The role of submersed macrophytes in river eutrophication and biogeochemical nutrient cycling

by

Jennifer Lynne Alice Hood

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Author's Declaration

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

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

Abstract

The goal of this work is to contribute to the understanding of eutrophication in large rivers with a detailed study of the Grand River, an impacted river in highly agricultural and urbanized Southern Ontario. It focuses on the role of nitrogen (N) and phosphorus (P) in the distribution and abundance of benthic submersed macrophytes, which are important actors in river N and P cycles.

Chapter 1 uses data from the Provincial Water Quality Monitoring Network to examine seasonal, long term and spatial patterns in total P (TP), soluble reactive P (SRP), nitrate and nitrite (NO_3^- + NO_2^-) and ammonium (NH_4^+). The monitoring of many sites in the Grand River began in 1965, and I examine data from the period from 1965 to 2009. The monitoring program began prior to the Canada-USA ban on the use of phosphate in detergents, which came into effect in 1973, and also before major improvements to municipal waste water treatment. The phosphate ban is analyzed as an example of a whole-system nutrient manipulation experiment, and the seasonal and long term response of the river system, from headwaters to mouth, is examined. TP and SRP declined over the monitoring period, with the greatest response found in TP, which declined by 120 µg/l/y immediately downstream of the of the watershed's largest treatment plant in the years 1972-1975. Thereafter, TP and SRP continued to decline over most of the lower river, with rates of decline in nutrient concentration accelerating with distance from the wastewater treatment plants (WWTPs). NO_3+NO_2 increased during the monitoring period in the upper portion of the river with the highest increase of 158 µg-N/l/y observed in the 10 year period of 1975-1985. It did not change in response to WWTP upgrades that occurred in the early 1970s. WWTPs were a clear source of TP, SRP and NH_4^+ to the river system, but not NO_3^-+NO_2^-, and the continual increase in NO_3^-+NO_2^- was due to increases in diffuse sources. The seasonal and spatial data suggest that non-point sources of N and P dominate in the Grand River watershed. However, the largest WWTP in the region at Kitchener is an important source of nutrients, and was an especially large source of P prior to changes in detergent standards and wastewater treatment.

The submersed macrophyte biomass in the Grand River was examined as a function of proximity to WWTPs in chapter 2. Spatial surveys were conducted in 2007 and 2009 on three reaches of approximately 10 km in length each, with two reaches having an upstream and

downstream section, separated by a WWTP. Macrophyte patches were mapped, biomass was estimated, and plants were analyzed for N and P. Tissue N and P were compared to published thresholds for evidence of nutrient limitation. Biomass was greater downstream of the WWTPs than upstream in both reaches and both years, indicating that nutrient loading leads to increased biomass downstream, evidence that even in a heavily agricultural watershed, point sources have a demonstrable effect on macrophyte biomass. Depth was important in explaining some of the variation, while river width and orientation were not important. Even though macrophyte biomass was elevated downstream of the WWTPs, there was no strong evidence of N or P limitation upstream based on tissue concentrations and a laboratory determined critical nutrient threshold, and I hypothesize that the nutrient limitation affecting biomass occurs earlier in the growing season, before peak biomass. This suggests that the eutrophication process in rivers is distinct from that in lakes, and future work should view eutrophication in rivers in the context of seasonal succession.

Drivers of seasonal and inter-annual variability in submersed macrophyte biomass were examined in chapter 3 with a multi-year, reach-scale spatial survey of three reaches near the WWTPs of Waterloo and Kitchener. Biomass differed among reaches, years and sites, and showed distinct seasonal patterns. The reach downstream of the WWTPs had the highest biomass, and peak biomass came soonest in the growing season, while the upstream reach had the smallest and latest peak biomass. Weather was significantly correlated to both the quantity and the time of the peak biomass, with higher temperatures associated with larger and earlier peak biomass and precipitation and higher flow associated with later and lower peak biomass. Therefore, the eutrophication response in rivers can depend on weather, and these drivers of variation should be accounted for when forecasting responses to future changes in nutrient loading.

The effect of nitrogen discharged by WWTPs on the riverine submersed macrophyte community, and the suitability of macrophyte tissues as indicators of point source impact, were quantified in chapter 4 using $\delta^{15}N$ as a tracer of WWTP effluent impact. Macrophytes and water for NO_3^- and NH_4^+ concentration and isotope analysis was collected by canoe along two 10 km reaches of the river, up and downstream of two WWTPs. Macrophytes incorporated effluent nitrogen into their tissues downstream of the WWTPs, using effluent NH_4^+ rather than NO_3^-. Impacts of the effluent on macrophytes can be traced as far as 10 km downstream, while

daytime chemical evidence of the plume disappeared much sooner. The $\delta^{15}N\text{-}NH_4^+$ value rapidly increased downstream of the WWTP, changing in one instance from +13‰ to +31‰ over 1 km, with macrophyte $\delta^{15}N$ values changing from +6‰ to +24‰ over 5 km, while $\delta^{15}N\text{-}NO_3^-$ values showed no such change. These data lead to the conclusion that riverine submersed macrophytes record the influence of WWTP effluent, specifically effluent NH_4^+, but that using two end-member mixing models to determine N sources would be inappropriate in such dynamic environments.

Nitrogen cycle processes such as nitrification and denitrification are influenced by dissolved oxygen (DO) and rapid transformations occur in environments with strong DO gradients. Because development of dense macrophyte beds in eutrophic rivers has the potential to greatly alter daily oxygen cycling, producing strong redox potentials, macrophytes could influence microbial nitrogen cycling. In Chapter 5, nitrogen uptake by macrophytes using a $^{15}N\text{-}NH_4^+$ tracer and N_2O production was investigated using *in situ* chamber incubations upstream and downstream of a WWTP. NH_4^+ uptake occurred in chambers, while measurable net N_2O production occurred in some chambers only. Neither N_2O production nor NH_4^+ uptake differed between chambers with and without PO_4^{3-} addition, nor did they differ between light and dark treatments. NH_4^+ uptake was higher at the upstream site, indicating that above the WWTP there was NH_4^+ demand in the macrophyte community. NH_4^+ uptake was a hyperbolic function of mean chamber NH_4^+ concentration. Turnover time for the macrophyte N pool due to NH_4^+ uptake was as long as 47 d, while the turnover of the dissolved NH_4^+ pool was as rapid as 14 h. Because net uptake was a small fraction of gross uptake, calculated release rates were almost as high as uptake rates, again indicating rapid NH_4^+ cycling.

Eutrophication of rivers has elements that make it a process distinct from that in lakes. I showed that, in the Grand River, N and P were both high in concentration throughout the river, with a distinct increase downstream of the largest WWTPs in the watershed. The biomass of benthic submersed macrophytes was elevated below the WWTPs, but there was no evidence of nutrient limitation upstream during the time of peak biomass. Macrophyte biomass development followed a seasonal pattern, but was also influenced by seasonal temperature and precipitation patterns. Thus, the riverine eutrophication process has an important seasonal component, much as the plants themselves do, peaking in the summer and senescing in the fall. As part of the eutrophication response, macrophytes altered the chemical cycles of nutrients

that fuel their growth. Though changes in benthic biomass themselves are part of riverine eutrophication, this thesis provides evidence that changes in macrophyte biomass produces chemical and ecological changes that are characteristic of increased trophic conditions.

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Dedication

This thesis is dedicated to my mom, Lynne Eileen Hood, born in Trenton Ontario, May 22 1952, and died November 10 2006. Though she was not here to see me through the completion of this work, her belief that I could accomplish anything has stayed with me throughout my graduate career. I will always continue to be inspired by her memory and the encouragement she gave me to be brave, imaginative, to never compromise what I believe in, and to make a difference in the world. She was proud of me in advance of my accomplishments, and I sincerely believe I would not be the person I am today without her love.

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Introduction

Eutrophication, a term used to refer to a suite of complex processes and effects in aquatic ecosystems, is the movement of an aquatic ecosystem towards increased primary production. It is the result of an increase in the biomass of algae or aquatic plants usually in response to an increased supply of the nutrients normally limiting for growth (Hecky and Kilham, 1988; Conley et al., 2009; Dodds et al., 1998; Dodds, 2006). It is a critical concept in freshwater science as it forms the historical and modern basis for how lakes are studied and classified. However, the term eutrophication is broadly and loosely defined and often misused (Wetzel, 2001). Eutrophication, as defined in two limnology texts is "the enrichment of waters with plant nutrients" (Kalff 2003); or "the alteration of the production of a lake along a continuum in the direction from low to high values" (Wetzel ,2001) which is perhaps the more fundamental definition of the processes. Other changes that occur as lakes become eutrophic are brought about as a consequence of the movement of a system to increased primary productivity, such as decreased hypolimnetic dissolved oxygen (DO) and proliferation of noxious algae.

The concept was formed just after the beginning of the 20th century in early work on lakes by Thienemann and Naumann, and formed the basis for much freshwater science that followed (Wetzel, 2001). Naumann identified the role of nutrients (phosphorus, nitrogen and calcium) in determining primary production in lakes, which was the basis for distinguishing an oligotrophic lake from a eutrophic lake. It was not until the 1960s and 1970s, when the concept of a single limiting nutrient was applied to explain the process of anthropogenic eutrophication and better techniques in quantifying chemical nutrients and algal biomass were developed, that empirical relationships between nutrient loads, particularly phosphorus loads, and algal biomass were developed (Vollenweider, 1968; Schindler, 1974; Schindler 1978). Eventually, eutrophication was understood as a process whereby lakes could evolve, predictably, based on the loading of the single limiting nutrient, from an unproductive lake to a productive lake.

Since the role of anthropogenic phosphorus loading in driving lake eutrophication was identified, freshwater researchers have applied the concepts of trophic states and eutrophication to other aquatic environments undergoing change resulting from human activity and, following the approach taken by Vollenweider, began investigating the role of anthropogenic phosphorus and nitrogen in producing the effects seen in lake ecosystems, as

well as other changes such as decreases in native species and changes to abundances of commercially important fisheries (Micheli, 1999; Duarte, 2002; Dodds, 2006). However, other ecosystems may respond to nutrient additions and other human activity differently than lakes, and our conceptualization of eutrophication as it occurs in lakes may not necessarily apply.

For rivers, there have been several models proposed to conceptualize the river as an ecosystem unit (Vannote at al., 1980; Newbold et al., 1982; Junk, 1989; Stanford and Ward, 2001; Hilton et al., 2006), however, many predictions of these models cannot be reproduced by observation (Statzner and Higler, 1985; Junk 1989) and they lack generality and applicability to the eutrophication concept. In order to adopt or modify existing conceptual models to be more inclusive of riverine eutrophication, basic questions regarding the eutrophication process in rivers need first be answered. To do so requires a return to a basic definition of eutrophication, which centers upon the role of primary producer as the agent of ecosystem change, and the relationship of the primary producer community to the environmental conditions that bring about that change.

The first logical step in formulating testable hypotheses regarding river eutrophication and the role of the primary producer community is to first identify how rivers differ from lakes, where the concept of eutrophication was originally developed, and ask how these differences might affect our predictions of how eutrophication would occur. Rivers are understood as lotic, or flowing, while lakes are lentic, or relatively stationary, in comparison. As the primary difference between the two systems, the effect of water movement, or a very low retention time, should be considered. Secondly, rivers are highly variable environments, both in space and in time, which can have several consequences for both understanding eutrophication in rivers and developing predictive models for the process. Thirdly, there are fundamental differences in the type of primary producer community that could come to dominate rivers rather than lakes; because of the much shorter retention times and shallow depths, rivers are dominated by benthic primary producers such as aquatic macrophytes, filamentous algae and other periphyton, rather than plankton. Finally, as lakes become eutrophic, additional aspects of the system also change, such as altered DO cycling. In rivers, the associated changes could be quite different and depend on features of the system other than primary productivity that allow those changes to occur, for example, the changes in lake oxygen as result of eutrophication often occur as a depleted hypolimetic oxygen. As rivers do not have depth stratification, the resultant

changes to oxygen manifest differently, as diel sinusoidal patterns of change. In conclusion, we may expect other aspects of a eutrophic river environment to undergo changes that are not apparent in eutrophic lake systems, due to fundamental differences in their physical characteristics.

Previous work on understanding riverine eutrophication has consisted mainly of site-scale studies attempting to link benthic biomass to water or sediment nutrient concentrations. These attempts have met with limited success, with some studies indicating a weak correlation and others finding no connection (Canfield and Hoyer, 1988; Carr and Chambers, 1998; Flynn et al., 2002; Sosiak, 2002; Mainstone and Parr, 2002; Carr et al., 2003; Dodds et al., 2006; Hilton et al., 2006; Demars and Thiebaut 2008; Demars and Edwards, 2009), thus the conclusion must be that eutrophication, as a response in the plant community to a change in limiting nutrients as we understand it to occur in lakes, is not the same process in rivers. Much research supports this idea by suggesting that other physical and chemical parameters, such as light availability, river current and substrate type explain the variation in benthic biomass better than nutrients (Chambers and Kalff, 1985; Barko and Smart, 1986; Sand-Jensen et al., 1989; Barko et al, 1991; Chambers et al., 1991; Riis and Biggs; 2003; Xie et al., 2005), however it is obvious that habitat and water quality degradation occurs in urban and agricultural rivers and is often associated with large quantities of benthic plant biomass. Thus it is still clear that rivers can become eutrophic but in different ways than for lakes, and that rivers can move towards increased primary productivity by changes to nutrient availability. Additionally, some authors have noted that riverine submerged macrophytes may have a special role in riverine nutrient cycling. Because they derive most of their nutrient from sediments and release them to the open water they act as a link and a conduit for sediment-sequestered nutrients to the water (Carignon and Kalff, 1980; Clarke, 2002; Hilton et al., 2006). Changes to macrophytes under eutrophic conditions will thus change the role of macrophytes in linking sediment and water biogeochemical cycles.

This thesis will examine nutrients and benthic macrophytes in an impacted river, using a basic definition of eutrophication as the movement towards the dominance by the benthic primary producer community made possible by increasing nutrient availability. I will explore the role of the submersed benthic macrophyte community in riverine eutrophication, both as the biomass is connected to increased nutrient loading, and how that biomass generates

changes to biogeochemical nutrient transformations that may be unique to rivers. I will focus this exploration of the concept of eutrophication on the inherent differences between lakes and rivers and develop a set of testable hypotheses that evolved from consideration of those differences. Additionally, I will approach the exploration so as to build on existing knowledge of eutrophication process in lotic systems, implementing suggestions and interpretations of previous findings. I have formalized these hypotheses into 5 different chapters which each tackle a set of related hypotheses towards the general aim.

Structure of the thesis:

Chapter 1 establishes the history of nitrogen and phosphorus conditions in the Grand River in order to place the investigation of the response of the submersed macrophyte community into the greater context of long-term environmental change. To accomplish this, long term nutrient monitoring history produced by the Provincial Water Quality Monitoring Network (PWQMN) was evaluated qualitatively and quantitatively to assess changes to N and P species in the Grand River, spatially and temporally over the history of the monitoring period.

Chapter 2 quantifies the biomass of macrophytes above and below nutrient point sources in the Grand River and, in order to establish a link between variation in community level biomass and nutrient concentrations, tissue nutrient concentrations will be used. The study uses methods intended to deal with the inherently high spatial and temporal variability of rivers, a feature of rivers which has confounded previous attempts to establish a relationship between increased biomass and nutrient loads, and will compare the variation produced by two common methods for sampling biomass.

Chapter 3 takes a closer look at the biomass of submersed macrophytes and examines inter-annual variation in biomass when site specific factors are controlled for, using 4 years of seasonal biomass monitoring data. The variation found was explored in relation to weather and climate drivers, examining the relationship between riverine macrophyte biomass and climate survey parameters such as air temperature and precipitation, as well as factors affected by weather such as water temperature and river discharge.

Chapter 4 explores the relationship of macrophytes to nutrient point sources, namely WWTP effluent source nitrogen, to determine whether individual contributions to riverine nutrient concentrations can be seen as influencing macrophytes, and will explore the utility of using

macrophytes as indicators of the presence of WWTP effluent in rivers. This was accomplished by the use of the natural abundance of a stable isotope, ^{15}N, in macrophyte tissue, to distinguish between recycled and effluent nitrogen.

Finally, chapter five explores the influence of macrophytes on the biogeochemical environment. Increased macrophytic biomass in eutrophic conditions may have an impact on the cycling of nutrients in rivers. The high biomass drives the daily change in oxygen, but because biogeochemical cycles are often tightly coupled in strong redox gradients, the possibility exists that macrophytes influence the geochemical cycling of other nutrients like P and N. This chapter illustrates the role of macrophytes and their epiphytes under phosphorus rich conditions on the geochemical cycling of nitrogen. In-situ incubations of macrophytes were conducted at two sites, up and downstream of a WWTP, to test hypotheses regarding the effect of elevated phosphorus on the nitrogen cycle. Changes in N_2O production were used as a proxy for changes to nitrogen cycle activities, and ^{15}N tracers were used to measure macrophyte NH_4^+ uptake as a likely cause for changes observed to the N cycle. Light and dark treatments were used to examine differences that might arise as a function of DO.

Chapter 1: Long term changes in nutrient dynamics in the Grand River, 1965-2009

1.1 Introduction

Anthropogenic phosphorus and nitrogen enrichment of the world's waterways has led to a host of ecological and human health problems such as eutrophication of lakes and coastal zones, oxygen-depleted dead zones, harmful algal blooms, and increased toxic contaminants such as nitrate and nitrite (Chambers et al., 2001; Schindler et al., 2006; Hecky and Schindler, 2009). The world's river and stream networks represent a major resource for drinking water, agriculture and economic activity as well being ecologically important for many aquatic and terrestrial species and are environments where globally important chemical cycling processes occur (Peterson et al., 2001; Fu et al., 2003; Meyer et al., 2007). It is estimated that the majority of rivers are affected by eutrophication or other contamination, and that fewer than 10% of rivers globally are in pristine condition (Walsh et al., 2005).

Eutrophication is a tendency for an aquatic system to move towards increased primary production. The proliferation of algae or aquatic plants in the aquatic system is initiated, in most instances, by fertilization with either nitrogen or phosphorus which are normally present in limited supply (Hecky and Kilham, 1988; Conley et al., 2009). In response to the increasing symptoms of eutrophication in the Laurentian Great Lakes basin, a Canada-US Great Lakes Water Quality Agreement was formed and, under the agreement, the International Joint Commission recommended a ban on phosphorus detergents for the region in 1972. This, along with improvements to wastewater treatment in the early-mid 1970's (Burian et al., 2000), led to a dramatic decline in P loading to the Great Lakes in the 1980s, and P concentration targets being met in the 90's (Hartig et al., 1982; Hecky et al., 2004; Auer et al., 2010). However the eutrophication problem and subsequent recovery in large rivers and tributaries has received much less attention than in lakes, even though rivers and streams are highly important for human activities and natural processes and are likely the most impacted of the world's ecosystems (Malmqvist and Rundel, 2002).

While anthropogenic nitrogen and phosphorus have been tied to the eutrophication of waterways (Dodds, 2006), it is still under debate in the literature whether phosphorus

limitation is ultimately the cause (Hecky and Schindler, 2009; Conley et al., 2009). Most attention has been paid to controlling phosphorus release and, as a result, reactive species of nitrogen have been allowed to increase unchecked in the environment. Even if its role in eutrophication is minor compared with phosphorus, excessive nitrogen has other detrimental effects such as acidification of precipitation, and N_2O (a greenhouse gas) production; additionally, reactive nitrogen species are toxic to humans and other species (Aber et al., 1989; Vitousek et al, 1997; Seitzinger et al., 2000; Harrison et al., 2005; Rosamond et al., 2011). It is estimated that global human industrial and agricultural activity has nearly doubled the amount of reactive nitrogen in the biosphere (Canfield et al., 2010), and the effects of adding nitrogen in these quantities to global nutrient cycles and ecosystems is only beginning to be understood.

Rivers and streams are important sites for nitrogen processing, with denitrification producing gaseous N_2 and representing a major sink for reactive nitrogen species. Roughly 50% of reactive nitrogen received by streams in the USA is lost to the atmosphere and the remainder is flushed to downstream reaches, ultimately to coastal wetlands, lakes and the oceans (Peterson et al, 2001; Seitzinger et al., 2002; Galloway et al., 2003) where it may continue to have ecological impacts (Rabalais and Turner, 2002). For highly developed catchments receiving large anthropogenic nitrogen loads, such as the Grand River and its tributaries, the proportion of anthropogenic N removed by the river may be much lower because removal processes become saturated (Earl et al., 2006), which increases the amount of N flux downstream and increases the areal extent of impact. Additionally, production of N_2O in nitrogen rich waters during removal processes can be substantial (Rosamond et al., 2011) turning natural systems from greenhouse gas sinks to sources. An inventory of changes to reactive nitrogen in Canadian waterways does not currently exist, making it difficult to assess the extent of the impact of nitrogen enrichment at the regional scale, and to develop targets and strategies for mitigating it.

River ecosystems are distinct from lakes and coastal areas with their unique characteristic of unidirectional flow of water, energy and nutrients (Vannote et al., 1980; Sedell 1989; Walsh 2005) which present a challenge to both understanding the functioning of the system and predicting its response to human development and management decisions. Rivers are also highly variable in space and time with chemical, physical and biological changes occurring seasonally and even daily. The inherent variability makes characterization of a river system

using only temporal and locational averages harder to justify, and the modeling of rivers as "well-mixed beakers" an even less satisfactory simplification than for lakes. The sampling required to appropriately characterize the dynamic nature of rivers is often expensive and labour intensive, thus a major hindrance to studying rivers has been the logistics of constructing meaningful sampling programs.

Nutrient concentrations measured in rivers result from loads originating at unknown points some distance upstream from where adverse effects are ultimately observed. As nutrients from multiple sources join the stream, cumulative impacts begin to occur. Sources of anthropogenic nutrients in rivers can be classified as diffuse sources or point sources, with point sources being proximate and exhibiting immediate and obvious impacts while diffuse sources are distal, show impact in a cumulative manner and are more difficult to monitor and control (Mainstone and Parr, 2002). In heavily populated watersheds, rivers are subject to a variety of diffuse and point source impacts which all eventually accumulate downstream in large, high order rivers and exit at the mouth. There has been debate over the importance of point sources versus diffuse sources (Hilton et al., 2006; Jarvie et al., 2006), and it has been argued that point sources are more important for biological production, thus eutrophication, mainly because of the timing of the delivery of nutrients, rather than the quantity delivered. Though the ability to distinguish individual sources of nutrients from each other is necessary to develop targeted strategies for nutrient management, it may be instructive to regard nutrient sources as existing along a continuum, to be consistent with the river continuum concept (Vannote et al., 1981) rather than having two distinct categories. All point sources eventually blend into the diffuse background, but the source of diffuse and distant nutrients is always a point, even if that point is a small patch of tile drained land. Thus, focus can be shifted to processes that may be common to all nutrient sources, and a broader predictive framework can be developed.

Much insight into the controls on stream and river nutrient dynamics has been achieved in recent decades as better tools, equipment and methodologies have evolved, such as stable isotopic methods (Peterson et al., 2001; Spoelstra et al., 2001; Mulholland et al., 2002), process blocking techniques (Triska et al., 1990; Teissier and Torre, 2002) and nutrient mass balance models (House and Warwick, 1998). These methods have the disadvantage of being expensive and labour intensive and focus mainly on short term and small scale processes, rather than long

term and reach scale, the scale at which the impacts of anthropogenic nutrient enrichment are observed.

As populations increase around already eutrophic waters, pressures are placed on freshwater resources and aquatic inhabitants, and it becomes increasingly difficult to reach management objectives. It is essential to begin to tie the small scale, short term understanding of nutrient cycle processes to those that occur over the long term, and at regional scales. Long term monitoring programs play an essential role in the ongoing and evolving understanding of aquatic nutrient cycling and anthropogenic effects.

Several analyses of long term monitoring in rivers exist (Fruget et al., 2001; Mitchell et al., 2001; Billen et al., 2001; Parr and Mason, 2003; Green et al., 2004; Chambers et al., 2006; Sileika et al., 2006; Billen et al., 2007; Duan et al., 2007; Lassaletta et al., 2009; Shen and Liu, 2009). However, none exist for highly urbanized and agricultural southern Ontario despite a long history of water quality monitoring exists. The Grand River is a eutrophic river system and is one of the largest in the region (Barlow-Busch et al., 2006; M. Anderson, personal communication) that receives both diffuse and point source nutrient inputs, and is an example of how current nutrient management strategies, policies and procedures are inadequate and unsuited to large river ecosystems. To better elucidate the nature of the problem of large river anthropogenic eutrophication, I analyzed the long term monitoring data produced by the Provincial Water Quality Monitoring Network program for this river since the late 1960's, and looked for historical and spatial trends. Specifically, I ask these questions:

Past to present

> What changes occurred in river nutrient concentrations through time? Have the concentrations of total and reactive phosphorus declined in the Grand River since the detergent ban in 1972? Have reactive nitrogen species increased over the period, and by how much?

Upstream to downstream

> Is there a longitudinal pattern to the changes in nutrient concentrations over this period, as predicted by the River Continuum concept?

Nutrient dynamics: Sources, processes and evidence of change

Are there hot spots and hot moments, and do they change? What are the seasonal changes in nutrients? Can we distinguish between diffuse and point sources using concentration data alone? Is there evidence of changes in nitrogen cycling? Does the river act a sink or source?

1.2 Materials and Methods

The Grand River, located in southern Ontario, is the largest Canadian tributary to Lake Erie and its basin is home to nearly 1 million people (887,408 by 2006 Conservation Authority Jurisdiction census) and the population is projected to reach 1.6 million by 2056 (GSP group, 2010). The main river is 310 km in length and spans an elevation of 362 m. Southern Ontario geography is shaped mainly by the most recent deglaciation, with the Grand River running through glacial gravel and sand kame moraine deposits in the upper reaches and lacustrine clay beds in the lower researches. The Grand River main channel is a 6th order stream by the time it runs through the cities of Kitchener and Waterloo (population of almost 300,000), varies from 43 m to 160 m in width along the survey area, and is an 8th order river at the mouth. The river flow is highly regulated, with over 100 constructed reservoirs in the watershed, 32 of which are operated by the Grand River Conservation Authority (GRCA) for flood management and other purposes. The land-use is about 85% agricultural, with the upper west portion of the basin closer to 95% agricultural. There are 29 WWTPs that discharge into the river and its tributaries, which vary in their capacity and treatment processes; table 1.1 gives the capacities for some of the major WWTPs in the Grand River watershed. Two WWTPs of concern in the basin are the Kitchener WWTP, the largest in the watershed with a rated capacity of 1.2×10^5 m^3/d, and current usage of 6.4×10^4 m^3/d, and the Waterloo WWTP, the third largest in the watershed with a rated capacity of 7.2×10^4 m^3/d and current flow of 4.6×10^4 m^3/d (Anderson, 2012) are a particular focus of this work. The Kitchener WWTP is the single largest point source of nutrients to the Grand River because of the high nutrient content of the effluent (fig 1.2) combined with a high volume of output.

Table 1.1 Capacities of some larger WWTPs in the WWTP

WWTP	Capacity (m^3/d)	Location (UTM)
St. Jacobs	9.55×10^2	536478, 4820295
Conestogo	1.48×10^2	540617, 4821286
Waterloo	7.27×10^4	41834, 4814382
Kitchener	1.22×10^5	546982, 4805530
Hespeler	9.32×10^3	554113, 4808104
Preston	1.69×10^4	552547, 4803895
Galt	3.86×10^4	555226, 4798486
Paris	7.04×10^3	550843, 4780626
Brantford	8.18×10^4	562669, 4774417

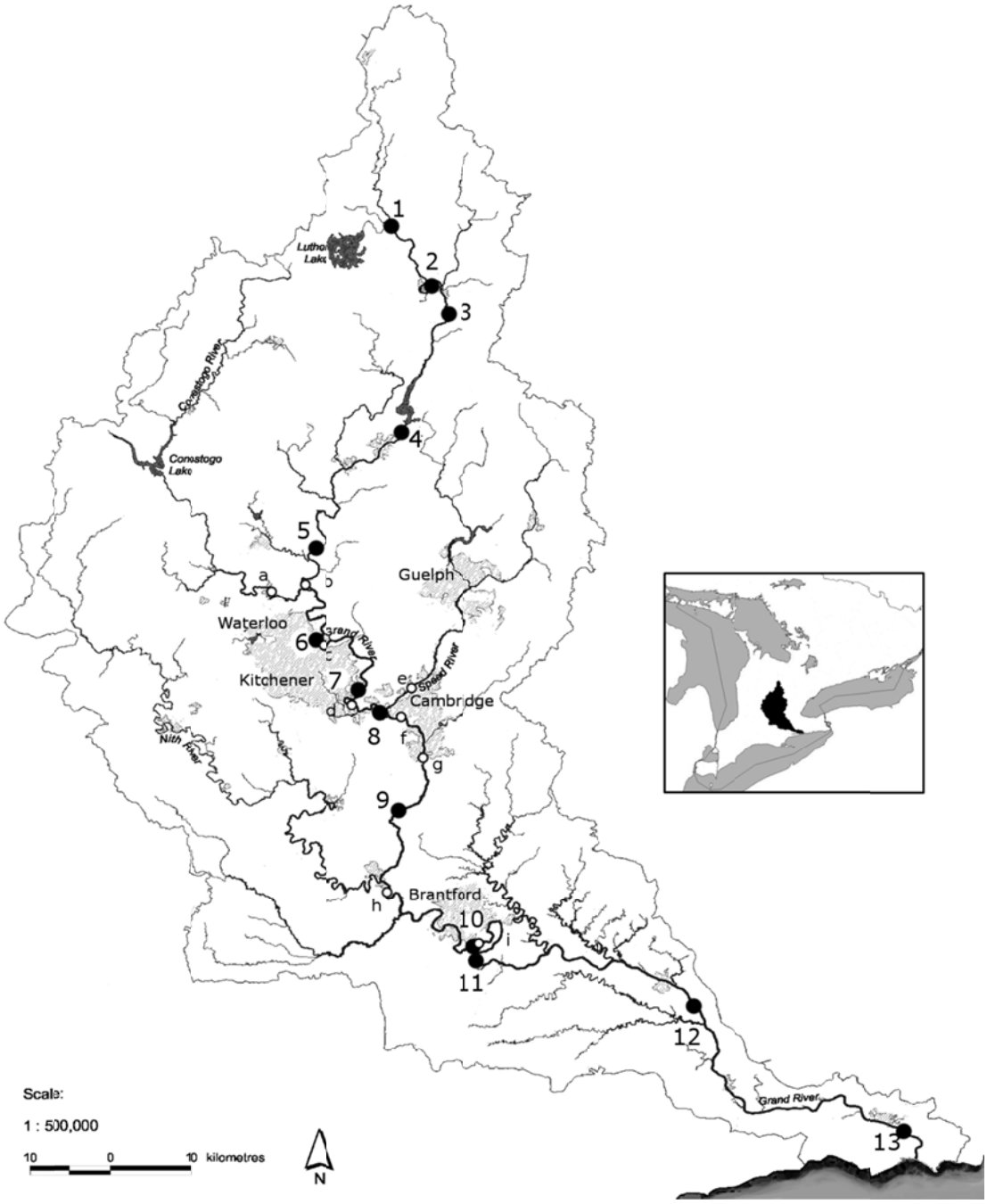

Figure 1.1 Map of the Grand River watershed, located in southern Ontario, Canada (inset map) showing the PWQMN sites used in this study (●) and major WWTPs (O). Sites are numbered from headwaters to mouth. 1. Sites: Leggatt, 0 km; 2. Amaranth/Grand Valley; 13 km; 3. Marsville, 20 km; 4. Belwood Lake outflow, 38 km; 5. West Montrose, 64 km;, 6. Bridgeport, 87 km; 7. Freeport, 102 km; 8. Blair, 113 km; 9. Glen Morris, 131 km; 10. Brantford, 169 km; 11. Newport, 184 km; 12. York, 221 km; 13. Dunnvile, 255 km. WWTPs: a. St. Jacobs; b. Conestogo; c. Waterloo; d. Kitchener; e. Hespeler; f. Preston; g. Galt; h. Paris; i. Brantford.

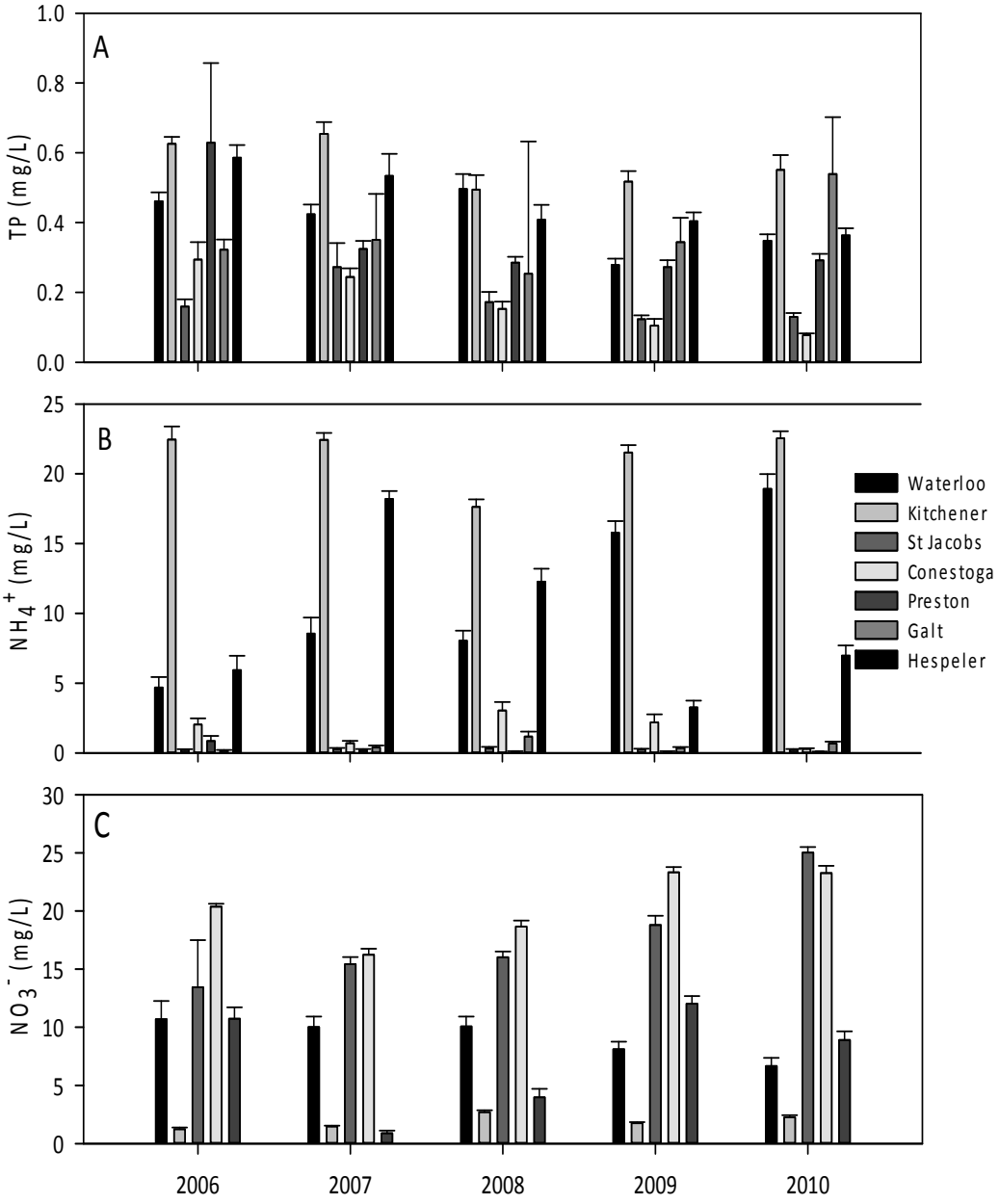

Figure 1.2 TP, NH_4^+ and NO_3^- concentrations in the treated effluent at 6 of the Grand River Watershed's largest WWTPs, including the Waterloo and Kitchener WWTPs, from 2006 to 2010. Yearly average concentrations represent an average of approximately 52 24-h averaged samples taken weekly throughout the year, with some exceptions (in 2006, Waterloo had on 25 weekly samples for TP and NH_4; Kitchener had only 41 for NH_4 and NO_3. 2007 Waterloo had 41 samples for NO_3. Error bars represent standard error of mean yearly concentration. No NO_3 data were available for the St. Jacobs and Conestoga WWTPs.

Nutrient data used for this study were obtained through the Provincial Water Quality Monitoring Network (PWQMN), which is a partner network made up of the Ontario Ministry of the Environment, Ontario Conservation Authorities, municipalities and Ontario Parks, formed with the mandate to monitor a variety of water quality parameters and provide the data freely and openly to the public. It began monitoring water quality in the Grand R. in 1964, and the monitoring period from 1965 to 2009 is used in this work. Data on river discharge was obtained through the Water Survey of Canada's hydrometric archive which is also a publicly accessible data archive. Parameters chosen for analysis were TP (total phosphorus), SRP (soluble reactive phosphorus), NO_3^- + NO_2^- (nitrate and nitrite) and NH_4^+ (ammonium), and were selected based on their relevance to cultural eutrophication and completeness of measurement and reporting. Sites were chosen for analysis among the nearly 400 monitoring stations in Ontario. Sites along the main channel of the Grand River were selected for analysis based on number of years covered, whether data coverage dated back to the early 1970s, and whether data were present for more recent years (since 2000).

Sampling consistency was an issue with this data set as no two sites were sampled over the same years, and many sites had years where data were absent, all sites included systematic bias towards summer months, and many lacked data in late winter months altogether. No two sites were sampled on the same day, and no individual sites were sampled on the same day of the month for consecutive years. In some years multiple samples were taken within the month, so sites were sampled as few as once per month and up to 35 times per month. For example, while the majority of sites were sampled only once per month, the Amaranth site was sampled 3 times in one month only once over the whole monitoring period while the Dunnville site was sampled 32 times in one month, but with approximately 25% of months sampled only once. To normalize temporal sampling inconsistencies, all sites were reduced to a monthly average concentration and when sites were sampled only once in a month, the monthly "average" is based on a single sample. Additionally, not every month was sampled. Sites were sampled between 8 and 12 months of the year, with the average sampling frequency being 11 months/ year. Typically, the sampling programs are biased towards summer months and, because of this, we removed December and January from the seasonal analysis for all years and sites in order to limit extrapolating interpretations to times where data are lacking.

To analyze temporal and spatial trends, qualitative and quantitative methods were used. Temporal trends were assessed visually first with an assessment of a 3D plot, produced using SigmaPlot 12.0, using year and month as x and y axis, respectively, with nutrient values represented by colour intensity. The data produced from discrete sampling events were made continuous through the interpolation algorithm applied by Sigma Plot. With this depiction of the data, seasonal trends and annual trends can be visualized, as well as the change in the seasonal trend with time. To evaluate trends identified in a visual assessment of the 3-D plots, a seasonal Mann-Kendal test was used, which computes whether the slope of the relationship between two variables is significant to a given level when there is a component of autocorrelation present in one of the variables. In this case, the change in nutrient concentrations with respect to time may have temporal autocorrelation. The method chosen to compute the Mann-Kendal test allows for blocking the data into seasonal components, so slopes from different seasons can be computed, and compared for differences. Software used to perform the Mann-Kendal test for trends in nutrient concentrations with time was provided by the USGS as a publicly available script, described in Helsel et al. (2006). For the spatial analysis, trends were identified qualitatively using an assessment of a 3-D plot, similar to those for the temporal trend analysis, with the y-axis representing season, and the x-axis representing distance downstream. To evaluate dissolved nutrient dynamics, a variety of representations and interpretations of the PWQMN data were used, and detailed descriptions of those are provided in the results sections.

1.3 Results

1.3.1 Temporal trends

Because the data are highly variable in space and time and because a wealth of data are available for analysis, it is useful to look at selected sites over the entire period of the data collection to gain a better understanding of trends in nutrients through time. Four sites were chosen for detailed analysis. Amaranth (site 2), located at km 13 from the designated 0 km point in headwaters, was used as a representative upstream site. Bridgeport (site 6), located at km 87, is upstream of both of the major treatment plants on the main stem of the Grand River, however it is located downstream of some major tributaries, including the Conestogo River which drains an intensively agricultural watershed. Blair (site 8), located at km 113, was the closest site immediately downstream of both Waterloo and Kitchener treatment plants. Dunnville (site 13), located at the mouth of the river was also examined, but the monitoring record at this location only begins in 1980 which limits a long-term trends analysis. Comparison between Amaranth and Bridgeport allows for an understanding of agricultural impact on the river through time, while a comparison between Blair and Bridgeport or Blair and Amaranth gives an understanding of the influence of the WWTP on the Grand River.

TP declined over time at all sites (table 1.2; fig. 1.3). TP was highest at the site immediately below the WWTP in Kitchener, Blair, with concentrations above 0.5 mg/l all year. TP decreased fastest at Blair over the monitoring period (2.8 µg/l/y) while the slowest decrease was at Amaranth (0.25 µg/l/y). In recent years TP appeared to have a seasonal pattern with highest values in March and April at both Blair and Bridgeport, but no seasonal trend was apparent at Amaranth. At the Blair site, there was an abrupt change in TP concentrations in 1974, and over the 4 years before and after this change, the TP decline was 120 µg/l/year.

After this initial drop, TP declined further, but only 0.897 µg/l/y when the data from 1975-2009 are used, and no trend was detected in recent years likely because of the small magnitude of change and the high variability in the data. The graphs of TP decline over time reflect the changes seen in the Mann-Kendall test, showing the large decline after 1973 and the further decrease. The graphs appear "spotty" with small and isolated peaks occurring at different times of the year, in different seasons all throughout the monitoring history. SRP was lowest at the upstream site, Amaranth, with significant slope of -0.05 µg/l/y (table 1.2). With the exception of

a few small peaks, the SRP has generally been below 0.05 mg/l over the monitoring period (figure 1.4). SRP was high all year, over 0.2 mg/l, with little seasonal variation before 1974. This pre-1974 high in SRP is absent from the Bridgeport location, illustrating the influence of the WWTP on SRP levels prior to improved wastewater treatment. SRP decreased by 1.5 µg/l/year at Blair (table 1.2), although the decline was not monotonic and there was an abrupt drop in SRP after the WWTP upgrade resulting in a change of 87.6 µg/l/y between 1972 and 1975.

Following the upgrade, SRP declined at a rate of 0.536 µg/l/y, a change which is only detectible over the 34 year period from 1975 to 2009. Even though it appears from figure 1.4 that SRP may be increasing in recent years, this was not supported by the results of the Mann-Kendall test for the period from 1995-2009. It is likely that an even more data are needed to see changes over this period because of the high variability in SRP. After 1974, a seasonal trend emerged at Blair and was also present at Bridgeport, with "spotty" high values that occurred in late winter-early spring (March) in 1982, 1987, 1992, and 1997 and in the 2000s, although results of Mann-Kendall test indicate there was no difference in long term trend by season. It is possible that the variability within seasons was still high enough to mask the seasonal trend, and was too high to be able to distinguish differences among seasons. The peak "spots" could be the result of high discharge, which is explored later in this chapter.

Table 1.2 P-values and slope results from Mann-Kendall seasonal tests for trends with time over the entire monitoring period. Where the trend was significant, a slope is provided. (*) indicates a significant seasonal difference

	Amaranth 1972-2007		Bridgeport 1965-2009		Blair 1965-2009		Dunnville 1980-2009	
	P	Slope (µg/l/yr)	P	Slope (µg/l/yr)	P	Slope (µg/l/yr)	P	Slope (µg/l/yr)
TP	0.0196	-0.25	< 0.01	-0. 943	< 0.01	-2.8	0.0105 0.0024*	-1.01
SRP	0.0024	-0. 05	< 0.01	-0. 275	< 0.01	-1.5	0.0015	-0. 561
NH_4^+	0.578		< 0.01	-1.65	0.825		0.367	
NO_3^-+NO_2^-	< 0.01	+11.1	< 0.01	+42.1	< 0.01	+51.2	0.701	

Ammonia concentrations were lowest at Amaranth and had no prominent trend through time at any site (table 1.2; fig. 1.5), although there was a significant decline of 1.65 µg/l/y at Bridgeport according to the Mann-Kendall test (table 1.2). At Blair there was no decline in NH_4^+ after the WWTP upgrade in 1974, but a significant decline of 15.6 µg l/y was observed in the 10 years following. Over the longer period between 1975-2009, NH_4^+ increased by 6.16 µg/l/y. The increase appears to begin in the mid-1980s (fig. 1.5), roughly 20 years after the WWTP upgrade was installed. Seasonally, concentrations appear to be highest in late winter-early spring (February and March) at Amaranth and Bridgeport, although they were high all year at Blair. However, no significant seasonal effect was determined with the Mann-Kendall test. Concentrations at Blair in recent years were almost always above 0.5 mg/l and in winter months were over 2 mg/l. At Amaranth, concentrations were rarely found above 0.1 mg/l.

Concentrations of NO_3^-+NO_2^- increased over time at the locations analyzed by the Mann-Kendall test, except for Dunnville (table 1.2), and the increase is readily apparent from heat-map plots of Amaranth, Bridgeport and Blair (table 1.2; fig 1.6). The increase in NO_3^-+NO_2^- was the largest long-term change observed relative to its concentration, with an increase in 11.1 µg/l/y at Amaranth, 42.1 µg/l/y at Bridgeport and 51.2 µg/l/y at Blair although no change was detectible at Dunnville. The increase at Blair was not noticeable over the 4 year period during the WWTP upgrade, however a change of 70.4 µg /l/y was observed in the 10 years following. In the longer term, from 1975 to 2009, a change of 32.1 µg /l/y was observed indicating that the increase was greater in the earlier years following the WWTP upgrades. We did not see that recent years had a different trend than the whole monitoring period; when data from the most recent years were used in the analysis (I tested most the recent 14 and 9) no trend was found. Concentrations in recent years were routinely above 5 mg/l at Bridgeport and Blair, and at times as high as 7 mg/l. Seasonally, patterns are observable at all three sites (fig. 1.6) although there were no differences in the long term trend in season according to the seasonal Mann-Kendall test. NO_3^-+NO_2^- were highest in winter to late spring, and lowest in late summer (July to August). The peaks are notably "spotty" with large concentrations occurring in the same years as the peaks in other dissolved nutrients.

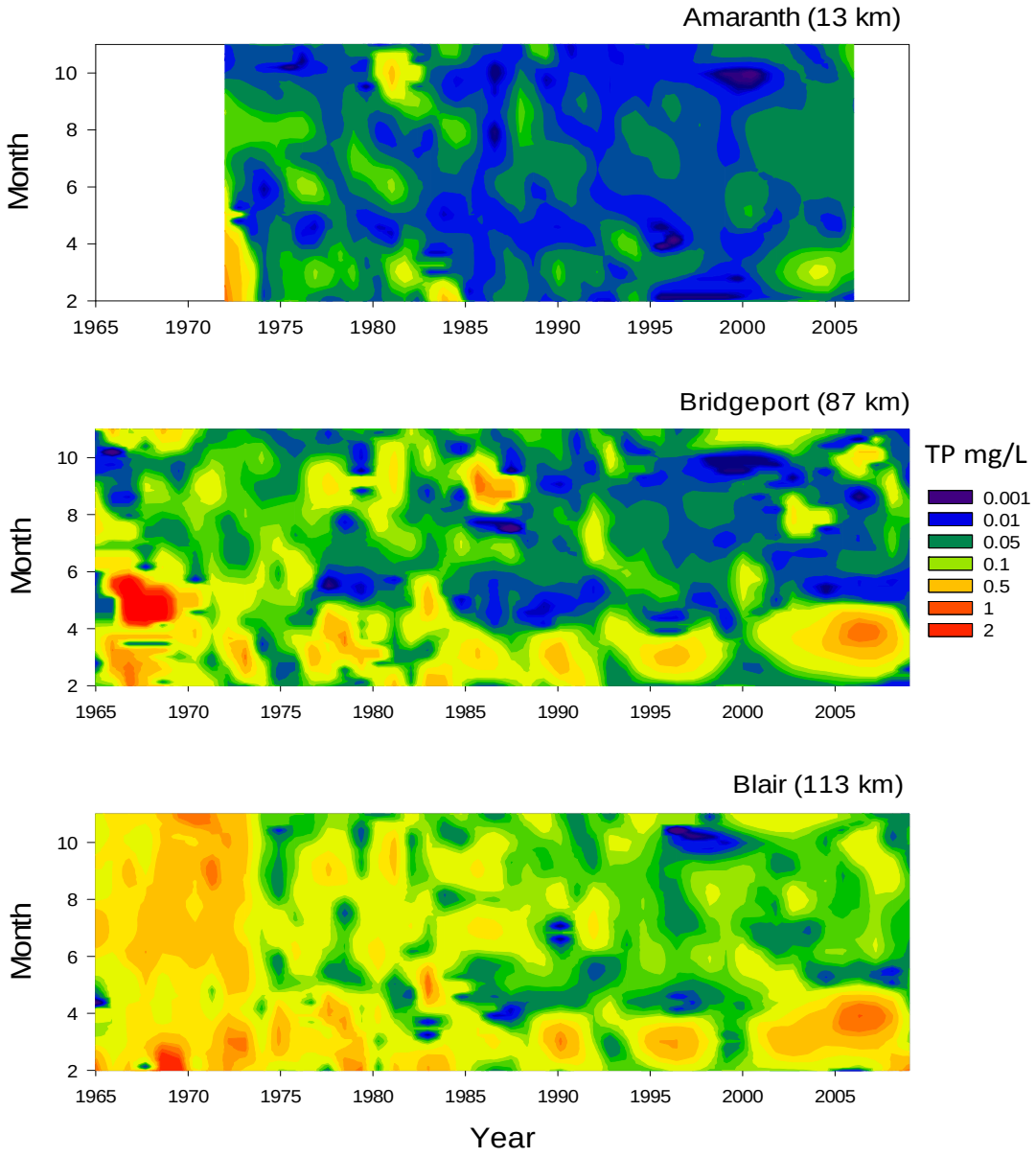

Figure 1.3 TP concentration in the Grand River at three sites, through time, displayed on a two dimensional axis to show both annual and seasonal distribution. The sites shown are Amaranth (site 2) located at 13 km downstream, Bridgeport (site 5) located 87 km downstream and Blair (site 8) located 113 km downstream and below the major WWTP in Kitchener .

