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Relationships between nitrogen, phosphorus and diel dissolved oxygen cycles in the Bow River, Calgary, Alberta before and after a century-scale flood

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by

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Abstract

High nutrient loading to rivers increases aquatic plant and algal growth resulting in low dissolved oxygen (DO) concentrations. The overall objective of this thesis was to examine the relationship of nitrogen (N) and phosphorus (P) in diel DO cycles induced by periphyton and macrophyte photosynthesis and respiration in the Bow River. I tested periphyton and macrophyte nutrient limitation status directly using fertilization experiments at multiple locations within Calgary's wastewater treatment plant (WWTP) effluent affected urban footprint. My results show that immediately after the 2013 flooding, primary production was limited by P (periphyton) or N+P (macrophytes). I investigated empirical relationships between periphyton, macrophytes and diel dissolved oxygen concentrations before and after a 1 in 100-year flooding event. The near complete removal of macrophytes was associated with only a transient decrease in the magnitude of diel DO oscillations at sites downstream of WWTPs.

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I offer my love and thanks to my family: to my parents who instilled a love of reading, and to my children, Samantha and Jack, who I hope will also develop a sense for their own curiosity. I am very grateful to Lisa Higham who provide much encouragement, grammar lessons, coaching and family breaks.

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Site name	Distance downstream (km)	Coordinates	Location
Bow 1	0	51.102502, -114.259224	Bowness
Bow 2	7	51.062141, -114.152499	Edworthy
Bow 3	20	51.038896, -114.012167	Inglewood
Bow 4	27	50.986683, -114.025933	Glenmore
Bow 5	31	50.964994, -114.022662	Riverbend
Bow 6	35	50.934767, -113.994183	Douglasdale
Bow 7	40	50.896283, -114.0107	Highway 22X
Bow 8	45	50.86245, -113.986267	Pine Creek
Bow 9	48	50.84195, 113.951533	Policeman's flats
Bow 10	57	50.879283, -113.84795	Cottonwood
Bow 11	71	50.820072, -113.793889	Upstream Highwood River
Bow 12	95	50.825241, -113.415048	Carseland
BBWWTP	23	51.007598, -114.020061	Bonnybrook wastewater treatment plant
FCWWTP	39	50.908445, -114.010277	Fish Creek wastewater treatment plant
PCWWTP	46	50.853696, -113.970108	Pine Creek wastewater treatment plant

Chapter 1: Introduction

1.0 Thesis motivation

Dissolved oxygen (DO) concentrations are used as an indication of water quality in rivers because they provide a summary measure of biological activity, which is what needs to be directly managed to maintain oxygen concentrations to be above a critical threshold (ESRD 2014a, Franklin 2014). Regulators often intend to improve low DO levels via nutrient reduction strategies (USEPA 2000, ESRD 2014a) because high nutrient loading causes higher rates of photosynthesis and respiration from increased periphyton and macrophyte growth (Sosiak 1990, 2002). Periphyton and macrophyte growth will have a diel impact on a stream's oxygen concentrations because photosynthesis augments oxygen and respiration depletes oxygen. During daylight, oxygen concentrations rise; during darkness, oxygen concentrations fall. As primary producer biomass increases, the magnitude of the dissolved oxygen oscillation increases. Although DO concentrations may be supersaturated during daylight, they can fall below the acute daily minimum threshold during darkness. The predominant concern is the maintenance of acceptable levels of DO relative to a regulator's guidelines at all times. For example, the Alberta Surface Water Quality Guidelines specify a one-day (acute) minimum level of 5 $mg \cdot L^{-1}$ and a 7-day mean (chronic) level of 6.5 $mg \cdot L^{-1}$ (ESRD 2014a). Dissolved oxygen concentrations have been measured below the minimum regulated values in the Bow River in the past (Iwanyshyn 2008, Chung 2013) and are hypothesized to be predominantly drive by macrophyte biomass (Golder 2007, Chung 2013). To improve low dissolved oxygen concentrations, as induced by primary producers, the nutrient that limits their growth must be reduced. Conceptually, the

