.2 Periphyton Sample Collection and Analysis

Following the collection of water samples at each transect, two cobbles (64-256 mm in maximum circumference) were selected from within a 1-m diameter plot at each of three distances (centered at 0.5 m, 3 m and 5.5 m) along the transect. The cobbles were placed individually into 20-L buckets partially filled with undisturbed lake water. Using methods established by Thomas et al. (2011), a Loeb sampler was used for approximately 30 seconds to remove periphyton from within a 28-mm diameter circle on each cobble (Loeb, 1981). This action created a slurry of periphyton and water that was trapped within the sampler, of which an aliquot of approximately 10-mL was collected by an attached syringe and placed into a 500-mL bottle. The process was then repeated, combining the resultant periphyton samples from the two remaining quadrants to produce one 'basin composite' sample for analysis by high taxonomic resolution diatom identification (approximately 400-450 mL). Samples were preserved in the field by adding 10 mL of 10% Lugol's solution to the basin composite samples and storing them in the dark until analysis.

Periphyton samples were processed and prepared for analysis of diatom community composition at the species level or higher, following methods outlined in Thomas et al. (2011). Composite samples were prepared by thoroughly mixing the preserved periphyton slurry and measuring 10 mL of each sample into individual test tubes. Contents were allowed to settle for 24 h before removing two-thirds of the supernatant and replacing it with deionized water. This process allowed for the removal of the Lugol's preservative, and was repeated until the solutions became clear. Samples were then treated with 30% hydrogen peroxide at 20˚C for one week to digest organic matter. Remaining hydrogen peroxide was removed by repeatedly siphoning off the top two-thirds of the sample supernatant and replacing it with deionized water, allowing contents to settle for 24 h between each cycle. The resulting cleaned diatom slurries were then dried onto circular coverslips and mounted onto microscope slides with Naphrax® mounting medium. Relative abundances of diatom taxa were determined for each sample by identifying and enumerating a minimum of 400 diatom valves using a Zeiss Axioskop 2Plus compound light microscope at 1000x magnification (numerical aperture =1.30). Taxonomic identifications were based on Krammer and Lange-Bertalot (1986–1991) and Lavoie et al. (2008b).

2.2.3 Data Analysis

Indirect gradient ordination analyses were used to explore patterns of variation among sites in anthropogenic stressors, water chemistry and diatom community composition, respectively. The method of ordination was selected for each dataset by first performing a preliminary detrended correspondence analysis (DCA), with detrending by linear segments, to calculate the gradient length for the first axis. Following criteria of Birks (2010), I used ordination methods with an underlying linear response model if the first axis gradient length was < 2 SD units, and ordination methods with an underlying unimodal model if the first axis gradient length was ≥ 2 SD units. Using this criterion, principal components analysis (PCA) was selected to investigate patterns in anthropogenic stressors and water chemistry; and DCA was used to investigate the patterns in diatom community composition.

2.2.3.1 Multivariate Analysis of Catchment Stressors and their Relations with Water Chemistry Conditions

As this study focuses on a multiple anthropogenic stressor approach, I summarized the nine identified stressors as a single value that represented the total magnitude of the stress acting on each lake basin. A PCA ordination was performed on the nine stressors using information regarding anthropogenic stressors (OMNR, 2000; OMNR, 2010) within and surrounding each lake basin (Fig. 2.1). For the PCA, the stressor variables were centered and standardized, and scaling focused on inter-variable correlations. The first axis of the PCA captures the greatest amount of variation associated with the nine anthropogenic stressors. Each basin was assigned a total stress score equivalent to its position along PCA axis 1 as a measure of the stress acting on each lake basin. Evaluation of stress in this manner effectively allows for the summation of percentage, abundance and binary data while preserving their relative variability. In subsequent graphs, we scaled the size of the symbol used for each lake basin according to the PCA axis 1 score as a way to visualize differences in the total magnitude of the multiple anthropogenic stressors.

A PCA was performed on the sixteen measured water chemistry variables to explore variation in water chemistry conditions among the sample sites. A redundancy analysis (RDA) was then run, constraining the sixteen water chemistry variables to the anthropogenic stressor attributes observed. This allowed for the investigation of relations among anthropogenic stressors and water chemistry variables. For each of these ordinations, water chemistry variables were centred and standardized, and scaling focused on inter-variable correlations.

2.2.3.2 Multivariate Analysis of Periphytic Diatom Community Composition and Relations with Water Chemistry and Multiple Stressors

Variation in diatom community composition among sites was explored using DCA. Detrending was performed by linear segments to avoid influence of a possible horseshoe effect which was observed in CA (Hill & Gauch, 1980).

The data were explored further using canonical correspondence analyses (CCA). CCA was first used to assess relationships between the distribution of diatoms and water chemistry variables. A second CCA was performed to investigate relations between the anthropogenic stressors and composition of diatoms communities among the lake basins. For both CCAs, bi-plot scaling focused on inter-species distances and environmental variables were centered and standardized.

Variation partitioning analysis (VPA; Borcard et al. 1992) was performed to decompose the variation in diatom community composition among sites due to unique effects of the anthropogenic stressors (percent of catchment covered by agriculture; percent cover by golf courses, percent cover by urban development; presence/absence of *Bythotrephes*; presence/absence of hydraulic control structures; density of unpaved roads; density of paved roads; proximity to roadways), natural lake/catchment attributes (lake area, catchment to lake area ratio, drainage density, slope; soil thickness, Strahler lake order, percent cover by wetland, percent cover by forest) and spatial factors (latitude; longitude; elevation), as well as that due to co-variation among the three categories of potential drivers. Variation partitioning of these three groups can identify the role anthropogenic stressors play in influencing diatom composition independent of, and in co-variation with, naturally-occurring phenomena. Natural factors and stressor variables were selected through a preliminary CCA using forward selection. For these categories, the seven variables which individually explained significant (p-value < 0.05) amounts of variation were included in the VPA. All three of the available variables in the spatial category were included in an attempt to have equal numbers of variables within each category; however, longitude was the only variable able explain a significant (p-value < 0.05) amount of variation. VPA was performed using multiple partial CCAs, and followed the steps outlined in Hall et al. (1999).

For ordinations involving the diatom data, taxon relative abundances were $ln(x+1)$-transformed to down-weight the influence of highly abundant taxa and equalize variances. Rare taxa (i.e., those with < 1% abundance in the samples) were excluded from analyses (Lavoie et al. 2009). All ordinations, including those used in the VPA, were performed using the program CANOCO 4.5 (ter Braak and Šmilauer, 2002). Significance of constrained axes was assessed using Monte-Carlo permutation test with 999 permutations under full model.

2.2.3.3 Univariate Analysis of Relations among Stressors, Water Chemistry and Diatoms

Relations among anthropogenic stressors, water chemistry and diatom community composition were explored by examining a series of scatterplots of the sample scores along axis one from the unconstrained ordinations of each data type (anthropogenic stressors, water chemistry, diatom community composition). Because the first axis of each unconstrained ordination explains the greatest amount of variation, this acts as the best single-dimension representation of the differences among sites for each data type. A preliminary Kolmogorov-Smirnov test revealed that the DCA axis 1 scores for the diatom community composition data were not normally distributed. Spearman's rank-correlation analysis, a non-parametric measure of correlation, was, thus, employed to assess bivariate associations between the main gradients of the anthropogenic stressor attributes, water chemistry and diatom community composition. In order to establish a strong basis for the further development of biomonitoring protocols, close association between axis 1 sample scores of diatom community composition and anthropogenic stressors must occur. To determine whether the association between stress and response variables (water chemistry, diatom community composition) was uniform along the full length of the gradients, I performed a Davies breakpoint analysis on each pairwise comparison of the three data types (Muggeo, 2008). Davies breakpoint analysis was used to explore the continuity of trends within each of the three pairwise comparisons. Spearman's rank-correlation analysis and Kolmogorov-Smirnov tests were performed using SSPS software (IBM Corp., 2011), Davies breakpoint analysis was performed using the R statistic package 'segmented' (R Development Core Team, 2008). For all significance tests, alpha was set at 0.05.

2.3 Results

2.3.1 *Anthropogenic Stressors*

The first two axes of the PCA ordination exploring the distribution of the anthropogenic stressors among sites explained 55.5% of the total variation (Fig. 2.1). The first axis explained 41.2% of the total variation and separated basins with higher amounts of anthropogenic land-use and those with *Bythotrephes* invasions and dams, positioned to the right (e.g., Lake Vernon (Hunter's Bay)-L197A, Peninsula Lake (West)-L743A and Lake Muskoka (Gravenhurst Bay)-L219C), from basins with relatively low values of those attributes (and correspondingly higher proportions of undisturbed land) which are positioned towards the left (e.g., West Dolly Lake-L353, Bear Lake-L363 and Ishkuday Lake-L719). The second axis of the PCA explains 14.3% of the total variation and captured mainly a gradient of percent cover by golf courses and agricultural land.

2.3.2 Water Chemistry

Analysis of the water chemistry data revealed that concentrations of TP (1.7-25 µg/L) and TKN (160-574 µg/L) spanned a fairly broad range across the 86 sampled basins, as did conductivity (9.80-232.0 µS/cm), and pH (5.23-7.47) (Table 1).

Ordination of the water chemistry data by PCA explained 63.6% of the total variation among lake basins along the first two axes (Fig. 2.2). The first axis explained 44.1% of the total variation, and the lake basins with relatively high anthropogenic stressor scores were positioned towards the right along axis 1. These basins had relatively higher Gran alkalinity, pH and concentrations of DIC, dissolved nitrogen species and major ions. Lakes with relatively low anthropogenic stressor scores were positioned to the left along axis 1, associated with lower values of these variables. Axis 2 explained 19.5% of the variation, and captured gradients of TP, TKN and DOC concentration. Lakes with relatively high values of these variables were positioned high along axis 2, and were associated with basins possessing relatively low values of the anthropogenic stressors but relatively high percentage of cover by wetlands.

2.3.2.1 Relations among Anthropogenic Stressors and Water Chemistry Variables

In an RDA with water chemistry variables constrained by the anthropogenic stressors, the first two axes explained 35.1% of the total variation among sites (Fig. 2.3). The relative orientation of sample scores and vectors of the water chemistry variables did not differ much between the unconstrained ordination with water chemistry variables alone (Fig. 2.2) and the RDA ordination with variation in water chemistry constrained to the anthropogenic stressors (Fig. 2.3). Eigenvalues of the first and second axes explain approximately half of variation in the constrained versus unconstrained ordinations with the water chemistry data. Although there is a greater proportion of unexplained variation, the nine anthropogenic stressors selected for this study appear to adequately capture the variation in water chemistry conditions among sites.

The first RDA axis captured 27.7% of the variation in water chemistry variables, and identified strong association between anthropogenic stressors and ionic content of lake water. Specifically, eigenvectors for road density, road proximity and percent cover by urban development and agricultural lands were positioned to the right along axis 1, associated with relatively high Gran alkalinity, conductivity, pH and concentrations of major ions (Ca, Na and Cl). Vectors for concentrations of TP, TKN and DOC were positioned positively along axis 2, and were uncorrelated to most of the other anthropogenic stressors. However, concentrations of TP, TKN and DOC were weakly and negatively correlated with the presence of *Bythotrephes* and hydraulic control structures.

2.3.3 *Variation in Periphytic Diatom Community Composition among Lakes*

A total of 313 diatom taxa were observed, of which 150 met the criteria to be included in the data analyses. Those taxa are listed in Appendix B. The first two DCA axes explained 22.2% of the total variation in the diatom data. The first axis explained 15.7% of the variation (Fig. 2.4). Although these values were low, they are not uncommon for large diatom data sets due to high frequency of zero values (Stevenson et al. 1991). Periphytic diatom communities of the lake basins were dominated mainly by *Achnanthidium minutissimum* (0% - 62.1%), *Brachysira microcephala* (0% - 28.1%), *Frustulia rhomboides* (0% - 11.9%), *Kobayasiella subtilissima* (0% - 18.2%) and *Tabellaria flocculosa* (0% - 79.6%). The two most abundant species, *A. minutissimum* and *T. flocculosa*, were rarely co-dominant. Rather, *A.*

minutissimum, along with *B. microcephala* and *Fragilaria capucina* (complex), tended to occur with highest relative abundance in lake basins with high values of the anthropogenic stressors, whereas *T. flocculosa*, *Cyclotella pseudostelligera*, *Frustulia rhombodies* and *Eunotia implicata* tended occur with the highest relative abundance in lake basins where the abundance of anthropogenic stressors was low. This ordination illustrated that despite a modest overlap between sites of differing total stressor levels, sample scores for basins with lower abundances of stressors within their catchment (small circles) tend to be positioned towards the left along axis 1, whereas sample scores for sites with higher levels of stressors (large circles) are positioned towards the right (Fig. 2.4).

2.3.3.1 Relations between Water Chemistry and Composition of Periphytic Diatom Communities

A CCA was used to assess relations between periphytic diatom community composition and water chemistry variables among sites. The first two CCA axes explained 10.9% of the total variation (Fig. 2.5). The first axis explained 6.8% of the total variation (p-value < 0.001) and separated diatom communities characteristic of sites with relatively high concentrations of major ions and high pH, positioned to the right, from communities associated with lower concentrations of major ions and lower pH, positioned to the left. Sites with relatively high ion content and high pH tended to have higher relative abundance of *Fragilaria* and *Achnanthes* taxa. These sites were characterized by higher levels of stressors, as illustrated by the relatively large size of the symbols for the site scores (Fig. 2.5, left panel). Conversely, sites with relatively low ion concentrations and low pH had higher percent abundance of *Tabellaria* and *Eunotia* taxa. These sites were characterized by lower levels of stressors, as illustrated by the relatively small size of the symbols for the site scores (Fig. 2.5, left panel). The second CCA axis explained 2.9% of the total variation and captured differences in diatom community composition along gradients of TKN, DOC and TP concentrations. The lack of a systematic pattern along CCA axis 2 in the size of symbols of the site scores suggests that axis 2 does not capture a gradient of anthropogenic stress. Of the 16 water chemistry variables assessed through CCA, 14 were found to explain significant directions of variation in the composition of the diatom communities (Table 2).

2.3.3.2 Relations among Anthropogenic Stressors and Composition of Periphytic Diatom Communities

The first two axes of the CCA with diatom community composition constrained to anthropogenic stressors explained 8.6% of the total variation between sites (Fig. 2.6). The first axis explained 5.2% of the total variation observed in diatom community composition (p-value < 0.001), and was correlated with most of the anthropogenic stressors. Specifically, sites positioned towards the right along axis 1 were associated with relatively close proximity to roads, high values of road density and percent urban area, as well as the presence of hydraulic control structures and *Bythotrephes*. Sites with high values of these stressors were associated with relatively higher abundance of the diatoms *A. minutissimum*, *Nitzschia palea*, *Fragilaria capucina* and *B. microcephala*. Sites plotting towards the left along axis 1 were associated with less disturbed basins and possessed higher percent abundance of *T. flocculosa*, *E. implicata*, *C. pseudostelligera*, and *Stenopterobia curvula*. The second axis explained 3.4% of the total variation and captured only modest changes in diatom community composition associated with differences in percent agricultural land cover.

2.3.4 ***Variation Partitioning Analysis***

The combination of natural attributes, anthropogenic stressors and spatial variables explained a total of 34.3% of the variation in diatom community composition among lakes (Fig. 2.7). Significant variation ($p < 0.05$) in the diatom community composition was explained by unique effects of all three categories (natural (11.6%), anthropogenic (11.3%) and spatial (5.3%)). Using forward selection, catchment area to lake area ratio and catchment slope were selected as the natural attributes that explained unique and significant directions of variation in diatom community composition. Percent urban development, road density and road proximity were selected for the anthropogenic stressors category. And, longitude was selected for the spatial category. Covariation among the explanatory categories did not capture a large proportion of the variation. For example, covariation between the anthropogenic stressors and natural attributes explained the greatest amount of covariation (2.6%) compared to the other covariation terms (Fig. 2.7).

2.3.5 *Correlation among Anthropogenic Stressor Scores and Response Variables*

Sample scores along axis 1 from the indirect gradient ordinations of anthropogenic stressors, water chemistry and periphytic diatom community composition demonstrated strong bivariate associations (Fig. 2.8). For all pairwise comparisons, the correlation was positive and statistically significant. Correlation between first axis scores for anthropogenic stressors and water chemistry variables showed the strongest correlation (r_s= 0.842, p = 3.31 x10^{-24}) and appeared linear in nature. The associations are strong, but two interesting trends became apparent in the form of the relations between diatom composition and water chemistry (r_s = 0.699, p = 7.00 x10^{-14}) and diatom composition and the abundance of anthropogenic stressors (r_s = 0.642, p = 2.80 x10^{-11}). First, the relation between diatom composition and water chemistry appears to be non-linear. Specifically, Davies breakpoint analysis detected a significant difference in the rate of change in diatom community scores across the gradient of water chemistry scores (t = 6.767; p-value = 1.81 x10^{-9}), where the rate of change becomes reduced at water chemistry PCA scores above zero. Second, the association between diatom composition and the abundance of anthropogenic stressors demonstrates higher variability at low abundances of anthropogenic stressors compared with higher levels of the stressors.

2.4 Discussion

Analyses of the 86 lake basins within the MRW identified that the magnitude of multiple anthropogenic stressors, especially the proximity and density of roadways, and percent cover by urban development in the catchment of lakes, exert a consistent, directional influence on water quality and the composition of periphytic diatom communities. These stressors resulted in altered water quality, where ionic content, conductivity and Gran alkalinity increased in tandem with the gradient of anthropogenic stressors. The close association observed between proximity and density of roadways, ion concentrations and periphytic diatom community composition, suggests that ions from road salt (NaCl), dust suppressant ($MgCl_2$ and $CaCl_2$) and roadway construction and maintenance may be influential in altering water chemistry and diatom community composition of basins in the MRW (Mattson & Godfrey, 1994). Partial CCAs identify Mg, Ca and Cl as explaining significant and unique directions of variance in diatom community composition. Concentration of Na was observed to increase with the abundance of anthropogenic stressors, although it was not found to explain significant amounts of variation in diatom community composition. Ion loading, especially Na and Cl, from anthropogenic sources within the catchment has been identified as an emerging threat to water quality within the MRW (Dixit et al. 1992; Palmer et al. 2011). Molot and Dillon (2008) suggest that Na export from lake catchments within the MRW has increased markedly since the mid-1980s, due to increased use of sand-salt road-safety agents during winter months. This finding has been echoed by Watmough and Aherne (2008) and Palmer et al. (2011), suggesting that excessive use of NaCl-based road salt may present a significant threat to water quality and biological integrity in developed areas of the MRW.

In addition to road salts, the use of dust suppressants on unpaved roadways has been found to be an important source of ion loading, specifically Ca, Mg and Cl, to nearby lakes (Palmer et al., 2011). This may present a paradox for lake managers, as anthropogenic Ca and Mg loading from dust suppressants may help combat declining Ca concentrations observed throughout the MRW as a result of past and ongoing acidification and logging activities (Cairns & Yan, 2009; Yao et al. 2011). Declines in Ca concentration have affected calcareous aquatic organisms in many softwater lakes (Jeziorski et al. 2008; DeSellas et al. 2011). Similar consequences have been observed as a result of declines in Mg (Molot &

Dillon, 2008). However, increasing Cl levels from anthropogenic loading may also reduce reproductive viability of these organisms (Gardner & Royer, 2010), although controlled bioassay experiments suggest that ion concentrations observed within the MRW (mean Cl concentration 4.35 mg/L) remain well below biological thresholds (Benbow & Merritt, 2004; Gardner & Royer, 2010; Palmer et al. 2011). Nevertheless, based on the observations made in this chapter, measurable shifts in the community structure of periphytic diatoms correspond closely with increases in ion concentration. Thus, periphytic diatoms may serve as important early-warning indicators of ecological changes resulting from this emerging stressor.

Nutrients TP, TKN and DOC, were found to explain a significant proportion of the variation in diatom community composition. Agricultural operations and golf courses can act as significant sources of these nutrients, contributing to water-quality and biological degradation of aquatic environments (Correll, 1998; Winter & Duthie, 2000; Winter et al., 2002). While there is a general perception that these stressors are influential to lakes within the MRW, neither was found to demonstrate strong correlation with nutrient concentrations. This may be in part because neither stressor is found in high density across the watershed (Tran & Brouse, 2009). Agricultural operations are limited within the MRW, because thin, nutrient-poor soils do not support sufficient crop growth in much of the region (Chapman & Putnam, 1984; Smit et al. 1991). Similarly, while golf courses may act as important point-sources of nutrient loading, golf courses cover a proportionately small portion of catchments within the MRW. As a result, the influence of these stressors on periphytic diatom community composition may be observed at a local scale (Winter et al. 2003), but do not account for important amounts of difference in diatom community composition across the MRW.

The presence of hydraulic control structures was negatively associated with TP, TKN and DOC concentrations. Quinlan et al. (2008) suggest that this may be a consequence of lake deepening, due to damming, effectively diluting nutrient inputs. By raising water levels or increasing (or decreasing) seasonal water-level fluctuations, dams should have a substantial effect on biota living in the nearshore zone of lakes. This has been observed to influence walleye (*Sander vitreus*) populations within the MRW, as construction of hydraulic control structures has been attributed to declines in breeding success (EGBSC et al., 2007). Additionally, the presence of *Bythotrephes* demonstrated co-variation with hydraulic control structures. Within the MRW, hydraulic control structures serve a number of purposes. However, the primary role for many dams is to maintain water levels in upstream and downstream water bodies for recreational use. As a result, accidental introduction of *Bythotrephes* into lakes with high amounts of recreational boating and fishing is common (Weisz & Yan, 2010). The establishment of *Bythotrephes* within the MRW has had a substantial influence on trophic structure of lakes by altering the community composition of zooplankton (Yan & Pawson, 1997; Yan et al. 2002; Weisz & Yan, 2011). Although the ecological impacts of both hydraulic control structures and *Bythotrephes* have been widely observed in other studies, forward selection suggests that these stressors do not account for significant amounts of variation in diatom community composition.

Total phosphorus concentration (TP) commonly limits primary production in Precambrian Shield lakes, and is often a strong driver of diatom community composition (Hall & Smol, 1996; Lavoie et al. 2006; Gottschalk & Kalhert, 2012). However, due to the narrow anthropogenic stressor gradient and limited land-use data available for the MRW, TP concentration was relatively weakly associated with total anthropogenic stress. Nutrient loading from natural sources may effectively mask the influence of the modest anthropogenic sources that may otherwise have been observed. Natural sources of TP, such as wetlands and forested areas, have been noted as important drivers of the TP gradient within the MRW (Dillon et al. 1991; Dillon & Molot, 1997).

Change in water chemistry was observed to respond linearly to increases in anthropogenic stressors. This was characterized largely by increases in ion concentration and pH. Additionally, examination of the pattern of change in diatom community composition along the gradient of water chemistry revealed a non-linear trend, with diatom composition changing at a higher rate at the low end of the water chemistry gradient. At moderate to high regions of the water chemistry gradient, the rate of diatom community composition response became reduced. The high rate of change observed at the low end of the water-chemistry gradient suggests that diatom communities are sensitive indicators of early water quality degradation. This feature suggests that water quality of the least disturbed lakes needs to be preserved in order to effectively guard against biotic changes as a result of human activities. Lakes of least disturbance may act as important refugia in the future for sensitive aquatic taxa unable to withstand ion enrichment.

The relation observed between the abundance of anthropogenic stressors and diatom community composition was found to be linear. However, higher variability in diatom community composition was observed among lakes situated at the low end of the stressor gradient. This suggests that diatom response may be less consistent at lower levels of anthropogenic stress. Analyses presented in Figure 2.9 suggest that the influence of pH in low-stressed lakes may account for the higher variability in diatom community composition, as a relatively wide range of pH values are observed in these lakes. In contrast, lake-water pH remains relatively constant among lakes with modest to high levels of anthropogenic stressors, which likely allows ion concentration to act as the primary driver of differences in community composition. Lakes with low pH (<6.45) tend to have lower DCA axis 1 scores for diatom community composition relative to sites with higher pH (Fig. 2.10). Thus, in lakes with lower abundances of anthropogenic stressors, acidity exerts important influence on diatom community composition and confounds the influence of variation in ionic concentration, which greater variability of diatom DCA scores. For example, Un-named Lake L289 (pH=5.86) reports a diatom score of 0, whereas Bear Lake L363 (pH = 6.81) has a diatom score of 1.976. The nearly ten-fold increase in acidity acts as an important determinant of the diatom community composition observed in these lakes, which are otherwise similar in their stressor abundance and location. This finding agrees with Hall & Smol (1996), who suggest that pH acts a principal driver of diatom community composition. Within the MRW, pH is influenced by both natural and anthropogenic factors (LaZerte et al., 1984; Aherne et al., 2006), with past acid precipitation and localized geochemistry acting as important drivers (Dillon et al., 1978). Catchments with thicker soils and surficial sediments provide a higher natural buffering capacity, allowing lakes to be more resistant to acidification (Dillon et al., 1978; Keller, 1992). However, these catchment attributes are found to be highly localized, limiting the predictability of pH based on geographical ordination. The pH of lakes should be noted as part of sampling protocols, as it may contribute substantial variation in diatom community composition, especially in low-stressed locations.