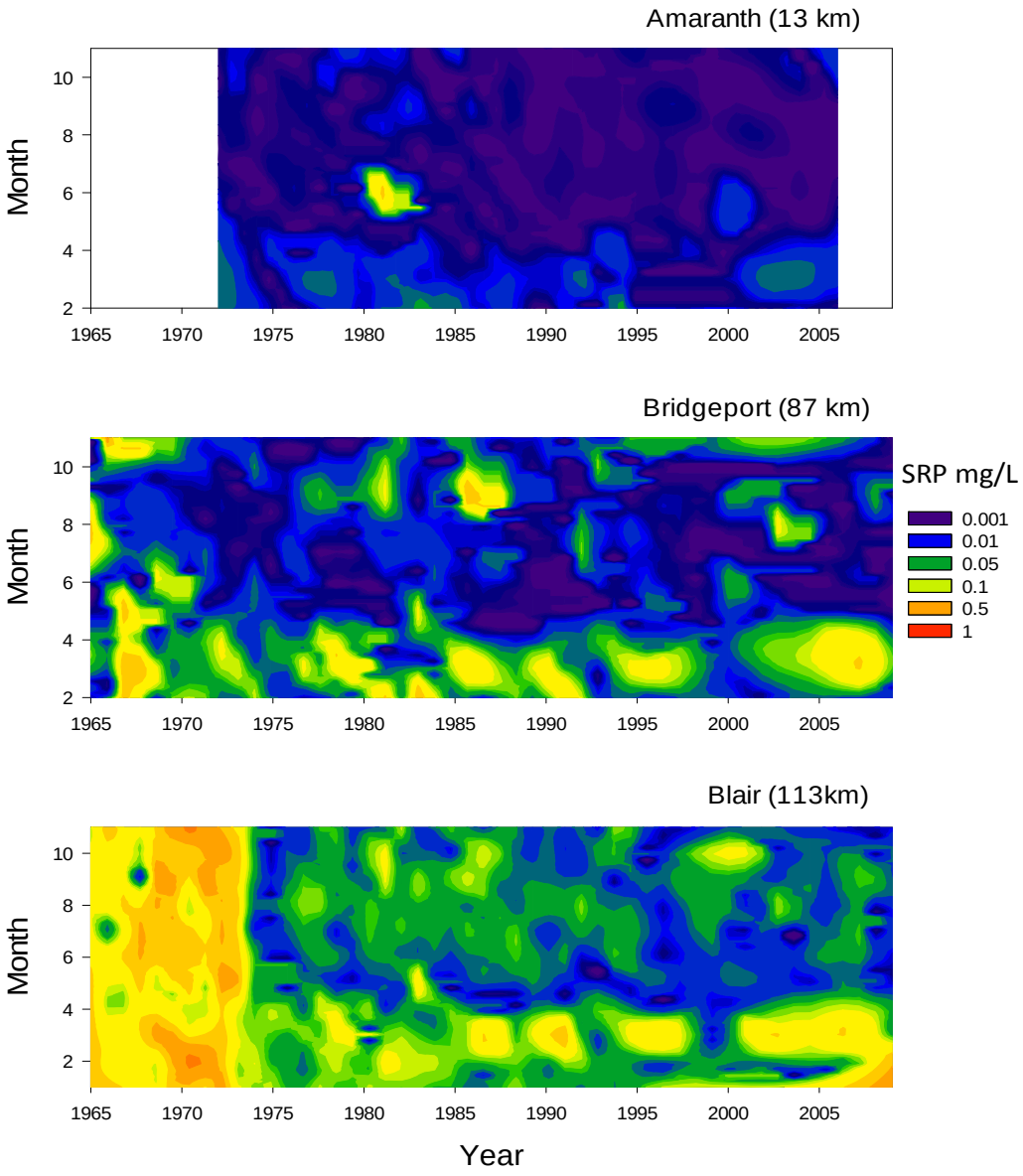

Figure 1.4 SRP concentration in the Grand River at three sites, through time, displayed on a two dimensional axis to show both annual and seasonal distribution. The sites shown are Amaranth (site 2) located at 13 km downstream, Bridgeport (site 5) located 87 km downstream and Blair (site 8) located 113 km downstream and below the major WWTP in Kitchener.

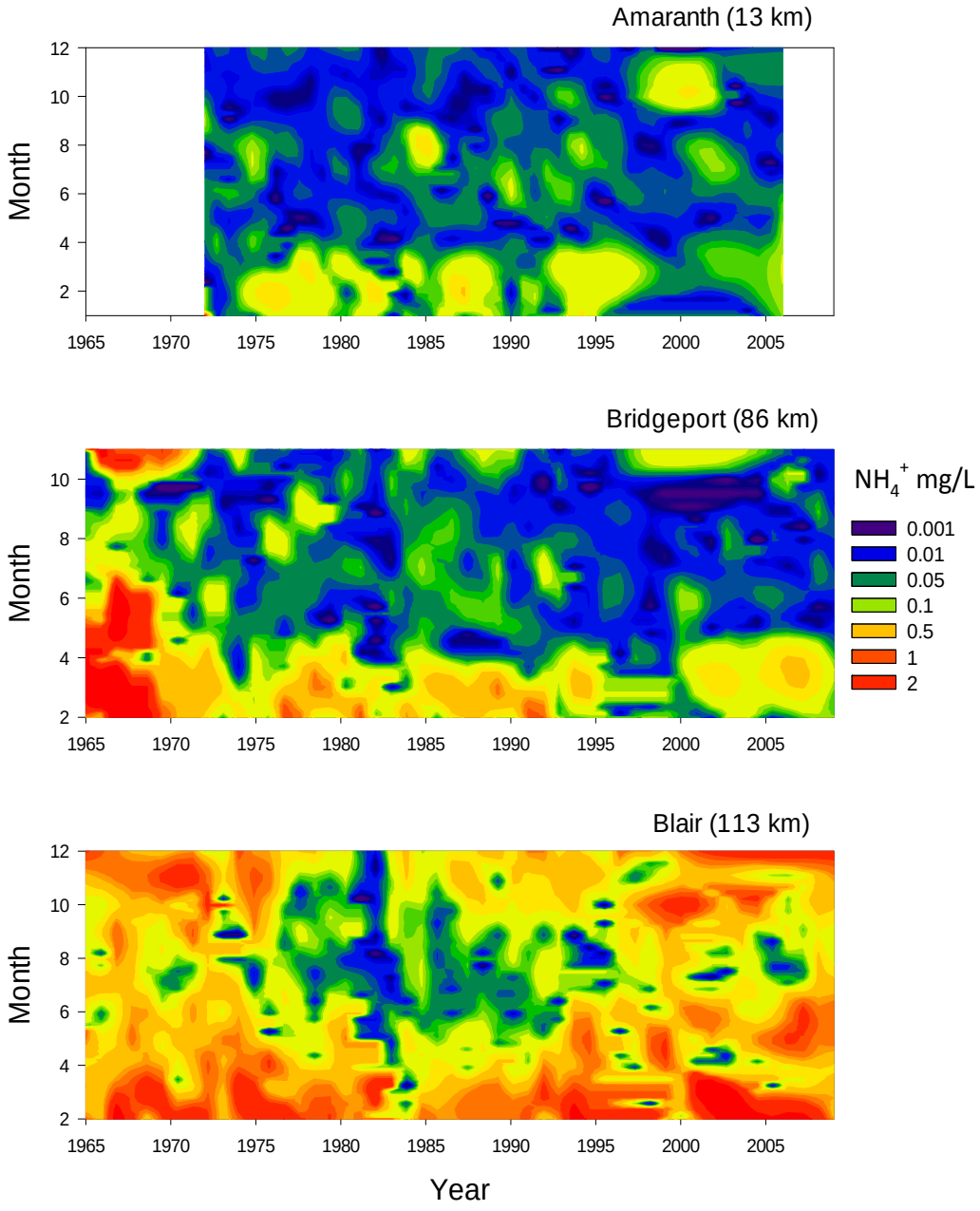

Figure 1.5 NH_4^+ concentration in the Grand River at three sites, through time, displayed on a two dimensional axis to show both annual and seasonal distribution. The sites shown are Amaranth (site 2) located at 13 km downstream, Bridgeport (site 5) located 87 km downstream and Blair (site 8) located 113 km downstream and below the major WWTP in Kitchener.

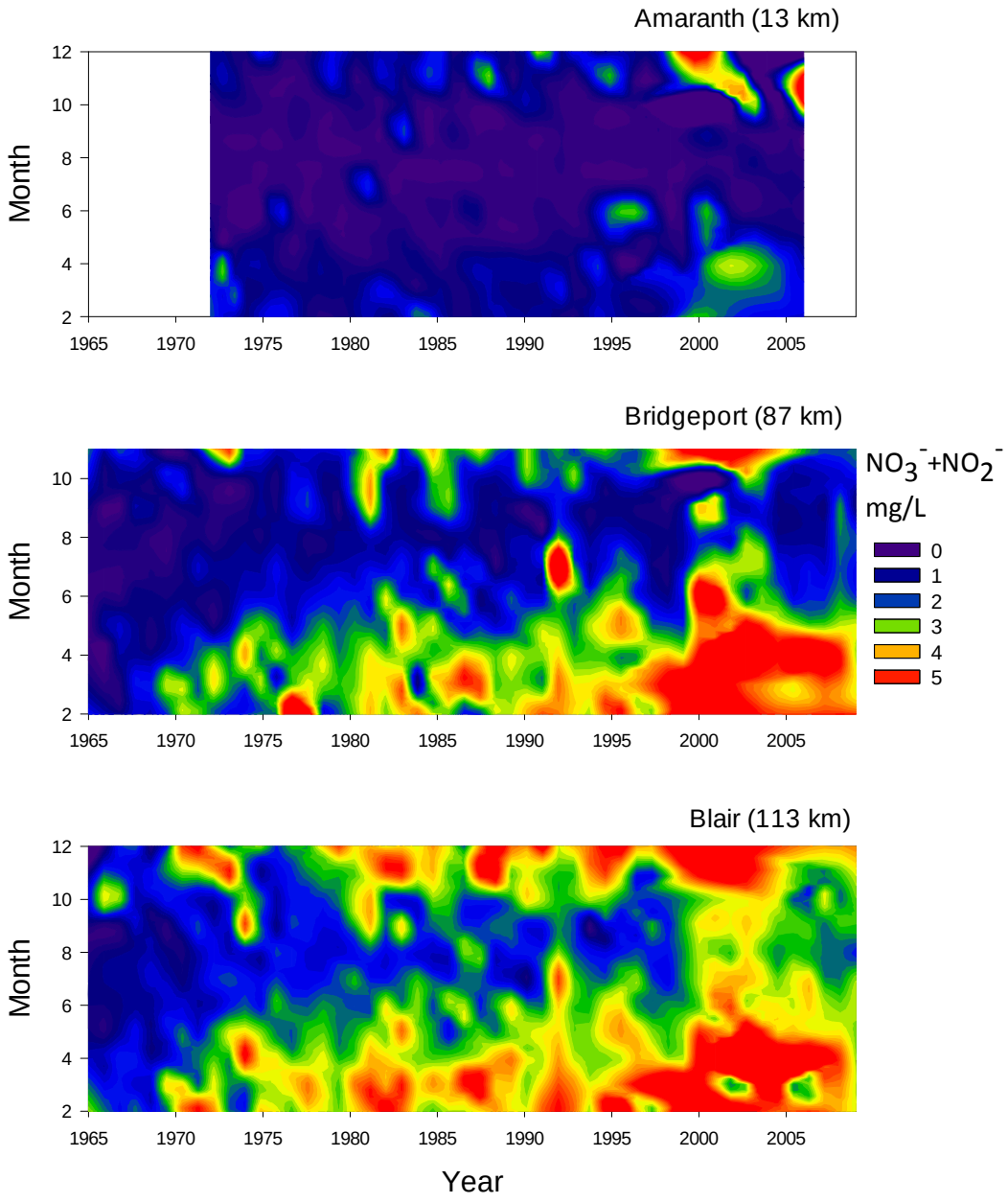

Figure 1.6 NO_3^- + NO_2^- concentration in the Grand River at three sites, through time, displayed on a two dimensional axis to show both annual and seasonal distribution. The sites shown are Amaranth(site 2) located at 13 km downstream, Bridgeport (site 5) located 87 km downstream and Blair (site 8) located 113 km downstream and below the major WWTP in Kitchener.

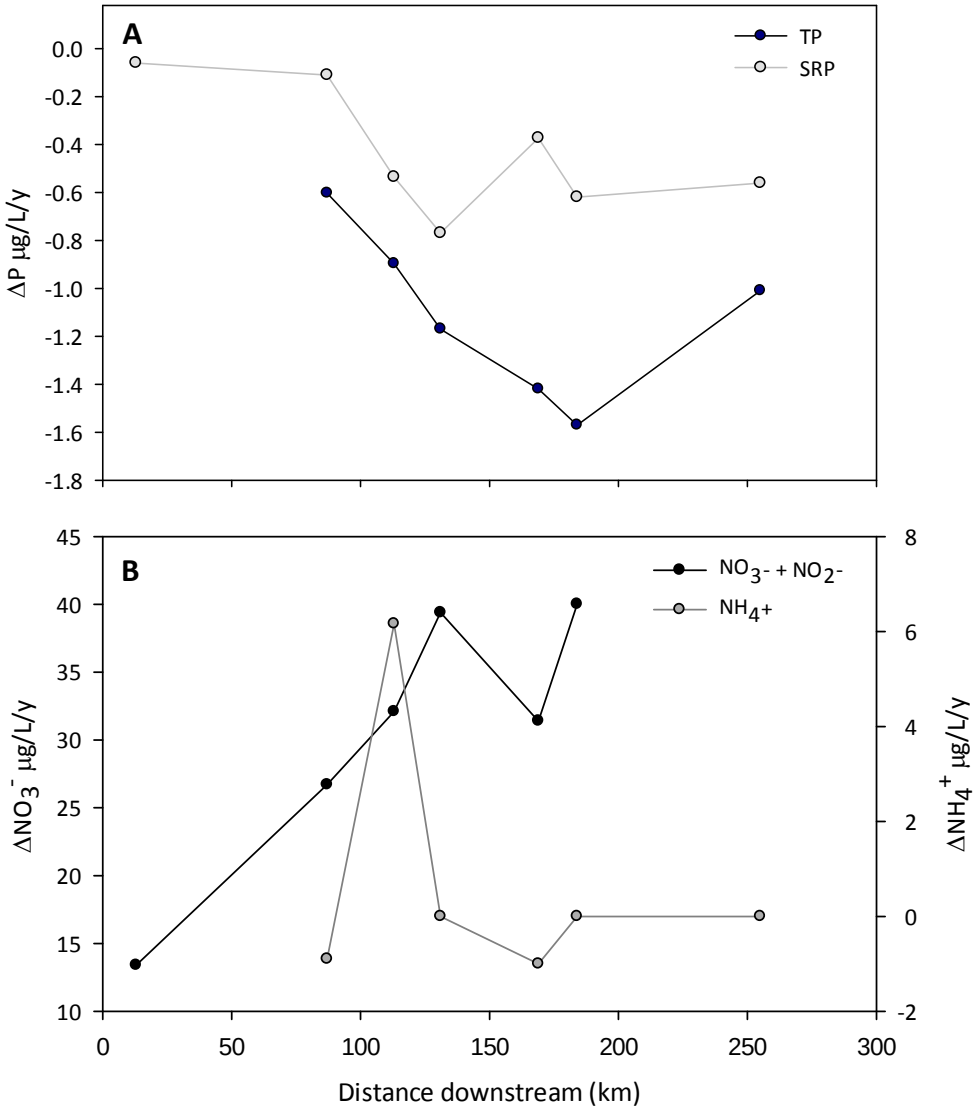

Figure 1.7 Rate of change (µg/l/y) of TP, SRP (A) and NO_3^-+NO_2^- and NH_4^+ (B) between 1975 and 2009, the 34 year period after the initial change resulting from detergent phosphate ban and WWTP upgrades. Rates were derived from significant trends resulting from Mann-Kendall analysis of monthly average data and are also represented in table 1.3. All SRP and TP declined (negative ΔP axis) over this period, NO_3^-+NO_2^- increased at all sites with a significant slope, while NH_4^+ increased measurable only at the first site downstream of the Kitchener WWTP.

Table 1.3 Results from the Mann-Kendall test for 7 sites divided up into time periods. 1972-1975, before and after WWTP upgrades, 1975-1985, the following 10 years, 1975-2009 entire period following recovery. Where the trend was significant, a slope is provided. The test was not run on sites with no apparent trend over time. In these tests, correcting for season did not improve the predictive value of the relationship.

		1972-1975		**1975-1985**		**1975-2009**	
		P	Slope (µg/l/yr)	P	Slope (µg/l/yr)	P	Slope (µg/l/yr)
Amaranth (2)*	TP	0.8102		-		0.1316	
	SRP	1.000		-		0.0016	-0.0623
	NH_4^+	-		0.739		0.9616	
	NO_3^-+NO_2^-	-		0.167		0.0000	+13.4
Bridgeport (6)	TP	0.471		-		0.0000	-0.603
	SRP	0.471		-		0.0426	-0.111
	NH_4^+	-		0.7408		0.013	+0.900
	NO_3^-+NO_2^-	-		0.0016	+115	0.0027	+26.7
Blair (8)	TP	0.0306	-120	0.378		0.0004	-0. 897
	SRP	0.0306	-87.6	0.508		0.0001	-0. 536
	NH_4^+	1.000		0.024	-15.6	0.0009	+6.16
	NO_3^-+NO_2^-	0.7842		0.024	+70.4	0.0035	+32.1
Glen Morris (9)	TP	0.0289	-73.0	0.680		0.0002	-1.17
	SRP	0.0289	-50.8	0.480		0.0000	-0.769
	NH_4^+	0.551		0.236		0.385	
	NO_3^-+NO_2^-	0.100		0.0135	+158	0.0000	+39.4
Brantford (10)	TP	0.0927		0.659		0.0000	-1.42
	SRP	0.0306	-52.8	0.0068	+2.24	0.0022	-0.373
	NH_4^+	1.000		0.581		0.0203	-1.00
	NO_3^-+NO_2^-	0.810		0.0009	+105	0.0004	+31.4
Newport (11)	TP	0.0927		1.000		0.0000	-1.57
	SRP	0.0306	-61.8	0.1858		0.0013	-0.62
	NH_4^+	0.810		0.409		0.317	
	NO_3^-+NO_2^-	0.810		0.0017	+91.7	0.0001	+40.0
Dunnville(13)**	TP	n/a		n/a		0.0024	-1.01
	SRP	n/a		n/a		0.0015	-0. 561
	NH_4^+	n/a		n/a		0.3670	
	NO_3^-+NO_2^-	n/a		n/a		0.7005	

1.3.2 Longitudinal Trends

TP increased from upstream to downstream in all years and was particularly high all year after the river passed by the cities of Waterloo and Kitchener, at 70 km downstream (fig. 1.8). Prior to 1974 a sudden increase in TP occurred downstream of the cities, but this was less noticeable through time. The results of the Mann-Kendall test for temporal trends at each site (table 1.3) indicate that TP decline in response to detergent phosphate bans and WWTP upgrades in the period of 1972-1975 was detectable at Blair (site 8) and Glen Morris (site 9). Although Brantford has its own smaller WWTP, no change in TP was detected downstream at the Newport location (site 11) during this period. TP declined by 120 µg/l/y at Blair, but at Glen Morris the decline was only 73 µg/l/y. There was no detectable change in TP in the 10 years immediately following the WWTP upgrades, possibly due to the high variation in nutrient concentrations and the lower *n* resulting from using a smaller time window. TP continued to decline in the 34 year period from 1975 to 2009, but not just downstream of the Kitchener WWTPs. Declines in TP over this period were detected in all sites in the lower half of the river, starting at the Bridgeport location (site 6) and Blair (site 8), Glen Morris (site 9), Brantford (site 10), Newport (site 11) to the mouth at Dunnville (site 13). From upstream to downstream the rate of TP decline accelerated until Newport (table 1.3; figure 1.7), with a maximum rate of decline of 1.6 µg/l/y, which is roughly two orders of magnitude less than the initial decline in TP in the early 1970s. Decline in TP at Dunnville (site 13) was detected, but was less than was observed at Blair. The seasonality of TP is also more prominent downstream, with a more obvious difference between summer months and the rest of the year. In most recent years sampled (2008-2009), TP was still above 0.1 mg/l downstream in early spring (April) and fall (September). The change in seasonal pattern appeared at 150 km downstream, after the city of Brantford.

Like TP, SRP increased downstream of the Waterloo and Kitchener WWTPs, and the increase was abrupt in years prior to the mid 1970's (fig. 1.9). The increase downstream of the Kitchener treatment plant appeared to be independent of season which is strongly indicative of the influence of point source impact. SRP declined substantially after the installation of WWTP upgrades (62 µg/l/y; table 1.3) and the decline was detected further downstream, as far as Newport (site 11), 76 km downstream of the Kitchener WWTP. After the immediate decline in concentration following the P detergent ban and WWTP upgrades, SRP continued to decline,

although this decline was slow and was only detected over the monitoring period 1975-2009. Similar to TP, the rate of change in SRP concentration generally increased with distance (fig 1.7), with a larger reduction further away from the largest WWTP. The biggest change in SRP over this period occurred at Glen Morris, with a change of 0.80 µg/l/y, which is nearly to two orders of magnitude less than the immediate drop in concentration following the phosphate detergent ban and WWTP upgrades. SRP changed seasonally, as mentioned in the previous section, and the seasonal pattern differed after the river passed by Kitchener-Waterloo. Higher concentrations were observed in late winter/early spring (fig.1.4), with lowest values in the summer. In recent years the seasonal pattern of SRP differed further downstream after the Brantford site, where SRP had a second peak in late summer (August-September). In 2008-2009 SRP was high in winter in upstream sections, unlike any other years in the past.

NH_4^+ increased after the Waterloo and Kitchener WWTP (at Blair), but then declined to nearly what it was upstream of the WWTPs (fig. 1.10). Otherwise, there was little longitudinal trend. The Mann-Kendal temporal trends (table 1.3) show that NH_4^+ increased over the long term at Blair and Bridgeport, but declined at Brantford, though only by 1.00 µg/l/y. In recent years, (1996 and later) NH_4^+ appeared to be increasing again in the river, with higher concentrations downstream of the Waterloo and Kitchener treatment plant being measured all year round, particularly in summer low flow months, as was indicated by the long-term analysis of Amaranth, Bridgeport and Blair sites.

NO_3^-+NO_2^- tended to be highest in mid to downstream sections in all years analyzed (fig 1.11), and increases in these locations were observed following WWTP upgrades in the 1970s. Increases in NO_3^-+NO_2^- through time were highest at most sites in the 10 years following the upgrades, from 1975-1985 (table 1.3), with the largest increase of 157 µg/l/y observed at Glen Morris, not at Blair immediately downstream of the WWTPs, nor in the upstream agricultural locations. Over the longer term, from 1975-2009, the rate of increase was lower, and grew with distance downstream, peaking at Newport, (and with a similarly high rate of change at Glen Morris) but with a decrease at the Brantford site, similar to SRP. The seasonal trend of high nitrate in the spring as seen in the temporal analysis of 3 sites appeared only downstream 60 km. The seasonal pattern appeared to emerge in NO_3^-+NO_2^- downstream of Glen Morris, at km 131, suggesting that NO_3^-+NO_2^- increase over time was not due to WWTP operation.

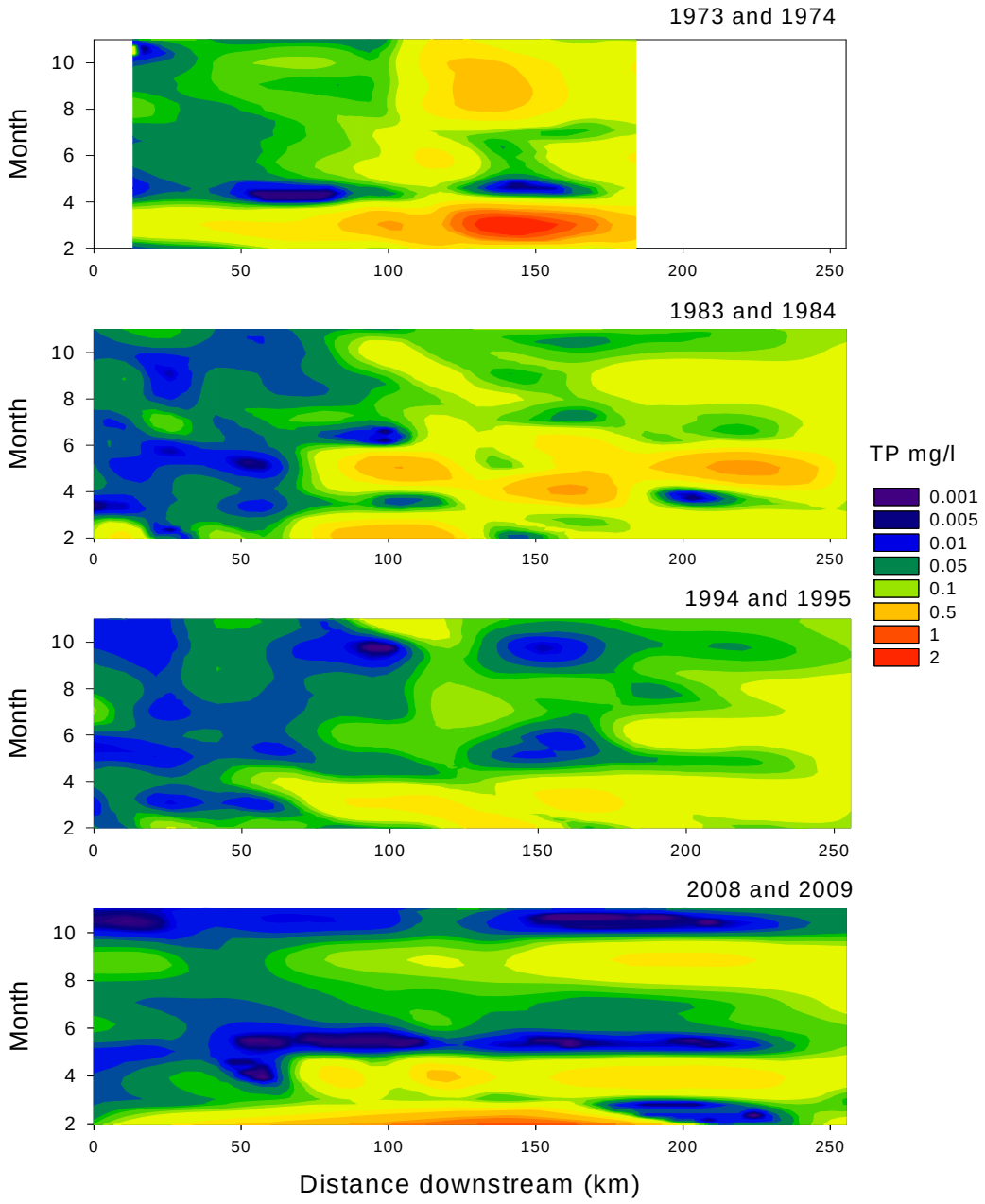

Figure 1.8 TP concentration from upstream to downstream and seasonally, interpolating data from the 13 sites at four periods of time within the monitoring data. Data from two years were combined to increase the amount of data available to construct the graph.

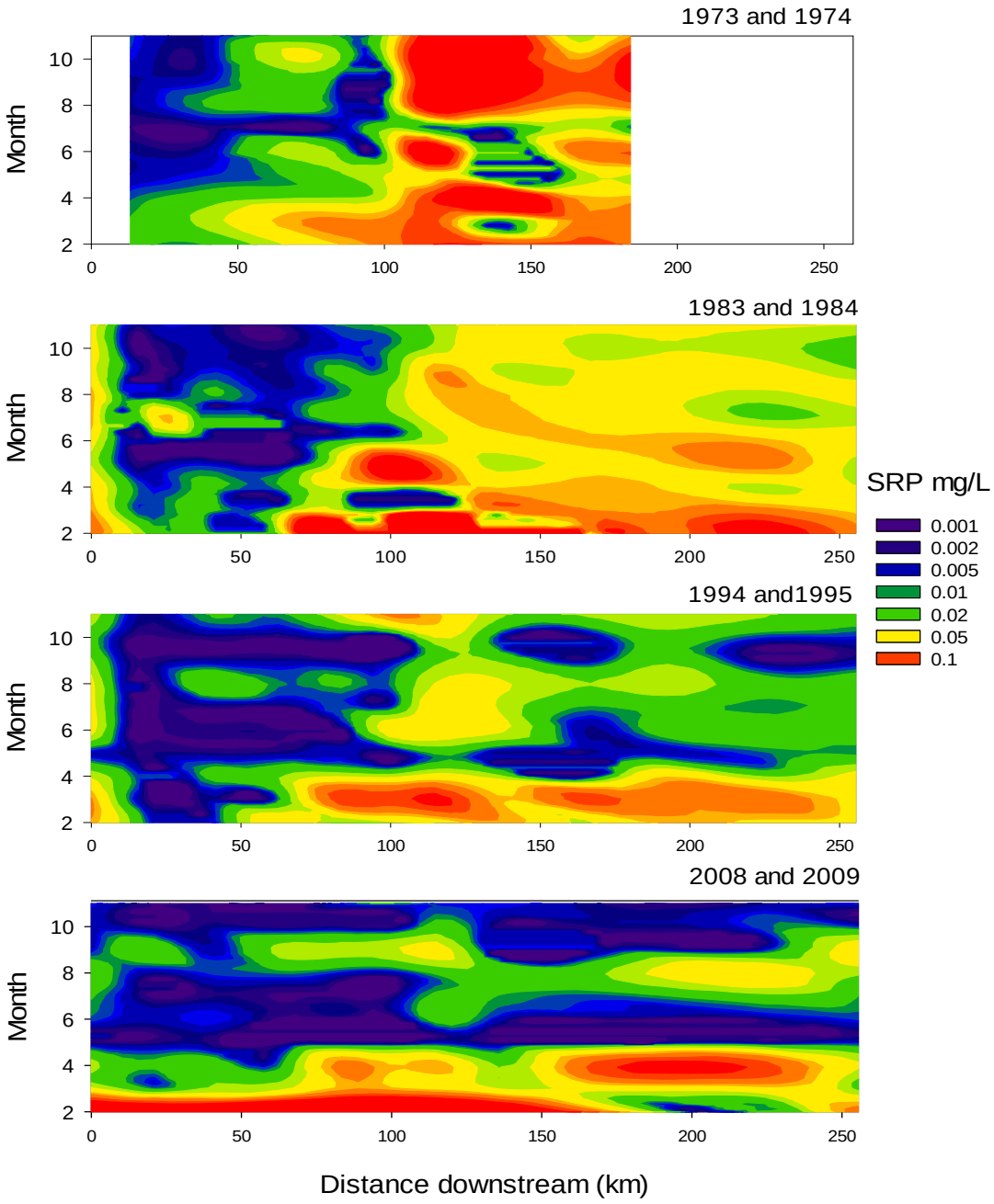

Figure 1.9 SRP concentration from upstream to downstream and seasonally, interpolating data from the 13 sites at four periods of time within the monitoring data. Data from two years were combined to increase the amount of data available to construct the graph.

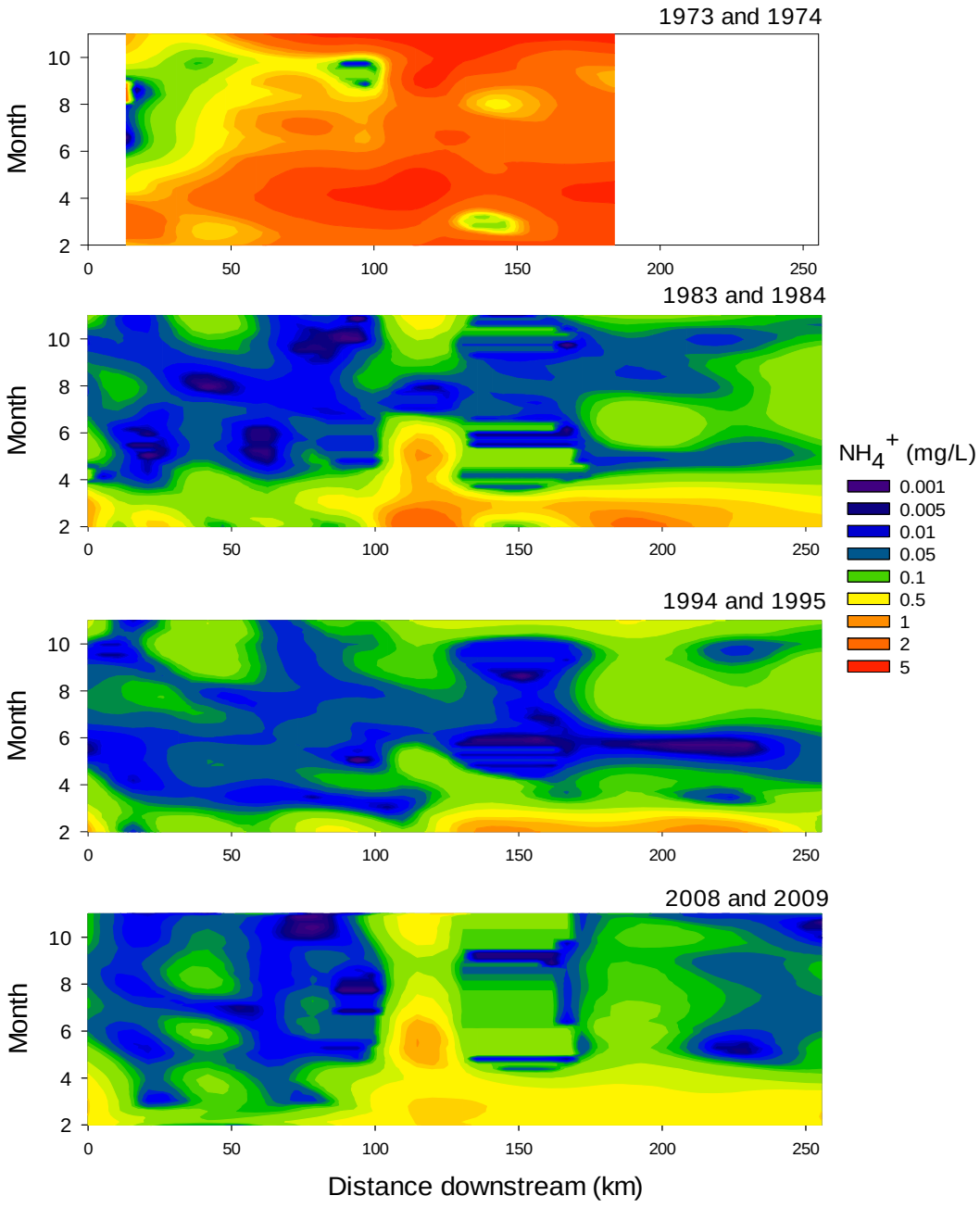

Figure 1.10 NH_4^+ concentration from upstream to downstream and seasonally, interpolating data from the 13 sites at four periods of time within the monitoring data. Data from two years were combined to increase the amount of data available to construct the graph.

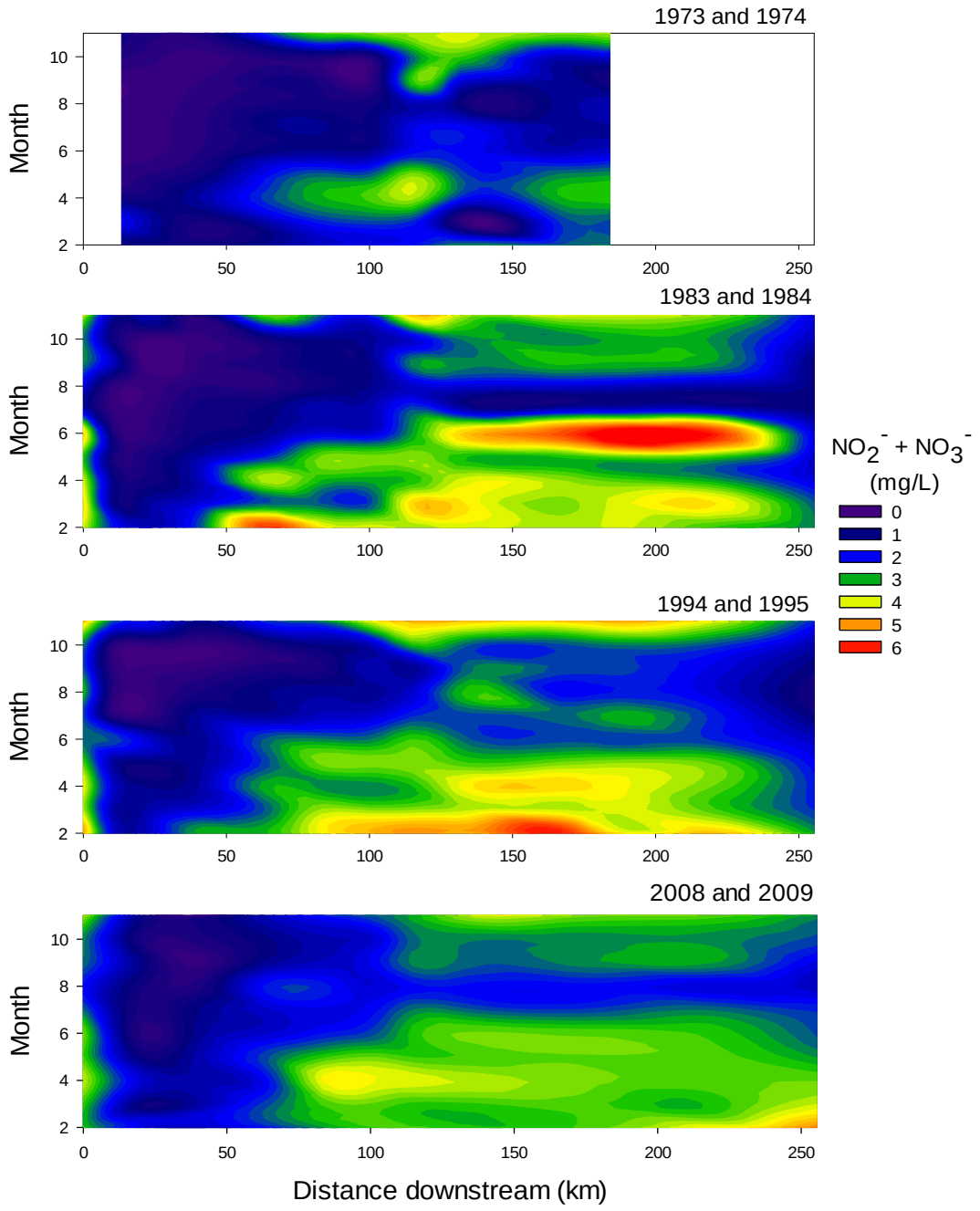

Figure 1.11 NO_3^- + NO_2^-concentration from upstream to downstream and seasonally, interpolating data from the 13 sites at four periods of time within the monitoring data. Data from two years were combined to increase the amount of data available to construct the graph.

1.3.3 Relative abundance of nutrients and relationship to discharge

SRP was a highly variable fraction of TP, accounting for 10% to 70% of TP over the monitoring period (fig. 1.12). SRP increased as a fraction of TP from upstream to downstream, and locations upstream of Waterloo had only 10% to 25% of TP as SRP. Over time, SRP decreased as a fraction of TP. In the most recent years (2008-2009) SRP accounted for about 15% of TP upstream of Waterloo, about 25% of TP immediately downstream of Kitchener and about 20% further downstream.

TIN relative to SRP in the river was also highly variable spatially and temporally. Upstream TIN: SRP values tended to be lower than downstream values, however TIN:SRP increased markedly before the WWTPs in Waterloo and Kitchener at km 70 (fig. 1.13) implicating a source for inorganic nitrogen other than the WWTPs, possibly the Conestogo River. After passing the WWTPs the TIN: SRP of the river decreased, indicating that effluent was a source of SRP, as was shown in the temporal and spatial analyses discussed in the previous sections. TIN: SRP increased through time, both due to decreasing SRP (fig. 1.4) and increasing NO_3^- + NO_2^- (figure 1.6). In recent years, TIN: SRP was highest after km 70, however in 2008-2009 the trend was not as pronounced.

NO_3^- and NO_2^- are often reported together in the PWQMN data, but they showed different trends through time relative to each other. At the Amaranth site (fig. 1.14) NO_2^- was highest in winter, spring, and early summer, and lowest in late summer, but the percent of NO_3^-+NO_2^- as NO_2^- was also highest in late summer because NO_3^- was lower. Both NO_3^- and NO_2^- increased through time, but the percent NO_2^- of NO_3^- + NO_2^- decreased; in years prior to 2000 the percent NO_2^- could reach over 40%. At the Bridgeport site (fig. 1.15), NO_3^- and NO_2^- were both at their highest in winter and spring and lowest in late summer, and the percent NO_2^- did not appear to change seasonally. The NO_2^- values at Bridgeport were much lower than at Amaranth, with concentrations mainly lower than 0.05 mg/l, while at Amaranth winter and early spring concentrations of nitrite reached over 2 mg/l. NO_3^- concentration at Bridgeport was generally greater than at Amaranth, especially in recent years (note the difference in scale between fig. 1.14 and fig. 1.15), with concentrations in winter reaching 6 to 8 mg/l at Bridgeport while at Amaranth NO_3^- concentrations rarely exceeded 2 mg/l. The percent NO_2^- declined over time, similar to Amaranth. At Blair (fig. 1.16), the NO_3^- concentration trend was similar to Bridgeport and Amaranth, with concentrations increasing over time, and highest concentrations being

observed in winter and spring, and lowest concentrations in late summer. However, NO_2^- did not share this pattern. Although the concentrations of NO_2^- were higher at Blair and more similar to Amaranth, the seasonal variation in concentration was very unlike Amaranth in that it appeared to be highest in the summer while at Amaranth NO_2^- concentrations were lowest in the summer. Over time, concentrations in NO_2^- and NO_3^- decreased then increased again, and in most recent years NO_2^- appeared to be high all year round. At Blair, NO_2^- concentration was found to be as high as 2 mg/l but not as frequently as at the Amaranth location. The percent NO_2^- was highest in summer, and appeared to have decreased through time until the 1990s where it began to increase again, following the trend in NO_2^- concentrations.

Because discharge data were not collected at the same time or in the all locations as nutrient data, nutrient concentrations at PWQMN sites cannot be "corrected" for discharge using a concentration versus discharge relationship. However some flow data were available for some of the sites and dates, although not for the same sites that fit the criteria appropriate for spatial and temporal trend comparison for the determination of point our diffuse source origin. Flow data for Marsville (site 3), 7 km downstream of Amaranth (fig. 1.17) and Blair (Site 8, fig. 1.18), were available for some of the sampling dates. Marsville had extensive discharge data dating back to the earliest nutrient sampling and as recent as the most recent nutrient data obtained. Blair discharge data dates back only as far as 2006.

Discharge was positively correlated to TP, SRP and NO_3^-+NO_2^- in the early (1975-1978) period and the more recent period (2007-2009) of the monitoring data. At Marsville, discharge was not correlated with NH_4^+ in the earlier period (1975-1978), but it was correlated in the more recent period (fig. 1.17). At Blair (fig. 1.18), discharge was not correlated with NH_4^+ in the most recent data. Discharge explains less variation in all parameters in the earlier data than in the later data, suggesting that the importance of diffuse sources has decreased over time. Between 41% and 68% of the variation in SRP concentration is explained by discharge, and 50-87% of the variation in TP concentration is explained at the Marsville and Blair sites (fig. 1.17, fig. 1.18). Some of the variation in NO_3^-+NO_2^- (22% to 64%) can be attributed to discharge (fig. 1.17 and fig. 1.18).

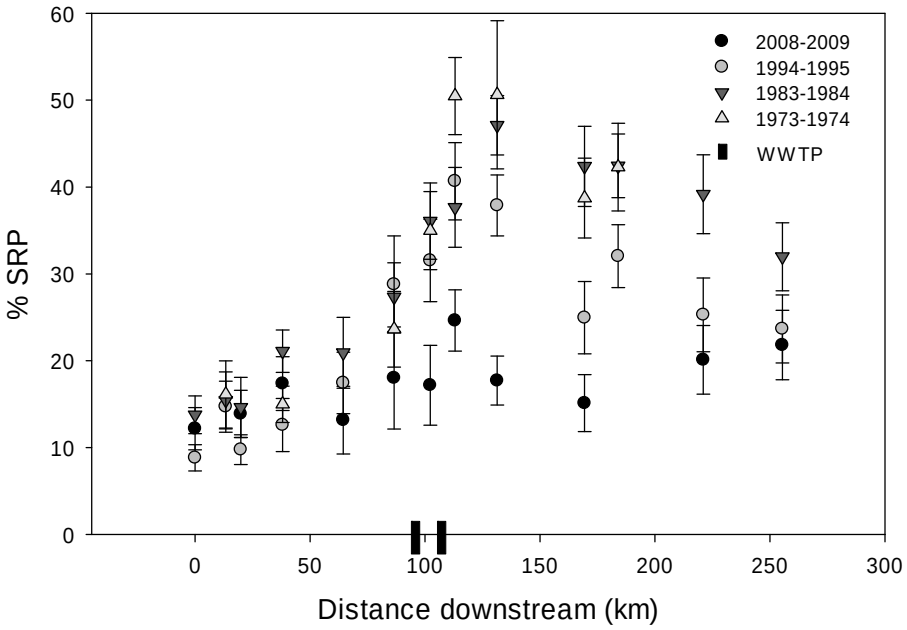

Figure 1.12 Percentage of TP as SRP at the 13 sites from upstream to downstream in 4 two-year periods. Location of the treatment plants in Waterloo (upstream) and Kitchener are marked on the x-axis with black boxes.

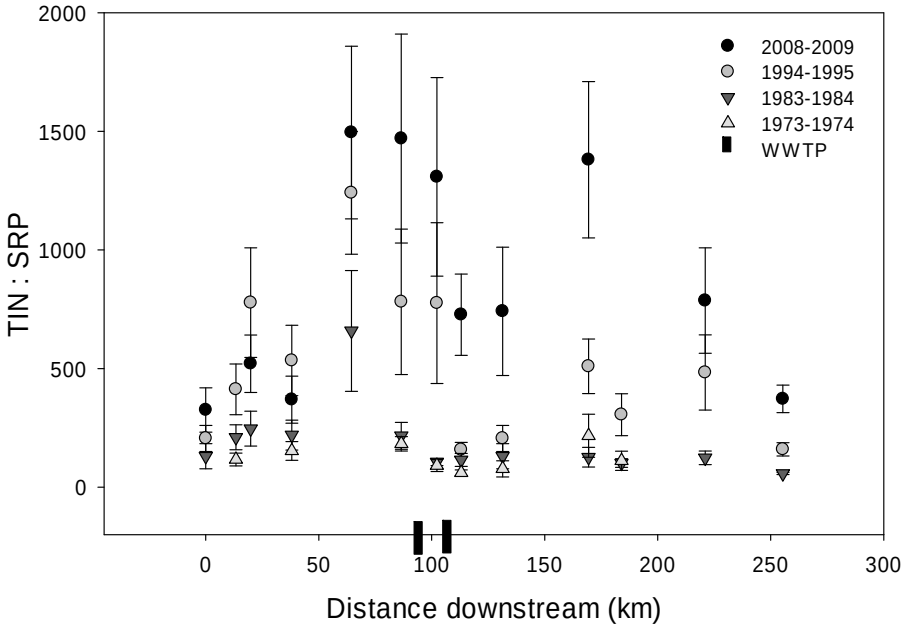

Figure 1.13 Mass ratio of TIN: SRP at the 13 sites from upstream to downstream in 4 two-year periods. Location of the treatment plants in Waterloo (upstream) and Kitchener are marked on the x-axis with black boxes.

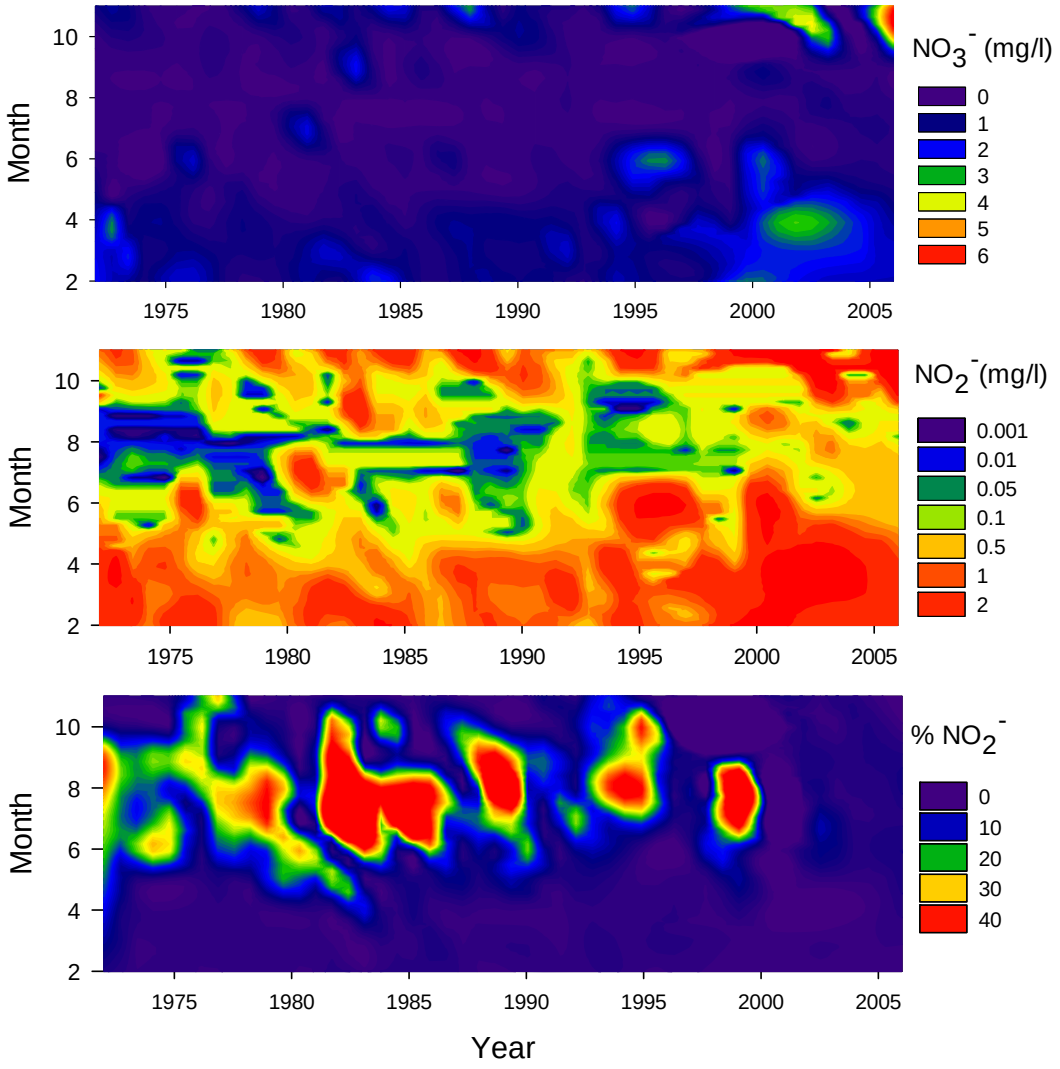

Figure 1.14 Changes in the concentration of nitrate (NO_3^-), nitrite (NO_3^-), and percent NO_2^- of NO_3^-+ NO_2^- (% NO_2^-) through time and season at Amaranth (site 2), 13 km downstream of the headwater PWQMN site 1. Colour represents NO_3^-, NO_2^- concentration and %NO_2.