assumption is that if the key limiting nutrient is controlled, whether it be nitrogen (N) or phosphorus (P), then primary production is limited and the cascading effects of eutrophication do not occur. But, is it fair to assume that periphyton and macrophyte growth will similarly respond to a change in N or P loading in rivers? Also, if dissolved oxygen concentrations are to be improved through reduced autotroph growth, is it also fair to assume that periphyton and macrophytes influence dissolved oxygen cycles to the same extent? Below, a literature review identifies factors in rivers that obscure the effects of N and P on periphyton and macrophyte growth, and factors that confound the effects of periphyton and macrophytes on dissolved oxygen concentrations.

1.1 Role of nitrogen and phosphorus in the control of river eutrophication

Discharge of anthropogenic N and P into flowing waters continues to be a global cause of freshwater quality degradation associated with excessive growth of nuisance algae and rooted aquatic plants (Holeton et al. 2011, Smith et al. 1999, Smith et al. 2003). For watershed management, P has historically been regarded as the primary limiting nutrient for nuisance algal growth in lakes (Hecky & Kilham 1988, Smith & Schindler 2009), and over the past five decades, P mitigation from wastewater sources has been adopted as the main watershed management tool to control freshwater eutrophication in lakes and rivers (Dodds & Welch 2000, Jarvie et al. 2013, Schindler et al. 2008).

Most literature supporting P limitation theory comes from studies of phytoplankton growth in lakes (Schindler 1977, 2006, 2012, Schindler et al. 2008), but there remains considerable controversy over the relative roles of N and P in controlling eutrophication (Conley

et al. 2009, Moss et al. 2012, Smith et al. 2006). Because river eutrophication science has often lagged that for lakes, the rationale for P-control concluded from lake science has been applied to rivers. However, reasons that support dual-nutrient reduction strategies in rivers have been identified (Dodds and Smith 2016, Lewis et al. 2011) and debated (Lewis et al. 2008, Schindler 2012) .

The basis of P control in lakes include the following arguments: (1) N fixation should ultimately compensate for N deficiencies in algal communities (Schindler et al. 2008); (2) bioassays are only run for short periods of time, whereas phytoplankton communities of whole lakes respond over multiple years, meaning that phytoplankton communities, given enough time, would shift to nitrogen-fixating species (Schindler et al. 2008); and (3) N-loading control should incur unnecessary expense to wastewater treatment plants in eutrophication control because technologies for wastewater treatment to reduce P versus N are substantially different (WEF 2015). Reasons in favour of N and P control in rivers include the arguments that (1) bioassay studies have frequently indicated N limitation or N and P limitation (Elser et al. 2007, Harpole et al. 2011), (2) N limitation is detected in streams more frequently than previously hypothesized (Francoeur 2001, Harpole et al. 2011, Keck and Lepori 2012); and (3) the biogeochemistry of P does not have a significant gaseous component and cannot be lost from an aquatic system to atmosphere, whereas dissolved inorganic N can be lost in the form of N_2 via microbial denitrification (Paerl et al. 2014). N availability for biological uptake would thus be reduced in the water column. N limitation of primary producers is more likely to occur if environmental factors limit nitrogen fixation from the large atmospheric N pool. In a recent review, Dodds and Smith (2016) conclude that N-fixing cyanobacteria are not sufficiently present in rivers and

streams to satisfy N limitation when P is present in excess of N. Evidence suggests that both nutrients should be considered in eutrophication management efforts for flowing waters.

