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Diel oxygen cycles in the Bow River: Relationships to Calgary's urban nutrient footprint and periphyton and macrophyte biomass

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Diel oxygen cycles in the Bow River:

Relationships to Calgary's urban nutrient footprint and periphyton and macrophyte biomass

by

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Abstract

The City of Calgary discharges wastewater effluent from three wastewater treatment plants. Although nutrient inputs from effluent increase fish growth, increased productivity may be detrimental to fish populations by increasing primary producer biomass, which subsequently affects the magnitude of diel oxygen (O_2) concentrations through photosynthesis and respiration. Overnight depressed O_2 concentrations can negatively impact local fish populations.

Changes in nitrogen and phosphorus concentrations, periphyton and macrophyte biomass, $\delta^{15}N$ isotopes and diel O_2 cycles were measured along the Bow River during summer months when primary producer metabolism and water temperatures are highest. A strong urban footprint associated with wastewater effluent inputs was detected. Primary producer biomass is dominated by periphyton upstream, while macrophytes dominate the river beginning downstream of Calgary's first effluent input. The transition from periphyton to macrophyte dominated communities leads to larger amplitude diel O_2 cycles, suggesting macrophytes are the primary driver of larger diel O_2 cycles.

Acknowledgements

I must first thank my supervisor, Dr. Leland Jackson, for his support and guidance throughout my M.Sc. degree. My time in the lab has been an incredible learning experience. My committee, Dr. John Post and Dr. Monique Dubé, also provided invaluable advice for which I must thank them. This research certainly would not be possible without the generous funding provided by the Canadian Water Network (CWN) and the Alberta Conservation Association (ACA) Grants in Biodiversity.

I would like to extend my thanks to the Jackson Lab, particularly Lisa Duke, Haley Tunna and Erick Elgin, for their advice, support, and countless enlightening discussions. I am also grateful for the numerous assistants who have helped me in the lab and the field, and all the volunteers who helped me churn through the seemingly endless samples—everything would have taken double the time otherwise! I sincerely thank Dr. Bernhard Mayer and Steve Taylor for their insights in using environmental isotopes, and Wendell Koning for providing historical data and background information on the Bow River. I would have been lost without their help.

Finally, I would like to offer my love and thanks to my family: to my parents for being so patient and understanding, and to Kelvin for being there all this time despite the ups and downs.

Without you all, this thesis would not exist.

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Site Name	Coordinates	Location
LOUS	51.443143, -116.213855	Highway 93-Jasper junction
CANM	51.085517, 115.365283	Canmore
COCH	51.18255, -114.486083	Cochrane
BWNS	51.09895, -114.207833	Bowness Park
EDWR	51.0644, -114.1532	Edworthy Park
10ST	51.050411, -114.0848	10th Street Bridge
INGL	51.0382, -114.010883	Inglewood
GLEN	50.986683, -114.025933	By Glenmore Trail
RIVB	50.964994, -114.022662	Riverbend
DOUG	50.934767, -113.994183	Douglasdale
H22X	50.896283, -114.0107	Highway 22X
PINE	50.86245, -113.986267	By Pine Ck. wastewater treatment plant
STIR	50.84195, -113.951533	Stier's Ranch
PRED	50.850867, -113.922217	By Predator Bay
CTNW	50.879283, -113.84795	Cottonwood
MCKN	50.80465, -113.698517	McKinnon Flats
CARS	50.8314, -113.4175	Carseland
BNBK	51.007598, -14.020061	Bonnybrook wastewater treatment plant
FISH	50.908445, -114.010277	Fish Creek wastewater treatment plant
PNCK	50.853696, -113.970108	Pine Creek wastewater treatment plant

CHAPTER 1: INTRODUCTION

Ecosystems are complex assemblages of living and non-living components. Hallmarks of ecosystem ecology include high variability in rates of ecological processes, relatively high noise: signal ratio, and complex, often non-linear interactions. Given such complexity of ecosystems, ecosystem ecologists often adopt a holistic approach to study how ecosystem structure affects ecosystem function. Structure refers to ecosystem composition, and includes biotic (e.g. species life history and distribution, biomass) and abiotic components (e.g. nutrients, water, geological morphometry). Function refers to the rate of processes, which can be binned into energetics (e.g. production/respiration rates), nutrient cycling (e.g. turnover/loss rates), and changes in community structure (e.g. species diversity and interactions). Observation of changing trends within these three groups could indicate perturbations or stress on the system, which causes shifts in structure and the resulting functions (Odum 1985), and potentially ecosystem services upon which we depend.

Disturbances That Affect Riverine Function

Anthropogenic disturbances affect riverine structure and function on top of natural alterations driven by geography and climate (Sabater 2008). Dams and weirs have altered flow regimes, stream channelizations have transformed riparian banks, and water withdrawals and returns have altered biotic and abiotic processes occurring within the river. The River Continuum Concept (RCC) proposed that the biological features of a stream are determined primarily by the physical system, which generates a series of biological changes that are driven by physical-geomorphic changes from headwaters to lower reaches (Vannote et al. 1980). If true, mitigation of anthropogenic disturbances may be attempted through manipulation of a stream's physical characteristics. However, this often proves to be difficult to execute because manipulation is labour-intensive and expensive. It may also be unfeasible due to increased disturbance as a result of the proposed mitigation. Stream channelization may improve flow, but have adverse effects on invertebrates and fish through loss of habitat, and increase sediment loads through increased erosion from exposed banks (Brooker 1985).

A change in chemical structure as a result of additional nutrient inputs from human activities has been of particular concern and a focus of riverine research. Nutrients, such as nitrogen and phosphorus, are major drivers of primary production, which subsequently affects other biotic and abiotic factors (e.g. eutrophication increases algal growth, leading to blooms that uses up the dissolved oxygen in the water). Manipulation of chemical discharge into streams may be easier to achieve relative to physical manipulation (e.g. manipulating nutrient concentrations in effluent as opposed to constructing a dam to regulate flow to control submerged aquatic plant growth), in which case it becomes a logical first step in mitigation projects. Derived from the longitudinal aspect of the RCC is the concept of nutrient spiralling, which explains nutrient cycling in fluvial systems, taking into account the coupled spatial-temporal aspects of nutrients flowing downstream during the nutrient cycling (Webster and Patten 1979, Ensign and Doyle 2006). This downstream transport subsequently affects the extent to which a stream can utilize available nutrients (Newbold et al. 1981). Stakeholders are thus concerned with not only what their section of the river is receiving in terms of point source and non-point source inputs, but also additional nutrients leaked from inefficient upstream processes. Nutrients from an upstream point that were not used by upstream biota will be transported downstream and can contribute to the growth of downstream biota. In managing wastewater effluent, upstream nutrients need to be considered to understand how effluent input will affect local biota.

Cumulative Effects

Canada's current policy and regulations require implementation of cumulative effects assessment (CEA) within the environmental impact assessment (EIA) framework to evaluate environmental consequences of development in a watershed (Smit and Spaling 1995, Baxter et al. 2001). CEA focuses on how an ecosystem function is affected by various influences (Therivel and Ross 2007). For example, fish populations may drastically respond to changes in predator abundance, food availability, and dissolved oxygen levels. CEA must then consider how each of those variables individually affect fish populations, and how individual effects that may appear insignificant on their own, interact to produce a significant cumulative effect. CEA systematically assesses the existing state of accumulating environmental change as a result of various interacting stressors. The change assessment, be it spatial or temporal, is vital to CEA; interest in the relationship between stressors and response is the direct result of observable, quantified change that suggests a response in the indicator of choice (e.g. a fish population).

CEA also incorporates a predictive aspect (Smit and Spaling 1995, Dubé and Munkittrick 2001, Dubé 2003, Duinker and Greig 2006), which allows the EIA performed to inform managers and practitioners about possible environmental consequences of a proposed development. However, the current predictive capabilities of

CEA are limited because of CEA’s emphasis on only additive effects. While cumulative effects may be generally additive, chemical changes, such as nutrient accumulation, may be synergistic instead (MacDonald 2000, Dubé et al. 2010). CEA for rivers thus requires improved understanding of stressor-response, or structure-function relationships to increase the accuracy of CEA predictions for environmental consequences. Another potential problem occurs when trying to make cumulative effect predictions because ecosystem processes tend to be nonlinear; therefore, an unit change in structure does not necessarily generate an unit change in function.

Alternate States in Riverine Systems

If point-source nutrient inputs create different water quality states, the system may be more analogous to separate states or regimes. The idea that ecosystems could exist in multiple states was first proposed by Lewontin (1969), who used the term "alternate stable states". Theory suggests an ecosystem may be able to adopt different states or configuration across the same range of driver conditions. While a current state will generally remain non-transitory (thus "stable") due to ecosystem resilience, which is the magnitude of disturbance a system can buffer against before being pushed into another regime (Gunderson 2000), the system may shift from one regime to another following perturbation (Folke et al. 2004). When shifts do occur, they are often sudden, unexpected and undesirable—not to mention difficult to mitigate (Scheffer et al. 1993). There are two primary theories as to how regimes shift, and both can be demonstrated with a ball-and-cup diagram (Fig. 1).

Numerous mathematical models have identified conceptually how alternate states could occur and supported their theoretical existence in nature (Holling 1973, May 1977). Experimental evidence for shifts was scarce and initially strongly criticized, with the belief that the theory worked well on paper but not so much in real life. However, recent studies have provided a strong empirical case for alternate stable states in nature (Scheffer et al. 2001), which have been extensively demonstrated in shallow lakes and marine ecosystems, although examples also include terrestrial systems (Blindow et al. 1993, Scheffer et al. 1993, Bayley and Prather 2003, Jackson 2003, Folke et al. 2004, Petraitis and Dudgeon 2004). The idea of alternate states is particularly well-studied in shallow lakes, with dramatic examples of sudden shifts between clear, low turbidity, low algal, abundant macrophyte(submerged aquatic plants) systems to turbid, high algal, low macrophyte systems (Scheffer et al. 1993, Scheffer et al. 2001). The shift from a clear to turbid state appears to be due to nutrient loadings and biotic relationships that bring water turbidity past a critical threshold (Fig. 2).

Documented cases of alternate stable states in riverine systems are rare. However, one example stems from reservoirs, which are known to alter riverine flow regimes. Experimental biannual flooding was initiated to test how reversion to a natural flow regime would change a currently regulated river. Although there was little change in the physicochemistry of the river, significant reductions in macroinvertebrate richness and biomass were recorded three years after the floods. Subsequent floods were found to have caused less disturbance than earlier floods, and it was concluded that the system had shifted to a regime more resilient to floods (Robinson and Uehlinger 2008). Nonlinear models that incorporate positive feedbacks and multiple states have been proposed for rivers—but it has been argued that rivers, unlike lakes, may be highly affected by geomorphology and hydrology and thus feedbacks must take into account more than just biotic factors (Dent et al. 2002).

An improved understanding of alternate states is critical for watershed management. If rivers do exhibit alternate states, management practices must be altered to prioritize avoidance of a state shift, given the drastic implications it has for a changed ecosystem and the difficulty involved with reversing a shift. Being unable to predict a potential state shift could also lead to undesirable surprises and costly restoration (Beisner et al. 2003).

The Bow River

The City of Calgary, located in Southern Alberta, Canada, relies heavily on the Bow River for drinking water, irrigation, hydroelectricity and recreation, to name a few. The Bow River sources from Bow Glacier in the Canadian Rockies, which flows into Bow Lake before flowing into Bow River. Wastewater treatment plants (WWTPs) are significant point-source stressors on the Bow River, as effluent discharge is a major nutrient contributor to streams and rivers (Nichols 1983, Nagumo and Hatano 2000). Through its course, the river passes through the Hamlet of Lake Louise, the towns of Banff, Canmore and Cochrane, and the City of Calgary. The Bow River is highly regulated, with 13 dams and weirs along its length, leading to an altered flow regime and fairly regular flows through its stretch. Municipal and industrial effluents place nutrient stress on the Bow River. There are six WWTPs directly discharging into the Bow River: one each for Lake Louise, Banff, Canmore, and three for Calgary (which also treats

Cochrane's wastewater). Most nutrient input into the Bow River is from effluent discharged from Calgary's three WWTPs (BRBC 2005); the composition of said effluent can be found in Appendix A. Nitrogen and phosphorus, the two most important nutrients in aquatic systems for biotic growth, have been found to be significantly higher on the right bank (where the WWTPs are located and effluent is released) relative to the left bank immediately downstream of Calgary's most upstream WWTP (Bonnybrook WWTP); in particular, the biologically available fractions of nitrogen and phosphorus made up major components of the nutrients found in the right bank (AENV 2011).

Macrophyte biomass peaks during late summer and early fall (Charlton et al. 1986). The right bank of the Bow River shows a much higher average macrophyte density compared to the right bank as of 2011, with higher biomass abundance around the WWTPs and reduced biomass abundance in reaches distant from the WWTPs (AENV 2011). This spatial trend in macrophyte density correlated to nutrient spatial trends, and thus increases in macrophyte density and biomass have been attributed to nutrient-rich effluent input from Calgary's WWTPs (Carr and Chambers 1998, Sosiak 2002, AENV 2011). Seasonally, macrophyte biomass peaked in September and began senescence in October (Sosiak 2002, AENV 2011). Periphyton density was opposite to that of macrophytes as the left bank had higher biomass relative to the right bank, but was similar to macrophytes in that higher densities were found in areas close to the WWTP rather than more distant from the WWTP (AENV 2011).

The Bow River, particularly downstream of Calgary, is a well known, world-class fishery for brown trout (*Salmo trutta*), rainbow trout (*Onchorhynchus mykiss*) and mountain whitefish (*Prosopium williamsoni*) (Government of Alberta 2010). Growth rates of rainbow trout in the Bow River are notably higher than in other areas of the province and the United States, and for all species, individuals were generally above-average in weight for their lengths (Council and Ripley 2006). However, while rainbow and brown trout appear to have benefited from nutrient enrichment from effluent input, other dominant fish species such as mountain whitefish appear to suffer in biomass downstream of WWTPs (Askey et al. 2007). Possible explanations include competition from rainbow trout, or macrophyte presence downstream of WWTPs having a negative effect on mountain whitefish foraging habitat (Askey et al. 2007) as there is a similar lag in fish response and macrophyte abundance below effluent input (Askey et al. 2007). The Bow River fishery is an important source of recreation and revenue for Alberta, and thus requires careful management to maintain a high quality. One period of concern is late summer, when primary productivity is high and may cause oxygen-stressed conditions. Although sportfish such as rainbow trout primarily spawn in the Highwood River, a tributary of the Bow, around 20% of rainbow trout spawn in the Bow River mainstem between mid-April and late June (Rhodes 2005). Rainbow trout eggs hatch in approximately 4-7 weeks (DFO 2013), which coincides with the beginning of this period of low oxygen conditions, putting young trout at risk of hypoxia.

Previous work on the Bow River had shown diurnal fluctuations in dissolved oxygen (DO), with the greatest magnitude of variation in nutrient enriched sites downstream of effluent input; the diurnal fluctuation curve was noted to resemble that of fluctuations found in eutrophic streams (Bathory et al. 2005). These fluctuations in DO concentration were greatest in August and September, and were attributed to higher degrees of photosynthesis and respiration from aquatic plants (Bathory et al. 2005). A major flood in 2005 decimated the macrophyte community in the Bow River, and drastically reduced the fluxes of DO concentration in the river (Robinson et al. 2009).

History of Wastewater Management in Bow River

Canadian wastewater treatment varies across the country. Coastal communities tend to have primary or no treatment while inland communities tend to have secondary or tertiary treatment (CCME 2006). Calgary's wastewater treatment plants (WWTPs) have tertiary treatment. Municipal governments are responsible for providing wastewater treatment, and sewer use bylaws (CCME 2006). Provincial and territorial governments are responsible for the operation regulations of WWTPs (CCME 2006). While the federal government does not directly legislate municipal effluent discharges, they do enforce the *Fisheries Act* and *Canadian Environmental Protection Act (1999)*, which protects Canadian waters; wastewater effluent does need to comply with those two legislations (CCME 2006). In 2003, the three levels of Canadian government began to collaborate to develop a national strategy to improve wastewater management and reducing effluent impacts on human health and the environment (CCME 2006). As of 2012, they have developed an approach for management of wastewater biosolids, sludge and treated septage (see http://www.ccme.ca).

Prior to construction of Calgary's three WWTPs, the city discharged raw sewage into the Bow River and assumed that dilution would negate environmental impacts(Armstrong et al. 2009). After downstream communities complained about degrading water quality there was a push for a sewage treatment. Bonnybrook WWTP was commissioned in the mid-1930s (Armstrong et al. 2009).

At the time, Bonnybrook WWTP provided primary treatment for up to 72 million litres of sewage per day (Armstrong et al. 2009), which involved screening large objects, settling suspended solids and bacterial break down of organic materials in a large digester tank, which produced residual sludge that was landfilled (CCME 2006). The treated effluent was released into the Bow River. While primary treatment was a great improvement compared to no treatment and removed approximately half of the suspended solids and reduced oxygen demand in the river by approximately 25%, not all sewage passed through the Bonnybrook WWTP, and a substantial amount of sewage from parts of Calgary flowed untreated into the Bow River (Armstrong et al. 2009). During times of heavy stress on the plant, such as heavy rainfall or clogging up of the sewage treatment works, operators would also simply allow the sewage to bypass the plant and flow untreated into the river (Armstrong et al. 2009).

Calgary's population continued to grow, which increased demand for additional sewage treatment. The city more than doubled the capacity of Bonnybrook in the late 1950s and built the Fish Creek WWTP. Fish Creek WWTP was also a primary treatment plant, and together the two plants removed about half the suspended solids, and 25% of organic content from domestic and industrial sewage (Armstrong et al. 2009). However, complaints began from fishermen, who enjoyed the developing recreational fishery downstream of the WWTPs as fish caught immediately downstream of the plants had a persistent oily taste that made them inedible (Armstrong et al. 2009). The oil boom at the time led to refineries releasing untreated wastewater that still had oil residues and phenols, which produced the oily taste (Armstrong et al. 2009). Thick growths of pondweed, resulting from increased phosphate inputs into the river from the increasing popularity of laundry detergents, provided an additional warning of the Bow River's waning water quality (Armstrong et al. 2009). Reports in 1965 showed depressed levels of dissolved oxygen that were further worsened by increasing weed growth driven by phosphate-laden waters (Armstrong et al. 2009). There were large fluxes in dissolved oxygen concentrations over the course of a day, with concentrations fatally low for fish. The reports concluded that secondary treatment was necessary to address these issues and removal of phosphate was particularly important (Armstrong et al. 2009).

Following these recommendations, Bonnybrook WWTP was quickly upgraded to secondary treatment by 1971. There was significantly reduced nutrient loading to the river observed post-operation of this upgraded plant (Armstrong et al. 2009). Fish Creek WWTP soon followed suit and was upgraded to secondary treatment in 1980, to further accommodate the increasing population (Armstrong et al. 2009). Secondary treatment is designed to remove biodegradable organic matter and suspended solids (CCME 2006). In this case, it also included nutrient removal. Both plants were upgraded again in the 1980s to tertiary treatment for chemical phosphorus removal (Armstrong et al. 2009). Tertiary treatment is added to secondary treatment to remove suspended, colloidal and dissolved constituents that remain after secondary treatment and often includes biological processes to remove nutrients (CCME 2006). Chlorination, which was implemented with secondary treatment, was replaced by UV disinfection. By the 1990s, Bonnybrook and Fish Creek WWTPs were removing 92% of suspended solids and 88% of phosphorus from Calgary's wastewater, returning high-quality effluent back into the Bow River (Armstrong et al. 2009). Fishermen's complaints changed from oily-tasting fish to fish in the Bow River being less numerous, smaller and thus harder to catch - a likely result of the greatly decreased nutrient loading (Armstrong et al. 2009). A third WWTP, Pine Creek WWTP, was opened in 2008 as a state-of-the-art tertiary treatment plant to handle increasing sewage treatment pressures from a fast-growing Calgary. While Calgary has taken much initiative in improving sewage treatment and maintaining the quality of the Bow River, and with a good degree of success, wastewater effluent and stormwater runoff (which remains largely untreated) still compromise water quality downstream of the WWTPs (BRBC 2005).