A combination of natural attributes, spatial factors, and anthropogenic stressors explained 34.3% of the total variation in diatom community structure observed among lake basins. Approximately equivalent amounts of the explained variation were attributed to the influence of natural basin attributes (11.6%) and anthropogenic stressors (11.3%). Both natural and anthropogenic factors play important roles in affecting diatom community composition. This finding is supported by the high degree of 'naturalness' (or, non-anthropogenic land cover) of the MRW. Forward selection revealed that catchment-to-lake area ratio and catchment slope accounted for significant amounts of variance in diatom community composition. As a result, lakes must be managed in a manner which recognizes the importance of natural lake and basin attributes. For example, due to its very high catchment to lake area ratio and high percent wetlands, Brandy Lake (L660) experiences high allochthonous inputs of DOC and nutrients from the catchment (Watmough et al., 2005; DeSellas et al., 2011). Proportionately, Brandy Lake features only moderate levels of anthropogenic stressors although, given its very large catchment area, the absolute abundance of stressors is high. Combined with natural sources of inputs, lakes with high catchment-to-lake area ratios may appear to be more degraded (Carignan and Steedman, 2000). Similarly, lake catchments with relatively steep slopes may export ions and nutrients at a faster rate than catchments which relatively little relief (D'Arcy and Carignan, 1997; Aitkenhead-Peterson et al. 2005; Kreutzweiser et al., 2008). For example, Lake of Bays (Trading Bay-L210C) is observed to be one of the more degraded lake basins within the MRW. Although this degradation is largely due to the influence of anthropogenic stressors found within the catchment, it also features the highest mean slope (17%). Managing anthropogenic stressors is important; however consideration should be made for naturally occurring features as well.

2.4.1 Application of Periphyton Biomonitoring

Strong relations were observed between the anthropogenic stressor scores and gradients of both water chemistry and diatom community composition. Relations with the stressor scores were slightly stronger for water chemistry than for the diatoms, but both relations are sufficiently strong to form the foundation for the development of monitoring programs. Use of a biological metric such as diatoms provides direct information about the ecological effects of anthropogenic stressors rather than simply the effects on chemical conditions. Diatoms as a bioindicator integrate information continually, and are able to convey information about influential short-lived events that can be missed by periodic water chemistry sampling (Lambert et al., 2008). Widespread use of periphytic diatom biomonitoring protocols for stream environments suggests that regulatory compliance agencies have gained sufficient confidence in their application (Biggs & Kilroy, 2000; Solak & Ács, 2011). Comparatively, periphytic diatom bioassessment in lakes has been largely absent (Thomas et al. 2011; Gottschalk & Kalhert, 2012). However, the analysis of diatoms within lakes of the MRW is particularly useful as extensive paleolimnological diatom data exists (e.g., Hall & Smol, 1996; Quinlan et al., 2008; Hadley et al., 2013), which can be used in conjunction with contemporary samples to provide a temporal understanding of ecological change dating back to pre-European settlement of the region. Periphytic diatoms are well suited for biomonitoring, requiring only minimal effort to collect samples, from which a well-trained technician can provide relative abundance information regarding a specific lake (Resh, 2008; Thomas et al. 2011). Diatom sample preparation leads to construction of permanent microscope slides that can be retained for re-analysis in the future (quality-control, taxonomic consistency, re-analysis to gain information that was missed initially but that comes to light in the future), a valuable feature in the early development of any biomonitoring program.

As mentioned previously, pH can have a confounding influence on the community composition at low end of the anthropogenic stressor gradient. Unlike ion concentration that varies closely with the abundance of anthropogenic stress, pH appears to vary naturally among lakes. Consequently, variation in diatom community composition due to pH does not aid in the assessment of biological response to the nine anthropogenic stressors identified in this study. Perhaps as a method of dealing with the confounding influence of pH, bioassessments should utilize stratified (by pH) sampling designs or account for pH effects during data analysis. Assessing lakes which are grouped by pH can allow for better detection of impairment due to loading of nutrients and ions. These improvements can reduce confounding variability and improve overall assessments of biological integrity.

Ordinations presented in this chapter were important to demonstrate the strong relations between diatom community composition, stressors and water chemistry. The findings outlined in this chapter, provide evidence which can be used to support the development and application of periphyton-based protocols for use in long-term biomonitoring programs. The combination of effectiveness and logistic feasibility make the assessment of periphytic diatom community composition a viable option when looking to improve lake biomonitoring programs. However, bioassessment based on community composition generates complex multivariate datasets that are challenging to use in practical ways by monitoring agencies (Resh, 2008). This is because information is communicated as complex multivariate ordination analyses and biplots that only highly-trained persons can understand and interpret. Biomonitoring programs are most effective for use in management if they can communicate complex observations of community composition through relatively simple and effective descriptions of ecosystem status. A lack of intuitive analytical tools for assessing algal assemblages in lakes has often been identified as an important barrier to employing biomonitoring methods (Resh, 2008). As a result, other biomonitoring methods, deficient in other regards, may be favoured if they are more amenable to application of intuitive analytical tools (Barbour et al., 1999; Mazor et al., 2006; Resh, 2008). Thus, when assessing periphytic diatom community composition in lakes, there is a need to convert the complex multivariate data to simpler univariate metrics, such as indices of biotic integrity. Periphyton assessments of stream environments have been successful in employing a variety of bioassessment indices to track the biological responses to anthropogenic stressors (e.g. Coste, 1982; Kelly & Whitton, 1995; Lenoir & Coste, 1996; Lavoie et al. 2006). The development of an efficient method of bioassessment, similar to what is employed for the assessment of stream periphyton is an important next step in advancing the use of periphytic diatom community composition in lakes. A tool such as this can provide lake managers with the ability to effectively establish the range of expected biological responses, from which future biomonitoring can be employed. The next chapter will address this need by developing simpler indices for application in a MRW lake biomonitoring program.

2.5 Figures & Tables

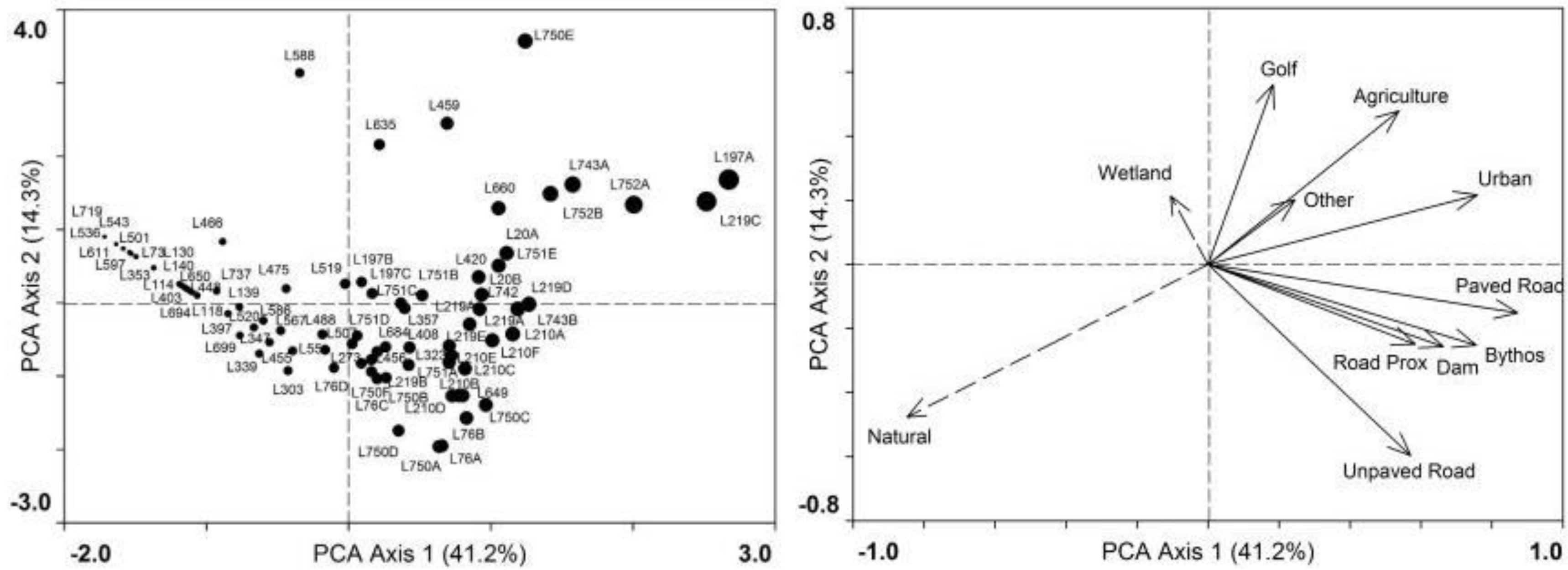

Figure 2.1. Principal Components Analysis ordination plots illustrating the distributions of sampled lake basins within the Muskoka River watershed with respect to the nine identified anthropogenic stressors. Left panel shows the distribution of sample scores along the first two axes. The right panel shows eigenvectors of the stressor variables. Because values of stressor variables increase along axis 1, the axis 1 sample scores were used to assess relations with water chemistry and diatom communities in subsequent ordinations. To do this, the size of the sample scores are scaled in proportion to their axis 1 scores, so that small symbols have low amounts of total stress, whereas large symbols have relatively higher amounts of stress. Lake names corresponding to the codes in the left panel are presented in Appendix A. Variables 'Natural' and 'Wetland' denoting natural land-cover are plotted as supplementary variables and illustrated as dashed vectors.

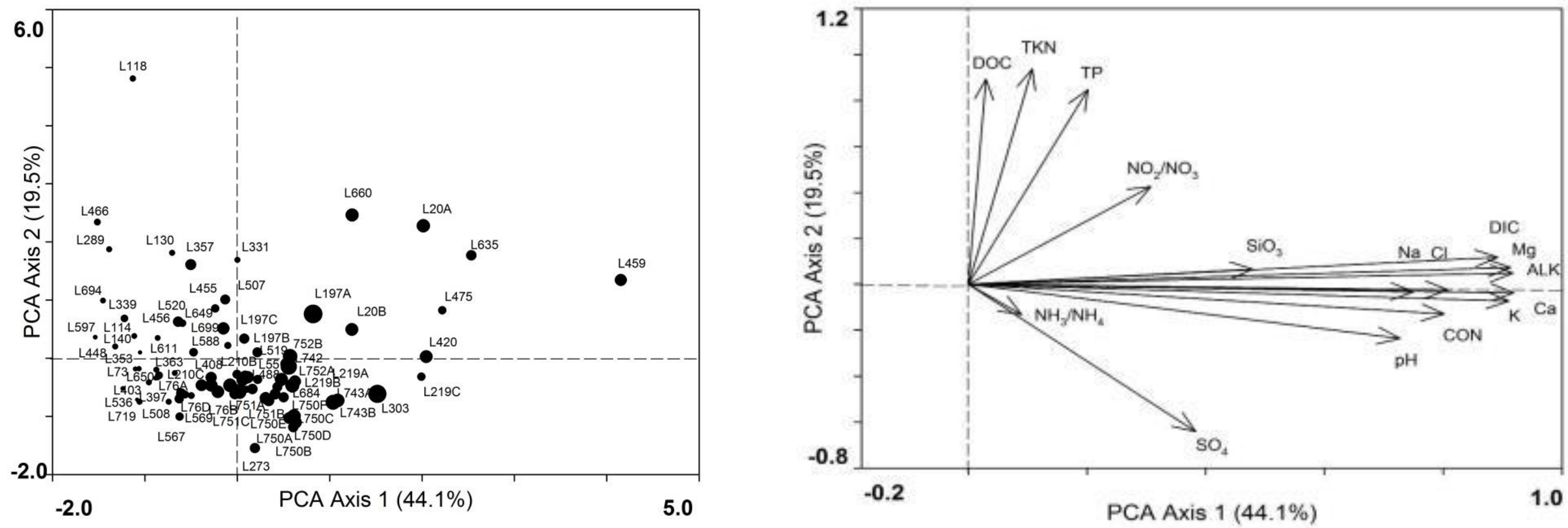

Figure 2.2. Principal Component Analysis ordination of sampled basins within the Muskoka River watershed with respect to the water chemistry variables measured in the nearshore reaches. Left panel: Lake scores are coded by size in proportion to first axis stress scores of PCA ordination of anthropogenic stressor variables illustrated in Fig. 1 (small symbols have low amounts of total stress, large symbols have relatively higher amounts of stress). Right panel: Ordination of water chemistry variables, all of which are plotted as active variables. Lake names corresponding to the codes in the left panel are presented in Appendix A.

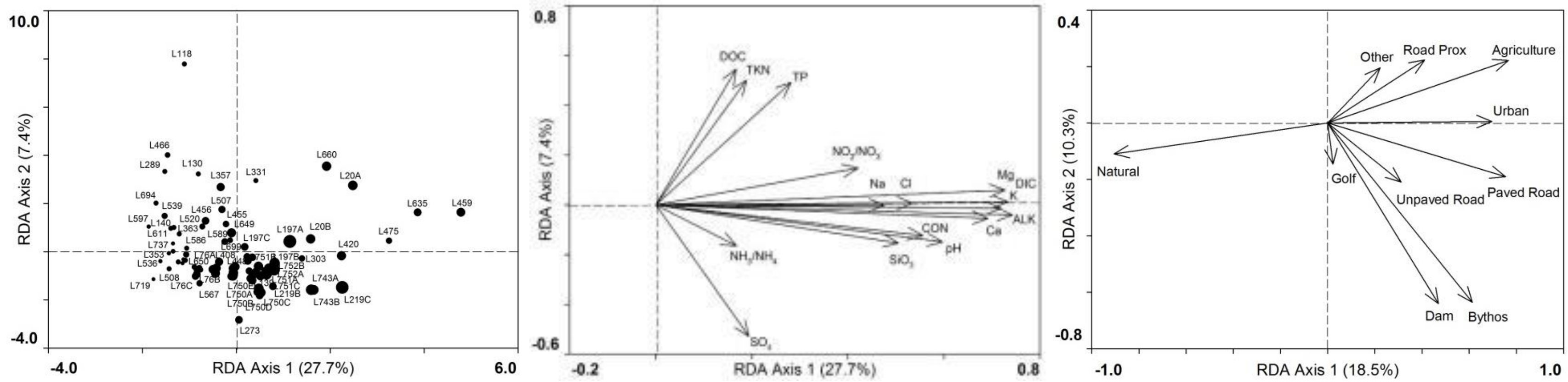

Figure 2.3. Redundancy Analysis ordination illustrating patterns of water chemistry variables among nearshore reaches of MRW lakes as they are constrained by anthropogenic stressors within their catchments. Left panel: Lake site scores are coded by size in proportion to first axis stress scores of PCA ordination of stressor variables illustrated in Fig. 1 (small symbols have low amounts of total stress, large symbols have relatively higher amounts of stress). Centre panel: Eigenvectors for each water chemistry variables. Right panel: Ordination of stressor attributes. Lake names corresponding to the codes in the left panel are presented in Appendix A.

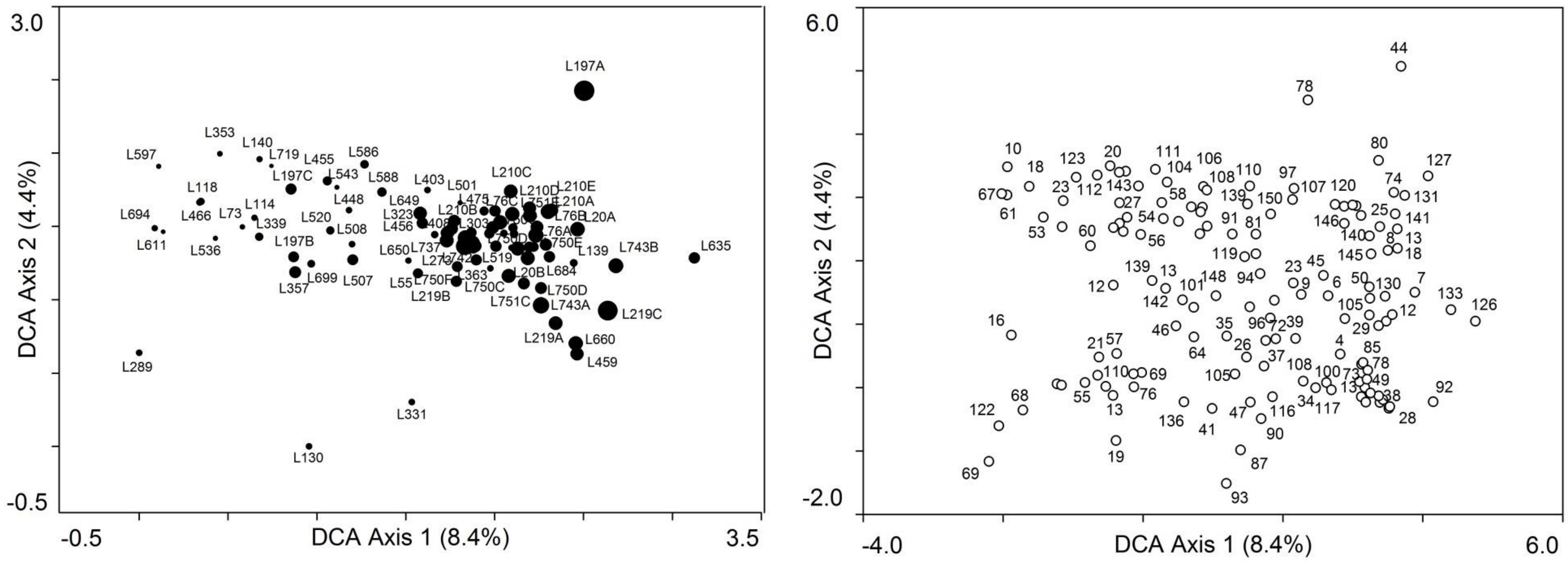

Figure 2.4. Detrended Correspondence Analysis ordination illustrating the variation in diatom community composition across the Muskoka River watershed. Left panel: Lake scores are coded by size in proportion to first axis stress scores of DCA ordination of stressor variables illustrated in Fig. 1 (small symbols have low amounts of total stress, large symbols have relatively higher amounts of stress). Right Panel: Distribution of diatom taxa. Taxon codes correspond with the taxa listed in Appendix B. Lake names corresponding to the codes in the left panel are presented in Appendix A.

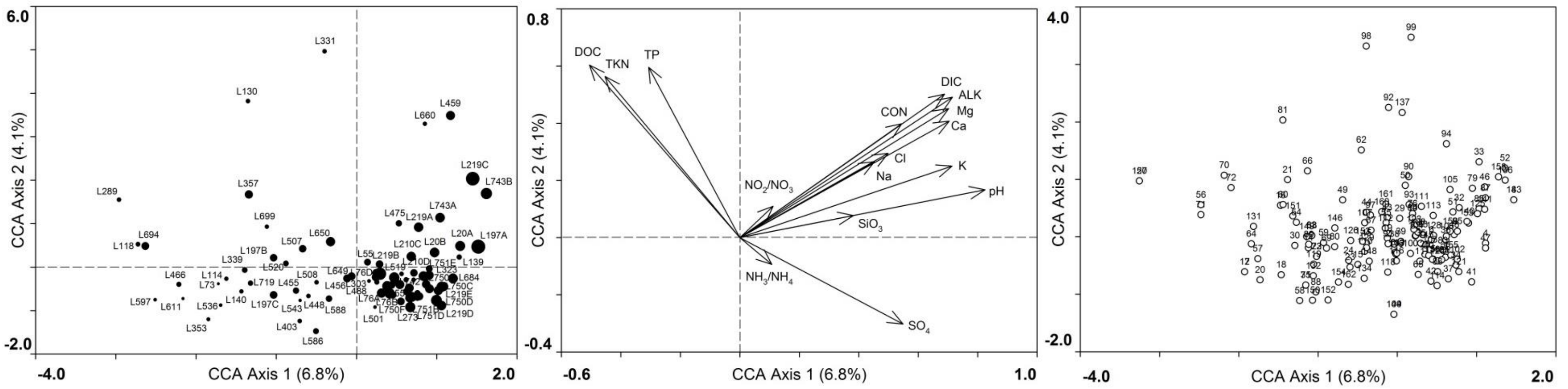

Figure 2.5. Canonical Correspondence Analysis ordination revealing patterns in diatom community composition constrained by water chemistry variables. Left panel: Lake scores are coded by size in proportion to first axis stress scores of PCA ordination of stressor variables illustrated in Fig. 1 (small symbols have low amounts of total stress, large symbols have relatively higher amounts of stress). Centre panel: Ordination of water chemistry variables. Right panel: Distribution of diatom taxa. Taxon codes correspond with the taxa listed in Appendix B. Lake names corresponding to the codes in the left panel are presented in Appendix A.

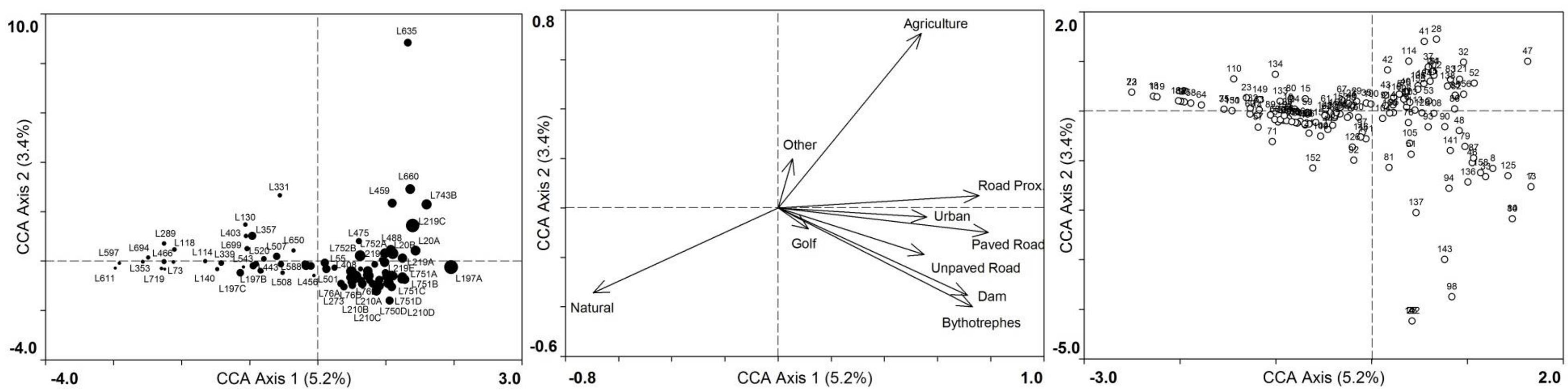

Figure 2.6. Canonical Correspondence Analysis ordination illustrating variation in diatom community composition among lakes of the MRW as they are constrained by anthropogenic stressors within their catchments. Left panel: Lake scores are coded by size in proportion to first axis stress scores of PCA ordination of stressor variables illustrated in Fig. 1 (small symbols have low amounts of total stress, large symbols have relatively higher amounts of stress). Centre panel: Ordination of catchment stressor attributes. Right panel: distribution of diatom taxa. Taxon codes correspond with the taxa listed in Appendix B. Lake names corresponding to the codes in the left panel are presented in Appendix A.

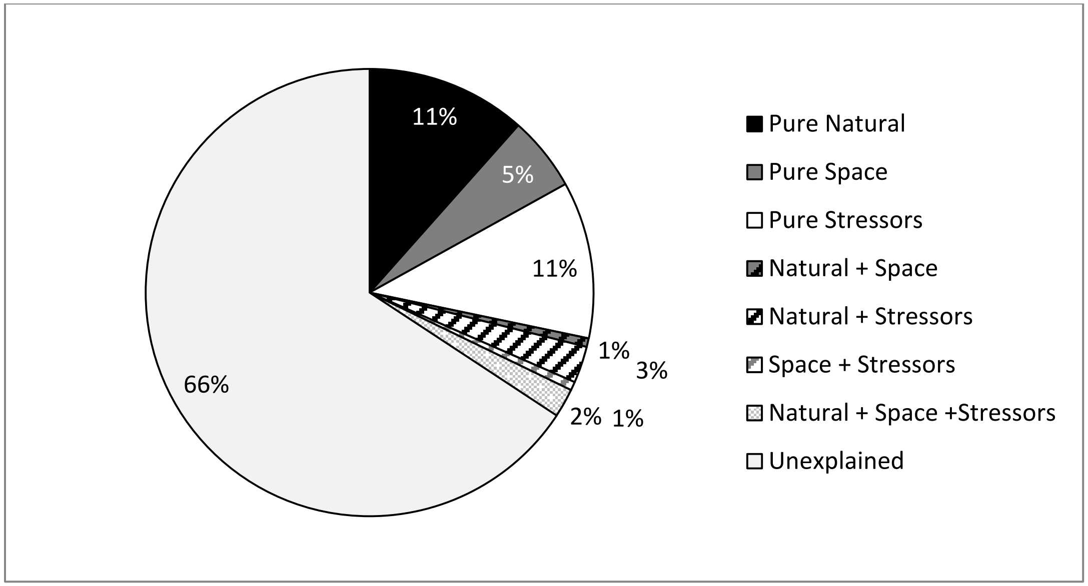

Figure 2.7. Variation Partitioning Analysis illustrates the relative amount of variation explained by anthropogenic stressors, spatial factors and naturally occuring physical catchment variables, as well as covariaton among them. A total of 34% of the total variation observed in diatom community composition among lakes within the Muskoka River watershed was explained by these factors, and 66% of the total variation in diatom commmunity composition among lakes remained unexplained.