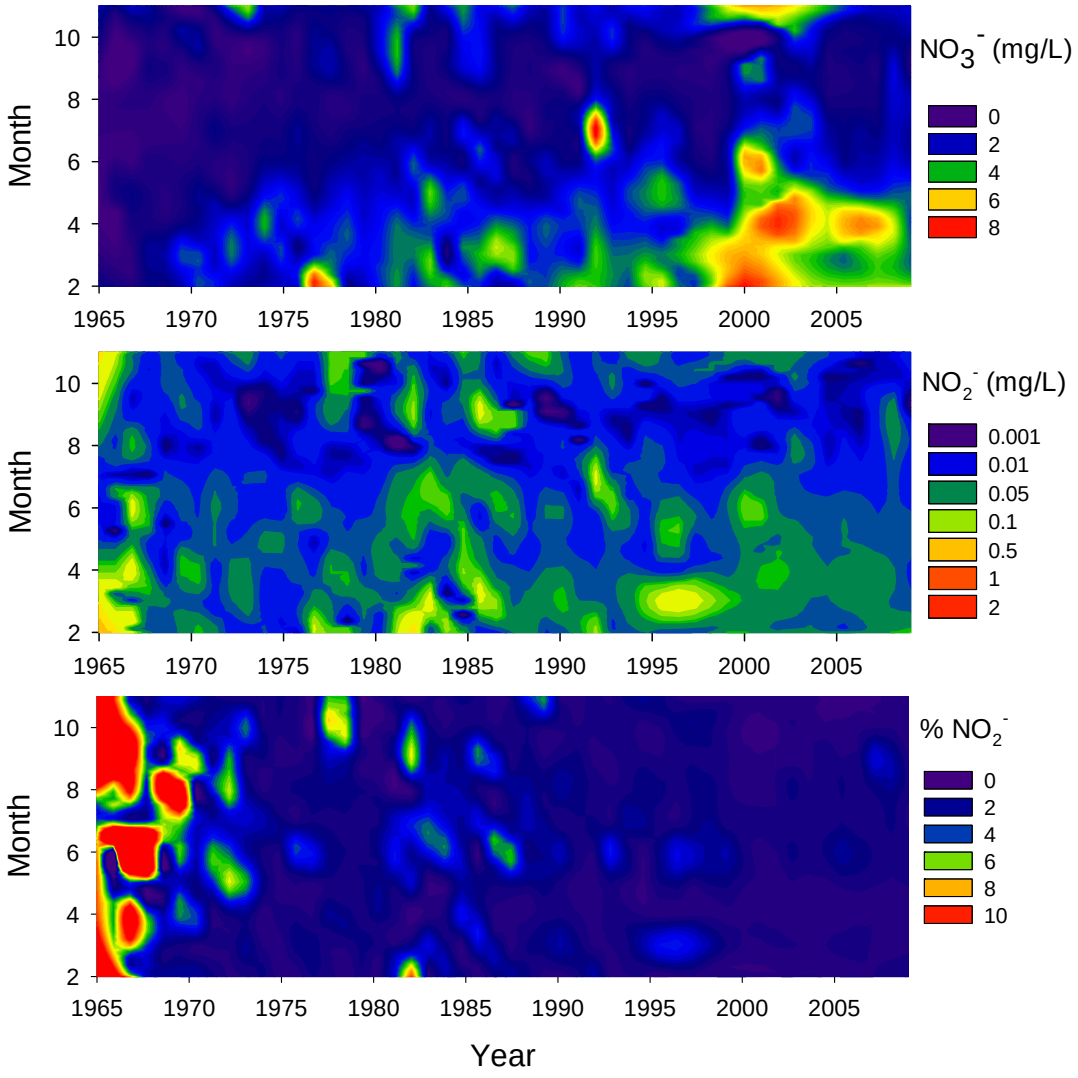

Figure 1.15 Changes in the concentration of nitrate (NO_3^-), nitrite (NO_3^-), and percent NO_2^- of NO_3^-+ NO_2^- (% NO_2^-) through time and season at Bridgeport (site 5), 87 km downstream of the headwater PWQMN site 1, and upstream of the Kitchener and Waterloo WWTP outfalls.

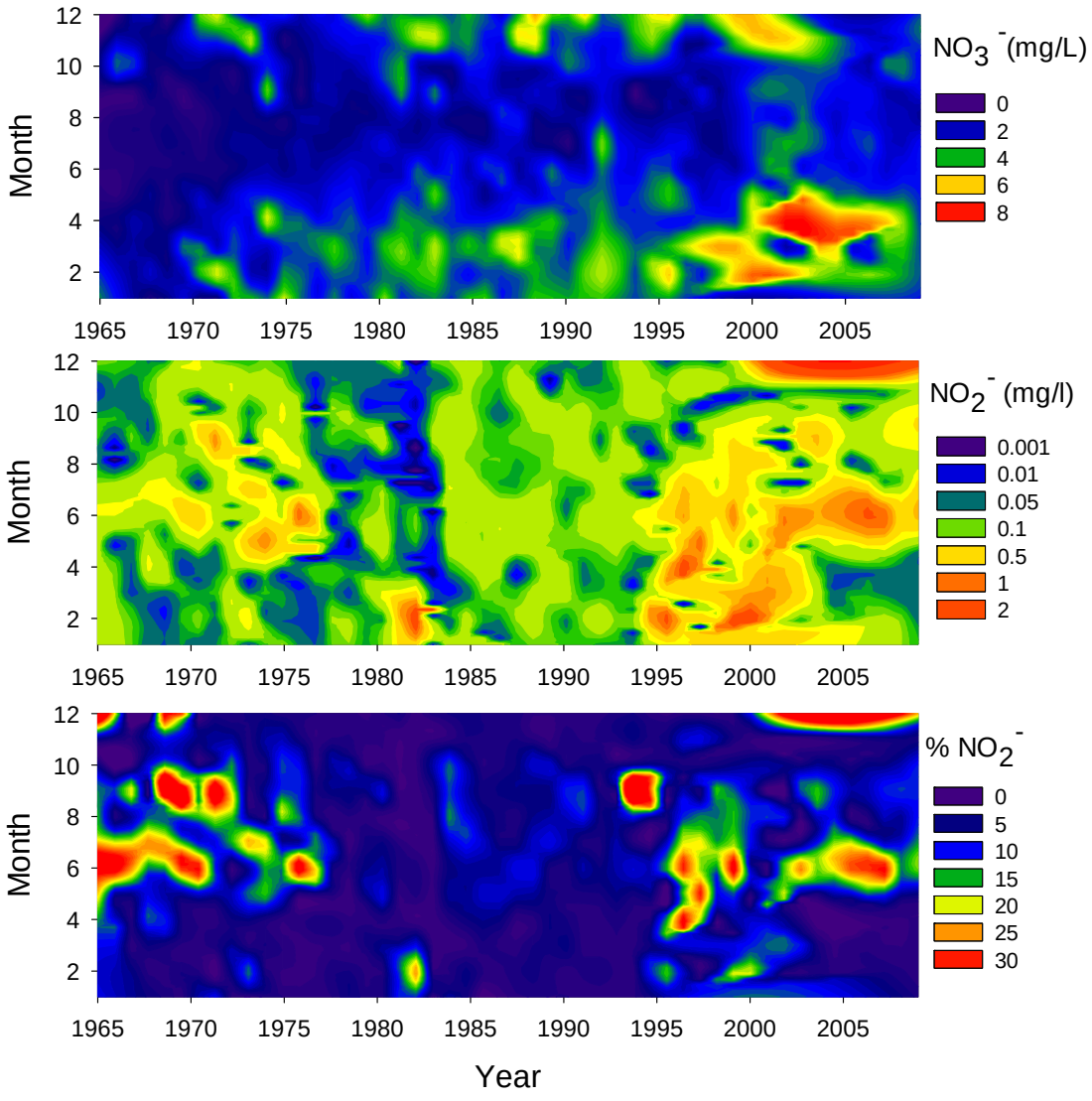

Figure 1.16 Changes in the concentration of nitrate (NO_3^-), nitrite (NO_3^-), and percent NO_2^- of NO_3^-+ NO_2^- (% NO_2^-) through time and season at Blair (site 6), 113 km downstream, and downstream of the Kitchener-Waterloo WWTP outfalls. Colour represents NO_3^-, NO_2^- concentration and %NO_2.

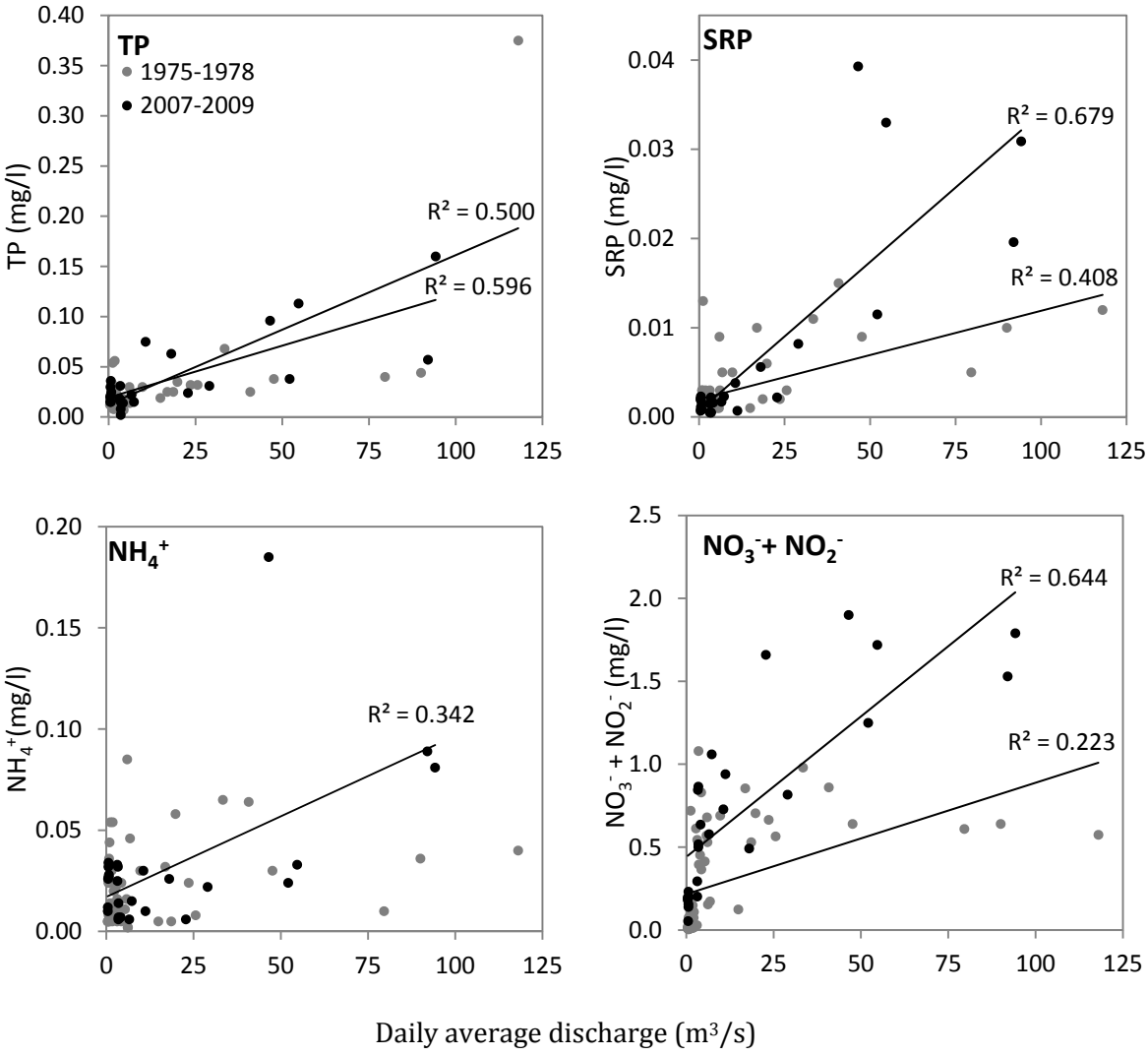

Figure 1.17 Relationships between discharge and TP, SRP, NH_4^+ and NO_3^-+NO_2^- at Marsville (site 3), Points represent individual nutrient samples and the average discharge measure for that day.

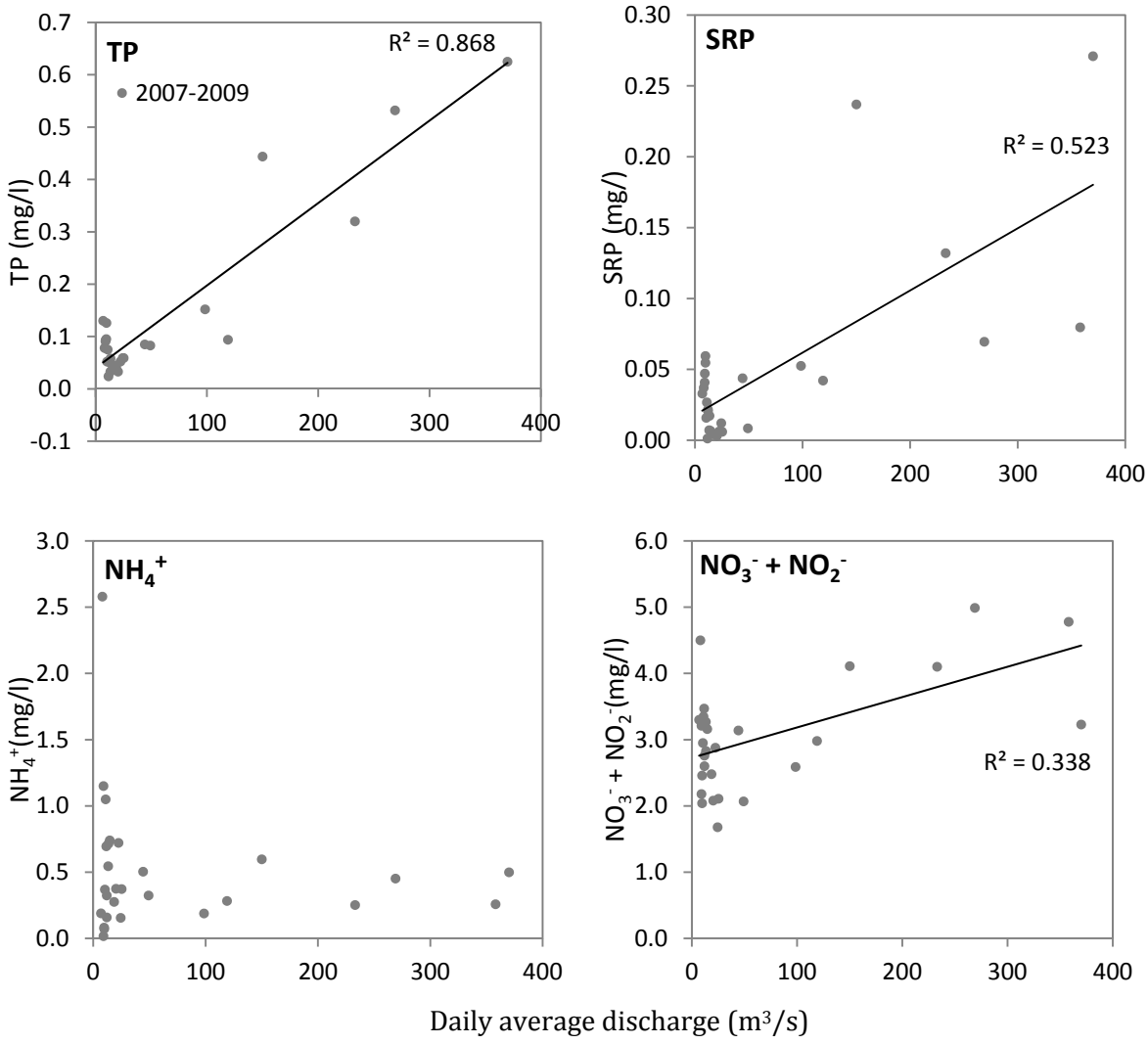

Figure 1.18 Relationships between discharge and TP, SRP, NH_4^+ and NO_3^-+ NO_2^- at Blair (site 6), 2007 to 2009. Points represent individual nutrient samples and the average discharge measure for that day.

1.4 Discussion

1.4.1 Past to Present

TP and SRP declined over the monitoring period at all locations, with a stepwise decrease at the mid-1970s, likely a result of the WWTP upgrades occurring at the time, and the introduction of legislation banning laundry detergents containing phosphate. Following the initial drop a smaller, but detectible, decline in TP and SRP was two orders of magnitude lower than the drop in the 1970s. This finding is somewhat puzzling; as populations increase and agriculture becomes intensified, phosphorus concentrations in surface waters might be expected to increase.

Other long-term studies of river nutrient concentrations have generally seen declines in both TP and SRP in recent decades. In the Nemunas River, located in Lithuania and Belarus, SRP has been decreasing over time (Sileika et al., 2006). SRP increased in several English lowland streams until 1980, then decreased (Parr and Mason, 2003). In both cases, the decreases were attributed to installation of WWTPs and the development of P restriction targets. Recent work on lakes on the Canadian Shield indicate the same trend of declining P (Eimers et al., 2009) and in headwater streams across Ontario (Stammler, 2012)

Typically lakes can take many years to recover after nutrient abatement (Phillips et al., 2005), while the Grand River appeared to show an immediate response to phosphorus removal. The continual decline in recent years is more difficult to explain, but could be a result of increased awareness and use of best management practices by farmers, but it could also be indicative of a larger change in landscape-scale biogeochemical cycles, such as long-term soil acidification which could enhance the capacity of soils to adsorb and retain free phosphate (McDowell et al., 2002).

The TP and SRP decline in the 1970s was not accompanied by significant change in the concentrations of $NO_3^- + NO_2^-$ and NH_4^+. Indeed, $NO_3^- + NO_2^-$ and NH_4^+ increased in the 10 year period after the WWTP upgrades for many locations on the Grand River, and increased over the whole monitoring period in most locations chosen for analysis. It is unlikely that these increases have much to do with the WWTP upgrades, but rather are related to agricultural intensification in the watershed. Although chemical fertilizers are often NH_4^+-based, the NH_4^+ quickly oxidizes to form NO_3^- in soil microenvironments and often very little NH_4^+ makes it to streams, while

NO_3^- concentrations of agricultural streams are often high (Mayer et al., 2002). NH_4^+ concentrations increased over the entire monitoring period, though only at the Bridgeport and Blair locations.

The increase in NO_3^- and NO_2^- over time is not unique to the Grand River. In the Changjiang (Yangtze) River in China, NO_3^-, NO_2^- and NH_4^+ increased over time due to increased urban effluent and increased chemical fertilizer use (Duan et al. 2007). Concentrations of NH_4^+ in the Seine, France, increased over time and downstream of urban centers, even after modern septic and sewage and treatment facilities were put in place (Billen et al., 2007; Billen et al., 2001) up until the mid-1980s when they started showing signs of decline. NO_3^- increased in the Tully River, Australia, between 1987 and 2000 (Mitchell et al., 2001), in the Nemunas River and tributaries in Lithuania and Belarus between 1986 and 2002 (Sileika et al., 2006), in the Ebro River and tributaries in Spain between 1981 and 2005 (Lassaletta et al., 2005), and at the mouth of the Seine, France (Billen et al., 2001). A model of global river N fluxes (Green et al., 2004) indicates that since the beginning of the industrial era, N fluxes from river basins have increased from 2 to 5 times. The increasing quantity of reactive nitrogen in rivers is consistent with the finding that reactive nitrogen loading to the global biosphere has roughly doubled (Green et al., 2004; Galloway et al., 2004) since pre-industrial times. The data are disconcerting as the increase in NO_3^- may mean increased denitrification and increased riverine production of the greenhouse gas N_2O (Seitzinger, 1988; Inwood et al., 2004; Mulholland, 2008). It is likely that the impact of chronically high nitrate on water resources and aquatic ecosystems is yet to be fully recognized.

1.4.2 Upstream to Downstream

All of the nutrients measured increased with distance downstream and were highest in the mid reaches of the river. These maxima were located near the cities of Kitchener and Waterloo, but not necessarily downstream of the major WWTP in Kitchener for all nutrients analyzed, indicating different sources or transformation mechanisms behind each nutrient. Early in the monitoring period SRP increased substantially after the river passed the Kitchener WWTP, while big increases in TP appeared before the Kitchener and Waterloo WWTP. It is likely that there were other important sources of TP at the time, such as from agriculture. The Conestogo River joins the Grand at about 70 km from the source and upstream of Waterloo, and drains a

heavily farmed region of the basin. Because of its close proximity to the WWTP and the spacing of the monitoring stations, it may appear that nutrients from the Conestogo came from the Waterloo and Kitchener WWTPs.

After the mid 1970's, TP and SRP were substantially lower at Blair, but the changes to TP and SRP were also evident further downstream. The change in TP in the mid 1970's was detected at Glen Morris and the change in SRP around the same time was detected at Newport, roughly 3 times as far downstream from the Kitchener WWTP as Glen Morris, possibly indicating that the changes to phosphate content of detergents and wastewater treatment technology occurring at the time had a larger effect on the dissolved component of the river P cycle. In the later period, 1975 to 2009, TP concentrations declined at most sites, but the largest decline differed spatially from the earlier period in that TP declined the fastest at the Newport site, while the largest decline in SRP occurred at Glen Morris. Nitrate increased with distance downstream to the mid-reaches of the river, with the biggest increase found at Newport, while NH_4^+ only showed modest changes, with an increase at Blair, downstream of the Kitchener WWTP.

SRP, a surrogate for PO_4^{3-}, the most biologically available form of P (Levine and Schindler, 1980), should diminish faster than TP from the effluent. The other dissolved components of TP, dissolved organic P, would require enzymatic hydrolysis to PO4 before being taken up. Particulate P would be lost to sedimentation or capture by suspension feeders. However, under "nutrient saturated" conditions during the late 1960s-early 1970s, phosphate supply may outstrip biological demand and thus phosphate removal from the water column may be controlled by physical processes rather than biological uptake.

The distance downstream to which a particle travels before it is removed through sedimentation is a function of particle size (Bursik, 1995), with smaller particles travelling farther on average than larger ones. This result could be viewed in the context of the nutrient spiraling concept of riverine nutrient cycling (Webster, 1975; Newbold et al., 1981) where nutrient retention, degradation, remineralization, and re-uptake govern the flow of nutrients from upstream to downstream in a fashion that resembles a spiral. High nutrient streams are characterized by longer spiral lengths (Webster et al., 2003; Mulholland et al., 2008) and, accordingly, PO_4^{3-} removal after a point source would occur over a longer distance.

The general longitudinal pattern of nutrient concentrations, as seen in phosphorus and nitrogen, is an increase with distance downstream. The increasing trend might be seen as a cumulative effect of nutrient loading from multiple sources, including tributaries and groundwater, upstream in the watershed (Seitzinger et al., 2002; Alexander et al., 2007). Physical and redox changes to the river channel with distance downstream would promote N loss through denitrification, while a deeper river channel and a slower current would reduce volatilization. While NH_4^+ had no discernable longitudinal pattern, there is an apparent hot-spot downstream of the major WWTP at Kitchener and after Brantford. Additionally, NH_4^+ was not correlated with discharge at Blair, a strong indication of point source dominance (Mainstone and Parr, 2002). Because NH_4^+ is readily volatilized (depending on the pH) and nitrified, a high concentration does not persist for very long or cover extensive areas. For this reason, NH_4^+ hotspots make good indicators for point source impacts on river system. A spatial examination of river monitoring data can give an indication of important sources of nutrients to river systems; however an examination of land-use may also be beneficial to this endeavor.

1.4.3 Nutrient dynamics: sources, processing and evidence of change

The temporal and spatial analysis of the long term data in the preceding sections indicates that both point and diffuse sources affect the nutrient chemistry of the Grand River. The data provide some means to distinguish sources as well, using a seasonal analysis of the relative abundance of nutrients and their relationship to discharge. The temporal analysis indicates that prior to upgrades in 1973-1974, the Kitchener WWTP was a major source of TP and SRP to the Grand River, and spatial analysis demonstrated the WWTP continues to be a source for NH_4^+. However these analyses do not implicate the WWTP as a major source of NO_3^- (NO_3^- + NO_2^-) even in the early monitoring data, before current wastewater treatment practices were in place. The seasonal pattern of nutrients also supports this conclusion. Prior to WWTP upgrades, TP, SRP and NH_4^+ at Blair showed a distinct pattern representative of point source impact, with high concentrations year round, especially during low-flow in summer months (Mainstone and Parr, 2002). After the mid-1970s, TP and SRP begin to exhibit a more diffuse source pattern, with higher concentrations during periods of high flow (late winter and early spring), a pattern that is also seen in upstream sites. NH_4^+ remained high year-round and the Kitchener WWTP continues to be an important source of NH_4^+. NO_3^-+NO_2^- concentrations, however, show the diffuse source pattern over the monitoring period at the sites above and below the WWTP,

corroborating previous interpretations that diffuse sources (which likely include the oxidation of NH_4^+ to NO_3^-) predominantly contribute to river NO_3^-.

A large and variable proportion of TP was present as SRP in the Grand River. Across lakes, PO_4^{3-} (measured by radiobioassay) increased linearly with TP, resulting in PO_4^{3-}:TP being roughly the same (Hudson et al., 2000), while in the Grand River this trend was not found. SRP as a proportion of TP varied anywhere from 10% to 70% over all sites and years. Hudson et al., (2000) did not use SRP to estimate PO_4^{3-}, as it generally produces over estimation artifacts; however, SRP is typically higher in rivers and is likely a better measure of PO_4^{3-}. SRP increased as a proportion of TP below the WWTP, indicating the influence of nutrient point sources on river P dynamics .

TP downstream of Kitchener-Waterloo, even after treatment upgrades, was composed of proportionally high levels of SRP compared with other species of phosphorus. Further downstream the proportion of SRP declined, possibly due to biotic demand for SRP, and returned to a similar proportion as upstream of the WWTPs. The proportion of TP as SRP could be useful in determining the distance downstream phosphorus concentration is influenced by a point source, and perhaps another useful tool for the determination of the assimilative capacity of a river for nutrients. Using data from the Grand River we can suggest that the proportion of TP as SRP indicative of a point source impact be above 50%, but a better value would be one derived from cross system comparison of SRP:TP values, backed up by other methods that determine the contribution of nutrient point sources.

The ratio of DIN to SRP may be useful as an indicator of spatial or temporal coupling of nutrient cycles (Kemp and Boynton, 1984). In the Grand R., DIN: SRP ratios increased over time and with distance downstream due to both increasing DIN concentrations and decreasing SRP concentrations. The increasing ratios may indicate that the Grand R. is on its way to becoming P limited, although the quantities of both DIN and SRP are likely too high to limit algal and macrophyte production (Wong and Clark, 1976; Mohamed et al., 1998, Dodds, 2006).

The ratio of nitrite to nitrate can also provide information about nitrogen cycle processes and how they might have changed temporally and spatially in the Grand river. During nitrification, ammonia is converted first to nitrite by ammonia oxidizing bacteria, typically the ammonia-

oxidizing bacteria, the Proteobacteria of the genera *Nitrosomonas, Nitrosospira*, and close relatives. The nitrite produced by these bacteria is then converted to nitrate by nitrite-oxidizing bacteria, namely *Nitrobacter* and three other distinct groups (Lees, 1952; Lees and Simpson, 1957; Teske et al., 1994; Cébron et al., 2003). The NO_2^- → NO_3- step occurs readily, leaving behind relatively little NO_2^-. Denitrification removes nitrogen from the aquatic environment by microbial conversion of NO_2^- or NO_3- to N_2 with N_2O and NO as intermediate steps, both of which are gaseous and can leave the aquatic environment before denitrification is complete (Tiedje, 1988). Because ammonia oxidation proceeds first to NO_2^- then to NO_3^-, while denitrification can remove either, changing NO_2^- relative to NO_3^- can indicate changes to NH_4^+ and NO_2^- oxidation rates as well as NO_3^- and NO_2^- uptake rates. Under conditions of elevated temperature, pH, and NH_4^+ and decreased dissolved oxygen, the two step process of nitrification changes such that the oxidation of NO_2^- by *Nitrobacter* spp. is limited or even inhibited (Kholdebarin, 1977; Bae et al, 2002; Ruiz et al, 2006) resulting in NO_2^- accumulation. In a culture experiment, Bae et al (2002) observed that the lowest rates of nitrite oxidation occurred at 30 C, pH of 8-9, DO of 1.5 mg/l and an NH_4^+ concentration of 4 mg/l or higher. They found nitrite accumulated and yielded concentrations of up to 25 mg/l when these conditions were met.

There is evidence of NO_2^- accumulation at some sites and times of the year in locations where high NH_4^+ concentrations may be found, for example Amaranth and Blair in late summer, but not Bridgeport. Amaranth and Blair may experience high levels of ammonia and hypoxia during the summer (although our data do not indicate Amaranth has high concentrations of ammonia) so it is conceivable that when temperatures are high and oxygen is low, inhibitory effects on *Nitrobacter* spp. and relatives may occur in these locations, producing NO_2^- accumulation. The inhibitory conditions may indicate regions of the river where the assimilative capacity for DIN is relatively low, where the spiral length for NH_4^+ is increased and the demand for O_2 is transferred further downstream. It may be interesting to conduct future work on the utility of the NO_2^- as an indicator of nitrification inhibition to understand catchment scale nitrogen processing.

1.4.4 Conclusions

The 30+ year monitoring history in the Grand River reveals several important long-term nutrient trends that are occurring in the river; firstly, that concentrations of TP and SRP have declined and are continuing to decline in many of the sites examined in this study. Nitrate (NO_3^- + NO_2^-) increased over the monitoring period in most locations, and appears to be diffuse in origin as concentrations did not seem greatly elevated in the "hot spot" region of the river downstream of the region's largest WWTP, and the seasonal pattern of NO_3^- + NO_2^- is indicative of diffuse source origin. The contrasting trends in P and N reflect both the P-centric focus of nutrient management and the shift in importance from nutrient point sources to diffuse sources in the Grand River watershed. Nutrient ratios, such as SRP:TP, SRP:DIN, NO_3^- + NO_2^- can provide information that is distinct from concentration data alone, and also indicate that while diffuse nutrient sources are becoming more important in the watershed, the large WWTP facility in Kitchener still contributes significantly to river P concentrations.

Chapter 2: Macrophyte response to nutrient point sources in a eutrophic lowland river in Southern Ontario

2.1 Introduction

Submersed macrophytes are important primary producers in lotic communities. They serve as refugia for fish and invertebrates, provide food for a number of aquatic species, and act as a substrate for periphytic algae. They also influence the water chemistry in synchrony with daylight through photosynthesis and respiration (Carpenter and Lodge 1986; Chambers and Prepas 1994; Carr et al. 1997; Chambers et al. 1999; Mainstone and Parr, 2002; Caraco and Cole, 2002; Lacoul and Freedman, 2006). For rivers located in densely populated areas, submersed macrophytes communities can achieve a summer biomass which is often considered to be a nuisance. The respiration associated with nuisance biomass can drive river DO down at night to hypoxic levels, which threatens aquatic life and reduce the suitability of river water as a resource for human populations (Davis, 1975; Chambers et al., 2006). Although it is generally understood that increased river macrophyte biomass occurs in response to nutrient enrichment, the problematic peak summer biomass in rivers is relatively understudied and there is no useful predictive relationship between macrophytes and nutrients to quantify river eutrophication (Carr and Chambers 1998; Dodds 2006; Hilton et al., 2006).

Conceptualizing and modeling the benthic macrophyte response to anthropogenic loading has been difficult. Although it is generally recognized that river macrophytes and benthic algae show a biomass response to nutrient loading, a clear empirical relationship has not been established, therefore the predictability of macrophyte communities and their responses to human activities is limited (Neilsen, 2003; D'aiuto et al., 2006; Hilton et al., 2006; Franklin et al., 2008). Correlations *in-situ* between dissolved nutrients and macrophyte biomass have not yielded a strong positive relationship and, in some studies, no relationship or a negative one was found (Canfeild and Hoyer, 1988; Carr and Chambers, 1998; Flynn et al.,2002; Sosiak, 2002; Carr et al., 2003; Hilton et al., 2006; Demars and Thiebaut 2008; Demars and Edwards, 2009).

In light of the empirical evidence, some have concluded that nutrients are not important in explaining the variation in macrophyte biomass in impacted river systems because physical conditions such as light availability, current velocity and substrate type and quality are much

more important in structuring habitats and influencing biota. In fact, studies that focus specifically on these individual parameters can demonstrate their roles in explaining some of the variation in macrophyte densities (Chambers and Kalff, 1985; Barko and Smart, 1986; Sand-Jensen et al., 1989; Barko et al, 1991; Chambers et al., 1991; Riis and Biggs; 2003; Xie et al., 2005). My work (Chapter 3) has demonstrated there to be high inter-annual variation which can be predicted to a certain degree by seasonal temperature and flow, however, physical factors alone cannot explain why some rivers located near urban populations and within agricultural catchments have such high productivity and biomass of benthic macrophytic plants and algae, and watershed managers are still left with the need to address problematic levels of macrophyte and macroalgal biomass associated with summer hypoxic conditions.

Some explanations for the discrepancy between theory and observation are that the environmental heterogeneity in space and time, recognized to be relatively high in river ecosystems (Sand-Jansen and Borum, 1990; Mainstone and Parr, 2002), confound the interpretation of results from field studies, which are typically short lived and with sampling schedules that are spatially and temporally unable to capture the full range of variation that occurs in rivers. Inability to represent the full range of variability within a dataset may lead to a lack of statistical power to detect real effects (Francoer, 2001) or lead to systematic biases in the data. Correlations between *in-situ* concentrations and biomass also do not take into account the rapid cycling that can occur (Mulholland et al., 2000). Uptake and release rates, which cannot be determined from concentration measurements alone, can better indicate the activity of benthic primary producers and their response to elevated nutrient conditions. Uptake and release rates can be highly variable among systems that could be characterized as having similar nutrient concentrations. Another problem is that benthic angiosperms, and to some extent filamentous benthic algae, are slower growing and longer lived than planktonic algae, making laboratory studies and bioassays more challenging to conduct .

An additional challenge to measuring the response of the benthic macrophytic community to nutrient additions is the lack of a consistent, agreed upon conceptual framework of the process of eutrophication in rivers. Hilton et al., (2006) present a framework for viewing river eutrophication based on a modern understanding of features and processes found in lake ecosystems important to eutrophication, and propose the application of Grime's stress-disturbance theory of species distribution and biomass. Their proposed framework provides a

number of testable hypotheses regarding the response of the aquatic producer community to increasing nutrient concentrations. The two main forces in the model, stress and disturbance, act to structure plant communities, with individual species having specific adaptations to allow them to occupy a space in the stress-disturbance plane. Eutrophication of rivers can be included within this theory as light and nutrients (or lack thereof) are considered stressors, and flood frequency is considered a disturbance. All other factors influencing biomass may be fit into these categories as well. Under this explanation, increased nutrients (high stress) of a eutrophic condition would lead to the disappearance of macrophytes due to light limitation as they are colonized by epiphytes, which are better adapted to higher nutrient concentrations. At lower nutrient availability, rooted submersed macrophytes will outgrow algae due to the ability of macrophytes to utilize sediment nutrient reserves (Carignon and Kalff, 1980). As nutrient levels increase, submersed macrophytes disappear, and epiphytic and filamentous algae and emergent species (if flow permits) take their place. The use of this framework as described by Hilton et al., (2006) does not provide for high and low biomass stands of macrophytes, merely that they should be present or absent, if the stress and disturbance are not optimal. Thus, this type of model does not provide a complete enough picture of the benthic community dynamics to be useful in understanding and quantifying the eutrophication of macrophyte dominated rivers.

Further explorations of the relationship between river macrophytes and anthropogenic nutrient effects are necessary to characterize eutrophication processes for rivers, and to resolve the apparent paradox that benthic macrophytic plants and algae may not be limited by nutrients, yet grow in luxuriant patches to the point of ecological degradation in many heavily populated and nutrient enriched river systems. These studies may include alternative explorations of plant response, such as measurements of nutrient deficiency, nutrient storage and plant stress. Plant tissue nutrient composition and critical nutrient concentrations necessary for maximum growth and biomass production may be useful tools to determine whether nutrient limitation is occurring in macrophyte species, and the level of exposure of macrophytes to bioavailable nutrients. The tissue approach has the advantage of being independent of factors that can influence nutrient bioavailability, such as current velocity, boundary layer effects and seasonal variations in nutrient supply.

In this study I work towards resolving the apparent paradox of the macrophytic response to nutrient enrichment in rivers by addressing some of the problems that may have plagued previous work. I attempt to adequately sample the highly variable benthic submersed macrophyte community using a more spatially resolved approach conducted at a regional scale, based on a mapping technique developed for streams by Butcher (1933), and refined by Wright et al., (1981) and compare this method to a common technique of taking transects to measure macrophyte biomass. I ask whether submersed macrophyte biomass was elevated in sections of the Grand River considered eutrophic as characterized by low oxygen concentrations periodically in the summer, particularly downstream of major waste water treatment plants (WWTPs). If the results of the study demonstrate the macrophyte community biomass was elevated downstream of WWTP compared with upstream reaches, it is assumed that nutrient limitation must be occurring in those upstream reaches. I recognize that this approach will not empirically tie enhanced macrophyte biomass to nutrient loading, but it will better characterize the macrophyte communities that thrive in rivers heavily utilized by human societies. As evidence of nutrient limitation, macrophyte tissue nutrient concentrations and nutrient ratios based on the findings of Koerselman and Meuleman (1996) and Demars and Edwards (2007) will be used to determine whether the macrophyte tissue N and P concentrations are below a critical level: one at which growth rates would be less than 95% of their potential maximum. The effect of current velocity, species composition and plant tissue type will be examined to determine whether these variables have an impact on the outcome of the determination of nutrient limitation using plant tissue composition.

2.2 Materials and Methods:

This study was conducted within the Grand River basin, located in southern Ontario, Canada. For a detailed description of the watershed, see the Materials and Methods section in Chapter 1. To measure the macrophyte biomass response to nutrient point sources, three reaches of the Grand River main-stem (fig. 2.1) each approximately 10 km in length, were surveyed. The first, most upstream reach was located upstream of the cities of Kitchener and Waterloo, and is referred to as West Montrose or WMR reach. It runs from the Weisenberg Rd. bridge to the covered bridge in the community of West Montrose. The second reach was located mainly within the city of Waterloo, and is referred to as the Waterloo reach. It runs from Snyders Flats Rd in Waterloo to Victoria St. in Kitchener. Two sub-reaches were distinguished within this reach; one upstream of the Waterloo WWTP, and one downstream of the Waterloo WWTP. The two sub-reaches are called the Waterloo upstream reach and the Waterloo downstream reach. The third and most downstream reach used in this study was the Kitchener reach, and is located within the cities of Kitchener and Cambridge, ON, and extends from the King St. bridge in Kitchener to the Parkhill dam in Cambridge. Similarly to the Waterloo reach, the Kitchener reach was also composed of sub-reaches, one upstream of the Kitchener WWTP and one segment downstream of the Kitchener WWTP to the Fountain St. bridge, and a third is distinguished further downstream, from Fountain St. to the Parkhill dam. The sub-reaches are called Kitchener upstream and Kitchener downstream and Kitchener downstream 2. In total, there were three segments of river considered "upstream" and three "downstream". The West Montrose reach was sampled only on July 25, 2007. The Waterloo upstream and downstream sub-reaches were sampled on July 26, 2007, and August 28, 2009. The Kitchener upstream and downstream sub-reaches were sampled on July 20, 2007, and on August 27, 2009, while the Kitchener downstream 2 reach was sampled only once on July 21, 2007.

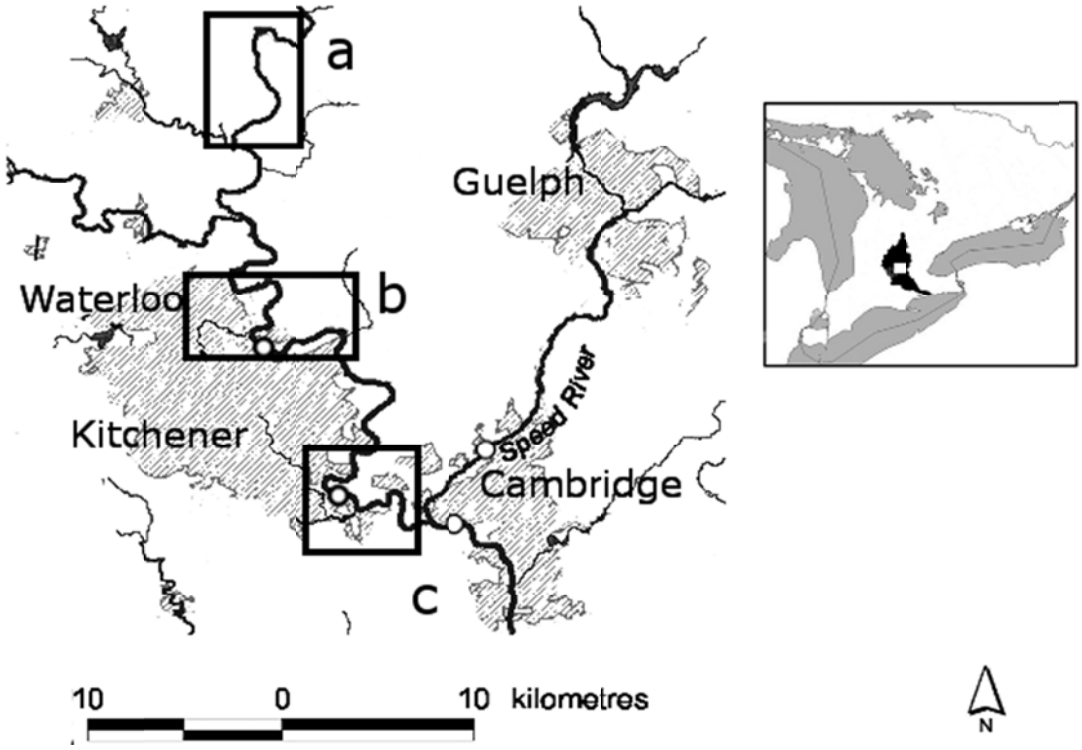

Figure 2.1 Site map showing the three reaches surveyed in this study. The West Montrose Reach (a) is located upstream of the cities of Kitchener and Waterloo, and their WWTP outflows. Downstream of the West Montrose reach, the Waterloo reach (b), was located within the city of Waterloo, and included an upstream and a downstream sub-reach, the border of the two marked by (O) the Waterloo WWTP. Similarly for the third most downstream reach (c), the Kitchener reach, with an upstream and a downstream sub-reach marked by (O), the Kitchener WWTP. The fourth reach, Kitchener downstream 2 (d), began where the Kitchener sub-reach ends, about 5 km further downstream of the Kitchener WWTP.

Mapping of macrophyte patches resulted in continuous, 2-dimensional spatial data, rather than discrete samples. This method had the advantage of including all patch information, as all patches, however small, and non-patch locations in the river were included in estimates of coverage and biomass. To obtain spatial patch information, the selected reaches were surveyed by canoe over several days during the peak biomass season (July to August) of 2007 and 2009. Exact dates of surveys in each year depended on weather- to obtain best patch visibility surveys were conducted after 3-4 days of no rain, when river flows were as close as possible to baseflow conditions. Maps were generated by canoeing a section of river by moving with the river flow, and bank-to-bank in a diagonal fashion to ensure the entire river bottom was mapped. In this work, maps were hand drawn on laminated printouts of GIS-generated maps of the chosen

reaches and then digitized for analysis by GIS software (Quantum GIS v 1.4.0 licensed under GNU General Public License). In the canoe, locations were verified by GPS coordinates and by the identification of landmarks, such as islands and buildings. Measurement error in the location and size of canoe-located and hand-drawn patches is estimated to be 1-5 m^2 based on several replicated drawings of patch size and location. As patches were mapped, each patch was given an estimate of density between 1 and 3 (1 being sparse and 3 being dense). The species composition of each patch was noted but, as mapping was done by canoe, only a coarse identification and estimate of species composition was obtained, with rare species and species of low representation in the community likely being missed; however for each patch a dominant species was always noted. Density estimates were converted to patch biomass by cropping where selected patches were sampled using a quadrat, taking up to 5 quadrats per patch for large patches. Above ground portions of macrophytes within the quadrat were harvested, then rinsed with river water, placed in Ziploc bags and transported back to the lab where they were cleaned again with DI, separated from detritus and benthic invertebrates, and sorted to species. Macrophytes were cleaned to remove epiphytes by shaking three times in a plastic bag with DI. Then they were dried in an oven in foil trays at 65 C for several days and subsequently weighed to obtain dry weight. After pooling all density estimates from 2007, 2009 and from several macrophyte biomass surveys conducted in 1997-2000 by the Grand River Conservation Authority (GRCA), discussed in detail in chapter 3 (fig. 2.2), the average biomass of the three density categories was applied to patches that were not sampled directly. During the canoe surveys, measurements of depth were taken off the side of the canoe using a large ruler with precision of 0.05m. Depth measurements were taken for every patch, and semi-regularly between patches (about 100-250m, depending on the patch coverage and location of the patches). Bank-full width of the river was obtained from the GRCA in the form of a map, and was not measured directly during field excursions.

Two methods were used to extract data from the maps produced in the biomass surveys. The first method, called the segment method, involved dividing the surveyed reach into segments of 500 m each in length, and determining the area of each segment length. The area covered by macrophyte patches within each 500m segment was then determined. Macrophyte patch areal coverage per 500 m segment was divided by the total area of the segment to get the percent cover per 500 m segment. The average biomass of each 500 m segment was obtained using the

patch area, total segment area and the density estimate for each patch within the segment. The density estimate of each patch was converted to an average patch biomass (g/m^2), based on the harvesting mentioned previously. Density estimate 1 (sparse) became 80 g/m^2, density estimate 2 (moderate) became 215 g/m^2 and density estimate 3 (dense) became 458 g/m^2. The average areal biomass of each patch (g/m^2) was multiplied by the total area of the patch to give the total patch biomass (kg), then patches were added together to get the total biomass (kg) per 500 m segment (fig. 2.2). Then the total segment biomass was divided by the total river bed area of the segment to get the areal biomass for the segment (g/m^2). This areal biomass per segment is distinguished from average patch biomass as it is not the density of a particular macrophyte patch that would be sampled by a quadrat, as is sometimes measured in other macrophyte studies, but it is the average macrophyte density for the entire streambed of a particular segment. The segment method thus takes into account both the patch density and the percent cover and gives a measure of the biomass that removes some variation in biomass estimates due to heterogeneity of macrophyte patch size and distribution. This method is also robust for reaches that differ in their patchiness because, ideally, all patches are mapped and contribute to the reach biomass estimate.

For the second method, the transect method, macrophyte maps and associated biomass data were sampled digitally using transects, which in this study consisted of accounting for the biomass present across a line drawn over a length of river. Biomass for each transect was calculated using % cover, their density and transect length (river width) using GIS tools. Average depth for each transect was also taken, and transects were drawn every 250. Transects were only taken from maps generated from surveys, not directly in the field. Though this is not exactly how traditional transect methods are applied in the field, whereby biomass is sampled along a transect using a quadrat to gather biomass, the transect method employed here samples the patchiness of macrophytes in a discrete manner, similarly to the traditional transect method.

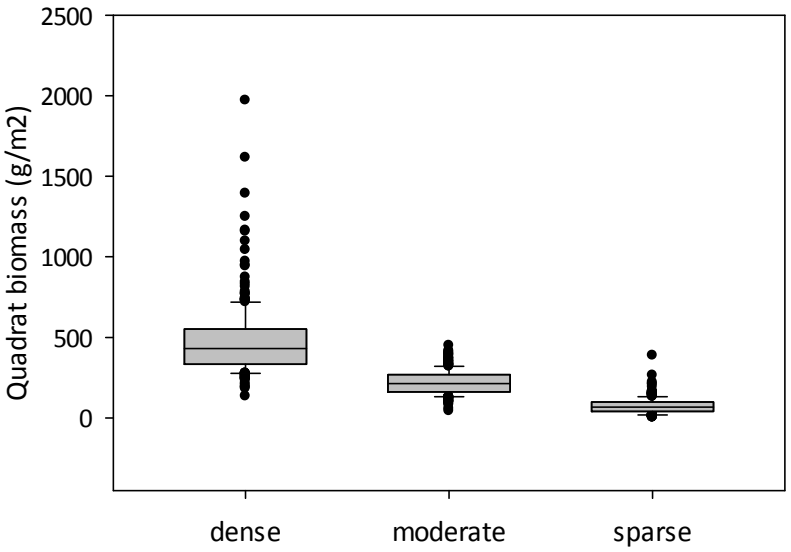

Figure 2.2 Biomass from each density category from pooled collections, 1999-2009, which form the basis for assigning an average patch biomass to each path mapped during macrophyte mapping survies. Boxes represent the interquartile range, whiskers representing 90th and 10th percentile, dots representing outliers.