Macrophytes proliferate in reaches downstream of effluent inputs, as those same reaches now receive higher nutrient loads from a growing population. Effluent inputs represent a substantial additional resource to the riverine food web through increase in production (deBruyn et al. 2003). This occurs also in large Canadian rivers as the stream flow is often slow enough that macrophytes are able to establish and utilize the wealth of nutrients (Chambers 1994). Macrophyte biomass has been shown to reduce dramatically as a result of improved wastewater treatment across Canadian rivers, including in the Bow River, suggesting a strong relationship between nutrient loadings and macrophyte biomass (Sosiak 1990, Chambers 1993, Sosiak 2002). There are also additional issues associated with fecal coliforms (which can cause severe illness in humans), biochemical oxygen demand (amount of dissolved oxygen used to break down organic materials), total suspended solids (organic and inorganic debris suspended in the water), and metals (found in relatively small quantities in effluent, but can cause severe illness in higher quantities) (CCME 2006). Emerging contaminants are of increasing concern, as many are persistent, bioaccumulative, biologically active or toxic. Emerging contaminants include natural and synthetic hormones, pesticides and surfactants, dioxins and furans, DDT and

PCBs; these chemicals are often found in commonly used products(CCME 2006). Fish populations are sensitive to contaminants and pollutants in the water, and thus can be one indicator for the health of the aquatic environment in which they reside (Bathory et al. 2005).

Study Objectives

Eutrophication remains a major global problem, plaguing not only freshwaters but also marine and terrestrial systems (Smith et al. 1999). Low nutrient levels are thus highly preferential to high nutrient levels; cities continuously pursue sewage treatment upgrades to minimize eutrophication (Mallin et al. 2005). Urban wastewater can be a huge contributor to nutrient inputs, leading to increased primary producer biomass, subsequently affecting the magnitude of diel oxygen fluxes in the water. Dissolved oxygen levels may be depressed to hypoxic values downstream of high nutrient concentrations before recovering, presenting an oxygen sag. As wastewater effluent can significantly alter a stream's chemical structure, it presents an excellent opportunity to observe subsequent changes in stream function. Phosphorus and nitrogen levels are expected to increase as the waters flow from relatively pristine headwaters through a large urban centre. A main goal of my research was to identify the drivers behind changes in nutrient, primary production and diel oxygen cycles in the river, and determine whether these drivers have significant effects on their own or only within an interactive feedback context. I also considered any discontinuities that may suggest the presence of alternate states or regimes. The goal was not to test for the existence of alternate states in the river system. Alternate states are but one possible form of non-linear relationships resulting from feedbacks (Anderson et al. 2006). Rather than considering clear and turbid states, I proposed the division of high and low quality conditions in the Bow River as described in Anderson et al. (2006). A high quality condition would be defined by low nutrient concentrations, low biomass and minor fluctuations in diel O_2 cycles; a low quality condition would be defined by high nutrient concentrations, high biomass and major fluctuations in diel O_2 cycles. I expected to see a high quality condition upstream of wastewater effluent inputs where macrophyte biomass is low due to low nutrient concentrations. I expected a low quality condition downstream of the wastewater effluent inputs where nutrient concentrations are high and macrophytes are able to increase their biomass. The transition between these two quality conditions is affected by flow and the transition may be linear or non-linear.

It was also interested in utilizing wastewater isotope tracing ($\delta^{15}N$) to identify the "footprint" of wastewater effluent, which can increase the nutrient heterogeneity between the two banks at a particular site in the river. Wastewater isotope tracing was used to estimate how much of the nutrient contribution to the river is attributed to wastewater discharge (and thus be able to identify the magnitude of perturbation). Algae in effluent-affected sites display significantly enriched values of $\delta^{15}N$ compared to untreated sites, suggesting that a high level of nutrient loading in the river may be attributed to wastewater discharge (Nagumo and Hatano 2000, Wayland and Hobson 2001).

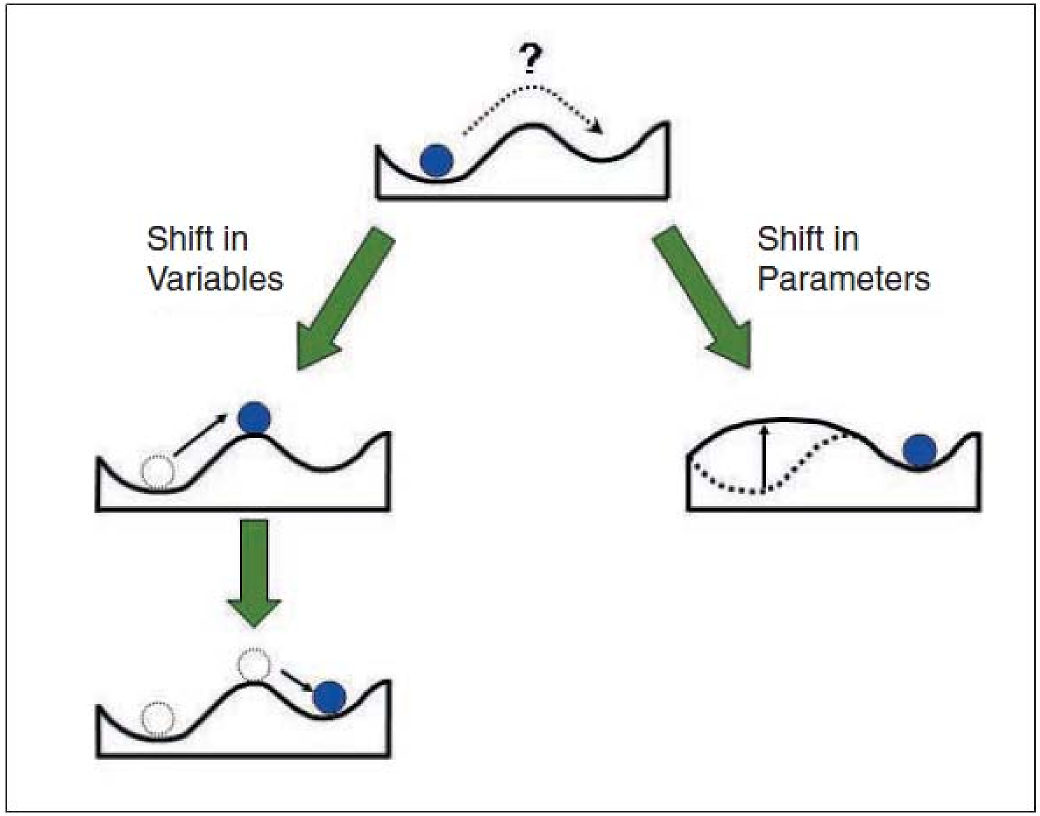

Figure 1. Ball-and-cup model demonstrating alternate state shifts. Figure from Beisner et al. (2003). The ball represents the system, and the valleys represent state equilibria. (Left) The system is pushed from one equilibrium to another by perturbation as a result of a shift in variables. (Right) The system is forced to move on to another equilibrium as the equilibrium it was previously in is no longer an equilibrium due to a shift in parameters.

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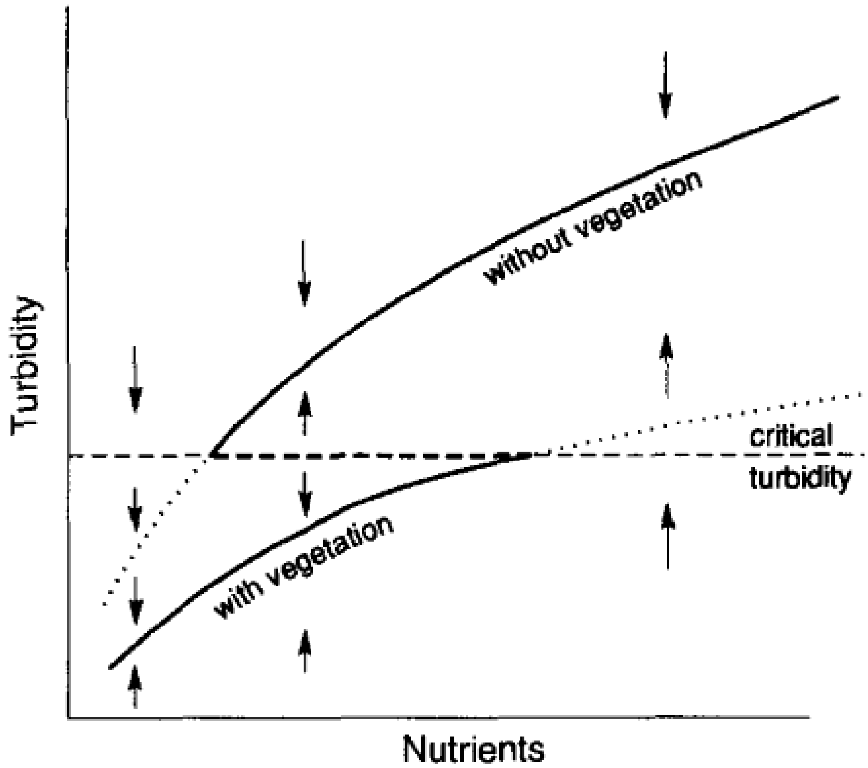

Figure 2. Graphical model of alternate stable states in a shallow lake. Figure from Scheffer et al. (1993). Macrophytes are referred to as vegetation in the model. The two curves represent the two equilibria or stable states the system may assume. The model makes three assumptions: 1) water turbidity increases as nutrient levels increase, 2) macrophytes help reduce turbidity, and 3) above the critical turbidity, light limitation occurs and macrophytes cannot persist in the system. When turbidity is below the critical level, macrophytes are present and will assist in keeping turbidity low through positive feedback; the system is then likely to remain below the lower equilibrium line. Should a significant perturbation occur (e.g. anthropogenic loading of nutrients) and turbidity exceeds the critical level, the upper equilibrium line dominates, macrophytes are unable to persist and the positive feedback is lost. A possible driver is the addition of a herbivore to the shallow lake, which would decrease macrophyte biomass. This decrease in macrophyte biomass would then increase water turbidity. Removal of the herbivore at this point (before it passes the critical turbidity) will allow the macrophyte population to slowly recover. However, if grazing pressure by the herbivore continues and macrophyte growth rates cannot recover the lost biomass, turbidity will continue to increase, eventually reaching and surpassing the critical turbidity level. At that point, the lake will shift from a clear regime to a turbid regime. In the turbid regime, macrophyte biomass is unlikely to recover even if the herbivore is removed, due to limited light penetration.

My thesis is organized into a general introduction (Chapter 1), two data chapters (Chapters 2 and 3), and a general conclusion (Chapter 4). Each data chapter has been written in manuscript style. In the first data chapter I examined nutrients, primary producers and diel oxygen cycles, while in the second data chapter I focused on the utilization of wastewater isotope tracing. The general conclusion summarizes my major findings.

CHAPTER 2: LONGITUDINAL STUDY OF NUTRIENTS IN THE BOW RIVER (CALGARY, ALBERTA)

Introduction

The City of Calgary (Alberta, Canada) has experienced immense growth in recent years as the result of a thriving oil and gas industry. Between 2006 and 2011, there was a 10.9% increase in the population, breaking the population milestone of one million (StatisticsCanada 2011) . An increase in people also meant an increase in effluent volume from the city's three wastewater treatment plants (WWTP). Wastewater effluent is a major source of pollution in Canadian streams, rivers and lakes, and although all three of Calgary's WWTPs possess tertiary treatment, enough nutrients remain in the effluent to have an effect on the receiving water of the Bow River.

Nutrient inputs into aquatic systems often lead to eutrophication, which can have severe ecological and financial impacts. Algal blooms not only decrease the aesthetics of a river, impacting tourism and recreational activities, but can also affect aquatic life through shading, toxic excretions, and depressed oxygen levels induced by senescence and decomposition. Communities downstream of effluent inputs may incur increased costs of drinking water treatment to remove particles, bacteria and dissolved contaminants.

Hypoxia as a Result of Eutrophication

Dissolved oxygen (DO) concentrations are monitored by the City of Calgary as an indicator for effluent—specifically phosphorus—effects (BRBC 2005). DO concentrations are affected by factors such as temperature and geomorphology, and fluctuate primarily as a function of photosynthesis and respiration by autotrophs. In the late 1970s to early 1980s, macrophytes and algae were found to be most abundant in the middle reaches of the Bow River (Figure 3), particularly downstream of wastewater input. This reach was also prone to nuisance levels of aquatic plants (Charlton et al. 1986). When WWTP upgrades were implemented, the subsequent six years saw a decrease in macrophyte biomass, presumably due to the reduced nutrient loading. Periphyton (algae attached to underwater substrate) biomass, however, did not change significantly (Sosiak 1990, 2002).

The province of Alberta currently establishes the dissolved oxygen guideline for the protection of freshwater aquatic life as 5.0 mg/L; lower concentrations may cause significant stress or even mortality (AENV 1997). Although high nutrient levels may benefit fish growth, high nutrients may actually be indirectly detrimental to fish populations. High nutrient concentrations encourage macrophyte growth. Depressed oxygen (O_2) concentrations thus pose major concerns during summer periods, when sunlight availability is high, primary producer metabolism is high, water flow is low and water temperature is high. Macrophytes also trap suspended sediments and reduce water flow, which along with the increased plant biomass, causes summer overnight oxygen depression to be heightened.

Depending on the severity of oxygen depression, fish populations may experience stress and mortality in several ways. In areas with moderate O_2 depression, fish may leave areas of high macrophyte biomass that had served as refuge and experience increased predation. Aerial respiration and/or lowering activity level help fish to alleviate the stress of low oxygen levels, besides shifting habitat (Kramer 1987). Surfacing for air may also increase predation risks. High oxygen depression may cause juvenile fish to suffer direct mortality because juveniles are more sensitive to low DO concentrations than adults (Doudoroff and Shumway 1970, Alabaster and Lloyd 1980, Johnson and Evans 1991). Should oxygen depression become severe and widespread, massive fish kills may occur (Anderson et al. 2006).

Current Efforts in Management

Bonnybrook WWTP is the oldest WWTP in Calgary, established in 1918 and expanded twicein 1958 and 1994. Fish Creek WWTP was built in 1960 to accommodate the growing city. Both WWTPs were eventually upgraded to secondary, then tertiary treatment. Pine Creek WWTP opened in 2008 and was designed to have enhanced nitrogen removal. All three WWTP have screening, sedimentation, activated sludge treatment, biochemical phosphorus removal, anaerobic digestion and ultraviolet-light disinfection. Bonnybrook and Pine Creek WWTPs utilize Biological Nutrient Removal (BNR), a biological nutrient process that removes phosphorus and nitrogen using microorganisms or chemicals. BNR has helped to reduce total ammonia concentrations. Fish Creek uses oxygen activated sludge, utilizing the Union Carbide Oxygenation System (UNOX), which improved the conventional activated sludge process by providing a high oxygen transfer rate. Alberta surface water quality guidelines dictate total phosphorus limits of 0.05mg/L, and total nitrogen should not exceed 1.0mg/L (AENV 1999). Calgary's WWTPs follow plant-specific guidelines for effluent nutrient levels. Bonnybrook WWTP has a total phosphorus limit of <1.0 mg/L, and ammonia-nitrogen limits of <5.0 mg/L (July 1 to Sep 30) and <10 mg/L (Oct 1 to June 30). Fish Ck. WWTP has a total phosphorus limit of <1.0 mg/L. Pine Ck. WWTP has a total phosphorus limit of ≤0.5 mg/L, total nitrogen limit of ≤15 mg/L, and ammonia-nitrogen limits of ≤5 mg/L (July 1 to Sep 30) and ≤10 mg/L (Oct 1 to June 30). Averaged annual final effluent quality information for all three plants in 2012 is in Appendix A.

Monitoring the quality of effluent can improve adaptive management and indirectly help avoid eutrophication-induced hypoxia. It had been previously found that nutrient levels and growth of submerged macrophytes were low upstream of Calgary, whereas downstream of the WWTPs the riverbed displayed substantial macrophyte growth (Charlton et al. 1986). There have been significant efforts invested to limit the input of organic materials and nutrients to the Bow River; reduction of municipal loading has indeed reduced macrophyte and periphyton biomass (Sosiak 2002).

Objectives

Several questions were addressed in this study to investigate and understand the effect of wastewater effluent on dissolved oxygen levels in the Bow River:

1) What is the spatial change in N and P concentrations throughout the Bow River from pristine headwaters to downstream of wastewater effluent inputs? The most upstream sites should show low nutrient levels that increase as the river approaches and enters the City of Calgary. Significant jumps in N and P are expected immediately downstream of wastewater effluent inputs.

2) Are spatial changes in nutrient concentrations reflected in local primary producer biomass? An increase in nutrients available for primary producer growth would be expected to increase primary producer biomass.
3) Do diel O_2 concentrations vary as a result of changing primary producer biomass? Are there areas of hypoxia? Increased primary producer biomass would suggest higher levels of photosynthesis during the day and higher levels of respiration during the night, leading to larger amplitudes in diel O_2 cycles. If fluctuations are large enough, there may be night-restricted hypoxic areas.
4) If there are hypoxic areas, are there any observable effects on the local fish population? Negative effects of hypoxia may be presented as decreased growth rates and lowered condition factors. Missing or significantly decreased abundance in younger age classes may also suggest increased mortality, whether directly through low DO concentrations or indirectly through predation.

Materials and Methods

Study Area

The Bow River originates at Bow Lake in the Canadian Rocky Mountains, and flows south-eastward across the Alberta foothills and prairies until it joins the Oldman River to form the South Saskatchewan River. Wastewater treatment plants (WWTPs) are significant point-source stressors on the Bow River, as effluent discharge is a major nutrient contributor to streams and rivers (Nichols 1983, Nagumo and Hatano 2000). Through its course, the river passes through the Hamlet of Lake Louise, the towns of

Banff, Canmore and Cochrane, and the City of Calgary. The Bow River is highly regulated, with 13 dams and weirs along its length, leading to an altered flow regime and fairly regular flows through its stretch. Municipal and industrial effluents place nutrient stress on the Bow River. There are six WWTPs directly discharging into the Bow River: one each for Lake Louise, Banff, Canmore, and three for Calgary (which also treats Cochrane's wastewater). Most nutrient input into the Bow River is from effluent discharged from Calgary's three WWTPs (BRBC 2005). Macrophyte biomass peaks during late summer and early fall (Charlton et al. 1986).

The Bow River, particularly downstream of Calgary, is a well known, world-class fishery for brown trout (*Salmo trutta*), rainbow trout (*Onchorhynchus mykiss*) and mountain whitefish (*Prosopium williamsoni*) (Government of Alberta 2010). Growth rates of rainbow trout in the Bow River are notably higher than in other areas of the province and the United States, and for all species, individuals were generally above-average in weight for their lengths (Council and Ripley 2006). This fishery is an important source of recreation and revenue for Alberta, and thus requires careful management to maintain a high quality fishery. One period of concern is late summer, when primary productivity and water temperatures are high and may cause oxygen-stressed conditions.

Sample Collection

Approximately 300 km of the Bow River, Alberta was studied between July 20-October 24, 2010 and July 19-September 22, 2011 to capture the period of maximum macrophyte biomass. Thirteen sites were sampled four times in 2010, and 16 sites were sampled three times in 2011. Site 10ST was only sampled in 2010, and sites GLEN, RIVB, H22X and PRED were only sampled in 2011 (Figure 3). The sites were chosen to identify changes in water column N and P, periphyton and macrophytes from undisturbed headwaters to downstream of Calgary's urban footprint. The most upstream site was located just east of the Highway 1-Highway 93 junction in Banff National Park, where the watershed is largely undisturbed. Subsequent sites should experience increasing nutrient inputs from tourism activities and small towns (low inputs),urban runoff, wastewater and agricultural disturbance. The largest point source of nutrient input into the Bow River are Calgary's three WWTPs. The most downstream sampling site was located east of Carseland, AB, where Highway 24 crosses the Bow River. Ranching and oil and gas extraction are the main land uses downstream of Calgary.

During the 2010 sampling, one bank at each site was sampled for water chemistry, periphyton and macrophytes. Three 1L water samples were collected from mid-water column for analysis of total phosphorus (TP), soluble reactive phosphorus (SRP), total suspended solids (TSS), and chlorophyll *a* (chl *a*). Three additional 125mL water samples were collected with the 1L samples and sent to the University of Alberta Biogeochemical Analytical Service Lab for total nitrogen (TN), nitrite+nitrate (NO_2^- $+NO_3^-$) and ammonium (NH_4^+) analysis. TN was analysed with a Shimadzu 5000A TOC analyzer and TOC-V CPH with TMN unit. NO_2^-+NO_3^- and NH_4^+ were analysed using flow injection analysis with a Lachat QuikChem 8500 FIA automated ion analyzer. A Shimadzu UV Spectrophotometer (model UV-1800, 120V) was used for the phosphorus analyses, which were performed using a simplified technique involving a single digestion reagent and a single "mixed reagent" for colour development (Eisenreich et al. 1975). Chl *a* was measured using the methanol extraction method with a Barnstead/Turner Quantech fluorometer (model QNT Wide 120V) (Holm-Hansen and Riemann 1978).