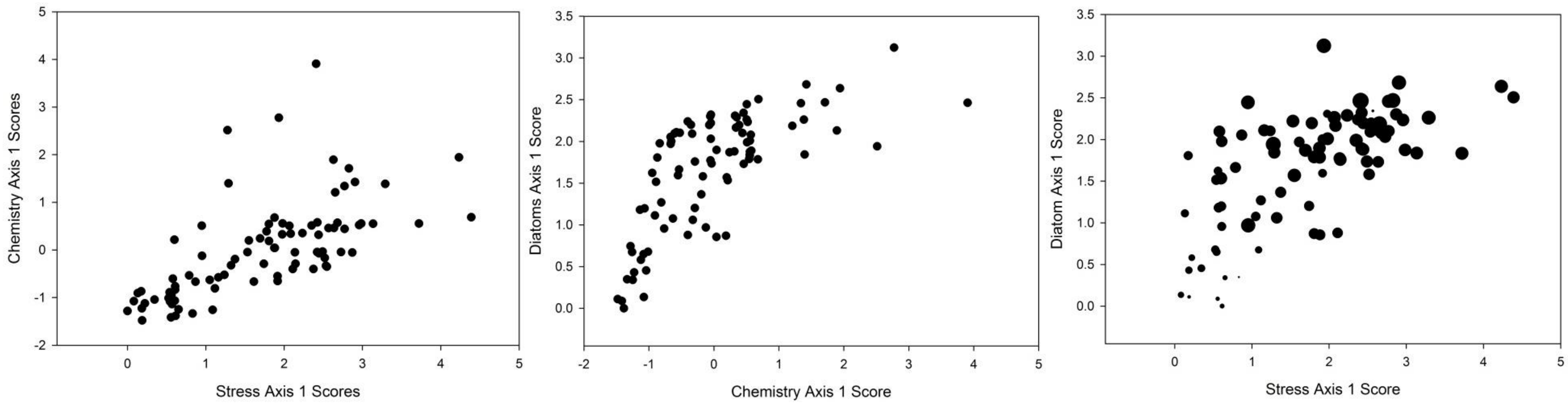

Figure 2.8a-c. Scatterplots illustrating relations among indirect-gradient axis 1 scores for lakes in the MRW of: (a) anthropogenic stressors acting within the catchment versus nearshore water chemistry (b) nearshore water chemistry versus diatom community composition, and (c) anthropogenic stressors acting within the catchment versus diatom community composition.

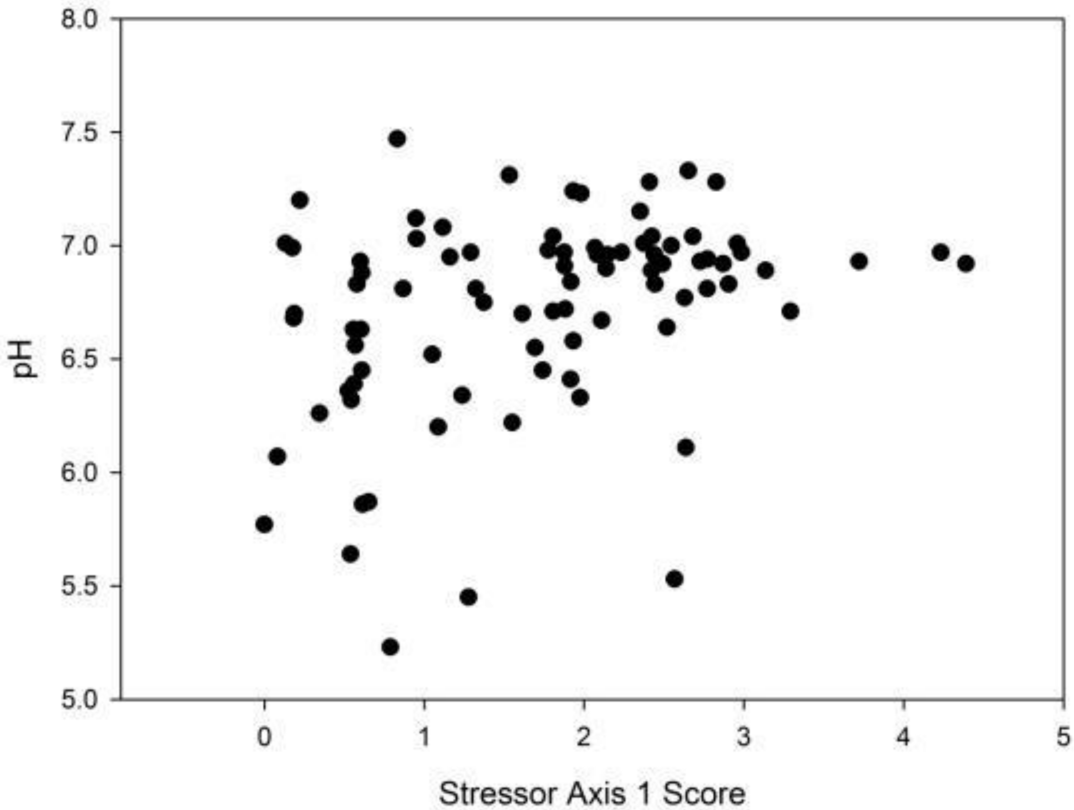

Figure 2.9. Regression illustrating the relation between stressor abundance of each lake and the observed lake-water pH. Lake-water pH appears to be much more variable in lake basins with low levels of the anthropogenic stressors.

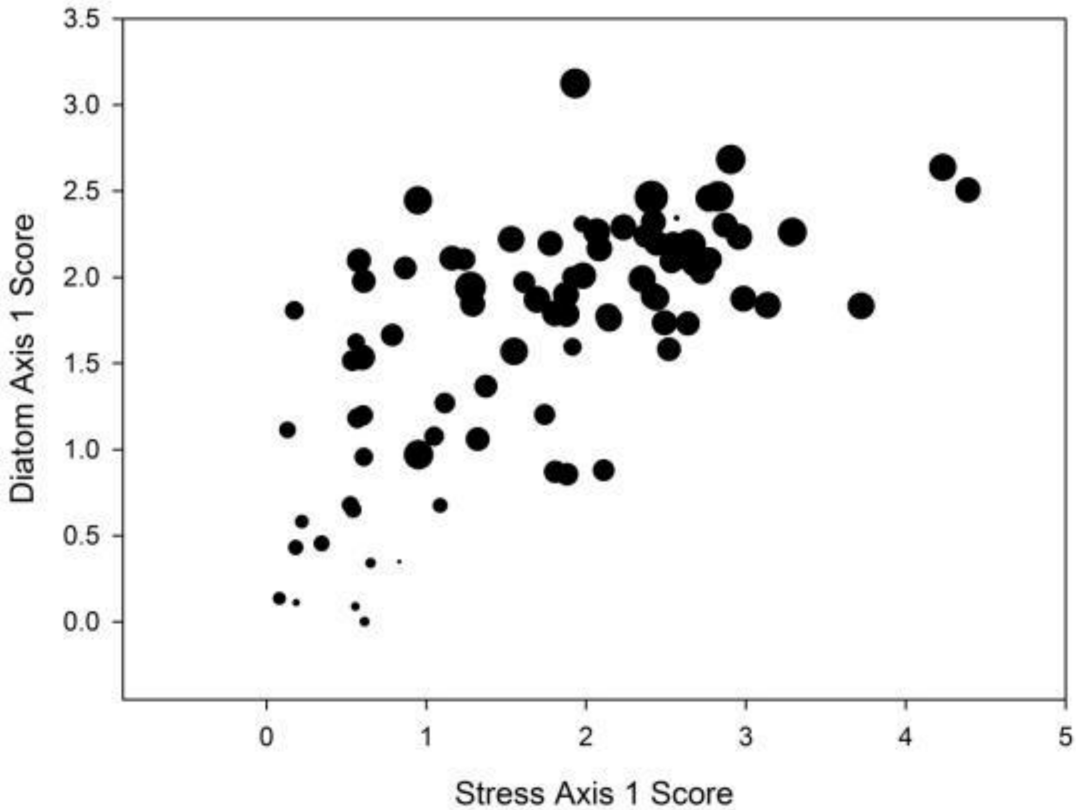

Figure 2.10. Scatterplot illustrating the relation between abundance of anthropogenic stressors (stressor PCA axis 1 score) and diatom community composition (diatom DCA axis 1 score). Symbols are sized in proportion to lake-water pH (small symbols represent sites with lower (acidic) pH; larger symbols represent sites with higher (circumneutral) pH.

Table 2.1. Central tendency and range for selected water chemistry variables based on composite water chemistry samples from nearshore reaches of 86 lakes within the Muskoka River watershed during July and August of 2012. For full water chemistry results for each site see Appendix C.

Parameter	Mean	Median	Minimum	Maximum
Gran alkalinity (mg/L of $CaCO_3$)	8.28	8.7	2.9	20.4
Ca (mg/L)	2.62	2.4	0.8	9.0
Cl (mg/L)	4.35	2.7	0.0	51.6
Conductivity (µS/cm)	48.80	35.2	9.8	232.0
DIC (mg/L)	2.34	1.35	0.4	49.7
DOC (mg/L)	4.90	4.4	2.0	16.1
K (mg/L)	0.47	0.5	0.1	1.3
Mg (mg/L)	3.26	2.6	0.2	26.2
Na (mg/L)	3.24	2.3	0.46	29.9
NH_3/NH_4 (µg/L)	13.50	8.0	2.0	286.0
NO_2/NO_3 (µg/L)	9.10	4.0	2.0	52.0
TKN (µg/L)	290.93	267.5	160.0	574.0
TP (µg/L)	6.75	4.7	1.7	25.0
pH	6.73	6.86	5.23	7.47
SiO3 (mg/L)	0.80	0.98	0.0	3.4
SO_4 (mg/L)	3.96	4.2	0.1	8.4

Table 2.2. Summary of partial canonical correspondence analysis (partial CCA) of periphytic diatom community composition to assess the variance explained along the first (**λ_1**) and second (**λ_2**) axes by selected water chemistry variables. CCAs were run with a single constraining variable at a time. First axis p-value is determined by Monte Carlo permutation tests (999 unrestricted permutations). P-values reported in bold and are significant to $\alpha = 0.05$.

Variable	**λ_1**	**λ_2**	**λ_1/λ_2**	**Axis 1 p-value**
Gran alkalinity	0.236	0.327	0.72	**0.001**
Ca	0.212	0.322	0.66	**0.001**
Cl	0.115	0.327	0.35	**0.020**
Conductivity	0.156	0.325	0.48	**0.001**
DIC	0.232	0.33	0.70	**0.001**
DOC	0.169	0.373	0.45	**0.001**
K	0.211	0.323	0.65	**0.001**
Mg	0.236	0.333	0.71	**0.001**
Na	0.097	0.339	0.29	0.054
NO_2/NO_3	0.191	0.381	0.50	**0.001**
NH_3/NH_4	0.047	0.388	0.12	0.496
TKN	0.161	0.375	0.43	**0.002**
pH	0.238	0.316	0.75	**0.001**
TP	0.141	0.386	0.37	**0.001**
SiO_3	0.131	0.355	0.37	**0.001**
SO_4	0.155	0.348	0.45	**0.001**

-Chapter 3-

The Development and Evaluation of Indices Based on Periphytic Diatom to Assess Anthropogenic Impairment of Lakes within the Muskoka River Watershed

3.1 Introduction

In the previous chapter, it was demonstrated that strong association exists between the composition of periphytic diatom communities and anthropogenic stressors which influence lake conditions. Given the strength of these associations, indices and metrics can be developed to assess the ecological impacts of cumulative effects for lakes within the Muskoka River watershed (MRW) based on periphytic diatom community composition. Biological indices, in one form or another, have been used for more than 160 years to assess water quality (Abbasi & Abbasi, 2013). It is only in the past three decades, however, that the use of biological indices has become widespread for management agencies (Hawkins et al. 2000; Rooney & Bayley, 2012). Currently, more than 90% of watershed management agencies within North America use some form biological index to inform management decisions (Barbour & Yoder, 2000). Indices effectively capture complex information about ecological condition by examining the presence and or abundance of multiple taxa (Karr, 1981; Rosenberg & Resh, 1993). Using this information, which describes community structure, indices can assign a single value or score to a sample (Freund & Petty, 2007). These index scores can be used in an intuitive manner to examine trends, inform management decisions and evaluate the effectiveness of stewardship programs (Abbasi & Abbasi, 2013).

The primary objective of this chapter is to develop bioassessment indices based on the observed association between diatom community composition and anthropogenic stressors, as identified in Chapter 2. The ability of the indices to effectively characterize biological integrity will be evaluated based on the strength of the association observed between the index scores and magnitude of anthropogenic stressors. Specifically, five variations of two classes of indices (biological index (BI) and index of biotic integrity (IBI)) were developed and assessed using lakes sampled from within the MRW. The first index, the Eastern Canadian Diatom Index (IDEC), is a BI which follows methods outlined by Lavoie et al. (2013b; IDEC 3.0). The IDEC was developed and tested in streams throughout Ontario, Québec and the Maritimes, including a number of sample locations from within the MRW (Lavoie et al. 2006, 2008a, 2013b). The performance of the current IDEC, as developed for rivers, plus a version modified to the specific range of conditions observed in our lake set within the MRW (mIDEC), will be assessed. Additionally, three variations of the Index of Biological Integrity (IBI; Karr et al., 1981) will be evaluated. Assessing multiple variations of IBI calculation is important as the method used to calculate and summarize indicator taxon scores can affect the performance of the index (Blocksom, 2003; Rooney & Bayley, 2012). The first variation of the IBI (IBI-1) uses a continuous scoring method, scoring each indicator taxon according to the relative abundance observed. In the second variation (IBI-2), abundances of indicator taxa are scored for each site using fixed integer values (1-5) representing 'bins', defined by ranges of percentile of the maximum relative abundance observed. The final index calculation method (IBI-3) also uses a continuous scoring method. However, it uses the formula developed by Blocksom (2003), whereby each indicator taxon is weighted proportionally to the maximum abundance of that taxon observed across the watershed. Evaluation of each index will be based on the ability to show strong correlation with the measured abundance of anthropogenic stressors influencing each basin (as described in Chapter 2). Based on index performance, recommendations will be made regarding the best index for use by natural resource managers to evaluate the impacts of anthropogenic stressors for lakes in the MRW.

3.2 Methods

To develop indices based on the responses of periphytic diatom community composition within the MRW along a gradient of stressor magnitude, biological samples and stressor attributes were obtained from 86 lake basins, representating of the full range of lakes present throughout the watershed. A PCA ordination was performed to summarize the influence of nine anthropogenic stressors within and surrounding each lake basin. Each basin was assigned a total stress score equivalent to its position along PCA axis 1, allowing for quantification of the stressor gradient while preserving their relative variability of each stressor variable. The lakes range from minimally impacted lake basins, including catchments that are currently absent from any permanent sources of anthropogenic activity, to heavily impacted lake basins within the MRW where at least 70% of the local catchment is altered by anthropogenic land-uses. Periphyton samples were collected from the 86 lakes, and high taxonomic resolution analysis of periphytic diatoms was performed to quantify differences in community composition among sites. The 86 lake basins were sampled across the MRW within an 8-week period during July and August, 2012. A full description of the data collection and analysis is outlined in Chapter 2.

3.2.1 *Development of Biotic Indices (IDEC, mIDEC)*

The IDEC was originally developed by Lavoie et al. (2006), and is based on the evaluation of diatom taxon relative abundances using ordination by correspondence analysis (CA). The IDEC is based on a 600-site training set collected from streams across southern Ontario, Québec and the Maritimes. Ordination by CA was used to calculate three important parameters (Lavoie et al., 2006; 2013b). First are species weights, which are a scaling factor that compensates for the rarity of a given taxon. It is necessary to 'weigh' or 'scale' each taxon on the basis of rarity, as variation in the abundance of a common species contributes less to the overall variation observed among sites than equivalent variation observed in rare taxa (Legendre & Gallagher, 2001). Species scores are the second parameter calculated.

Species scores are the position which each taxon orients along the first CA axis, and are proportional to the amount of variation each taxon demonstrates. Finally, the position in which each site orients along the first axis is calculated. Position of each site is determined by summarizing the scores of all of the weighted taxa observed at a given site (ter Braak & Smilauer, 2002). In their dataset, Lavoie, et al. (2013b) demonstrated that variation along the first CA axis captured mainly a trophic gradient and separated oligotrophic streams from eutrophic streams. Position along the first CA axis (x_i^*) for additional samples can be determined by the observed diatom community composition at each site using equation 1 (ter Braak and Smilauer, 2002):

$$x_i^* = \lambda^{a-1}\left[\frac{\sum_k w_k^* y_{ik} u_k}{\sum_k w_k^* y_{ik}}\right]$$

[Equation 1]

where, x_i^* is the position along the first CA axis for a lake basin, λ is the first axis eigenvalue, α refers to the scaling method in CANOCO 4.5, however for each index presented here α=1 (ter Braak and Smilauer, 2002). w_k^* are species weights, u_k are species scores along the first CA axis, and y_{ik} is the relative abundance for taxon *k* at site *i*. x_i^* can be scaled using equation 2, to develop a more intuitive index where values range between 0 and 100 and where a score of 100 represents a site in best possible condition.

$$IDEC\ Score = \frac{(x_i^* + 1.0451)}{(2.1618 \cdot 100)}$$

[Equation 2]

Using these methods, all 86 lake basins were assigned index scores using the IDEC, examining the appropriateness of an established index for the assessment of lake environments.

In the previous chapter it was demonstrated that pH exerts influential control on diatom community composition in lakes of the MRW. Variability in lake water pH due to past acid precipitation and localized geomorphology can confound signals attributed to anthropogenic stress. Lavoie et al., (2013b) suggests that sub-indices should be developed to reduce the influence of pH variation, grouping together sites with similar pH. This method, in effect, attempts to remove confounding influence of pH, and focus the assessment of community response to the nine anthropogenic stressors of interest. Using Ward's cluster analysis, lake basins were sorted into two primary groupings based on pH. Lake basins with pH 5.23-6.45 (n=20) form an acidic group, and basins with pH 6.52-7.47 (n=66) form a circumneutral group.

In an attempt to focus the IDEC index on the assessment of taxa found within the MRW, the IDEC was modified (mIDEC) by replacing the initial training set from Lavoie et al. (2013b) with the lake data set from the MRW. A benefit of BIs is that they employ a large proportion of the taxa observed to develop indices based on a substantial amount of biological information. However in large data sets, taxa which demonstrate weak association with the abundance of anthropogenic stress can reduce the effectiveness of the index by introducing noise. As a result, a preliminary CCA was performed, constrained only by anthropogenic stressor scores. Using the cumulative fit per species as a fraction of variance of species, taxa which explained less than the median value were excluded from further analysis. Of the 150 taxa observed within the MRW, only 75 were retained to develop the mIDEC sub-indices. The removal of such a large number of taxa was necessary as the distribution of taxa was highly skewed, with a high proportion of taxa contributing very little to the overall variance explained. A CA was performed to develop the mIDEC for the circumneutral lakes (mIDEC-Circumneutral), in which site scores, species scores and weights for each lake were retrained, providing index scores for each lake basin. The process was repeated using the 20-acidified lakes to develop the mIDEC-Acidified. Additional lakes were assessed and assigned index scores using the values generated by these CAs and equation 1. All mIDEC values were scaled using equation 3 so values range between 0-100.

$$mIDEC\ Score = \frac{(x_i^* + |minimum\ observed\ value|)}{(maximum\ observed\ value + |minimum\ observed\ value|)}$$

[Equation 3]

In all indices (IDEC, mIDEC-Circumneutral, mIDEC-Acidic), significance of relations between index scores and anthropogenic stressor scores were evaluated using Spearman's correlation analysis at a = 0.05.

3.2.2 *Development of Indices of Biotic Integrity (IBIs)*

Using the IBI method, all taxa observed in the training set act as potential metrics describing responses to anthropogenic stressors. However, unlike the IDEC or mIDEC, which employs multivariate ordination analysis on a substantial proportion of the taxa observed to calculate index scores for each site, an IBI uses only a small subset of 'indicator' taxa that respond significantly to the known anthropogenic stressor gradient. Significant associations for both the circumneutral and acidified lake basins were determined using Spearman's correlation analysis for each of the 162 candidate taxa (Hill et al., 2000). For the circumneutral lakes, 16 taxa were found to have significant correlation with the stressor gradient and were used to calculate IBI-circumneutral scores for each lake (*Achnanthidium minutissimum, Asterionella formosa, Brachysira brebissonii*, *Brachysira microcephala*, *Cyclotella bodanica, Eunotia bilunaris*, *Eunotia incisa*, *Eunotia praerupta*, *Eunotia pectinalis var. curta*, *Fragilaria capucina, Fragilaria construens var. venter*, *Frustulia rhomboides*, *Nitzschia linearis, Nitzschia palea*, *Tabellaria flocculosa* and *Tryblionella angustata*). The acidified lake set had only 4 taxa which are significantly correlated with the abundance of anthropogenic stressors (*Brachysira brebissonii*, *Eunotia naegelii*, *Frustulia rhomboides* and *Pinnularia subostrata*). Scatterplots illustrating the relation between the indicator taxa and the abundance of anthropogenic stressors for both circumneutral and acidified lakes can be found in Appendix A.

With the indicator taxa identified, IBI scores for each lake were calculated to develop circumneutral and acidified sub-indices for three different variations of IBI calculation methods. The first method (IBI-1) calculated raw index scores based on a continuous scoring system. Relative abundance data for each of the indicator taxa were standardized and then summed together. Taxa which had a positive relationship with stressors were multiplied by -1 prior to summation to achieve a final index score in which a high value indicated a higher level of biological integrity. The second calculation method (IBI-2) uses a binned scoring method. For each site, each indicator taxon was placed into one of five ordinal categories (bins) based on the relative abundance observed. Each bin represented a range of 20 percent abundance, based on the maximum percent abundance observed for each taxa among the training lakes. For each site, taxa were placed in bin 1 if maximum percent abundance fell between 81and 100 percent, bin 2 if maximum percent abundance fell between 61and 80 percent, bin 3 if between 41and 60 percent, bin 4 if between 21and 40 percent and bin 5 if between 1and 20 percent. Indicator taxa which exhibited a negative association with stressors were scored oppositely, such that a high index score represented a site with high biological integrity. Bin scores for each metric were summed forming an index score for each site. The third method of IBI calculation (IBI-3) was based on methods illustrated by Blocksom (2003), where the relative abundance of each indicator taxon is converted into a raw index score using equation 4.

$$IBI - 3\ score = \sum_{i=17} \frac{(observed\ relative\ abundance\ of\ indicator\ taxa\ i)\ \cdot 100}{maximum\ observed\ relative\ abundance\ of\ taxa\ i}$$

[Equation 4]

Similar to the other methods of IBI calculation, indicator taxa which were positively correlated with anthropogenic stressors were inverted (subtracting by 100). Values for indicator taxa were summed forming an index score for each lake. Scores for all IBI variations were transformed using equation 5, adjusting values to range between 0-100. This allowed for intuitive comparisons between each metric.

$$Adjusted\ IBI\ Score = \frac{IBI_{yj} + |Min\ IBI_y\ score|}{Max\ IBI_y\ score + \left|Min\ IBI_y\ score\right|}$$

$$[Equation\ 5]$$

Where *y* is the method of IBI being examined and *j* is the individual lake score.

The ability for each IBI to assess the influence of anthropogenic stressors was evaluated by comparing IBI scores to the anthropogenic stressor score for each lake basin. Correlation between index and anthropogenic stressor score was assessed by Spearman's correlation analysis performed to test significance, at $\alpha = 0.05$.

3.3 Results

3.3.1 *Biotic Index (IDEC, mIDEC) Scores*

IDEC scores were significantly and negatively correlated with the abundance of anthropogenic stressors (r_s= -0.415, p-value = 7.08 x 10^{-5}). The IDEC, as developed from diatoms in streams of eastern Canada, ranges from 0-100 when applied to stream sites. However, 60% of the lake sites in this study produced values that exceeded 100 (Fig. 3.1), indicating that these sites were assessed to be of higher quality than even the best stream sites in the original IDEC training set. To improve the evaluation of lakes, additional indices were developed, separating lakes on the basis of pH and focusing indices on taxa observed within lakes of the MRW. The IDEC formula was modified (mIDEC) using diatoms from the MRW, such that all MRW lake scores fell between 0 and 100. Scores based on the mIDEC-Circumneutral, were significantly and negatively correlated with the abundance of anthropogenic stressors (r_s = -0.320, p-value = 9.34×10^{-3}; Fig. 3.2a), but mIDEC-Acidified lakes were not found to be significantly correlated with the abundance of anthropogenic stressors (r_s = -0.409, p-value = 0.073; Fig. 3.2b). The mIDEC-Circumneutral demonstrated relatively consistent amounts of variability of index scores along the gradient of anthropogenic stressors. Scores produced by the mIDEC-Acidified demonstrated a general decreasing trend over the anthropogenic stressor gradient, but there was a high variability among sites with a low abundance of anthropogenic stressors.

3.3.2 *Index of Biological Integrity (IBI) Scores*

All IBIs produced useful metrics for biomonitoring of human impacts (Table 1). IBI-3 produced scores which showed the strongest correlation with the anthropogenic stressor gradient (IBI-3-Circumneutral: r_s = -0.748, p-value = 5.45×10^{-13}; Fig. 3.3e; IBI-3-Acidified: r_s = -0.709, p-value = 4.62×10^{-4}; Fig. 3.3f) as well as the lowest amount of variability. IBI-2 also demonstrated strong correlation and variation (IBI-2-Circumneutral: r_s = -0.723, p-value = 6.92×10^{-12}; Fig. 3.3c; IBI-2-Acidified: r_s = -0.698, p-value = 6.22×10^{-4}; Fig. 3.3d). IBI-1 scores were found to exhibit weaker correlation with the anthropogenic stressor gradient than the other IBI indices (IBI-1-Circumneutral: r_s = -0.397, p-value = 1.07×10^{-3}; Fig. 3.3a; IBI-1-Acidified: r_s = -0.648, p-value = 2.00×10^{-3}; Fig. 3.3b). Furthermore, IBI-1-Circumneutral shows high variability along the anthropogenic stressor gradient, which combined with its relatively weak correlation, suggests that it would provide a weaker measure of biological integrity compared to the other IBIs developed.