1.2 Nutrient limitation

How nutrient limitation occurs in plants and algae has been described by two theories that offer different predictions about organism growth response to nutrient addition and fall into single resource limitation (Liebig's law of the minimum) and multiple resource limitation. Single resource limitation states that growth is limited by a single resource at a time (Salisbury 1992, Gorban et al. 2011). It is only after the demand is met for one resource than another resource may become limiting. Multiple resource limitation states that newly acquired resources are differentially allocated to new plant organ biomass, and the extent of the allocation strongly affects the continued uptake of further resources. For example, a plant may increase its root-shoot ratio to improve nitrogen uptake if nitrogen is scarce, and thus the plant may shift its morphology to balance resource uptake and become co-limited by all resources. Multiple resource limitation predicts that an increase in any one nutrient should increase growth. In contrast, single resource limitation predicts that only an increase in supply of the limiting resource would increase growth. Growth limitation by nutrient deficiency is fundamentally similar in phytoplankton, periphyton and macrophytes. Multiple resource limitation may better describe the biomass response of biologically diverse communities of plants to nutrient addition than single resource limitation, which was developed to describe constraints on production of

individual crop plants because of the diversity of nutrient requirements among primary producers (Danger et al. 2008).

To predict which nutrient limits growth, without testing nutrient limitation directly, freshwater ecologists often assume that there is a critical ratio between N and P supply rates that maximizes the growth of primary producers. Redfield (1934) analysed marine phytoplankton species and showed that the mean optimal atomic ratio of carbon to nitrogen to phosphorus is 106:16:1. Kahlert (1998) however, showed the optimal ratio in periphyton was 158:18:1. If the ratio of elements in the environment deviates widely from the optimum ratio, then the element present in excess should not limit growth. However, the nitrogen to phosphorus ratio has often failed to predict the nutrient that limits primary production in streams (Keck and Lepori 2012).

The limiting nutrient concept is more complex for an entire community or ecosystem than it is for a single organism. After a large-scale meta-analysis that indicated N and P limitation frequently occurs, Elser (2007) advocated that Liebig's law of the minimum is founded on the principle of single crop species, and may not apply simultaneously to all primary producers in a river environment. The likelihood is that growth limited by nutrient deficiency likely changes depending on plant type (macrophyte, benthic algae, epiphyte, etc.), location and time (Townsend et al. 2008). There have been many studies that question the adequacy of the Redfield ratio to predict nutrient limitation in rivers unless the specific organism's cellular carbon, nitrogen and phosphorus stoichiometry is known (Dodds 2003, Townsend et al. 2008, Keck and Lepori 2012). The wide variation in N:P for growth among phytoplankton species (~8:1 – 45:1) means that it is possible in a mixed species community for some species to be

limited by P while others may be limited by N, and for an environment to be very near the nutrient limitation thresholds for N and P simultaneously (Townsend et al 2008). To identify whether N or P becomes limiting may depend on local nutrient supply and organisms' nutrient requirements. However, factors arise from the spatially heterogeneous characteristics of rivers that confound the relationship between nutrients and primary producer growth and may create a highly patchy environment.

1.2.1 Non-nutrient related growth factors

The response of periphyton and macrophyte growth to changes in nutrient concentrations is obscured by abiotic factors that vary over the run of a river (Clarke and Wharton 2001, Bornette and Puijalon 2010, Keck and Lepori 2012, Mebane et al. 2014). Light is required for photosynthesis, but is strongly reduced by water column characteristics such as turbidity and shading (Bornette and Puijalon 2010). Net photosynthetic rates often decline with increasing temperature because respiration increases more rapidly than photosynthesis with temperature (Carr et al. 1997). Two factors contribute to the availability of carbon dioxide for photosynthesis. One is related to gas exchange between the atmosphere and water at the surface. Another is pH, which controls the forms of inorganic carbon in equilibrium with carbon dioxide (carbon dioxide, carbonic acid, bicarbonate, and carbonate) (Wetzel 2001). The physical texture of the substrate varies greatly according to flow constraints that scour, transport and deposit fine sediment (Clarke and Wharton 2001). Current velocity affects macrophyte growth independently of any indirect effects on sediment texture (Chambers et al. 1991). Periphyton response can be

modified by flow (Biggs and Close 1989), substrate size (Cattaneo et al. 1997), shading (Welch et al. 1992), and temperature (Welch et al. 1989).