To obtain periphyton samples, three scrapes within a 4 cm internal diameter circular template were performed on each of five rocks taken from the riverbed around each bank. One scrape was used to determine ash-free dry mass, one scrape was used to determine periphyton chl *a* (by methanol extraction), and one scrape was preserved in 0.5% glutaraldehyde for algal enumeration. Macrophytes were harvested from establishment to end of season to capture maximum seasonal biomass. One to five 936.36cm^2 quadrats were sampled along each bank. Macrophyte samples were identified to species and measured for fresh and dry biomass. Dissolved oxygen (DO) loggers (RBR DO-1050) were calibrated to 100% O_2 using Ruskin 1.5.25 (RBR). The DO loggers were deployed once at each site with a temperature logger (Alpha Mach iBCod type Z) for 24 hours in order to capture diel oxygen concentrations. Water temperature, flow, depth, pH and dissolved oxygen concentration also were measured at each sampling event.

A backpack electrofisher (Smith-Root Backpack Model 12B) was used to collect 30-50 longnose dace (*Rhinichthys cataractae*) at each site with exception of sampling sites upstream of BWNS due to lack of abundance, and PRED due to site access limitations. Each dace collected was measured for forklength, weight, gonad weight, liver weight, sex and age (determined by removal and processing of sagittal otoliths). Calculations were made from these data to determine condition factor, gonadosomatic index (GSI), hepatosomatic index (HSI), and length and weight growth rates (determined by dividing forklength and weight by age). Dace were also assessed for external deformities, eroded fins, lesions and tumours (DELTs) as a broad indicator of chronic, sublethal environmental stress (OEPA 1995).

The 2011 field sampling was revised slightly based on 2010 results, incorporating the following changes: sampling both banks at each site instead of one to take into account mixing issues, reducing the number of periphyton samples from five to three, and chl *a* for water and periphyton samples were not taken. Two DO loggers and two temperature loggers were deployed at each site instead of one, with one pair (one DO + one temperature) on each bank.

Statistical Analysis

To determine the change in dissolve oxygen concentrations ([DO]) within a 24-hour period, I calculated expected [O_2] (mg/L) at 100% saturation based on Mortimer's (1942) temperature (°C) and atmospheric pressure (mm Hg) relationship:

$$\ln(O_2 \text{ saturated}) = 7.7117 – 1.31403 * \ln(T + 45.93) – \ln(P/760)$$

O_2 saturated = [O_2] at 100% saturation (mg/L)
T = water temperature (°C)
P = atmospheric pressure (mm Hg)

Water temperature was obtained from the temperature logger deployed alongside the DO logger, and atmospheric pressure was calculated using site altitudes taken from Google Earth and a table for partial pressure correction factors and dissolved oxygen solubility factors at different altitudes(Kalff 2002). ΔDO is referred to in this thesis as the difference between the temperature-adjusted maximum and minimum [DO].

Scatterplot matrices and Pearson's ranked correlation test were used in R 2.15.2 to identify significantly correlated variables. First differences were used in the Pearson's correlation test as it is a common time series method to create a detrended series, and thus allowed correlation testing of autocorrelated variables. From the Pearson's correlation results, some variables were found to be correlated and therefore excluded from the "beyond optimal model" to reduce model complexity and avoid convergence problems. From the nutrients measured, only SRP and dissolved inorganic nitrogen (DIN, includes NO_2^-, NO_3^- and NH_4^+) were retained in the model as they represent the biologically available N and P. Flow was significantly correlated with depth ($p=9.32\times10^{-5}$), but retained in the model as water flow contributes to the presence or absence of macrophytes(Madsen et al. 1993).

To account for fixed and random effects, as well as spatial autocorrelation, I created a linear mixed-effects model (LMM) with a Gaussian spatial variance-covariance structure in SAS 9.3. An autocorrelation function was plotted and the data showed no apparent temporal autocorrelation. Based on correlated variables and biological reasoning, the follow LMM was established and tested:

PP biomass = SRP + NO23 + NH4 + flow + pH + temperature + interactions

PP = primary producer (periphyton, macrophyte)
SRP = soluble reactive phosphorus
NO23 = nitrite + nitrate
NH4 = ammonium

Stepwise selection was used to reduce the model to the following two final selected models:

Periphyton biomass = NO23 + NH4 + flow + interactions

Macrophyte biomass = SRP + NO23 + NH4 + flow + temperature + interactions

The model was run to identify significant relationships in three river stretches—(1) upstream of WWTP: LOUS to INGL, (2) within WWTP effluent area: GLEN to

CTNW, and (3) downstream of effluent mixing zone: MCKN to CARS. The effluent mixing zone length was assumed to be around 30 km based on previous study (Hogberg 2004).

Spatial autocorrelation associated with sampling different points of the river violates the assumption of independence, making many traditional statistical approaches, such as ANOVA, problematic. One alternative approach is to look for discontinuities, which suggests an abrupt change in structure and can be identified as outliers in the data. Dixon's Q test and generalized ESD test were used in R. 2.15.2 to detect outliers. However, the tests were not successful in detecting outliers where large value jumps in constituent concentrations were seen. This may be due to the large variability associated with field data. The uncertainty regarding the reliability of Dixon's Q test and generalized ESD test thus led to the decision to not use those two tests for outliers. An appropriate test to compare a single variable measured across spatially autocorrelated sites could not be found, so qualitative assessment of the data was performed instead based on plotted data.

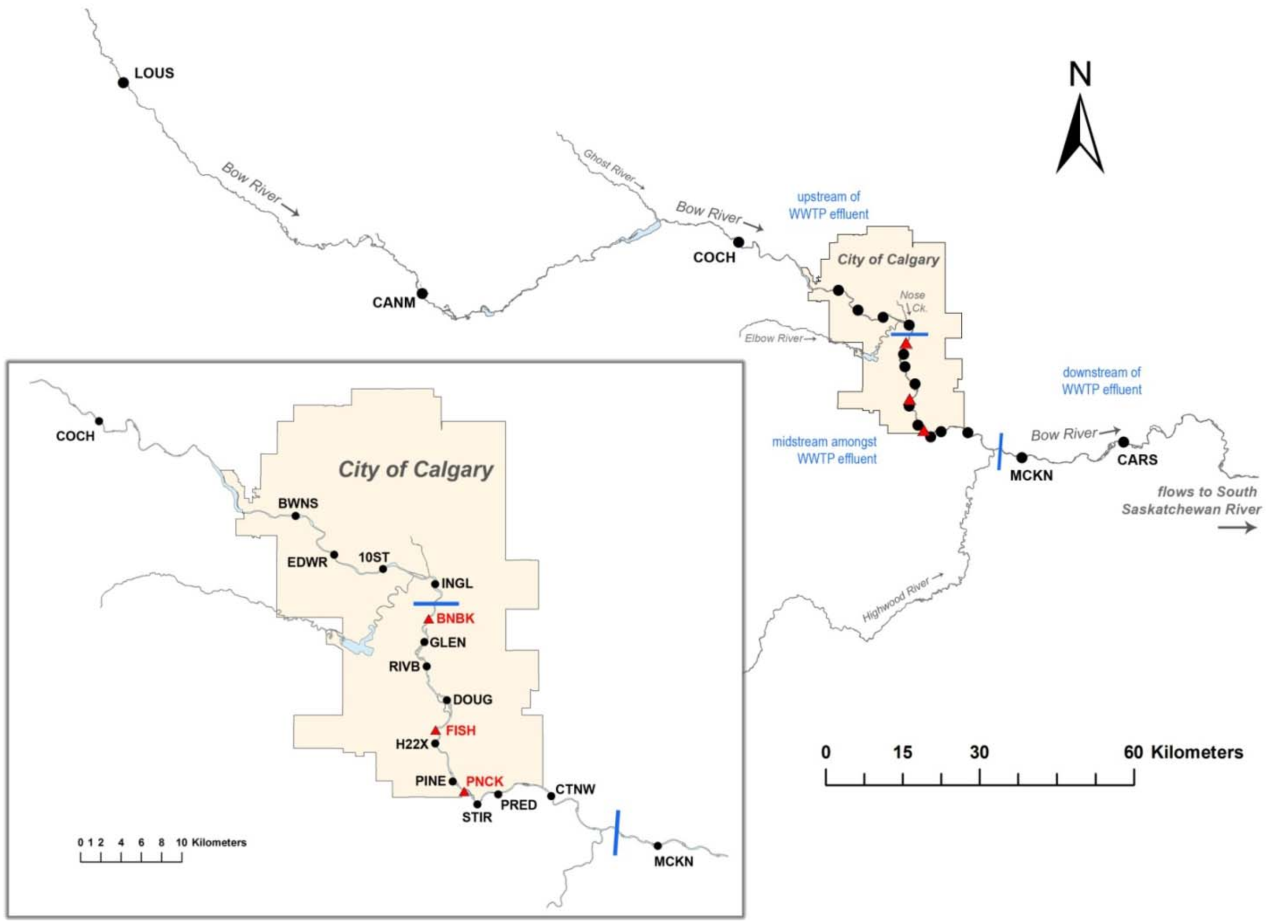

Figure 3. Map of 2010 and 2011 sampling sites on the Bow River, AB. Inset box shows a close up of finer spaced sites within the City of Calgary. Black circles indicate sampled sites and red triangles indicate Calgary's three wastewater treatment plants. Blue lines divide the sampled river stretch into upstream of effluent, midstream amongst effluent, and downstream of effluent for comparisons.

Results

Spatial patterns of measured variables

Water temperature increased by almost five degrees Celsius (8.9°C to 13.3°C) in the most upstream reach of the Bow River (site LOUS to COCH; Figure 4). Upon entering the City of Calgary (downstream of COCH, beginning with BWNS), water temperature remained fairly constant with slight fluctuations between sites (max. 16.0°C and min. 12.6°C; Figure 4). There were no observed trends in water temperature associated with wastewater effluent input points. Stream flow varied a bit across the 300 km sampled stretch of the Bow River, with a minimum of 0.36 m/s at RIVB and a maximum of 0.97 m/s at PRED (Figure 4). There was no consistent trend in stream flow from upstream to downstream. There were notable drops in flow rate after the first two WWTP effluent inputs (Figure 4). Water pH also showed slight drops in pH (<0.5 in change) downstream of WWTP effluent inputs, but remain fairly consistent with low variability (Figure 4).

Total suspended solids (TSS) showed little variability between sites (max. 2.65 mg/L and min. 0.68 mg/L), while turbidity had a relatively greater variability (max. 5.56 NTU and min. 1.78 NTU; Figure 5). There are small drops in TSS downstream of WWTPs, but turbidity levels remained constant from just upstream of the WWTPs (site 10ST) to downstream of the Calgary, beyond the expected mixing distance (site MCKN; Figure 5). Periphyton chlorophyll *a* was low (<500 mg/m^2) in upstream of Calgary sites and sites just entering Calgary (sites LOUS to EDWR), but rapidly increased upon the first WWTP input at Bonnybrook WWTP (Figure 5). Periphyton chlorophyll *a* then remained around 2000 mg/m^2 for much of the downstream portion of the Bow River

(Figure 5). Water chlorophyll *a* showed a similar trend to periphyton chlorophyll *a*, with relatively low values upstream of the WWTPs and then increasing chlorophyll *a* downstream of the WWTPs (Figure 5).

Total phosphorus (TP) and soluble reactive phosphorus (SRP) both showed increases downstream of the Bonnybrook WWTP on the right bank (the WWTP is located on the right bank and the effluent is also released closer to the right bank; Figure 6). TP reached its highest averaged concentration at site H22X (downstream of Fish Ck. WWTP), and SRP reached its highest averaged value at site GLEN (downstream of Bonnybrook WWTP; Figure 6).

Total nitrogen (TN), nitrite+nitrate ($NO_2^- + NO_3^-$) and ammonium (NH_4^+) all showed large increases in averaged concentration downstream of WWTPs (Figure 7). TN and $NO_2^- + NO_3^-$ showed almost identical trends on both banks, peaking just downstream of Bonnybrook WWTP at site GLEN (Figure 7). NH_4^+ increased slightly after Bonnybrook WWTP, and did not experience a large jump until downstream of Fish Ck. WWTP at site H22X (Figure 7). Nitrogen concentrations also showed much smaller seasonal variability than phosphorus concentrations (see error bars; Figure 6, Figure 7).

Periphyton AFDW displayed a very different pattern relative to macrophyte fresh biomass, [SRP] and [DIN] ($NO_2^- + NO_3^- + NH_4^+$), achieving its peak weight upstream of the Bonnybrook WWTP (Figure 8). Macrophytes were not found upstream of the first wastewater effluent input in Calgary, with exception of the site immediately upstream of Bonnybrook WWTP at INGL (Figure 8). The biomass present at INGL was very low, however; it is not until the site after the first WWTP (site GLEN) that macrophytes begin to flourish. Neither periphyton AFDW nor macrophyte fresh biomass showed differing patterns between left and right banks (Figure 9). It is also interesting to note that the macrophyte community really only began to establish and thrive as (i) the wastewater inputs entered the river and (ii) when the periphyton community began to dwindle in biomass. Diatoms were the most common class of algae found in the periphyton samples. The predominant macrophyte species found at sample sites was *Stuckenia pectinata* (formerly *Potamogeton pectinatus*).

The difference between observed ΔDO and expected (based on temperature and altitude) ΔDO increased downstream of the first WWTP input, which is where macrophytes begin to dominate (Figure 10). ΔDO was much larger on the right bank relative to the left bank, corresponding to larger nutrient peaks on the right bank (Figure 10).

Relationships between nutrients and plants

Periphyton ash free dry weight (AFDW) was unrelated to any of the measured physical or chemical variables (AppendixB). Macrophyte fresh biomass appears to be driven heavily by DIN concentration in the water (LMM: F=9.32, df=248, p=0.0025; Figure 9). SRP, flow and temperature did not independently explain macrophyte fresh biomass, but were significant when interacting with DIN (

Table 1; Appendix C).

Relationships between plants and dissolved oxygen

Cumulative periphyton AFDW and cumulative macrophyte fresh biomass were highly significant in explaining dissolved oxygen concentrations downstream of the first wastewater treatment plant (i.e. downstream of site INGL) (LMM: within effluent input stretch: periphyton: F=91.04, df=224, p=0.0001; macrophyte: F=Inf., df=5.12, p=0.0001; macrophyte: F=213.00, df=81, p=0.0001;Figure 10). While the Bow River generally stays above 5 mg O_2/L, there were quite a few sites, particularly those downstream of WWTPs, where the night time minimum fell below 5 mg O_2/L (Table 2).

Condition of longnose dace

Averaged condition factor (CF) increased from upstream to downstream, with a slight decrease downstream of Pine Ck. WWTP (Figure 11). Averaged fish age varied from site to site, with no consistent trend (Figure 11). Length growth rates (LGR) and weight growth rates (WGR) both experienced quite a bit of variation from site to site, but also displayed no consistent pattern (Figure 11). There were increases in LGR and WGR downstream of Bonnybrook WWTP, but a decrease can be seen in LGR and WGR downstream of Fish Ck. WWTP (Figure 11). LGR also decreased a bit downstream of Pine Ck. WWTP, while WGR experienced a very slight increase (Figure 11). Averaged gonadosomatic index decreased downstream of WWTPs, whereas hepatosomatic index increased downstream of WWTPs (Figure 12). No DELTs were found in any of the fish sampled and examined. A summary of collected fish data can be found in Appendix D.

Table 1. Significant explanatory variables for macrophyte fresh biomass as a function of biologically available nutrients (SRP, DIN) and physical parameters (flow, temperature). Asterisks (*) indicate interaction between listed variables.

	p-value	**df**	**F-value**
DIN	0.0025	248	9.32
SRP*DIN	< 0.0001	240	18.87
DIN*flow	0.0009	247	11.35
DIN*temp	0.0190	247	5.57
SRP*DIN*flow	0.0004	249	13.01
SRP*DIN*temp	< 0.0001	250	17.57
DIN*flow*temp	0.0035	246	8.71
SRP*DIN*flow*temp	0.0004	249	13.04

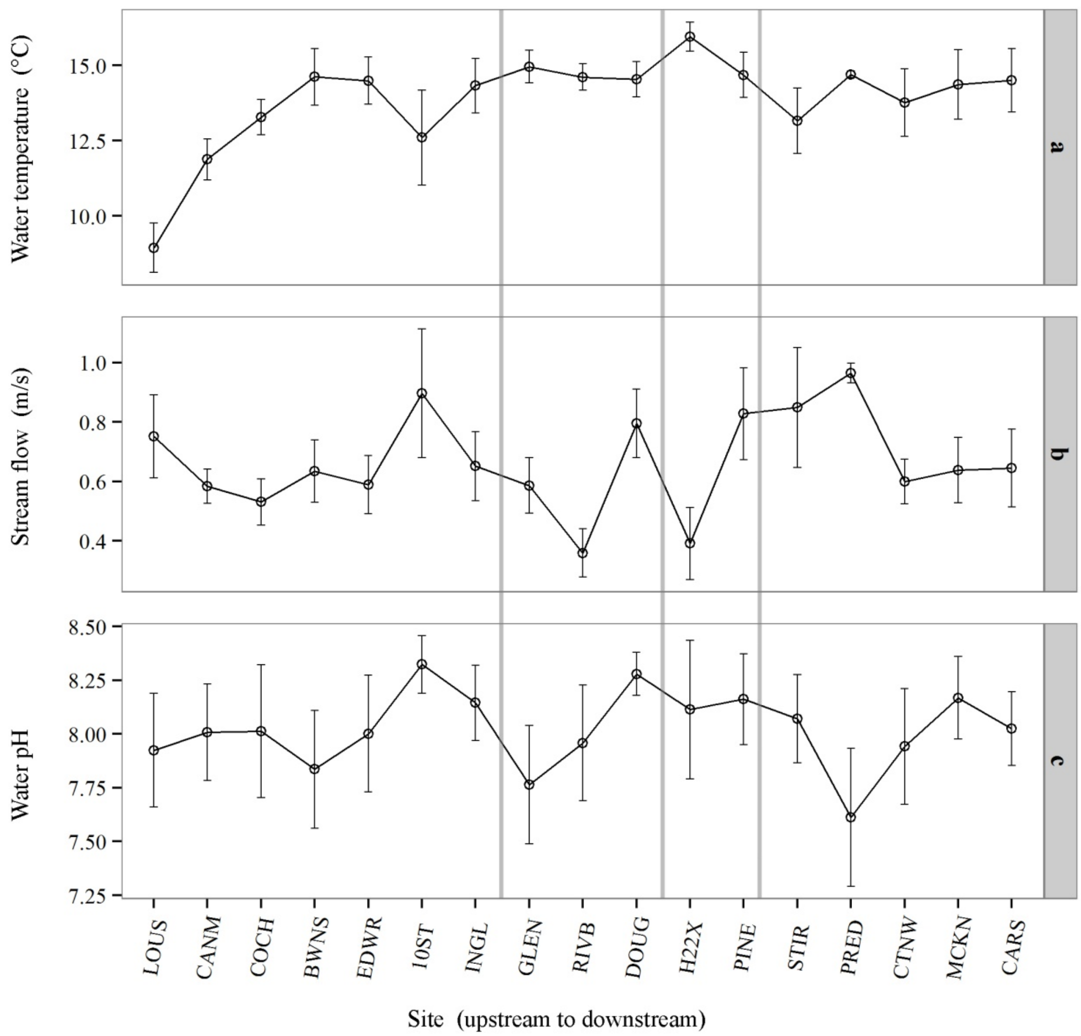

Figure 4. Longitudinal pattern of water temperature (a), stream flow (b) and water pH (c) along 300 km of the Bow River, AB. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence intervals at the respective site over late summer-early fall 2010 and 2011.