Areal biomass for each of the survey reaches was summarized and compared. Areal reach biomass (g/m^2) was determined in a similar way as areal segment biomass (g/m^2), instead of adding up the total patch biomass (kg) in each segment and dividing by the segment area, the total of all patch biomass was obtained for the entire reach, then divided by the total reach area. Reach total biomass, reach percent cover, average segment biomass, average patch biomass, average patch size and number of patches in each reach were compared to quantify the influence of the WWTPs on macrophyte biomass and patch dynamics. Within-reach variability in areal biomass was compared among reaches, and the two methods for sampling the survey data, the segment and the transect methods were also compared. Depth and width were examined as possible predictors of within-reach macrophyte biomass using linear regression analysis with width and depth as independent variables, and t-tests were used to examine differences in width and average depth among transects with and without biomass patches. To test for the effect of shading by river-bank vegetation, a shading factor was calculated for each segment based on the orientation of the reach at that location. Because the sun is in the southern sky during the early growing season, we predict that rivers could be

oriented such that some sections receive more sunlight over the day than others. Rivers oriented in the N/S direction would have shading during sunrise and sunset, but be fully exposed during the day. Rivers with E/W orientation may be shaded or not, depending on the height of the bank vegetation. Therefore we calculated a shading factor to look for an effect of river orientation on macrophyte biomass. A shading factor of 1 was assigned for N-S direction as it would receive the least amount of shade, 5 for E-W, with 2, 3 and 4 for headings in between. Late growing season exposure may differ in each of these headings however, we would predict the early growing season exposure to have a bigger effect, because the early season occurs around the summer solstice when there are more daylight hours and because this period is dominated by rapid growth, while late season, at and after peak biomass, is characterized by slower growth and senescence. The calculated shade factor was then related to segment and transect average areal biomass as an independent variable in a linear regression analysis.

Macrophytes were harvested from these reaches for tissue N and P concentration analysis during the 2009 survey to assess the occurrence of nutrient limitation. Macrophyte tissue P was sampled again in the Waterloo and Kitchener reaches on July 15 and 16, 2010, to look for an effect of current velocity on the P content of macrophytes. In both 2009 and 2010, Waterloo and Kitchener upstream and downstream reaches were sampled with a focus on the effect of the WWTP on tissue nutrient concentrations. In the 2010 survey, macrophytes were collected from the reach between the downstream end of the Waterloo reach and the start of Kitchener upstream reach, extending the length of each of those sub-reaches. Because there is only an arbitrary distinction between the downstream of the Waterloo reach and the beginning of the upstream Kitchener reach, for the 2010 data we set the boundary between the Waterloo downstream and the Kitchener upstream to be exactly half way between the Waterloo WWTP and the Kitchener WWTP. The two WWTPs are 20.5 km apart, thus the boundary between the Waterloo reach and the Kitchener reach was 10.25 km downstream of the Waterloo WWTP.

Sites for harvesting plant tissue for nutrient concentration analysis were chosen within each reach, approximately 1 km apart, where a large and representative patch of macrophytes was growing. There were 4 to 5 suitable sites within each reach with proximity to the WWTPs, resulting in a total of 17 sites in 2009, and 15 in 2010, within the Waterloo and Kitchener reaches combined. In 2010, locations within sites were selected based on current velocity;

macrophytes from a “high velocity” patch and a “low velocity” patch were sampled, based on a visual assessment of whether the current was faster than approximately 0.5 m/s or slower than 0.2 m/s. The current velocity was assessed visually with a stopwatch; the gap between “high velocity” and “low velocity” was to ensure no overlap between categories and accidental mischaracterization. In 2009, no attempt was made to characterize current velocity. After patches were chosen, 3-4 macrophytes were cropped by taking the top 10 to 15 cm of actively growing shoots, rinsed with river water, and stored in plastic bags in a cooler on ice. Upon return to the lab macrophyte samples were rinsed thoroughly by vigorously shaking three times with DI in the collection bag to remove biofilm, epiphytes, invertebrates and other material. After rinsing, macrophytes that were not free of debris were cleaned manually by hand to remove any remaining invertebrates and invertebrate housing (such as black fly pupal cases) and detritus. Macrophytes sampled in 2009 and 2010 were separated into species and were additionally separated into two tissue types in 2010: leaves and stems. Only a small quantity of root tissue was collected and was insufficient for analysis. Processing was done within two days following sample collection. Macrophytes waiting for processing were kept in open bags in a refrigerator, and were processed in random order to reduce the possible effect of the two day storage on results. After cleaning, macrophytes were dried at 65 C for several days. Dried plant material destined for N analysis was weighed into tin cups and sent to the Environmental Isotope Lab where N content was determined. P analysis was conducted on dried macrophyte tissue using a modified ascorbic acid- phosphomolybdate spectrophotometric method which involved reducing the plant material to ash by combustion at 500C to remove organic C and release organic bound P, followed by oxidation by acid digestion using a 2.5% persulphate solution, then assayed as described by Murphy and Riley (1962). Additional macrophyte tissue data was obtained from an earlier survey of the Grand River from headwaters to mouth, conducted by our research group, where macrophytes were collected and tissue P concentrations were measured by the previously described methods.

2.3 Results

Macrophyte biomass responded downstream of WWTP outfalls, as indicated by an increase in areal biomass from upstream reaches to downstream reaches (figs. 2.3, 2.4, 2.5, 2.6; 2.7), whether determined by the transect or the segment method and in both 2007 and 2009. The increase in biomass in sub-reashes downstream of WWTPs is evident when viewing the maps generated from the spatial survey (figs. 2.3, 2.4) as well as in the numerical biomass data extracted from them. Both downstream sub-reaches had higher biomass than the WMR reach (table 2.1) as determined by both segment and transect methods. Immediately downstream of the WWTPs, macrophyte biomass appears to be inhibited (figs. 2.3, 2.4, 2.6; 2.7) for 1 or 2 km, and this inhibitory effect was most pronounced in the Kitchener reach in 2007. When comparing reaches, the inhibitory effect had an impact on the t-test, resulting in no difference between Kitchener up and downstream sub-reaches in 2007. Only when the 2 km stretch of river where macrophyte biomass appeared to have been inhibited by the WWTP was removed from analysis did the Kitchener downstream reach have significantly more biomass than upstream sub-reach and the upstream WMR reach. There were no consistent up/down stream patterns when percent cover was used (table 2.1). In 2007, the Waterloo reach had higher percent cover in the downstream sub-reach, but Kitchener did not. In 2009, Kitchener's downstream sub-reach had higher percent cover than upstream, but the Waterloo reach did not. The average patch biomass differed up and downstream. Similarly to percent cover, patch size and the number of patches showed no consistent differences between upstream and downstream of the WWTPs. Patch size was larger downstream of the Kitchener WWTP in both 2007 and 2009, but it was not different downstream of the Waterloo WWTP in 2007 or in 2009 (Mann-Whitney test, $P > 0.05$). The numbers of patches also had no discernable upstream-downstream pattern. The reach with the most patches was the WMR reach, while the reach with the fewest patches was the Kitchener upstream sub-reach.

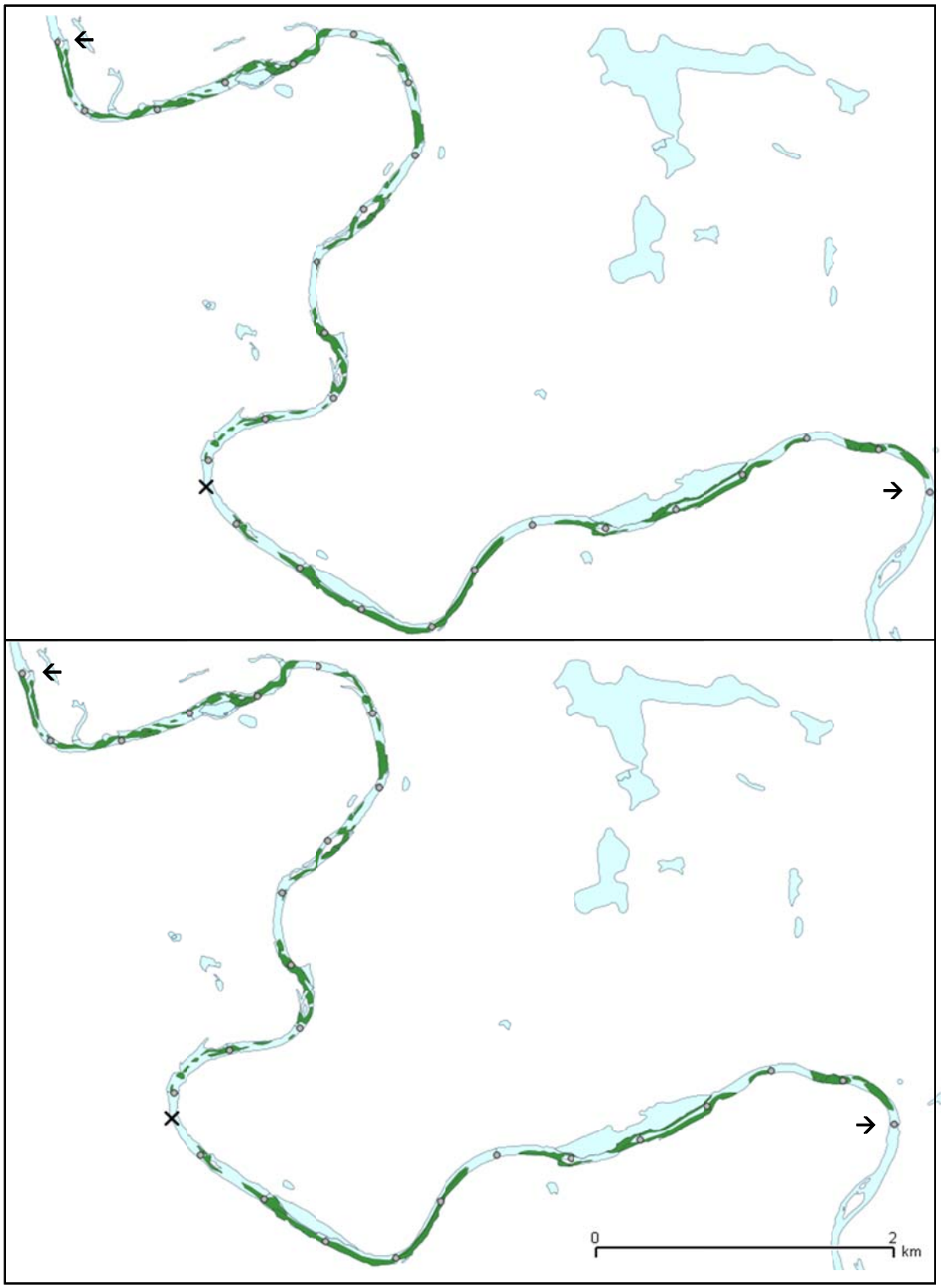

Figure 2.3 Maps of the 2007 (A) and 2009 (B) macrophyte mapping survey of the Waterloo reach. Patches appear in green, WWTPs represented as (**X**) and segments division (•). Start and end of reaches marked by arrows (→), and always land on a segment border

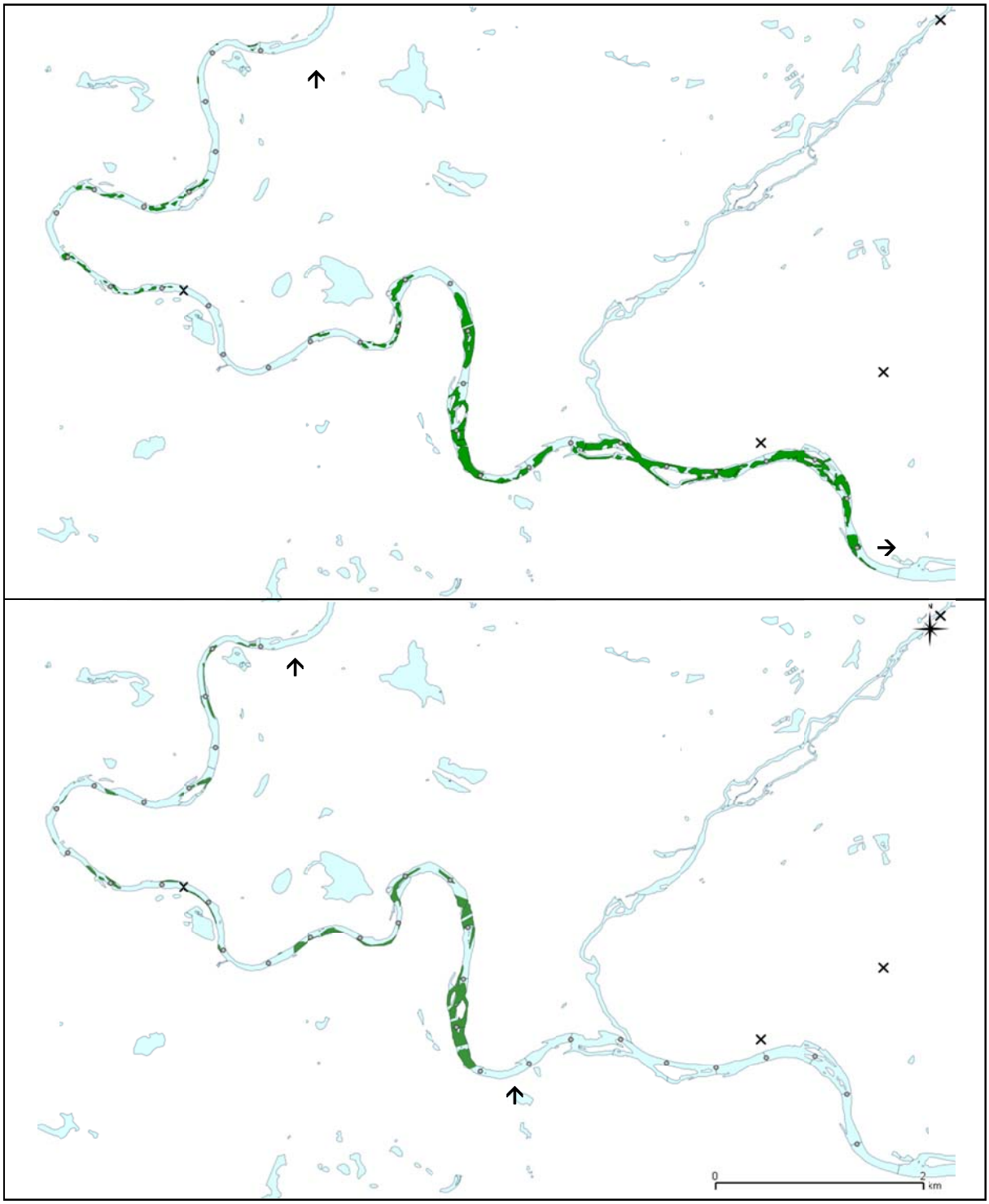

Figure 2.4 Maps of the 2007 (A) and 2009 (B) macrophyte mapping survey of the Kitchener reach. Patches appear in green, WWTPs represented as (**X**) and segment division (•).Start and end of reaches marked by arrows (↑), and always land on a segment division.

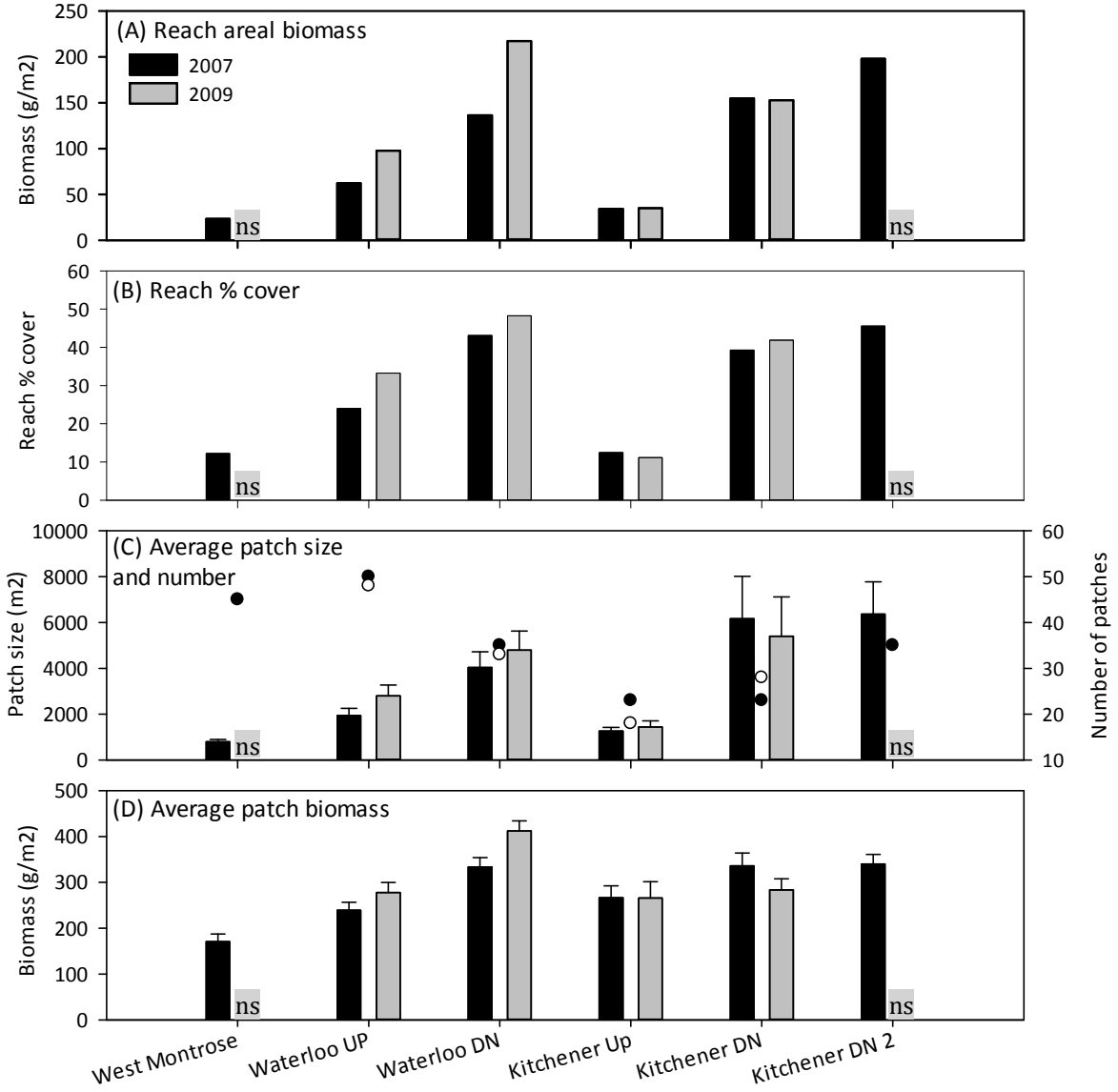

Figure 2.5 Macrophyte patches and biomass by reach. Average reach biomass, (A) which incorporates both patch size and patch density; % cover (B); Average patch size (bars) and number of patches (circles) in each reach (C). Error bars show standard error of the average patch size; Average patch biomass (D) of patches in each reach. Error bars show standard error of the mean density of each patch, which was assigned to each patch based on the density estimate. Reaches marked as "ns" were not sampled (West Montrose and Kitchener DN 2 in 2009).

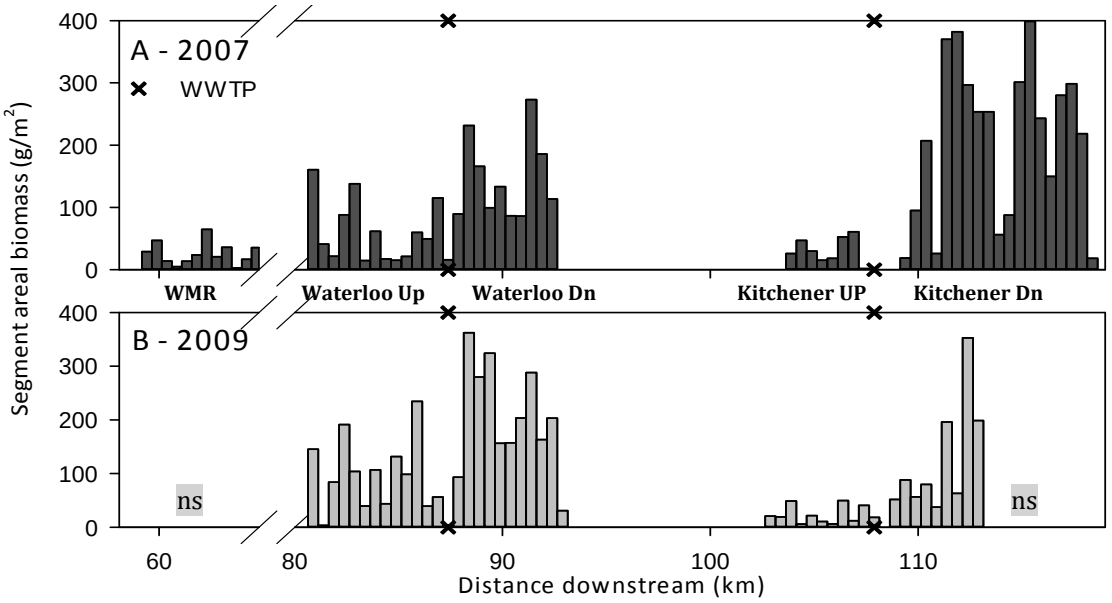

Figure 2.6 Biomass of macrophytes in the Grand River from August 2007 (A) and August 2009 surveys (B). Biomass represented as average segment biomass (g/m^2) derived from the segment method. The sub-reach from 113.4 km to 118.4 km is the Kitchener Downstream 2 reach, and was only sampled in 2007, not 2009, as indicated by "ns". Segments are 500 m in length

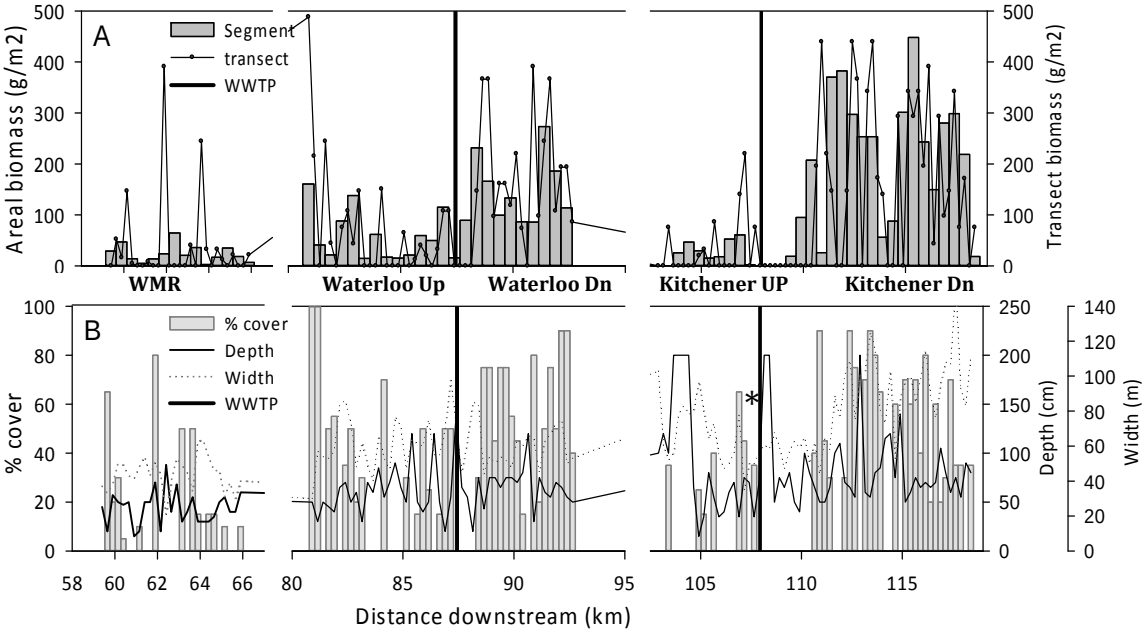

Figure 2.7 Comparison of areal biomass estimates produced by transect and segment method from 2007 mapping data, (A) with distance downstream in all three reaches; the percent cover, width and depth (B) with distance downstream as derived from the 2007 transect method. The locations of the WWTPs (|) and a small reservoir for drinking water intake (*) are indicated on the graph.

Table 2.1 Differences in macrophyte biomass upstream and downstream sub-reaches of the Kitchener, Waterloo and West Montrose (WMR) reaches from surveys conducted in 2007 and 2009. Student's t-test was performed on all segments from each reach when assumptions of normality and equal variance were not violated. Mann-Whitney test was used when the compared reaches did not have equal variances.(*) Indicates the P value obtained when the first 2 km downstream of the Kitchener WWTP were removed

	Waterloo Up/Down	**Kitchener Up/Down**	**WMR-Waterloo Down**	**WMR- Kitchener Down**
2007 segment biomass	t-test; *P=0.009*	Mann-Whitney; P=0.767 **P=0.043*	Mann-Whitney; *P=0.001*	Mann-Whitney; P=0.455 **P=0.004*
2007 transect biomass	Mann-Whitney; *P= 0.010*	Mann-Whitney; P=0.244 **P=0.015*	Mann-Whitney; *P=<0.001*	Mann-Whitney; P=0.684 **P=0.048*
2009 segment biomass	t-test; *P= 0.005*	Mann-Whitney; *P=0.008*	N/A	N/A
2007 % cover	Mann-Whitney; *P= 0.049*	Mann-Whitney; P=0.204	Mann-Whitney; *P=0.001*	Mann-Whitney; P=0.073
2009 % cover	Mann-Whitney; P= 0.132	Mann-Whitney; *P= 0.011*	N/A	N/A
2007 patch biomass	Mann-Whitney; *P=0.009*	Mann-Whitney; P=0.090 **P=0.021*	Mann-Whitney; *P=<0.001*	Mann-Whitney; *P=0.005*
2009 patch biomass	Mann-Whitney; *P=0.013*	Mann-Whitney; *P=0.025*	N/A	N/A

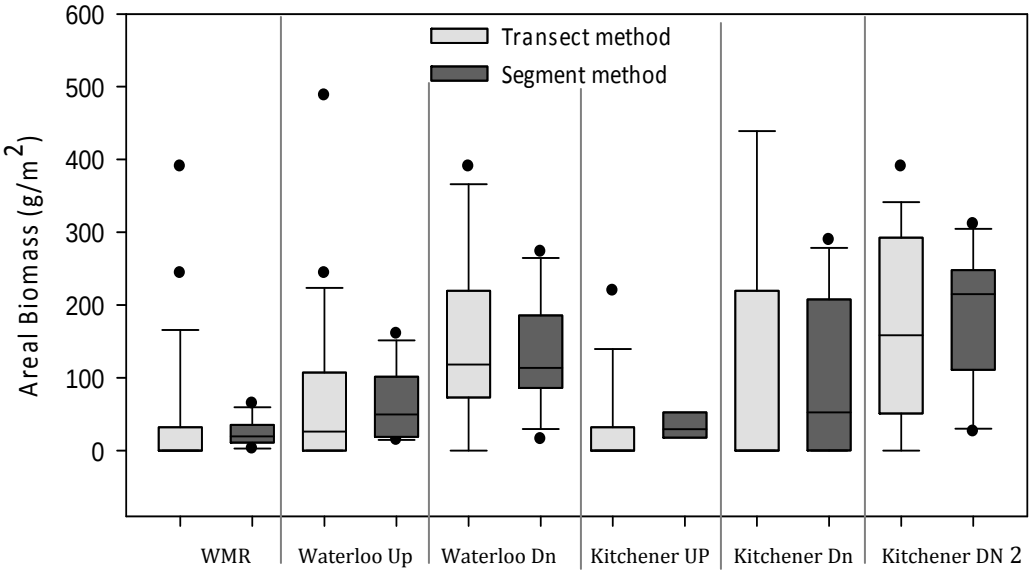

Figure 2.8 Comparison of segment and transect methods to determine reach biomass. Transects and segments are methods used for interpreting map data. Transects and segments give similar average reach biomass in most cases, but transects have higher variation, even when sampled twice as frequently (every 250 m) than segments (every 500 m)

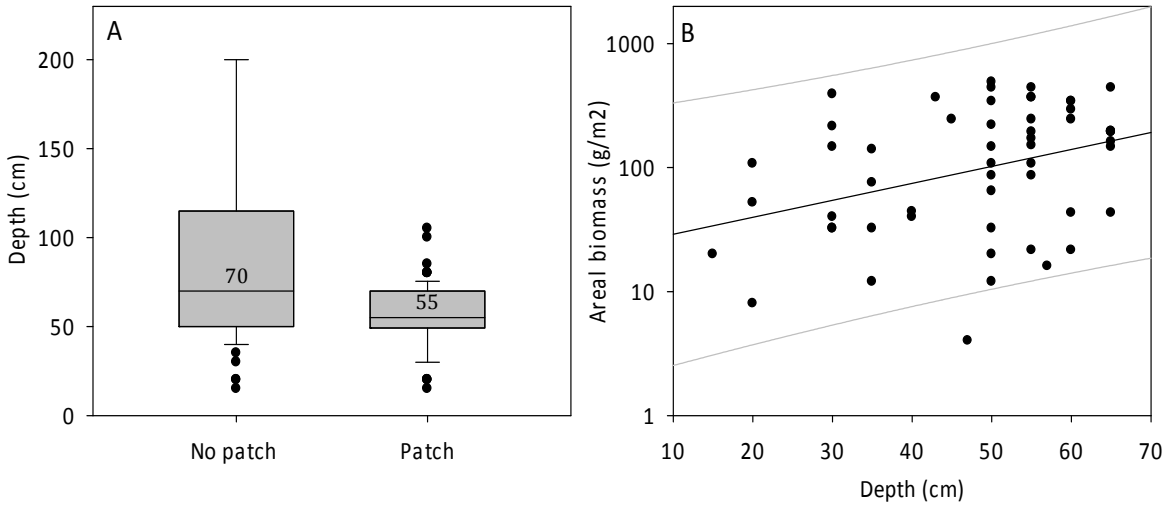

Figure 2.9 Effect of river depth on the presence of macrophyte patches. The depth where patches are located (A) is lower (55 cm on average) than locations where patches were absent (70 cm on average). Boxes represent the interquartile range; whiskers represent the the 10th and 90th percentile; dots are outliers. For patches located in areas less than 60 m of depth, the biomass is weakly but positively related to depth (B).

Reaches displayed high spatial variation in areal biomass (fig. 2.8) when they were analyzed using both the segment and the transect method. Although the segment and transect method both produced a similar average areal biomass for most reaches, except Kitchener downstream 2, the variation in macrophyte biomass within reaches was much higher for the transect method. This point is especially emphasized because the number of transects taken was double the number of segments, and as standard error is equal to the standard deviation over square root of *n*, the higher *n* in of transect method should decrease the variation.

The within reach variation was partially explained by depth, but not width. The depth for which no patches were found was higher on average than the depth where patches were located (students t-test of depths, P=0.001; fig. 2.9 A). The mean depth for transects through locations where there were no patches was 70 cm, while the mean depth for transects through macrophyte patches was 55 cm. For patches located at depths less than 60 cm, areal macrophyte biomass (as determined by the transect method, in 2007) was weakly positively related to depth (fig. 2.9 B; R^2=0.129, P=0.018). This suggests that there is non-linear (inverse parabolic or discontinuous) relationship between biomass and depth, where deeper water produces higher biomass, to a certain depth (somewhere between 55 and 70 cm) beyond which biomass is inhibited. There was no major difference in macrophyte biomass limiting depths between upstream and downstream of the Waterloo and Kitchener reaches (table 2.2), West Montrose had fewer transects that were deep enough to limit biomass, while the downstream sub-reaches had only 2 to 4 more transects deeper than 55 cm, but the same number of transects that were deeper than 70 cm. Width was not related to the presence or macrophyte patches (P=0.151; student's t-test), nor was width related the quantity of biomass found in the river (linear regression analysis, R^2= 0.0353, P=0.099). This analysis was not conducted for segment method data, as the average depth for an entire segment could not be accurately determined. The shade factor was not significantly related to macrophyte biomass, meaning that river orientation (N-S versus E-W) did not influence within-reach variation in late summer macrophyte biomass.

If macrophyte biomass is elevated in response to increased nutrient loading from WWTPs, it is expected that macrophytes are nutrient limited upstream of WWTPs. However, there is little indication that macrophytes collected from these reaches, in August of 2009 or July of 2010

were limited by N or P (figs. 2.10; 2.11 A, B, C) upstream of the WWTPs. Macrophyte tissue N and P concentrations on August 27 and 28, 2009 were all higher than the critical growth limiting thresholds for submersed aquatic described by Demars and Edwards, (2007), with the exception of one value found upstream of the Kitchener WWTP. Macrophyte N and P values in general were higher than the range of values found by Demars and Edwards, (2007), and higher than those found in a meta-analysis of wetland macrophytes tissue nutrient concentrations (Koerselman and Meuleman, (1996)), which proposed that macrophyte nutrient limitation could be determined by an N:P ratio specific to vascular macrophytes. By the latter method, macrophytes in the Grand River in August of 2009 might be interpreted as being mainly N limited, or because of the exceedingly high N and P values, more likely to become N limited if N and P reserves were to be drawn down. Macrophytes collected on July 14 and 15, 2010, had generally lower tissue P concentration than macrophytes collected in 2009, but not all upstream values were below the critical tissue P concentration. Some values were below this level, but they came from specimens found both up and downstream of the WWTPs in Kitchener and Waterloo reaches.

Table 2.2 Biomass inhibiting depths in each reach. Number of transects which have an average depth greater than 55 cm, the average depth that patches are found and 70 cm, the average depth where there is an absence of patches.

Reach	# of transects with average depth > 55	# of transects with average depth > 70
WMR	5	1
Waterloo Upstream	11	4
Waterloo Downstream	15	9
Kitchener Upstream	13	9
Kitchener Downstream	15	9

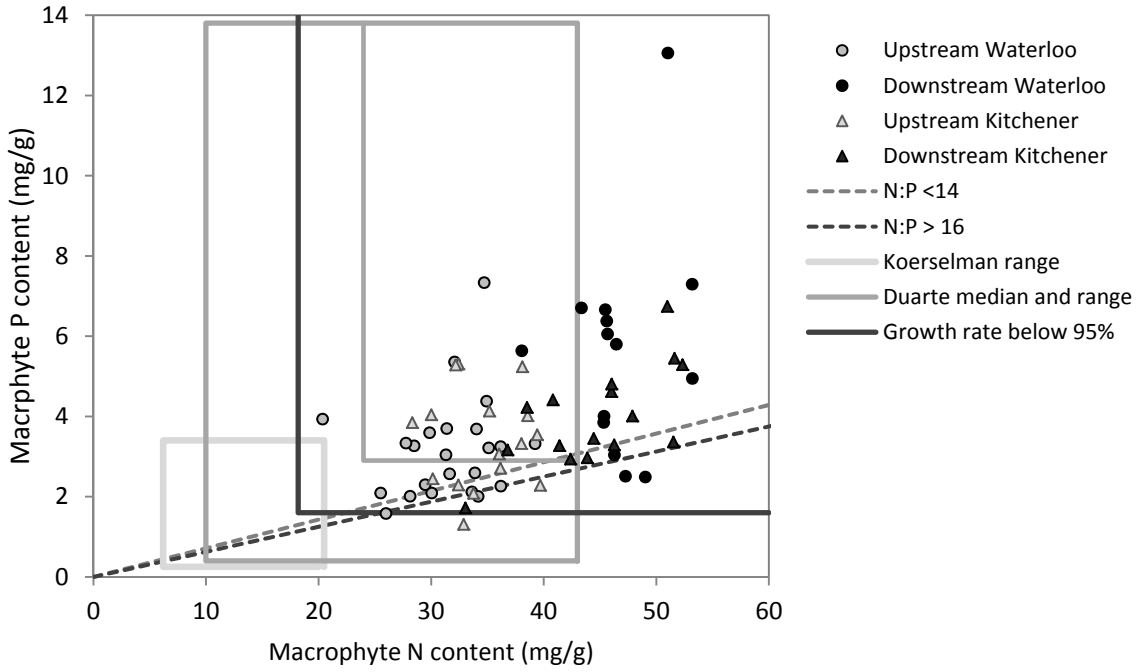

Figure 2.10 Macrophyte N and P content for samples collected in 2009. The lines on the graph indicate conclusions of previous work. The dotted lines represent the N:P mass ratios for nutrient limitation where plants found above the N:P < 14 line are N limited while below the N:P>16 line are P limited (Koerselman and Meuleman, 1996). The "Koerselman range" box at the bottom left indicates the range of values found by Koerselman and Meuleman (1996).The "Duarte range" is the range of values found in a review of aquatic plant nutrient concentrations (Duarte, 1992). The threshold boxes indicate the critical growth rate threshold below which growth rates are 95% of their maximum (Gerloff, 1975; Demars and Edwards, 2007). Most all the data in Koerselman and Meuleman should be limited by either N or P, while plants in this study should be limited by neither because they are above the critical limit. All data are within the range previously found for P, but much higher than previously found for N.

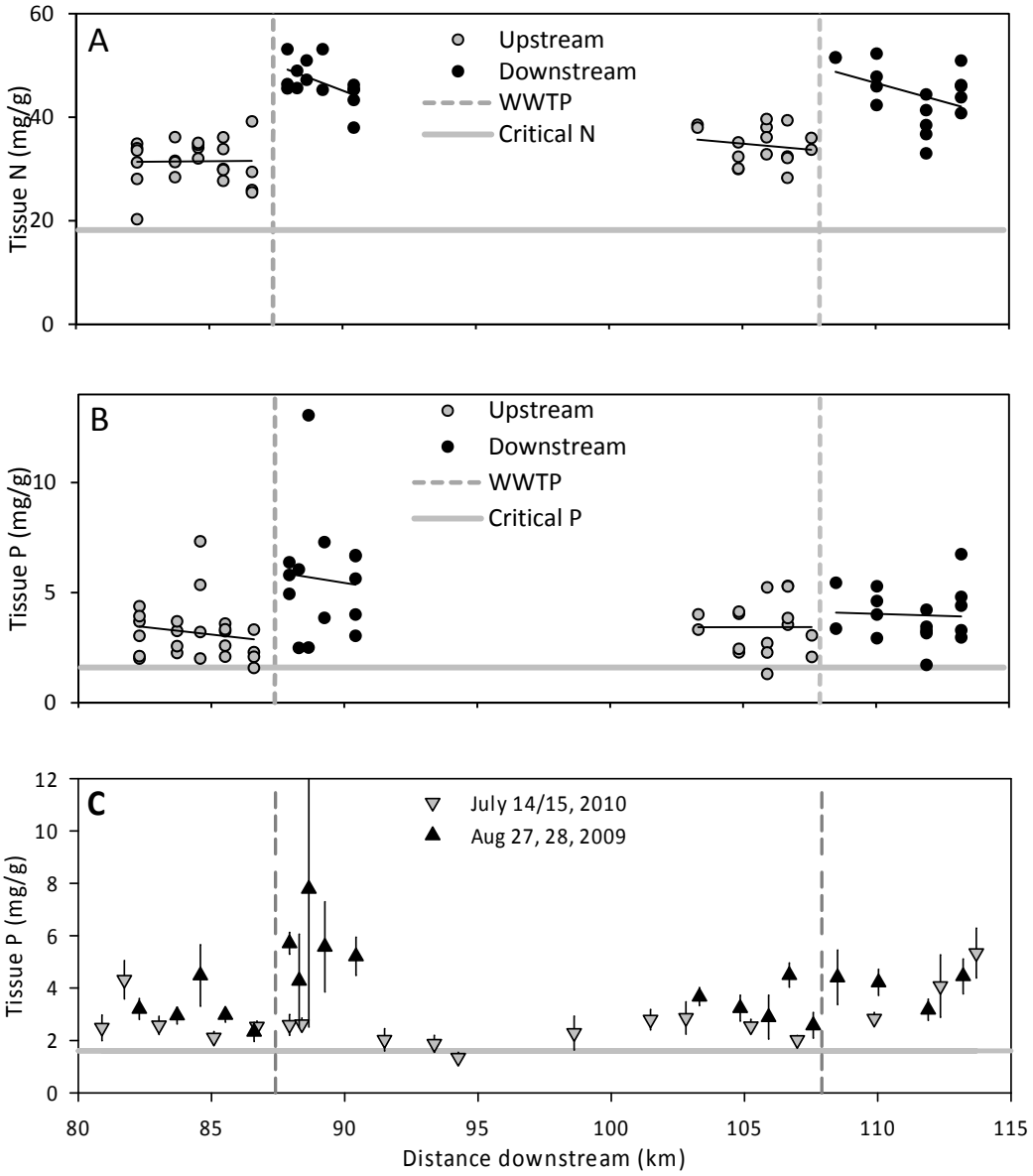

Figure 2.11 N (A) and P (B) content in macrophytes from 2009, and P data found in both 2009 and 2010 (C), up and downstream of two WWTPs. N content is significantly different up and downstream of both WWTPs and the relationship of decreasing N content with distance downstream of the WWTP is significant only in plants downstream of the Waterloo WWTP. P content is only significantly higher downstream of the Waterloo WWTP (using a one-way ANOVA with a Shapiro-Wilk normality test). P content from July 2010 is lower on average than August 2009 in the up and downstream segments. Critical N and P tissue concentrations, from Demars and Edwards (2007)), are tissue nutrient concentrations below which growth rates are below 95% of the maximum.

Macrophytes were not below the critical threshold determined in laboratory studies for submerged aquatic macrophytes (Demars and Edwards (2007); figs. 2.10; 2.11). However there were differences in the tissue nutrient concentration between up and downstream of WWTPs. Macrophytes had higher N content downstream of both Waterloo and Kitchener WWTP (Students t-test, $P<0.001$ for both Waterloo and Kitchener; fig. 2.11 A), with N content increasing about 15 mg/g over the 1 km distance spanning the location of the WWTP for both locations, and declining significantly downstream with distance from the plant (linear regression with distance, both downstream reaches combined, $P>0.001$). By the Kitchener upstream reach, the tissue N concentration was closer to Waterloo upstream values, but was still higher (student's t-test, $P=0.022$). Macrophyte P content was higher downstream of the Waterloo WWTP (student's t-test, $P<0.001$) (fig 2.11 B), but not downstream of the Kitchener WWTP (student's t-test, $P=0.211$). Unlike tissue N, the decline in macrophyte tissue P concentration with distance from the WWTP was not significant (linear regression with distance, both downstream reaches combined, $P = 0.190$).

Samples collected for macrophyte tissue P concentration on July 15 and 16, 2010, showed that macrophytes growing in fast (>0.5 m/s) locations in the river had significantly less P content than those growing in slow (<0.2 m/s) locations (fig. 2.12). This pattern did not change spatially; fast patches were consistently lower in tissue P concentration in both upstream and downstream locations. Tissue P concentration varied among taxonomic groups (fig. 2.13 A), with *Myriophyllum spicatum* being lower in P content in than the 3 other selected species in the fast patches, and lower only than *Stuckenia pectinata*, formerly *Potamogeton pectinatus* (Crow and Hellquist, 2000; Lindqvist et al., 2006), in the slow patches while *S. pectinata* had the highest P concentrations only in slow patches (ANOVA on ranks, Dunn's Pairwise test on differences among groups with $P < 0.05$). There were differences in P content between stems and leaves of *M. spicatum* (fig. 2.13 B), with stems having higher tissue P concentration than leaves, but only in fast sections of river (signed-rank test $P<0.05$). Tissue P concentration from macrophytes collected July 14 and 15, 2010 was lower than for samples taken in August 2009 at the same distance downstream, except for the furthest downstream locations within the Kitchener downstream sub-reach (fig. 2.11 C). The July 2010 tissue concentrations did not show the large P increase downstream of the Waterloo WWTP that the August 2009 samples did.

Macrophyte tissue P concentrations were highly variable when viewed over the spatial scale of the whole Grand River (fig. 2.14). Tissue P concentrations from the more spatially resolved surveys in 2009 and 2010 had did not have a greater range of variability within the 30 km of surveyed mid-reaches than across the entire length river, as surveyed on September 5th, 2007. The values immediately downstream of the Kitchener WWTP in the September 5th, 2007 are higher than those found at the same location on August 27, 2009.

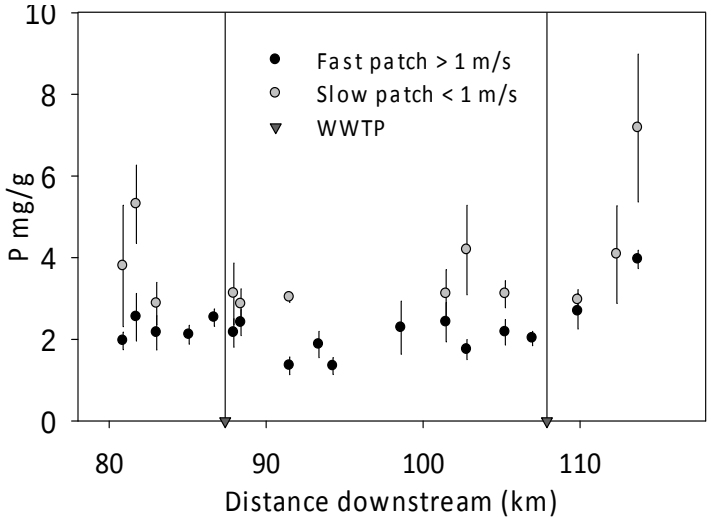

Figure 2.12 Tissue P concentration of macrophyte separated by relative current velocity, slow and fast, at the location of the patch. WWPTS in the Waterloo and Kitchener reach indicated by (|), as well as a (▼). Points represent macrophyte tissue P average of all macrophytes found in the patch, lines represent standard error.

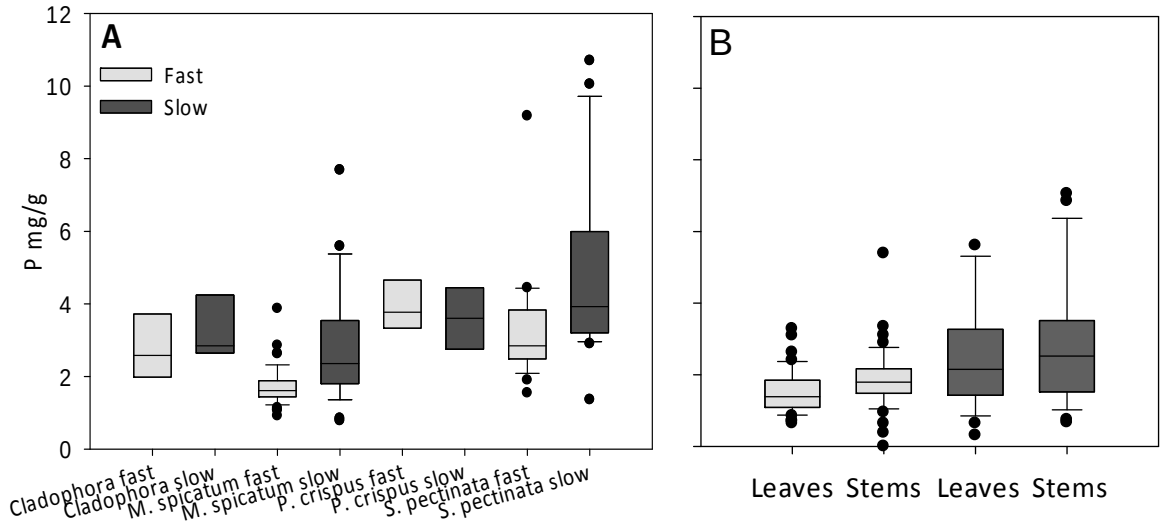

Figure 2.13 Difference in tissue P concentration among species (A) and for two different types of tissue for the selected species *M. spicatum* (B).Data are from macrophytes sampled July 14 and 15, 2010. *M. spicatum* has lower P than *Cladophora* spp., *P. crispus*, and *S. pectinta* in fast patches, while among the slow growing patches *M. spicatum* is only lower than *S. pectinata* (ANOVA on ranks, Dunn's Pairwise test on differences among groups with $P < 0.05$). Only plants growing in fast sections of the river have differences in leaves and stems (signed-rank test $P<0.05$).

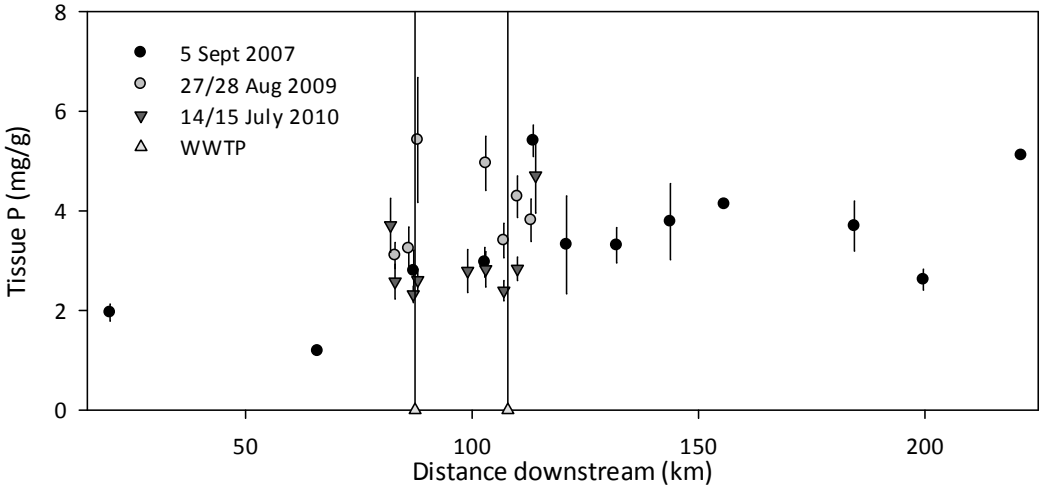

Figure 2.14 Macrophyte tissue P concentration in 3 different surveys of the Grand River. The 2009 and 2010 surveys were conducted at smaller scale, covering 20 and 30 km of river, respectively, while the 2007 was conducted over the distance of the entire river, starting at just downstream of PWQMN site. Points are averages of all macrophytes collected at the site, bars are standard Error. WWTPs located on the graph at the (△)

2.4 Discussion

2.4.1 Biomass relationship to WWTPs

This study demonstrates that macrophyte biomass, determined by both methods employed, is greater downstream of both the Kitchener and the Waterloo WWTPs of the Grand River. Although there are 29 WWTPs discharging effluent into the river in the Grand River watershed, the watershed land-use is highly agricultural, and the two WWTPs in question are only separated by 20 km of river distance, so the impact of a single point source on the biomass of the macrophyte community can be detected.