3.4 Discussion

Index scores from the IDEC metric were significantly correlated with the anthropogenic stressor gradient. This association suggests that the simplified measures of multivariate predictor and response variables, as estimated by this index, provides a useful method of quantifying relations between stressors acting within lake basin and the responses of periphytic diatom communities. However, the

IDEC scored a majority of MRW lake basins in excess of the expected maximum of 100. The IDEC was designed for the assessment of stream environments (Lavoie et al., 2006; 2013b), and prior to this study had never been tested using lake periphyton samples (Dr. Michelle Palmer, Ontario Ministry of the Environment, Toronto, ON, Personal Communication). While periphyton communities in lake and stream environments are largely controlled by similar factors (e.g., water chemistry, light availability, grazing pressure), additional factors specific to streams, such as variation in stream flow and differences in microhabitats may exert strong control on periphytic community composition (Patrick, 1977; Soininen & Weckstöm, 2009). As a result, the relative abundance of some diatom taxa can differ substantially between stream and lake habitats (Ludlam et al., 1996). Such differences in diatom community composition may reduce the effectiveness of the IDEC for assessing lake environments. Additionally, the IDEC was designed to capture variation in periphytic diatom community composition based primarily on a gradient in trophic status (due largely to agricultural inputs; Lavoie et al., 2006; 2013b). Consequently, IDEC scores from the nutrient-poor lakes of the MRW used in this study should score relatively high. The combination of these factors may have led to the IDEC formula providing inflated index scores.

Modification of the IDEC, which included recalculating species scores, weights and scaling factors using samples obtained from lakes within the MRW, attempted to better describe relations between IDEC scores and the abundance of anthropogenic stressors for lakes within the MRW. Although the mIDEC-Circumneutral site scores were significantly correlated with anthropogenic stressors, the association was weaker than was observed with the IDEC. Furthermore the mIDEC-Acidified was unable to capture a significant association between diatom community composition and abundance of anthropogenic stressors in lakes with low pH. Natural and unexplained variability can introduce substantial error into the evaluation of a lake, influencing the performance of an index. Taxa which are weakly associated with the abundance of anthropogenic stressors can confound index scores, increasing variability and reducing the reliability of bioassessments (Hering et al. 2006). The mIDEC analysis excluded many of these taxa from both sub-indices. Even with these measures in place to limit natural variability, poor association between diatom community composition and anthropogenic stressor abundance limits the overall usefulness as a bioassessment tool.

The three variations of IBI scoring illustrated varying abilities to track the level of stressors among lake basins. This finding is consistent with reports by a number of studies, which have cautioned that IBI scores are highly sensitive to the method employed to score taxa and calculate index scores (Blocksom 2003; Dolph et al., 2010; Abbasi & Abbasi, 2012). Historically, IBIs categorized the continuous raw data (percent abundances) of the indicator taxa into discrete bins or numerical categories (similar to the method employed for IBI-2) to simplify the quantification and interpretation of index scores (Karr, 1981). However, this method has been criticized because data can become truncated or oversimplified, and it reduces the amount of biological information captured in samples and decreases accuracy of the IBI scores (Minns et al. 1994; Dolph et al. 2010). Although this is a valid criticism, IBI-2-Circumneutral and IBI-2-Acidified both demonstrated that a binned scoring approach can provide consistent index scores. A binned approach has the important advantage of being able to minimize the influence of confounding taxa by limiting the influence any single taxon can have on the final index score. This is an important feature, as Blocksom (2003) suggests that IBI performance may be strongly influenced by the method in which taxa are scored and summarized. If the distribution describing the relations between individual indicator taxa and stress scores is not representative of what is actually observed, IBI scores will be poor descriptors of biological condition (Blocksom, 2003). Furthermore, if the method of index calculation employed allows individual taxa to become overly influential in the final score, relatively small shifts in the relative abundance due to temporal or spatial variation can equate to large variations in IBI scores.

Continuous taxa scoring methods such as IBI-1 and IBI-3 are often preferred over binned formulae, as they retain a greater amount of information (Dolph et al. 2010). However, this is only one consideration, as for instance IBI-3-Circumneutral was found to be the best measure of biological integrity among lakes with a circumneutral pH, whereas IBI-1-Circumneutral was the worst. The similar continuous approach employed by both IBI-1 and IBI-3 deviated in the amount of influence each taxon was allowed to have on the final index score. IBI-1 standardized the relative abundance of each indicator taxon, allowing each taxon to influence the final index score in proportion to its rareness and variance. Samples with a higher abundance or variance of rare taxa produced higher scores, increasing the variability observed in index scores and in some cases decreasing the association observed with the anthropogenic stressor gradient. Conversely, IBI-3 valued each taxon equally, while scoring them as continuous values. This method provided the best measure of biological integrity, for both circumneutral and acidified lake basins providing an accurate depiction of diatom community composition.

In Chapter 2, it was observed that at the low end of the stressor gradient the relation between diatom community composition and the abundance of anthropogenic stressors was confounded by the influence of pH. Lakes with circumneutral pH follow the linear relation observed at higher values of the stressor gradient; however, a set of acidified lakes had relatively lower diatom DCA axis 1 scores. Lavoie et al. (2013b) found that variation in pH among sample sites was a significant impediment to effectively assessing the response of periphytic diatoms illustrated to anthropogenic stressors related to nutrient supply. To avoid the confounding influence of pH, Lavoie et al. (2013b) suggested that sites be first grouped together on the basis of pH and then assessed, effectively partitioning out the influence of natural pH variation. Following this example, the sub-divided indices presented in this chapter illustrated relations with the anthropogenic stressor gradient, with a reduced influence of pH. Without employing this partitioned or stratified design, index scores could mirror the relationship observed in Chapter 2, in which diatom communities, although driven by the influence of anthropogenic stressor through much of the gradient, demonstrate high variability where pH is variable.

For the preservation of ecological integrity within the MRW, it is important that the biomonitoring methods evolve to match the growing number of stressors (Yan et al., 2008a). All five indices tested demonstrated beneficial qualities for bioassessment, and with the exception of mIDEC-Acidified, were significantly correlated with the anthropogenic stressor scores. Having multiple adequate indices to assess ecosystem status is not unusual (Resh, 2008), but it does underscore the challenge of selecting a single method of assessment for implementation into a biomonitoring program. Based on the Spearman's correlation analysis, IBI-3 demonstrated the strongest correlation for both circumneutral and acidified lakes. Correlation strength between the scores of the best performing indices and the abundance of anthropogenic stressors is comparable to what has been observed in other established diatom-based indices. For example, Lavoie et al. (2013b) examined the association observed between IDEC scores and amount of agricultural land-use (the greatest driver of anthropogenic stress among sites in their study), finding only moderate correlation across 154 sites (Pearson $r = 0.56$, p-value < 0.001). Similarly, Hill et al. (2000) demonstrated that IBI correlation with stressors ranged greatly (Pearson $r = 0.40$-0.83) depending on the type of stressor that was compared.

IBIs are designed to rely on the information provided by only a handful of indicator taxa, focusing exclusively on taxa which demonstrate significant association with the anthropogenic stressor gradient (Karr et al., 1981; Blocksum, 2003). This is designed to greatly limit the influence of taxa driven largely by confounding factors. Furthermore, it provides a bioassessment platform which is easy to comprehend and interpret. However, because IBIs only employ a handful of taxa, assessments are geographically limited to the range in which the indicator taxa exist, or may be found. Lakes found outside of this range may provide biased assessments, as the absence of an indicator species does not correlate with the abundance of anthropogenic stressors. This is of specific concern in acidified lakes, where only four taxa demonstrated a significant association with anthropogenic stressors. The absence of even one of these taxa can significantly reduce the effectiveness of the assessment (Beck & Hatch, 2009). Comparatively, BIs such as the IDEC and mIDEC incorporate biological information from a larger number of taxa, allowing for the absence of a number of taxa due to restrictions in their range, without greatly impacting the quality of the index assessment (Cao & Hawkins, 2005). As a result, BIs are able to be employed over a larger natural range in environmental conditions. It is recommended that the IBI-3 index be employed within the MRW, as it demonstrated the best overall performance. However, the IDEC may act as a suitable backup for use in lakes where the indicator taxa are not sufficiently abundant.

3.4.1 *Future Directions*

This study focused on observing variation in anthropogenic stressors acting in catchments across a large spatial gradient to evaluate and identify indices that can be used for biomonitoring of ecological degradation. However, biomonitoring programs ideally implement indices which are also able to track changes over time within individual lakes (Schindler, 1987). Hoagland et al. (1982) suggest that seasonality may play an important role in determining the establishment of periphyton community composition, as colonization and growth demonstrate a succession-like pattern. However, they further suggest that this pattern is highly predictable, with a progression beginning with the early colonization of bacteria, followed by the establishment of small diatoms, ending with large pennate diatoms which dominate mature end-communities (Hoagland et al., 1982). Additional studies suggest that, while establishment of new or disturbed substrates may demonstrate a successional pattern, naturally occurring temporal variation in mature communities appear to only have a modest influence in eutrophic systems (King et al., 2002). Oligotrophic lakes such as those found in the MRW exhibit negligible variation between sampling periods relative to the variation observed among water bodies (Jones & Flower, 1986; Nygaard, 1994; King et al., 2006). My study attempted to minimize temporal variation by sampling during the mid-summer maxima of periphyton biomass, a state in which the mature periphyton community is thought to be temporally stable (King et al., 2006). However, to improve understanding of within-lake fluctuations in community structure, future steps should make an effort to quantify seasonal shifts. While it is not expected to greatly influence the ability of indices to distinguish stressor influence among lakes, it will assist in the understanding of the expected within-lake changes as a result of natural fluctuations and shifts in management practices.

Major challenges that watershed monitoring programs face include anticipating ecological degradation from unknown or non-point sources stressors and identifying the synergistic interaction of identified stressors (Christensen et al., 2006). Uncertainty caused by these features often limits the effectiveness of managment stewardship policies, as the source of environmental degradation may not be fully addressed (Frissell & Bayles, 1996). By employing programs that incorporate the assessment of both physico-chemical properties, and a biological component, managers can begin to map out the interacting drivers of biological degradation. The use of biotic indices in collaboration with assessment of water chemistry can provide important information about cumulative effects and non-point source stressors within a watershed (Wang, 2001). Unlike many regions, management agencies of the MRW are well positioned to employ a periphyton-based assessment index, as land-use data currently exist, and both chemical measurements and biological assessments of other assemblages are well established (Yan et al. 2008; Tran & Brouse, 2009). Inclusion of periphyton-based biomonitoring indices can build upon existing assessment metrics such as evaluation of zooplankton or benthic macroinvertebrates, providing an assessment metric that can detect the early onset of ecological degradation. This is an aspect which is not found in existing biomonitoring approaches within the MRW (Bowman et al. 2006; Thomas et al. 2011). As management agencies move from passive or reactionary management regimes to ones that are able to proactively reduce and prevent ecological degradation, assessments of highly sensitive biotic assemblages such as periphytic diatoms will become increasingly important (Vos et al. 2000; Gerhardt, 2002).

3.5 Figures and Tables

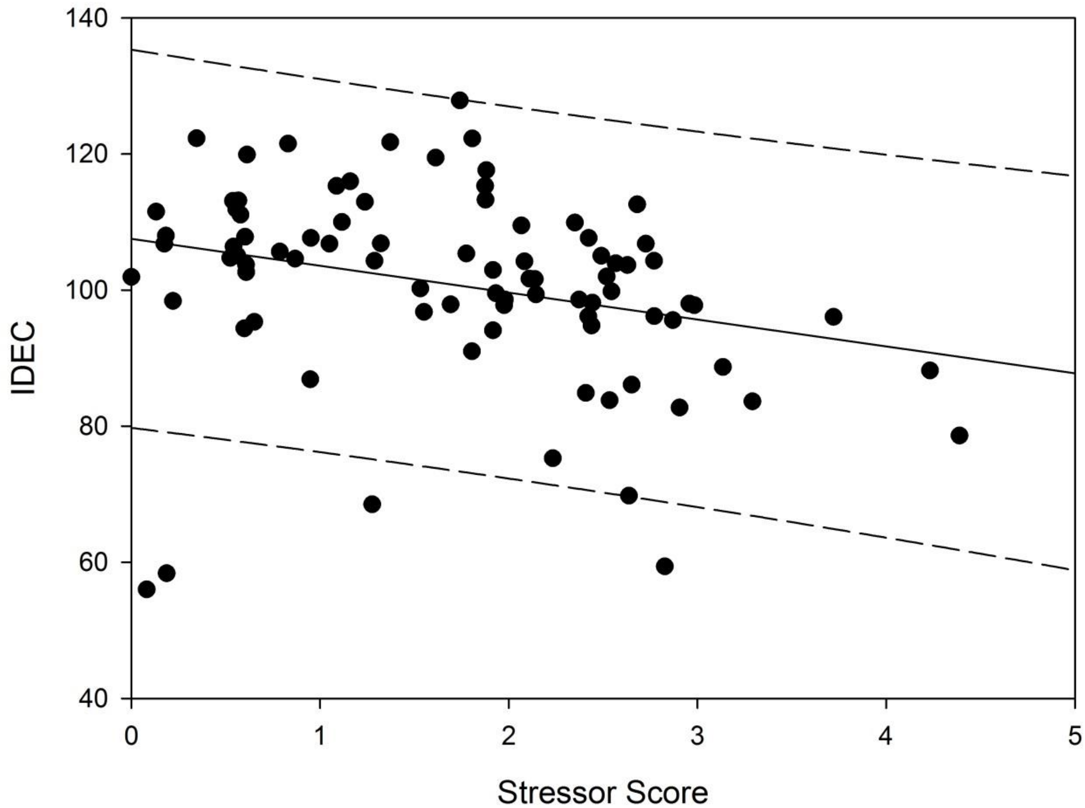

Figure 3.1. Relation of IDEC index scores for each lake basin with respect to basin stress score. Regression line (solid) and 95% confidence intervals (dashed) depicted for the development set of scores.

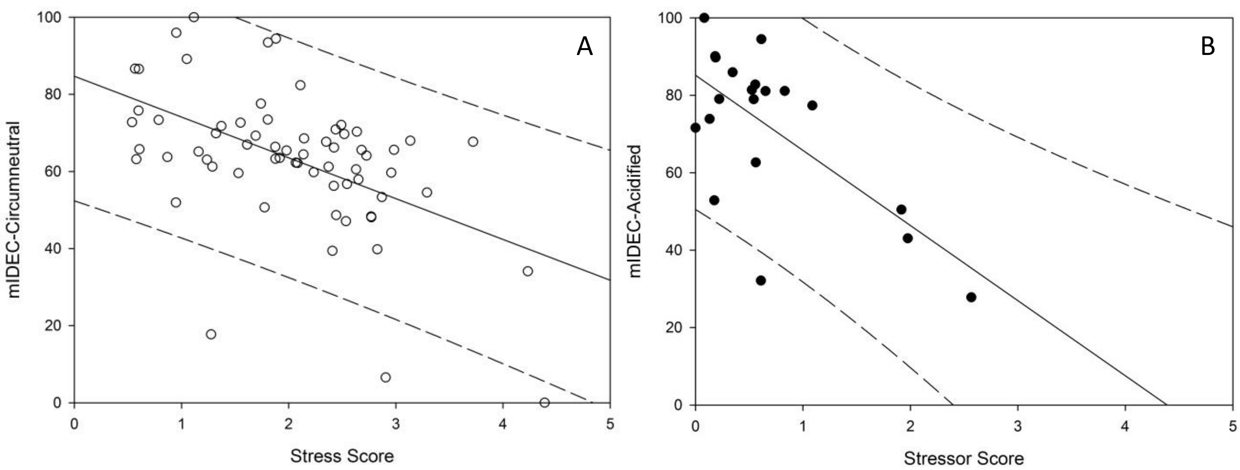

Figure 3.2a-b. Relation of mIDEC index scores for each lake basin with respect to basin stress score. Left Panel (A): Open circles represent the 66 mIDEC-Circumneutral lakes used to develop index values for lakes with a pH range of 6.52-7.47. Right Panel (B): Closed circles represent the 20 mIDEC-Acidified lakes, used to develop index values for lakes with a pH range of 5.23-6.45. Regression line (solid) and 95% confidence intervals (dashed).

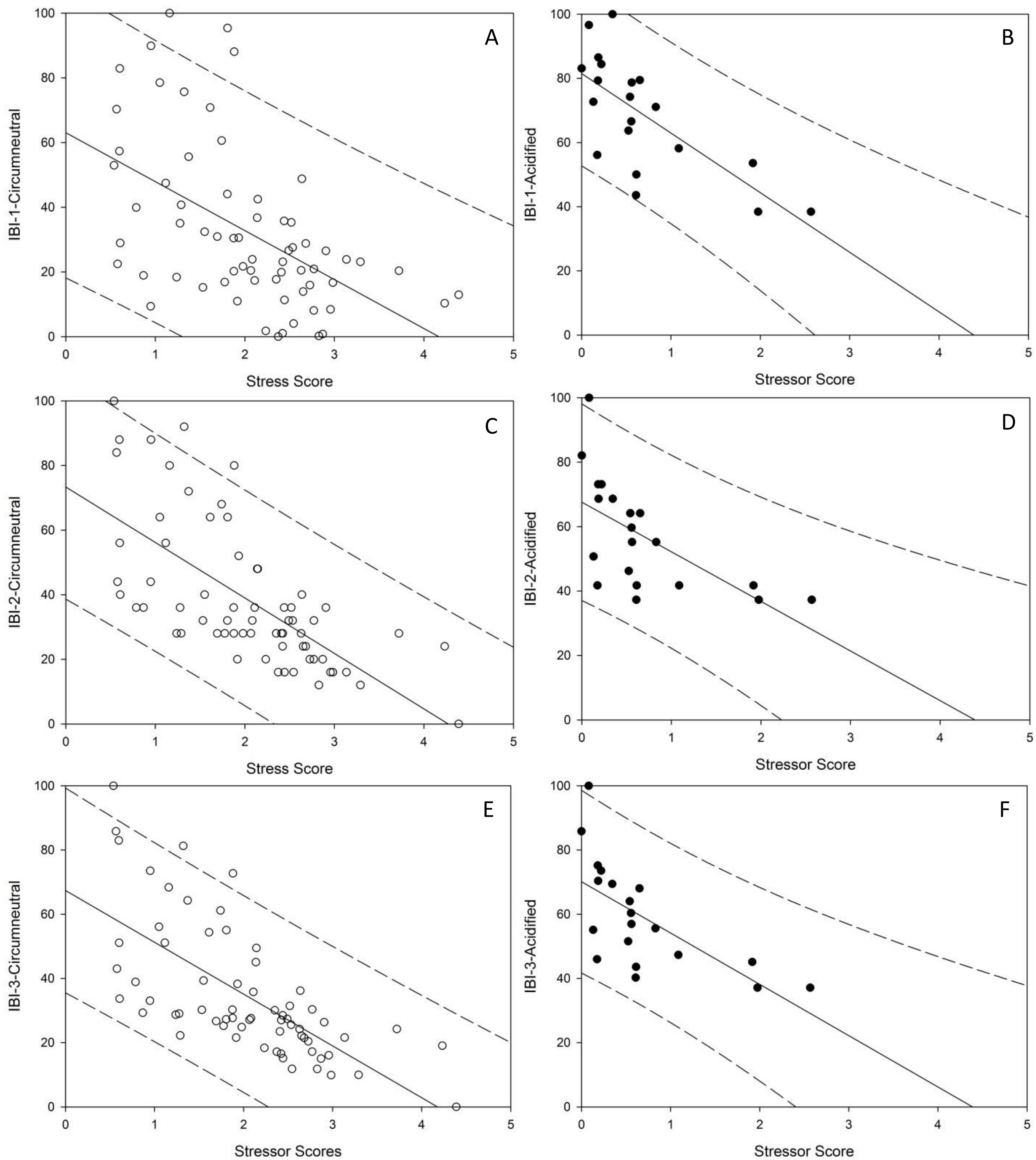

Figure 3.3a-f. Relation of (A) IBI-1-Circumneutral index scores, (B) IBI-1-Acidified index scores, (C) IBI-2-Circumneutral index scores, (D) IBI-2-Acidified index scores, (E) IBI-3-Circumneutral index scores and (F) IBI-3-Acidified index scores for each lake basin with respect to basin stress score. Open circles represent index scores for the 66 IBI-Circumneutral lakes used to develop index scores for each lake with a pH range of 6.52-7.47. Closed circles represent the 20 IBI-Acidified lakes, develop index scores for each lake with a pH range of 5.23-6.45. Regression line (solid) and 95% confidence intervals (dashed).

Table 3.1. Pairwise correlation analysis between lake-basin index scores and corresponding stress scores. Values illustrated in bold are significant α = 0.05, * values are significant α=0.01.

Index	Circumneutral Lakes Pearson's rho (p-value)	Acidified Lakes Pearson's rho (p-value)
mIDEC	**-0.320*** **(9.34×10^{-3})**	-0.409 (7.32×10^{-2})
IBI-1	**-0.546*** **(2.15×10^{-6})**	**-0.666*** **(1.36×10^{-3})**
IBI-2	**-0.723*** **(6.92×10^{-12})**	**-0.698*** **(6.22×10^{-4})**
IBI3	**-0.748*** **(9.34×10^{-13})**	**-0.709*** **(4.62×10^{-4})**

	All Lakes Pearson's rho (p-value)
IDEC	**-0.415*** **(7.08×10^{-5})**

-Chapter 4-

Synthesis and Recommendations

In areas of growing populations and increasing anthropogenic activity, cumulative effects of multiple stressors are beginning to be recognized as a significant threat to ecosystems as a consequence of their largely unknown or unanticipated impacts (Christensen et al., 2006; Duinker et al., 2013). Long-term environmental monitoring projects, such as those carried out within the MRW (Muskoka River watershed; e.g. Recreational Water Quality Monitoring Program; DMM, 2014), are vital for shaping our understanding of trends and changes in environmental conditions, and providing a foundation for informed policy development and management decisions. Without adequate environmental monitoring data, spanning sufficient spatial and temporal scales, teasing apart naturally occurring fluctuations from those due to anthropogenic stressors is difficult. Limited funding for effective environmental monitoring programs is a concern throughout North America (Kerckhove et al., 2013; Lindenmayer et al., 2013). However, within the MRW there is a concerted effort to ensure environmental quality is maintained (Yan et al., 2008a; Tran & Brouse, 2009). The natural environment of the MRW is crucial in driving the tourism-based economy. Future economic growth of the region hinges on maintaining the perceived natural beauty or esthetics of the watershed (Smith, 1987; Winter et al., 2002; Dillon et al., 2007). Ensuring that effective environmental monitoring programs are in place to guide management decisions is pivotal to safeguarding ecological integrity.

The overarching goal of this thesis was to investigate the ability of periphytic diatom community composition to track the influence of multiple anthropogenic stressors for the purposes of biomonitoring. It is anticipated that the development of this novel approach will improve the environmental monitoring capacity of management agencies within the MRW. In Chapter 2, distributions of water chemistry, and periphytic diatom community composition in lakes of the MRW were explored in relation to an observed gradient of multiple anthropogenic stressors. Chapter 3 tackled the second goal of developing and recommending a practical bioassessment index able to efficiently and reliably assess changes in ecological integrity using observations of periphytic diatom community composition. The following synthesis summarizes the primary results of this study, providing context for the recommendation of an effective biomonitoring platform making use of periphytic diatom community composition.

Across the watershed, 86 lakes were examined that span a broad gradient of anthropogenic stressors. Water chemistry within these lakes was found to show strong correlation with the abundance of anthropogenic stressors. Ionic content (Mg, Na, Ca, Cl) increased with the abundance of stressors. This association has been observed by a number of other studies within the MRW, suggesting that anthropogenic sources of ion loading such as road salt (NaCl) and dust suppressants ($MgCl_2$, $CaCl_2$) may threaten water quality (Molot & Dillon, 2008; Palmer et al., 2011; Palmer & Yan, 2013). However, road salt and dust suppressants are important road safety agents, and as such a significant restriction on their usage is not recommended. However, to guard against further environmental degradation it is recommended that effects of their use be closely monitored and the use of viable alternatives be explored.

4.1 Periphytic Diatoms are Sensitive to Low Levels of Anthropogenic Stress in the MRW

Community composition of periphytic diatoms was driven by large numerical response in a few dominant taxa. While this response is somewhat limited, overall community composition could be observed to respond to shifts at even low abundances of anthropogenic stressors. This is a beneficial property of periphytic diatoms which allows biologists to track ecological change before effects are observed in organisms at higher trophic levels which may have a greater societal regard. This relation agrees with numerous other studies which have suggested that diatom communities are able to respond quickly to changes in environmental condition (Lambert et al., 2008; Thomas et al., 2011; Gottschalk & Kalhert, 2012). Other biota that are widely used in biomonitoring, such as fish or benthic macroinvertebrates, can demonstrate a delayed response to the influence of stressors (Resh, 2008). This research is unique in that it is the first to demonstrate the relation between periphytic diatom community composition and the abundance of anthropogenic stressors in a spatially diverse series of lakes within North America.