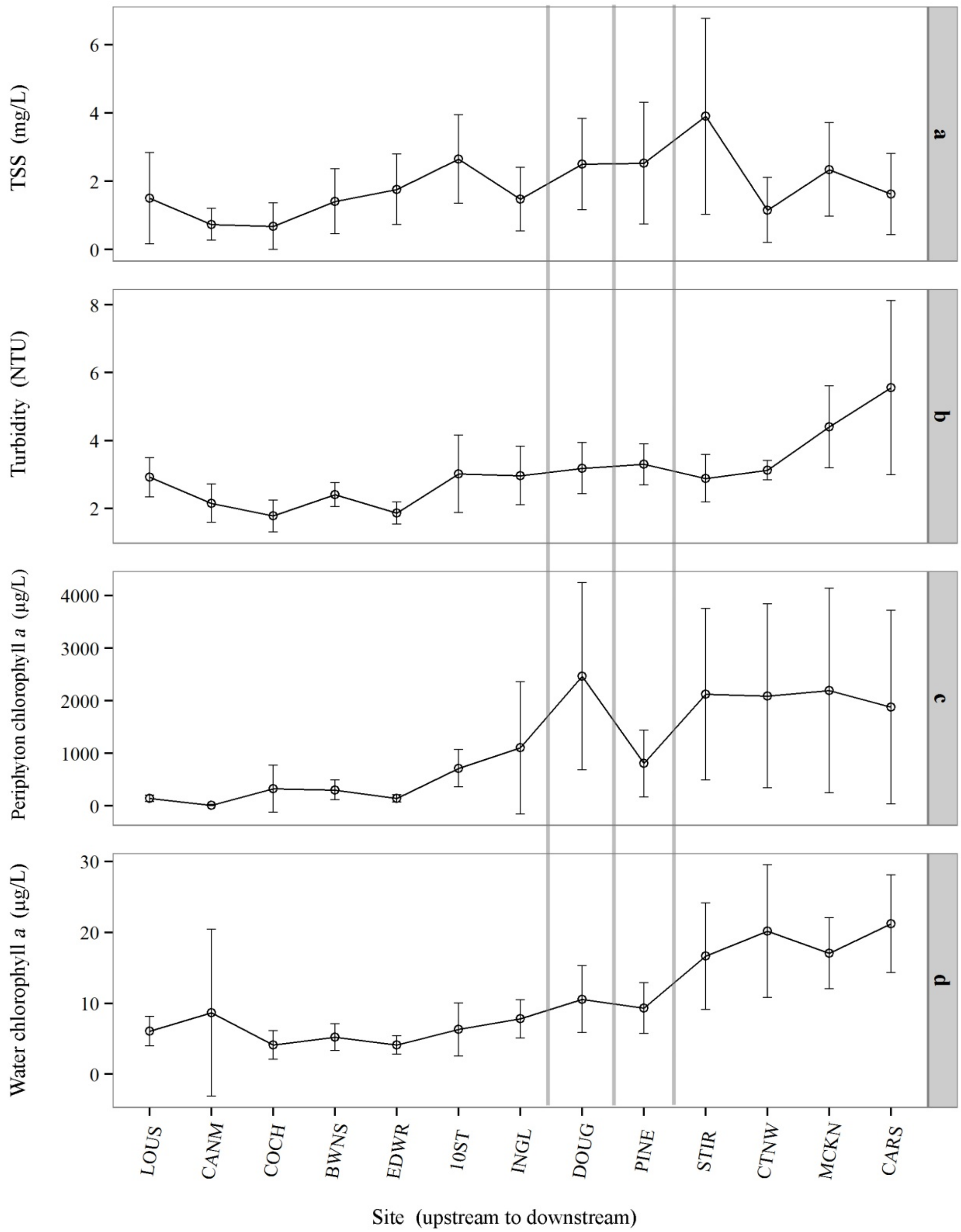

Figure 5. Longitudinal pattern of total suspended solids (a), turbidity (b), periphyton chlorophyll *a* (c) and water chlorophyll *a* (d) along 300 km of the Bow River, AB. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010.

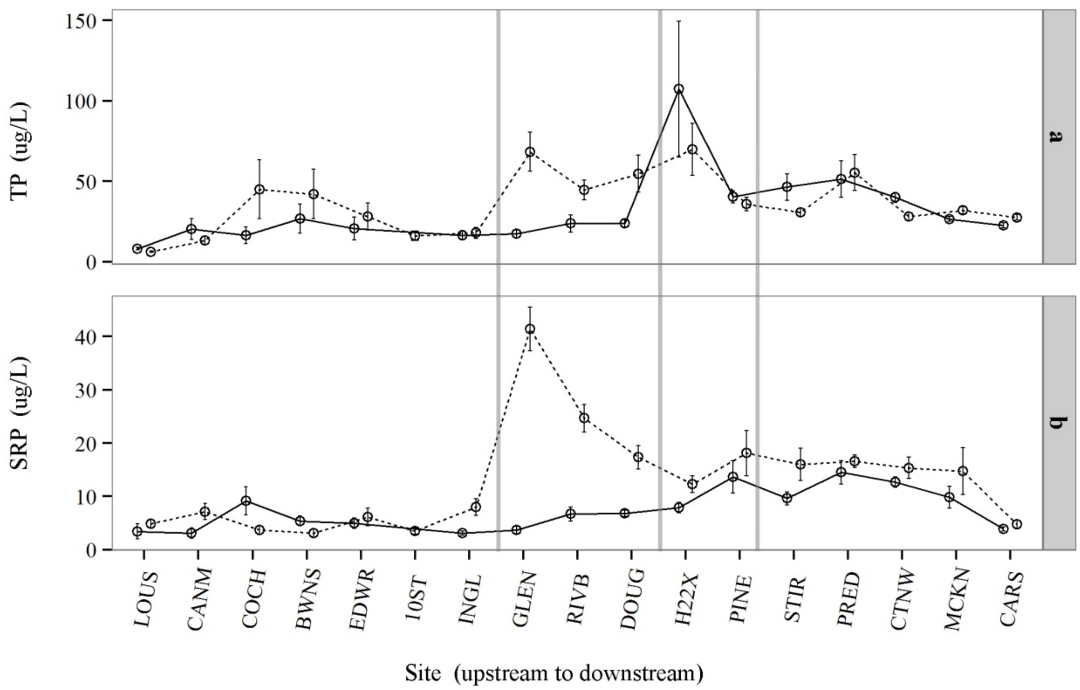

Figure 6. Longitudinal pattern of total phosphorus (a) and soluble reactive phosphorus (b) along 300 km of the Bow River, AB, on both left and right banks (facing downstream).Right bank is indicated by the dashed line, and left bank is indicated by the solid line. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010 and 2011.

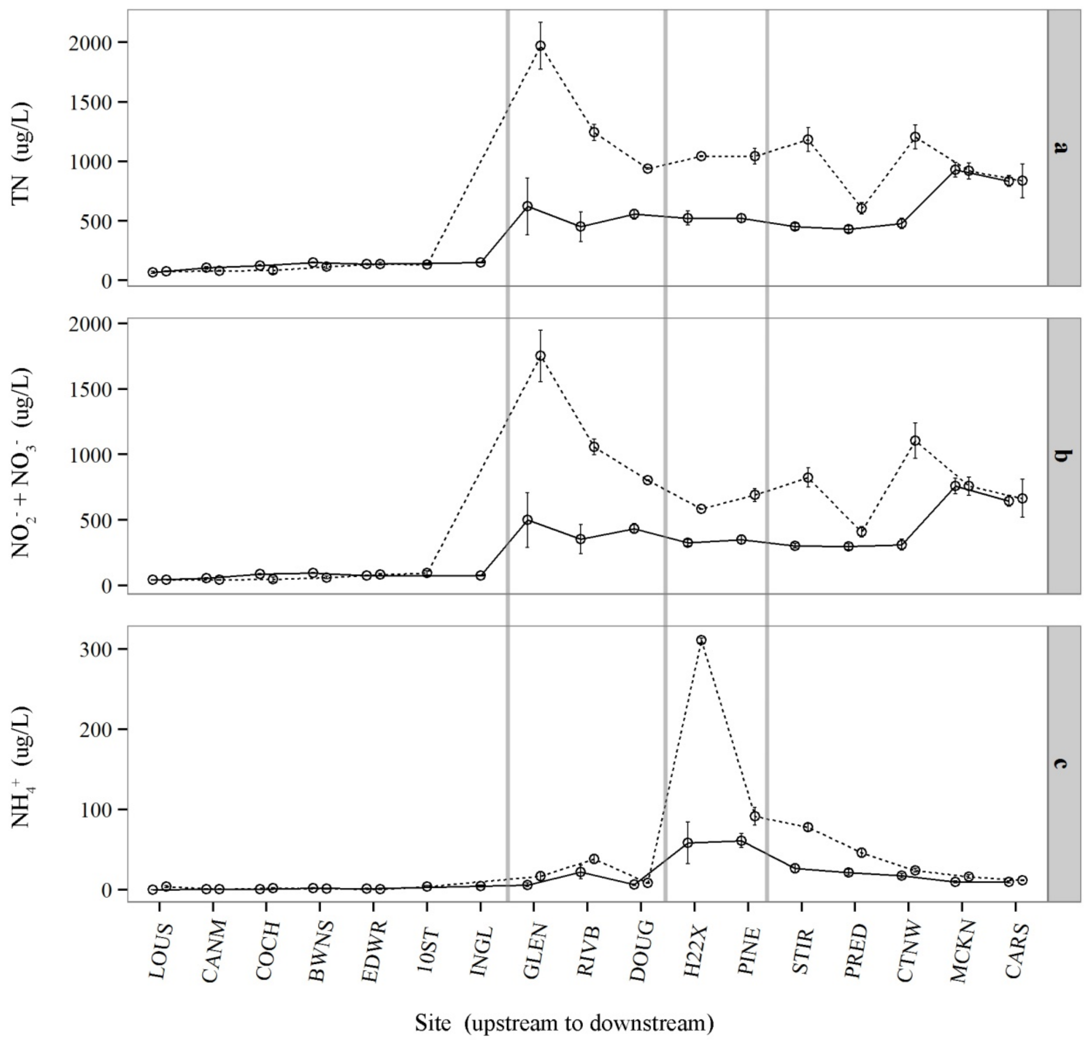

Figure 7. Longitudinal pattern of total nitrogen (a), nitrite + nitrate (b) and ammonium (c) along 300 km of the Bow River, AB, on both left and right banks (facing downstream). Right bank is indicated by the dashed line, and left bank is indicated by the solid line. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010 and 2011.

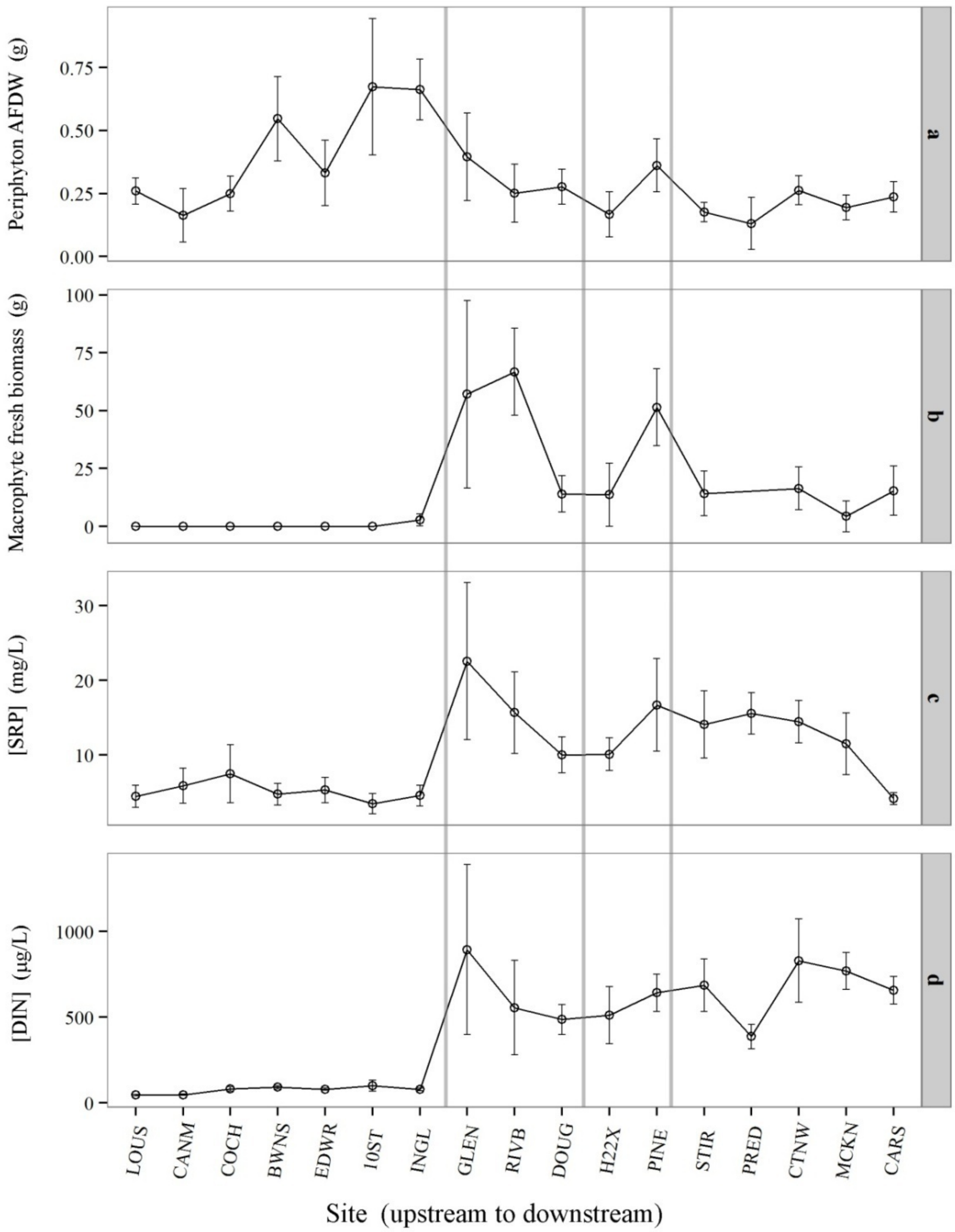

Figure 8. Longitudinal pattern of periphyton ash-free dry weight (a), macrophyte fresh biomass (b), soluble reactive phosphorus (c) and dissolved inorganic nitrogen (d) along 300 km of the Bow River, AB. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010 and 2011.

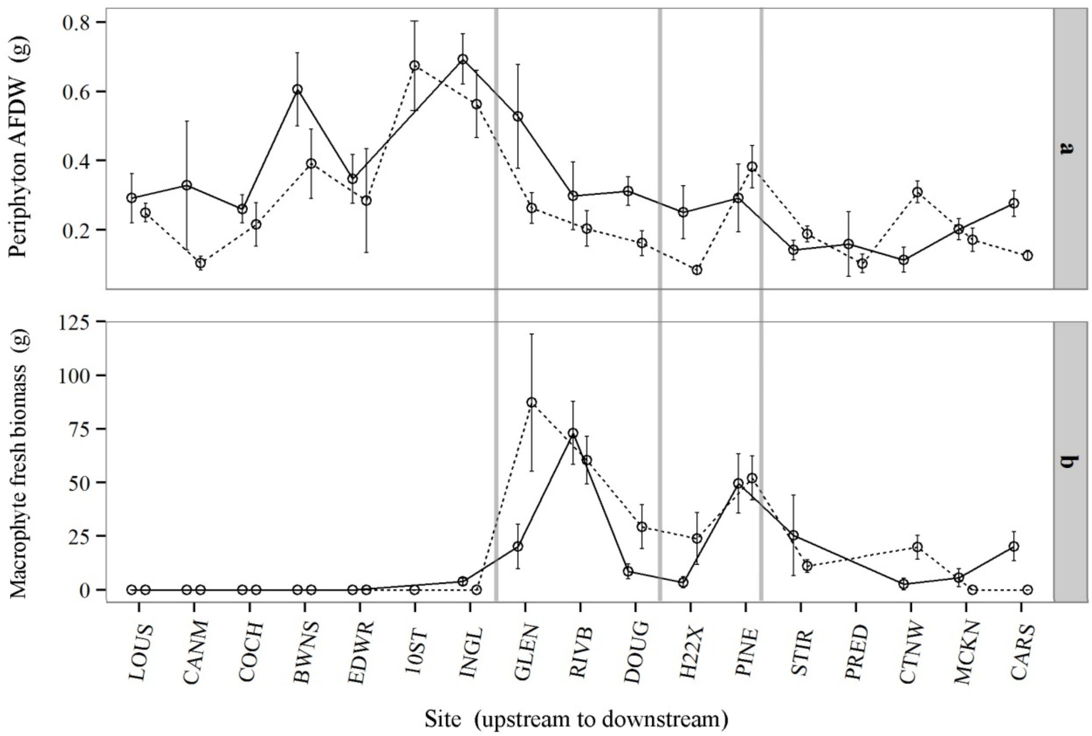

Figure 9. Longitudinal pattern of periphyton ash-free dry weight (a) and macrophyte fresh biomass (b) along 300 km of the Bow River, AB, on both left and right banks (facing downstream). Right bank is indicated by the dashed line, and left bank is indicated by the solid line. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010 and 2011.

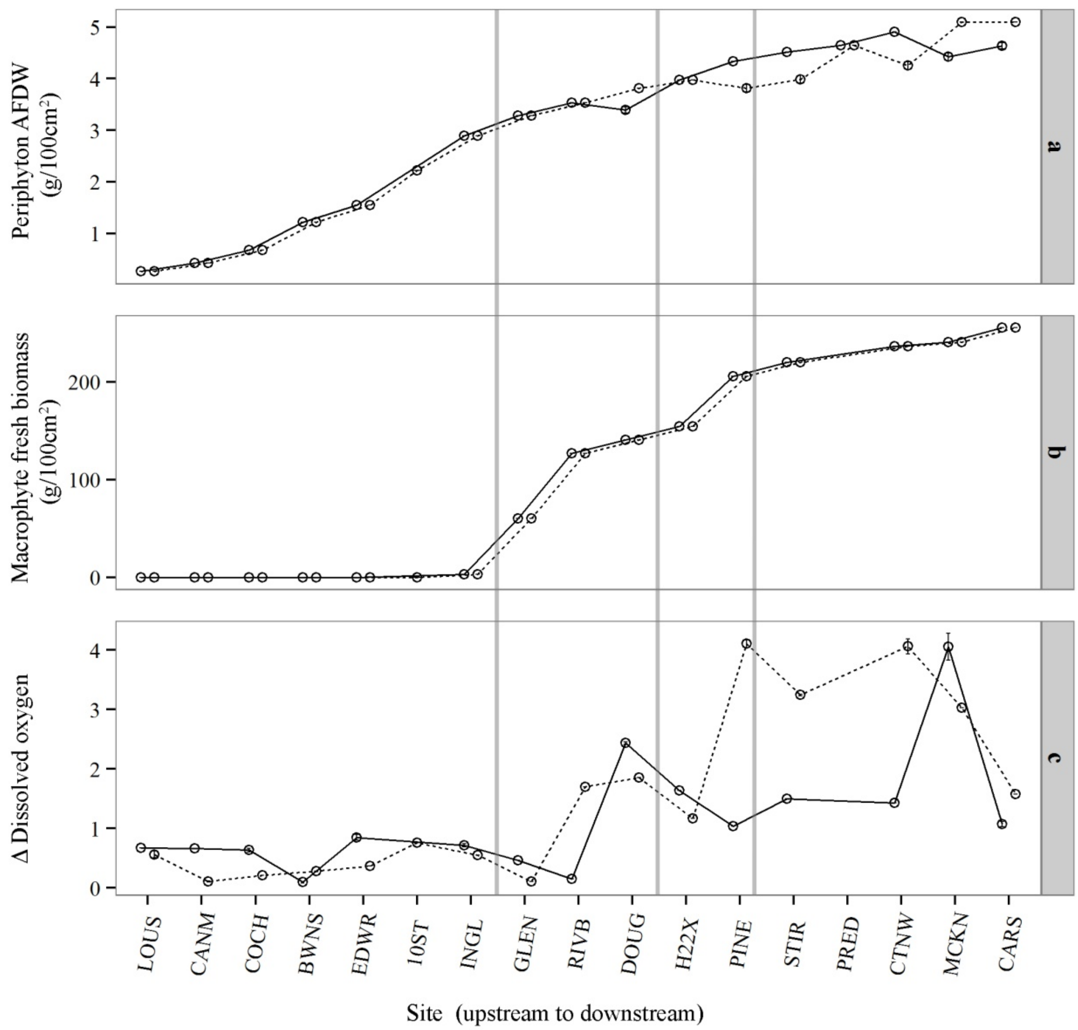

Figure 10. Longitudinal pattern of cumulative periphyton ash-free dry weight (a), cumulative macrophyte fresh biomass (b) and temperature-corrected change in dissolved oxygen (c) along 300 km of the Bow River, AB, on both left and right banks (facing downstream). Right bank is indicated by the dashed line, and left bank is indicated by the solid line. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010 and 2011.

Table 2. Maximum and minimum dissolved oxygen concentration by site. Values were obtained from three rounds of 24-hour logged values using a dissolved oxygen logger. Starred values indicate dissolved oxygen levels of <5 mgO_2/L, which is below the Alberta guideline for viability of aquatic freshwater organisms (AENV 1997). Double starred values indicate dissolved oxygen levels of <2 mg O_2/L, which are defined as hypoxic zones.