Other work has demonstrated that increased submersed macrophyte biomass is associated with proximity to WWTP effluent discharge sites, providing direct evidence of the influence of the WWTP, however, only circumstantial evidence indicates that nutrients are the cause. It is well documented in chapter 1, that both the Waterloo WWTP and the Kitchener WWTP are sources for both dissolved inorganic N and P, while submersed macrophytes could be limited by either N or P (Carr et al., 2003; Dodds, 2006). The Kitchener downstream sub-reach can have higher turbidity on some occasions than the upstream sub-reach (Mark Anderson, personal communication), but this would not lead to an increase in biomass; instead, the opposite effect would be expected. Similarly, there were no differences in the number of transects running through biomass-limiting depths between up and downstream of the WWTPS that might explain why reach biomass is greater downstream. Although substrate type can influence the growth of macrophytes (Barko and Smart, 1981), we did not quantify substrate in our reach scale surveys. Although substrate type may vary within reaches, it is not expected to differ among reaches. Previous efforts to demonstrate the relationship between macrophyte biomass and N and P loading exist; Sosiak (2002) quantified a decrease in the river macrophyte biomass downstream of the Calgary WWTP when upgrades in the 1980s occurred to improve P and, later, N removal. Both N and P reductions from effluent resulted in a reduction in macrophyte biomass downstream. The data on macrophyte biomass they provide in their work show that not only was the peak biomass downstream of the WWTP influenced by nutrients, but so was the distance downstream to which macrophyte biomass was elevated above a background level. Before WWTP upgrades, macrophyte biomass was elevated a distance downstream of the WWTP as far as they surveyed, (approximately 51 km) downstream and, after P and N

upgrades, the distance downstream to which biomass was elevated above upstream values decreased to approximately 45 km. There are studies that have failed to find a response of macrophytes to nutrient removal from WWTPs (Chambers, 1993; Terrell and Canfield, 1996 as cited by Sosiak, 2002), but it is possible that nutrient removal in these cases was not sufficient to produce nutrient limitation in the submersed macrophytes, thus no reduction in biomass occurred.

In this survey of Grand River macrophytes, the reach included in the survey was not large enough to capture the entire downstream effect of WWTPs on biomass, as this distance was not known *a priori*. However, the Kitchener upstream sub-reach, located about 15 km downstream the Waterloo WWTP, was not different than the biomass at the WMR reach in 2007, and the Waterloo upstream sub-reach, in both 2007 and 2009, giving an indication of the distance downstream of the Waterloo WWTP that biomass remains elevated. The biomass downstream the WWTP was elevated, and then declined to upstream levels by 15 km downstream.

It might be useful to able to characterize the macrophyte biomass response to nutrient point source loading as a total the crop "grown" by the nutrient load of a nutrient point source. This would entail taking the integral of macrophyte biomass over the distance downstream it is elevated above background levels, a value which would present the total biomass yield. With several load-yield pairs, a the relationship between nutrient loading and biomass yield could be constructed and may indicate the biomass response to nutrient loading that is tailored for rivers and analogous to the spring P load/Chl a model for lakes (as in Dillon and Rigler, 1974). The distance downstream that macrophyte biomass is elevated from some baseline level might also be a useful method to assess the areal extent of point source impact, though the it may vary by river with differences in discharge, substrate and other factors, and it could be a useful parameter because it would require fewer field and laboratory resources to obtain.

Macrophyte biomass was greater in the downstream sections of the Waterloo and Kitchener reaches, however immediately downstream of the WWTPs macrophyte biomass was greatly reduced, even lower than upstream levels. In 2007, immediately downstream of the Kitchener WWTP for 2 km the biomass was zero, a condition not found anywhere else in the surveyed portions of the river. This is perhaps not unexpected, as unmixed wastewater effluent is likely toxic with high levels of NH_4^+, Cl_2 or chlorinated compounds, as well as high turbidity. These

results might also be explained in the context of the conceptual model based on Grime's theory of succession as proposed by Hilton et al., (2006), where macrophyte growth might be inhibited under conditions of high nutrient stress. Under extremely high water column nutrient concentrations, as would be found directly downstream of a WWTP outflow, macrophytes would be outcompeted by periphyton that would grow rapidly on the shoot and leaf surfaces of the plants, producing a thick layer and cause them to be light limited. We did see evidence of a thick biofilm in the immediate downstream sections of the WWTP, along with abundant moss which we did not quantify (though some authors consider aquatic moss to be macrophytes). However, 2 km downstream of the WWTP the plume is not well mixed into the full channel, so complete inhibition across the entire river by the effluent plume is still difficult to explain.

Depth explains some of the variation in macrophyte biomass within reaches, but biomass is only weakly related to depth. Higher biomass may not be found in deeper sections of a reach due to light limitation with depth (Barko and Smart, 1981; Rooney and Kalff, 2000) especially during times of high turbidity such as after rain events. However the biomass relationship with depth is only significant for locations that are 60 cm or less, and driven mainly by values at the low end of the range indicating macrophytes are limited by depth at only the most shallow depths, less than 20 cm deep based on data collected in this study. At depths less than 20 cm, macrophytes are not likely inhibited by light availability, but rather by scouring and drag produced by higher velocities often associated with shallow riffle areas. There is substantial evidence to suggest that current velocity is one of the main factors influencing the performance of macrophytes in streams, once communities have been established (Chambers et al., 1991; Biggs et al., 2005; Franklin et al., 2008). The positive relationship between biomass and depth shows that slower current velocities associated with slightly deeper regions promote macrophyte growth. Other studies of the effect of current velocity on various aspects of macrophyte communities, such as abundance, diversity and biomass support these findings. Chambers et al. (1991) demonstrated that increased current velocity in the Bow River, Alberta, was related to decreased macrophyte biomass between 0.01 and 1 m/s. Riis and Biggs (2003) showed that peak macrophyte abundance also occurred with median velocities, from 0.3 to 0.5 m/s. Our results given in Chapter 3 also support these findings, as they show that peak biomass is inversely correlated with spring-time discharge, indicate the inhibitory effect of flow on the growth of macrophytes in the spring and ultimately influencing the summer-time peak biomass.

Consistent high flow velocity over a long period of time means increased drag, and can produce a tensile stress on macrophytes (Biggs et al., 2005), and very high flow events associated with flashy hydrographs could cause sediment disturbance and uprooting events (Riis and Biggs, 2003), which would have an effect on biomass over the growing season. Higher flows may also have an impact on the diffusive boundary layer, and alter the rates of exchange of gas and nutrients (Madsen et al., 2001). In our study, there was an effect of current velocity on tissue P concentration, with macrophytes growing in regions of higher velocity having lower P content than macrophytes growing in slower regions in the same area within the river. This could mean that macrophytes growing in regions of higher current velocity take up fewer nutrients, as macrophytes meet at least some of their nutrient demand through open water uptake, but this explanation is unlikely as macrophytes take up nutrients from roots in the sediment, when sediment reserves are greater (Carignan and Kalff, 1980).The same findings could result from there being more available sediment P to macrophytes growing in slower regions because of enhanced residence time in macrophyte stands leading to higher sediment deposition rates and retention times (Clarke, 2002; Schulz et al., 2003). A third interpretation could be that macrophytes in higher velocities grow faster, and have reduced P content as a result of growth dilution. However the latter explanation is less likely because all other evidence suggests that higher flows impede the growth of submersed macrophytes, and the improvement to nutrient and inorganic carbon uptake rates in macrophytes is only present at current velocities of 0 to about 0.1 m/s, which is lower than our "low velocity" value (Madsen and Søndergaard, 1983; Madsen et al., 2001). Our work indicates that upper and lower thresholds likely exist for both depth and velocity on optimal macrophyte biomass production and tissue nutrient concentration.

The effect of WWTPs on the biomass of macrophytes in two reaches of the Grand River is shown in this study. However, to increase the generality of conclusions regarding the effect of nutrient point sources on the biomass of riverine macrophytes, studies in other river systems need to be conducted. The enriching effect on macrophyte biomass was clear in the Bow River downstream of Calgary in Alberta (Sosiak, 2002), and in the South Saskatchewan River downstream of Saskatoon (Carr et al., 2003), but there are few other studies to compare our results to.

2.4.2 Transect method and segment method compared

There was substantial within-reach variation in biomass, even when attempts to account for this heterogeneity were undertaken. Variation was high among segments, such that segments with very high biomass were immediately adjacent up and downstream to segments with little or no biomass. This can obscure any response if an inappropriate scale or sampling method is used. The difference in the variation in biomass between the segment method and the transect method illustrates this point, especially since *n* is double for the transect method than for the segment method. In using the transect method, the patchiness of the macrophytes is expressed as high variance, as a transect may or may not go through a seemingly arbitrarily located patch of vegetation. The segment method, which integrates biomass over a given spatial scale, accounts for all patches and reduces the sample variance. Therefore, when high heterogeneity is expected to affect the outcome of the study, we suggest employing a sampling method similar to our segment method, which has the ability to account for all patches and remove the patchiness effect from the sample set. The advantages of this method are clearly demonstrated, but there are disadvantages as well. The segment method requires a larger amount of effort than sampling by simple quadrat or transect, and that may require a loss of detail. Because not all patches were sampled directly, the density of most patches was estimated. A 3 level scale of density was used, which is a coarse level of detail. The density levels could easily be increased to produce finer resolution within segments if necessary, with some additional effort in sampling, though it cannot be quantified how doing so would affect variance using data produced in this study. There is also the problem that each patch was assigned one density value and it is likely that patches vary in their density. Because each patch was not sampled directly, a boat-side estimate of species composition was made which results in the loss of representation of uncommon and rare species from the data set, and possible errors in species identification. This type of survey would not be appropriate when detailed information on taxonomic representation and community composition is required. The variation observed in this type of study thus reflects the macrophyte assessment method chosen.

The segment method is very similar to the "rectangles method" by Wright et al., (1981), the recommended macrophyte mapping method among three that they compared. The rectangles method involved reducing the river bed to a grid, and for each box of the grid noting certain macrophyte characteristics such as percent cover, dominant species and density. Although the

segment method used in this study provides only a one-dimensional grid over the length of the river, the method of accounting for all patches, patch density and dominant species provides similar information for each linear segment, and both methods also have the same type of informational disadvantages such as lack of representation of rare species, low density resolution and potential effects of observer bias. While the rectangle method arguably provides better informational resolution than the segment method, it is also much more time consuming and is probably ill-suited to quantifying macrophyte biomass at the reach scale of large rivers. While we covered large river reaches of approximately 10 km in length, Wright et al. (1981) covered stream beds 50 m in length. The segment method also has practical disadvantages as well which should be considered when choosing this method, it requires canoe navigation which can be challenging if there are shallow areas and impoundments, long days and possible fatigue of the survey team. Additionally, the observer bias must be accounted for especially if there are multiple technicians generating hand drawn maps. The segment method is likely to be most advantageous to use when covering a large spatial area, when need for large spatial coverage is worth the trade-off in sampling effort, measurement error and low informational resolution.

Evidence of nutrient limitation

The analysis of tissue nutrients does not indicate that macrophytes are nutrient limited upstream of the WWTPs, based on the critical threshold determined by Demars and Edwards (2007). Therefore it is difficult to make a conclusive argument that nutrients from the WWTP produced the increase in biomass observed. However, there is no other compelling reason for why macrophyte biomass should be elevated downstream.

Our results show that macrophyte tissue N and P concentrations in the August 2009 collections were very high, higher than any other tissue N and P concentrations we are currently aware of, and the macrophytes located in the downstream sub-reaches had the highest N and P content. Tissue N and P concentration in 2009 were higher than those reported for wetland macrophytes (Koerselman and Meuleman, 1996) and for aquatic angiosperms (Duarte, 1992) including those studies in which tissue nutrient concentration of the same species as in this work were used; *M. spicatum* and *S. pectinatus* (Carr and Chambers; 1998; Demars and Edwards, 2007). These results suggest that submersed macrophytes growing in

nutrient rich conditions have a high capacity to absorb and store nutrients, both N and P, through "luxury uptake", which is nutrient uptake in excess of immediate growth or metabolic demands. Luxury uptake of N and P by aquatic macrophyte species has been documented by other researchers (James et al., 2006), and it has been shown that N and P content of macrophytes is a function of nutrient input (Portielje and Roijackers, 1995). The capacity for luxury uptake would make macrophytes effective seasonal sinks for anthropogenic N and P from nutrient point sources, and is the one of the reasons for their use in constructed and treatment wetlands for wastewater nutrient reduction (Bishop and Eighmy, 1989; Sooknah and Wilkie, 2004).

The reason for luxury uptake and storage of N and P could be to allow macrophytes to escape nutrient limitation when N and P are less available in the environment or during times of rapid growth when photosynthesis and growth rates out-pace nutrient uptake. It is possible that the unusually high levels of N and P were a result of storage due to high uptake rates and a slower growth rate, which would allow nutrients to accumulate in plant tissues. Because we sampled at or past the peak in biomass in 2009, macrophyte patches may not have been accumulating biomass as rapidly as earlier in the season and, if uptake rates were unchanged, N and P tissue concentrations would be increasing. Dissolved nutrient concentrations are higher in early spring upstream of the Waterloo WWTP (chapter 1), but the spring peak in nutrients drops off rapidly by May, when macrophytes begin to rapidly accumulate biomass. The implication is that nutrient limitation may only exist during periods of rapid growth, which is usually late spring to early summer when temperature is high and the photoperiod is longest (May and June, for the temperate Southern Ontario region). Therefore, nutrient limitation may only occur in the early season of growth, and not after the biomass peak. Peak biomass, after all, represents not instantaneous conditions but a season's worth of net growth and washout, a sum of conditions over a period of time. Nutrient limitation in the upstream sections of the Grand River in the early season might account for the observed differences in biomass during the late summer biomass peak, just as early season temperature, precipitation and flow seem to influence the later season peak in macrophyte biomass (Chapter 3). Results in this study provide some evidence of this, as the 2010 samples, taken earlier in the growing season (July 14/15, 2010) have lower P than the later season 2009 samples (Aug 27/28, 2009) samples, some which were below the critical threshold for P and could be considered nutrient limited. Samples taken

downstream of the Kitchener WWTP (which corresponds to a location the end of the Kitchener Downstream reach) on September 5th, 2007, had higher P content. The July, 2010, macrophytes did not show the same increase in P downstream of the WWTP as the macrophytes taken in August, 2009, indicating that perhaps less of the P was either taken up or stored.

This work focused on above-ground biomass and tissue nutrient concentration, but the role of roots in macrophyte nutrition and response to increases in nutrient loads is well documented and has been debated for several decades (eg.; Nichols and Keeney, 1976; Carignan and Kalff, 1980; Madsen and Cedarwood.). It is possible that root tissues may be better indicators of general nutrient status in macrophytes, though this has not been demonstrated. Additionally, root uptake gives macrophytes an advantage when nutrient concentrations are low, allowing them to access nutrients in sediment, where concentrations are often higher than in water. Thus, in low nutrient environments, macrophytes accessing sediment nutrients might reach a higher biomass then would be predicted based on open water nutrient concentrations alone. However, our results showed that above-ground biomass was greater downstream of known nutrient point sources, and it is not necessary to invoke the role of roots to explain this outcome. However, as shoot and leaf tissues differed in their P concentration, roots may differ as well, and this might be worth consideration in future investigations.

Based on the results of this work it cannot be firmly demonstrated that nutrients are limiting for growth in the reaches upstream of the Waterloo or Kitchener WWTPs based on the thresholds that I used, however, the evidence suggests macrophyte biomass does respond to point source nutrient loading. Substantial and prolonged limitation in the early growing season could be responsible for the differences between up and downstream of the WWTPs, and sampling late in the season and measuring nutrient concentrations in the newest growth did not capture this. I also showed in this work that other factors can influence macrophyte nutrient content, such as current velocity, tissue type and macrophyte species. Thus, several factors should be considered when using macrophyte tissue nutrient concentrations as a means to assess nutrient limitation. For example, because macrophytes from lower velocity sites have a higher nutrient content, sampling macrophytes only from slow moving sections of river may lead to a significantly higher estimate, which may lead to a mistaken conclusion that nutrient limitation is not occurring in the river. Though in 2009 we did not intentionally select macrophytes for cropping to remove a potential bias from current velocity, we did not select

sites only in slow flowing reaches. Nor did we sample one patch at a location, one species, or only a particular type of tissue. A continuation of this work to investigate nutrient limitation in rivers using macrophyte tissue nutrient concentrations should implement a sampling strategy to remove the potential bias inflicted by these parameters in the study design. Nutrient limitation may be more likely to occur earlier in the season before peak biomass (fig 2.15), when nutrient uptake rates are higher than release rates. Conversely, macrophytes may be less likely to be nutrient limited later in the season when senescence begins and release rates are high. I hypothesize that the duration of early-season nutrient limitation may affect the peak biomass observed in the season. Conceptualization of the eutrophication process in rivers should not only consider long term temporal ecosystem succession, but a spatial and seasonal continuum as well.

It is important to note that the critical nutrient concentrations determined in laboratory studies may not be strictly applicable to field studies. Macrophytes growing in the field must contend with sub-optimal light, current velocity, sediment composition and the stresses of competition and herbivory, all of which may change the physiological demand for nutrients. Thus, the nutrient thresholds used in this work might be better applied as general guidelines. Until appropriate thresholds are determined by manipulation of nutrient concentration in the field, it may not be possible to demonstrate nutrient limitation in macrophytes based on nutrient quotas. Evidence for increased biomass in response to increased nutrient loads is provided in this work, thus advancing the understanding of eutrophication in riverine systems.

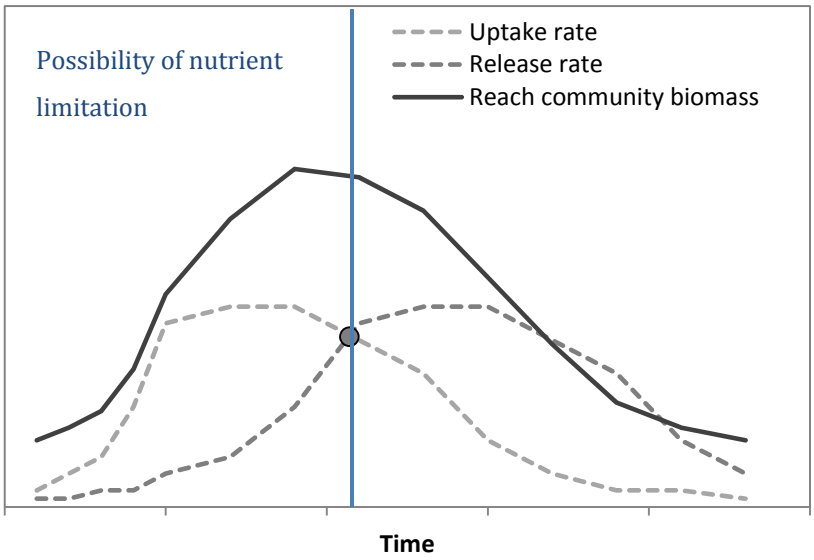

Figure 2.15 Suggested seasonal pattern of macrophyte community biomass development, uptake and release rates and predicted temporal range of nutrient limitation in submersed macrophyte communities. Nutrient limitation may only be possible when growth rates are high and uptake rates exceed release rates. During peak biomass, when growth rate slows and nutrient release is equal to uptake and greater than uptake thereafter, nutrients may no longer be limiting due to reduced demand, the availability of regenerated nutrients from the community itself, or from upstream patches.

Chapter 3: Inter-annual variation in submersed macrophyte biomass its and relationship to weather and climate

3.1 Introduction

Submersed vascular macrophytes are prominent primary producers in riverine ecosystems and have been known to develop nuisance levels of biomass in urban and agricultural settings in response to elevated nutrient loads, mainly nitrogen and/or phosphorus, as shown in chapter 2. The increased primary productivity leads to changes in the diel cycling of oxygen and often results in depleted DO levels at night which can threaten aquatic animal species and can compromise the suitability of the river as a freshwater resource (Davis, 1975; Smith et al., 1999; Chambers et al., 1999; 2006;).

Studying the relationship between river macrophytes and anthropogenic nutrient enrichment is difficult because of the spatially and temporally heterogeneous nature of rivers. Rivers and streams are highly dynamic environments, with conditions such as depth, water velocity, temperature, turbidity, dissolved oxygen and CO_2 varying on short spatial and temporal scales. Rivers are highly sensitive to physical processes and changes in weather in the catchment, particularly precipitation (Dent et al. 2002). Additionally, macrophyte communities are highly patchy in their spatial distribution (Mackay et al. 2003; Lacoul and Freedman 2006). These features make it difficult to design field scale studies of the relationship of macrophyte biomass to dissolved nutrients in rivers. Although some researchers have found that variation in sediment and water nitrogen and phosphorus concentrations are sometimes important in explaining the peak summer biomass of macrophytes (Carr and Chambers 1998; Sosiak 2002; Carr et al., 2003; Bernez et al. 2004), there are many other important factors controlling the benthic biomass of rivers, such as current velocity, light and substrate type (Barko and Smart, 1981; Barko and Smart 1986; Nilsson 1987; Chambers et al. 1991; Madsen et al. 2001; Riis and Biggs, 2003; Barendregt and Bio 2003; Franklin et al. 2008).

Long term evaluations of river macrophyte biomass development over several years currently do not exist, and as a result we have little understanding of the year-to-year variation in biomass and what the drivers of that variation might be. However, some studies indicate that weather-dependent factors such as light, temperature and precipitation are important in

influencing the peak biomass of aquatic macrophytes. For example, macrophyte biomass in eel-grass communities in estuaries was elevated during an El Nino year when temperatures were higher, average irradiance was greater and dissolved nutrient concentrations were lower Nelson (1997). Carter et al., (1994) found that increased clarity of a tidal river produced increased total coverage of macrophytes, and high turbidity led to decreases in total coverage. Similarly, Rooney and Kalff (2000) found that light was the most important factor influencing the total macrophyte biomass of a lake; as the clarity of the lake increased, the depth of colonization increased allowing for greater habitat area. Other research has indicated the positive effect of temperature on submersed macrophyte productivity (Beal et al, 2004; Duarte and Kalff 1986, Lacoul and Freedman 2006; Shafer, 2008), though it is often it is impossible to separate the effects of temperature and irradiance on growth.

Because rivers are highly dynamic, with flow regimes and nutrient concentrations that are strongly influenced by catchment processes and local weather events, it is likely that inter-annual variation in submersed macrophyte biomass is influenced by seasonal and annual weather patterns. Factors such as the number of daylight hours, daily and seasonal temperature, precipitation and discharge are expected to have an influence on macrophyte growth rates, and the trend in average weather over the growing season is likely to influence the total amount of biomass that will eventually develop. Specifically, we expect that years with higher precipitation, and thus higher discharge, will lead to lower peak biomass in riverine submersed macrophytes over the growing season due to a greater number of cloudy days with reduced light reaching macrophyte beds, increased depth further reducing light penetration to the river bed, increased turbidity resulting from catchment sediment runoff and greater tensile stress creating the biomass washout associated with high flow rates (Franklin et al. 2008) during major storm events. Conversely, we expect that years with warmer and sunnier growing seasons will lead to higher peak biomass since the increased temperature and light can fuel faster growth (Kemp et al. 1987), allowing macrophytes to recover more quickly from washout events. From the point of view of science-based management, year-to-year changes in weather are problems that obscure changes due to increased loading or remediation efforts.

We investigated the inter-annual variation in biomass of riverine macrophytes and the influence of weather and climate on peak biomass development of submersed macrophyte communities using 4 years of reach-level monitoring data in Grand River. We used macrophyte

biomass surveys along with river monitoring and meteorological data to test seasonal peak biomass, and the timing of peak biomass development, with water and air temperature, precipitation and discharge. We expected increased biomass, and earlier seasonal biomass maximum biomass development, in years with higher seasonal temperatures and a lower biomass and later maximum in years with higher precipitation and higher discharge. We also probed for differences in maximum development in three dominant species and compared reaches to detect an effect that could be attributed to WWTP nutrient loading.

3.2 Materials and Methods

This study was conducted in the Grand River, in southern Ontario. See the Materials and Methods section of Chapter 1 for a full description of the study area. Three reaches of river, approximately 10 km in length, were included in the study (fig. 3.1). The upstream reach, Bridgeport (BP), is located just upstream of the contiguous cities of Waterloo and Kitchener and the large sewage treatment plant outfalls associated with them and is 7 x 10^5 m^2 in area. It corresponds exactly to the Waterloo upstream sub-reach from chapter 2. Blair (BL) is located about 20 km downstream below the Kitchener WWTP , is 3.11 x 10^5 m^2 in area and corresponds to the Kitchener downstream sub-reach in chapter 2. The downstream reach, Glen Morris (GM) is located another 20 km downstream of the BL reach, is 4.91 10^5 m^2 in area and is considered by the Grand River Conservation Area (GRCA) to be within a "recovery zone" with groundwater discharge that dilutes river water (fig. 2.1).

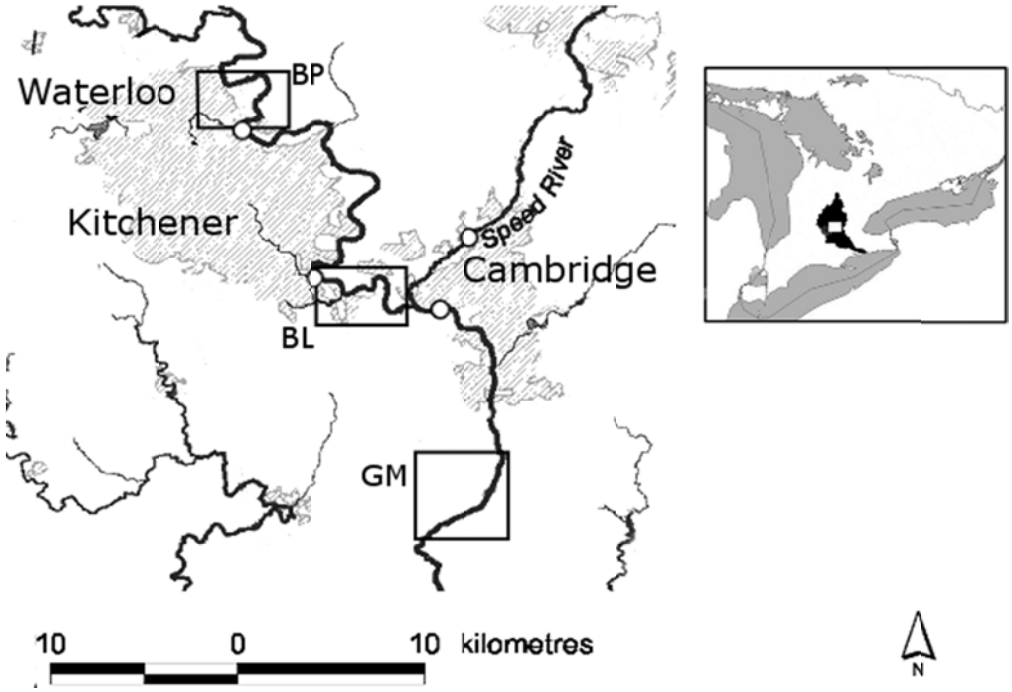

Figure 3.1 Map of the study area showing the location of the three survey reaches, BP, BL and GM, where the submersed macrophyte biomass was surveyed from 1997-2000. The BP reach is entirely upstream of the cities of Kitchener and Waterloo, while BL and GM are both downstream. WWTPs, indicated by the (O) symbol, are located immediately downstream of the BP reach and immediately upstream of the BL and GM reach, with the largest at Kitchener being located immediately upstream of BL.

The reaches were surveyed for macrophyte biomass on 3 to 6 occasions over the growing season for 4 successive years, from 1997 to 2000 by employees of the GRCA. Two types of surveys were conducted, a "cropping" survey and a "mapping" survey. The mapping survey was conducted 3 to 4 times throughout the growing season and the cropping survey occurred more frequently. The mapping portion consisted of canoe surveys of the study reaches, moving from bank to bank while drifting downstream. Because no precision is provided, we assume an error of 10% of the patch area was made when locating and drawing the macrophyte patch. The location of macrophyte patches was documented by hand-drawn spatial maps, with assistance of a GPS unit and detailed satellite maps of the river. Individual patches were assigned to one of three density categories: "sparse", "moderate" or "dense" based on a subjective assessment. The hand-drawn maps of the macrophyte patch distribution were digitized using ArcGIS, and individual patch areas for each reach were extracted using GIS toolkits. Each patch was assigned a patch area and used in combination with the cropping surveys to determine the average patch biomass, total patch biomass and finally average reach biomass.

On cropping surveys, 10-20 large patches were chosen at random and sampled with 5 randomly selected replicates within the patch, using a surber sampler which samples an area of 0.0929 m^2. Due to the nature of the substrate where macrophyte patches were located, only the above ground biomass was sampled, making the surber equipment as effective as sampling by hand using a quadrat, the biomass collection method used in Chapter 2. After collection, plant material was rinsed in river water, collected in plastic bags, and brought back to the lab where it was sorted to into species categories based on the three most dominant taxa, *M. spicatum,* and a group we call "*Potamogeton* spp*."* which includes several similar looking species of the family Potamogonacea that are difficult to identify to species without having flower or fruit present on the specimen. The category *Potamogeton* spp. includes also the highly abundant species *Stuckenia pectinata*. Even though it is not in the genus *Potamogeton*, *Stuckenia* is very closely related and were formerly included in that genus (Crow and Hellquist, 2000). *Cladophora* sp*.* was also collected, though it is not a vascular macrophyte, but because it has a very dominant presence in the Grand River the GRCA mapped and collected it along with macrophytes. Less common species were not included in this work, but comprised less than approximately 15% of the total biomass of each patch. These taxa contributed to the mass estimates derived from quadrats, but their taxonomic representation was not quantified. After sorting, macrophytes

were oven dried at 80 C for 5 days, then weighed to obtain an average patch density measurement (g dry weight/m^2). The dry weight measurement obtained for each patch was multiplied by the patch area to obtain a total patch biomass (kg). The average biomass from each patch that was cropped also had a density estimate associated with it, so an average patch biomass could be given to each density estimate. The biomass associated with each density estimate was applied to patches in the reach that were not sampled directly. The patches that were not cropped also had an approximate species composition associated with them from the mapping surveys, allowing an average biomass for the species found in the patches that were not sampled by surber sampler to be obtained as well. Thus a total biomass (kg) for each species in each patch for all reaches could be determined. The total biomass of all the patches were summed up to obtain a total biomass per reach, and then divided by the reach area to obtain an average reach biomass (g/m^2). Average reach biomass was calculated for all macrophytes, and in each of the three species categories. It is worth noting that the average reach biomass (g/m^2) determined in this work is a different measurement from the biomass (g/m^2) measured in studies which report an average biomass obtained by taking the average dry mass from several quadrats within a patch or along a transect. We refer to a biomass measurement taken in such a fashion as average patch biomass (g/m^2).

River and weather data for the region were collected from several monitoring sources. The air temperature and precipitation data were obtained from 3 stations within the watershed near the study area using the Canadian National Climate Data and Information Archive. The three monitoring stations were chosen both for their proximity to the sample reaches and for the completeness of data coverage for all years. Data obtained from the national archive included average daily temperature, daily maximum temperature, daily minimum temperature, and daily precipitation accumulation for the months April- August. Average temperature and precipitation amounts were averaged for each calendar month and for 3, 5, 10 and 30 days prior to individual sampling dates. Water temperature data were obtained from the GRCA and were collected with continuous sensors installed permanently at stations located within the 3 survey reaches. Discharge data were provided from two sources, the Water Survey of Canada and the GRCA. Discharge data were in a form uncorrected for plant growth; Water Survey of Canada often corrects their discharge data for ice cover and submerged macrophytes while the GRCA does not. Discharge data were missing for the GM location for 1997, 1998, 1999, however

discharge at this location could be predicted using discharge data from the BL location upstream (fig. 3.2) using data from 2001-2011. Comparing predicted GM discharges for the year 2000 against measured GM discharges indicates that this method of obtaining the missing data is highly accurate with an R^2 for the relationship between reaches of 0.992 and an R^2 for prediction of 0.9995.

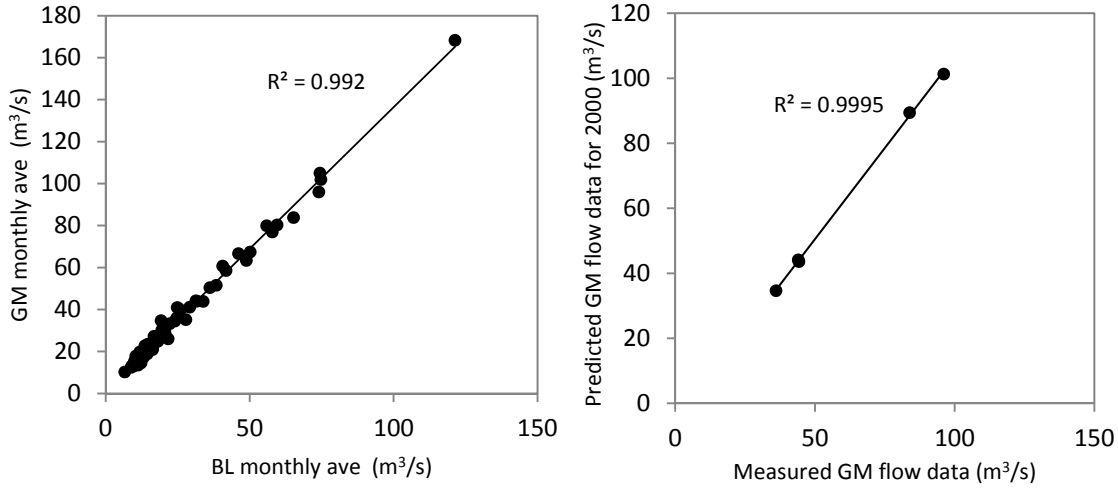

Figure 3.2 Using BL discharge data to predict GM discharge where GM discharge data are missing (A), monthly discharge average (m^3/s) for years 2001-2010. GM discharge data are missing for years 1997-1999, but the data from 2000 can be validated against model predicted discharge (B) Model is constructed using monthly averages during ice-off season, from April to October, using available data from GRCA from 2000 to 2010. Actual discharge data from reach GM is used for year 2000. Validated against monthly average discharge data for 2000 (B).

ANOVA and t-tests were used to test whether average reach biomass differed among reaches over the period of study, rather than multivariate tests as limited data from only 4 years limits the statistical power of multivariate analysies. To analyze the effect of climate and weather-driven parameters on the inter-annual variation in macrophyte biomass, two metrics were derived from the seasonal response curve, the peak seasonal biomass (B_{max}) and the day the peak occurred (in Julian days), (D_{max}). The observed maximum biomass was used for B_{max} and D_{max}, rather than an estimate of the maximum based on interpolation based on a fitted curve, as it has yet to be demonstrated that seasonal macrophyte biomass accumulation follows a unimodal growth curve, though this is a typical operating assumption. Different statistical methods were employed to determine whether inter-annual variation in these features of the seasonal growth pattern of submersed macrophytes is explained by weather-related factors (air

and water temperature, discharge and precipitation). An ANCOVA (conducted through Systat's General Linear Model protocol) was used, where reach was a categorical variable, so that the effects of the climate-driven parameters on biomass could be determined after the effect of site-specific differences among the three different reaches were removed. For the ANCOVA analysis to successfully compare the dependent variable at the three reaches, the slope of the relationship between biomass and the independent variable must be the same (no significant interaction between the reach and the independent variable), otherwise ANCOVA assessed differences among sites.

In the case of an interaction, ANCOVA cannot be used. However, a second method to evaluate the regression that is independent of the categorical variable (reach) can be used when slopes are not parallel. This method computes a predictability score (P_s) which weighs all of the significant linear correlations as a proportion of the number of possible perfect linear correlations. This score allows an evaluation of the amount of variation in inter-annual biomass that is explained by the different weather and climate related variables. The predictability score (Ps) is calculated by equation 2.1:

$$Ps = \frac{\sum_{i=1}^{N} Ri^2}{N} \qquad (2.1)$$

Where N is the total number of possible linear correlations, in integer values, and R_i^2 is the R^2 of each significant linear correlation. For example, if all of the relationships were significant with an R^2 value of 1, then the P_s score would be 1. If half of the relationships were significant with an R^2 of 0.658 each, then the P_s would be 0.329.

3.3 Results

Weather parameters varied over the four years surveyed, with average may-august temperatures being highest in the year 1998, lowest in 2000. The wettest year was 2000, and the driest was 1997 (fig. 3.3). The average reach biomass of submersed vascular macrophytes varied over the season, and between years (fig. 3.4), with an apparent seasonal progression from May, when sampling began, to a August for each year, when was when it was assumed that maximum biomass had been achieved. Mean average reach biomass varied by year, with the most productive year 4.9 to 6.1 times as much biomass as the least productive year. Reach biomass also reached its peak at different times each year, and the peak did not coincide with the maximum day light hours of the growing season.

The reaches differed in biomass (log-transformed biomass, ANOVA, $P < 0.001$); the upstream reach, BP, had the lowest biomass, while the BL reach, immediately downstream of the Kitchener WWTP, had the highest biomass and GM, the reach furthest downstream, had an intermediate biomass. Seasonal progression appeared do differ among the three taxa, *Cladophora* sp. appeared earlier than either *M. spicatum* or *Potamogeton* spp., and declined in biomass while the *M. spicatum* and *Potamogeton spp.* Increased. In the GM location, M. spicatum and Potamogeton spp had slightly different seasonal patterns, with M. spicatum increasing in biomass over the growing season; and all three reaches appeared to have a higher biomass of Potamogeton spp than M. spicatum throughout the growing season. There appeared to be some difference in the relative abundance of macrophyte species between years, though we did not have enough data to evaluate this possibility statistically.

Over the part of the growing season that was sampled, May to August, day of the year was a good predictor of biomass when all species data from all three reaches and all years were combined in an General Linear Model (GLM) with reach as a categorical independent variable ($P=0.002$). Because reaches appeared to have different seasonal growth patterns (fig 3.4 and fig. 3.5), it was sensible to assess whether day of the year was useful in predicting biomass at each reach individually, as well for each species individually. In this case, day of the year was linearly related to biomass only for the BP reach ($P < 0.001$). The biomass at BL and GM reaches appeared to have a unimodal relationship with date, with B_{max} occurring at some point between the beginning and end of the sampling period (fig 3.4; fig 3.5).

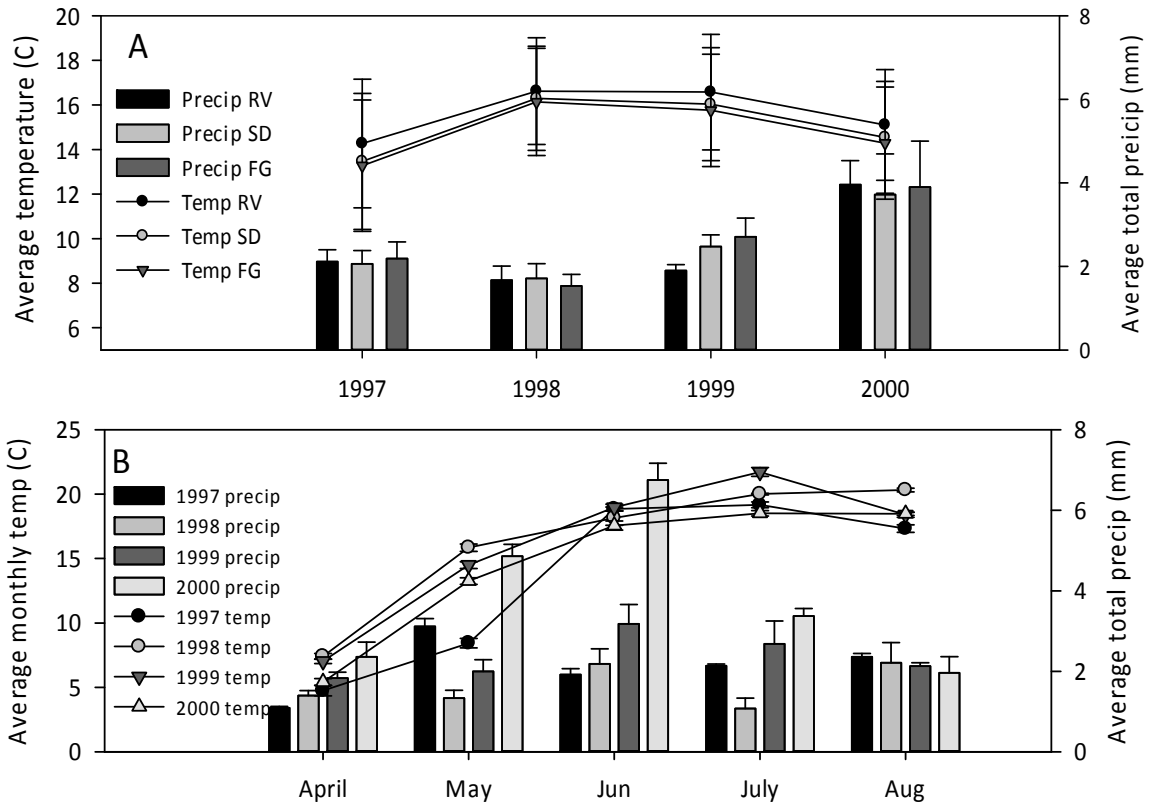

Figure 3.3 Summary of temperature and precipitation at the three weather stations, over the Apr-august growing season in the 4 survey years. Data are displayed in two ways: average temperature and the average of total monthly precipitation for the period between April-August by year for each of the three stations (A); Site average temperature and total precipitation by month for each year (B) are shown.

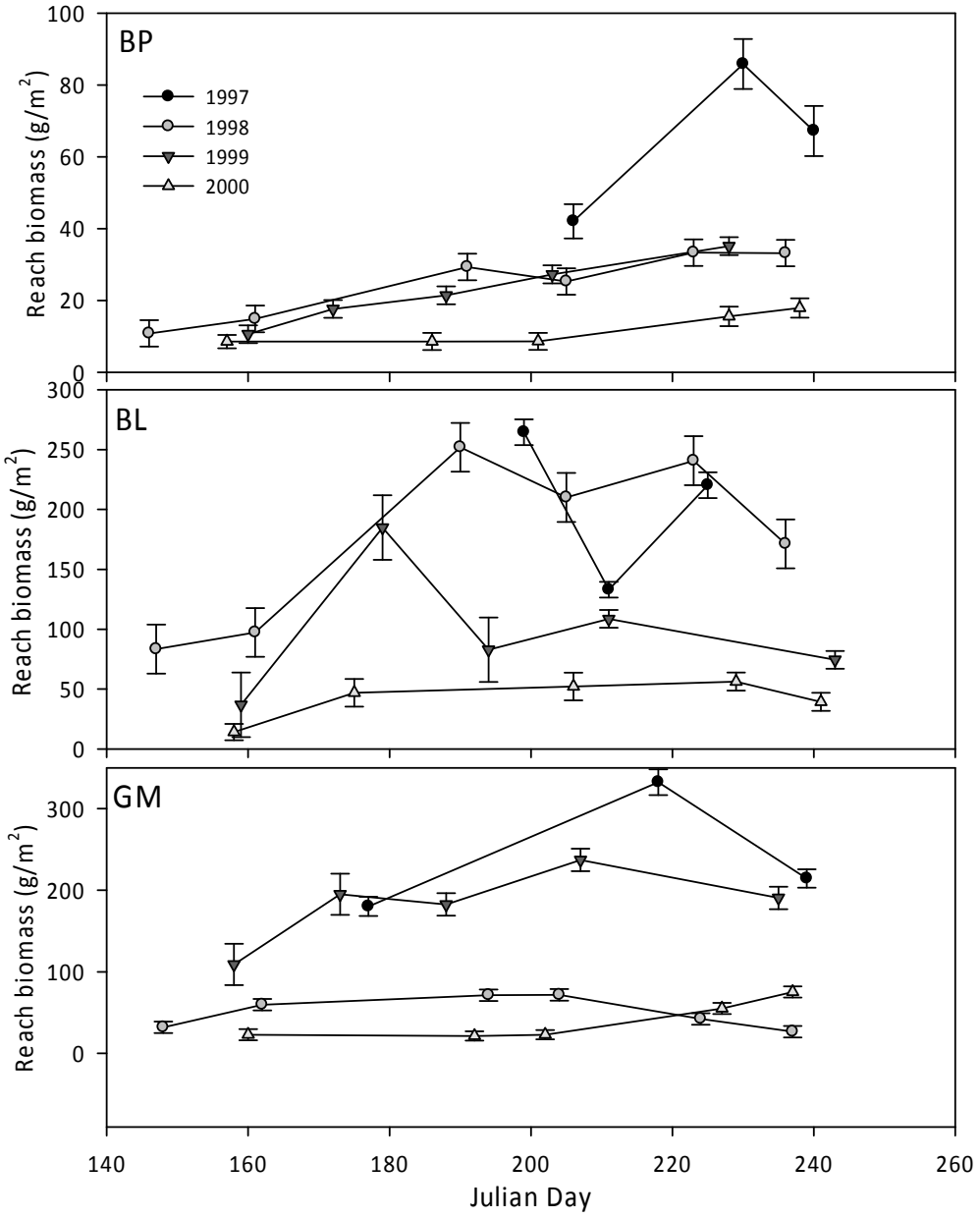

Figure 3.4 Seasonal average reach biomass (g/m^2) of all vascular macrophytes (excluding *Cladophora* sp.), for three reaches, BP, BL and GM for four years, 1997-2000. Each point represents a biomass estimate for the sampling date in Julian Day. Error bars represent range of estimation based on error propagation of a 10% inaccuracy in determining patch area.

Short-term changes in weather did predict some of the variation in biomass, with average water temperature for 5 days leading up to biomass harvest significantly related to with biomass (GLM, reach as a categorical variable, P=0.007).Other short-term parameters, air and water temperature for the previous 10 days, and average precipitation for the previous 5 and 10 days, were not significant predictors of biomass.

Differences in B_{max} and D_{max}, among reaches were observed. BL reached its maximum (D_{max}) much sooner than BP and GM (paired student's t-tests, P=0.034; 0.027, n=4) and BP produced less biomass at its peak than BL, but not GM (paired student's t-tests, P=0.027; 0.079, n=4). BP, the upstream reach, was thus the most different, with less peak biomass and a peak occurring later in the season than BL, which had the highest biomass and earliest production (fig 3.6).

Years differed not only in their B_{max}, but D_{max} as well, and both might be influenced by weather. The weather parameters examined for relationships in this study, mean monthly river discharge (m^3/s); monthly total precipitation (mm); monthly average water temperature (C); and monthly average air temperature (C) were tested separately for each month, April- August as some of these parameters might be correlated with each other and thus explain a similar portion of variation in biomass. Additionally, there were only four years of data to use in any analysis making the threshold for statistical significance high and the resulting degrees of freedom low, which limited the use of a multivariate approach. ANCOVAs conducted on the B_{max} and $D_{max,}$ (using reach as a categorical variable) revealed that for all four weather related parameters spring values were the strongest predictors. The interaction term, which indicates whether the relationship between biomass and climate parameters depends on reach, was not significant except in the case of the effect of discharge on D_{max}. Thus, the effect of air temperature, water temperature and precipitation on biomass was independent of site. B_{max} was related to June average water temperature (P=0.028), June average air temperature (P=0.005), and June total precipitation (P=0.010), but not to discharge for any month, though June average flow resulted in the best correlation (P=0.069). D_{max} was also influenced by June average water temperature (P= 0.007), June average air temperature (P=0.045) and may total precipitation (P=0.013). June average air and water temperature had a positive influence on B_{max} and negative on D_{max}, and average June/May precipitation had a negative effect on B_{max} and a positive one on D_{max}.

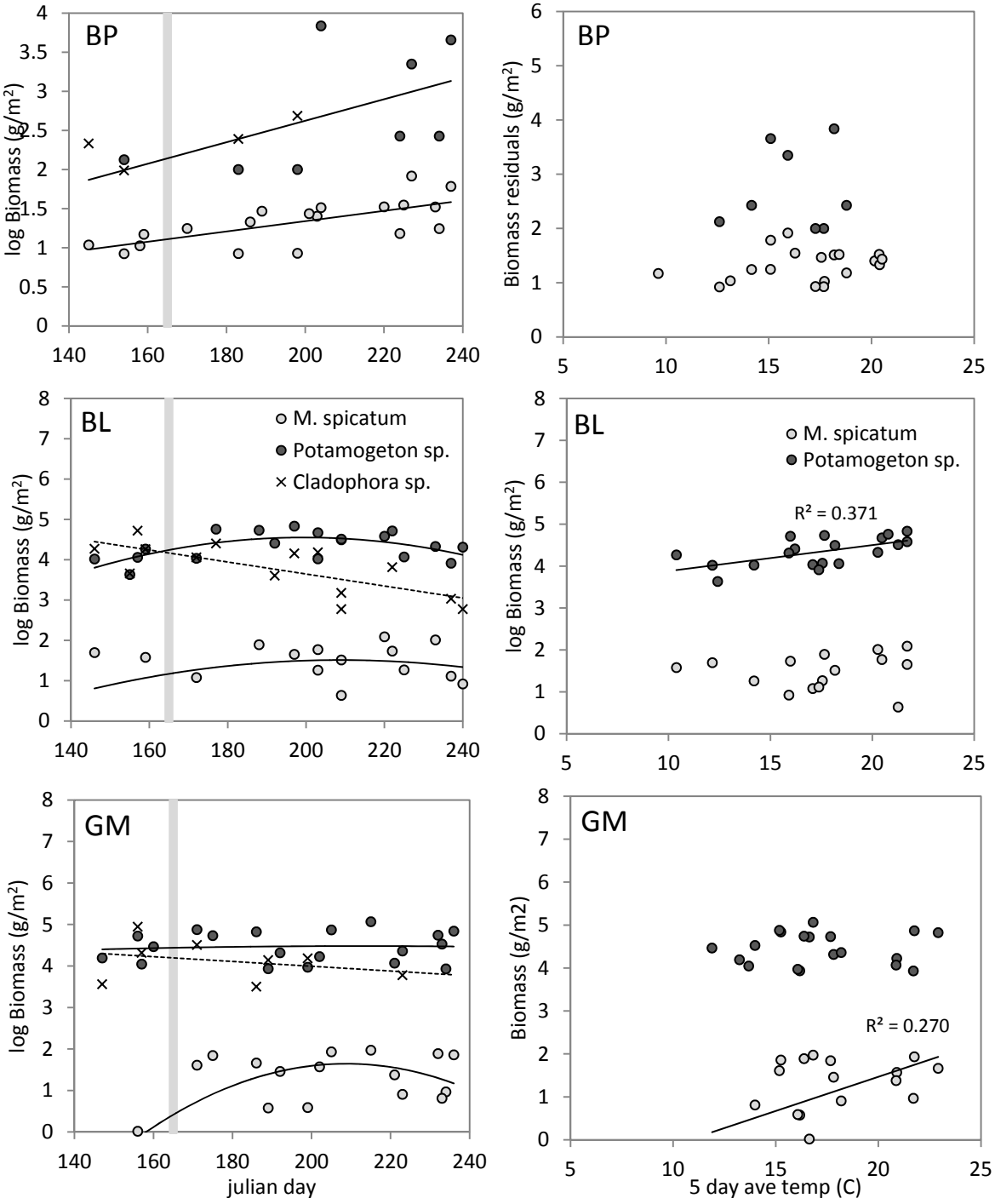

Figure 3.5 Biomass relationship with Julian day and temperature for 5 days prior to biomass harvest, for all dates sampled in 1997-2000, for reaches B, BL and GM. The grey line on the three Julian day graphs indicates the longest day of the year.