4.2 The Influence of pH and the need for a Stratified Assessment Approach

Changes in diatom community composition in response to watershed disturbances were confounded by the influence of pH. This was not unexpected as numerous studies have suggested that pH can drive diatom community composition (Dixit et al., 1992; Hall & Smol, 1996). The confounding influence of pH is problematic in a region such as the MRW, which has a relatively wide range of pH observed among lakes (pH 5.23-7.47), as a result of past acid precipitation and localized variation in surficial geology. As a result, it is necessary to partition out the influence of pH by employing a stratified sampling approach. In Chapter 3, lakes were divided in to two groups on the basis of pH (Acidified pH 5.23-6.45; Circumneutral pH 6.52-7.47), such that lakes with similar pH can be compared to each other, reducing the influence of pH variation. Management agencies looking to employ diatom biomonitoring should be mindful of significant natural variation among sites which should be reduced through a stratified sampling approach.

4.3 Bioassessment Indices as an Effective Management Tool

Using the direct relations observed between the abundance of anthropogenic stressors and periphytic diatom community composition, a series of bioassessment indices were developed and evaluated. These indices are designed to improve the ability for biologists to interpret complex community data by providing a single assessment value or grade for each lake. Of the five variations of indices tested, both IBI-3-Circumneutral and IBI-3-Acidified were found to be the best methods of describing biological integrity in relation to the abundance of anthropogenic stressors for both groups of lakes within the MRW. The IBI-3 approach, an index of biological integrity, is an intuitive and widely accessible assessment method, which can be easily used by even those who are unexperienced with manipulating or interpreting biological data. This user-friendliness is a valuable feature, as end-users may have a wide range of backgrounds and experience. The recommendation of a complex bioassessment method may deter its usage. These principles allow for IBI-3 to provide consistent assessments of ecological integrity based on the observed periphytic diatom community composition within the MRW. However, because IBIs base their assessments on only a handful of indicator taxa (Karr, 1981), they may become unreliable in lakes where the indicator taxa are naturally absent. As a result, although the performance of IBI-3 is found to be the best among the indices tested, a more robust index such as the IDEC, may be more effective, where indicator taxa are limited. Ensuring that an index can consistently evaluate similar basins is important to developing a tool which can reliably be used to inform policy development and landuse planning able to effectively mitigate ecological degradation (Karr et al., 1991; Bonada et al., 2006; Angerer et al., 2006).

4.4 Considerations for Developing a Periphytic Diatom Based Monitoring Strategy

The research outlined in this thesis highlights a number of advantages to employing periphytic diatom community composition as a method of biomonitoring. However, it also sheds light on the limitations of this study design as well as with the employment of a diatom based biomonitoring protocol. Diatoms are a complex group of organism which are directly influenced by their surrounding environmental conditions (Lambert et al., 2008). Both natural and anthropogenic factors play a role in defining the community composition at any given site. Therefore, understanding the relative influence of natural and anthropogenic attributes is important in identifying the specific factor(s) responsible for driving the observed distribution of diatom communities across the MRW. Only 11.3% of the observed variation in diatom community composition among sites could be attributed solely to the influence of anthropogenic stressors. Although this value is low, for a large portion of the anthropogenic stressor gradient a clear association between abundance of anthropogenic stressors and diatom community composition can be observed.

A factor which may contribute significantly to the low amount of variability in diatom community composition among lakes being explained by anthropogenic stressors is that the MRW features a relatively small anthropogenic stressor gradient. Compared with watersheds found in southern Ontario, which may have upwards of 80-90% anthropogenic land-use (OMNR, 2000; OMNR, 2012), across the MRW, only 4% of the total land use can be classified as anthropogenic (Tran, 2007). As a consequence, diatom communities may display a close association to the influence of natural attributes (Lambert et al., 2008). Care must be taken to consider the role of naturally occurring attributes when developing a management strategy for a lake.

4.5 Application of Biomonitoring Methods

The research presented in this thesis provides an effective protocol for bioassessment of lakes at a watershed scale, via analysis of periphytic diatoms. This protocol built upon the findings of Thomas et al. (2011), as well as established protocols for assessment of periphyton in stream habitats (Biggs & Kilroy, 2000; Barbour et al., 2000). In addition to the sampling protocol, the IBI-3 bioassessment index demonstrates the most effective analytical method for the evaluation of biological integrity among lakes within the MRW. This index, paired with the sampling protocol, form the platform for a highly effective biomonitoring program that can be easily adopted by end-users. Using this protocol and bioassessment index, information has been gathered on 86 lakes, which span the full range of anthropogenic stressors observed within the MRW. These lakes provide baseline information able to illustrate the contemporary relations between biological integrity and the abundance of anthropogenic stressors observed within the watershed. Future monitoring of these lakes by end-users can be compared to these baseline values, allowing the progress of management efforts to be tracked.

Of the 86 lake basins sampled, 26 were selected in consultation with end-users. These 26 lake basins are of strategic importance to end users as a result of nearby population, recreational use, local tourism revenue, and/or having significant existing monitoring data (DMM 2014; Ingram et al. 2006). These 26 lake basins are expected to form the backbone of future sampling efforts and provide information on the lakes of greatest societal interest. It is recommended that these 26 basins be sampled annually as anthropogenic stressors are expected to show the greatest increase in close proximity to many of these lakes. Additionally, effort should be made to ensure that a series of least-impacted lakes are sampled. This will provide reference information regarding how basins are changing naturally over time, as well as a result of regional changes due to large scale stressors such as climate change. A hypothetical monitoring design may look to examine the 26 special interest basins which are of greatest concern to the end-users as well as 10 low impact basins and an additional 10-20 randomly selected basins, ensuring all fit into the same category of circumneutral or acidified. The 26 special interest basins and 10 low-impact basins would be sampled on an annual basis to provide insight into temporal changes at each basin as a result of both natural fluctuations and changes in anthropogenic stressors. The additional 10-20 randomly selected basins could be randomly divided into two groups and each group sampled on a bi-annual basis. Alternating sampling on a bi-annual basis can provide further understanding of wider patterns observed across the watershed, while reducing the resources necessary. This would see approximately 45 lake basins sampled annually, striking a balance between strategic interests, watershed scale impacts and resource availability.

To begin to prepare for the adoption of a biomonitoring program based on the assessment of periphytic diatoms, management agencies should establish a set of priorities outlining what aspects of environmental monitoring they perceive to be important. This includes ensuring that the lakes being sampled accommodate their interests, such that the knowledge garnered from the lake assessments can be effectively used to develop environmental policy. As the employment of any new biomonitoring program comes at a significant cost, end-users and management agencies must have a firm understanding of what resources are available and how they will be effectively allocated between the sampling and analysis of diatoms. Many of the challenges which face the adoption of a new biomonitoring program in the MRW as well as in other regions deal with logistical concerns. As a result, management agencies must establish a plan which identifies how samples are to be collected, analyzed and then translated into usable information. As an additional benefit, information produced from diatom assessments can be incorporated into the District of Muskoka's Watershed Report Card. This widely circulated report provides both decision makers and residents with an overview of the current state of the watershed. Since it offers such a wide range of information, this report is held in high regard.

4.6 Future Directions

The Muskoka River Watershed Monitoring and Management consortium will be tasked with translating research findings into a comprehensive report, able to inform future management directions. This report will provide insight into the future of environmental management within the MRW. As part of this consortium, research presented in this thesis presents important findings which contribute to developing an effective biomonitoring program to track the cumulative effects of multiple anthropogenic stressors. However, to effectively develop an informative biomonitoring program for use by management agencies within the MRW, the analysis of diatom community composition must be effectively integrated with existing programs, as well as parallel research. Future work will look to investigate agreement between diatom communities and benthic macroinvertebrates which are being examined in a related research project. As both diatoms and benthic macroinvertebrates can be seen as complementary sources of biomonitoring metrics, it is of specific interest to understand what aspects of these two monitoring approaches are unique and where there is overlap. By understanding the congruency between biomonitoring metrics, end-users will be able to efficiently collect information regarding a wide range of stressors which are degrading the MRW.

Partnering biomonitoring metrics with a thorough investigation of the anthropogenic land use and natural characteristics of the MRW will provide an informative method of examining the interaction between natural and anthropogenic features. In another related research project, the interaction between land use patterns and natural characteristics is being examined, with the hope that lake environments most vulnerable to the impacts of stressors can be identified. Research presented in this thesis, combined with research investigating benthic macroinvertebrates and the investigation into land use patterns, may shed light on the mechanisms which drive ecosystem structure and help to develop targets for future development and watershed planning. The ultimate goal of this combined research effort is to develop predictive models able to forecast ecological change under future multiple-stressor scenarios.

A comprehensive biomonitoring approach employing periphytic diatom community composition provides a number of benefits over existing biomonitoring practices. These benefits have been detailed throughout this thesis and include the ability of diatoms to effectively incorporate information about the environment around them. This efficient approach allows for the collection of important ecological information and, thus, end-users are able to effectively develop and refine environmental and planning policy. Increasing ecological monitoring capacity allows management agencies to be better prepared to deal with the cumulative effects of ever-evolving stressors, such that future ecological degradation can be mitigated. By maintaining effective monitoring programs, management agencies can ensure that future growth within the MRW is done in a manner which is ecologically sustainable. This ensures that the beauty of the MRW is preserved, sustaining property value, economic development and biological integrity.

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Appendix A. Sampled Lake Basin information

Table B.1. Description of the location of each of the 86 lakes sampled for periphytic diatom community composition and water quality as well as their designation based on pH for use in the indices presented in Chapter 3.

Lake ID	Lake Name	Coordinates		Selection Grouping	Index Grouping
L114	Green's Lake	-79.16258459	45.44435611	Least-Impact	Acidic
L118	Un-named Lake 118	-79.49516476	44.95926367	Random	Acidic
L130	Un-named Lake 130	-79.54166733	44.99270197	Least-Impact	Acidic
L139	Long Line Lake	-78.96435485	45.25327846	Random	Circumneutral
L140	Birch's Lake	-79.19741487	45.41571113	Least-Impact	Acidic
L197A	Lake Vernon (Hunter's)	-79.23229621	45.32469712	High-Impact	Circumneutral
L197B	Lake Vernon (Main)	-79.28401924	45.32632584	Random	Circumneutral
L197C	Lake Vernon (North)	-79.32082766	45.34493792	Random	Circumneutral
L20A	Three Mile Lake (Main)	-79.45852781	45.17297688	High-Impact	Circumneutral
L20B	Three Mile Lake (Hammel's)	-79.46776571	45.18910001	High-Impact	Circumneutral
L210A	Lake of Bays (Portage)	-79.07062911	45.30679107	Special Interest	Circumneutral
L210B	Lake of Bays (Dwight)	-79.02373523	45.31506522	Special Interest	Circumneutral
L210C	Lake of Bays (Trading)	-79.89087133	45.24248329	Special Interest	Circumneutral
L210D	Lake of Bays (Rat)	-79.05092543	45.31424186	Special Interest	Circumneutral
L210E	Lake of Bays (Haystack)	-79.03778532	45.29035622	Special Interest	Circumneutral
L210F	Lake of Bays (Ten Mile)	-78.97971972	45.27882683	Special Interest	Circumneutral
L219A	Lake Muskoka (Main)	-79.40272623	44.97680123	Special Interest	Acidic
L219B	Lake Muskoka (East)	-79.40393922	45.03393019	Special Interest	Circumneutral
L219C	Lake Muskoka (Gravenhurst)	-79.39785272	44.92844892	Special Interest	Circumneutral
L219D	Lake Muskoka (Bala)	-79.60006371	45.00921907	Special Interest	Circumneutral
L219E	Lake Muskoka (Dudley)	-79.61047129	45.04023678	Special Interest	Circumneutral
L273	Skeleton Lake	-79.50099569	45.22527313	Special Interest	Circumneutral
L289	Un-named Lake 289	-79.55507737	44.96733493	Least-Impact	Acidic
L303	Tooke Lake	-79.14636820	45.17667427	Random	Circumneutral
L323	Harp Lake	-79.12705212	45.37609127	'A' Lake	Circumneutral
L331	Boot Lake	-79.78991463	44.97235614	Least-Impact	Circumneutral
L339	Moot Lake	-79.17873884	45.14706130	Random	Acidic

Lake ID	Lake Name	Coordinates		Selection Grouping	Index Grouping
L347	Emsdale Lake	-79.19056694	45.51042329	Random	Circumneutral
L353	West Dolly Lake	-78.93850423	45.64527547	Least-Impact	Acidic
L357	Fawn Lake	-79.24522591	45.17633826	'B' Lake	Circumneutral
L363	Bear Lake	-79.59392206	44.98803558	Least-Impact	Circumneutral
L397	Bigwind Lake	-79.07385911	45.04874273	'B' Lake	Circumneutral
L403	Rain Lake	-78.95281733	45.60328235	Least-Impact	Acidic
L408	Bay Lake	-79.21001323	45.49907824	Random	Circumneutral
L420	Henshaw Lake	-79.59621745	45.10046589	High-Impact	Circumneutral
L448	Surprise Lake	-79.16403029	45.45573727	Least-Impact	Circumneutral
L455	Foote Lake	-79.18295321	45.47144746	Random	Circumneutral
L456	Dickie Lake	-79.09493720	45.14312601	'A' Lake	Acidic
L459	Penfold Lake	-79.28480720	45.26495797	High Impact	Circumneutral
L466	Sims Lake	-79.4509350	45.33843779	Least-Impact	Acidic
L475	Fish Lake	-79.24098147	45.44583132	Random	Circumneutral
L488	Waseosa Lake	-79.27410451	45.41634725	Random	Circumneutral
L501	Hot Lake	-78.90097433	45.63066198	Least-Impact	Acidic
L507	Healey Lake	-79.19770299	45.08490053	'B' Lake	Circumneutral
L508	Westward Lake	-78.77219880	45.48664428	'B' Lake	Circumneutral
L519	Walker Lake	-79.09162174	45.37241415	'B' Lake	Circumneutral
L520	Jessop Lake	-79.26779969	45.39035715	Random	Circumneutral
L536	Islet Lake	-78.88163408	45.63964650	Least-Impact	Acidic
L543	Sawyer Lake	-78.90373324	45.64226011	Least-Impact	Acidic
L55	Ripple Lake	-79.13443772	45.45900605	Random	Circumneutral
L567	Solitaire Lake	-79.01154325	45.39975936	'B' Lake	Circumneutral
L569	Buck Lake	-78.98425166	45.39033833	'B' Lake	Circumneutral
L586	Brush Lake	-79.75497077	45.26921331	Random	Circumneutral
L588	Armishaw Lake	-79.77222017	45.23194536	High-Impact	Circumneutral
L597	Weed Lake	-78.88489654	45.61222183	Least-Impact	Acidic
L611	Jubilee Lake	-78.90233283	45.65918422	Least-Impact	Acidic
L635	Robinson Lake	-79.28010093	45.33982406	Random	Circumneutral
L649	Leach Lake	-79.10148357	45.05820909	'B' Lake	Circumneutral
L650	Little Arrowhead Lake	-79.19142920	45.40706529	Least-Impact	Circumneutral
L660	Brandy Lake	-79.52007963	45.10333198	'B' Lake	Circumneutral

Lake ID	Lake Name	Coordinates		Selection Grouping	Index Grouping
L684	Grandview Lake	-79.05723602	45.20007714	Random	Acidic
L694	Tongua Lake	-79.36446244	45.22942598	Least Impact	Acidic
L699	Chub Lake	-78.98474558	45.21084624	'A' Lake	Circumneutral
L719	Ishkuday Lake	-78.87294617	45.63088584	Least-Impact	Acidic
L73	North Rainy Lake	-78.91211066	45.63874962	Least-Impact	Acidic
L737	Smoke Lake	-78.70769898	45.52246407	Random	Circumneutral
L742	Mary Lake	-79.27535715	45.21724415	Random	Circumneutral
L743A	Peninsula Lake (West)	-79.11216927	45.34660281	High-Impact	Circumneutral
L743B	Peninsula Lake (East)	-79.08498251	45.33600018	High-Impact	Circumneutral
L750A	Lake Joseph (Little Lake Joseph)	-79.68626563	45.20176388	Special Interest	Circumneutral
L750B	Lake Joseph (North Main)	-79.75313621	45.21007283	Special Interest	Circumneutral
L750C	Lake Joseph (Hammer)	-79.77419200	45.22239763	Special Interest	Circumneutral
L750D	Lake Joseph (Burnegie)	-79.75607021	45.23851128	Special Interest	Circumneutral
L750E	Lake Joseph (Pinelands)	-79.62261734	45.10965882	Special Interest	Circumneutral
L750F	Lake Joseph (Main)	-79.71318762	45.14510321	Special Interest	Circumneutral
L751A	Lake Rosseau (North)	-79.61256210	45.21903881	Special Interest	Circumneutral
L751B	Lake Rosseau (East Portage)	-79.55352517	45.14293381	Special Interest	Circumneutral
L751C	Lake Rosseau (Main)	-79.60939821	45.15316022	Special Interest	Circumneutral
L751D	Lake Rosseau (Skeleton)	-79.57052012	45.21447984	Special Interest	Circumneutral
L751E	Lake Rosseau (Brackenrig)	-79.54506234	45.11645633	Special Interest	Circumneutral
L752A	Fairy Lake (North)	-79.18126743	45.32772679	High-Impact	Circumneutral
L752B	Fairy Lake (South)	-79.19429835	45.31138024	High-Impact	Circumneutral
L76A	Kawagama Lake (Fletcher's)	-78.79338821	45.31367827	Special Interest	Circumneutral
L76B	Kawagama Lake (Minden)	-78.74783214	45.29609801	Special Interest	Circumneutral
L76C	Kawagama Lake (Main)	-78.78602743	45.27415783	Special Interest	Circumneutral
L76D	Kawagama Lake (North East)	-78.71552823	45.32137890	Special Interest	Circumneutral

Appendix B-List of Observed Periphytic Diatom Taxa

Table E.1. List of diatom taxon names comprising ≥ 1% of at least one sample.

Taxon Number	Taxon Name (Authority)	Maximum Relative Abundance	Number of Occurrences
1	*Achnanthes exigua* (Grunow) Cleve & Grunow	0.02	1
2	*Achnanthes hostii* Cleve	0.015	1
3	*Achnanthes microcephala* (Kützing) Grunow	0.012	1
4	*Achnanthes pseudohungarcia* Cholnoky-Pfannkuche	0.015	1
5	*Achnanthes sp*.1	0.01	1
6	*Achnanthes sp*. 2	0.019	2
7	*Achnanthes stolida* Krasske	0.01	1
8	*Achnanthidium miuntissimum* (Kützing) Czarnecki	0.384	82
9	*Achnanthidium minutissimum* var. *scotica* (Carter) Lange-Bertalot	0.05	31
10	*Amphipleura pellucida* Kützing	0.01	1
11	*Anomoeoneis seriens* (Brébisson ex Kützing) Cleve	0.01	2
12	*Anomoeoneis styriaca* (Grunow) Hustedt	0.034	16
13	*Asterionella formosa* Hassall	0.086	7
14	*Actinella punctata* Lewis	0.015	1
15	*Aulacoseira alpigena* (Grunow) Krammer	0.029	1
16	*Aulacoseira ambigua* (Grunow) Simonsen	0.02	1
17	*Aulacoseira canadensis* (Hustedt) Simonsen	0.024	6
18	*Aulacoseira distans* (Ehrenberg) Simonsen	0.043	2
19	*Aulacoseria granulata* (Ehrenberg) Simonsen	0.022	1
20	*Aulacoseria italica* (Ehrenberg) Simonsen	0.105	2
21	*Aulacoseira pfaffiana* (Reinsch) Krammer	0.197	16
22	*Brachysira brebissonii* Ross	0.135	48
23	*Brachysira microcephala* (Grunow) Compère	0.221	75
24	*Caloneis bacillum* (Grunow) Cleve	0.029	1
25	*Cavinula cocconeiformis* (Gregory ex Greville) Round	0.015	7
26	*Caloneis undulata* (Gregory) Krammer	0.01	1
27	*Chamaepinnularia mediocris* (Krasske) Lange-Bertalot	0.031	11
28	*Chamaepinnularia soehrensis* (Krasske) Lange-Bertalot & Metzeltin	0.012	1
29	*Cymbella aequalis* (Smith) Greville	0.022	10
30	*Cymbella falaisensis* (Grunow) Krammer & Lange-Bertalot	0.041	1
31	*Cymbella gaeumannii* Meister	0.017	2
32	*Cymbella mesiana* Cholnoky	0.077	20
33	*Cymbella perpusilla* Cleve	0.041	7

Taxon Number	Taxon Name (Authority)	Maximum Relative Abundance	Number of Occurrences
34	*Cymbella proxima* (Reimer) in Patrick & Reimer	0.015	2
35	*Cocconeis placentula* Ehrenberg var. placentula Ehrenberg	0.017	2
36	*Craticula accomoda* (Hustedt) Mann	0.01	1
37	*Craticula molestiformis* (Hustedt) Lange-Bertalot	0.012	3
38	*Cyclostephanos dubis* (Fricke) Round	0.029	7
39	*Cyclotella bodanica* var. *bodanica* Eulenstein ex Grunow	0.022	13
40	*Cyclotella bodanica* var. *lemanica* (O. Muller ex Schroter) Bachman.	0.033	3
41	*Cyclotella ocellata* Pantocsek	0.017	3
42	*Cyclotella pseudostelligera* Hustedt	0.057	38
43	*Cyclotella stelligera* (Cleve & Grunow) in Van Heurck	0.078	15
44	*Denticula tenuis* (Kützing)	0.01	1
45	*Diploneis marginestriata* (Hustedt)	0.012	2
46	*Encyonema neogracile* Krammer	0.137	21
47	*Encyonema minutum* (Hilse ex Rabenhorst) Mann	0.044	5
48	*Encyonema silesiacum* (Bleisch) Mann	0.038	15
49	*Eunotia bilunaris* (Ehrenberg) Mills	0.052	14
50	*Eunotia elegans* Østrup	0.012	1
51	*Eunotia exigua* (Brebisson) Rabenhorst	0.024	2
52	*Eunotia faba* (Ehrenberg) Grunow	0.019	3
53	*Eunotia formica* Ehrenberg	0.014	1
54	*Eunotia implicata* Nörpel, Lange-Bertalot & Alles	0.047	28
55	*Eunotia incisa* Gregory	0.142	17
56	*Eunotia mondon* var. *major* (Smith) Hustedt	0.01	2
57	*Eunotia monodon* Ehrenberg	0.01	1
58	*Eunotia naegelii* Migula	0.034	11
59	*Eunotia nymanniana* (Grunow) van Heurck	0.017	3
60	*Eunotia praerupta* var. *curta* (Grunow) Van Heurck	0.028	7
61	*Eunotia pectinalis* (Dyllwyn) Rabenhorst	0.027	8
62	*Eunotia praerupta* Ehrenberg	0.082	21
63	*Eunotia paludosa* var. *trinacria* (Krasske) Nörpel et Alles	0.01	1
64	*Eunotia pectinalis* var. *undulata* (Ralfs) Rabenhorst	0.169	15
65	*Eunotia rhynchocephala* (Hustedt)	0.01	1
66	*Eunotia serra* var. *diadema* (Ehrenberg) Patrick	0.015	2
67	*Eunotia sp.*	0.01	1
68	*Eunotia serra* Ehrenberg	0.01	1
69	*Eunotia veneris* (Kützing) A.Berg	0.012	1
70	*Eolimna minima* (Grunow) Lange-Bertalot	0.02	4

Taxon Number	Taxon Name (Authority)	Maximum Relative Abundance	Number of Occurrences
71	*Epithema adnata* (Kützing) Brébisson	0.02	1
72	*Eucocconeis flexella* (Kützing) Meister	0.02	13
73	*Ecyonopsis microcephala* (Grunow) Krammer	0.214	44
74	*Fragilaria capucina* var. *capucina* Desmazieres	0.171	67
75	*Fragilaria capucina* var. *mesolepta* Rabenhorst	0.01	1
76	*Fragilaria exigua* Grunow	0.017	3
77	*Fragilaria lata* (Cleve-Euler) Renberg	0.015	1
78	*Fragilaria nanana* Lange-Bertalot	0.04	4
79	*Fragilaria neoproducta* Lange-Bertalot	0.129	3
80	*Fragilaria oldenburgiana* Hustedt	0.017	1
81	*Fragilaria sp.* 1	0.015	1
82	*Fragilaria sp.* 2	0.019	3
83	*Fragilaria ulna* var. *acus* (Kützing) Lange-Bertalot	0.019	2
84	*Frustulia pseudomagliesmotana*	0.059	1
85	*Frustulia rhomboides* (Ehrenberg) De Toni	0.106	40
86	*Gomphonema acuminatum* Ehrenberg	0.01	3
87	*Gomphonema angustatum* (Kützing) Rabenhorst	0.012	1
88	*Gomphonema clevei* (Fricke) Schmitd et al.	0.067	14
89	*Gomphonema clavatum* Ehrenberg	0.2	16
90	*Gomphonema entolejum* Østrup	0.034	7
91	*Gomphonema gloiferum* Meister	0.015	4
92	*Gomphonema parvulum* Guttinger	0.076	19
93	*Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot	0.015	2
94	*Gomphonema sp.* 1	0.019	3
95	*Gomphonema sp.* 2	0.019	2
96	*Gomphonema truncatum* Ehrenberg	0.017	1
97	*Kobayasiella subtilissima* (Cleve) Lange-Bertalot	0.133	80
98	*Navicula bottnica* (Grunow) Cleve & Grunow	0.029	8
99	*Navicula cryptocephala* Kützing	0.031	13
100	*Navicula cryptotenella* Lange-Bertalot	0.129	63
101	*Navicula halophile* (Grunow) Cleve	0.048	11
102	*Navicula jaernefeltii* Hustedt	0.022	5
103	*Navicula leptostriata* Jørgensen	0.017	1
104	*Navicula lapidosa* Krasske	0.036	9
105	*Navicula numannii*	0.011	2
106	*Navicula nugalis* Hohn & Hellerman	0.019	2
107	*Navicula radiosa* Kützing	0.036	22
108	*Navicula rotunda* Hustedt	0.05	12
109	*Navicula soehrensis* var. *hassiaca* (Krasske) Krammer	0.019	1