Site name	**Maximum**	**Minimum**
LOUS	7.0529	4.0588*
CANM	14.0010	4.9946*
COCH	8.9649	5.7533
BWNS	7.7892	6.5192
EDWR	8.0379	6.2917
INGL	8.9412	6.3156
GLEN	8.4102	5.9558
RIVB	9.5299	1.2466**
DOUG	9.8210	3.1133*
H22X	9.6386	2.2392*
PINE	12.7606	2.4823*
STIR	10.6676	2.5564*
CTNW	12.5661	1.7915**
MCKN	10.6695	2.5201*
CARS	9.4326	4.1873*

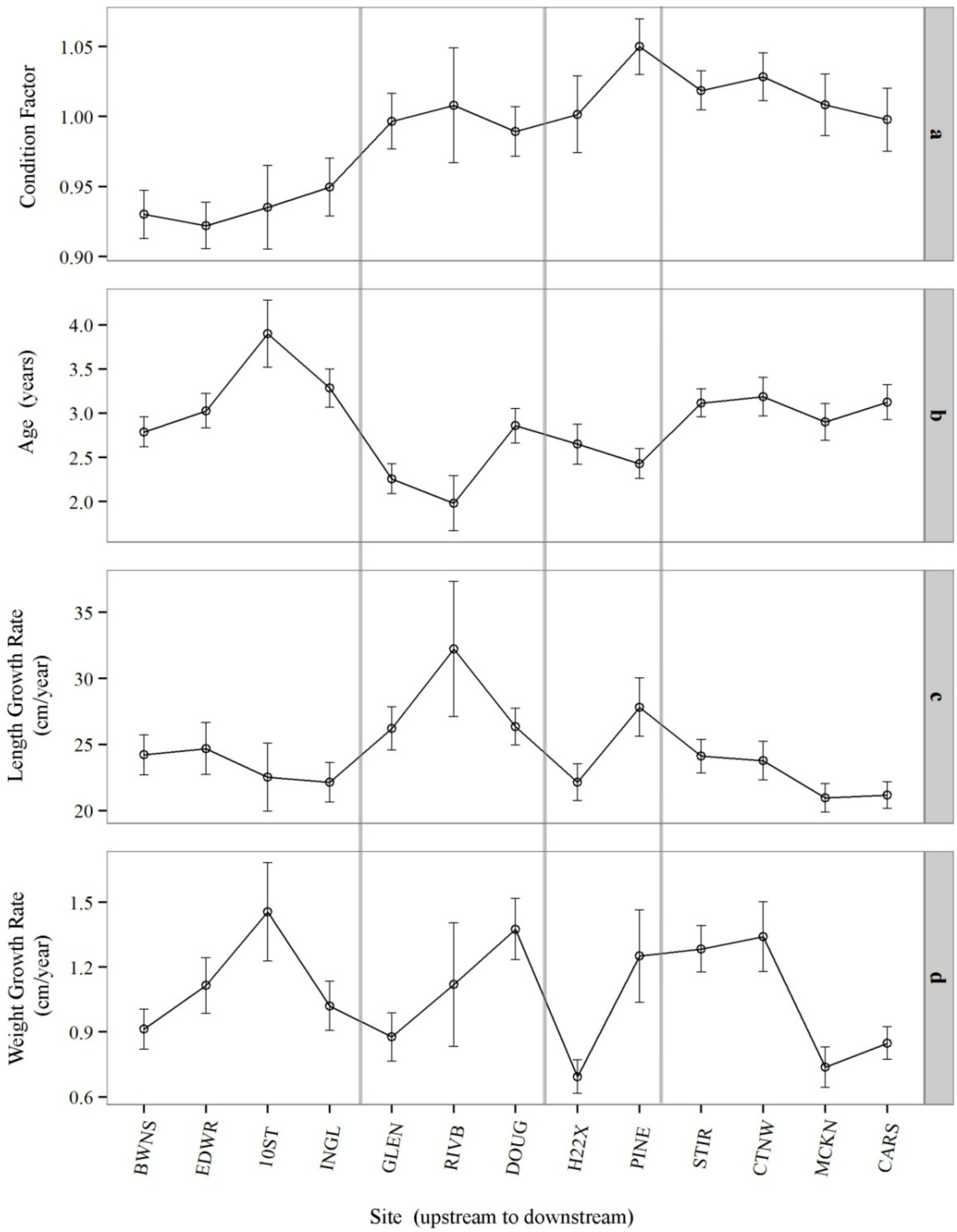

Figure 11. Condition factor (a), fish age (b), length growth rate (c) and weight growth rate (d) of longnose dace by site. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010 and 2011.

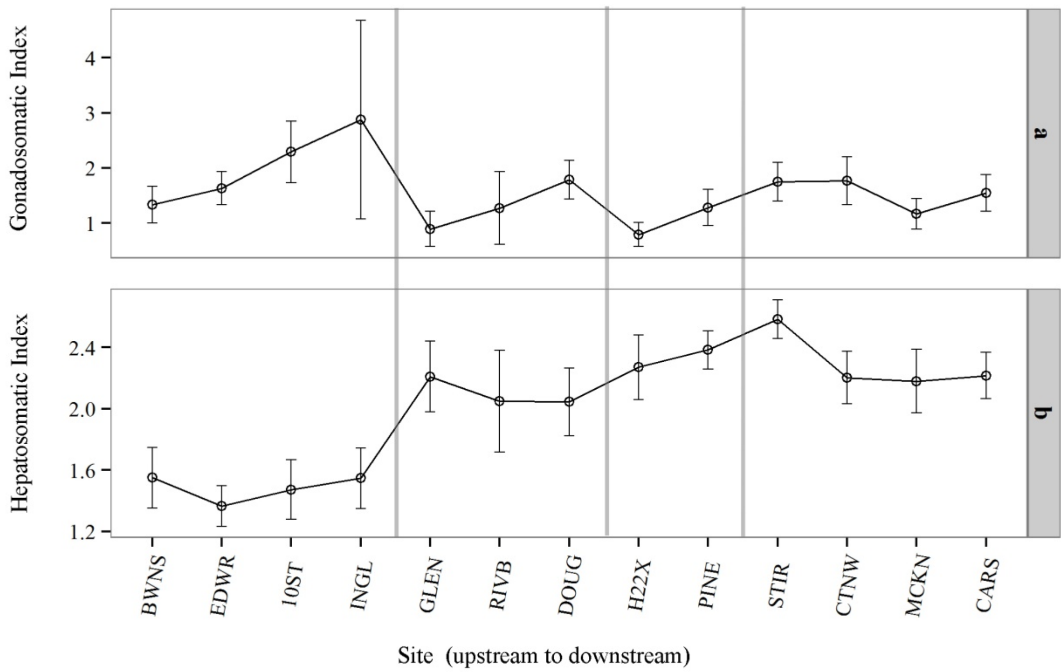

Figure 12. Gonadosomatic index (a) and hepatosomatic index (b) of longnose dace by site. Gray vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP and Pine Creek WWTP from upstream to downstream respectively. Plotted values are averages of all measured values with 95% confidence interval bars at the respective site over late summer-early fall 2010 and 2011.

Discussion

In rivers, periphyton and macrophytes are fixed in space, yet the oxygen they produce moves unidirectionally with water flow. As a given mass of water moves downstream, the sedentary plants (and other organisms that use oxygen) will add or remove oxygen depending on whether photosynthesis or respiration dominates local processes (Figure 13). I considered primary producer biomass and dissolved oxygen concentration ([DO]) to display cumulative behaviour from upstream to downstream, in order for the primary producer biomass and [DO] to be comparable. Cumulative periphyton biomass and cumulative macrophyte biomass are both unsurprisingly major drivers behind the diel oxygen swings—but only downstream of the wastewater treatment plants (WWTPs), as the river upstream of WWTPs is void of macrophytes with exception of very small, highly localized patches. The highest biomass of periphyton was found upstream of the WWTPs, yet diel [DO] are small compared to downstream of WWTPs. Therefore, it appears that macrophytes, rather than periphyton, drive the diel oxygen variation in the Bow River, particularly downstream of Bonnybrook WWTP. Macrophyte beds account for substantially higher biomass than periphyton, which would provide more physiologically active tissue to photosynthesize and respire. Phytoplankton was ignored as suspended chlorophyll *a* in the Bow River was always low, indicating low biomass. Phytoplankton density most positively contributes to dissolved oxygen at intermediate densities where chlorophyll *a* values are around 200 μg/L (Smith and Piedrahita 1988), yet the highest chlorophyll*a* value I found was approximately 20 μg/L, a factor of ten less.

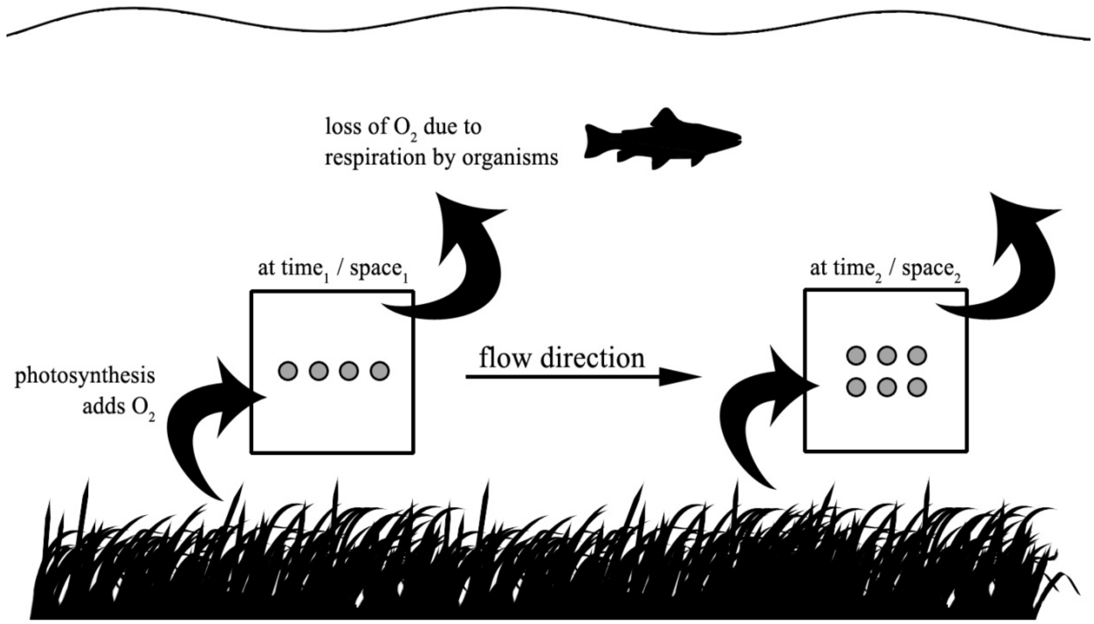

Figure 13. Conceptual model of a block of water moving downstream in a river, with constant addition and removal of oxygen through photosynthesis and respiration. In this example, photosynthesis initially adds 4 units of O_2 to the block of water when it is at time$_1$/space$_1$. At the same time, respiration removes 2 units of O_2, leaving 2 units of O_2 remaining in the block as it travels to time$_2$/space$_2$. At time$_2$/space$_2$, photosynthesis adds another 4 units of O_2 for a total of 6 units of O_2; respiration consequently removes another 2 units for a total of 4 units as it moves to time$_3$/space$_3$. As photosynthesis exceeds respiration in this example (4 units added and 2 units removed), the block of water will accumulate units of O_2 as it travels downstream.

I considered fish "health" metrics as possible indications of negative effects that could be from low dissolved oxygen levels, using longnose dace as a sentinel species. A few sites had night-time minimum [DO] below 5 mg/L, the Alberta guideline for viability of aquatic freshwater organisms (AENV 1997). Two sites downstream of effluent input—RIVB and CTNW—measured overnight minima that fit the requirement for hypoxia (<2 mg O_2/L).The increase in condition factor from upstream to downstream may be attributed to increases in N and P that provide resources that facilitate growth, as also shown by the increase in hepatosomatic index, which suggests large livers in dace (sign of a nutrient-rich environment).However, gonadosomatic indices were low downstream of effluent input, which suggests that although nutrients are rich, water quality may be poor and dace may be affected by other pollutants (Thomas 1988, Leblanc et al. 1997). Macroinvertebrate abundance increases with TP enrichment (Askey et al. 2007), and dace feed on aquatic insect larvae (Nelson and Paetz 1992). Low [DO] may increase mortality; however, without a long-term study following cohorts, it is not possible to determine whether there was mortality due to hypoxia. The average age of longnose dace is lower downstream of RIVB, which happens to also be where minimum [DO] below 5 mg O_2/L begins to be observed and persists downstream. My results are inconclusive as average fish age increases again downstream while [DO] remains below 5 mg O_2/L, but this perhaps warrants future investigation to identify the underlying cause. Predation by larger fish (e.g. trout) could explain this discrepancy in average age from upstream to downstream. Average age is lower immediately downstream of the wastewater treatment plants, where rainbow trout and brown trout have been shown to have higher biomass (Askey et al. 2007). While there is notable variation between sites for length and weight growth rates, there is no observable pattern that suggests negative effects of low dissolved oxygen levels. A lack of DELTs found in the fish collected suggest that although low [DO] zones were observed in the river, its effects are not manifested as external abnormalities in fish. DELT results have been observed and attributed to low DO conditions before (OEPA 1995, Baumann et al. 2000, OEPA 2000). I thus argue that low [DO] in the Bow River are transient and do not persist long enough to cause chronic sublethal effects on the fish population, even if those transient low [DO] periods occur nightly. The maximum and minimum [DO] data corroborate this idea; while there are consistently low [DO] measurements during the night minima measured downstream of RIVB, the day maxima are well over the 5 mg/L O_2 requirement.

Although it appears that low [DO] does not have a negative effect on fish population, hypoxia has been shown to increase susceptibility of fish to contaminants (Barton and Taylor 1996, Pollock et al. 2007). It is thus important to be aware of these possible interactions of hypoxia with other variables not measured in this study. [DO] found in the Bow River may not be persistently low enough to be of direct concern yet, but a moderate decline in [DO] may already be sufficient to affect fish through other sublethal interactions.

Rapid increases in P and N concentrations were observed immediately downstream of Bonnybrook and Fish Ck. WWTP, which is consistent with effluent inputand very low upstream nutrient concentrations. STIR, the sampling site immediately downstream of the Pine Ck. effluent input, was not as different in P and N concentrations from PINE, the sampling site immediately upstream of the Pine Ck. effluent input, when compared to differences in upstream and downstream sites for the other two WWTPs. Pine Ck. WWTP's use of diffusers to release the final effluent may help increase the amount of mixing between final effluent and river water immediately upon effluent release, thus minimizing changes in nutrient concentration. SRP and NO_2^-+NO_3^- were higher at RIVB than at H22X, and may explain the higher macrophyte biomass as SRP and NO_3^- are biologically available. However, there was also a relatively high NH_4^+ concentration on the right bank at H22X, presumably from the Fish Ck. WWTP that did not correspond to the local macrophyte biomass. Macrophyte biomass appears to be affected not so much by single characteristics, but by the interaction of DIN with other variables, with DIN as the primary driver. Based on a 16 year study, Sosiak (2002) concluded that the Bow River is N-limited. My data show a high correlation between DIN and macrophyte biomass, and are consistent with Sosiak's (2002) conclusion.

There is a surprising lack of relationship between nutrients and periphyton biomass throughout the studied stretch of the river, regardless of whether the variables are considered individually or interactively, with or without other environmental variables. This contrasts well-known relationships between nutrients and periphyton biomass (Lohman et al. 1992, Chételat et al. 1999). In the Bow River upstream of WWTPs, there was higher flow and lower nutrients with increasing periphyton biomass. The midstream stretch from the first effluent input to and including the estimated mixing zone downstream of the last effluent input has lower flows and higher nutrients, and declining periphyton biomass. Downstream of the mixing zone there was medium flow, low nutrients and low periphyton biomass. The decrease in nutrient concentrations in the downstream stretch is likely due to dilution with distance and the Highwood River (Bow River's largest tributary) entering the main stem.

A previous study on the contribution of flow and nutrients in regulating periphyton biomass observed that flow and nutrients were equal contributors (Biggs and Close 1989). My results suggest that if flow and nutrients are indeed equally contributing to periphyton biomass, there are likely other variables not considered that affect periphyton growth, such as light (Schiller et al. 2007) or top-down control by grazers (Bourassa and Cattaneo 1998), or changes in substrate, as visual observation during sampling noted that substratum characteristics vary throughout the Bow River and could explain the variation in periphyton biomass. If certain areas have a predominantly siltbased streambed and do not have enough suitable substrate for periphyton to colonize, periphyton biomass would be unsurprisingly low regardless of resource abundance and otherwise suitable water chemistry. Herbivores and grazers could also potentially have unrecognized substantial effects, both as single characteristics or interactively, on biomass that could also explain the highly variable relationships (Welch et al. 1992, Hillebrand and Kahlert 2001). Periphyton biomass has been shown to be primarily affected by light and nutrient variability as opposed to temperature and substrate; the more light and nutrients available, the higher the biomass (Hansson 1992). However, addition or removal of grazers had the largest effect on periphyton biomass (Rosemond et al. 2000).

A shift from a periphyton-dominated system to a macrophyte-dominated system is observed downstream of Bonnybrook WWTP. While it is unclear why this shift occurred, competition may be a possible explanation. Macrophytes have lower nutrient requirements than microalgae, and have access to not only nutrients in the water, but more importantly, nutrients in sediments (Sand-Jensen and Borum 1991, Carr and Chambers 1998). If macrophytes are indeed N-limited (Sosiak 2002), macrophytes may have been able to flourish as a result of the increase N input from the WWTP effluent (which has a N:P of ~30:1) (Jarvis Singer, City of Calgary, personal communication), which may subsequently lead to a decrease in periphyton biomass through other competitive aspects such as shading (Sosiak 1990). Substantial streambed scouring as a result of a 2005 flood in the Bow River also decreased macrophyte biomass in subsequent years; it was hypothesized that the reduction in macrophytes allowed increased sunlight penetration, allowing increased periphyton biomass (Barry Kobryn, City of Calgary, personal communication).

Conclusion

The City of Calgary has made much effort to reduce its effluent impact on the Bow River through WWTP upgrades. Currently, there appear to be no direct negative effects of effluent input through the use of sentinel fish species due to lack of long-term data, yet the increasing diel oxygen cycles and minimum observed dissolve oxygen concentrations are cause for concern. Addition of anthropogenic nutrient inputs through wastewater effluent contribute to increases in primary producer biomass, macrophytes in particular. These increases in macrophyte biomass are likely the primary driver behind the increasing diel oxygen cycles downstream. While there currently does not seem to be a need for urgent concern, it is worrying that minimum dissolved oxygen levels below 2 mg/L have been observed, given that 2 mg/L is classified as "hypoxia" and proposed minimum dissolved oxygen concentration for aquatic freshwater organisms is 5 mg/L. Nutrient inputs provide resources to increase fish growth, yet if in excess, nutrients may drive the river to areas of hypoxia which can cause stress and even mortality to those very same fish. It is possible that over time, average nutrient levels will increase. As phosphorus is highly particulate-reactive, much of it can settle in sediments, which allows phosphorus to accumulate locally.

Should hypoxia become an issue, primary producer biomass management must be carefully considered. Based on the results of this study, decreasing macrophyte biomass is one proposed approach to mitigating hypoxia. However, macrophytes do not solely contribute to dissolved oxygen levels. It is important to also consider side effects of minimizing macrophyte biomass, such as loss of habitat and refuges for aquatic organisms, effects on stream flow and sedimentation, and subsequent alterations of temperature and water chemistry (Chambers et al. 1999).

CHAPTER 3: ISOTOPIC TRACING OF WASTEWATER EFFLUENT IN THE BOW RIVER, ALBERTA

Introduction

Point source water pollution, such as wastewater treatment plants (WWTPs), continue to contribute greatly to eutrophication in many freshwaters globally. Although non-point sources (e.g. agriculture, urban runoff) are gaining notice after previously being overlooked due to their difficulty in measurement and regulation (Carpenter et al. 1998), the growing global population's increasing demand for clean water and relative ease of treating point sources mean point sources are targets for mitigation. Much effort has been made to reduce pollution from point sources, including important successes that have lead to better wastewater treatment.

To further improve effluent quality, it is helpful to understand the effluent's fate once it is released into the river to identify what characteristic of the effluent requires management. In this study, I investigated the spatial scale of Calgary's WWTP effluent incorporation into the Bow River and the spatial variability in isotopic signatures of aquatic primary producers via stable isotopes tracing.

Stable Isotopes

Atoms of the same element can differ in the number of neutrons they possess; these variants with differing number of neutrons are called isotopes. Isotope values use the δ notation, which signifies a difference from the standards used during analysis. Units of δ are measured as "per mil (‰)", which is parts per thousand, calculated as follows:

$$\delta^{H}X = [(R_{sample} / R_{standard} - 1)] * 1000$$

where X = a particular element (e.g. nitrogen)

H = heavy isotope mass of element X

R = ratio of heavy to light isotope for element X.

Positive δ values indicate heavier isotopes relative to the standard, whereas negative δ values indicate lighter isotopes. Δ value of 0‰ equals the standard. Two isotope behaviours that are relevant to tracing studies are mixing and fractionation. Mixing combines two or more sources into one common pool; fractionation acts opposite to mixing and separates isotopes through alteration of the heavy-light isotope ratio. The two work in conjunction to continually recycle isotopes in the natural system (Peterson and Fry 1987, Fry 2006).