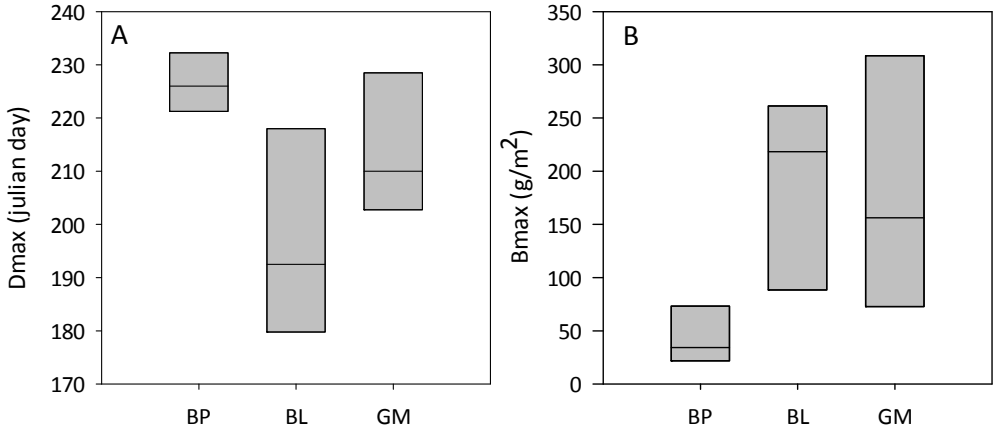

Figure 3.6. Biomass parameter D_{max} in each reach, in Julian days (A); and parameter B_{max} in each reach (g/m^2) (B) in the years 1997 to 2000, excluding *Cladophora* sp. Reaches differ marginally in D_{max} and significantly in B_{max} (ANOVA, P= 0.54 for D_{max} and P=0.031 for B_{max})

Because the slope of D_{max} and B_{max} against discharge varied depended on reach, ANCOVA could not be used to determine the explanatory value of discharge on biomass, so the Ps was used. Only relationships with a R^2 of 0.66 were included, which is the level of signicance regression of n=4.

The best predictor of biomass (B_{max}) using the P_s statistic was precipitation, with a P_s of 0.557, and secondly discharge with a P_s of 0.312, (fig 3.8) while the best predictor of D_{max} was also precipitation (Ps=0.930) and secondly discharge (Ps=0.918) (fig 3.8). Average June air and water temperature predicted the least amount of variation (fig. 3.9). Even though we could not use ANCOVA to assess the effect of discharge on macrophytes because of an interaction between reaches, discharge does appear to be important in explaining variation in biomass.. Discharge and precipitation were better predictors than water or air temperature of both B_{max} and D_{max}, and D_{max} was generally better predicted by climatic parameters than was B_{max}. The predictability score (P_s) of each of the 4 parameters indicated that May and June averages of discharge, precipitation, air and water temperature, over those of other months (analysis not shown) were the best predictors of the maximum biomass and the time of max biomass. Combined, these results indicate that increased spring precipitation and discharge (which are often related) delay the timing of the peak seasonal biomass and also lead to lower biomass production, while higher spring temperatures can lead to earlier B_{max}

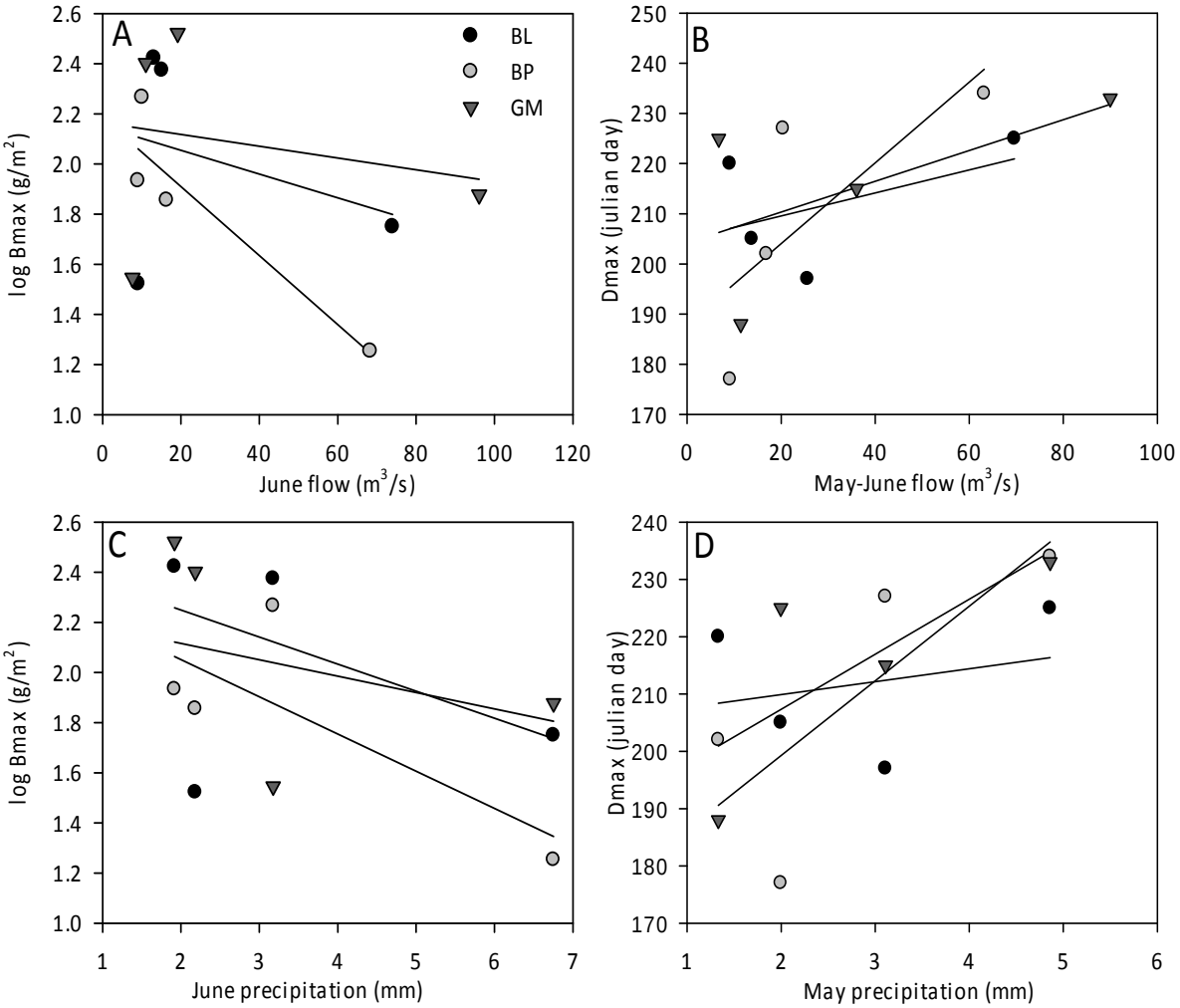

Figure 3.7 Inter-annual biomass variation, excluding *Cladophora* sp., for 1997-2000, as it relates to June or May +June average discharge (A and B), and June and May average precipitation (C and D). Inter-annual biomass variation is explored in two ways, using B_{max} (A and C) and D_{max} (B and D).Monthly average discharge and monthly average precipitation which produced the strongest correlation were chosen. Significance at α=0.05, df=4, is an r^2=0.658

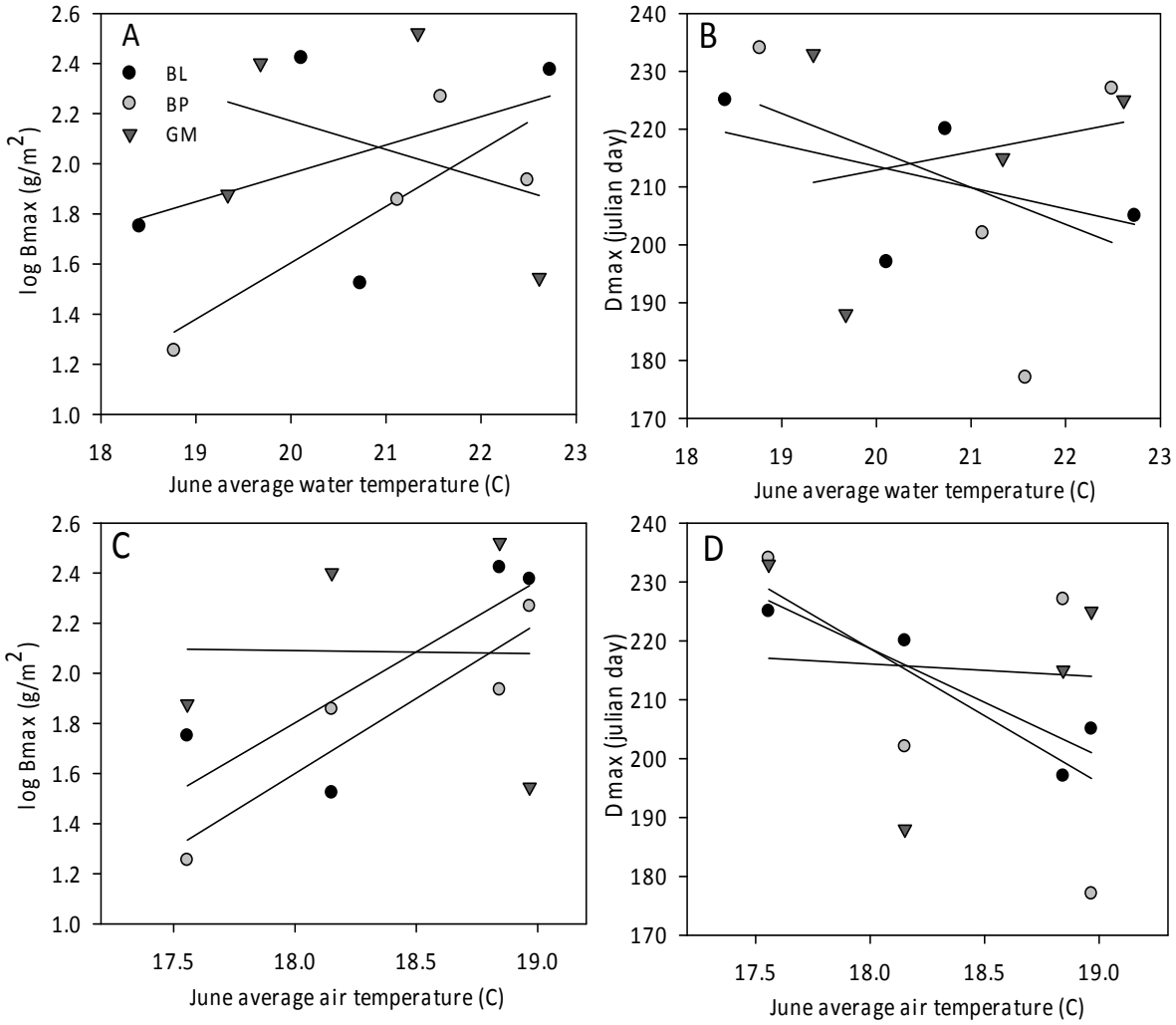

Figure 3.8 Inter-annual biomass variation, excluding *Cladophora* sp.*,* for 1997-2000, as it relates to June average water temperature (A and B), June average air temperature(C and D). Inter-annual biomass variation is explored in two ways, using B_{max} (A and C) and D_{max} (B and D). Monthly average discharge and monthly average precipitation which produced the strongest correlation were chosen. Significance at α=0.05, df=4, is an r^2=0.658.

3.4 Discussion

Biomass differed among reaches and dates, and much of the temporal variation could be explained by the parameters chosen for analysis. The three surveyed reaches appear to be distinct in their patterns of biomass production, with the upstream reach BP producing less biomass later in the growing season, the downstream reach BL peaking sooner and producing a greater quantity of biomass at the peak, and the recovery reach GM somewhere in between. The differences in biomass among reaches are probably due the effect of the effluent discharged by the Kitchener and Waterloo WWTPs, located upstream of the BL reach, which are sources of NH_4^+ and PO_4^{3-}. Nutrient point sources have been shown to explain differences in biomass among locations in other studies (Carr et al., 1998; Sosiak et al., 2002). The timing of peak biomass is another layer of complexity in the story of the response of river environments to human influence and, to my knowledge, has not been examined in previous work on riverine submersed macrophytes. In attempting to understand the response of macrophytes to nutrient enrichment, the temporal aspect of nuisance biomass development needs further exploration.

Biomass increased over the sampling period between May and August, but only when all years and reaches were pooled; when reaches were examined individually, biomass did not increase predictably with date, and only at the upstream reach did biomass relate linearly to date. Our first sampling of macrophyte biomass in May or early June was not early enough to capture the entire period of active growth. However, because the upstream reach BP achieves seasonal peak biomass later than the other two locations, some of the rapid growth period was captured by May and early June sampling, producing a linear relationship with Julian day. The biomass of *M. spicatum* at BP was best explained by a linear relationship, whereas the biomass of *M. spicatum* at the other two locations the seasonal pattern was not approximately linear; it peaked at some intermediate point and then declined, roughly approximating a hyperbolic function. We did not assess the decline of biomass in this study, as sampling did not continue long enough into the season, but it is likely that the decline in biomass may also be an important parameter in describing the seasonal pattern of submersed macrophyte biomass in large rivers.

The quantity of biomass present at any reach did depend on the date in some cases, linearly or otherwise, but weather variables explained more of the variation and are likely more useful in predicting macrophyte biomass. The strongest relationships between peak biomass (Dmax

and Bmax) and climate parameters were found for May and June weather, even though the peak biomass always occurred later, in July or August, indicating that conditions earlier in the season are important for late season biomass production. Both temperature and precipitation were significant in explaining inter-annual differences in biomass. Average early-season precipitation, air and water temperature were significant predictors of inter-annual peak biomass, and the timing of the peak. However, it is possible that there are other important weather-related parameters that we did not test, such as wind speed, insolation and ice-cover. I can conclude from this work that river discharge and precipitation are important in accounting for some of inter-annual variation in biomass in this system, although the effect of discharge is different depending on the reach.

Higher discharges and higher precipitation earlier in the season will result in a lower average reach biomass, and a later peak in biomass. This is expected as river flow rate and current velocity have been shown to influence the establishment, growth, and abundance of riverine macrophytes at different temporal and spatial scales. Very high discharges can uproot and remove biomass and propagules (Riis and Biggs, 2003; Franklin et al., 2008) and negatively impact the season's biomass (Sosiak, 2002). As well, high discharge events in winter may produce ice-scouring which can uproot and remove macrophyte beds. In rivers where the level of flood-related disturbance permits macrophyte biomass development, current velocity remains an important determining factor, as macrophyte biomass tends to peak at intermediate velocities, i.e., 0.3-0.5 m/s. Lower velocities tend to limit the delivery of dissolved substances to the macrophytes by influencing the boundary layer, and higher velocities result in tensile stress due to drag and eventually lead to stem breakage and washout (Sand-Jensen 1989; Riis and Biggs 2003; Madsen et al. 2001). Beyond 1 m/s macrophytes are unable to survive (Chambers et al., 1991). Very low discharges would also negatively influence the seasonal biomass production of submersed macrophytes, and at low flow floating leaf plants begin to dominate, shading out submersed ones. The range of discharge values in our work, in combination with a small sample size, may not have made detection of this effect possible and, in addition, we did not map or sample floating-leaved or emergent vegetation, although these plants are present in the Grand River.

There are some confounding influences on biomass that may vary with precipitation and discharge. With increasing discharge, depth and velocity increase. Bernez et al. (2004) found

depth to be an important factor in explaining variation in macrophyte distribution across three French streams. Precipitation events can also lead to higher turbidity in rivers; as discharge increases, sediments run off the landscape (Lawler et al., 2006) and previously deposited sediment may be re-suspended. In deep pools, prolonged periods of high discharge and associated turbidity may be enough to cause light limitation in macrophytes. Higher precipitation in the active growing season may lead to light limitation, which could be driving the negative relationship found between biomass and precipitation. Additionally, precipitation is associated with cloud cover, and the associated decrease in solar radiation may also contribute to light limitation. Rain events can lead to mobilization of nutrients from the landscape and deliver them during the active growing season, resulting in nutrient uptake followed by rapid growth, particularly in shallow areas that would not experience light limitation during high discharge periods. However, in an agricultural and urbanized watershed like the Grand River, it is possible that nutrient delivery through storm events is not an important influence on the biomass of macrophytes as they may not be nutrient limited during periods of rapid growth (as in Chapter 2). The upstream reach could be more susceptible to the positive, fertilizing effect of rainfall events, than the downstream reaches but our data do not support this idea.

Warm temperatures produce high growth rates (Barko and Smart 1981; Beal et al.; Lacoul and Freedman 2006; Shafer, 2008) and we found evidence that warmer years produce more biomass, and sooner. Although air and water temperatures did not explain as much of the variation as discharge and precipitation did, temperature can still contribute to the inter-annual variation in macrophyte biomass. With only 4 years of data the statistical powers of the tests are low and it is possible that there is a relationship although we did not detect one (type II error). Expanding the dataset by continued monitoring could allow for better resolution of the influence of temperature on macrophyte biomass.

Although it is often difficult to distinguish the influence of temperature from solar radiation, evidence for lakes suggests that light is an important determinant for whole lake seasonal macrophyte biomass. Increased light penetration into lakes can increase the colonization depth of macrophytes, and therefore the macrophyte biomass (Roony and Kalff, 2000; Collins et al., 1987). Seasonal and inter-annual changes to lake turbidity are primarily responsible for changes in lake macrophyte biomass, and drivers of pelagic production that contribute to

turbidity are thus important mechanisms. However, light penetration and depth of colonization are probably less important in rivers because they tend to be shallow environments so light penetrates to the substrate over most of the width of the river, and many of the dominant macrophyte species form canopies that float at or just below the water surface (Madsen et al., 2001). In years with higher temperature and fewer cloudy days during the growing season, increased irradiation may result in higher photosynthetic rates and increased biomass production, however, light may not be as important as temperature over the long term for seasonal biomass production as river macrophyte communities can be light saturated, especially in larger, lowland rivers with little canopy cover (Chen et al., in prep). In our work the timing of the peak biomass did not coincide with the longest day of the year, June 20-22; it came much later in July or August. However, the maximum growth rate might coincide with the longest day. In other work conducted by our lab, it was found that ecosystem photosynthesis in some locations in the Grand River and the Speed River became light-saturated well before noon (Chen et al., in prep). Other evidence suggests that temperature, regardless of irradiance, is important. Duarte and Kalff (1987) found that macrophyte biomass is a function of latitude in lakes of similar transparency, thus growing season length, impacting both light and temperature, can have an important influence on macrophyte biomass not just clarity of the lake. Experimental evidence indicates that macrophytes can become light saturated at irradiances far below daily maxima observed in southern Ontario summers, suggesting that the high irradiance on long sunny summer days may not be as important for rapid growth as the temperature. *Littorella uniflora* was saturated at 850 $\mu E/m^2/s$ (Robe & Griffiths, 1994) and *Hydrilla* sp. was saturated at 1050 $\mu E/m^2/s$, although *Egeria* sp. was not saturated at the highest irradiation used (Barko and Smart, 1981). Maberly (1985), in measuring the interaction between irradiance, temperature and CO_2 concentration, found that at the highest CO_2 concentrations (1 mmol/L) photosynthesis of *Fontinalis antipyretica* was saturated between 300 and 700 $\mu mol/m^2/s$. They also found a significant positive relationship between maximum gross photosynthesis and the log of temperature between 3 and 20 C.

Results from this study have implications for the understanding, modeling and management of macrophyte dominated temperate rivers. My work demonstrates the influence of weather on the inter-annual and seasonal development of macrophyte biomass in a large river ecosystem and, as a result, we should expect biomass to vary strongly among years. Measurements of

macrophyte biomass need to be done in context of the range of natural variation in submersed macrophyte biomass and account for the role of weather in producing some variation. My results provide evidence that macrophyte biomass in the Grand River is likely to increase in response to a warming climate, and especially under a warmer and drier climate, which is one of the forecasted climate change scenario for the southern Ontario region (OMNR, 2007). Future work should attempt to better quantify the impact of climate drivers and assess the generality of the seasonal and inter-annual variation described in this work.

Chapter 4: Submersed macrophytes as indicators of waste water effluent ammonia using the $^{15}N/^{14}N$ stable isotope ratio in a large lowland river

4.1 Introduction

Anthropogenic eutrophication of rivers is a problem of global magnitude leading to altered aquatic dissolved oxygen (DO) cycles which degrade water quality and render the environment inhospitable for many species (Vollenweider, 1968; Smith et al., 1999). As in other aquatic ecosystems, phosphorus and nitrogen loading to rivers from domestic, agricultural and industrial sources can lead to excessive growth of macrophytes or algae (Dodds, 2006). Although accepted as a general phenomenon, empirical investigations, so far, do not demonstrate a strong link between increased nutrient loads and nuisance plant growth in rivers (Hilton et al., 2006). River systems are highly variable environments and important growth factors such as water depth, sediment type, current velocity, temperature, canopy cover and light exposure, can vary greatly over short temporal and spatial scales making the study of nutrient loading on riverine macrophyte biomass challenging.

Researchers have sought various tools and proxies to study the link between nutrients and macrophyte growth in rivers and streams. Lab studies (e.g. Barko et al., 1986), field studies (e.g. Carr et al., 2003) construction of artificial streams (e.g. Carr and Chambers, 1998), long term monitoring after nutrient reduction (e.g. Sosiak, 2002), tissue stoichiometry (e.g. Koerselman and Meuleman, 1996), and stable isotope analyses have all been used to study macrophyte relationships with nutrients. However, in complex river environments, multiple tools are likely necessary for understanding eutrophication, effects on the macrophyte community, and the sources and sinks for important plant nutrients.

Natural variation in stable isotope ratios has been used to study nutrient dynamics in many environments. Isotopes are particularly useful for studying nitrogen cycles because of strong chemical and biological discrimination that favours ^{14}N over ^{15}N, leading to N processes that are traceable through the environment even under highly variable conditions. Specifically, human sources of nitrogen to aquatic environments can be identified using stable isotope analysis.

Typically, treated sewage tends to have high $\delta^{15}N$ values in the residual nitrogen released to the environment due to the effect of volatilization and nitrification on NH_4^+, and denitrification on NO_3^-. The $\delta^{15}N$ values for treated sewage can range from 8‰ to 20‰ higher than atmospheric N_2 (Kendall et al., 1998). Another important anthropogenic nitrogen source, inorganic fertilizers, is lower, or closer to 0‰, due to the Haber-Bosch process of atmospheric N_2 fixation (Kendall et al., 1998; Bateman and Kelly, 2007). High levels of inorganic fertilizer inputs to the landscape can lead to lower $\delta^{15}N$ values due to soil N cycling processes that typically yield $\delta^{15}N$ values of leachable-form NO_3^- in the range of 3‰ to 6‰.

The concentrations of the various nitrogen species in the environment alone cannot indicate their origin and, therefore, cannot link anthropogenic nitrogen enrichment to biological eutrophication. Nitrogen can be rapidly cycled among bacteria and algae, as fast as minutes in some streams (Mullholland et al., 2000; Tank et al., 2000). Concentration values are more akin to snapshots in time of continuous processes and eutrophic aquatic environments are not always high in dissolved nutrients. The difficulty in using concentration values to assess eutrophication is particularly acute for rivers where environmental heterogeneity greatly influences the fate of reactive N species. Many researchers have used macrophyte and macroalgal tissue $\delta^{15}N$ values in an attempt to assess the biological fate of isotopically distinct anthropogenic sources of nitrogen. Isotope values yield information regarding nutrient sources, rather than just nutrient quantities, if sources have distinct values. Macrophytes and macroalgae have been used in previous work to indicate the presence of wastewater derived nitrogen in a variety of environments such as coastal areas and marine bays (Rogers et al., 2003; Savage et al., 2004; Derse et al., 2007) estuaries (Grice et al., 1996; Dillon and Chanton, 2008), mangroves (Fry et al., 2000), and coral reefs (Yamamuro et al., 2003, Marion et al., 2005; Lin et al., 2007; Risk et al., 2009).

The use of macrophytes and macroalgae as sentinels of human N sources requires a number of assumptions. One assumption is that no fractionation occurs during plant uptake and assimilation. This assumption can be safely made in nutrient limiting conditions, where cells often have a high demand for nitrogen and do not discriminate between N isotopes. For example, Derse et al. (2007) found no evidence that macroalgae fractionate N, and Savage and Elmgren (2004) successfully used $\delta^{15}N$ isotopes to determine the extent of sewage N use in *Fucus vesiculosus* without accounting for fractionation because marine systems are frequently

nitrogen limited. However phosphorus, not nitrogen, often limits productivity in rivers and streams (Francoeur, 2001). Immediately downstream of waste water treatment plants (WWTPs), nitrogen and phosphorus are both in abundant supply and it may not be realistic to assume that macrophyte or macroalgae do not isotopically fractionate N sources during uptake. Yoneyama et al. (1991) describe substantial fractionation by wetland rice plants growing under N fertilized conditions, suggesting that isotopic discrimination may occur when N uptake is not limited by N concentration.

Using macrophytes as sentinels of N sources and constructing 2-source mixing models using macrophyte tissue often requires the assumption that no processing of the source N occurs between the N source and N uptake. If a mixing model is used where 2 (or more) N sources are suspected, typically the end members of the model are source values, and the tissue N values are said to result from proportional use of one source or the other. This assumption may not be valid if there is a time lag or distance between emission from the source and uptake by the plant which would allow for N processing. Because the processes that distinguish sources in the first place (fixation, nitrification, denitrification, volatilization) occur readily in aquatic environments, the N reaching the plant can be isotopically modified with many different factors controlling the degree of source processing. Fry et al. (2000) used $\delta^{15}N$ values to trace sewage impact on mangrove tree dwarfism. They suggested 2 possibilities to explain the difference in tree tissue $\delta^{15}N$ and source $\delta^{15}N$. If the tree $\delta^{15}N$ values were lower than source values, then fractionation at the plant level must be occurring. However, if tree $\delta^{15}N$ values were higher than source values, then processing of N must be occurring before it is received by the tree. It is likely that both of these situations occur and plant values lower than source N values may indicate fractionation, but it does not preclude that processing occurred prior to plant fractionation, or that another unidentified lighter source of N might have been involved. Given the complexity of N-cycle processes, it may be impossible to determine nitrogen sources using end-member values in some cases.

In many aquatic environments, including rivers, submersed macrophyte beds exist as mixed-species communities. There are exceptions, such as eutrophic coastal waters, where often a single species of algae dominates during a bloom event. Nitrogen uptake and isotope discrimination may vary among taxa depending on growth rate, growth stage and life cycle. Differential nitrogen use between species would have important implications for the use of

macrophytes as sentinels, especially when comparing results between systems or regions of the same system where species composition differs. However, species differences in nitrogen uptake may be useful for understanding macrophyte community responses to WWTPs and elevated N concentrations.

In this study I investigated the suitability of macrophyte tissue $\delta^{15}N$ values as sentinels of WWTP effluent presence in a eutrophic river environment. I tested 3 common assumptions made when using plant tissue N for tracking nitrogen sources: 1) that no important amount of nitrogen processing occurs between N source and uptake by plants, such that mixing models can be constructed from source and tissue $\delta^{15}N$ information; 2) that fractionation of isotopes during uptake and assimilation does not occur or is not important in river environments; and, 3) there are no differences in $\delta^{15}N$ among river macrophyte species at the same location.

4.2 Materials and Methods

This study was conducted in the Grand River, in southern Ontario. See the Materials and Methods section in Chapter 1 for a full description of the study area.

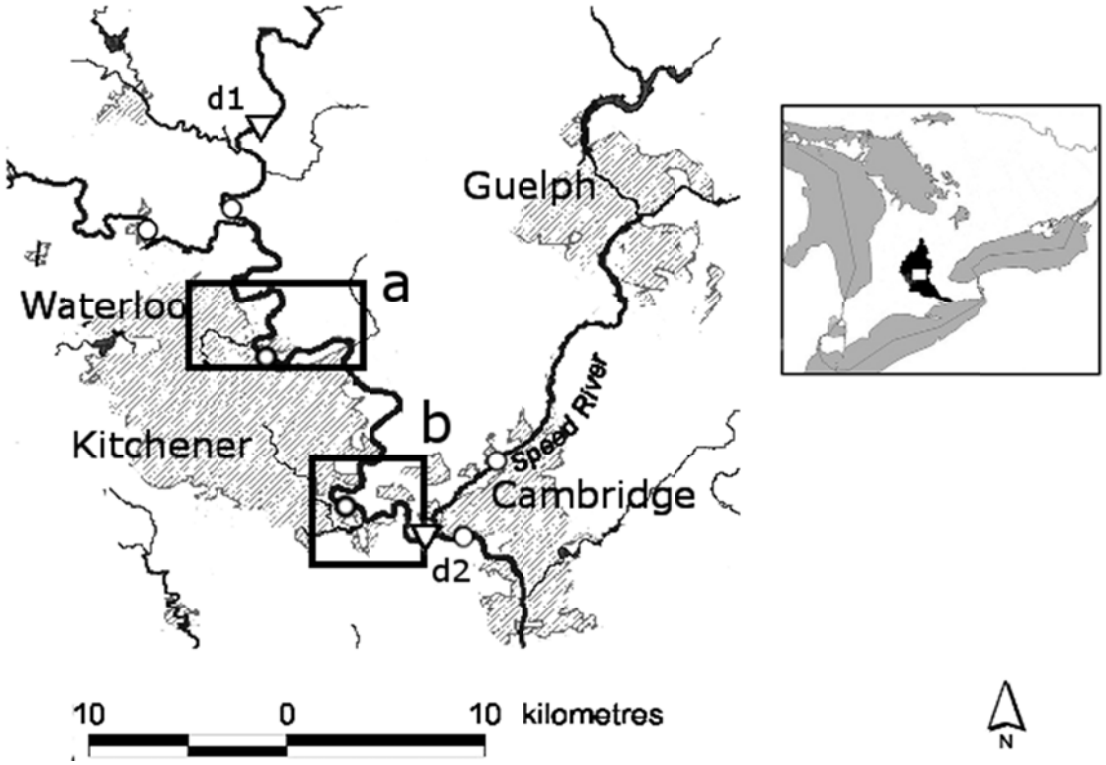

Figure 4.1 Site map showing the 2 survey reaches located within the Grand River watershed, Ontario, Canada. The reaches are both 10 km in length, with the more upstream of the two, the Waterloo reach (a) roughly 10 km upstream of the Kitchener reach (b). Both reaches include a large WWTP effluent outfall location, indicated by (O), in the center of the reach. Flow data was obtained from two Water Survey of Environment Canada discharge monitoring sites (D1 and D2), indicated by (▽).

Two reaches of approximately 10 km in length along the mid-reaches of Grand River starting 82 km downstream of a headwater monitoring station, were chosen for intensive study (fig. 4.1). This monitoring station is located on the Grand River shortly after it becomes a second order stream, and is located at 43.96723, -80.35494. Both the chosen reaches include an upstream and a downstream segment of approximately equal length, with a WWTP marking the boundary between upstream and downstream. The more upstream of the two reaches, the

Waterloo reach, is located within The City of Waterloo and flows past the Waterloo WWTP outfall. This WWTP is the 3rd largest in the watershed and has a discharge capacity of 7.3×10^4 m^3/d, with secondary treatment and partial nitrification of the effluent. The second reach, the Kitchener reach, is located about 15 km downstream of the Waterloo outfall and flows past the Kitchener WWTP outfall. The Kitchener WWTP is the largest in the watershed with a capacity of 1.2×10^5 m^3/day. It has secondary treatment with no specific treatment stage to remove ammonia.

Macrophytes were sampled in 10 approximately regularly-spaced locations in each reach. The top 10 cm of the vegetative growing tip of randomly selected macrophytes was sampled by hand and put into plastic zip lock bags and stored in a cooler. At each location of sampling, one macrophyte patch was chosen and each species found within the patch was sampled. Two to three replicate samples of abundant species were taken. In the downstream section of each reach, the plume was located using conductivity measurements, and water samples were taken at the centre of the plume. Where possible, macrophytes were sampled at the same location as water samples, but because macrophytes often were not found growing directly in the undiluted plume, a patch as close as possible to the plume was chosen for sampling. Water samples were collected for NH_4^+, NO_3^-, and Cl^- concentration measurement and N isotope analysis in HDPE bottles. For NH_4^+-N isotope analysis, samples were acidified upon collection. Water for isotope and concentration analysis was filtered immediately upon returning to the lab.

Table 4.1 Summary of daily river discharge m^3/s on each of the plume sampling occasions. Data provided by the Water Survey of Environment Canada. Two locations are provided, Site 1 (WMR) is 25 km upstream of both of the survey reaches, and site 2 (DN) is located downstream of the Kitchener WWTP, at the end of the Kitchener survey reach

Location	Plume sampling Date	Daily Discharge (m3/s)	
		D1: 541799, 4825956	D2: 550460, 4803999
Waterloo reach	2007-Aug-22	4.86	9.2
	2007-Oct-30	2.12	4.6
	2008-Jul-9	7.57	11.3
	2009-Aug-27	6.37	9.91
Kitchener reach	2007-Aug-27	4.86	9.2
	2007-Oct-23	2.37	6.83
	2008-Ju/l8	7.75	11.1
	2009-Aug-28	7.38	12.3

After collection, macrophytes were cleaned extensively with tap water, which was a combination of treated Grand River water and ground water. Surface deposits such as periphyton, calcium carbonate, both living and dead invertebrates (such as blackflies and caddis flies) and their retreats were removed by hand. After thorough cleaning, acidification of the macrophyte material was not necessary. Macrophytes were then identified to species and dried in industrial quality tin foil trays at 60 C for at least 48 h. After drying, macrophyte tissue was ground by hand with a mortar and pestle and weighed into tin cups for tissue $\delta^{15}N$ analysis.

Water collected for NH_4^+ and NO_3^- concentrations was filtered through cellulose acetate 0.2-µm pore-size membranes. NH_4^+ analysis followed Holmes et al. (1999). NO_3^- and Cl^- concentrations were determined by ion chromatography using a Dionex ion chromatograph composed of a GP50 pump, ASRS-4mm suppressor, an IonPac® AS22 column and CD25 conductivity detector. Water chemistry parameters were not corrected for plume dispersal downstream because of some locations lacked good measurements of Cl^- data for 2009 and 2008, therefore, concentrations downstream will be a result of dilution and other loss processes, and an estimate of a range of possible concentrations resulting from dilution will be given for the end of the survey reach.

Samples for $\delta^{15}N$ -NH_4^+ and $\delta^{15}N$ -NO_3^- were analyzed at the Environmental Isotope Lab located at the University of Waterloo, Ontario, Canada. Samples for $\delta^{15}N$-NH_4^+ analysis were prepared and analyzed using a modified acidified disk - PTFE trap method on a Finnigan Delta Plus Continuous Flow Stable Isotope Mass Spectrometer (Brookes et al., 1989). This method collects both NH_3 and NH_4^+ for isotope analysis, so values reported as $\delta^{15}N$-NH_4^+ are actually for both NH_3 and NH_4^+. Samples collected for $\delta^{15}N$-NO_3^- were analyzed with the silver nitrate method on the same instrument, following the method of Silva et al. (2000). Average error for both methods was 0.3 ‰, verified by analysis of duplicate samples.

The downstream Kitchener reach was surveyed by canoe in the afternoon on August 27th, 2009 and the upstream Waterloo reach was surveyed similarly on the afternoon of the following day, August 28th, 2009. Additional data used in this analysis were collected and analyzed in the downstream sections of the 2 reaches on August 22, August 27, October 23, October 30 of 2007, and July 1, 2008, using the same methods. As part of the survey effort in 2007 and 2008, the treated effluent was sampled before it was released to the river multiple times throughout the day on 2 occasions in August and October and analyzed for $\delta^{15}N$-NO_3^- and $\delta^{15}N$-NH_4^+ to obtain the range of $\delta^{15}N$ values produced by the WWTPs. Cl^- concentration was used to estimate what concentration values would be if dilution were the only processes changing values downstream, thus undiluted values and an estimate of the diluted values are reported in this study. Discharge data were obtained from Environment Canada's water survey hydrometric data archive. Discharge data (table 4.1) from 2 sites, upstream (D1) and downstream (D2) of the surveyed section are provided for each of the survey dates. River discharge varied among sampling dates with 01 July 2008 having the highest discharge and 30 October 2007 having the lowest discharge.

4.3 Results

The WWTPs at both Waterloo and Kitchener were significant sources of NH_4^+ on all sampling dates (fig. 4.2). The Waterloo WWTP was also a source of NO_3^- at some times of the year but the Kitchener plant was not (fig. 4.3). The concentration of NH_4^+ in the plume declined with distance from both WWTP on all sampling dates, but returned to background levels between the two treatment facilities, as seen by the near zero concentrations upstream of the Kitchener WWTP measured in 2009. The Waterloo WWTP released NO_3^-, and further downstream NO_3^- increased slightly and then did not decline over 10 km. The Kitchener plume NO_3^- concentrations, however, increased with distance, over the 5 km sampled downstream of the WWTP outflow. There was only one sampling location upstream of either WWTP in 2007 or 2008, so an upstream-downstream comparison was not possible for these years.

The trends in NO_3^- and NH_4^+ concentrations within the plume were similar on all sampling dates, with the exception that immediately downstream of the treatment plant there was no spike in NH_4^+ in August 2009 at the Waterloo or Kitchener WWTPs. It is likely that the water samples taken immediately downstream of the outfalls were not directly in the plumes, as the as indicated by Cl- data which was highest at the second site downstream of the plume. The Cl^- concentration allowed determination of what the NH_4^+ and NO_3^- concentrations would be downstream of the WWTP outfall had plume dilution been driving changes (also shown on fig. 4.2 and fig. 4.3). Without specifically correcting for dilution, these values indicate that processes other than dilution were altering NH_4^+ and NO_3^- concentration, as NH_4^+ values were lower than expected had only dilution been acting, while NO_3^- concentrations were much higher.

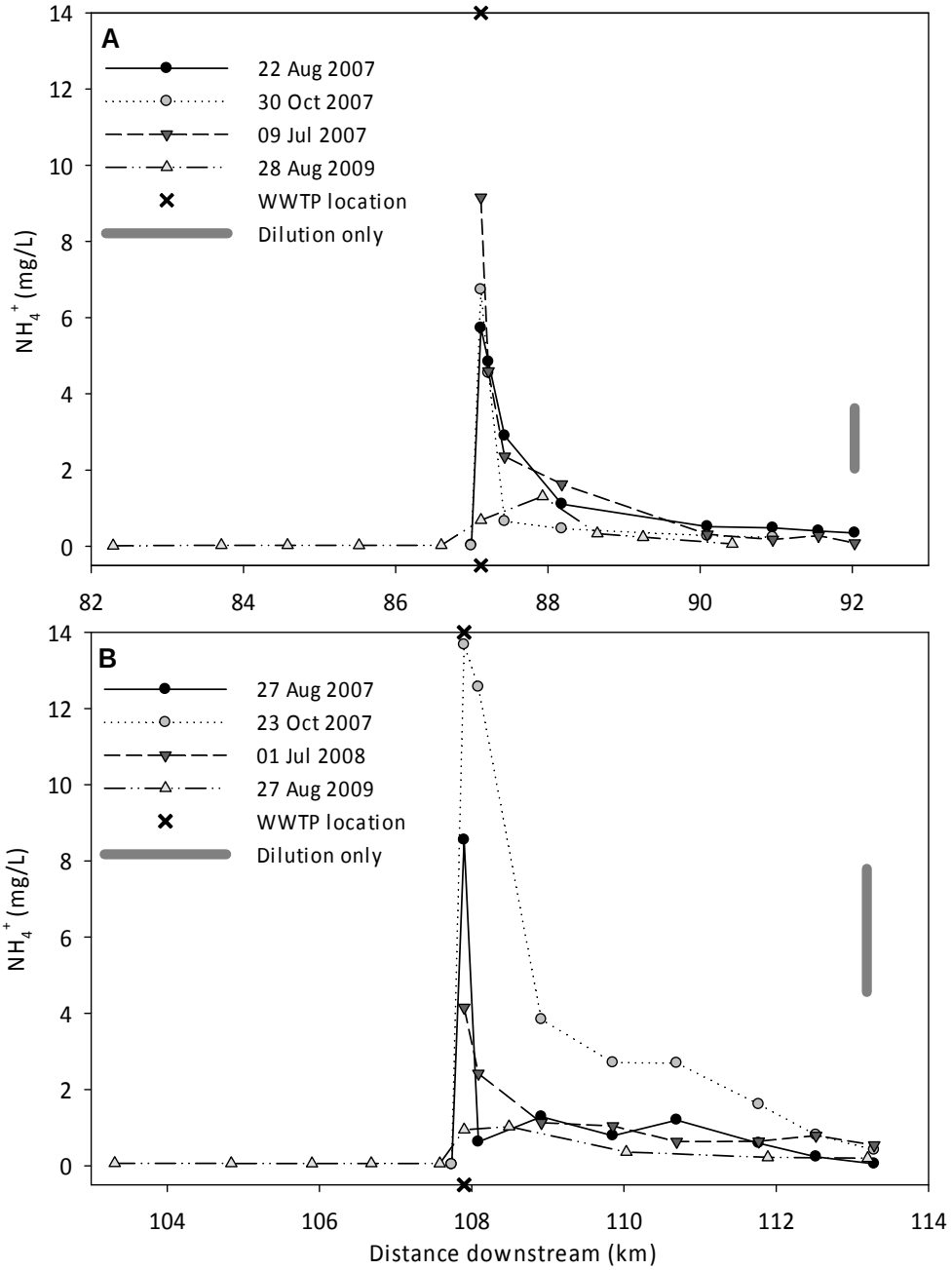

Figure 4.2 Ammonium concentrations in the surveyed reaches. Data from the upstream Waterloo (A) reach and downstream Kitchener (B) reach from 2007, 2008 and 2009 surveys is displayed as a function of distance downstream of headwaters site. The WWTP location (x) divides each reach between upstream and downstream segments. The grey bar represents what the NH_4^+ concentration would be if dilution was the only process acting to change WWTP effluent concentrations, and is based on Cl^- concentration.

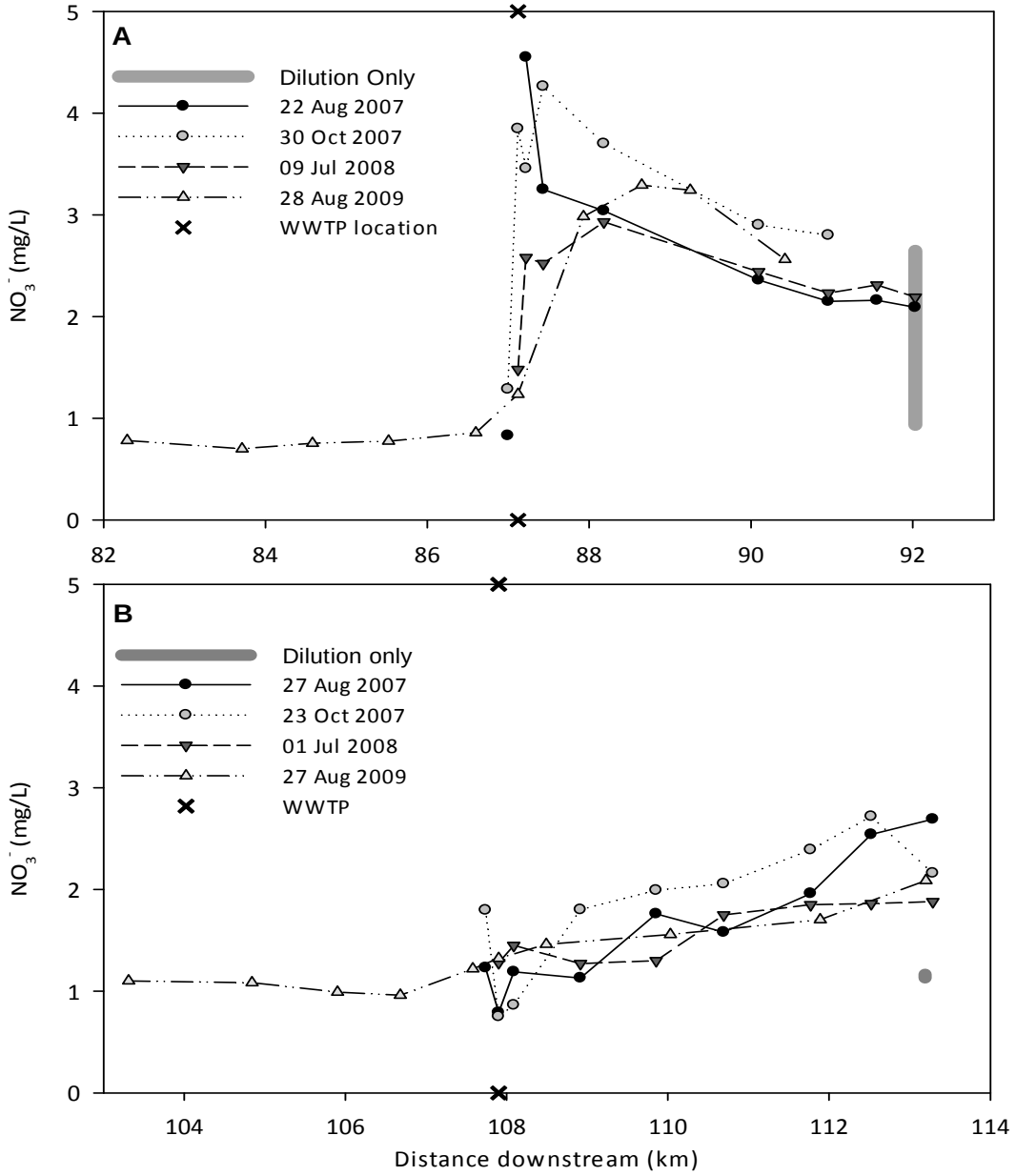

Figure 4.3 Nitrate concentrations in both the surveyed reaches. Data from the Waterloo reach (A) and Kitchener reach (B) from the 2007, 2008 and 2009 are displayed as a function of distance downstream of the headwaters site. The WWTP location (x) divides each reach between upstream and downstream segments. The grey bar represents what the NO_3^- concentration would be if dilution was the only process acting to change concentrations, and is based on Cl^- concentration.

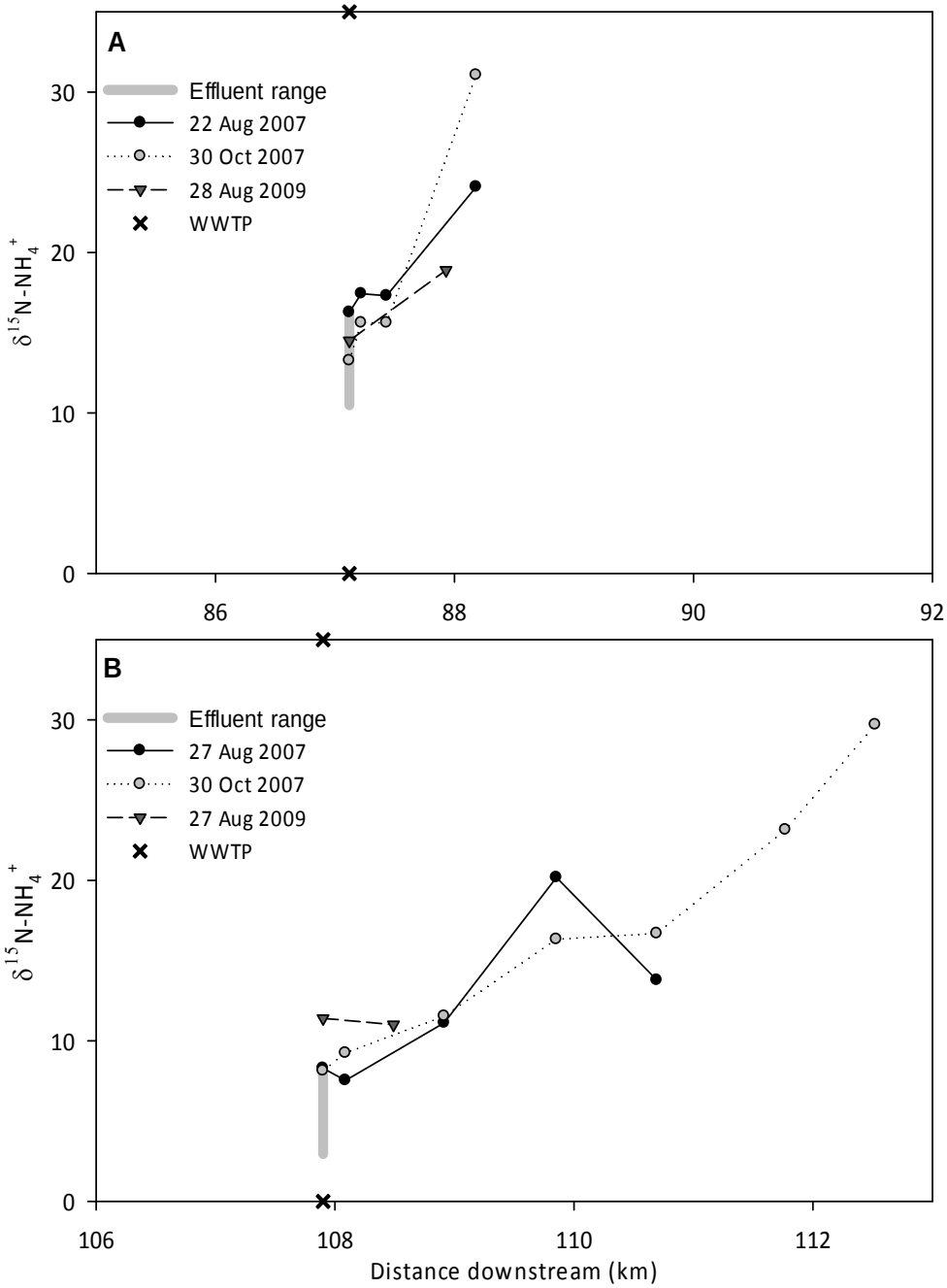

Figure 4.4 δ^{15}N-NH_4^+ in the Waterloo reach (A) and Kitchener reach (B) with distance downstream of the headwaters site, collected in 2007 and 2009. Values from the July 2008 survey were not available. The WWTP location (x) divides each reach between upstream and downstream segments. The bar at the location of the outfall indicates the range of values of the effluent measured at the outfall pipe for the Waterloo WWTP, and within the treatment plant itself for the Kitchener WWTP.