Taxon Number	Taxon Name (Authority)	Maximum Relative Abundance	Number of Occurrences
	& Lange-Bertalot		
110	*Navicula sp.* 1	0.021	1
111	*Navicula striolata* (Grunow) Krammer & Lange-Bertalot	0.019	2
112	*Neidium affine* (Ehrenberg) Pfizer	0.024	1
113	*Neidium ampliatum* (Ehrenberg) Krammer & Lange-Bertalot	0.026	2
114	*Neidium densestriatum* (Østrup) Krammer & Lange-Bertalot	0.012	1
115	*Nitzschia agnita* Hustedt	0.021	1
116	*Nitzschia amphibia* Grunow	0.017	1
117	*Nitzschia frustulum* var. *frustulum*(Kützing) Grunow	0.06	10
118	*Nitzschia gracilis* Hantzsch	0.099	2
119	*Nitzschia homburgiensis* Lange-Bertalot	0.015	2
120	*Nitzschia linearis* (Agardh) Smith	0.031	14
121	*Nitzschia palea* (Kützing) Smith	0.08	32
122	*Nitzschia perminuta* (Grunow) Peragallo	0.076	32
123	*Pseudostaurosira brevistriata* (Grunow) Van Heurk	0.01	2
124	*Pseudostaurosira parasitica* (W.Smith) Morales	0.017	3
125	*Pseudostaurosira robusta* (Fusey) Williams & Round	0.012	1
126	*Placoneis pseudangelica* Cox	0.01	1
127	*Pinnularia braunii* (Grunow) Cleve	0.012	2
128	*Pinnularia microstauron* (Ehrenberg) Cleve	0.012	3
129	*Pinnularia subrostrata* (Cleve) Cleve-Euler	0.012	5
130	*Pinnularia viridis* (Nitzsch) Ehrenberg	0.01	2
131	*Planothidium rostratum* (Østrup) Round & Bukhtiyarova	0.015	3
132	*Planothidium sp.* 1	0.087	10
133	*Psammothidium bioretii* (Germain) Bukhtiyarova & Round	0.015	4
134	*Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round	0.036	19
135	*Puncastriata lancettula*	0.048	6
136	*Raphoneis surirella* (Ehrenberg) Grunow	0.109	1
137	*Rhopalodia gibba* (Ehrenberg) Müller	0.015	2
138	*Sellaphora capitata* (Mann & McDonald) Mann et al.	0.01	1
139	*Stauroforma inermis* Flower & Jones	0.026	2
140	*Stenopterobia curvula* (Smith) Krammer	0.043	15
141	*Stenopterobia delicatissima* (Lewis) Brébisson ex Van Heurck	0.033	6
142	*Staurosira construens* var. *venter* (Ehrenberg)	0.057	11

Taxon Number	Taxon Name (Authority)	Maximum Relative Abundance	Number of Occurrences
	Hamilton et al.		
143	*Stauroneis anceps* Ehrenberg	0.01	1
144	*Tabellaria binalis* (Ehrenberg) Grunow	0.019	2
145	*Tabellaria binalis* var. *elliptica* Flower	0.015	1
146	*Tabellaria fasciculata* (Agardh) Williams & Round	0.114	2
147	*Tabellaria flocculosa* (Roth) Kützing	0.461	81
148	*Tabellaria ventricosa* Kützing	0.132	2
149	*Tryblionella angustata* Smith	0.031	17
150	*Ulnaria ulna* (Nitzsch) Compère	0.012	4

Appendix C-Water Chemistry Attributes

Table D.1. Selected water chemistry characteristics obtained at each of the 86 lake basins sampled within the Muskoka River Watershed. 'ALK' refers to Gran alkalinity and is expressed in units of mg/L of $CaCO_3$. 'CON' refers to conductivity.

Lake ID	ALK mg/L	Ca mg/L	Cl mg/L	CON µS/cm	DIC mg/L	DOC mg/L	K mg/L	Mg mg/L	Na mg/L	NO_3 µg/L	NH_4 µg/L	TKN µg/L	pH	TP µg/L	SiO_3 mg/L	SO_4 mg/L
L114	5.6	1.16	0.38	17	0.72	5.8	0.28	0.425	0.56	10	4	330	6.32	5.5	0.08	2.95
L118	4.35	1.24	0.1	9.6	0.86	13	0.08	0.355	0.205	14	4	971	5.87	30.2	0.16	0.2
L130	6.5	1.6	3.53	29.6	0.86	9	0.25	0.445	2.99	10	4	439	6.45	17.1	0.2	1.7
L139	11.2	3.52	1.94	40	2.2	4.7	0.715	1.25	1.54	4	8	228	7.12	3.9	0.8	4.35
L140	5.6	1.44	0.19	17.8	0.68	5.2	0.22	0.325	0.755	18	4	315	6.36	7.3	0.22	3.8
L197A	9.9	3	4.28	46	1.8	6.2	0.685	0.935	3.49	40	78	308	6.92	9.1	2.28	4.1
L197B	8.75	2.4	3.46	40.4	1.4	6	0.635	0.85	2.75	10	80	294	6.71	8.2	2.24	4.35
L197C	7.15	2.5	3.19	38.4	1.24	6.2	0.575	0.825	2.45	12	64	330	6.72	8	2.18	3.9
L20A	18	2.9	8.7	73.4	3.72	6.1	0.675	1.28	6.05	36	4	557	7.28	25	2.08	3.55
L20B	16.3	2.86	7.04	66.8	3.38	5	0.715	1.16	5.04	6	6	352	7.33	12.5	1.88	3.15
L210A	8.3	2.44	2.71	36.2	1.28	4.1	0.47	0.745	2.57	12	16	224	6.92	3.8	1.68	4.35
L210B	8.85	2.28	3.03	37	1.4	4.3	0.45	0.77	2.47	12	12	254	6.92	4.5	1.6	4.3
L210C	8.65	2.04	1.11	27	1.32	3	0.415	0.645	1.2	8	8	221	6.83	6.2	1.28	3.9
L210D	8.25	2.26	2.84	36.8	1.34	4.4	0.455	0.75	2.4	14	10	214	6.93	4.5	1.6	4.25
L210E	9.25	2.28	2.67	35.8	1.36	4	0.465	0.765	2.35	12	8	199	6.89	3.7	1.68	4.35
L210F	9.2	2.32	2.85	36.2	1.3	3.8	0.465	0.775	2.38	10	6	207	6.91	3.7	1.72	4.35
L219A	11.6	3.3	6.74	130	1.74	4.4	0.62	0.92	4.73	12	56	266	5.53	-	1.72	4.4
L219B	10.6	3.22	6.46	126	1.72	4.2	0.605	0.895	4.58	8	42	258	6.97	-	1.72	4.3
L219C	15.5	5.66	12.1	200	2.94	4.3	0.785	1.31	8.12	10	2	284	7.04	-	1.08	4.75
L219D	9.8	3.38	6.58	55	1.78	4.2	0.645	0.955	4.52	8	8	284	6.97	3.9	1.7	4.9
L219E	9.7	3.52	6.46	55.2	1.76	4.4	0.63	0.975	4.52	12	2	278	6.97	4	1.62	5
L273	7.45	2.58	4.81	49	1.54	2	0.48	0.71	3.27	8	286	160	6.97	1.7	0.42	5.15

Lake ID	ALK mg/L	Ca mg/L	Cl mg/L	CON µS/cm	DIC mg/L	DOC mg/L	K mg/L	Mg mg/L	Na mg/L	NO_3 µg/L	NH_4 µg/L	TKN µg/L	pH	TP µg/L	SiO_3 mg/L	SO_4 mg/L
L289	4.25	0.8	0.24	9.8	0.62	7.5	0.205	0.235	0.46	18	6	413	5.86	14.3	0.48	0.65
L303	7.25	3.1	30.7	171	1.36	3.5	0.54	0.705	26.2	6	4	269	6.9	4.3	0.76	2.25
L323	7.85	2.6	2.38	35	1.3	4.6	0.495	0.835	2.06	4	2	238	6.93	3.2	1.76	4.8
L331	13.5	4.28	0.49	35.4	2.8	11.1	0.35	0.92	0.89	2	2	474	6.95	10.8	0.08	1.7
L339	3.3	1.22	0.27	16.4	0.68	6.1	0.21	0.33	0.585	8	4	343	6.2	8.9	0.28	2.55
L347	7.5	2.12	1.45	24.6	1.12	3	0.425	0.425	0.965	8	4	208	6.88	3.2	0.32	3.55
L353	4.85	1.28	0.25	18	0.62	4.6	0.37	0.37	0.78	4	2	272	6.26	5.2	0.6	3.9
L357	7.7	2.32	1.18	26.2	0.96	8.6	0.495	0.575	1.25	16	4	418	6.67	18.7	0.06	3.2
L363	7.6	1.52	0.28	18	1.1	3.7	0.295	0.445	0.625	10	2	235	6.81	3.9	0.02	2.5
L397	6.9	1.82	0.51	21.8	1	3.8	0.35	0.545	0.86	6	2	223	6.77	3.8	0.56	4.15
L403	5.6	1.26	0.16	18.4	0.62	3.6	0.38	0.395	0.725	4	2	254	6.39	4.9	0.88	4.1
L408	8.85	2.38	1.37	28.2	1.32	4	0.465	0.555	1.21	10	2	250	6.96	3.5	1	3.55
L420	16.9	7	16.9	108	3.3	6.1	0.665	0.895	11	8	2	344	7.28	5.2	0.58	4.1
L448	5.75	1.38	0.19	18.4	0.82	3.5	0.26	0.41	0.635	10	4	236	6.56	3.1	0.26	0.05
L455	7.75	2.16	0.61	24	1.1	7.5	0.435	0.525	0.975	22	4	374	6.81	6.1	1.74	3.25
L456	5.35	2.34	4.34	34	0.96	6.1	0.475	0.53	2.16	14	2	339	6.41	7.6	0.16	2.75
L459	20.4	6.6	33.6	173	3.8	7.9	1.18	2.23	22.5	12	2	508	7.47	22.3	1.1	4.95
L466	3	1.66	0.53	18.6	0.52	16.1	0.265	0.38	1.13	8	10	574	5.45		0.78	1.7
L475	15.2	5.72	11.9	86.6	2.86	7.8	1.31	1.93	7.54	8	4	420	7.31	16.9	3.4	5.65
L488	8.65	2.6	1.64	31.6	1.34	4.4	0.525	0.675	1.62	10	14	255	6.99	5.8	1.76	4.25
L501	5.7	1.46	0.25	20.2	0.8	4.6	0.36	0.455	0.85	4	4	261	6.45	3.1	0.94	3.95
L507	6.45	2.3	6.85	44.8	1	7	0.375	0.56	4.75	14	2	416	6.63	10.5	0.52	2.9
L508	4.4	1.28	0.22	18.2	0.88	2.3	0.31	0.4	0.51	6	4	185	6.55	3	0.02	3.5
L519	9.25	3.04	5.42	45	1.72	4.5	0.68	0.96	3.05	6	2	286	7.01	4.1	0.24	3.8
L520	6.4	1.82	1.05	23.8	0.86	7.6	0.385	0.54	1.21	4	4	356	6.52	9.5	1.26	3.8
L536	4.3	0.98	0.19	15.6	0.54	3.2	0.32	0.32	0.565	4	2	216	6.22	3.7	0.36	3.6
L543	4.75	1.24	0.23	18.8	0.6	4.3	0.4	0.375	0.765	8	4	237	6.34	4.4	1.42	4.3
L55	9.45	2.86	1.89	35	1.6	4.4	0.595	0.785	1.93	10	8	244	7.08	5.8	1.92	4.6
L567	7.55	1.92	0.83	25.2	1.12	2.5	0.52	0.57	0.785	4	2	181	6.7	3.9	0.16	5

Lake ID	ALK mg/L	Ca mg/L	Cl mg/L	CON µS/cm	DIC mg/L	DOC mg/L	K mg/L	Mg mg/L	Na mg/L	NO_3 µg/L	NH_4 µg/L	TKN µg/L	pH	TP µg/L	SiO_3 mg/L	SO_4 mg/L
L569	6.5	2	0.38	24.8	1.38	2.8	0.46	0.58	0.775	2	2	205	6.83	2.5	0.2	3.45
L586	4.6	1.96	1.64	24.8	0.94	4	0.39	0.44	1.12	4	2	254	6.58	3.9	0.54	3.2
L588	6.55	2.04	1.79	31.4	1.56	5.6	0.74	0.83	1.38	8	2	332	6.75	4.3	0.24	3.65
L597	3.85	0.78	0.07	12.8	0.42	5	0.26	0.29	0.525	6	4	323	5.64	7.1	0.04	3.1
L611	4.35	1.18	0.14	17.6	0.62	5.3	0.35	0.355	0.755	6	10	299	6.07	7.2	0.8	4.15
L635	20	5.62	7.86	79.6	4.14	7.7	1.1	2.21	4.66	52	6	405	7.24	15.4	2.76	4.35
L649	9.25	3.02	0.62	28.6	1.5	5.1	0.45	0.575	1.08	24	4	303	6.81	9.6	0.4	3.55
L650	6	1.3	0.25	17.8	0.76	3.6	0.225	0.32	0.705	12	4	234	6.63	5.1	1.52	3.6
L660	12.7	4.32	8.23	62.8	2.72	11.8	0.835	1.38	5.61	10	6	552	7.03	23.5	1.68	3.25
L684	5.1	3.86	12	16.8	0.54	3.8	0.765	1.62	7.35	8	2	257	6.33	4.7	0.18	4.8
L694	2.85	1.08	0.18	14	0.5	7.1	0.24	0.315	0.555	6	2	338	5.77	11.5	0.34	2.35
L699	13.2	1.26	0.24	78.4	2.48	6.3	0.345	0.42	0.67	6	2	293	7.2	6.5	0.2	3
L719	2.95	1.84	0.01	24.4	0.36	3.4	0.05	0.54	0.725	6	2	319	5.23	6.3	0.12	8.35
L73	4.35	1.1	0.15	17.2	0.46	4.5	0.34	0.355	0.74	6	2	280	6.11	4.9	0.8	4.25
L737	5.65	1.8	3.3	32.4	1.06	3.7	0.4	0.61	2.42	6	2	206	6.71	3.5	0.58	4.2
L742	8.5	3.02	5.95	52	1.52	5	0.63	0.94	4.07	16	122	297	6.83	5.7	2.14	4.3
L743A	14	3.78	9.03	161	2.4	3.6	0.755	1.28	5.95	6	2	284	7.15	-	1.52	4.65
L743B	14	3.86	9.06	163	2.34	3.2	0.76	1.27	5.98	6	4	324	7.23	-	1.6	4.7
L750A	12.3	3.6	7.11	63.4	1.74	3.2	0.59	0.8	4.91	6	2	206	7.04	3.5	0.3	5.6
L750B	10.8	3.84	8.76	67.6	1.66	2.7	0.625	0.815	5.99	8	8	184	6.99	2.4	0.16	5.65
L750C	11.1	3.82	8.7	68	1.68	2.9	0.615	0.815	6.08	8	6	206	7.01	2.4	0.16	5.65
L750D	8.75	3.76	8.73	66.4	1.7	3	0.615	0.815	6.03	10	6	206	7.04	2.4	0.16	5.8
L750E	10.2	3.68	8.49	64.4	1.78	3.2	0.615	0.805	6	8	2	211	6.97	2.9	0.28	5.6
L750F	10.6	3.68	8.73	65.6	1.74	2.8	0.62	0.8	6.18	8	6	203	6.96	3.2	0.2	5.8
L751A	10.6	3.06	5.69	52.8	1.8	3.5	0.58	0.745	4.12	10	8	225	6.96	3.6	0.76	4.8
L751B	10.8	3.08	5.77	53.2	1.84	3.2	0.58	0.75	4.23	8	4	234	6.98	4.3	0.84	5
L751C	10.3	3.12	5.92	53.2	1.82	3.7	0.58	0.775	4.29	10	6	239	6.94	3.9	0.8	4.85
L751D	11.1	3.26	5.77	53.6	1.86	4	0.585	0.785	4.23	10	4	249	6.93	4.7	0.8	4.75
L751E	11.2	3.26	5.81	54.4	2.08	3.7	0.59	0.835	4.32	4	4	276	6.89	8.7	0.84	4.85

Lake ID	ALK mg/L	Ca mg/L	Cl mg/L	CON µS/cm	DIC mg/L	DOC mg/L	K mg/L	Mg mg/L	Na mg/L	NO_3 µg/L	NH_4 µg/L	TKN µg/L	pH	TP µg/L	SiO_3 mg/L	SO_4 mg/L
L752A	10.2	3.04	5.13	50.2	1.56	5.6	0.655	0.96	3.91	12	122	286	7.01	6	2.24	4.35
L752B	10.1	2.86	4.97	50	1.58	6.2	0.67	0.95	3.99	12	114	287	7	8.2	2.24	4.45
L76A	7.45	1.94	0.62	25.6	1.22	3.2	0.38	0.67	0.92	12	2	232	6.84	4.5	1.32	4.2
L76B	7.1	1.92	0.43	23.8	2.28	3.1	0.39	0.585	0.915	10	10	172	6.7	3.6	1.36	3.75
L76C	5.45	1.72	0.5	23.8	1	2.9	0.365	0.545	0.8	8	16	236	6.64	3.2	1.4	4.2
L76D	5.45	1.76	0.49	23.8	0.98	3.2	0.36	0.55	0.795	6	12	201	6.68	3.2	1.4	4.15

Table D.2. Selected water chemistry characteristics obtained as part of the quality assurance sampling. Values represent the water chemistry attributes observed at individual locations within each lake. See Appendix F for detailed description of quality assurance sampling protocol and rationale.

Lake ID	ALK mg/L	Ca mg/L	Cl mg/L	COND µS/cm	DIC mg/L	DOC mg/L	K mg/L	Mg mg/L	Na mg/L	NO_3 µg/L	NH_4 µg/L	TKN µg/L	pH	TP µg/L	SiO_3 mg/L	SO_4 mg/L
L139-2	11.3	3.56	1.94	40.6	2.24	4	0.705	1.22	1.55	2	2	231	7.15	3.7	0.72	4.35
L139-3	11.5	3.52	1.89	40.2	2.16	4	0.705	1.21	1.5	2	4	231	7.15	3.7	0.8	4.3
L210C-1	8.1	2.14	1.09	26.4	1.3	2.8	0.415	0.65	1.18	10	16	201	6.75	6	1.28	3.75
L210C-2	7.85	2.2	1.06	27.8	1.3	3	0.45	0.67	1.34	4	6	226	6.78	7.4	1.3	3.9
L210C-3	8.2	2.24	1.05	27.6	1.3	2.9	0.425	0.655	1.34	8	6	211	6.74	5	1.3	3.75
L569-1	6.65	1.94	0.32	24.8	49.7	3.1	0.46	0.575	0.765	2	2	209	6.85	2.4	0.2	3.95
L569-2	6.65	2	0.01	24.8	1.3	3.5	0.46	0.57	0.78	2	4	196	6.85	2.4	0.18	0.05
L569-3	6.35	2	0.38	24.8	1.36	3.4	0.46	0.575	0.765	2	2	196	6.81	2.6	0.2	3.75
L737-1	5.65	1.78	3.12	31.8	1.06	3.5	0.395	0.615	2.39	8	2	208	6.69	3.4	0.6	4.2
L737-2	5.4	1.76	2.95	32.2	1.04	4	0.39	0.61	2.44	2	10	190	6.72	2.8	0.56	4.1
L737-3	5.4	1.78	3.32	33.6	1.06	3.8	0.395	0.625	2.65	4	4	190	6.76	2.6	0.56	4.45
L743B-1	14	4.02	9.04	163	2.38	3.8	0.745	1.32	6.15	4	2	309	7.2	-	1.6	4.7
L743B-2	13.2	4.08	9.07	162	2.26	3.7	0.75	1.34	5.91	4	8	297	7.28	-	1.6	4.7
L743B-3	14	3.78	9.04	164	2.36	3.9	0.755	1.29	6.11	4	4	314	7.23	-	1.56	4.6

Appendix D: Investigation of Alternative Methods of Periphyton Composition Analysis

The following briefly outlines the analysis of three levels of measurement employed to investigate the relations between anthropogenic stressors and the biological response illustrated by the sampled periphyton. Each of these three levels of measurement were identified by Thomas et al. (2011) as either effective at detecting anthropogenic stress (High taxonomic resolution diatom analysis) or potentially effective (High Performance Liquid Chromatography and Chlorophyll *a*). Each method below illustrates the observed relations with stress each method demonstrated as well as a general description of the rationale used to decide the best levels of measurement employed in the remainder of this thesis.

Measurement Level 1: High Performance Liquid Chromatography (HPLC) Algal Pigment Analysis

Pigment analysis by HPLC is designed to yield information about total algal biomass, as well as relative production from the major groups of periphytic algae (e.g., green algae, blue-green algae, diatoms) for each site. Thomas et al. (2011) suggested that high performance liquid chromatography (HPLC) illustrated potential as a biomonitoring metric within the MRW, and is a desirable approach as samples are relatively easy to analyze and cost-effective (Thomas, 2013). Thomas et al. (2011) suggested that the stressor gradient observed was not great enough to produce a significant trend in HPLC samples.

Samples taken from 86 lakes within the MRW, demonstrated no significant association with stressors, suggesting that HPLC analysis is not effective for biomonitoring in lakes of the MRW. The first two PCA axes of the HPLC pigment data explains 52.5% of the total variation observed among lakes within the MRW. However, there is no apparent correlation with stressors, as sites appear to be scattered, with poor to no association between pigments and total stressor scores (represented by coloured points) (Fig. A.1). As a result, HPLC analysis provides an unreliable measure of anthropogenic stressors. To support this finding, there appears to be poor association between composite lake samples (e.g. L210C) and quality assurance samples (see Appendix F) taken at individual points within each basin (e.g. L210C-1, L210C-2 and L210C-3). Site composites should act as an average of individual quality assurance samples if they are to be used as reliable indicators of overall lake status. We do not observe this pattern; rather these results suggest that HPLC samples are influenced by localized effects rather than lake-wide stressors leading to high variability among sub-samples.

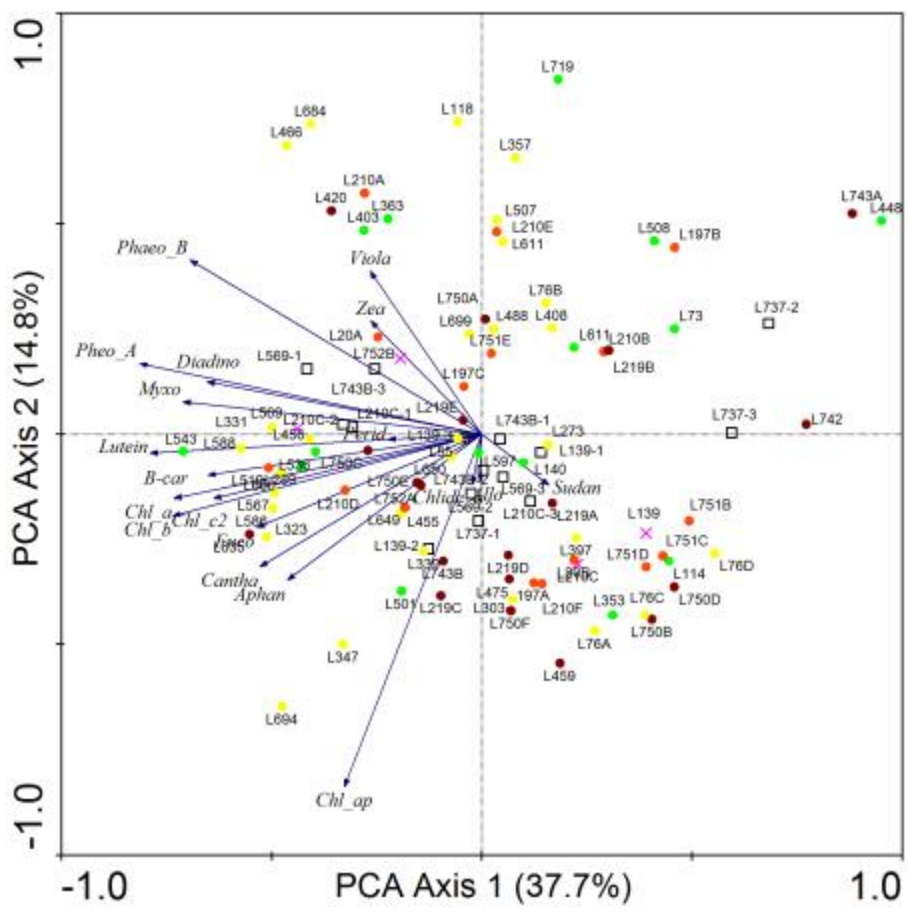


Figure D.1. PCA illustrating the ordination of sites based on HPLC pigment analysis. Symbols are coloured relative to the total amount of anthropogenic stress observed (Green-Low stress, Yellow-Moderate stress, Orange-High stress and Brown-Very High stress). For HPLC to be an effective metric in assessing periphyton response to anthropogenic stressors, similarly coloured sites should position themselves in close proximity to each other.