$\delta^{15}N_{nitrate}$ in Water and Plants

The two stable isotopes of nitrogen, ^{14}N and ^{15}N, have an overall fixed global proportion of approximately 273 ^{14}N atoms to one ^{15}N atom. The specific ratio in different N pools in the environment, however, varies. The different pools thus have distinct isotopic signatures that allow the pools to be identified, as $\delta^{15}N$ values are calculated based on the ratio between the two stable N isotopes (Peterson and Fry 1987, McClelland et al. 1997, Fry 2006). Wastewater effluents have distinct isotopic signatures due to each plant's unique treatment procedures, leading to different fractionating processes. These distinct isotopic signatures can be used to assess the contributions of effluent to submerged aquatic plants. $\delta^{15}N$ in aquatic ecosystems has been an effective and low-cost way to identify potential eutrophication, or to assess relative contribution of sources (Mayer et al. 2002, Cole et al. 2004).

Macrophytes excel as an indicator for wastewater tracing as macrophytes are typically abundant and long-lived, and there is a direct linear relationship between macrophyte $\delta^{15}N$ value and percent of wastewater contribution (Cole et al. 2005). Algae in sewage treatment ponds have been shown to have significantly enriched $\delta^{15}N$ values relative to algae in untreated sites, and may also be an useful indicator for wastewater tracing(Wayland and Hobson 2001). Analysing the $\delta^{15}N$ signatures in biota helps map the distribution and extent of an effluent's footprint, and provides an estimate for the bioavailable N from wastewater (Benson et al. 2008).

Materials and Methods

Study Area

Originating from Bow Lake in the Canadian Rockies, which is fed by Bow Glacier, the Bow River flows south-eastward across the Alberta foothills and prairies until it joins the Oldman River to form the South Saskatchewan River. Wastewater treatment plants (WWTP) are a significant point-source stressor on the Bow River, as effluent discharge is a major nutrient contributor to streams and rivers (Nichols 1983, Nagumo and Hatano 2000). The Bow River is highly regulated, with numerous dams and weirs along its stretch, leading to an altered flow regime and fairly regular flow throughout. The City of Calgary, with a population of over one million, places substantial stress on the Bow River from three WWTPs. The effluent from these three plants is the primary source of nutrient input into the Bow River (Chapter 1).

Calgary's Wastewater Treatment Plants

Calgary's three WWTPs have a combined annual return flow of 162,180,000m^3 (BRBC 2005). Bonnybrook WWTP is the most upstream plant and is also the oldest (built in the 1920s). It was expanded between 1954 and 1958 to increase capacity, though it still provided only primary treatment. In 1971, Bonnybrook WWTP was upgraded to secondary treatment to maintain good water quality in the Bow. Just over a decade later, Bonnybrook WWTP was upgraded to tertiary treatment with the addition of chemical phosphorus removal. Another expansion in the 1990s added other tertiary treatments such as primary sludge fermentation, biological nitrogen and phosphorus removal, and ultraviolet (UV) disinfection. Bonnybrook WWTP releases 365 ML (1 ML = 1 000 000 L) of final effluent per day.

The original Fish Creek WWTP, a primary treatment plant, was completed in 1960. It was upgraded to secondary treatment in 1980, and to tertiary treatment at the same time Bonnybrook WWTP was upgraded to tertiary. The tertiary upgrade added chemical phosphorus removal. The most recent upgrade in 1996 implemented UV disinfection as well. Fish Creek WWTP releases 34 ML of final effluent per day.

To meet with the demands of a growing population, the Pine Creek WWTP was opened in 2008 and is one of Canada's most advanced wastewater treatment plants. It isequipped with four conventional primary clarifiers, four secondary clarifiers, and two bioreactors to perform Biological Nutrient Removal (BNR). There are also 12 effluent filtration modules in place for tertiary filtration, and UV disinfection completes the final step in the treatment process. Pine Creek WWTP releases 74 ML of effluent per day.

Sample Collection

Approximately 300 km of Bow River, Alberta was studied between July 20-October 24, 2010 and July 19-September 22, 2011. Thirteen sites were each sampled four times in 2010, and 16 sites were each sampled three times in 2011. Site 10ST was only measured in 2010, and sites GLEN, RIVB, H22X and PRED were only measured in 2011 (Figure 14). The sites were chosen to provide a reference for water quality upstream of the WWTPs, and to include a good distance downstream of the last wastewater treatment plant in an attempt to detect any returns to pre-WWTP values. Sampling sites were spatially clustered closer in the city to achieve finer resolution, as the three WWTPs are located fairly close to each other.

During the 2010 sampling, one bank at each site was sampled for water chemistry, periphyton and macrophytes. Three 125 mL water samples were collected using acid washed HDPE Nalgene bottles and sent to the University of Alberta Biogeochemical Analytical Service Lab for TN, NO_2^-+NO_3^- and NH_4^+ analysis. Water samples were obtained by submerging the Nalgene bottle midway down the water column, filling it with water and capping off the bottle instream to prevent any bubbles from being trapped in the container. The sample was then transported on ice in a cooler back to the university laboratory. Additional 125 mL water samples were collected (also using HDPE Nalgene bottles) during August 2011 for water isotope analysis.

To obtain periphyton, a 4 cm circular template was used to delineate one scrape from each of five rocks taken from the riverbed around each bank. Macrophytes were harvested from establishment to end of season to capture maximum seasonal biomass. One to five 936.36 cm^2 quadrats were sampled randomly along each bank, depending

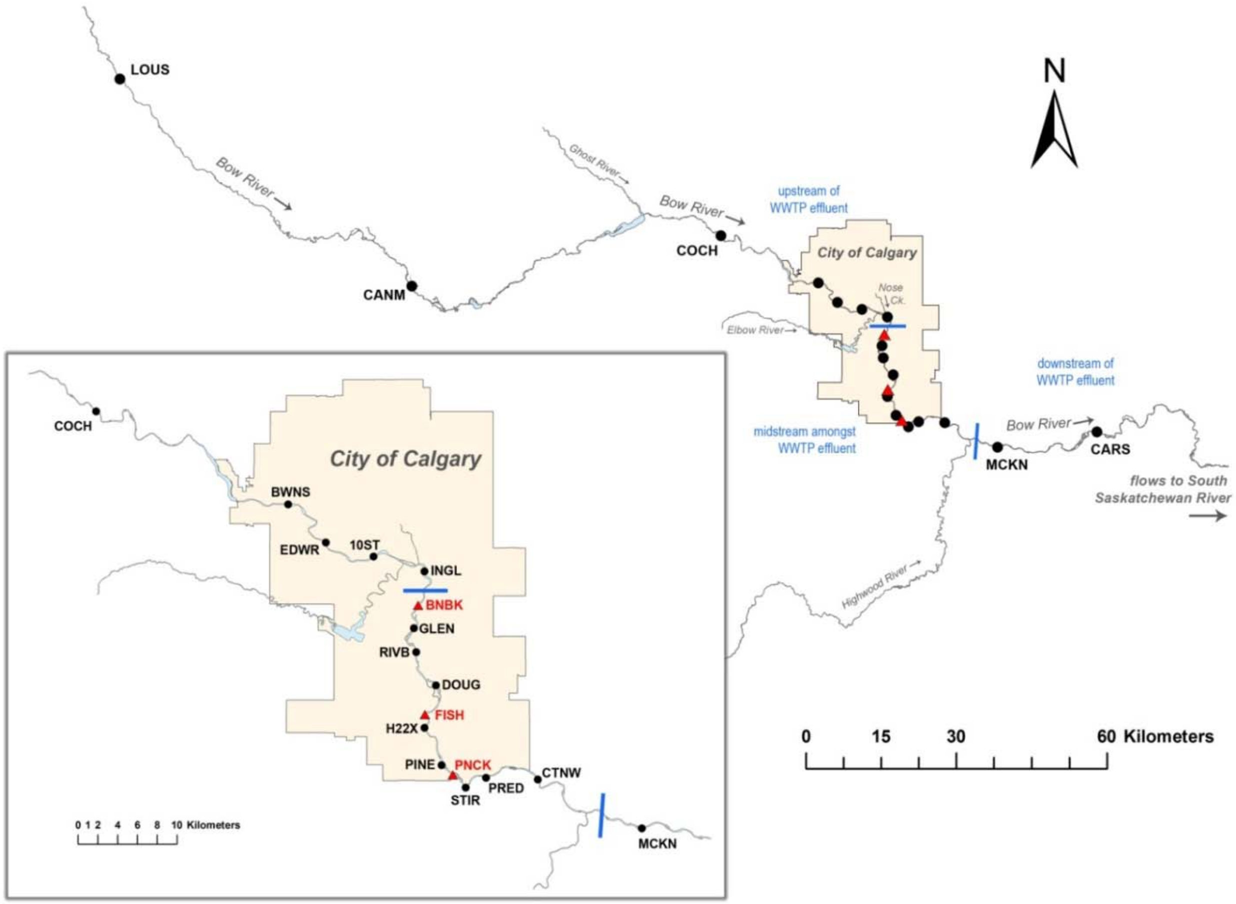

Figure 14. Map of 2010 and 2011 sampling sites on Bow River, AB. Inset box shows close up of finer spaced sites within the City of Calgary. Black circles indicate sampled sites and red triangles indicate Calgary's three wastewater treatment plants. Blue lines divide the sampled river stretch into upstream of effluent, midstream amongst effluent, and downstream of effluent for comparisons.

on macrophyte abundance. Macrophytes were stored in small plastic garbage bags and transported on ice in coolers back to the university laboratory.

The 2011 field sampling was revised slightly based on 2010 results, incorporating the following changes: sampling both banks at each site instead of one to take into account mixing issues and reducing the number of periphyton samples from five to three.

Stable Isotope Analysis

Water, periphyton and macrophyte $\delta^{15}N$ signatures were analysed at the Isotope Science Lab at the University of Calgary, Alberta, Canada during April 2011, December 2011 and May 2012. Water samples were also analysed for $\delta^{18}O$ values. Periphyton and macrophyte samples were analysed for $\delta^{15}N$ signatures for both 2010 and 2011, and water samples were analysed only for 2011.

Periphyton and macrophyte samples were prepared for isotope analysis by drying at 60°C to constant mass, then ground and homogenized using a ceramic mortar and pestle. The powdered sample was then packed into analytical tin cups and weighed to obtain 10 mg for periphyton and 8 mg for macrophytes. Peach leaves (SRM #1547) were chosen as one of the standard reference materials as it has similar physical properties as the grounded plant matter in the samples. The prepared samples were analysed using Continuous Flow-Elemental Analysis-Isotope Ratio Mass Spectrometry technology with a Finnigan Mat Delta Plus+XL mass spectrometer coupled with a Costech 4010 Elemental Analyser. Samples were taken over July-October in 2010 from one bank, and over July-August in 2011 from both banks. All periphyton samples collected in 2010 were analysed, and the August round of periphyton collected in 2011 were analysed

(August was chosen in order to allow comparison with the water isotope samples). All macrophyte samples collected were analysed.

Water samples were analysed using the "denitrifier method", in which the bacterium *Pseudomonas aureofaciens* reduces nitrate to nitrous oxide. Reduction of N_2 does not occur as *P. aureofaciens* lacks nitrous oxide reductase. Nitrate concentration data obtained from University of Alberta Biogeochemical Analytical Service Lab were used to determine the amount of bacteria required for each sample. After approximately 16 hours, the bacteria were lysed with NaOH, and the sample vials were mounted in a 24-vial autosampler interfaced to a HP 6890 gas chromatogram with PreCon(R) device interfaced to a Finnigan Mat Delta+XL mass spectrometer. Determination of $\delta^{15}N$ and $\delta^{18}O$ values of the sample NO_3^- was then performed by measuring the $\delta^{15}N$ and $\delta^{18}O$ values of the sample N_2O and using the instrument software (ISODAT 2.63) for calculations. One sample from each site+bank combination was analysed. All results are expressed using δ notation in per mil (‰) relative to internationally accepted standards.

Statistical Analysis

Spatial autocorrelation associated with sampling different points of the river violates the assumption of independence, making many traditional statistical approaches (such as ANOVA) problematic. One alternative approach is to look for discontinuities, which suggests an abrupt change in structure and can be identified as outliers in the data. Dixon's Q test and generalized ESD test were used in R. 2.15.2 to detect outliers. However, the tests were not successful in detecting outliers where large value jumps in constituent concentrations were seen. This may be due to the large variability associated with field data. The uncertainty regarding the reliability of Dixon's Q test and generalized ESD test thus lead to the decision to not use those two tests for outliers. An appropriate test to compare a single variable measured across spatially autocorrelated sites could not be found, so qualitative assessment of the data was performed instead based on plotted data.

Values were averaged between years if a particular site and bank were sampled in both 2010 and 2011. Figures were also plotted in R 2.15.1 (2012-66-22) with the ggplot2 package.

Results

Wastewater effluent from the three wastewater treatment plants (WWTPs) generally had higher $\delta^{15}NO_3^-$ values than sites upstream of the WWTPs (Bonnybrook: 8.4‰; Fish Ck.: 11.7‰; Pine Ck.: 11.8‰; see Figure 15 for upstream values). WWTP effluent generally enters the river right by the WWTP building (locations as marked in Figure 14). While the Bonnybrook and Fish Ck. WWTPs did not significantly alter the $\delta^{15}N$ value of the river water from immediately upstream the effluent to immediately downstream of the effluent, there was a difference in $\delta^{15}N$ value upstream to downstream of Pine Ck. WWTP (Figure 15). $\delta^{15}N$ values of river water increased fairly linearly with increasing distance, and showed continual increase across the three WWTP (Figure 15). There appears to be no recovery to pre-WWTP values by 46 km downstream of the WWTPs (Figure 15).

Periphyton showed a similarly linear increase in $\delta^{15}N$ values from upstream to downstream (Figure 16). Right bank values were higher than left bank values for sites upstream of the City of Cochrane (COCH).Upon approaching Cochrane and subsequently Calgary, right bank values dropped to below left bank values (Figure 16). Right bank values were again higher immediately downstream of Bonnybrook and Pine Ck.

but not immediately downstream of Fish Ck. WWTP (Figure 16). Fish Ck. WWTP altered the $\delta^{15}N$ of periphyton located downstream (relative to upstream), to a greater degree than Bonnybrook or Pine Ck. WWTP. Although $\delta^{15}N$ values remain high for both left and right bank downstream of the WWTP, the two most downstream sites revert back to the left bank having higher $\delta^{15}N$ values than their respective right bank, as observed upstream of the WWTPs within the city (from COCH to Bonnybrook WWTP) (Figure 16).

The lack of macrophytes upstream of WWTPs and the patchy abundance downstream caused difficulties in analysis, as macrophytes were not found at every site, and not every bank. $\delta^{15}N$ values of macrophytes found on the right bank dropped drastically immediately after Fish Ck. WWTP, and returned to pre-Fish Ck. WWTP values just downstream of Pine Ck. WWTP (Figure 17).

The $\delta^{15}N$ of river water was lighter upstream, where [NO_2^-+NO_3^-] were low, and heavier downstream of WWTP effluent inputs (Figure 18). Downstream post-WWTP $\delta^{15}N$ values (downstream of Pine Ck. WWTP) tend to be higher than values found within the middle river stretch where WWTP effluents are added to the river (from Bonnybrook to Pine Ck. WWTP), though both river sections have similar ranges in [NO_2^-+NO_3^-].

$\delta^{15}N$ values of periphyton when measured across a range of [NO_2^-+NO_3^-] also showed a rapid increase in $\delta^{15}N$ value from 0-200 μg NO_2^-+NO_3^-/L, but continued to slowly increase past 200 μg NO_2^-+NO_3^-/L rather than plateau (Figure 19). As seen with

$\delta^{15}N$ value of the water samples, upstream pre-WWTP samples showed high variability in $\delta^{15}N$ value within a relatively narrow range in [NO_2^-+NO_3^-]. There are notably two samples, both from upstream sites, that have lighter isotopes than the standards.

Macrophyte $\delta^{15}N$ values showed no apparent trend in $\delta^{15}N$ value across the range of [NO_2^-+NO_3^-] (Figure 20). Macrophytes were present at only one site upstream—INGL (immediately upstream of Bonnybrook WWTP). There was large variability in $\delta^{15}N$ values regardless of location or [NO_2^-+NO_3^-].

Discussion

Treated wastewater $\delta^{15}NO_3^-$ is generally 10‰ to 20‰ (McClelland et al. 1997) and are thus notably higher than other major contributors to riverine nitrate levels, such as natural soil nitrate (2‰ to 8‰) (McClelland and Valiela 1998), fertilizers (0‰ to 5‰) (McClelland et al. 1997), atmospheric deposition (-10‰ to 8‰) and rainwater (approximately 0‰) (Hoering 1957, Heaton 1987). The jump from 0‰ at site LOUS (the most upstream, pristine site used as reference condition) to around 4‰ at the next site (CANM) suggest an urban signature from CANM onwards. The consistent increase in river water $\delta^{15}NO_3^-$ values upstream to downstream post-CANM suggests natural soil nitrate, fertilizer runoff and/or atmospheric deposition (i.e. non-WWTP sources) to be the dominant nitrate source for much of the Bow River. Post-WWTPs, $\delta^{15}NO_3^-$ values approach 9‰, with the highest value being 8.8‰. While this is clearly lighter than the 10‰ to 20‰ found in wastewater, there may still be notable contribution from wastewater to the post-WWTPs $\delta^{15}NO_3^-$ values as Bonnybrook WWTP effluent was

8.6‰, also below the typical values in wastewater. The high nitrate concentrations from downstream of effluent input is also the source of $\delta^{15}NO_3^-$ values greater than 7.5‰.

Immediately downstream of Bonnybrook WWTP and Pine Ck. WWTP, the right bank shows higher $\delta^{15}NO_3^-$than the left bank, which may be an indication of the entry of high $\delta^{15}NO_3^-$ effluent. Water with a high $\delta^{15}NO_3^-$ value such as WWTP effluent will see a decrease in isotopic ratios if mixed with water that has a lower $\delta^{15}NO_3^-$ value (in this case, river water). The volume of effluent discharged by the WWTPs each day is relatively small compared to the overall river flow, making up only 3% to 6% of total flow (Wendell Koning (AENV) and Lal Amatya (City of Calgary), personal communication). Sites immediately downstream of the WWTPs are not at the effluent source, but anywhere from 1.7 to 4.4 km downstream of the plant, providing plenty of water and time for dilution to occur.