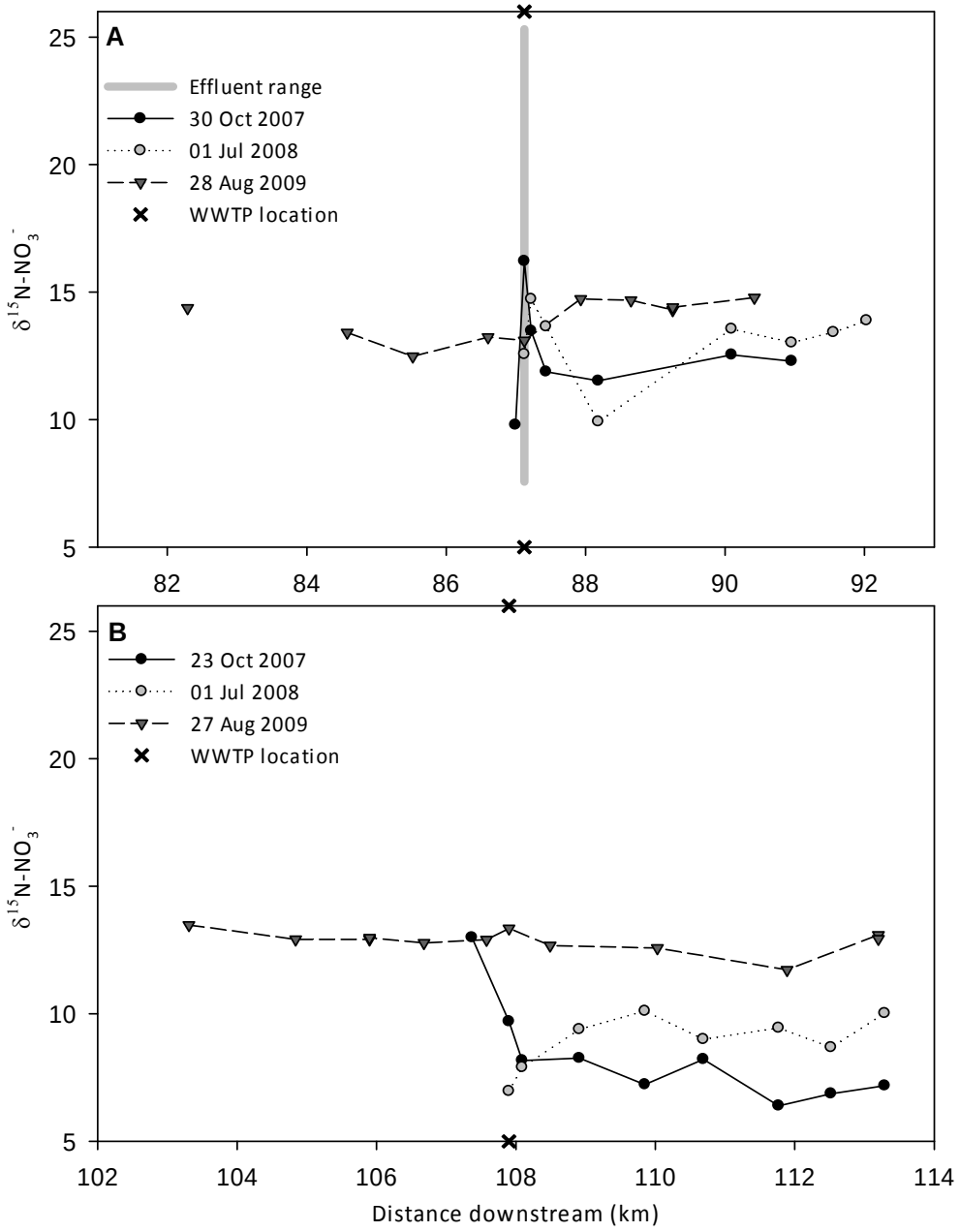

Figure 4.5 $\delta^{15}N$ -NO_3^- in the Waterloo reach (A) and Kitchener reach (B) with distance downstream of the headwaters site, collected in 2007, 2008 and 2009 surveys. Values from the August 2007 survey were not available. The WWTP location (x) divides each reach between upstream and downstream segments. The bar at the location of the outfall indicates the range of values of the effluent measured at the outfall pipe, before contact with river water.. Values were only obtained for nitrate of the Waterloo WWTP effluent because there is no nitrate release at the Kitchener WWTP.

The $\delta^{15}N\text{-}NH_4^+$ for both the Waterloo and Kitchener reaches (fig. 4.4) increased downstream of the WWTP in all years surveyed. Data only extends 1.5 km of the WWTP in the Waterloo reach because concentrations further downstream were below detection limits. In the downstream section of the Waterloo reach, values ranged from +16‰ to +24‰ in August, 2007, and +13‰ to +31‰ in October, 2007. The latter is an enrichment of 18‰ over 1 km. In 2009, only 2 samples were high enough in concentration to determine $\delta^{15}N\text{-}NH_4^+$ but over a distance of 0.72 km between these 2 sites values increased by 4.4‰. For the Kitchener reach, $\delta^{15}N\text{-}NH_4^+$ values increased downstream from +8.3‰ to +20‰ in August, 2007, and from +8.2‰ to +30‰ in October, 2007, an increase of 22‰ over 4.62 km. In 2009, the 2 sites with enough NH_4^+ to measure $\delta^{15}N$ were the same, +11.0‰ showing no trend over this 0.67 km distance, however the center of the plume was not sampled, and it is possible that directly in the plume the $\delta^{15}N\text{-}NH_4^+$ values were lower. Where NH_4^+ concentrations were high enough for $\delta^{15}N$ analysis, a trend of rapidly increasing $\delta^{15}N\text{-}NH_4^+$ values was found in the effluent plume downstream of both Waterloo and Kitchener WWTPs in all seasons and years sampled.

The Kitchener and Waterloo reaches both had similar trends in $\delta^{15}N\text{-}NO_3^-$. Both reaches showed high temporal variability with as much as 7‰ difference downstream of the Kitchener WWTP between October, 2007, and August, 2009 (fig. 4.5). Variability between seasons in 2007 was less, but also apparent at an average of about 2‰. In 2007, the $\delta^{15}N\text{-}NO_3^-$ increased at the outflow of the Waterloo WWTP, but then dropped within a few hundred meters. The average $\delta^{15}N\text{-}NO3$ value in August 2009 of the Waterloo plume was 1.3‰ higher than the upstream values and, in the Kitchener plume average $\delta^{15}N\text{-}NO_3^-$ values were 0.6‰ lower than upstream, but given the precision this difference is not significant. The $\delta^{15}N\text{-}NO_3^-$ in the Kitchener plume tended to be lower at each sampling date compared to Waterloo plume values. The effluent at the Waterloo WWTP appeared to have an increasing effect on $\delta15N\text{-}NO_3^-$ however the effluent at the Kitchener WWTP did not change the $\delta^{15}N\text{-}NO_3^-$, which is as expected as the Kitchener WWTP does not produce NO_3^-.

The $\delta^{15}N$ values of macrophyte tissue in both reaches of the 2009 survey followed a similar trend (fig. 4.6). Upstream of the WWTP at Waterloo, macrophyte $\delta^{15}N$ ranged between +9.2‰ and +13‰ with an average for all taxa at all sites of +12‰. Downstream of the Waterloo

WWTP, tissue $\delta^{15}N$ values showed an initial decrease immediately downstream, but with further distance they increased beyond the upstream values towards the end of the surveyed section. In the Waterloo reach, values ranged from +7.0‰ immediately downstream of the WWTP to +24‰ at the last site surveyed in the reach, a change of 17‰. The upstream section of the Kitchener reach, though considered "upstream" with respect the section immediately downstream of the Kitchener WWTP is still just 12 km downstream of the end of the Waterloo downstream reach, so we expected macrophyte tissue $\delta^{15}N$ values might be similar to those of the Waterloo downstream section, however the tissue $\delta^{15}N$ values were more similar to the values found upstream of the Waterloo plant; ranging from +12‰ to +16‰, 2‰ higher on average than the upstream values found in the Waterloo reach.

Immediately downstream of the Kitchener WWTP, tissue values were lower than those immediately downstream of the Waterloo WWTP. They declined to +5.7‰ then increased with distance downstream to +27‰, a change of 21‰ over 3.3 km. Because sampling stopped at the 113 km mark, approximately 5 km downstream of the Kitchener WWTP treatment plant, it is unknown whether values were even greater further downstream and at what point they began to decline, but it is possible that an even greater disparity between up and downstream existed had sampling continued just a few more kilometers downstream. Although it was apparent that in 2009 water sampling immediately below the Kitchener WWTP missed the center of the effluent plume, the lowered $\delta^{15}N$ values of macrophyte tissue, close to 6‰, were within the range of values found in the treatment plant (of 4‰ to 6‰) and were substantially lower than those immediately upstream of the WWTP outfall, indicating that while macrophytes were not growing directly in plume as determined at the time of sampling, at least some of the time, macrophytes must reside within the effluent plume and incorporate effluent N into their tissues. Effluent plume can migrate based on river flow and temperature, but it is unknown in the Grand River how much lateral migration can be expected.

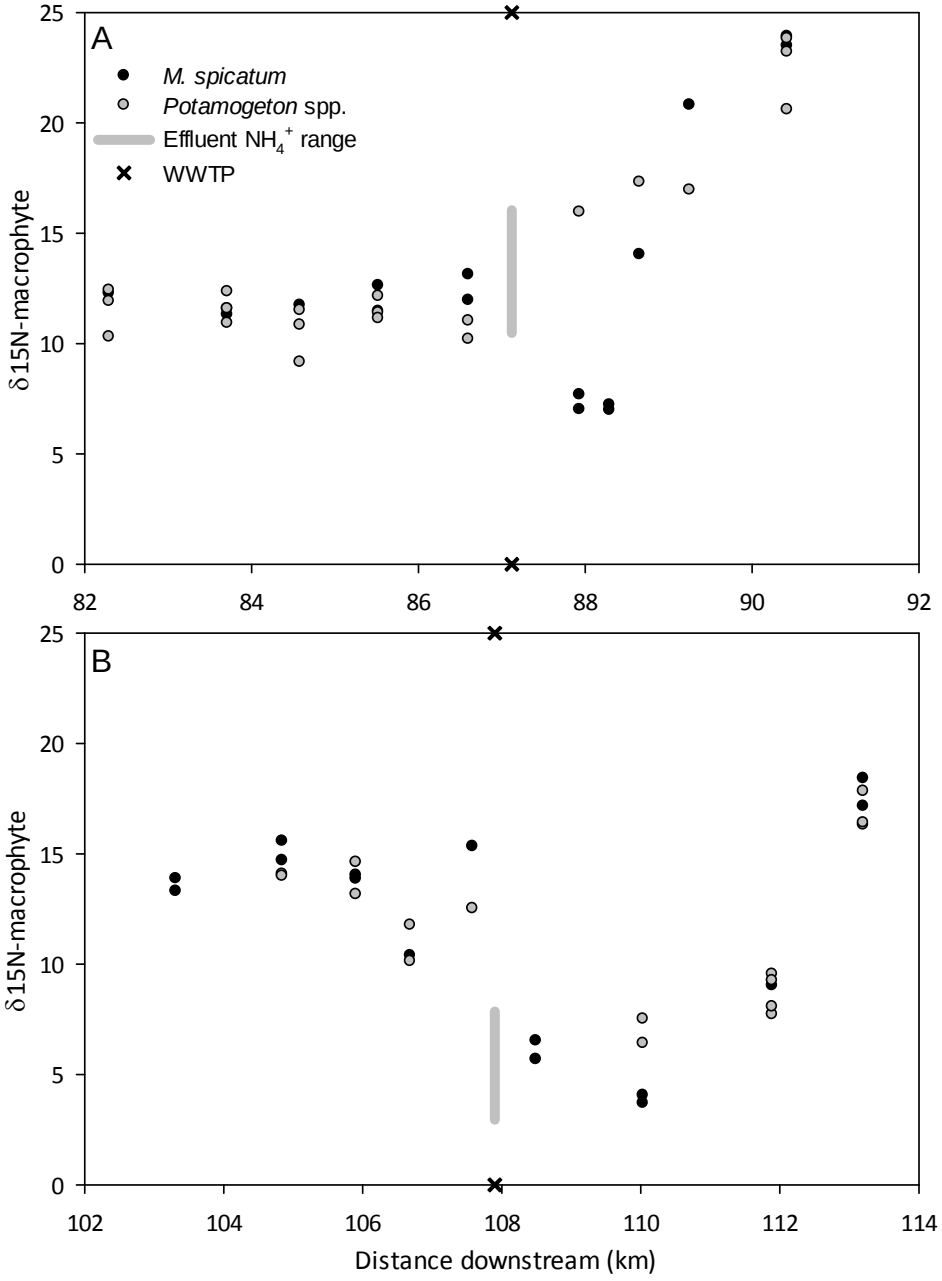

Figure 4.6 δ15N of Potamogeton spp. and M. spicatum collected in the Waterloo reach (A) and Kitchener reach (B) with distance downstream of the headwaters site. Samples were collected in August 27 and 28, 2009. The δ15N value of WWTP effluent NH4+, before it contacts river water, is indicated on the graph with a grey bar at the location of the WWTP, which is also indicated on the graph (x) on the x-axis.

The $\delta^{15}N$ values of macrophytes exhibited high variation within and among species, particularly downstream of WWTPs (fig. 4.6). Not all species were found at each sampling location, so for ease of comparison we pooled all members of the Potamogetonaceae, which includes all Potamogeton and Stuckenia genera (Crow and Hellquist, 2000; Lindqvist et al., 2006) into 1 group called "*Potamogeton* spp." (table 4.2). The remaining species with low representation, E. canadensis, was left out of the analysis but it was not an outlier from the general spatial trends. The range of macrophyte $\delta^{15}N$ values at each site was small compared to the overall range found in the data set, and was also smaller than the range of values within species across sites. The rapid and strong increase in $\delta^{15}N$ values downstream of the WWTP was the most prominent trend observed in these data. Looking only at the upstream sites, where site differences in $\delta^{15}N$ values were much less pronounced, significant differences between *M. spicatum* and *Potamogeton* spp. at upstream sites were found (students t-test, P = 0.02), with *M. spicatum* having higher $\delta^{15}N$ values than *Potamogeton spp.,* however the difference is small and may not be ecologically relevant. It was not possible to determine if there were species differences downstream due to the prominent enriching trend making individual sites different enough from each other to mask any differences among species.

Table 4.2 List of taxa sampled in the plume survey in the Waterloo and Kitchener reaches, and the number of locations they along the survey they were found to be growing.

Taxon	Number of locations
E. canadensis	2
M. spicatum	18
P. crispus	5
P. foliosus	3
P. zosteriformis	10
S. pectinata	13

4.4 Discussion

Concentrations of NH_4^+ and NO_3^- downstream of both the Kitchener and Waterloo WWTPs indicate that substantial processing of nitrogen occurred in the plume. Both of the WWTPs released large quantities of NH_4^+, which declined rapidly downstream, but even after 5 km were still 3 to 7 times higher than upstream values. Because the decrease in NH_4^+ was much greater than might be expected from dilution alone, I infer that chemical and biological loss processes such as volatilization, nitrification and biological uptake drove this change. In the Grand River, the daytime pH can reach 9.0 and, with NH_4^+ having a pKa value of 9.3, volatilization is likely an important process. The increasing NO_3^- concentration found downstream the WWTP outfalls is also indicative of nitrogen processing. The NO_3^- concentration in the Waterloo plume was higher than upstream values, and this was expected as the Waterloo plant partially nitrifies the effluent. The further increase in NO_3^- downstream of the outfall was likely due to nitrification occurring in the plume, as some NH_4^+ is also released in the effluent. The Kitchener WWTP however, does not nitrify wastewater, so the WWTP is not an important source of NO_3^-. Although the river below the Kitchener outfall can at times become hypoxic, even during the day (Rosamond et al., 2011; M. Anderson, GRCA, personal communication) this doesn't appear to inhibit nitrification from producing nitrate in the plume during the day.

The increasing trend in $\delta^{15}N$ -NH_4^+ downstream of the WWTPs (a change in 18‰ at Waterloo and 22% at Kitchener) is most likely due to the strong isotopic fractionation associated with nitrification and volatilization (Delwiche and Steyn, 1970; Mariotti et al., 1981; Brandes and Devol, 1996; Peterson et al., 2001) and possibly biological uptake (Yoneyama, 1991). With DO changing dramatically on a 24 h cycle in the Grand River (Rosamond et al., 2011) nitrification, and denitrification can readily occur. With daytime photosynthesis of macrophytes providing ample oxygen for nitrification, and turbulent flow over shallow riffle environments providing for high rates of gas exchange, NH_4^+ released by WWTPs can be quickly converted into NO_3^-, volatilized as NH_3 or taken up by river organisms. Although the trend of increasing $\delta^{15}N$-NH_4^+ was present at every sampling event, the trajectory of the trend differs among sampling dates for both $\delta^{15}N$-NH_4 and $\delta^{15}N$-NO_3^-, demonstrating the highly variable nature of $\delta^{15}N$ in rivers and the variability of N in effluent output.

Macrophyte tissue $\delta^{15}N$ changed markedly over the sampled reaches becoming more positive with distance below the WWTP at both locations. This change is large compared to other studies of $\delta^{15}N$ values, and the largest range we are aware of in the literature. The values are among the most enriched found in general for aquatic plants and algae. Hesslein et al. (1990) found lake macrophyte and moss $\delta^{15}N$ values of +3.4‰ and +5.8‰ in the northern Mackenzie River basin, while Grice et al. (1996) found values in Moreton Bay seagrasses between -4.5‰ and +8.8‰, with the most enriched values at sites nearest WWTPs and the variation being related to distance from the WWTP. Fry et al. (2000) found mangrove leaf $\delta^{15}N$ values range from +2‰ to +12‰, with higher values closer to human effluent sources. Marine macroalgae were found to range from 8‰ to 9‰ near a WWTP in Himmerfjärden Bay, Sweden (Savage and Elmgren, 2004), and -4‰ to +4‰ in Hanalei Bay, Hawaii (Derse et al., 2007). The large range in river macrophyte $\delta^{15}N$ values demonstrates that river reaches below nutrient point sources are highly dynamic and variable environments. It is evident in this study that the changes in macrophyte tissue $\delta^{15}N$ reflect the changing values of their N source with distance from the WWTP, and that source values rapidly change due to chemical and biological transformations of nitrogen.

The rapid increase of macrophyte $\delta^{15}N$ downstream of the WWTP reflects the same trend in $\delta^{15}N\text{-}NH_4^+$. As well, the concentration of NH_4^+ decreases rapidly downstream of WWTPs to levels similar to upstream but macrophyte $\delta^{15}N\text{-}NH_4^+$ values continue to increase to well above that of $\delta^{15}N\text{-}NO_3^-$ indicating that macrophytes were incorporating primarily NH_4^+ as their N source downstream of the WWTPs. I could not use a mixing model to determine the contribution of NH_4^+ and NO_3^- to macrophyte $\delta^{15}N$ values due to the rapid increase in effluent $\delta^{15}N\text{-}NH_4^+$ downstream, and the variability in that increase, nor could I determine the proportion of N that came from WWTP effluent due to a lack of $\delta^{15}N$ data from upstream locations.

The preferred source of N to algae and aquatic plants is generally NH_4^+ (Yoneyama, 1991; Wyman and Bird, 2007). Although the concentration of NH_4^+ is analytically low in the upstream reaches of the Grand River, macrophytes may still be using it as their primary source of N. Concentration values alone cannot provide information on which form of DIN is most important as, despite low concentration, rapid cycling of NH_4^+ upstream of N sources is likely (Mulholland et al., 2000). The similarity of macrophyte $\delta^{15}N$ to $\delta^{15}N\text{-}NO_3^-$ values could indicate that

macrophytes were using NO_3^- as a source of N upstream of the WWTPs, but as $\delta^{15}N\text{-}NH_4^+$ values are not known it is not possible draw this conclusion with certainty. It is just as possible that $\delta^{15}N\text{-}NH_4^+$ values upstream were quite similar to $\delta^{15}N\text{ -}NO_3^-$ values, especially if rapid cycling between sediment, biota and water was occurring, and similar source $\delta^{15}N$ values would not allow for determination of proportional usage of NH_4^+ versus NO_3^-.

Although low concentrations of NH_4^+ produce a condition that might be described as "ammonia limited" (chapter 5) where NH_4^+ uptake is a linear function of concentration and where macrophyte N uptake would include use of NO_3^-, the presence of active transport enzymes in plant cells for the purpose of ammonia uptake (Herrero et al., 2001) would allow macrophytes to be biased in their N source and lead to a greater use of NH_4^+ than might be predicted from relative concentrations of DIN species. NH_4^+ concentrations in upstream sections of the river are so low it that it may be reasonable to assume that no substantial fractionation occurs during uptake, and $\delta^{15}N$ values of macrophytes reflect that of their N source. If macrophytes have a strong preference for NH_4^+, even when concentrations of NH_4^+ are low, their $\delta^{15}N$-values might be good surrogates for $\delta^{15}N\text{-}NH_4^+$, assuming that any NO_3^- use by plants is small and is relatively constant. However, without a more complete set of $\delta^{15}N\text{-}NH_4^+$ values it is difficult to make an inference about how much NO_3^- is being used macrophytes in the Grand River, and this should be determined before the use of macrophytes as indicators of $\delta^{15}N\text{-}NH_4^+$ can be recommended.

Many studies using $\delta^{15}N$ tracers of wastewater effluent in aquatic systems report a decreasing trend in $\delta^{15}N$ values with distance from emission source (eg. Savage and Elmgren, 2004; Lin et al., 2007; Barile and Lapointe, 2007; Risk et al., 2009) with only one other study reporting an increasing trend with distance away from the WWTP. Rogers (2003) found macroalgae tissue $\delta^{15}N$ in a coastal zone of New Zealand increased from +2.3‰ to +5.7‰ over a distance of about 500 m from the WWTP outfall. Based on our findings, we believe that in all of these studies an increasing trend away from the source may have been occurring, but at a much smaller spatial and temporal scale than was sampled. In this study, an increasing trend was still observed in macrophyte tissue $\delta^{15}N$ values after 5 km, however, a gradual decrease in $\delta^{15}N$ values further downstream would be expected as the point source is diluted by new DIN inputs. The upstream end of the Kitchener reach was located 10 km downstream of the last sampling location of the Waterloo reach, and macrophytes $\delta^{15}N$ values in the Kitchener

upstream were only slightly higher than those observed in the upstream section of the Waterloo reach. Thus, over the 10 km of distance between these 2 locations, macrophyte tissue $\delta^{15}N$ values gradually decreased with distance from the WWTP, as the influence of point source derived nitrogen diminished and recycling became more dominant. This finding is similar to other studies of $\delta^{15}N$ tracers of wastewater in aquatic systems, where the increasing effect of sewage $\delta^{15}N$ disappeared with distance from source (Savage and Elmgren, 2004; Lin et al., 2007; Barile and Lapointe, 2007; Risk et al., 2009).

This study suggests that macrophytes downstream of the WWTPs may discriminate against δ15-N-NH_4^+ upon uptake. Macrophyte $\delta^{15}N$ values are much lower than the $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-NO_3^- found at the same site in all locations, and the lowest values in the Waterloo reach are lower than the values found in effluent before it leaves the WWTP. In these downstream locations, it is highly unlikely that there are other sources with lower $\delta^{15}N$ values that would not be also available to macrophytes at other sites. Evidence from other work conducted by our lab suggests that night time values in the Kitchener effluent plume are not lower than the lowest macrophyte values. In July 2010, in similar locations downstream of the Kitchener WWTP as were sampled in this study, night values of $\delta^{15}N$-NH_4^+ were +10 ‰ to +12‰ immediately downstream and +14‰ to +16‰ further downstream (E. Cejudo et al., personal communication). The lowest $\delta^{15}N$-NH_4^+ values measured in this study came from effluent directly within the Kitchener WWTP at +3.0‰. These values are not likely indicative of typical $\delta^{15}N$-NH_4^+ values in locations where macrophytes grow because, as discussed previously, the processes that occur immediately at the outfall of the WWTP lead to a rapid increase in $\delta^{15}N$-NH_4^+ values, making this source value of 3.0‰ an unlikely end-member value. We did not measure $\delta^{15}N$-NH_4^+ values of pore water accessible to macrophyte roots.

Where NH_4^+ concentrations were high downstream of the WWTPs, macrophyte $\delta^{15}N$ values were lower than the river $\delta^{15}N$-NH_4^+ at the sample location indicating that some fractionation of NH_4^+ during uptake by macrophytes occurred. Fractionation and assimilation of NH_4^+ by wetland macrophyte species was documented in fertilization studies (Yoneyama et al., 1991; Yoneyama, 1995) with fractionation being stronger at higher concentrations of NH_4^+. Although some sources claim that aquatic macroalgae do not fractionate N (Costanzo et al., 2001) these findings for marine systems are likely due to nitrogen limitation. Under N limitation, macrophytes and algae would use any NH_4^+ present and fractionation would be minimal. But

even in some marine situations, fractionation during NH_4^+ uptake does occur, as was found in marine diatoms (Waser et al., 1998). In eutrophic rivers such as the Grand River, isotopic fractionation of NH_4^+ by macrophytes below WWTPs where NH_4^+ is abundant is a more likely scenario as uptake is likely not nitrogen limited. It is possible that isotopic fractionation varies spatially and temporally with the availability of NH_4^+, and that it is minimal in upstream sections when NH_4^+ concentrations are very low. Fractionation by macrophytes should be strongest when macrophyte growth is slow due to some other factor besides N-limitation (MacLeod and Barton, 1998; Goericke et al., 1994 in Fry et al., 2000). The effect of macrophyte fractionation on river nitrogen compared to other processes acting on the $\delta^{15}N$ value of NH_4^+ is beyond the scope of this work, however its effect on the interpretation of $\delta^{15}N$ values of macrophyte tissue is an important consideration when using $\delta^{15}N$ of macrophyte tissue as indicators of WWTP effluent in rivers. Obtaining a value for a maximum expected fractionation under fertilized conditions in effluent plumes might be useful.

Fry et al. (2000) highlighted the importance of fractionation when using plant tissue for tracing point source impacts. The first scenario they described is one of plant-level regulation where plants fractionate $\delta^{15}N$ upon uptake. The conditions that promote or deter discrimination, such as nitrogen limitation, would then be most important in explaining the $\delta^{15}N$ variations that are measured. If strong fractionation occurred in some locations, then plant $\delta^{15}N$ should be lower than their source N. The second scenario is of system-level regulation of $\delta^{15}N$ values where processes occurring outside of the plant are the most important in explaining the variation in $\delta^{15}N$ values. Under this scenario, tissues would be higher than source $\delta^{15}N$ values because volatilization, nitrification and denitrification acting before plant uptake would leave behind residual substrates with higher $\delta^{15}N$ values. Plants using this processed N would then have higher $\delta^{15}N$ values. In our study of river macrophytes, it is apparent that both scenarios apply. Immediately downstream of the WWTPs, macrophytes are depleted and are lighter than any N found in the river which suggests fractionation. However, macrophytes became more enriched further downstream and their tissue reflects nitrification and volatilization as water moves downstream, showing system level influence on tissue $\delta^{15}N$ values.

This study indicates that there are modest differences in tissue $\delta^{15}N$ values among macrophyte species found in the same sampling location, however significant differences were

only found among species in locations upstream of the WWTPs while no differences were found downstream. Because macrophyte $\delta^{15}N$ values increased so rapidly downstream of the WWTPs, sites were different enough from each other such that, variation with species but across sites was too high for species differences to be detected. Even upstream, the variation in tissue $\delta^{15}N$ values within a site was lower than the variation within species across all sites. Spatial variation is thus highly important, even when patches fairly close to one another are sampled and there are no major changes in sources of nitrogen. Even though the spatial variability was large, *M. spicatum* and *Potamogeton* spp. were shown to have different $\delta^{15}N$ values in upstream sites. Differences in $\delta^{15}N$ values could be the result of physiological differences in N uptake or recycling rates, fractionation effects, and preferences for NH_4^+ over NO_3^-. Tilman (1987) suggests that to avoid direct competition, species will perform optimally under different environmental conditions. In this situation, the differences in $\delta^{15}N$ values of macrophytes found over the small distance surveyed may be indicative of these kinds of differences in macrophyte resource-use strategies. Without further study it is difficult to say how differences in ^{15}N translate into differences in ecology.

Rivers are active environments with N-cycle processes acting rapidly or concurrently on NH_4^+ and NO_3^- (Kelso and MacCrimmon, 1969) resulting in highly altered $\delta^{15}N$ values of the substrates, furthermore, $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-NO_3^- values change seasonally and even daily (Schiff et al., unpublished data), possibly with variations in temperature, flow, and diel O_2 cycles, thus creating complex pathways into which stable isotopes may provide only limited insight. However, stable isotope tracers can nevertheless be useful tools for understanding N-cycle processes and the link between autotrophs and nutrients in river environments when interpreted cautiously and when used in combination with other tools. In this work we evaluated the use of macrophytes as indicators of DIN from WWTP in a large lowland river by looking at three common assumptions made when using macrophytes or macroalgae. First we found that substantial nitrogen processing, likely in the form of nitrification and volatilization, occurs from the site of effluent discharge to the site of macrophyte uptake resulting in a substantial increase in $\delta^{15}N$-NH_4^+ values within a short distance from the effluent outfall. Rapid changes in $\delta^{15}N$ after source emission invalidates the application of a two end-member mixing model because end member values are no longer relevant by the time effluent reaches the macrophyte bed. Second, we found evidence for fractionation of N immediately downstream of

WWTP outfalls, where DIN concentrations were high. In environments where N is not limiting, it is unwise to make the assumption that fractionation is not occurring, and consideration of fractionation is necessary when using macrophytes as indicators of point source DIN $\delta^{15}N$ values. Third, this study provides evidence for differences in $\delta^{15}N$ values by taxon, but only in upstream locations when the variation among taxa was detectable against the spatial variation. Thus, data from multiple species should be pooled with caution.

In general, macrophytes record the presence and pattern of WWTP effluent N, particularly $\delta^{15}N\text{-}NH_4^+$. Macrophyte $\delta^{15}N$ values can be useful tools for tracing the downstream distance wastewater effluent NH_4^+ can reach, as an integrated measure of $\delta^{15}N\text{-}NH_4^+$ at reach scale or smaller, when consideration is given to the possibility of fractionation and differences among taxa. It is important to consider the possibility that macrophytes may use NO_3^- when NH_4^+ concentration is low, and it is still unknown under what conditions macrophytes will be reliable indicators of $\delta^{15}N\text{-}NH_4^+$. Traditional two-end-member mixing models are inappropriate under the conditions where the source values change rapidly over small distances and short periods of time. Macrophyte tissue $\delta^{15}N$ has the advantage of being relatively inexpensive and easy to sample, process, analyse and store compared to $\delta^{15}N\text{-}NH_4^+$ and $\delta^{15}N\text{-}NO_3^-$. Macrophyte tissue also integrates $\delta^{15}N$ values over time, smoothing over some temporal variation and providing information about average $\delta^{15}N$, although the period of integration is unknown. Macrophyte tissue isotopes can supplement other types of information and enhance our understanding of N-cycling and anthropogenic nutrient enrichment of complex river environments.

Chapter 5: Changes to N cycle processes in a macrophyte dominated river: a closed chamber experiment

5.1 Introduction

Eutrophication of a river can result in excessive algal and plant growth, depressed O_2 and a host of biological changes such as changes to benthic and planktonic communities, fish kills, proliferation of toxic species and dominance of tolerant and invasive species (Davis 1975; Smith et al., 1999; Chambers et al., 2006; Tyler et al., 2007; Hecky and Schindler, 2009), but few studies exist on the effect of altered trophic conditions on biogeochemical cycling in large river networks. Biogeochemical transformations of nitrogen species are intense in environments with oxic/anoxic interfaces and strong redox potentials, such as in the metalimnia of lakes with an anoxic hypolimnion, the interface between oxic water and anoxic sediments (Chan and Campbell, 1980; Rysgaard et al., 1993), or at terrestrial-aquatic interfaces (McClain, 2003). The redox discontinuities in rivers and streams are also strong, due to adjacent air/sediment/water interfaces, and thus they are highly active environments for nitrogen cycle transformations (Hill, 1979; Laursen and Seitzinger, 2004). Macrophyte roots are also active sites for denitrification activity, due to oxic zones that roots generate in anoxic sediments, as well as the carbon they provide to bacteria (Howard-Williams, 1985). Due to the change in dissolved oxygen (DO) conditions resulting from eutrophication, riverine N cycle processes of nitrification and denitrification may be altered through a variety of mechanisms.

Denitrification and nitrification, important biological processes of the nitrogen cycle of freshwater systems, can be tightly linked to oxygen cycling. Denitrification occurs as an alternative to respiration when DO is low (Rysgaard et al., 1994; Seitzinger, 1988). Nitrate is reduced to N_2O and then to N_2 by organisms capable of using nitrate as a terminal electron acceptor in the electron transport chain. These organisms are often facultative anaerobes and, given the availability of oxygen, would metabolize aerobically. Nitrification involves the conversion of NH_4^+ to NO_3^-, and is an oxidative process. While NH_4^+ is generally found to be most abundant in anoxic regions of lakes or streams, nitrification activity requires oxygen and is greatest when steep oxygen gradients are found. Nitrification may be co-limited by the availability of ammonia, the absence of DO and the presence of labile organic carbon (Hall and

Tank, 2003; Kemp and Dodds, 2001). Nitrogen cycle processes are also influenced by physical factors such as current velocity and temperature, and by pH.

In a large river, N-cycle bacteria largely exist in the sediment and in epiphytic or epilithic communities where they must compete for space and resources while being grazed by bacterivores, resulting in multiple ecological controls determining their response to eutrophication. Microbial biomass in streams is controlled by both top-down and bottom-up effects (Hillebrand and Kahlert, 2002) so one may expect the biomass of organisms involved in N-cycle transformations to also be affected by these processes. Hall and Tank (2003) found N uptake to be coupled with metabolism and photosynthesis via carbon uptake, such that enhanced primary productivity led to greater N uptake in the benthic community. It has also been demonstrated that oxygen availability, which is also controlled by community metabolism, can influence N-cycle process by altering coupled nitrification-denitrification. Increased DO can lead to increased rates of coupled nitrification-denitrification, resulting in increased N loss from the stream system (Rysgaard et al., 1994; An and Joye, 2001), and other studies have shown that high DO can be associated with decreased nitrification in competition with NH_4^+ uptake by benthic algae (Rysgaard, 2001; Dong et al., 2000). In eutrophic waters with high quantities of labile carbon and inorganic nitrogen, it is unlikely that nutrients or competition for N would limit the growth and activity of nitrifiers and denitrifiers, However there are many times and locations in the Grand River where ammonia is close to zero, and could limit the activity of nitrifiers. If nutrients are not limiting, it is possible that other ecological factors such as grazing and competition for space with other non-nitrifying and denitrifying organisms are important, and may explain some of the contradictory findings (Rysgaard, 2003; Muylaert et al., 2002). Rosamond et al. (2011) found coupled diel patterns of DO and N_2O the eutrophic Grand River, but N_2O was not simply a function of available NO_3^-, indicating that other factors may be involved in the regulation of nitrification and denitrification. Understanding ecological interactions among members of the benthic microbial community is thus important for N-cycle processes in the riverine nitrogen cycle.

In lakes, the addition of the nutrient most limiting to pelagic algal biomass, usually phosphorus, can lead to a massive response in these communities (Schindler, 1976). However no such experiment has been carried out for large rivers, and there is no consensus on a conceptual model of the eutrophic response in rivers (Hilton et al., 2006). However there is

evidence that anthropogenic addition of either N or P fuels nuisance macrophyte and filamentous algal biomass (Carr et al., 1998; Sosiak 2002; Chapter 2).

Many aquatic primary producers are P or N limited at times (Schindler, 1977; Hecky and Kilham, 1988; Grimm 1986; Francoer, 2001; Suttle and Harrison, 1988), and the biomass of the heterotrophic microbial community may be limited as well. Heterotophic microbial communities involved in leaf litter decomposition in streams have been found to be N or P limited (Aumen et al., 1983; Elwood, 1981; Grimm, 1986). Although evidence suggests that river biota can be either N or P limited (Francoer, 2001), in eutrophic waters such as the Grand River where the focus of nutrient management efforts is heavily weighted on P control and removal, and reactive N species like nitrate continue to increase, it is likely that microbial communities are more frequently P limited rather than N limited unless they are unable to utilize NO_3^-.

Nuisance macrophyte biomass development may be an agent of biogeochemical change in eutrophic rivers. They are already considered ecosystem engineers for their ability to alter flow regimes (Riis and Biggs, 2003; Franklin et al., 2008), diel oxygen cycles (Caraco and Cole, 2002), sediment stability (Schulz et al., 2003; Sand-Jensen et al., 1989), and habitat for benthic invertebrates and fish (Mainstone and Parr, 2002), and for their effect on the heterotrophic microbial community could also be important. Macrophytes increase the amount of surface area for biofilms that contain organisms capable of nitrification and denitrification. Thus as macrophyte biomass increases, the area of biofilm and its population of heterotrophic bacteria also increases, which could result in increased river system DIN uptake, nitrification and denitrification. Through the photosynthesis and respiration of the increased macrophyte biomass, larger diel fluctuations in river DO are produced in the summer, leading to stronger redox cycles and a diel pattern of nitrification and denitrification that follows the diel cycling of oxygen (Laursen and Seitzinger, 2004; O'Brien et al., 2007; Thuss, 2008). Conversely, increased macrophyte biomass may have inhibitory effects on microbial nitrogen cycle processes. Macrophyte biomass in a river represents a seasonal sink for P, as well as for NH_4^+ and NO_3^-, placing macrophytes in competition for substrates with microbes involved in the nitrogen cycle. River trophic conditions may have consequences for N storage and downstream transport as well as N loss to the atmosphere as either N_2 or N_2O (a potent greenhouse gas), with macrophytes playing an important role in how this processes will be altered.

The alteration of N cycle processes resulting from increased river trophic condition, coupling with P cycling and primary producer biomass, was investigated using *in-situ* chamber incubations, nutrient manipulations, and ammonia stable isotope tracers. The response in N cycle processes was measured as changes to assimilative N-uptake and N_2O production.

5.2 Materials and Methods

This study was conducted in the Grand River, in southern Ontario. For a detailed description of the watershed, see Materials and Methods in Chapter 1.

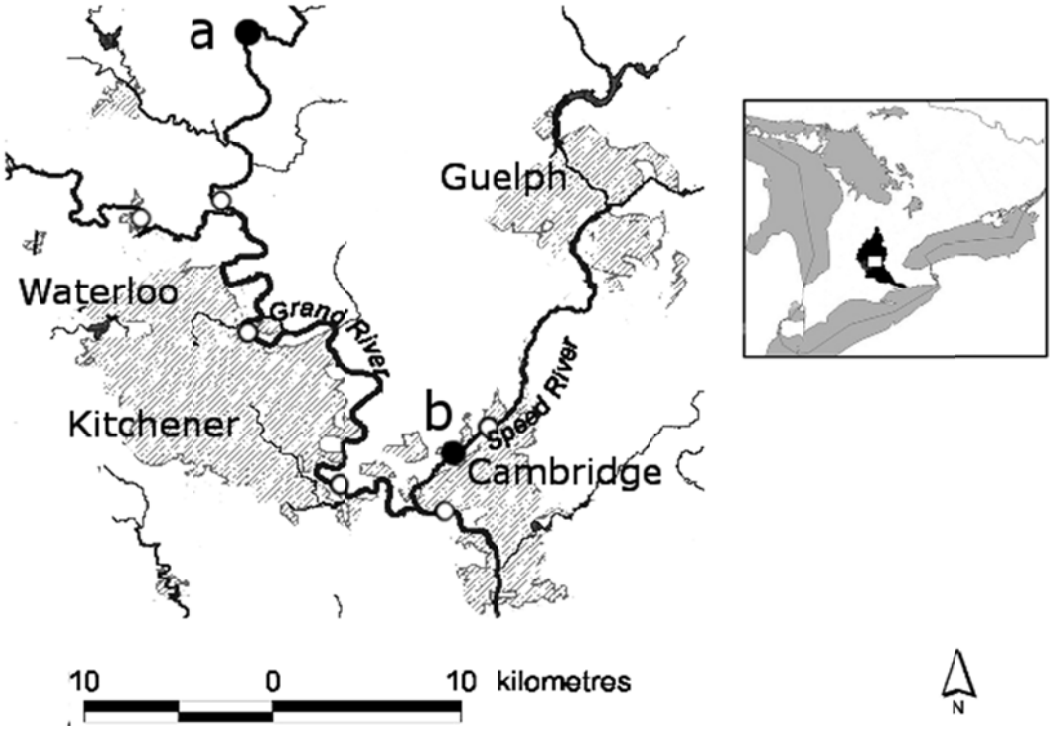

Figure 5.1 Map of the location of the incubation experiments. Two sites were used for incubations; Site a is located on the Grand River at Westmontrose, upstream of the cities of Kitchener and Waterloo and the large WWTPs (O) in the watershed, while site b is located on the Speed R. in Cambridge, downstream of the Hespler WWTP (O).

To address the role of macrophytes in altering nitrogen cycle processes, *in-situ* chamber incubations were run at 2 locations in the grand river watershed, one upstream and one downstream of a large WWTP (fig. 5.1). Site a, located on the Grand River main channel upstream of Kitchener and Waterloo, was the low impact upstream site, and was not dominated high levels of macrophyte or filamentous algae during the times that we sampled. Eurasian milfoil (*Myriophyllum spicatum)* was abundant at this location, and other submersed macrophytes such as *Ranunculus longirostrus* , *Potamogeton crispus* and *Stuckenia pectinata* were present. Site b was located downstream of the Hespler WWTP on the Speed R and in late summer was covered from bank to bank with dense growth of *S. pectinata* mixed with invasive

Eurasian milfoil, *M. spicatum*. There were 5 experiments in total, each having 6 to 8 chamber incubations of different treatment types (Table 5.1).

Chamber experiments containing river water and macrophytes and a tracer, $\delta^{15}N\text{-}NH_4^+$, were subjected to different levels of PO_4^{3-} enrichment (Table 1) to test the effect of increased P supply on N cycle transformations. The N cycle response variables chosen for this experiment were the net change in N_2O (% saturation) and gross macrophyte NH_4^+ uptake (*U*), measured by incorporation of the ^{15}N isotope into macrophyte and periphyton tissue. PO_4^{3-} concentration and light were the controlled variables in this experiment and all other variables expected to impact the experimental results, such as temperature, DO, SRP, NH_4^+ and NO_3^-, were measured.

The 5 *in-situ* chamber experiments were run from 6 August, 2009, to 3 September, 2009, in mid-morning to early afternoon. Each experiment consisted of a set of 6 or 8 20-l cylindrical chambers with an open top to allow for sample collection and treatment addition, and a closed bottom to exclude the effect of sediment processes. The chambers were not circulating or aerated as turbulent mixing and loss of NH_3 and N_2O to the atmosphere was not desired. The Grand River is a large and diverse river with many quiescent reaches. Although these chamber incubations may not be representative of faster, more turbulent sections, they are representative of extensive reaches of the river.

Chambers were placed randomly in the river in each experiment. Each chamber contained river water, a large cluster of the above-ground parts of submersed macrophytes from the reach, and a rock from the nearby river bottom, which was needed to hold down the macrophytes and to anchor the chamber from floating downstream (fig 5.2). The quantity of macrophytes selected for each chamber varied somewhat for each chamber and rocks selected were of similar size. The species used in the chambers were mainly composed of a tangled mix of *Myriophyllum spicatum* and *Stuckenia pectinata*, as these species were dominant members of the macrophyte community at study locations, and they are generally abundant throughout the Grand River and tributaries.

Table 5.1 Experimental set up for P addition chambers run in summer 2009. Experiment numbers are in chronological order of their being conducted in the field, and the table provides the number of chambers run in that experiment and the type of treatments applied

Experiment site, date	**Treatment**	**# of chambers**	**$PO4_3^-$ Treatment level**	**Light/dark**
#1 Grand R. (Site a)	Control	2	0 µg/l	Light
Aug 6	Treatment level 1	2	5 µg l	Light
	Treatment level 2	2	40 µg /l	Light
#2 Speed R. (Site b)	Control	2	0 µg /l	Light
Aug 13	Treatment level 1	2	5 µg /l	Light
	Treatment level 2	2	40 µg /l	Light
#3 Speed R. (Site b)	Control	2	0 µg /l	Dark
Aug 19	Treatment level 1	2	5 µg /l	Dark
	Treatment level 2	2	40 µg /l	Dark
#4 Speed R. (Site b)	Control	4	0 µg /l	2 Light and 2 Dark
Aug 25	Treatment 3	4	200 µg /l	2 Light and 2 Dark
#5 Grand R. (Site a)	Control	4	0 µg /l	2 Light and 2 Dark
Sept 03	Treatment 3	4	200 µg /l	2 Light and 2 Dark

Figure 5.2 In-situ chamber incubation setup in the Speed River showing light and dark chambers.

Macrophytes and rocks were not cleaned prior to placement in the chamber, but clean-looking (green, as opposed to brown and encrusted with periphyton) macrophytes and clean rocks (with low mass of periphytic biomass) were selected, briefly rinsed of any loose debris and added to the chambers. Although sestonic and epilithic organisms were present in the chamber, the dominant communities present were macrophytes associated epiphytes. Chambers were set up in a random sequence, and sampling from the chambers followed that same sequence. The chambers were filled to the 20-l mark with river water, the harvested above-ground parts of macrophytes and a rock were added, some chambers received a PO_4^{3-} addition and all received an ^{15}N-NH_4^+ tracer addition. PO_4^{3-} was administered as a Na_2HPO_4 solution, and the ^{15}N tracer was added as 2 mg of 10 atom percent (AP) ^{15}N-$(NH_4)_2SO_4$, dissolved in 50 ml of water such that when added to the chamber, the NH_4^+ concentration was increased by 100 µg N/l. This quantity of ammonia was intended to greatly increase the ^{15}N value of NH_4^+, but only slightly increase the concentration of NH_4^+. However, because ambient

NH_4^+ concentrations were lower than anticipated, between 24 and 200 µg /l, the addition of the ^{15}N-NH_4^+ tracer did significantly increase the NH_4^+ concentration in the chambers. Each chamber was sampled 3 times over the course of the experiment, approximately 30 minutes after start time (the time it took to finish setting up all chambers), then again 80 to 100 minutes after that, and finally after 200 to 300 minutes. Exact sampling times for each sample and each chamber were recorded, but times varied for each experiment and were slightly longer for the last 2 experiments, having 8 chambers to be sampled rather than 6. Before the chambers were set up, initial water and macrophyte samples were taken from the sampling location to determine the pre-treatment conditions.

Chambers were sampled at time intervals for temperature, DO, conductivity, and concentrations of SRP, NH_4^+, NO_3^-, N_2O and, at the end of the experiment, macrophytes were collected from the chambers for biomass and tissue ^{15}N analysis. Samples for dissolved ions were collected in 250-ml Nalgene bottles and were filtered the same day immediately upon returning to the lab, then stored frozen in separate vials for each type of analysis. Samples for SRP, NH_4^+ and NO_3^- concentrations was filtered through cellulose acetate 0.2-µm pore-size membranes, SRP was analyzed using the ascorbic-acid and phosphomolybdate colourimetric method (Murphy and Riley, 1962), NH_4^+ analysis followed Holmes et al. (1999) and NO_3^- was determined by ion chromatography using a Dionex ion chromatograph composed of a GP50 pump, ASRS-4mm suppressor, an IonPac® AS22 column and CD25 conductivity detector. Samples for N_2O were collected in gas-tight 60-ml serum bottles and preserved with 1% HgCl solution. N_2O was analyzed by gas chromatography. Macrophytes were not rinsed to keep their epiphytic communities intact, and were dried at 60C overnight then weighed. A portion of the biomass was ground and packed in tin cups for ^{15}N analysis, performed in the Environmental Isotope Lab located at the University of Waterloo, ON, Canada using a Finnigan Delta Plus Continuous Flow Stable Isotope Mass Spectrometer.

Uptake rate of NH_4^+ by the macrophytes and their epiphytes in each chamber incubation was calculated in three steps, based on equations of Dugdale and Wilkerson (1986) for uptake of ^{15}N in incubations of marine phytoplankton. The ^{15}N value of macrophyte tissue present at the end of the incubation estimates uptake in the following set of equations:

$$15N_{xs} = 15N_s - 15N_i \quad (5.1)$$

where $15N_{xs}$ is the excess ^{15}N of macrophyte tissue after the incubation, in atom percent (AP), and is a result of taking the ^{15}N ($15N_s$) harvest macrophyte tissue sample and subtracting an initial tissue $15N_i$ obtained from macrophytes at the site prior to the incubation.

$$V_f = \frac{15N_{xs}}{(15N_{enr} - 15N_s) \times T} \quad (5.2)$$

Where V_f is the specific uptake rate (min^{-1}), the fraction of NH_4^+ taken up by the macrophytes from the chamber per time. $15N_{enr}$ is the initial ^{15}N-NH_4^+ in the chamber after addition of the tracer which was calculated using the tracer ^{15}N, the ambient ^{15}N, and the relative concentration of each. V_f can be multiplied by the NH_4^+ concentration and divided by biomass to calculate NH_4^+ uptake velocity (U) per mass of macrophyte, i.e., µg N/g Dry Weight (DW)/min.

$$U = \frac{V_f * N_{mac}}{M} \quad (5.3)$$

This method assumes that the uptake of all isotopes of NH_4^+ can be approximated by the uptake of the tracer ^{15}N-NH_4^+, with no significant isotopic discrimination. If this assumption is not true, and macrophytes do discriminate in favour of the lighter isotope (Yoneyama et al. 1991), uptake values may be underestimated. However, because uptake rate would be underestimated all treatments if this assumption is violated, this method is still adequate testing my hypotheses concerning light and PO_4. treatments. This method also assumes that ammonia excretion from biomass does not discriminate among isotopic varieties of NH_4^+ over the duration of this experiment, and that the change in ^{15}N-NH_4^+ is a result of uptake only. Again, if macrophytes preferentially eliminate ^{14}N-NH_4^+, uptakes may be over-estimated but this would affect all treatments similarly. . The linear model for calculation of NH_4^+ uptake does not account for other processes that alter the ^{15}N value of the tracer, such as volatilization, which would cause tracer ^{15}N values to increase over the incubation period, and tracer dilution, which would result in lower ^{15}N values over the incubation period. I estimated the possible influence of these effects by calculating an upper and lower value of U with 15% of the tracer being volatilized (Gross et al., 1999) and 15% tracer dilution through release and ammonification (Dugdale and Wilkerson, 1986).