Measurement Level 2: Algal Biomass Assessment through Analysis of Chlorophyll *a* (Chl a) Concentration

Similar to HPLC, chlorophyll *a* (Chl *a)* analysis is designed to assess the total biomass of algae present in a sample though the estimation of pigment concentration. However, unlike HPLC which analyzes a wide spectrum of pigments, including those unique to individual algal groups, Chl *a* analysis acts as a summary of total biomass, as the pigment Chlorophyll *a* is found in all photosynthetic cells (i.e., all algae cells). Assessments of Chl *a* have been widely used in lakes to effectively assess primary production, because it is quick an inexpensive. Thomas et al. (2011), suggested that analysis of Chl *a* has potential for evaluating periphytic growth in the nearshore reaches of lakes, but they were unable to demonstrate its ability to consistently correlate with the abundance of anthropogenic stressors among their lake sites within the MRW. Samples taken in support of this thesis, illustrate similar results to Thomas et al. (2011), as significant correlation between stress score and Chl *a* was not found. Similarly, no significant correlation was found between total phosphorus concentration, the limiting nutrient to primary production in MRW lakes, and Chl *a* concentration. The concentration of Chl *a* in aquatic environments is often observed to be positively linked to the gradients of stressors, especially environments where cultural eutrophication is the dominant stressor (Schindler, 1974). Our samples suggest no relationship, if not the opposite association as there is a slight negative association observed between total phosphorus concentration and periphytic Chl *a* concentration in MRW lakes (Fig. A.2). Biomass of primary producers is often influenced by stressors, however, in regions where stress gradients are not driven by eutrophication or do not span a broad gradient, stressor influence on primary production may be muted by localized effects or naturally occurring factors such as light availability, wave action or local geochemistry. As a result, like the analysis of pigments by HPLC, Chl *a* analysis appears to be insufficiently effective at tracking the influence of anthropogenic stress.

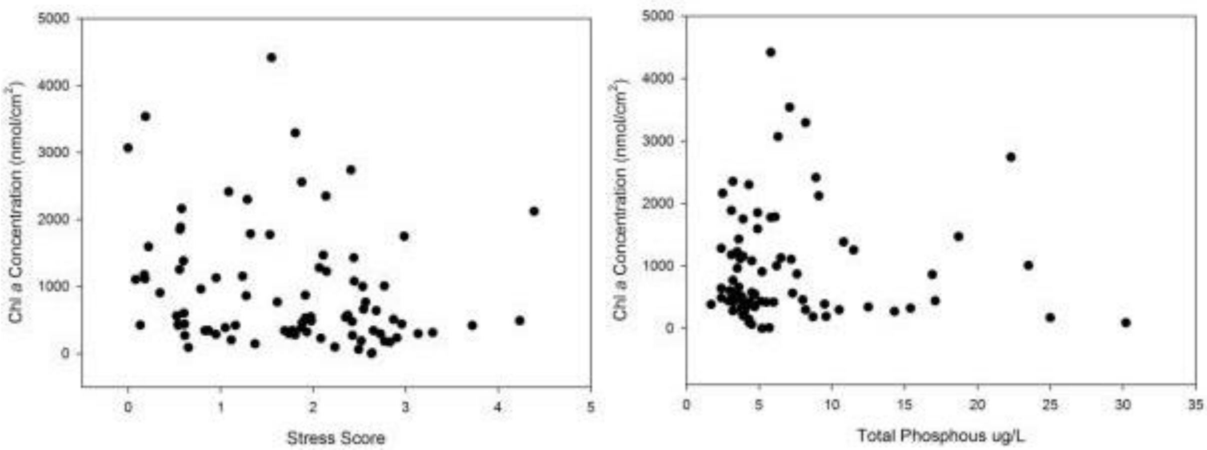


Figure D.2. Left Panel: Scatterplot illustrating the relation between the abundance of stressors (stress score) and the concentration of Chlorophyll *a*. Right Panel: Scatterplot demonstrating the relation between total lakewater phosphorus and Chlorophyll *a*. In both cases, a positive relationship is expected for Chlorophyll *a* to at as a good metric for the assessment of anthropogenic stressors.

Measurement Level 3: High Taxonomic Resolution Analysis of Diatom Community Composition

Thomas et al., (2011) suggested that above all other methods of assessing nearshore periphyton, high taxonomic resolution diatom analysis performed the best. On the basis of diatom community composition, they were able to distinguish between high and low anthropogenically stressed sites. They further suggest that the use of a larger stressor gradient, as well as more locations, may yield a stronger signal. As a result, high taxonomic resolution of diatom community structure demonstrates great promise for assessing the influence of stressors on lake environments.

By incorporating the study improvements suggested by Thomas et al. (2011), samples taken from the MRW in 2012, were found to be very effective at demonstrating an ability to track biological response to the influence of anthropogenic stressors. There is a strong horizontal separation exhibited between periphytic diatom communities found in low total stress sites (small circles) and high total stress locations (large circles; Fig. A.3). Placement along this axis was found to be highly correlated to the total stress scores ($r_s = 0.642$, $p = 2.80 \times 10^{-11}$). This strong correlation suggests that high taxonomic resolution analysis of diatom community composition, is a highly effect method of tracking the biological response to the influence of anthropogenic activity. As a result this metric, being the best method examined is used throughout the rest of the thesis, and the development of future biomonitoring programs based on this method is recommended. As a result, this level of measurement was effectively employed in Chapter 2 of this thesis.

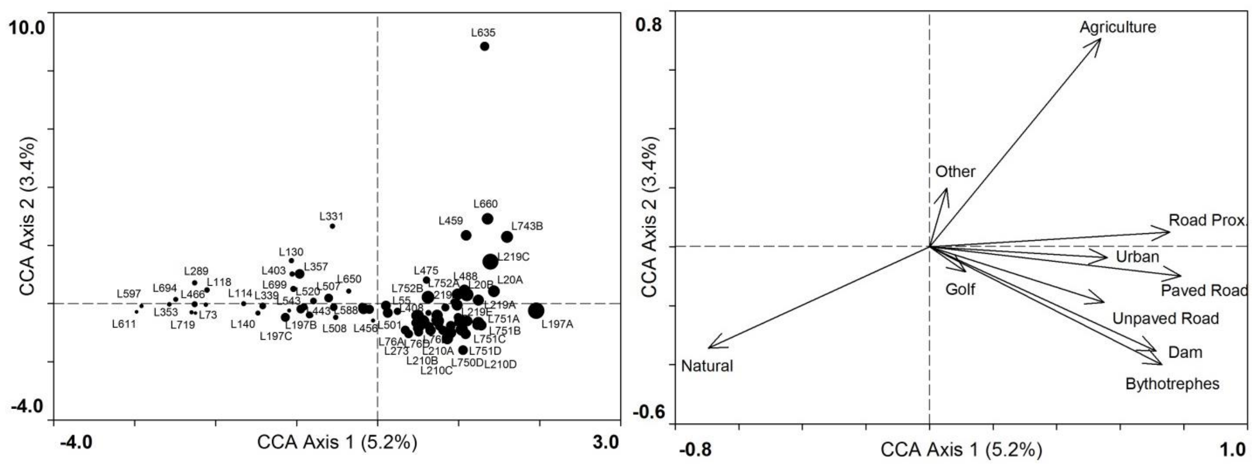


Figure D.3. CCA demonstrating the relation between anthropogenic stressors and the distribution of sites within the MRW. Right Panel: Relative positioning of lakes based on diatom community composition. Symbols are sized in proportion to the total stress observed at each site respectively. Left Panel: Ordination of the nine anthropogenic stressors which are of interest for this study.

Appendix E-Environmental Variables Available

Table C.1. Physical and chemical variables acquired for each location. "X" indicates the variables included in numerical analysis. Note: Values marked with * were unavailable for some lake basins.

Variable	Units	Used in Analysis
Physical Lake Attributes		
Latitude	Quantitative (Decimal Degrees)	X
Longitude	Quantitative (Decimal Degrees)	X
Elevation	Quantitative (m)	X
Lake Order	Categorical (Strahler)	X
Physical Catchment Attributes		
Drainage Density (Catch. Area/Lake Area)	Quantitative (m^{-1})	X
Wetland Area	Quantitative (m^2)	X
Rock/Rubble Area	Quantitative (m^2)	X
Exposed Area	Quantitative (m^2)	X
Scrub Forest Area	Quantitative (m^2)	X
Coniferous Forest Area	Quantitative (m^2)	X
Deciduous Forest Area	Quantitative (m^2)	X
Water (<8 ha) Area	Quantitative (m^2)	X
Downslope Distance Gradient	Quantitative (log())	
Wetness Index	Quantitative	
Mean Slope	Quantitative (m/m)	X
Overburden Thickness	Quantitative (m)	X
Catchment Area	Quantitative (m^2)	X
Total Natural Area	Quantitative (m^2)	X
Sample Attributes		
Julian Day	Quantitative	X
Air Temperature	Quantitative (°C)	
Water Temperature	Quantitative (°C)	
Wind Speed	Quantitative (m/s)	
Wind Direction	Categorical (Compass)	
Precipitation	Categorical (None-Storm)	
Cloud Cover	Quantitative Estimate (%)	
Site Shade	Categorical (1-3)	
Substrate	Qualitative (Description)	
Macrophytes	Categorical (1-3)	
Algae	Categorical (1-3)	
Detritus	Categorical (1-3)	
Riparian Vegetation Community	Categorical (1-6)	
Visual Site Stressors	Qualitative (Description)	
Anthropogenic Stressor Attributes		
Developed (Urban/Rural) Area	Quantitative (m^2)	X
Agricultural Area	Quantitative (m^2)	X
Golf Course Area	Quantitative (m^2)	X

Variable	Units	Used in Analysis
Other Area (Utilities/Waste etc.)	Quantitative (m^2)	X
Road Density	Quantitative (m^2)	X
Unpaved Road Density	Quantitative (m^2)	X
Roadway Proximity	Quantitative (m)	X
Distance to Hydraulic Control Structure*	Quantitative (m)	
Presence of Hydraulic Control Structure	Binary (Presence/Absence)	X
Presence of *Bythotrephes longimanus*	Binary (Presence/Absence)	X
Boat Traffic*	Categorical (1-3)	
Water Chemistry Attributes		
Silver (Ag)	µg/L	
Alkalinity-Total Fixed Endpoint	mg/L CaCO3 (Equivalent)	
Alkalinity-Granular	mg/L CaCO3 (Equivalent)	X
Aluminum (Al)	µg/L	
Anions	meq/L	
Arsenic (As)	µg/L	
Barium (Ba)	µg/L	
Boron (B)	µg/L	
Beryllium (Be)	µg/L	
Calcium (Ca^{2+})	mg/L	X
Cadmium (Cd)	µg/L	
Chloride (Cl^-)	mg/L	X
Colour-True	TCU	
Conductivity	µS/cm	X
Conductivity-Estimated	µS/cm	
Cobalt (Co)	µg/L	
Chromium (Cr)	µg/L	
Copper (Cu)	µg/L	
Carbon-Dissolved Organic (DOC)	mg/L	X
Carbon-Dissolved Inorganic (DIC)	mg/L	X
Iron (Fe)	µg/L	X
Cations	meq/L	
Ion Balance	%	
Potassium (K^+)	mg/L	X
Langeliers Index	-	
Magnesium (Mg)	mg/L	X
Manganese (Mn)	µg/L	
Molybdenum (Mo)	µg/L	
Sodium (Na)	mg/L	X
Nickel (Ni)	µg/L	
Nitrogen-Ammonium + Ammonia (NH_2/NH_3)	µg/L	X
Nitrogen-Nitrate + Nitrite (NO_2/NO_3)	µg/L	X
Nitrogen-Total Kjeldahl (TKN)	µg/L	X
Lead (Pb)	µg/L	
pH	-	X
pH-Saturation Estimated	-	

Variable	Units	Used in Analysis
Total Phosphorus (TP)	µg/L	X
Solids-Dissolved Estimate	mg/L	
Antimony (Sb)	µg/L	
Selenium (Se)	µg/L	
Reactive Silicate (SiO_3)	mg/L	X
Strontium (Sr)	µg/L	
Sulphate (SO_4^-)	mg/L	X
Titanium (Ti)	µg/L	
Thallium (Tl)	µg/L	
Uranium (U)	µg/L	
Vanadium (V)	µg/L	
Zinc (Zn)	µg/L	

Appendix F-Quality Assurance

At five sampling basins (selected on the basis of ease in transporting extra gear), individual water chemistry and diatom samples were collected from each sampling site in addition to the sampling basin composite samples. Water chemistry quality-assurance samples were sampled following the same methods as composite samples (See *Methods* in Chapter 2), however water from each of the three sample sites within each basin were kept isolated and submitted for chemical analysis as individual samples. Similarly periphyton samples from each of the three quadrants sampled were collected following the same methods as composite samples, however the samples were collected from an additional set of cobbles at the 0m, 3m and 6m sections of the transect. These quality assurance periphyton samples, were not combined with samples from the other sampling sites within the basin, allowing samples to be analyzed individually. Comparisons of individual samples to composite samples collected to represent the whole basin provide assurance that basin estimates from composite samples are indeed accurate representations of mean sampling site values. Furthermore, we can observe variation in sampling sites and identify whether specific sampling locations have a greater or lesser influence on the overall lake assessment. This analysis will also provide insight into the differences between this study and Thomas et al. (2011), which was limited to assessing localized stressors rather than lake level conditions. By taking quality assurance measures we can understand whether analysis can be accurately expanded to basin level analysis (rather than sampling site level). It would be expected that individual samples at any location within a basin may show some unique characteristics; however the overall trend in water chemistry and biological assemblage should be dictated by the specific environmental properties and stressors of the basin.

Appendix F.1-*Quality Assurance-Water Chemistry*

Key water chemistry attributes from each of the quality assurance sites were compared to the composite sample, and the percent difference for each variable was calculated. For full water chemistry results for composite and quality assurance samples, see Appendix C.

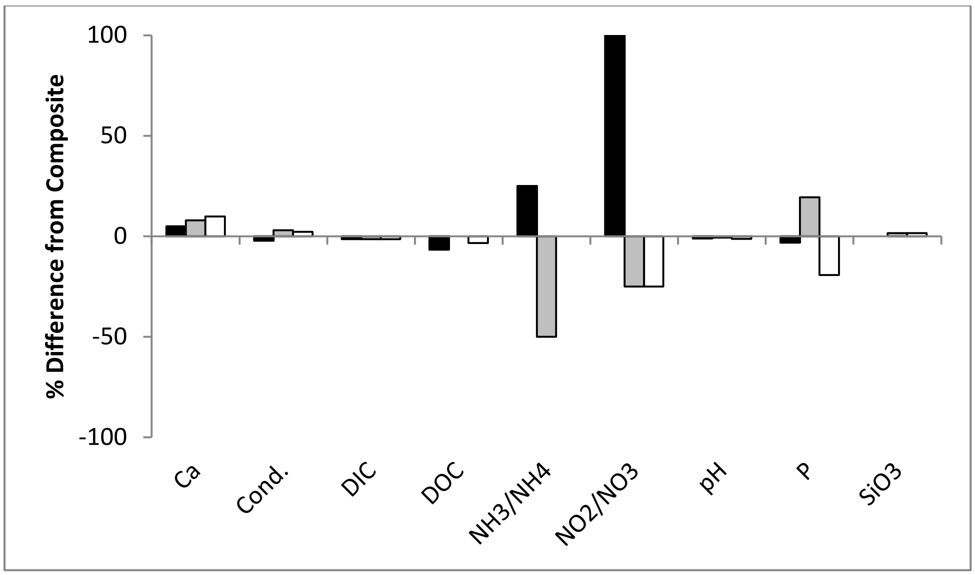


Figure F.1.1. Plot of water chemistry attributes (Ca-Calcium; Cond.-Conductivity; DIC-Dissolved Inorganic Carbon; DOC-Dissolved Organic Carbon; NH3/NH4-Ammonia + Ammonium; NO2/NO3-Nitrite + Nitrate, pH-Acidity; P-Total Phosphorus; SiO3-Reactive Silicate), for L210C (Lake of Bays, Trading Bay) quality assurance assessment. Graph presents the percent difference of values for the quality-assurance samples compared to the site composite sample. Black-quality assurance sample 1 (L210C-1); Grey-quality assurance sample 2 (L210C-2); White- quality assurance sample 3 (L210C-3).

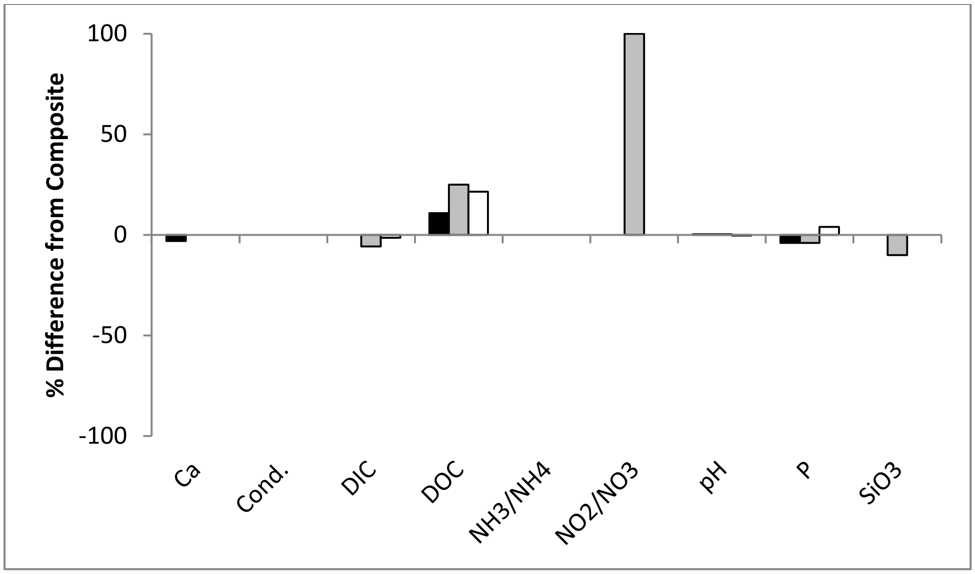


Figure F.1.2. Plot of water chemistry attributes (Ca-Calcium; Cond.-Conductivity; DIC-Dissolved Inorganic Carbon; DOC-Dissolved Organic Carbon; NH3/NH4-Ammonia + Ammonium; NO2/NO3-Nitrite + Nitrate, pH-Acidity; P-Total Phosphorus; SiO3-Reactive Silicate), for L569 (Buck Lake) quality assurance. Graph presents the percent difference of values for the quality-assurance samples compared to the site composite sample. Black-quality assurance sample 1 (L569-1); Grey-quality assurance sample 2 (L569-2); White- quality assurance sample 3 (L569-3).

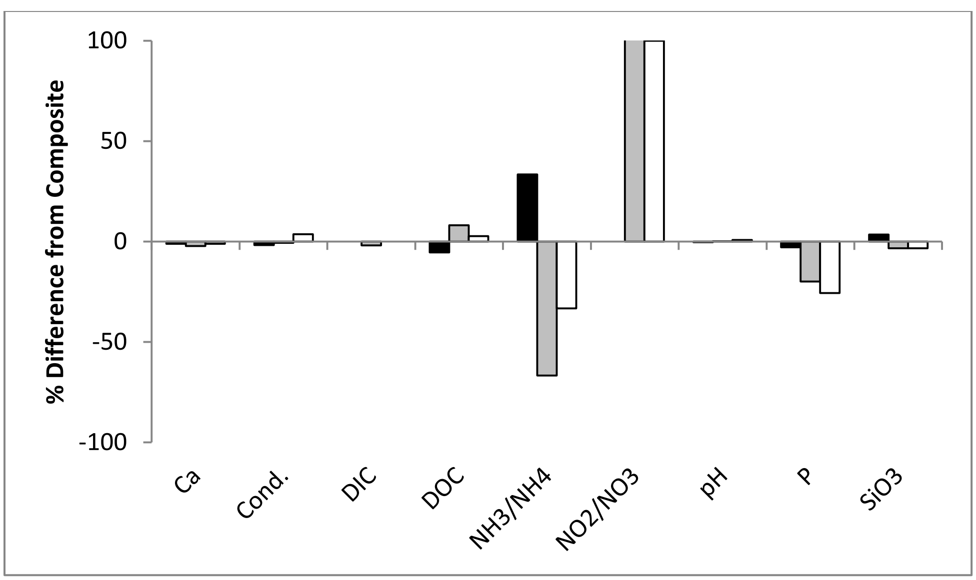


Figure F.1.3. Plot of water chemistry attributes (Ca-Calcium; Cond.-Conductivity; DIC-Dissolved Inorganic Carbon; DOC-Dissolved Organic Carbon; NH3/NH4-Ammonia + Ammonium; NO2/NO3-Nitrite + Nitrate, pH-Acidity; P-Total Phosphorus; SiO3-Reactive Silicate), for L737 (Smoke Lake) quality assurance. Graph presents the percent difference of values for the quality-assurance samples compared to the site composite sample. Black-quality assurance sample 1 (L737-1); Grey-quality assurance sample 2 (L737-2); White- quality assurance sample 3 (L737-3).

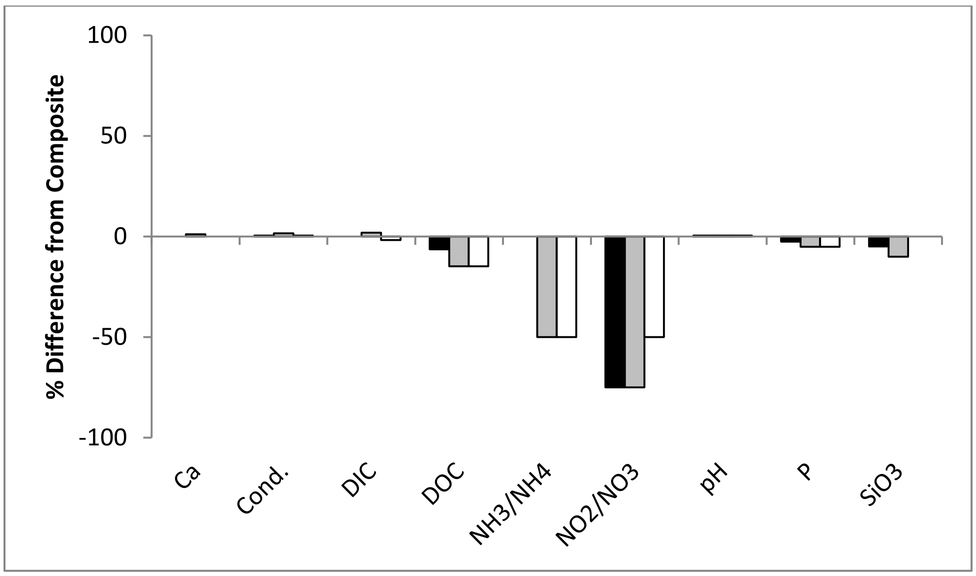


Figure F.1.4. Plot of water chemistry attributes (Ca-Calcium; Cond.-Conductivity; DIC-Dissolved Inorganic Carbon; DOC-Dissolved Organic Carbon; NH3/NH4-Ammonia + Ammonium; NO2/NO3-Nitrite + Nitrate, pH-Acidity; P-Total Phosphorus; SiO3-Reactive Silicate), for L139 (Long Line Lake) quality assurance. Graph presents the percent difference of values for the quality-assurance samples compared to the site composite sample. Black-quality assurance sample 1 (L139-1); Grey-quality assurance sample 2 (L139-2); White- quality assurance sample 3 (L139-3).

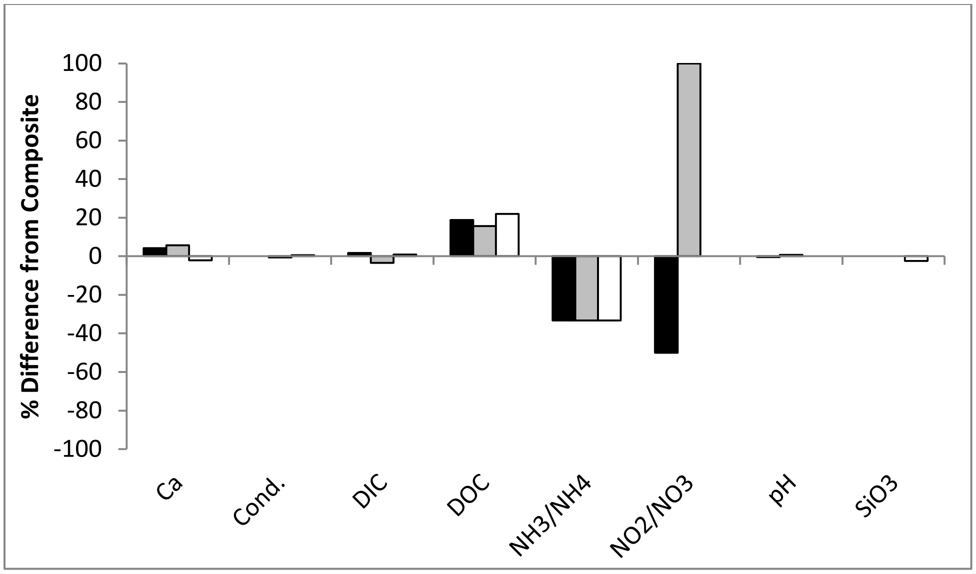


Figure F.1.5. Plot of water chemistry attributes (Ca-Calcium; Cond.-Conductivity; DIC-Dissolved Inorganic Carbon; DOC-Dissolved Organic Carbon; NH3/NH4-Ammonia + Ammonium; NO2/NO3-Nitrite + Nitrate, pH-Acidity; SiO3-Reactive Silicate), for L743B (Peninsula Lake, East Basin) quality assurance. Graph presents the percent difference of values for the quality-assurance samples compared to the site composite sample. Black-quality assurance sample 1 (L743B-1); Grey-quality assurance sample 2 (L743B-2); White- quality assurance sample 3 (L743B-3).