Higher $\delta^{15}NO_3^-$values closer to the 10‰ to 20‰ typical of wastewater are seen in the periphyton and macrophytes. These primary producers may be better indicators of wastewater contribution as their isotopic values are averaged over their growth. Periphyton also receive most of their N from the river water, and has been successfully used as an indicator of N sources to rivers (Toda et al. 2002). However, primary producers preferentially take up lighter isotopes rather than heavier isotopes, and will express lower $\delta^{15}NO_3^-$values than their sources (Wada and Yoshioka 1996). Periphyton and macrophyte samples collected in this study did indeed show $\delta^{15}NO_3^-$values consistently below effluent values (Bonnybrook: 8.6‰; Fish Ck.: 11.7‰; Pine Ck.: 11.8‰). Although fractionation during the process of nitrate uptake by primary producers is often deemed negligible (Mariotti et al. 1988), when comparing the values, 1‰ to 3‰

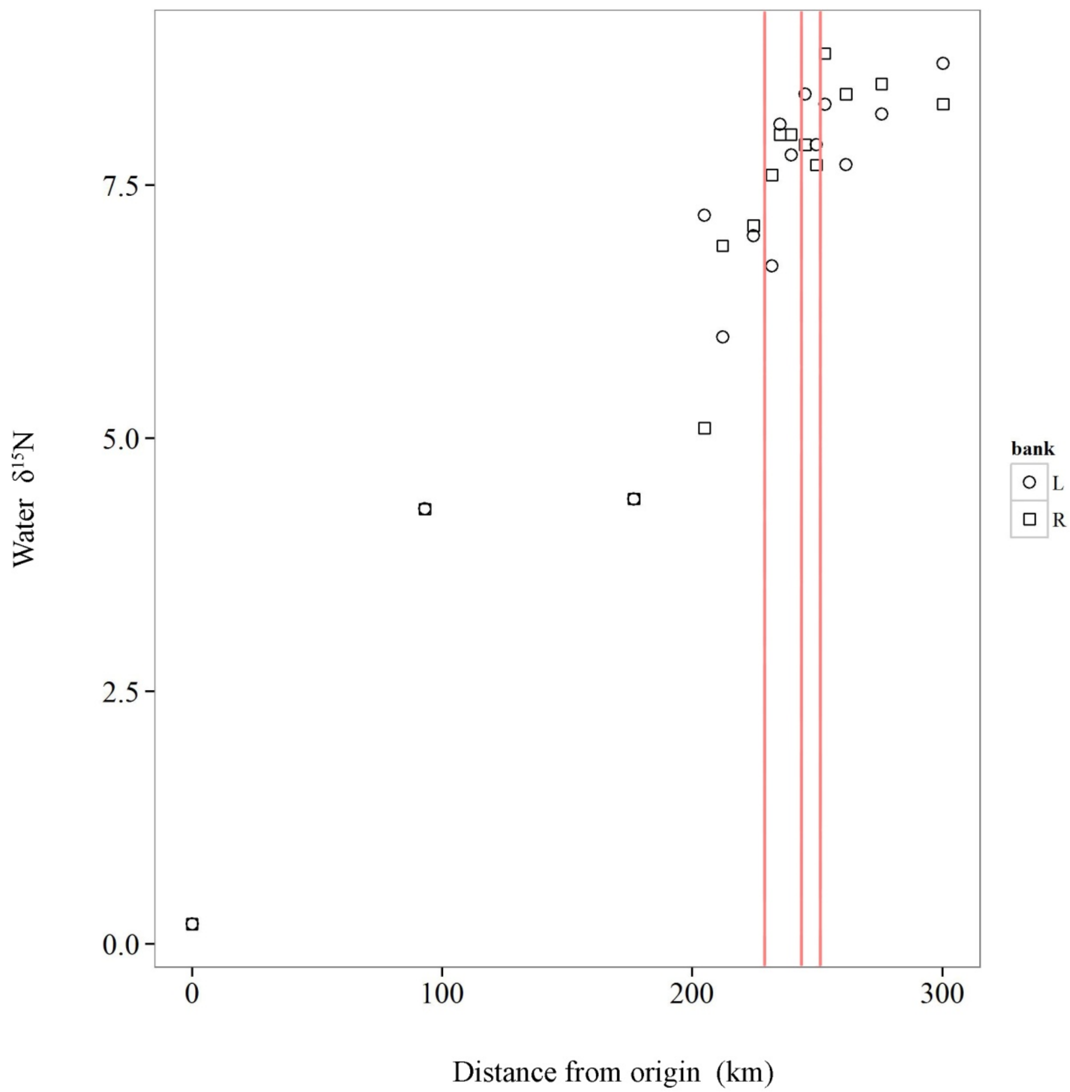

Figure 15. $\delta^{15}N$ values of water samples by distance from origin. Origin is the most upstream sample site, LOUS. Red vertical lines represent the location of the three wastewater treatment plants in order – Bonnybrook WWTP, Fish Ck. WWTP and Pine Ck. WWTP.

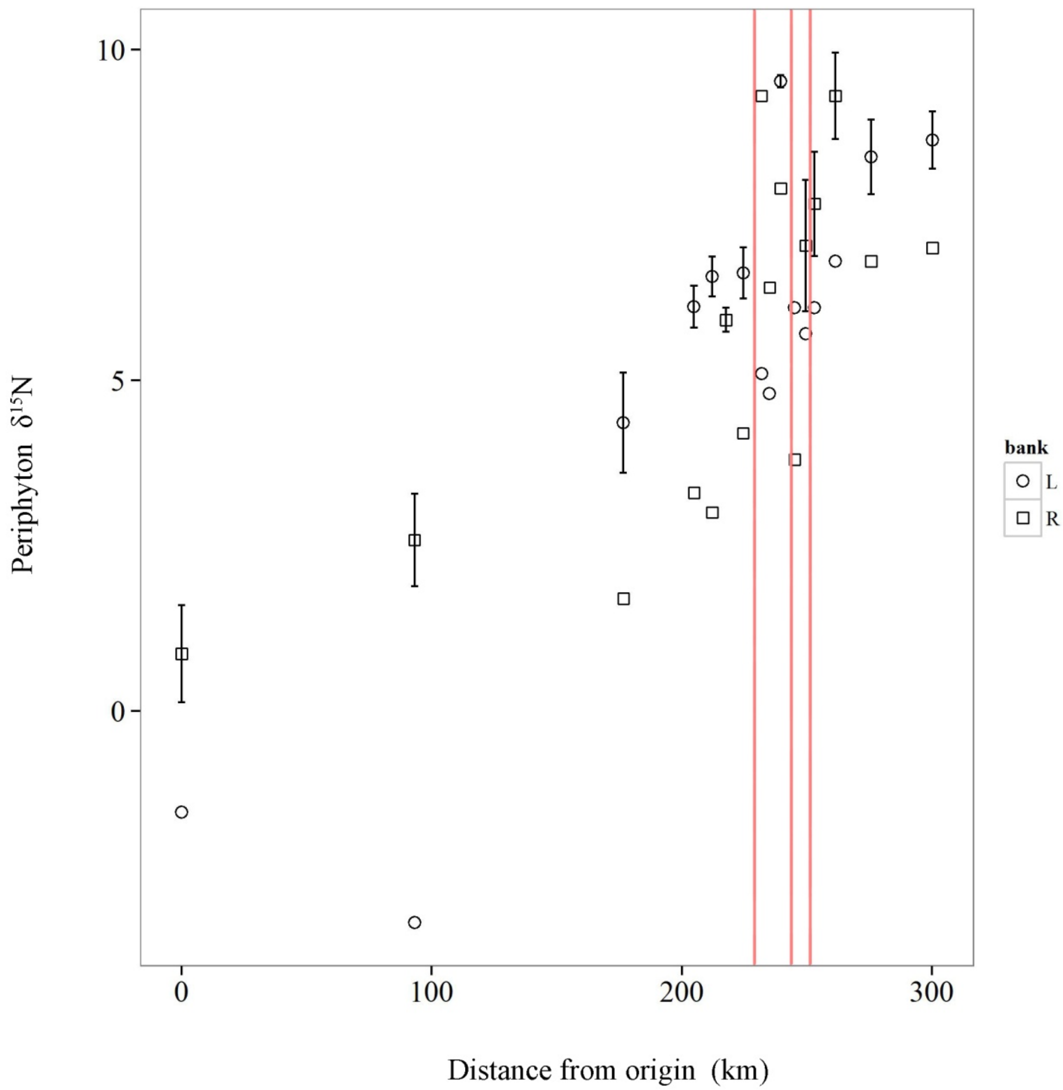

Figure 16. $\delta^{15}N$ values of periphyton samples by distance from origin (mean ± standard error). Origin is the most upstream sample site, LOUS. Redvertical lines represent the location of the three wastewater treatment plants in order – Bonnybrook WWTP, Fish Ck. WWTP and Pine Ck. WWTP.

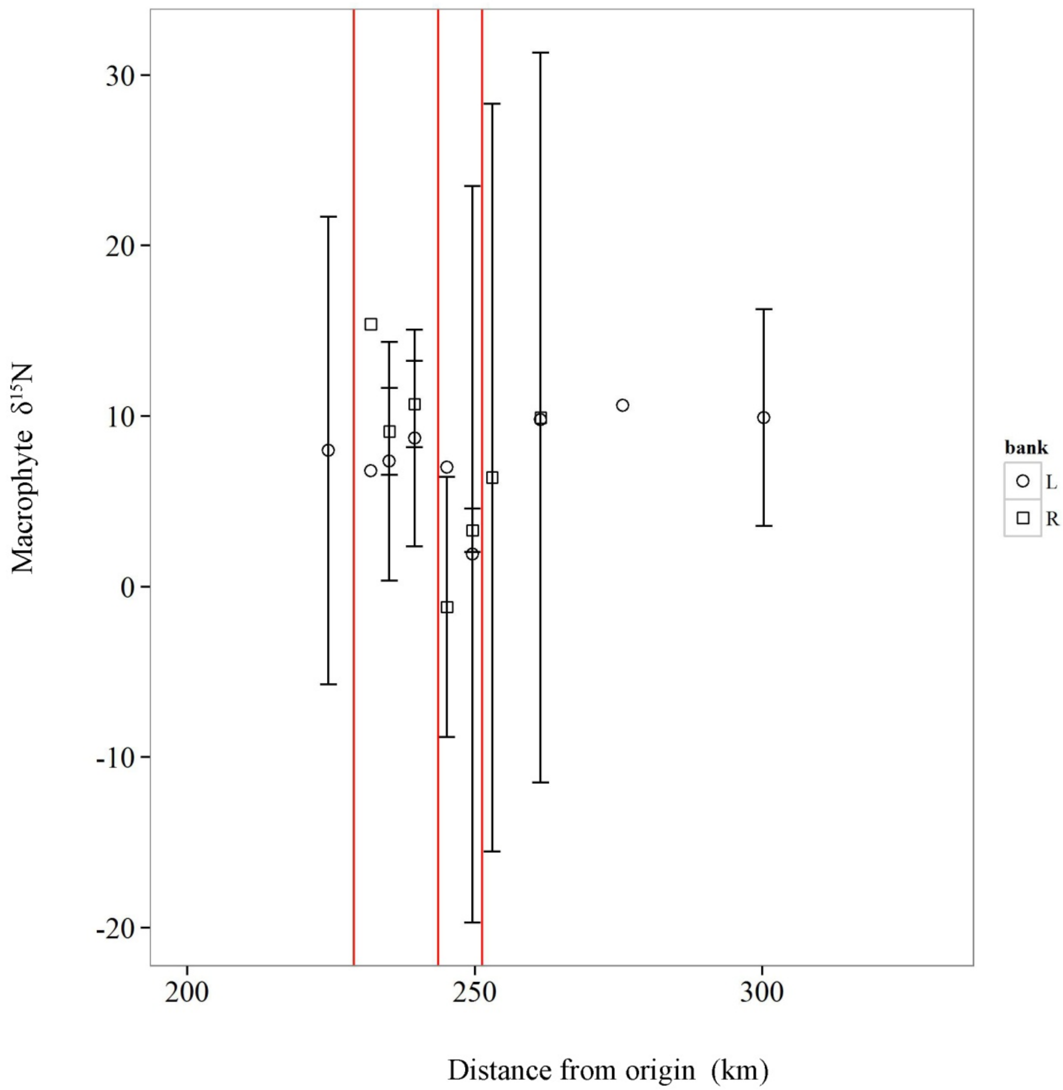

Figure 17. $\delta^{15}N$ values of macrophyte samples by distance from origin (mean ± standard error). Origin is the most upstream sample site, LOUS. Redvertical lines represent the location of the three wastewater treatment plants in order – Bonnybrook WWTP, Fish Ck. WWTP and Pine Ck. WWTP.

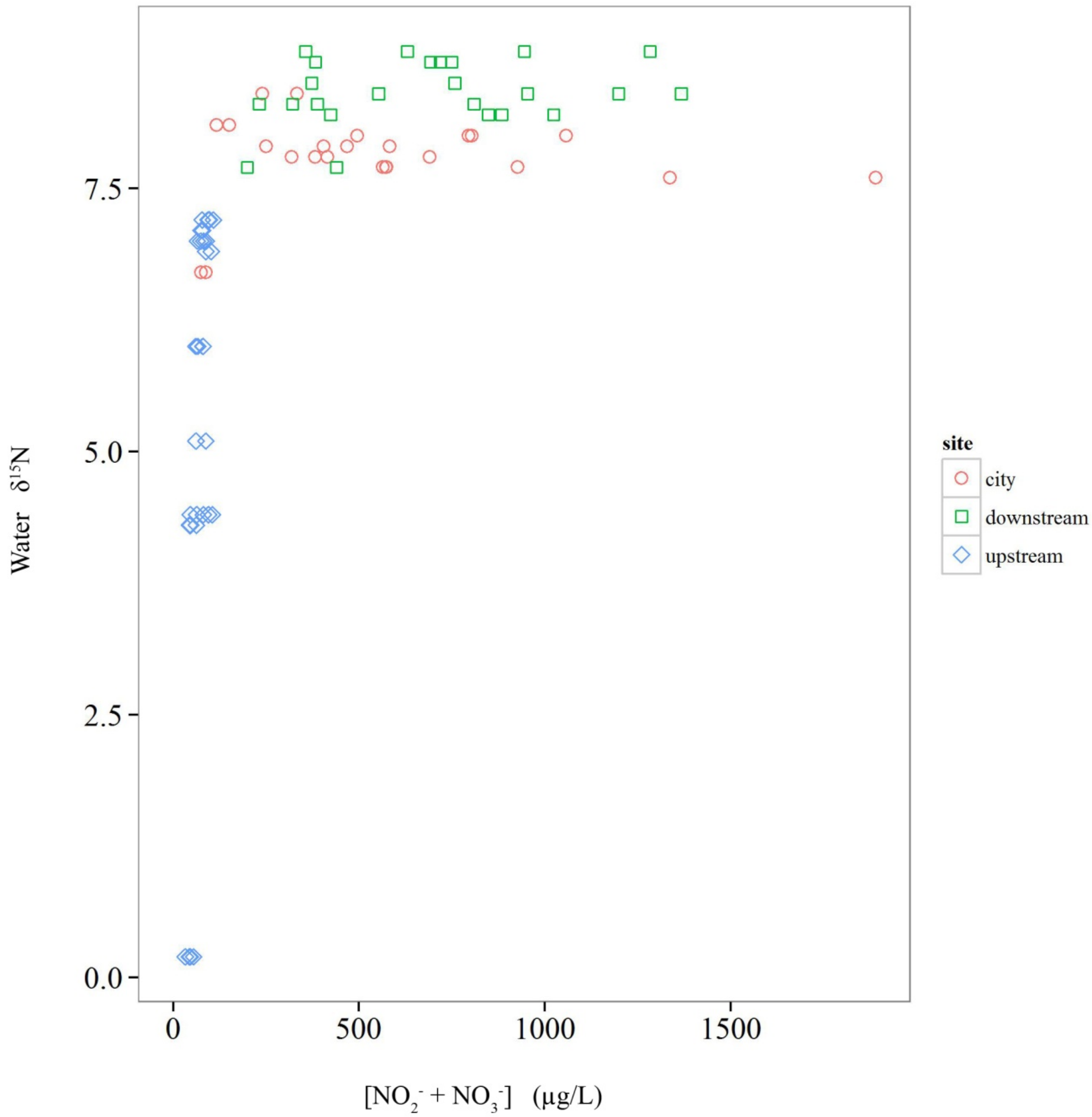

Figure 18. $\delta^{15}N$ values of water samples by nitrite+nitrate concentration. Origin is the most upstream sample site, LOUS.

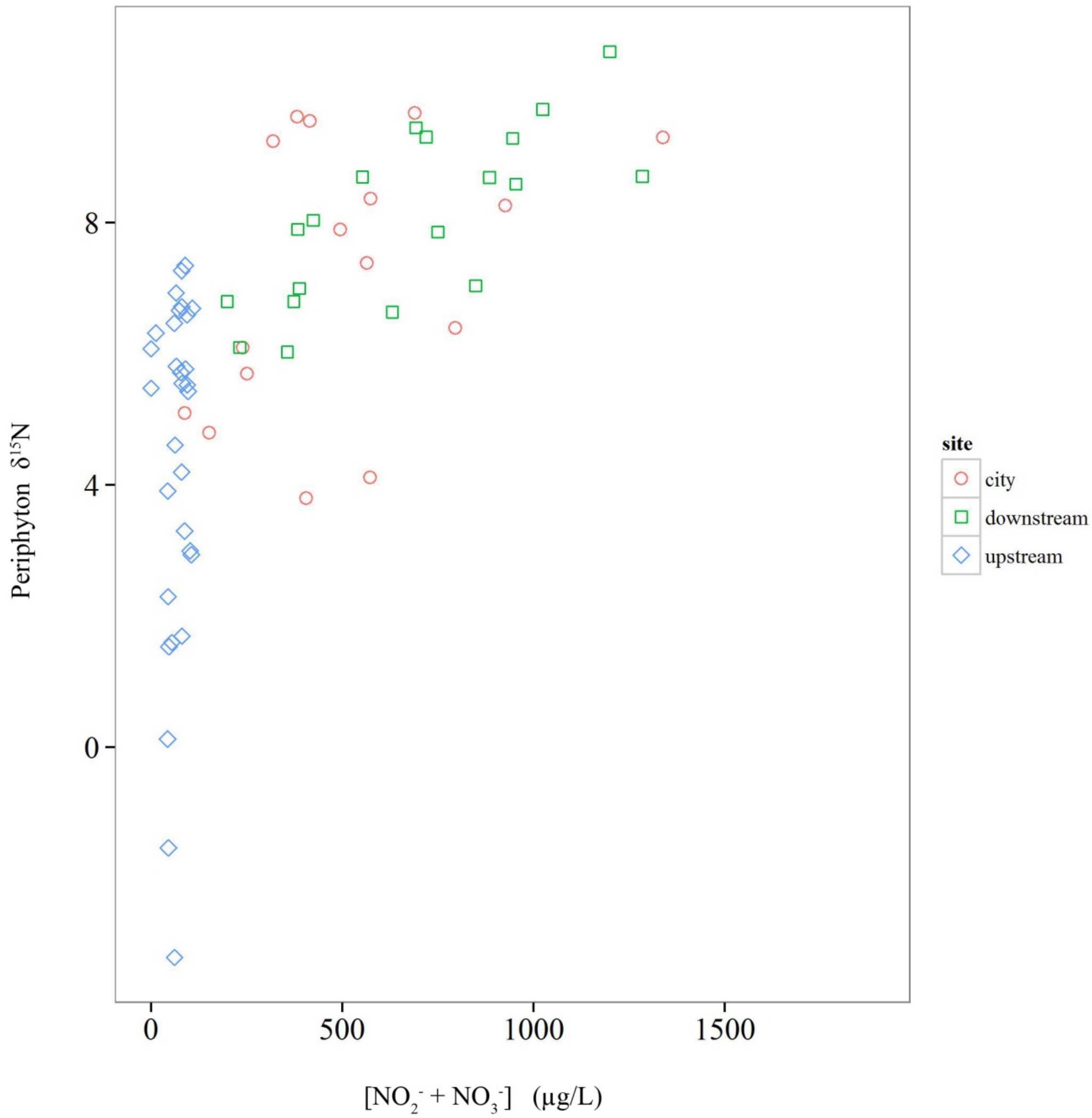

Figure 19. $\delta^{15}N$ values of periphyton samples by nitrite+nitrate concentration. Origin is the most upstream sample site, LOUS.

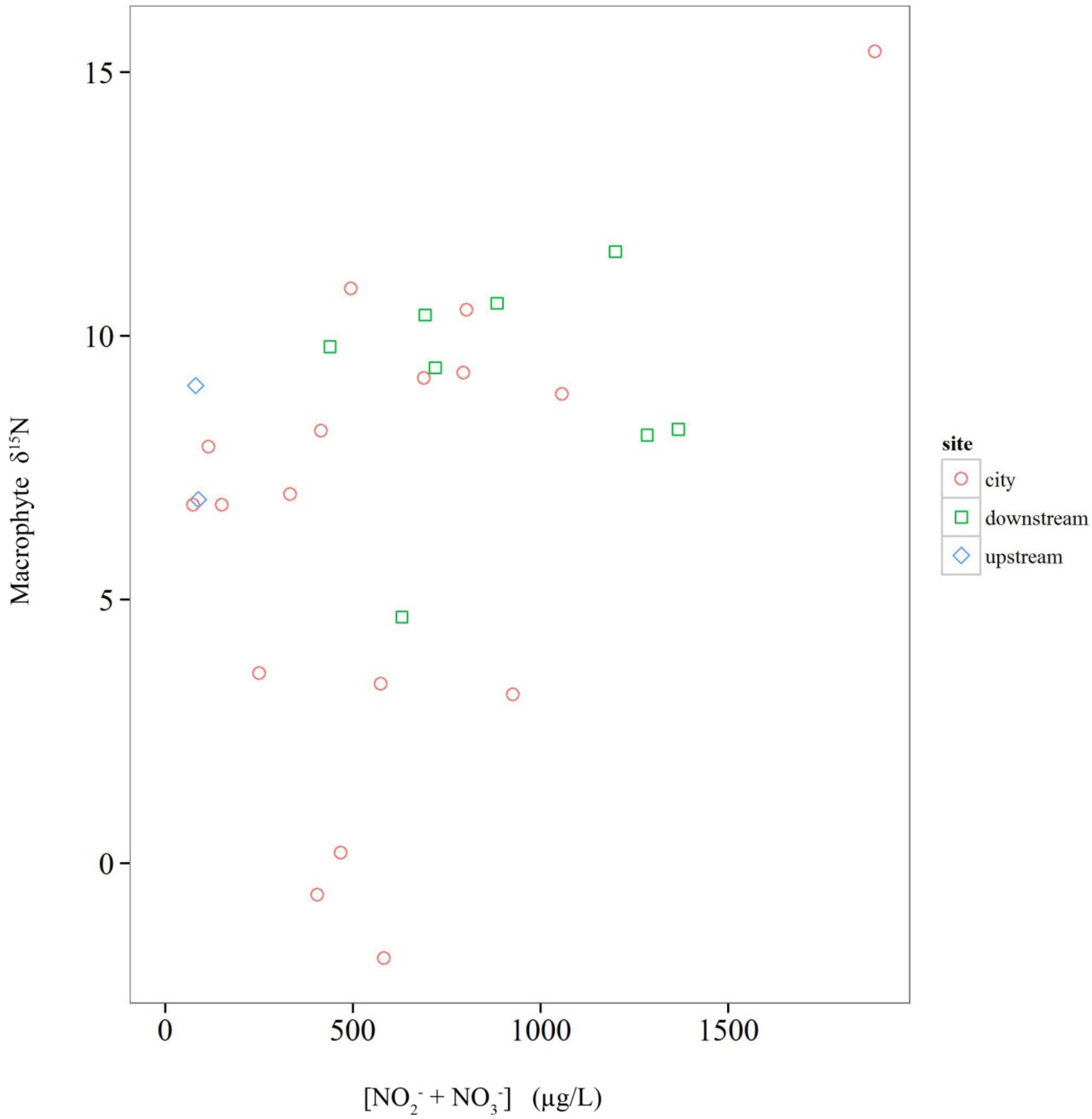

Figure 20. $\delta^{15}N$ values of macrophyte samples by nitrite+nitrate concentration. Origin is the most upstream sample site, LOUS.

may actually be quite significant due to the small differences between $\delta^{15}NO_3^-$ values in my samples. Periphyton $\delta^{15}NO_3^-$ in particular displayed very similar trends to water $\delta^{15}NO_3^-$, confirming the uptake of nitrate from the water column and subsequent assimilation into periphyton$\delta^{15}NO_3^-$. Given that effluent released from the three WWTPs cause large increases in river $[NO_2^-+NO_3^-]$, it is likely that increases in periphyton$\delta^{15}NO_3^-$ may be attributed to wastewater effluent as well.