Nutrient concentrations and DO in each chamber were recorded and were used either as a time-weighted average concentration for the chamber or as a rate of change over the incubation period. When used as an independent variable, NH_4^+, NO_3^- and DO concentrations were

represented as time-weighted average concentrations. When used as the dependent variable, they were analyzed as rates of change. This is because, for an independent variable, absolute concentration matters more than rates of change; however, as a response variable for biological processes, rates of change are more relevant. NH_4^+ release rate in the chambers was calculated from the 2 measurements of uptake obtained in this experiment by the following relationship:

$$U_{net} = U - R \tag{5.4}$$

where U_{net} is the net uptake of NH_4^+ by macrophytes, in µg N/g DW/min, U, is total or gross uptake of NH_4^+ in the chamber, in µg NH_4^+/g/min, and R, the release rate of NH_4^+ by macrophytes, in µg N/g DW/min. Gross uptake (U) was obtained from ^{15}N-NH_4^+ incorporation into plant tissue, and the net uptake (U_{net}) was the change in NH_4^+ over the incubation period, so release (R) can be calculated. Net NH_4^+ uptake assumes no loss of NH_4^+ to the atmosphere through volatilization, and insignificant uptake and release by the seston and rock within the chamber. If there are unaccounted losses, then net uptake calculated using change in NH_4^+ concentration will be overestimated, and calculated release rates will be underestimated.

From U (e.g. equation 5.3) turnover time (T) of macrophyte N can be calculated as the inverse of the uptake:

$$T = \frac{1}{U} \times \frac{N_{mac}}{M} \tag{5.5}$$

Turnover time (T) is in units of time, typically expressed in days.

5.3 Results

NH_4^+ uptake occurred in all chambers as indicated by an increase in the ^{15}N of macrophytes and by a decrease in NH_4^+ in the chambers over the incubation period (fig. 5.3; fig. 5.4 A). N_2O was produced in most chambers, as N_2O increased over the incubation period (fig. 5.3; fig. 5.4 B and C).

The light/dark treatment had an effect on DO in the chambers, with dark chambers having a lower average DO over the incubation period than the light chambers (fig. 5.5 A, student's t-test, $P<0.001$), but U did not differ between light and dark treatments (Mann-Whitney rank-sum test, $P=0.119$). Chamber N_2O production was affected by the light/dark treatment, with dark chambers having a higher mean % N_2O saturation than light chambers (Students t-test, $P = 0.016$; Fig. 5.6 A).

NH_4^+ uptake rate (U) did not differ with PO_4^{3-} addition (fig 5.4), nor did U differ between light/dark treatments (Mann-Whitney U test $P= 0.882$). However, NH_4^+ uptake was different between sites (fig. 5.4 A) with the Grand River upstream site having higher uptake rates for all treatments than the Speed River site (Mann-Whitney U test $P <0.001$). N_2O production in the chambers was not affected by PO_4^{3-} addition (fig. 5.4 B). Although there were no site differences in N_2O production over the incubation period (Student's t-test $P=0.706$), there were site differences in average % N2O saturation. The Grand River incubations had lower % N_2O saturation than the Speed R. locations (Student's t-test $P <0.001$; Fig. 5.4 B). We conclude that PO_4^{3-} addition did not alter 2 aspects of nitrogen cycle processes: macrophyte community NH_4^+ uptake and community N_2O production.

Uptake was influenced by other factors measured, but not controlled for, in the chamber experiments (Fig. 5.5). Although the same quantity of NH_4^+ tracer was added to each chamber, variation in NH_4^+ concentration occurred due to differences in ambient concentration among sites and days, and even among chambers. This variation resulted in a range of mean NH_4^+ across the experiments, from 68 to 204 µg/l, enough to determine a relationship to uptake. Uptake was related to the mean NH_4^+ concentration in the chamber over the length of the incubation (Fig. 5.5 C) for incubations when the mean chamber NH_4^+ was low. In total, the data suggest a hyperbolic function for which we can determine the half-saturation constant (K_s) for both upstream and downstream incubations as 55.8 µg N/l, and a V_{max}, which appears to be

different for the two sites; 0.6 µg N/g DW/min for the Speed River incubations and 1.3 µg/g DW/min for the Grand River incubations.

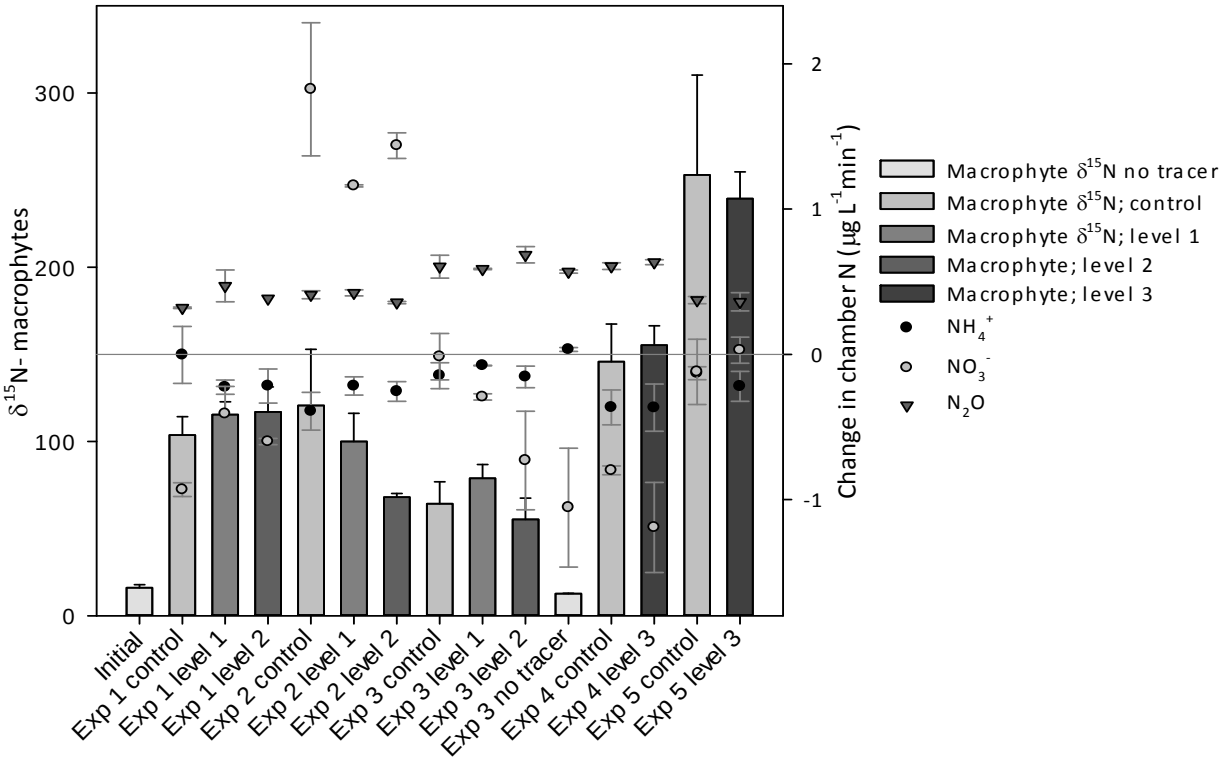

Figure 5.3 Macrophyte δ15N content before the experiments (initial) and after incubation, and the rate of change for NH_4^+, NO_3^- and N_2O, ordered by experiment set. Treatment levels for experiments 1-3 had two replicates, while experiments 4 and 5 had 4 replicates per treatment. The data presented are from light and dark incubations combined, as light/dark treatment had no effect on N uptake by macrophytes.

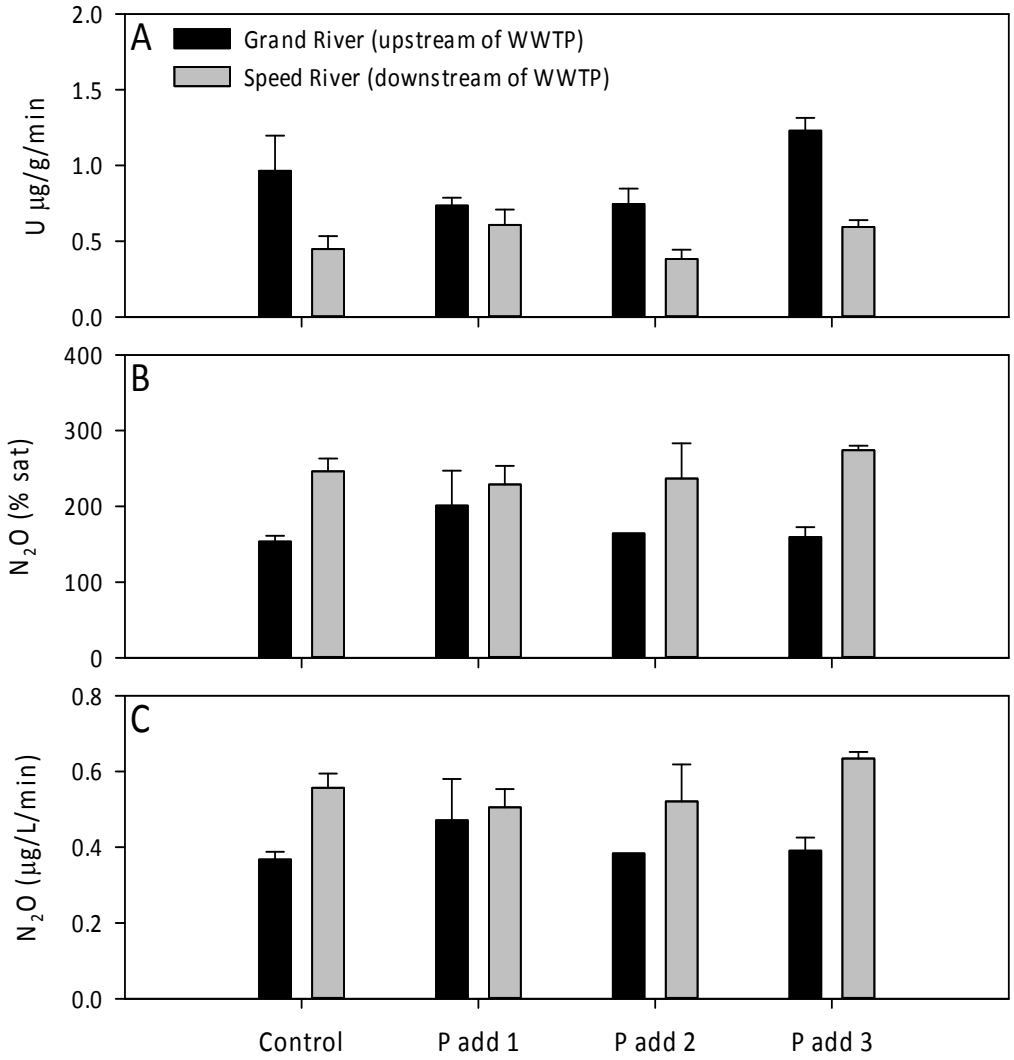

Figure 5.4 Results from chamber incubation experiments with PO_4 addition treatments. Gross NH_4^+ uptake (*U*) by macrophytes and attached epiphyton and biofilm (A) show differences between sites but not by P treatment. Time-weighted N_2O % saturation (B) and N_2O production (C) are different at each site, but show no difference based on P treatment level. Light and dark chambers are combined in this figure, though there were some differences in N_2O % saturation between light and dark incubations.

As with NH_4^+ concentration, it was difficult to control quantity of macrophyte biomass added to the chambers in the field. The chambers ranged from having 4 g to 20 g of macrophyte biomass, producing enough variation to enable the quantification of the effect of biomass on uptake. Macrophyte biomass was negatively related to uptake (fig. 5.5 B). Curiously, macrophyte biomass was negatively related to DO in both light and dark chambers (fig. 5.5 A).

Nitrate varied among experiment sets (fig. 5.3; fig. 5.5 D) and appeared to have a negative relationship with NH_4^+ uptake. Within experiments, NO_3^- changed only slightly, no more than 0.6 mg/l, over the incubation period. In some incubations NO_3^- concentrations increased while in others it decreased (fig. 5.3), so the relationship between *U* and NO_3^- across all experiments likely only reflects the differences in ambient NO_3^- concentration at each site, and should be interpreted cautiously. NO_3^- differences between incubation sets did not appear to affect the relationship between NH_4^+ and uptake. For example, experiment 3 had some of the highest NO_3^- values but a very strong relationship between NH_4^+ and uptake, suggesting that the presence of abundant NO_3^- did not reduce NH_4^+ uptake. SRP also changed in the chambers over the incubation period (data not shown), however it was unrelated to any other parameters measured, and the negative relationship between initial SRP and uptake may also reflect site and date differences.

Percent N_2O saturation increased during most of the incubations, and N2O production in chambers occurred, but due to the volatility of N_2O it is not possible to accurately quantify gross N_2O production from the methods used in this study. Mean N_2O was over 100% saturation in all chambers, so it is likely that some N_2O was lost to the atmosphere during these experiments. Oxygen influenced N_2O to some degree; in both light and dark chambers, the mean percent N_2O saturation was negatively related to the mean DO in the chambers, except for dark chambers of experimental set 5 (Fig. 5.6 A). Percent N_2O saturation was also negatively related to the average NO_3^- and to NH_4^+ uptake (Fig. 5.6 B&C). Individual experiments differed so greatly in NO_3^- that it is difficult to interpret the meaning of this kind of relationship, although it is likely that the concentration of NO_3^- in the chamber was related to the quantity of N_2O produced, as the rate of change in NO_3^- (µg /l/min) was related to N_2O % saturation (Fig. 5.6 D). The only other factor found to correlate with N_2O was *U* (Fig 5.6 B).

Macrophyte uptake of NH_4^+ was not the only cause for change in NH_4^+ concentration in the chambers, so net uptake rate and, thus, release rate can only be an approximation and probably an underestimate. Because none of the treatments appeared to influence NH_4^+ uptake, the values from each experiment can be pooled and compared (Fig. 5.7). In these experiments, gross uptake rate was not balanced by net uptake, and the calculated release rate was roughly 1/2 to 2/3 of the gross uptake rate, and in one incubation, the net uptake rate was greater than the gross uptake rate, indicating other processes consuming NH_4^+, such as nitrification and volatilization could were occurring. Turnover times for the macrophyte N pool are calculable as the reciprocal of gross uptake. They ranged from 16 to 158 d, with the longest turnover time found in the experiments run on 19 August 2009, and the shortest run on 3 September 2009. Although there are site differences in the gross uptake rate by macrophytes (Fig. 5.4 A), there does not appear to be a temporal pattern over the late summer when these experiments occurred (Fig. 5.7).

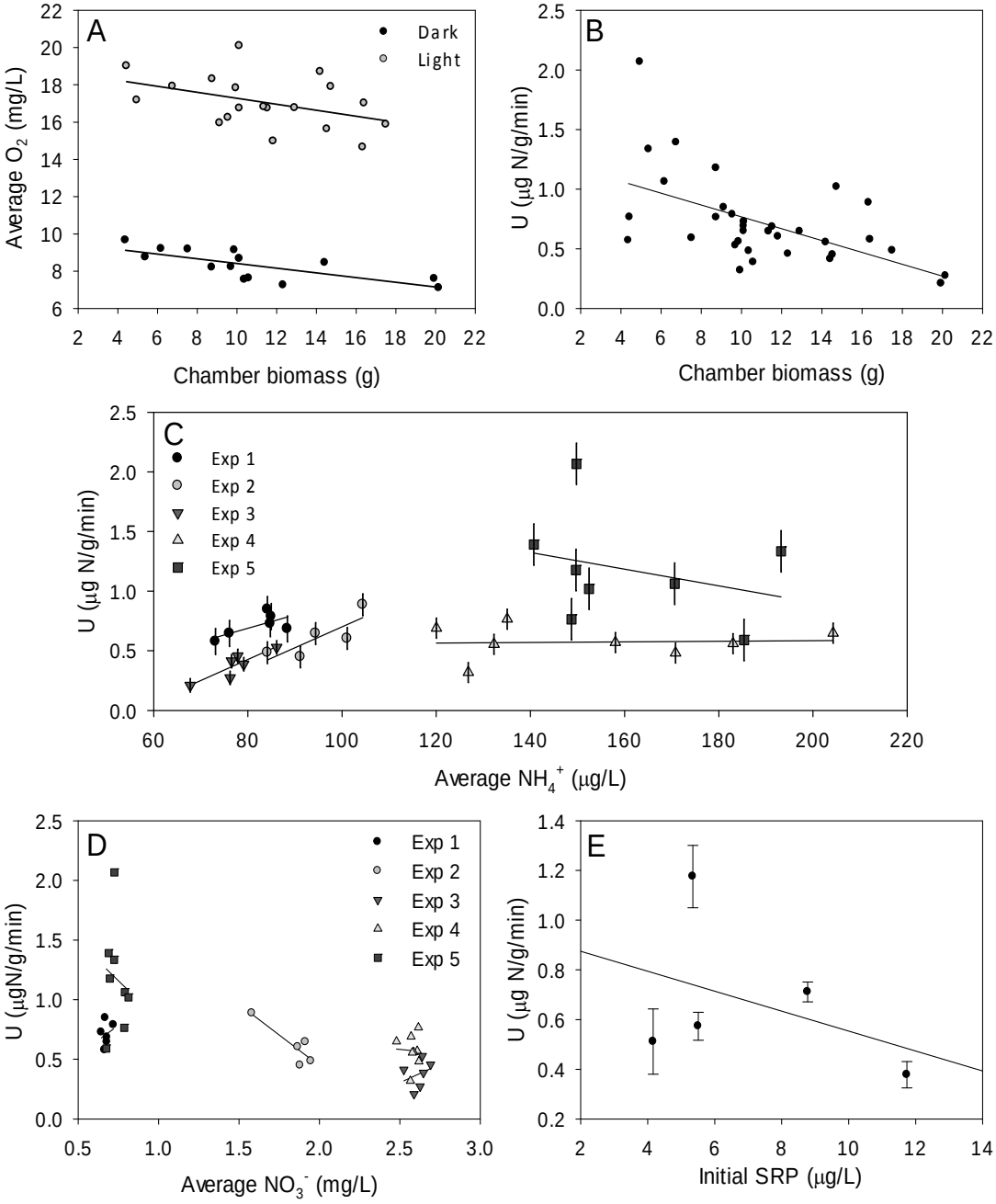

Figure 5.5 Factors influencing gross NH_4^+ uptake (*U*) in chamber incubations. Time-weighted average DO was negatively related to macrophyte biomass (A), R^2 = 0.358 in the light, and 0.545 in the dark; *U* was negatively related to biomass, R^2 = 0.310 (B); U as a function of time-weighted average chamber NH_4^+(C), with individual experiments in the lower concentration range linearly related, R^2 = 0.464 for exp 1, 0.662 for Exp 2, 0.782 for Exp 3, error bars representing uncertainty in U from estimates of volatilization and tracer dilution; U related to time-weighted average NO_3^- (D), R^2=0.390; U related to initial site SRP concentration (E), R^2=0.198.

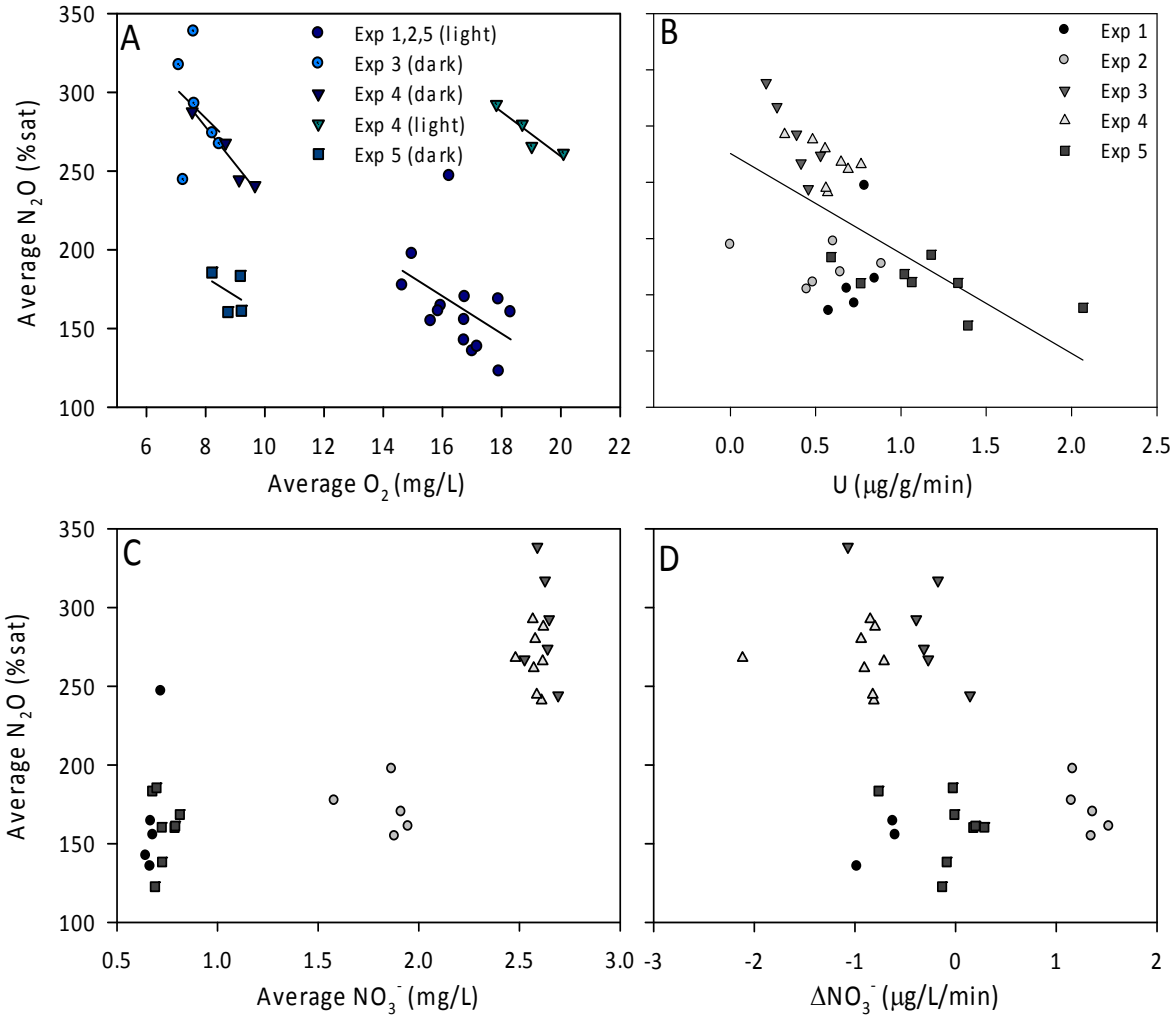

Figure 5.6 Factors influencing the concentration of N_2O in chamber experiments. N_2O was a negative function of DO in most treatment (A) with R^2=0.346 in combined light Exp 1+2+5 of, R^2 =0.401 for dark Exp 3, and R^2 =0.897 in dark chambers of Exp 4; N_2O was negatively related to U (B), R^2=0.405; N_2O was related to NO3- (C); R^2=0.755; N_2O was negative function of NO_3^- consumption (µg /l/min), R^2=0.264.

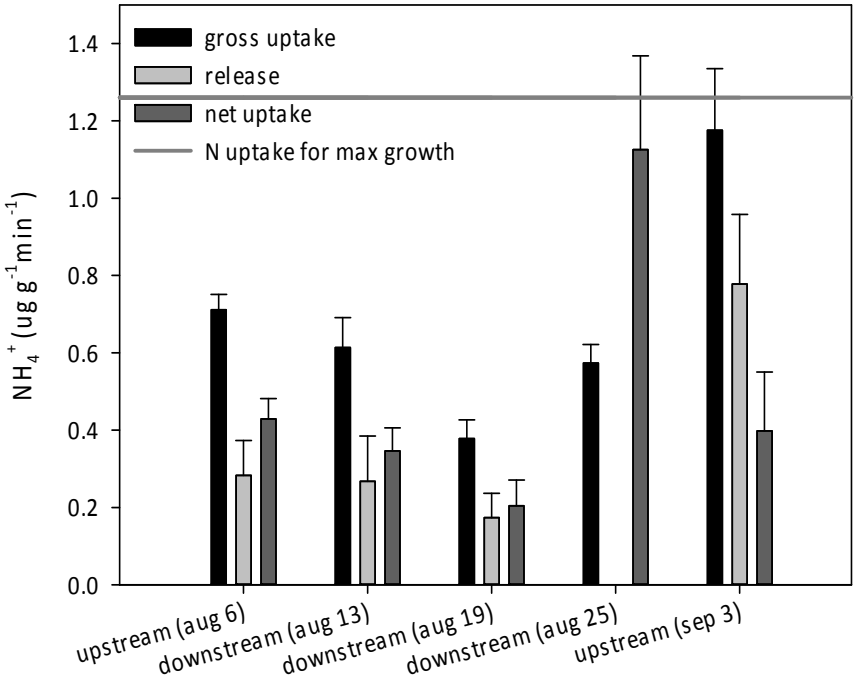

Figure 5.7 Gross NH_4^+ uptake (*U*), net uptake (U_{net}), release rate (*R*) and the nitrogen uptake required for maximum growth rates. N requirement is based on a maximum relative growth rate of 0.10 d^{-1} from Nielsen and Sand-Jansen (1990, 1991) who found a range of 0.007-0.109 d^{-1} for submersed species *M. spicatum*, *Potamogeton* spp. and *E. Canadensis*, and a critical N tissue concentration of 1.82%, beyond which macrophyte growth becomes limited (Demars and Edwards, 2007).

5.4 Discussion

5.4.1 Nitrogen and phosphorus interactions.

This study did not find any effect of PO_4^{3-} addition on NH_4^- uptake or N_2O production at Grand River or Speed River locations. Ambient SRP concentrations before additions were 3.7 to 11.9 µg/l, and may not have been limiting the growth or metabolic functions of riverine biota. The negative relationship between initial SRP and NH_4^+ uptake is unexpected, and may reflect that NH_4^+ uptake was highest when nutrients were low. Because the variation in NH_4^+ concentration and biomass in the chambers had unintended impacts on the NH_4^+ uptake rates of macrophytes, it may have been difficult to detect any effect of P addition on NH_4^+ uptake.

A meta-study of nutrient limitation experiments (Francoer et al., 2001) found that a very small response in benthic epiphyte biomass was present in N-limited streams for P addition, and vice-versa, but that these responses would be undetectable given the statistical power of most of these experiments. Subtle effects on NH_4^+ uptake due to the PO_4^{3-} treatment are possible, but future studies will need to carefully control the NH_4^+ concentration and the biomass of macrophytes, and increase the statistical power of the experiment to find this effect. It is also debatable whether detecting small effects statistically is biologically or ecologically relevant. N and P cycles may interact at the physiological level in streams, as PO_4^{3-} uptake can be inhibited by high concentrations of NH_4^+ through the interference of extracellular NH_4^+ on anion co-transport as demonstrated by Wolfram et al. (1984) for P-starved *Lemna gibba* in laboratory culture. However PO_4^{3-} uptake was not determined in our experiment, so this type of interaction cannot be specifically addressed by this study. If an effect of PO_4^{3-} on NH_4^+ uptake or transformation was present in the Grand and Speed Rivers, an experiment to detect it would need to select a site with lower background SRP concentration, operate the experiment with a higher number of treatments and add smaller quantities of NH_4^+ carrying the tracer.

5.4.2 NH_4^+ Uptake rates

The 10 AP ^{15}N-NH_4^+ tracer addition affected gross NH_4^+ uptake (U), as indicated by the relationship between U and concentration in experiments where the ambient NH_4^+ was low. There appeared to be a hyperbolic relationship between U and concentration with an estimated half saturation constant (K_s) of 56 µg NH_4^+/l when data from all chamber experiments were

pooled. This is within the range of previously published values, such as a K_s of 7-70 µg N/l found for some marine algae (Eppley et al. 1969) and within the 3 order of magnitude range found for several algal species (Kemp and Dodds, 2002b), but greater than the 0.6-7.6 µg /l they found for various stream benthic algae and microbial habitats. Vmax for riverine submersed macrophytes in this study (0.6 ug N/g/min at the Speed site and 1.3 ug N/g/min at the Grand R. site) was lower than a V_{max} of 4.097 ugN/g/min for benthic algae (Kemp and Dodds (2002b), but higher than a V_{max} of 0.039 ug N/g wet weight/min found for a marine macroalgae (Haines and Wheeler, 1978).

Webster et al. (2003) found that ammonium uptake by stream epilithic and filamentous algae was best explained by NH_4^+ concentration in streams across the United States, and no other physical or chemical parameters they examined explained the remaining variation. Similarly, in my work gross NH_4^+ uptake appeared to be related to NH_4^+ concentration below 110 µg/l. However, uptake limitation does not mean that growth is N limited. All of the uptake rates measured in each experiment would be able to support theoretical maximal growth rate of 0.1 d^{-1} determined for macrophyte species (Neilsen and Sand-Jansen, 1990; 1991) and maintain tissue N concentrations above a critical limitation threshold of 1.82% (Gerloff, 1975 as cited in Demars and Edwards, 2007). Uptake rates in chamber-incubated macrophytes exceeded N requirements to support the theoretical maximum growth rate in only one chamber incubation, experiment 5, at the Grand River site on September 3rd 2009. However it is likely that macrophytes may not meet all of their assimilatory N demands through NH_4^+ uptake in natural river settings, given that the concentration of NH_4^+ is normally much lower, below 10 µg/l, and the maximum laboratory growth rate determined in laboratory studies may not ever be reached in rivers even given ample nutrients. Because the chambers were elevated in NH_4^+ concentration by the addition of the ^{15}N-NH_4^+ tracer, actual river macrophyte *U* could be determined using K_s and V_m and ambient NH_4^+ concentrations. On the days chamber experiments were run, ambient NH4+ was always 60 µg/l or lower, meaning that ambient NH_4^+ uptake was < 0.01 µg N/g/min

Another consideration for this experiment is the assumption that the net change in NH_4^+ does not include losses such as volatilization and nitrification, both of which fractionate and enrich the remaining NH_4^+ (Mariotti, 1981; Högberg, 1997). Though the chambers were quiescent and volatilization was minimized, the pH range of the river water was 7.6-8.8 during the time of the

incubations so some volatilization likely occurred. Additionally, nitrification may have occurred because the chambers were oxic, had ammonia added to them, produced N_2O, and because the NO_3^- concentration increased in some chambers. Volatilization strongly fractionates dissolved NH_4^+ through both the equilibrium reaction involving NH_3, and the movement of NH_3 from water to air though volatilization (Högberg, 1997). Any volatilization that occurred in the chambers would enrich NH_4^+ isotope values due to fractionation effects and bias our determination of *U*. However, as the U_{net} was smaller than *U*, violating this assumption would produce minor effects. I determined the possible implications of volatilization on calculating *U* using a simple model. If volatilization caused a decrease in NH_4^+, and increased the 15N value through preferential loss of ^{14}N, an estimate of loss due to volatilization on the tracer and the calculated *U* can be made. If 15% of the loss of NH_4^+ in the chamber was due to volatilization, *U* would be 5-16% reduced. However it is likely that less than 10% of NH_4^+ would have been volatilized (Gross et al., 1999) making our estimates of uncertainty generous overestimates.

5.4.3 Macrophyte Biomass and NH_4^+ uptake

Macrophyte biomass in the chambers was negatively related to gross macrophyte NH_4^+ uptake rate (*U)*. As a closed system, a chamber has a finite amount of nutrient available for uptake, and chambers with more biomass may use up the amount of NH_4^+ available to them. A possible explanation is that the macrophytes in the chambers depleted the tracer, and released NH4+ with lower 15N values, leading to a smaller quantity of tracer assimilated per unit biomass over time. This dilution effect depends on release rate, which we did not know prior to the experiments. We made an estimate of the impact of tracer dilution by assuming that, for incubations of less than 5 h, 15% of the tracer is can be replaced by release, an estimate given by Dugdale and Wilkerson (1986) for incubations of marine phytoplankton. The difference between net and gross uptake of NH_4^+ indicates that recycling of ammonia was rapid in the chambers, and possibly in the river.

Chambers with more macrophyte community biomass would have a larger amount of tracer dilution, possibly resulting in a greater effect on *U* and producing an apparent negative relationship between chamber biomass and *U*. Hall and Tank (2003) found that increased rates of stream metabolism were positively correlated with uptake of NO_3^- and NH_4^+, with both GPP and CR being significant predictors of NH_4^+ uptake and GPP significantly predicting NO_3^- uptake.

There was a positive correlation between macrophyte community biomass and gross NH_4^+ uptake as would be expected. To correct the experiment for the influence of tracer dilution by NH_4^+ release, ^{15}N- NH_4^+ would need to be measured throughout the experiment.

Over the chamber biomass range present in this study, which was equivalent to 94 to 377 g m^{-2}, uptake of the ^{15}N-NH_4^+ tracer was influenced either directly by exhaustion of the tracer NH_4^+ with increased biomass, or indirectly by the effect of high recycling rates and dilution of the tracer. Either explanation of the data indicates a role for macrophytes and their attached epiphyton on the concentration of riverine NH_4^+, and indicates that NH_4^+ is in demand even in large eutrophic rivers.

Under a scenario of increased NH_4^+ loading, the turnover rate of NH_4^+ would be lower and the uptake length would be longer (Kemp and Dodds, 2002a; 2002b; Mulholland and Rosemond, 1992; Kemp and Dodds, 2001a; Peterson et al., 2001). High rates of NH_4^+ recycling, as was found in this study, mean that NH_4^+ is not retained, but is rapidly spiraling downstream with relatively high turnover. Rivers with high macrophyte biomass may have a higher community uptake, but with rapid cycling they may not have a higher assimilative capacity for new sources of NH_4^+ because the contribution of recycled NH_4^+. Additionally, the influence of macrophytes and their epiphyton would be seasonal, as attaining high biomass would require net nutrient uptake, not release, but in late season there would be net release of nutrients. Macrophyte communities may change from a sink for nutrients to a source of nutrients seasonally as their growth pattern changes. Higher rates of net uptake might occur earlier in the growing season when macrophytes are actively adding biomass. In this case, rivers that can support a higher biomass of macrophytes would have a higher assimilative capacity for new nutrients in the spring, while later in the season higher release rates would cause the standing stock of biomass to act as a source, reducing the assimilative capacity for rivers that host a higher biomass of macrophytes. Hill (1979) found that aquatic macrophytes in streams and rivers decreased in N and P content over the summer growing season, implying that net uptake of nutrients slows over the growing season, and that nutrients are being translocated to roots for storage. Because this study was conducted in August-September, when macrophyte community biomass was at its peak or beginning to decline, the high rate of recycling and low retention of N (seen by low U_{net}) could be due to a seasonal growth and nutrient uptake pattern of submersed macrophytes. Hill (1979) also reported rapid breakdown and release of macrophyte N and P during their late

summer sampling period, which also supports our findings. To determine the influence of macrophytes on river N cycling, future work on seasonal variation in N uptake will be necessary.

5.4.4 N_2O in chamber incubations

N_2O increased in most of the chambers, which could indicate that nitrification, denitrification or both were occurring in the chambers, as both can contribute significantly to N_2O production depending on the conditions (Mathieu et al., 2006). N_2O saturation was negatively correlated with DO, and was related to average chamber NO_3^- concentration indicating that denitrification may be occurring in the chambers even though DO was above the hypoxic levels usually required for denitrification activity. Further evidence of denitrification activity is provided by the relationship between the rate of NO_3^- consumption (ΔNO_3^-) and N_2O percent saturation. It is likely that microenvironments of low DO existed in the chambers within the large cluster of macrophytes and are possibly associated with older, brown, non-photosynthetic parts of macrophytes and their biofilm. This is supported by the finding that biomass was negatively related to DO in both dark and light chambers, indicating repiration exceeded photosynthesis regardless of light. Other work has demonstrated increased denitrification activity associated with macrophytes in lakes and ponds (Eriksson and Weisner, 1996; Eriksson, 2001), estuaries (Caffrey and Kemp, 1992; An and Joye, 2001) reservoirs (Eriksson and Weisner, 1999) in shallow streams (Schaller et al., 2004; Forshay and Dodson, 2011) and in filamentous algal mats (Kemp and Dodds, 2002b). This study shows that denitrification activity can be associated with the benthic macrophyte plants themselves, and that this activity is higher for incubations at the site located downstream of the Hespler WWTP on the Speed River which receives a high N load associated with WWTP effluent. Further work is needed to establish the significance of macrophyte-associated N_2O production in large rivers, particularly for reaches downstream of anthropogenic N sources.

The lack of correlations between N_2O and NH_4^+ and net NH_4^+ consumption do not necessarily indicate a lack of nitrification activity in the chambers. Both NH_4^+ and N_2O are volatile, and while attempts were made to minimize loss to the atmosphere by not aerating or vigorously stirring chambers, these losses were not completely prevented. Loss of NH_4^+ and N_2O through volatilization could explain some of the variation in N_2O production in the chambers.

Nitrification is often found occurring with denitrification in tightly coupled nitrification-denitrification processes at strong DO gradients, as the nitrate substrate required for denitrification is often generated by nitrification (DeLaune et al., 1991). Nitrification may have occurred in the chambers because all chambers remained oxic, above 2 mg /l, and because nitrate increased in some chambers over the experiment.

N_2O was negatively correlated with gross macrophyte NH_4^+ uptake (*U*), and this could be evidence of competition for NH_4^+ between macrophyte N uptake and nitrifiers. As macrophyte demand for NH_4^+ increases, the concentration of NH_4^+ is drawn down, resulting in competition for NH_4^+ substrate, possibly resulting in lower nitrification activity and less N_2O production. Additionally, at lower NH_4^+ concentrations macrophytes may use more NO_3^- to supplement their N demand, and the lower nitrate would result in lower denitrification rates and less N_2O production as well. Denitrification activity might be controlled by availability of NO_3^- (Peterson et al., 2001; Kemp and Dodds 2002a) but macrophyte N uptake will not likely be controlled by nitrate concentration, as nitrate concentrations are high at both locations. Denitrification is controlled by DO, so the macrophyte effect on DO could cause an indirect on N-cycle behaviour. This may also be a result of experiment 5 having high values for *U*, low N_2O saturation and lower than average macrophyte biomass compared to the other experiments.

It is generally understood that NH_4^+ is the preferred form of inorganic nitrogen to plants because of its reduced form and, given the generally higher availability of NH_4^+ in aquatic environments compared with terrestrial environments, aquatic macrophytes generally assimilate more N from NH_4^+ than the more energy-demanding NO_3^- (Saskawa and Yamamoto, 1978; Yoneyama et al., 1991; Fang et al., 2007; Wyman and Bird, 2007). Assimilation of NH_4^+ by plants can even inhibit assimilative NO_3^- uptake in some instances (Wolfram et al., 1984). Because this experiment was not originally designed to distinguish effects of both nitrification and denitrification and the effect of macrophyte N demand on these processes, it is difficult to interpret these observations with any certainty. These results may help in forming hypotheses to be tested in future experiments involving the role of macrophytes on nitrogen cycle processes in rivers.

5.4.5 Conclusions

Although there was no effect of PO_4^{3-}-uptake on U in the in-situ chamber incubations, the experiment did yield some interesting findings. U was a function of NH_4^+ concentration below approximately 100 µg/l NH_4^+, even when NO_3^- was high. When macrophyte biomass in the river is high, and NH_4^+ concentrations are low, macrophytes will help to maintain low NH_4^+ concentrations (along with volatilization, nitrification, and uptake by other communities). N_2O in the chambers increased, indicating that nitrification and/or denitrification were occurring. Macrophytes may increase the quantity of N_2O produced because of their large surface area for nitrifying and denitrifying organisms. Thus as rivers become eutrophic, the biomass of macrophytes may accelerate N cycling in rivers both directly and indirectly.

Summary

Aspects of nutrient dynamics and biomass response were examined in this thesis to gain a better understanding of the processes that characterize eutrophication in rivers. First, to put the modern Grand River into a historical context, I examined long-term nutrient data available through the Ontario Provincial Water Quality Monitoring Network and looked for indicators of changing nutrient cycles spatially and temporally, and for ways to distinguish point source impacts from diffuse impacts. TP and SRP declined over the 34 year monitoring period at all sites in the Grand, with the biggest declines occurring in the 1970s. TP and SRP continued to decline slowly in the decades following. The continual decline of TP in rivers is puzzling, as population increased and agricultural likely intensified over this period. These results are similar to findings for long term phosphorus trends in other watersheds in Ontario and around the globe (Parr and Mason, 2003; Eimers, 2004; Sileika et al., 2006).

The trends for nitrate (NO_3^-+NO_2^-), however, stand in stark contrast. Nitrate concentration increased in the Grand River over the 34 y monitoring period. The increase in nitrate was seemingly unrelated to proximity to WWTPs, and no important changes to nitrate concentration occurred in the early to mid-1970s. Temporal, spatial and seasonal patterns of NO_3^- concentration show evidence of a diffuse origin for Grand River nitrate, which could be a result of the intensification of agriculture; increased chemical fertilizer use and increase in livestock in the watershed. Long-term changes in NO_3^- in the Grand River are similar to those of other watersheds around the globe, where nitrate concentrations increased over time (Mitchell et al., 2001; Lassaletta et al., 2005; Duan et al., 2007; Billen et al., 2007) and is in line with the finding that reactive nitrogen in the global environment has roughly doubled since the beginning of the industrialized era (Galloway et al., 2004). These findings thus corroborate evidence of a global phenomenon from which the water quality of the Grand River is no exception. Phosphorus concentrations in rivers have been declining over the long-term, while NO_3^- has increased. Water quality and eutrophication in the Grand River might be said to be improving, if primary producers of the river are primarily P limited, however evidence provided in this thesis suggests that nitrogen, particularly NH_4^+, is also important for primary producers.

Additional ways to examine the monitoring data, such as nutrient stoichiometry; TP:SRP, DIN:SRP and NO_3^-: NO_2^-, can provide additional information as to the relative importance of nutrient sources and the ability of the river to handle nutrient loads. The changing TP:SRP indicates that the WWTP had an impact on nutrient concentrations, while DIN:SRP mainly reflects the long-term decrease in P and increase in NO_3^-. NO_3^-: NO_2^- highlights regions where denitrification was occurring. These uses of nutrient ratios in long-term monitoring are unique to this thesis and were explored as methods to derive novel information regarding changing water quality from a standard set of parameters. This information provides a long-term view of the nutrient chemistry of the Grand River and highlight areas where management efforts could be focused, such as the reach below the largest WWTP, and the rising NO_3^- concentrations likely the result of increased diffuse nutrient sources.

I explored the evolving trophic condition of the Grand River further by looking at macrophyte biomass as a manifestation of eutrophication resulting from nutrient loading. Although long term trends show declining P, TP exceeds the provincial standard for rivers of 30 ug P/l over much of the middle and lower Grand River. The Grand River is still considered eutrophic by regional resource managers because the river becomes hypoxic in many locations in the summer, and submersed aquatic macrophytes attain biomass considered to be a nuisance for water quality, recreation and industrial use. Although it is generally accepted that nuisance biomass of macrophytes is caused by anthropogenic nutrient loading, and there is some evidence for this view (Carr and Chambers, 1998; Sosiak, 2002; Carr et al., 2003), the relationship has not been empirically established, and the response of the benthic environment to increased nutrient loads does not fit clearly into conceptual models of river ecosystem structure and function (Hilton et al., 2006). I found strong evidence that increased nutrient loads to rivers produce elevated macrophyte biomass. For two reaches downstream of WWTPs, macrophyte biomass was significantly greater than upstream. I was able to demonstrate this mainly because I chose an appropriately large scale for study, and a method that reduces the effect of habitat variability on biomass estimates, two aspects important in studying large river environments. Although my study does not specifically link nutrients to biomass, the fact that the WWTPs are a significant source of nutrients to the river gives circumstantial evidence of this effect. Tissue nutrient content of macrophytes was higher in both downstream reaches, however macrophytes in upstream reaches were not demonstrably nutrient limited relative to

laboratory-determined critical nutrient thresholds for aquatic macrophytes. This presents a paradoxical finding of a biomass response to nutrient point sources, yet no clear evidence of nutrient limitation upstream. It is possible that the time we chose to sample, during peak summer biomass, offers an explanation. Macrophytes may not be nutrient limited during peak biomass when growth begins to slow and plants begin to grow storage organs and senesce. Future work demonstrating the effect of anthropogenic nutrient loading on macrophyte biomass should test the hypothesis that nutrient limitation, as indicated through tissue nutrient concentrations, occurs in upstream reaches during the spring growing season, rather than at a time when plant maturity and peak biomass has already been achieved. Further investigation of the empirical link between increased nutrient loading to rivers and enhanced benthic primary producer biomass may entail relating the total biomass yield of a reach to nutrient loading of a point source across multiple river systems.

As high chemical, physical and biological variability is an important and distinguishing characteristic of river environments, an understanding of the inter-annual variation present in macrophyte biomass may be necessary to detect changes due to nutrient enrichment. Causes of inter-annual variation in macrophyte biomass in the Grand River were explored. Four years of reach-level biomass data were examined in relation to factors hypothesized to explain year-to-year variation; average air and water temperature, average precipitation and average discharge. These parameters influenced both the maximum quantity of macrophyte biomass produced in a growing season, and the seasonal pattern of biomass development as characterized by the time the biomass maximum was reached. The finding that increased temperatures lead to higher peak biomass occurring earlier in the growing season, while increased flow leads to lower peak biomass later in the growing season, is in line with our current understanding of the factors that influence macrophyte biomass production (Barko and Smart, 1981; Chambers et al., 1991; Carr et al., 1997). This work makes a novel contribution to the understanding of the riverine response to nutrient loading in several ways. Long term data on macrophyte biomass are relatively rare in lakes and rivers, and none so far have examined the effect of seasonal and inter-annual variation in weather patterns on macrophyte biomass. It is also a novel finding that the seasonal pattern of macrophyte biomass development is affected by weather; many studies of biomass occur during the “peak” summer biomass, and I have shown that this peak varies year to year and can be predicted by weather patterns. These

findings can inform future attempts to understand the conditions leading to enhanced benthic primary production in rivers, lead to the improvement of macrophyte biomass production models used by water resource managers, and the implementation of these findings is the natural next step for this research.

Stable isotopes can provide information regarding sources of nutrients that concentrations cannot, and the link between macrophytes and WWTP nitrogen loading was investigated using this tool. Samples of ammonia, nitrogen and macrophyte tissue taken downstream of two WWTPs on the Grand River revealed that macrophyte tissue $\delta^{15}N$ reflected the enriching trend found in $\delta^{15}N\text{-}NH_4^+$ downstream of both WWTPs. Although the use of macrophytes and macroalgae as sentinels for presence of waste effluent has been explored in other aquatic systems around the world (Grice et al., 1996; Fry et al., 2000; Rogers et al., 2003; Savage et al., 2004; Derse et al., 2007; Dillon and Chanton, 2008; Yamamuro et al., Risk et al., 2009), no work has been done for riverine macrophytes, particularly in eutrophic rivers. We tested three common assumptions made when using stable ^{15}N isotope values of macrophyte or macroalgal tissue as effluent indicators and demonstrated that some of these may not apply in highly dynamic environments. Rapid nitrogen processing downstream of WWTP poses a problem in the use of mixing models, and in high nitrogen environments macrophytes discriminate during N-uptake. However, the results showed that macrophytes record the presence of the new $NH4_+^-$, and might be suitable sentinels when NH_4^+ concentration is too low for isotope analysis or varies on a diel basis. The strong preference for $NH4_+^-$ over NO_3^- in macrophytes growing below WWTPs also demonstrates the importance of NH_4^+ to macrophyte communities, even in rivers of high nutrient concentrations and multiple nutrient sources. Future work to improve the suitability of macrophytes as sentinels would be to determine at what concentration of NH_4^+ macrophytes will use NO_3^- proportionally such that the limitations of using macrophytes to indicate effluent NH_4^+ can be established.

In-situ chamber incubations with macrophytes, $\delta^{15}N\text{-}NH_4^+$ tracers, and PO_4^{3-} additions indicated that, during peak biomass in the mid-reaches of the Grand River, short-term incubations with added PO_4^{3-} had no detectible effect on macrophyte N uptake or N_2O production, however the difference in N uptake rate and N_2O production between upstream and downstream sites indicated that the riverine macrophyte community responded to continuously elevated nutrients which resulted in changes to nitrogen. NH_4^+ uptake increased

with of NH_4^+ concentration, up to a concentration of approximately 100 µg N/l, illustrating macrophyte preference for NH_4^+ even when NO_3^- is available, and supports similar findings from the previous chapter. The work also indicated that rapid recycling of NH_4^+ occurred in the chambers, which also supports the idea of ammonia limitation of macrophyte and epiphyton. The idea of ammonia limitation implies that not only is the quantity of nutrient loading important in influencing river benthic communities, but that the chemical form of the nutrient and the processes that convert nutrients between forms are important as well. Results from this work also provide some evidence that N_2O production in the chambers was influenced by the activity of macrophytes and identifies a role for macrophytes in community level denitrification and nitrification activity. Eutrophication of rivers thus results in changes to the benthic plant community as well as changes to biogeochemical cycling mediated through macrophyte communities. Future studies should quantify the effect of different forms of nitrogen added to benthic habitats on biogeochemical cycling mediated by macrophytes, research which could demonstrate the value of nitrification of waste effluent in mitigating impacts to macrophyte dominated river ecosystems.

This thesis contributes to the understanding of the eutrophication processes in rivers by demonstrating the influence of anthropogenic nutrients on the biomass of the submersed macrophyte community in the Grand River at the reach. It shows that spatial and temporal variation is an important feature of the biomass response, and that some of the former can be predicted by weather. It also suggests an important role for NH_4^+ in the macrophyte nutrient cycling and the riverine eutrophication process, even in river reaches where the concentration of nitrate is high. The thesis also provides evidence of consequences of eutrophication that may be unique to rivers, and that macrophyte biomass may be able to influence nitrogen cycle processes and alter the fate of reactive N species. Eutrophication in rivers results in increased primary producer biomass in response to elevated nutrients, and thus the most general definition of eutrophication, a movement of an aquatic system towards dominance by the primary producer community, can be extended to rivers. However, the process of eutrophication is likely to have different biogeochemical consequences for rivers than for lakes, thus for water and habitat quality. Eutrophication is thus a process that both brings unity to our understanding of disparate aquatic ecosystems as well as illustrates their diversity and complexity.

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