At each of the lakes examined, very little difference was observed between individual quality assurance sites and their respective composite samples. Nitrogen species, however, are an exception, as quality assurance sites showed up to a 100% difference from the composite samples. This would suggest that the concentration of nitrogen species is regulated by highly localized attributes, leading substantial within lake variation. Within-lake differences in water chemistry, large differences in nitrogen species is not expected to markedly affect community composition, because nitrogen species were not identified as strongly associated with variation in diatom community composition. On the basis of the quality-assurance analysis, the composite samples appear to represent well the average water chemistry conditions within each lake basin.

Appendix F.2-*Quality Assurance-Periphytic Diatom Community Composition*

Diatom community composition from each of the quality assurance sites were compared to the composite sample.

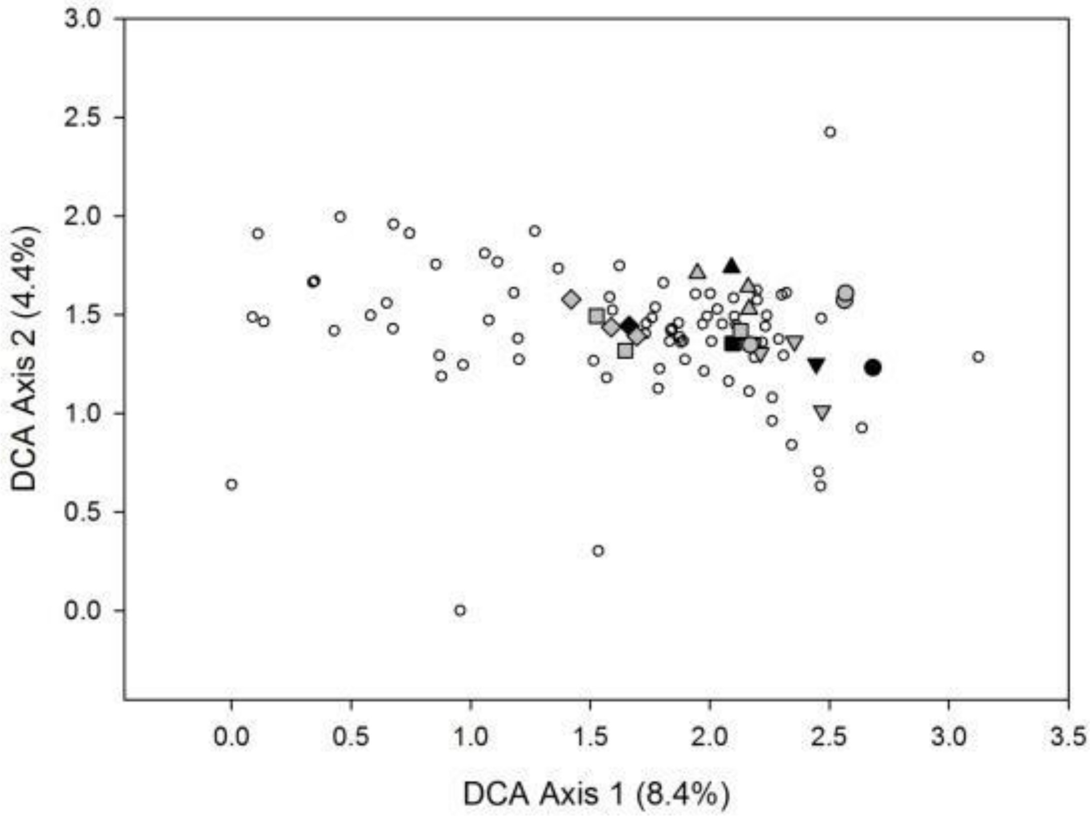


Figure F.2.1. DCA ordination plot of the site-composite samples for the quality-assurance sites (solid black Symbols), quality-assurance samples (Grey Symbols) and composite samples for the other sites within the 86-lake dataset (Empty Symbols). Each quality-assurance sample and corresponding composite lake sample are illustrated by: Down triangle (L139), Up triangle (L210C), Square (L569), Diamond (L737) Circle (743B)

For each lake assessed, diatom community composition of quality-assurance sites was found to be very similar to communities found in composite samples. This is an important finding, because it suggests that within-lake differences are less than between-lake differences. Furthermore, it suggests that the composite lake sample provides an adequate assessment of the 'average' periphytic diatom community composition for each lake-basin. It does, however, also suggest that a single sample from one sampling location may provide an equivalent assessment of community composition as the site-composite samples.

Appendix G-Method of Index Scaling

For each of the indices presented in Chapter 3, except the IDEC, index values were scaled to vary between zero and 100, as a way of making score more intuitive. While the Biotic indices (IDEC and mIDEC) and Indices of Biological Integrity (IBI-1, IBI-2, IBI-3) used a different formulae to summarize the periphytic diatom community composition, the formula to produce final index scores was constant. The formula used to scale each index is briefly summarized below (for details on how index scores were developed and tested see *Methods* in Chapter 3).

Index Scaling

Summarization of periphytic diatom community composition by each index produced values which varied greatly (e.g. IBI-1 values ranged between -2 and 1, whereas IBI-3 values ranged between 400 and 1700). This made comparisons between indices difficult to perform. These values are also not intuitive to unexperienced users of the index. As are result, these initial values scores are scaled for each index using equation G1.

$$Index\ Score = Scaled\ Raw\ Index\ Scores = \frac{k_{ip} + |k_{\wedge p}|}{\left(\left(|k_{\vee p}| + |k_{\wedge p}|\right) \cdot 100\right)}$$

Equation G1

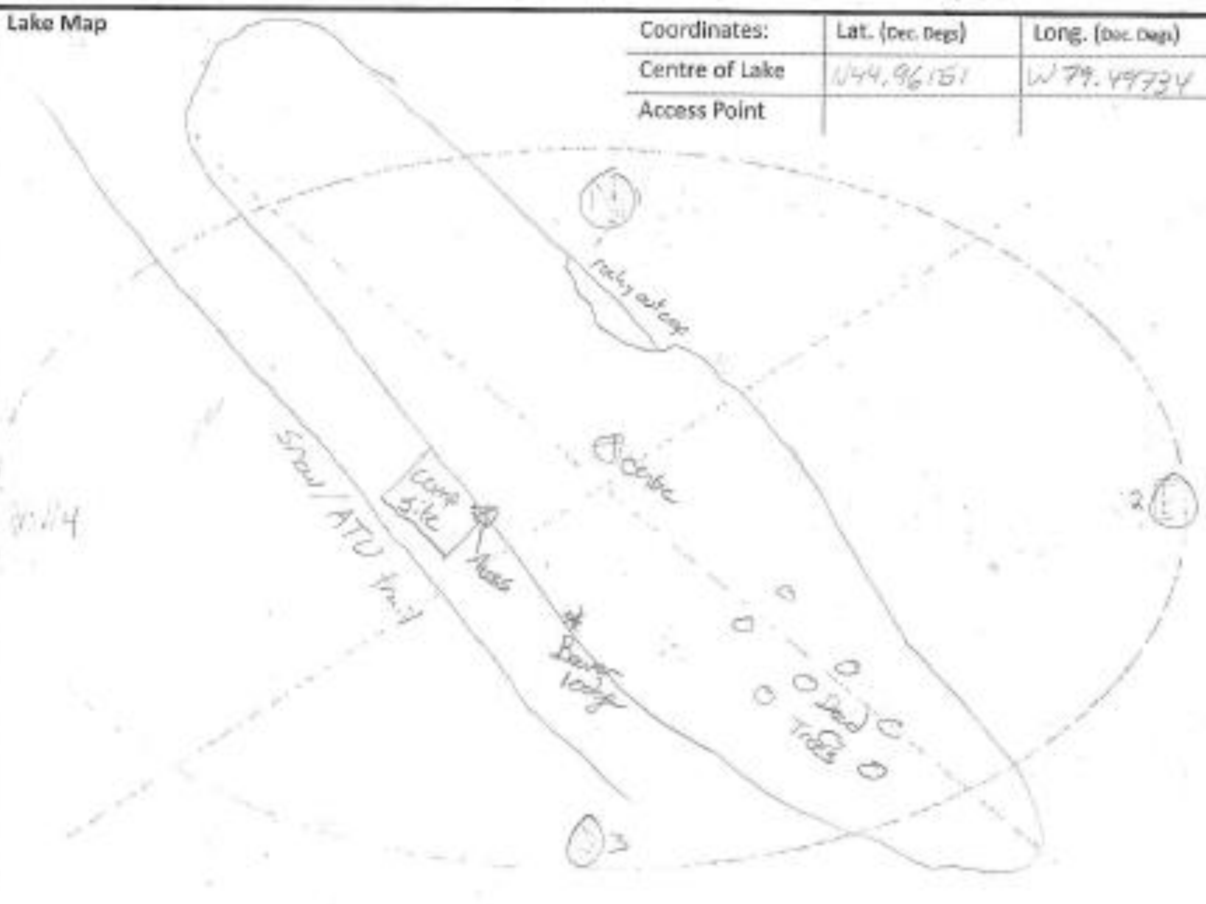
Where k_i^* represents the initial summarized value of site$_i$, $k_\wedge$ is the minimum initial summarized value observed for a selected index$_p$ and $k_\vee$ is the maximum initial summarized value observed for a selected index$_p$. This scaling factor allows each index to range between 0-100 based on the actual values observed (i.e. the highest quality lake basin in the MRW is scored as 100, the poorest quality basin is scored as 0).

Appendix H-Example Field Sheet

CWN-LAKES FIELD SHEET		Lake ID: L118
Lake Name: Unnamed Lake - Off Clear Lake Rd. Torrance		Chemistry Box #
Date Sampled: (dd-mmm-yyyy) 04-JUL-2012	Secchi Depth: 0.8m	
Start Time: 10:50	Lake Depth: 0.8m	
Crew #: A	Crew Leader: MM	
Wind Speed: Calm Direction:	Air Temp: 27.0°C Water Temp: 27.0°C	
Precipitation: None ☑ Light ☐ Moderate ☐ Heavy ☐ Storm ☐	Cloud Cover: 20 %	

Lake Map

Coordinates:	Lat. (Dec. Degs)	Long. (Dec. Degs)
Centre of Lake	N44.96151	W79.49734
Access Point		



Dam at outlet: Yes ☐ No ☐	Boat Traffic: None ☐ Light ☐ Moderate ☐ Heavy ☐

Memorable features:
- Coyote at access
- Lots of lilly pads
- lots of leeches!
- water snake

General Comments:
- lilly pads cover entire surface of lake
- old beaver pond?

SITE #2

Quadrant: N ☐ E ☑ S ☐ W ☐

Time: 1145

Site Shaded: Yes ☐ No ☐ Partially ☐

Diatom Transect Coordinates: *(from centre, decimal degrees)*
N 44.96106 W79.49586

Substrate:

	Sample 1	Sample 2	Sample 3
Dominant	silt		
2nd Dominant	bedrock		

Class	Description	Class	Discription
1	Clay (hard pan)	4	Gravel (2-65mm)
2	Silt (gritty, <0.06mm diameter)	5	Cobble (65-250mm)
3	Sand (grainy, 0.06 – 2mm)	6	Boulder (>250mm)
		7	Bedrock

Pebble Count:

Substrate Type (*silt, sand, gravel, etc.*)

	Sample 1	Sample 2	Sample 3
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Photographs:	Photo File Name	Description
Quadrant		
Sample 1		
Sample 2		
Sample 3:		
Benthos:		
Other 1:		
Other 2:		

Benthos Collection Waypoint 013.

Lat. (Dec. Degs)	Long. (Dec. Degs)	Sampling Distance(m)	Time (min)	Max Depth (m)
N 44.96111	W079.49596	2.20m	0:35s	0.35

Organic Matter-Aerial Coverage:
1 (Abundant), 2 (Present), 3 (Absent)

	Sample1	Sample 2	Sample 3
Woody Debris	2		
Detritus	1		

Aquatic Macrophytes & Algae (circle dominant type)

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	2		
Rooted Floating	1		
Submergent	2		
Free Floating	3		

Algae	Sample 1	Sample 2	Sample 3
Floating	3		
Filaments	3		
Attached	2		
Slimes/Crusts	2		

Riparian Vegetative Community
1 (none), 2 (cultivated), 3 (meadow), 4 (scrubland),
5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (*dist. from waters edge, in m*)	Onshore
1.5-10	Bedrock island – goes from
10-30	rock straight into water –
30-100	see pictures.

Site Features:
Contamination sources: None

Garbage/Waste: None

Adjacent land use:
Cottage/House ☐ Agricultural ☐ Urban ☐ Golf Course ☐ Road ☐ Utility ☐
Other: Forested.

Checklist:

Chemistry Collected ☑
Diatom Sample ☑
Diatom Sample ☑
Chl a ☑
Pictures ☑
HPLC ☑
Labels ☐

Site Comments:
all samples on bedrock island.

SITE #3

Quadrant: N ☐ E ☐ S ☒ W ☐ | Time: 12:30

Site Shaded: Yes ☐ No ☒ Partially ☐

Diatom Transect Coordinates: *(from centre, decimal degrees)*
Waypoint 14 N 44.96104 W 79.49778

Substrate:

	Sample 1	Sample 2	Sample 3
Dominant	Silt		
2nd Dominant	Be		

Class	Description	Class	Discription
1	Clay (hard pan)	4	Gravel (2-65mm)
2	Silt (gritty, <0.06mm diameter)	5	Cobble (65-250mm)
3	Sand (grainy, 0.06 – 2mm)	6	Boulder (>250mm)
		7	Bedrock

Pebble Count:

Substrate Type *(silt, sand, gravel, etc.)*

	Sample 1	Sample 2	Sample 3
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Photographs:	Photo File Name	Description
Quadrant		
Sample 1		
Sample 2		
Sample 3:		
Benthos:		
Other 1:		
Other 2:		

Benthos Collection WPT 15

Lat. (Dec. Degs)	Long. (Dec. Degs)	Sampling Distance(m)	Time (min)	Max Depth (m)
N 44.96107	W 79.49781	2.30	0:17	0.32

Organic Matter-Aerial Coverage:
1 (Abundant), 2 (Present), 3 (Absent)

	Sample1	Sample 2	Sample 3
Woody Debris	2		
Detritus	1		

Aquatic Macrophytes & Algae (Circle dominant type)

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	2		
Rooted Floating	1		
Submergent	3		
Free Floating	3		

Algae	Sample 1	Sample 2	Sample 3
Floating	3		
Filaments	3		
Attached	2		
Slimes/Crusts	3		

Riparian Vegetative Community
1 (none), 2 (cultivated), 3 (meadow), 4 (scrubland),
5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone *(dist. from water edge, in m)*	Onshore
1.5-10	1
10-30	5
30-100	1

Site Features:
Contamination sources: None
Garbage/Waste: None
Adjacent land use:
Cottage/House ☐ Agricultural ☐ Urban ☐ Golf Course ☐ Road ☐ Utility ☐
Other: Back country campsite / ATV trail

Checklist:

Chemistry Collected ☑
Diatom Sample ☑
Diatom Sample ☑
Chl a ☑
Pictures ☑
HPLC ☑
Labels ☐

Site Comments:

How many leeches is too many?
Not too mention Snakes

Sampled Bedrock ~ 0.3m

Periphyton Sampling Protocol-Lakes

General Assessment

- **a.** From the launch point, paddle out to the centre of the lake and take a depth reading and recorded it on the field sheet.
- **b.** Anchor the boat to eliminate drifting, and fill out the general information section of the field sheet
- **c.** Secchi depth should be taken by lowering a disk on the shaded side of the boat until it is no longer visible. Raise the disk until it is visible again. Take the average of these two values as the secchi depth.
- **d.** Draw a map of the lake with the help of a compass and randomly assign three quadrants to sample

Site Assessment

- **a.** Paddle to the centre of the quadrant and choose the first site you come across with sufficient cobble
- **b.** Within this area find a section of water that is 'undisturbed'. Mark out a 6m transect parallel with the shoreline. The centre of this transect should line up with the most undisturbed section of water. Each transect is divided into six contiguous 1 m diameter circular plots in which periphyton will be sampled at 3m intervals (0, 3 and 6m) **(Figure 1).**
- **c.** Using a 500mL collection bottle, collect 1L of water for chemistry analysis approximately half way down the water column.
- **d.** This site will represent **1/3** of the total composite lake sample

Diatom/HPLC/Chlorophyll *a* Collection

Sampling From the Substrate-<u>*Sampling from cobbles is preferred*</u> (however may not be possible in all situations; in which case bedrock or boulders should be sampled *in situ*)

Sampling Cobbles/Boulders/Bedrock- To reduce unwanted material in the sample from a disturbed bottom, place cobbles *face up* into a bucket filled with 'clean' lake water deep enough for the rock to be fully submerged. Other substrates should all be sampled *in situ* with minimal sediment disturbance

- **i.** At each sample plot (0, 3, 6m) identify the first reasonable sized cobble to sample from enough for 3 replicates (ie. 3 circles). Place the cobble in the bucket for sampling.
- **ii.** Place the syringe (with the brush) firmly against the top surface of the cobble. Apply pressure to the end of the plunger and rotate against the surface of the rock for 30 seconds.
- **iii.** Collect the resulting slurry with the attached syringe, by drawing approximately 25mL of fluid
- **iv.** Eject the 25mL slurry into a 500mL PET jar and use a squirt bottle to clean any remaining periphyton off of the brush.
- **v.** Repeat steps **i-iii** for a second replicate (circle) on the **SAME** cobble or rock surface (do not overlap subsamples). Rather than placing these samples in PET jars, place them in Brown Nalgene bottles for HPLC and Chlorophyll *a* analysis respectively

Repeat steps **i-v** on an additional cobble or rock area within the plot, than repeat the entire sample procedure for the 2 remaining plots (3m and 6m).

At the end of each site you should have sampled 6 cobbles; each cobble sampled 3 times for a total of 18 circles per site.

Field Sheets-Ensure that the field sheets outlining site specific data are completed and placed with the sample bottles in the cooler, for each site.

Repeat Chemistry, Diatom collection and Benthic Sampling at the remaining two quadrant sites

Analysis Preparation

- **a.** Ensure that the 3 diatom subsamples are combined into one 500mL PET jar which will then be treated as one lake composite sample for analysis. Fix this sample by adding approximately 1.0 ml of Lugol's solution, making it a light tea colour
- **b.** When finished keep all bottles in a closed cooler with ice packs. <u>**Ensure all bottles are properly labeled**</u>

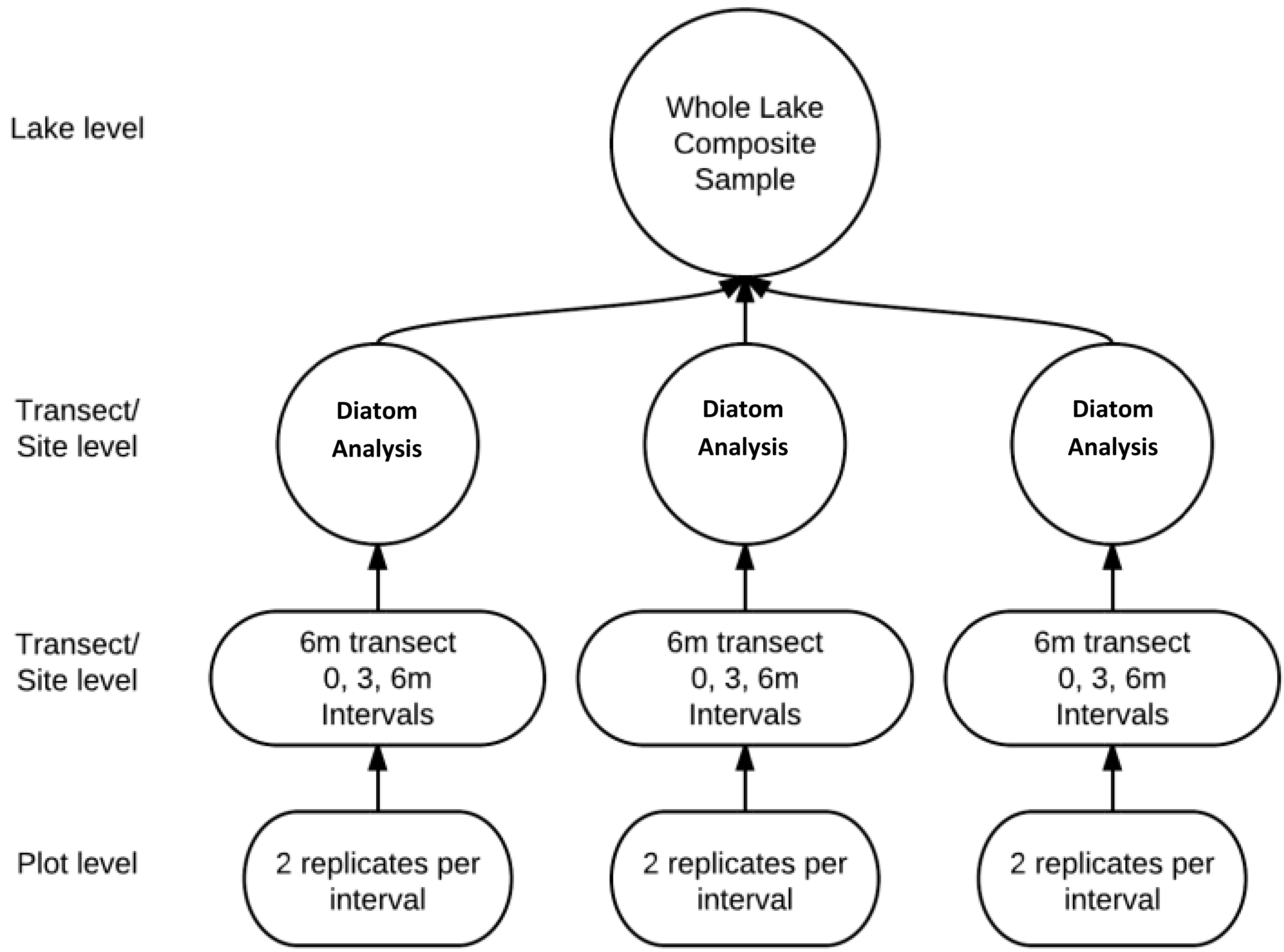


Figure I.4. This figure illustrates the progression of diatom analysis samples from plot replicates at 3m intervals for each transect, which is combined for the entire transect (site samples) which are then combined to create one composite sample for the entire lake.

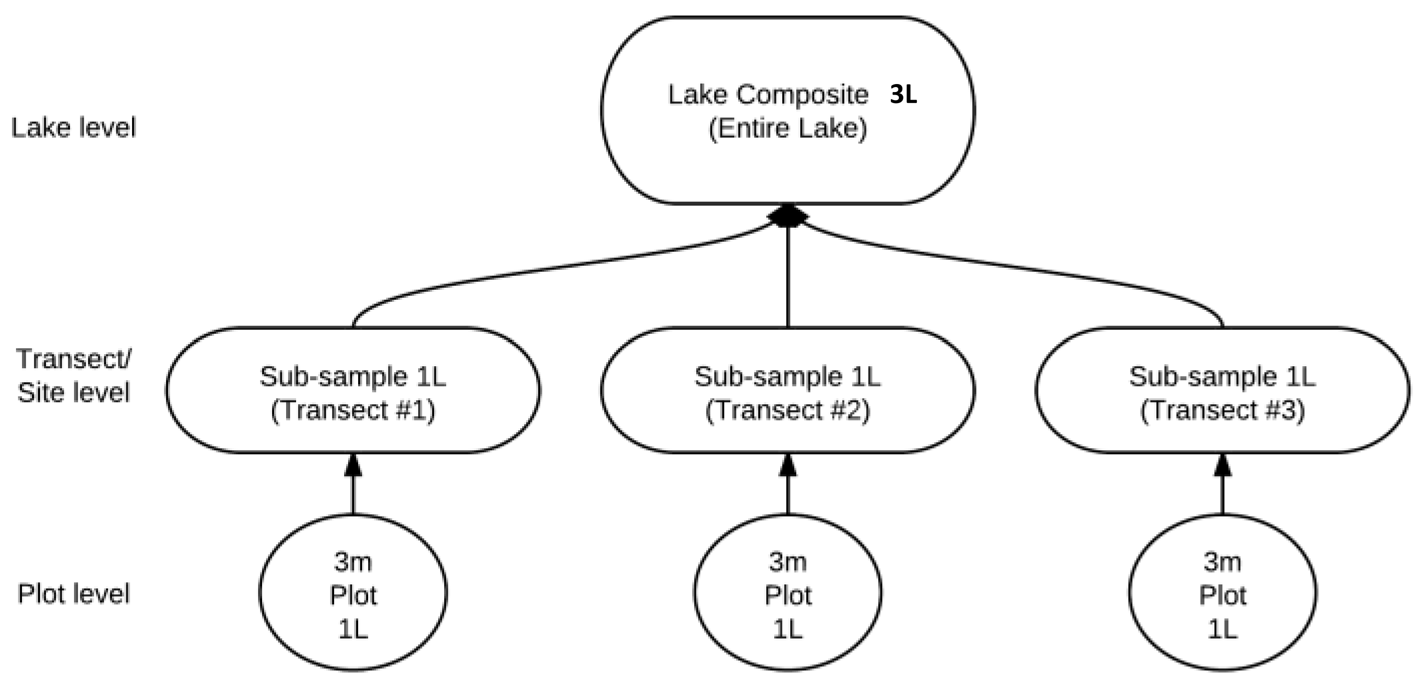


Figure I.5. This figure illustrates the sample progression for collecting chemistry samples. 1L of water will be collected at the 0, 3 and 6m plots along each transect and then filtered and combined to form one 3L composite sample for the entire lake**.**