Macrophytes displayed higher $\delta^{15}NO_3^-$values than periphyton downstream of the WWTPs. While this could result from fractionation during uptake, macrophytes may be using a different N source. Plants will preferentially take up^{14}N and yet when the system is N-limited, they are forced to take up anything available and assimilate ^{14}N and ^{15}N indiscriminately, and could therefore end up with a ratio producing a higher $\delta^{15}NO_3^-$value (Wada and Hattori 1978, Wada and Yoshioka 1996). The higher $\delta^{15}NO_3^-$signature seen in the macrophytes could indicate a stronger N limitation in macrophytes than in
As macrophytes obtain much of their nutrients from the sediment (as opposed to periphyton, which use the water column) (Barko et al. 1991, Jackson et al. 1994), sediment $\delta^{15}NO_3^-$could be a larger contributor to macrophyte$\delta^{15}NO_3^-$and any N limitation in the sediments may be reflected in the macrophytes(Jones et al. 2004). Sediment $\delta^{15}NO_3^-$is also expected to have a different signature than the water column due to the various nitrifying and denitrifying processes within the hyporheic zone. Heterogeneity in sediment beds along the river could provide an explanation for the lack of trends seen in the macrophyte$\delta^{15}NO_3^-$values, if sediments were the primary nitrate source. The predictive powers of macrophyte$\delta^{15}NO_3^-$values for water $[NO_2^-+NO_3^-]$ (and subsequently

N limitation) is currently low, but increasing the sample size may produce a more obvious relationship as seen with periphyton and water column samples.

Table 3. Percentage of total volume in the Bow River, Alberta from July to September 2011. Background source is considered to be at site INGL, the site immediately upstream of all three WWTPs. Percentage of total flow and $\delta^{15}N$ values of WWTP is derived from its monthly averaged final effluent input into Bow River.

	Percentage of total flow			
	July2011	Aug 2011	Sept 2011	**$\delta^{15}NO_3^-$ value**
Background source	97.18%	95.98%	94.24%	7.1
Bonnybrook WWTP	2.17%	3.16%	4.41%	8.6
Fish Ck. WWTP	0.21%	0.29%	0.40%	11.7
Pine Ck. WWTP	0.43%	0.57%	0.95%	11.8

Table 4. Proportion of monthly nutrient (TP and NO_2^-+NO_3^-) contribution by natural background sources and by wastewater effluent as determined by a mass mixing model. NO_2^-+NO_3^- September 2011 contributions are unavailable due to missing data. Flow data supplied by Alberta Environment and City of Calgary. Nutrient data sourced from this study and from City of Calgary.

Total Phosphorus			
	July 2011	August 2011	September 2011
Natural sources	67.03%	41.66%	21.88%
WWTP effluent	32.97%	58.34%	78.12%
Nitrite+Nitrate			
	July 2011	August 2011	
Natural sources	~0.00%	~0.00%	
WWTP effluent	~100.00%	~100.00%	

Neither water, periphyton nor macrophyte $\delta^{15}NO_3^-$ values recovered to pre-WWTP levels within the sampled area, up to approximately 46 km downstream of the last wastewater treatment plant. I conclude that the Bow River requires greater than 46 km to fully mix and revert to pre-WWTP conditions, which is corroborated by results from Hogberg (2004), who suggested a distance of 60-120km based on isotopic indicators. Other findings in a study done on the Bow and South Saskatchewan Rivers (Canada) revealed in an effluent footprint greater than 50 km, further supporting my conclusion (Wassenaar et al. 2010). An isotopic mixing model was not effective for several reasons. Bonnybrook WWTP was the biggest volume contributor volume-wise out of all the WWTPs, but its effluent $\delta^{15}NO_3^-$ signature was difficult to distinguish from the background signature (Table 3). Macrophyte $\delta^{15}NO_3^-$ was originally intended to reflect wastewater contribution, but macrophytes had a much heavier signature than the background source and the effluent sources. This is likely because macrophytes are taking up nitrate from the sediments as well, which could have a heavier signature from denitrification (bacteria prefer lighter isotopes). Periphyton $\delta^{15}NO_3^-$ values better reflected the background and effluent sources, showing similar trends, though periphyton $\delta^{15}NO_3^-$ values were still a little heavier than the sources. This could also be explained by denitrification, which may occur within the periphyton mat and will leave behind heavier isotopes (Triska and Oremland 1981). Given the shortfalls of the isotopic mixing model in this study, a mass mixing model was used to show that wastewater effluent is indeed a sizeable contributor, particularly during lower flows (i.e. September 2011) (Table 3, Table 4).

Conclusion

From a [NO_2^-+NO_3^-] perspective, Calgary's three WWTPs have a significant impact on the Bow River. From an isotopic signature perspective , the results are less clear. Water column and periphyton$\delta^{15}NO_3^-$values reflect an increase in ^{15}N over ^{14}N as the river flows downstream; this is likely a result of a combination of natural soil nitrate, atmospheric deposit and (in the most downstream sections) wastewater effluent input, which is also high in $\delta^{15}NO_3^-$. Nitrogen limitation may also be a contributing factor upstream of the WWTPs. Unless intense sampling is possible, macrophytes in this study were shown to be poor indicators in the Bow River due to their spatial patchiness, which in turn greatly reduces the sample size and further muddles any potential relationships involving macrophyte $\delta^{15}NO_3^-$. Two of the three WWTPs provided isotopic signatures that were clearly distinct from river water, but the small difference in $\delta^{15}NO_3^-$value between the two makes it difficult to attribute contributions specifically to one or the other. Although $\delta^{15}NO_3^-$ has been shown to be an effective effluent tracer in previous studies (Tucker et al. 1999, Savage 2005), in this study it presented ambiguous results regarding the contributions of wastewater effluent. There were numerous factors that were not quantified and thus could have possibly increased the ambiguity of the results, such as the contributions of other N sources other than effluent (e.g. groundwater, urban stormwater, agricultural run-off). Non-point sources of N pollution may be a bigger component in $\delta^{15}NO_3^-$dynamics than expected.

CHAPTER 4: GENERAL CONCLUSIONS

Anthropogenic inputs of nutrients from wastewater effluent can adversely affect the chemical structure of a riverine system. This change in chemical structure subsequently changes the local biological structure, because primary producers are inextricably linked to nutrients. Altered biological structure produces altered ecological function; photosynthesis and respiration contribute to fluctuations in dissolved oxygen concentrations. Valued ecosystem services, such as fisheries, may be at risk if ecological function is no longer capable of being supporting. This study investigated the quality state of the Bow River as described by nutrient concentrations, primary producer biomass and degree of fluctuation in diel oxygen (O_2) cycles, and attempted to describe their relationships using biochemical and isotopic measurements.

The largest dissolved oxygen (DO) swings were observed in the lower reaches of the Bow River, downstream of wastewater effluent inputs. Periphyton and macrophytes both drive the magnitude of these DO swings. The largest amplitudes in DO swings appear immediately downstream coincident with the highest macrophyte biomass, suggesting macrophytes to be the primary contributor to DO swings. Phytoplankton densities were very low, and considered negligible in their contribution to diel O_2 fluxes. Sediment biological oxygen demand (BOD) was not measured in this study, but may also contribute to the magnitude of O_2 fluxes.

Phosphorus and nitrogen concentrations increased significantly downstream of Bonnybrook and Fish Ck. wastewater treatment plants (WWTPs), as predicted. There was a surprising lack of relationship between periphyton and nutrients measured, which may be due to unmeasured environmental factors such as streambed composition having a larger effect on periphyton biomass than nutrients in some river reaches. This lack of relationship may also be due to changes in total dissolved phosphorus (TDP), which was not measured in this study but takes into account dissolved hydrolysable phosphorus (DHP) in addition to soluble reactive phosphorus (SRP). Previous work on the Bow River had suggested TDP to be a more accurate indicator of biologically available phosphorus (Cross et al. 1986), and Sosiak (2002) noted a decrease in periphyton where [TDP] measured below 10 µg/L. Macrophytes were found to be significantly correlated to dissolved inorganic nitrogen (DIN) in the water column; macrophytes are likely also taking up nutrients from the sediments, but sediments were not measured in this study. There was a noted periphyton-dominated to macrophyte-dominated shift around the first WWTP; while this shift was not specifically studied in this study, one potential explanation is that macrophytes have lower nutrient requirements than microalgae due to their ability to access nutrients in the sediments (Sand-Jensen and Borum 1991, Carr and Chambers 1998).

Isotopic measurements of $\delta^{15}NO_3^-$ revealed somewhat consistent increases in $\delta^{15}NO_3^-$ values from upstream to downstream in the Bow River, with no significant change in signature downstream of WWTPs. Non-point sources such as natural soil nitrate, fertilizer runoff and/or atmospheric deposition, which have similar signatures to that measured in the river water, may be a dominant nitrate source. This does not mean effluent is not be a significant contributor to nutrient concentrations. The significant increase in nitrite+nitrate concentration ($[NO_2^- + NO_3^-]$) immediately downstream of Bonnybrook WWTP and significant increase in ammonium concentrations ($[NH_4^+]$)

strongly suggest that effluent is indeed a main contributor to nitrogen concentrations in the Bow River. The lack of significant change in signature may simply be due to Bonnybrook effluent having a very similar signature (8.6‰) to river water, rather than the heightened values suggested by literature (10-20‰) (McClelland et al. 1997), despite its treatment processes.

Periphyton and macrophytes, which may be more accurate indicators of wastewater contribution than values measured in river water as their isotopic values are averaged over their growth period, presented higher $\delta^{15}N_{nitrate}$ values closer to the 10-20‰ values expected in wastewater. While periphyton and macrophyte samples consistently showed values lower than those obtained from effluent, this is likely due to primary producers preferentially uptaking lighter isotopes as opposed to heavier isotopes. Trends in periphyton$\delta^{15}NO_3^-$greatly resemble trends in water $\delta^{15}NO_3^-$,suggesting that periphyton access [NO_3^-] in the water column and assimilate the NO_3^-. Nutrient data, which showed a large increase in [NO_2^-+NO_3^-] downstream of Bonnybrook WWTP, supports the idea that periphyton $\delta^{15}N_{nitrate}$ may be attributed to wastewater effluent despite not seeing large jumps in isotopic signature downstream of WWTPs. Macrophyte samples had higher $\delta^{15}NO_3^-$values than periphyton samples, and this may be attributed to N-limitation in macrophytes; in an N-limited system, primary producers will cast aside their preference for lighter isotopes and take both lighter and heavier isotopes indiscriminately.

Based on phenotypic observations using longnose dace (*Rhinichthys cataractae*) as a sentinel species, local fish populations do not currently seem to be affected by low dissolved oxygen levels in the Bow River. Low O_2 levels have been associated with negative effects on local fish population, such as indirectly through increased predation on younger fish as they are forced to leave hypoxic refuges, or directly through heightened mortality from localized hypoxia or anoxia (Anderson et al. 2006). Younger fish are also more susceptible to low O_2 levels than older fish (Doudoroff and Shumway 1970, Alabaster and Lloyd 1980, Johnson and Evans 1991). My data revealed no missing age classes or age classes with significantly lower abundance in the younger age classes, which suggests that younger fish are not being exposed to higher predation or direct mortality through hypoxia or anoxia. The sites directly downstream of the Bonnybrook WWTP effluent output showed a significantly lower average fish age, but lack of correlation with oxygen levels suggest that this lower age average is likely due to non-O_2-related causes. Rough calculations of growth rates, which have been shown to decrease with lower O_2 levels, were also found to have no relationship with O_2 levels. Fishes do appear to benefit from increases in nutrient levels downstream of the WWTPs, as shown by their increasing condition factor from upstream to downstream.

I conclude that while results regarding the effects of low dissolved O_2 concentrations on local fish populations are inconclusive, the presence of low O_2 and even a couple of hypoxic zones is worthy of concern. While the increase in phosphorus and nitrogen concentrations downstream of WWTPs do appear to produce well-nourished, rounder and thicker fish, hypoxic zones also appear to be ultimately nutrient-induced. Should these hypoxic zones increase in occurrence and become more widespread, the local fishes may begin to display signs of oxygen stress. There may already be increased susceptibility of fish to contaminants as a result of hypoxia, which would require more than the phenotypic observations used in this study to identify

(Barton and Taylor 1996, Pollock et al. 2007). Should mitigation of oxygen issues (an ecological function) become necessary to protect valuable ecosystem services (the fish), it is worthwhile to put efforts into altering the chemical structure (nutrients) or biological structure (primary producers) of the river, particularly in controlling macrophyte biomass. While the use of nutrients in this study have pointed at WWTPs as the primary source of nutrient contribution in the Bow River, $\delta^{15}N$ isotopes suggest that it is also important to look at non-point-sources of nitrogen input. Neither point-source or non-point-source contributors can be ignored in managing nutrient concentrations.

My study aimed to further our understanding of the stressor-response or structure-function relationships related to the impact of effluent input on primary producer biomass, and the subsequent changes in diel O_2 cycles. These relationships are cumulative as the river flows downstream and cannot be considered on their own, but needs to be considered in the context of all the variables involved. While dissolved inorganic nitrogen (DIN) is the single major explanatory variable for macrophyte biomass, its interactions with soluble reactive phosphorus (SRP) and flow also significantly explain macrophyte biomass. Manipulating just DIN will likely not produce a direct, linear response in macrophyte biomass, as the interaction with SRP and flow will also be affected. Understanding these structure-function relationships will also increase the predictive powers of CEA; if one wishes to predict the magnitude of diel O_2 cycles at $time_n$ or $space_n$, the chemical structure would first need to be extrapolated based on existing relationships, followed by the biological structure. While no conclusions can be made from this study about synergistic effects, given the experimental design, this study has confirmed the interactivity of several chemical and physical variables in their contribution to primary producer biomass. An additive effect is also observed in the effect of primary producer biomass on diel O_2 fluxes.

The Bow River appears to display a discontinuity in nutrients around the first effluent input where nitrogen increases rapidly in concentration, coincident with the discontinuity between periphyton-dominance and macrophyte-dominance. While this discontinuity does not appear to translate to the magnitude of diel O_2 cycles, two out of three of my definition for high-low water quality conditions have been met. The river upstream of wastewater treatment plants does present significantly lower nutrients and primary producer biomass, and is dominated by periphyton (which also explains the low primary producer biomass). The river downstream of the first wastewater input shows a jump in nutrients and a sudden increase in primary producer biomass. The abruptly higher primary producer biomass is the result of the periphyton to macrophyte shift in community dominance. These results are not necessarily indicative of alternate states and may simply be another form of non-linear response; more investigation would be necessary to determine the reasons behind this observed shift from periphyton-dominance to macrophyte-dominance.

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APPENDICES

Appendix A: Wastewater Effluent Summaries

Table 5. Annual averaged final effluent concentrations for numerous constituents and parameters, for City of Calgary's three wastewater treatment plants, in 2012. Values averaged from monthly values in the Wastewater Treatment Plants 2012 Historical Data Report(CoC 2013).

	Bonnybrook	**Fish Ck.**	**Pine Ck.**
Flow (m^3/d)	364,250	32,581	70,595
$cBOD_5$(mg/L)	3	NR (17)	2
TSS (mg/L)	9	NR (18)	2
TP (mg/L)	0.50	NR (0.49)	0.11
NH_3-N (mg/L)	0.88	NR(31)	0.21
NO_3-N (mg/L)	15.22	NR (<0.07)	5.93
TN (mg/L)	NA	NA	7.29
F. coli (CFU/100mL)	NA	NA	4

NA = Data not available.
NR = No results due to sampling errors; values in parentheses are estimated values.

Legend

$cBOD_5$ = 5-day Carbonaceous Biochemical Oxygen Demand
TSS = Total Suspended Solids
TP = Total Phosphorus
NH_3-N = Ammonia-Nitrogen
NO_3-N = Nitrate-Nitrogen
TN = Total Nitrogen
F. coli = Fecal Coliform counts (monthly geometric mean)

Appendix B: Linear Mixed Model Results for Periphyton

Table 6. Linear mixed model results from SAS 9.3 for periphyton biomass. Asterisks between variables (e.g. SRP*DIN) indicate interaction. p-values followed by (***) indicate significant results ($p<0.05$).

	p-value	**df**	**F-value**
$NO_2^- + NO_3^-$ (NO23)	0.9631	278	0.00
NH_4^+ (NH4)	0.9579	164	0.00
flow	0.2247	294	1.48
NO23*NH4	0.6857	235	0.16
NO23*flow	0.6505	292	0.21
NH4*flow	0.8799	247	0.02
NO23*NH4*flow	0.6113	259	0.26

Appendix C: Linear Mixed Model Results for Macrophyte

Table 7. Linear mixed model results from SAS 9.3 for macrophyte biomass. Asterisks between variables (e.g. SRP*DIN) indicate interaction. p-values followed by (***) indicate significant results (p<0.05).

	p-value	**df**	**F-value**
SRP	0.4164	241	0.66
DIN	0.0025***	248	9.32
flow	0.7128	244	0.14
temperature (temp)	0.8156	245	0.05
SRP*DIN	<0.0001***	250	18.87
SRP*flow	0.3378	241	0.92
SRP*temp	0.3932	242	0.73
DIN*flow	0.0009***	247	11.35
DIN*temp	0.0190***	247	5.57
flow*temp	0.8027	242	0.06
SRP*DIN*flow	0.0004***	249	13.01
SRP*DIN*temp	<0.0001***	250	17.57
SRP*flow*temp	0.2957	241	1.10
DIN*flow*temp	0.0035***	246	8.71
SRP*DIN*flow*temp	0.0004***	249	13.04

Appendix D: Summary of Collected Fish Data

Table 8. Summary of longnose dace data collected in 2010. Values are averaged over all fish collected from that particular site in 2010.

Site	Catch per Unit Effort (fish/min)	Condition Factor	Age (years)
BWNS	2.3	0.94	2.9
EDWR	1.0	0.92	2.8
10ST	1.3	0.94	3.9
INGL	1.1	0.94	2.0
DOUG	2.2	0.99	2.9
PINE	0.8	1.02	2.4
STIR	3.6	1.01	3.1
CTNW	0.9	1.06	2.8
MCKN	0.5	1.01	3.1
CARS	3.2	0.98	3.6

Table 9. Summary of longnose dace data collected in 2011. Values are averaged over all fish collected from that particular site in 2011.

Site	Catch per Unit Effort (fish/min)	Condition Factor	Age (years)
BWNS	2.2	0.92	2.6
EDWR	2.4	0.93	3.2
INGL	2.1	0.96	3.5
GLEN	2.5	1.00	2.3
RIVB	0.8	1.01	2.0
DOUG	2.1	0.98	2.8
H22X	3.3	1.00	2.7
PINE	4.7	1.07	2.4
STIR	5.6	1.03	3.1
CTNW	1.7	1.03	3.4
MCKN	2.1	1.01	2.8
CARS	5.4	1.02	2.7