1.2.2 Nutrient pool access

Macrophytes access different nutrient pools than periphyton. Macrophytes absorb nutrients through their roots or stems, or both to varying proportions depending on the sediment:water nutrient ratio (Carignan 1982). In most cases, macrophytes assimilate the majority of their nutrients (Carignan and Kalff 1980, Chambers and Prepas 1989, Carr and Chambers 1998) and trace elements (Jackson et al. 1994) from the sediments, whereas periphyton acquire nutrients from the water column (Sand-Jensen and Borum 1991). Macrophyte biomass has been shown to be related to sediment P concentrations (Carr and Chambers 1998), but it is likely that the nutrient that limits growth varies with plant type (e.g. macrophyte versus periphyton) and the trophic state of the river (Hilton et al. 2006). If nutrients are to be managed to reduce aquatic macrophyte abundance, then reductions of sediment nutrients will be an important component (Carignan and Kalff 1980, Carr and Chambers 1998, Thomaz et al. 2007). Periphyton nutrient reduction will likely be driven primarily by water column nutrient impoverishment (Keck and Lepori 2012).

1.2.3 Nutrient forms and bioavailability

Phosphorus and nitrogen occur in various forms, but not all forms are able to be used by periphyton and macrophytes. Total P is the measure of all fractions of P, which include the dissolved fraction and the particulate fraction. Dissolved P can be further broken down into

soluble organic P and soluble inorganic P. The same organic and inorganic fractions are also true of particulate P. The measure of soluble reactive P, which consists largely of inorganic orthophosphate and some organic polyphosphate, is the best short-term indicator of bioavailable P (APHA 1995). Yet, total P has sometimes been the best long-term predictor of biomass production, presumably because it reflects the true size of the nutrient pool that becomes available as the result of decomposition (Dodds 2003). Similarly, N occurs in freshwater in a variety of dissolved, particulate, organic and inorganic forms that sum to equal total N. Organic nitrogen is present as amino acids and proteins. Inorganic N consists of dissolve molecular N (N_2), and the bioavailable ammonium (NH_{4+}), nitrite (NO_2^-) and nitrate (NO_3^-). Most nitrogen transformations, such as nitrification, denitrification, nitrogen fixation and ammonia volatilization are organism-mediated, not physically mediated as is P (Wetzell 2001). Denitrification is the microbial anoxic reduction of NO_3^- to N_2, and actively removes biologically available forms of nitrogen from water. The ammonium ion is the preferred nitrogen source for plant growth (James et al. 2004). Nitrate is taken up, but nitrate assimilation requires more energy than that of ammonium (Miller and Cramer 2005).

1.2.4 Nutrient spiralling

Nutrients move downstream within running waters and are subject to physical, chemical and biological processes that alter their form and availability for biotic uptake. Nutrient spiralling (Elwood et al. 1981, Newbold et al. 1981) describes the binding and assimilation of dissolved substances for a period of time before they are later released for further movement downstream (Newbold and Elwood 1984, Stream Solute Workshop 1990). A nutrient atom may be used repeatedly as it travels downstream as it cycles between dissolved and particulate forms. The

shorter the spiral length, the quicker an element cycles through dissolved and organic forms, and the more limiting the nutrient is likely to be (Newbold et al. 1981).

Sediments have a greater affinity for phosphorus than nitrogen. The main physicochemical controls on nutrient spiraling for P are precipitation and sorption to sediments. P is highly particle reactive, readily absorbing to particles and forming P-complexes with ions such as iron and manganese. Particulate P can undergo desorption, which releases P back into the water column as PO_4^{3-}. River sediments can act as an important sink or source of P depending on mechanisms associated with mineral-water equilibria (Wetzel 2001). For example, measurements of exchangeable phosphorus in sediment of the Pembina River found that the highest concentrations were in the finest sediments, with the lowest in sandy sediments (Chambers et al. 1992). Interstitial P was consistently higher than values in the open water (Chambers et al. 1992). However, N is far more soluble and mobile than P and does not bind to sediment (Wetzel 2001). Sediments, because they are often anoxic, promote denitrification, thus N to P ratios in sediments are usually low (Moss et al. 2012). Ammonium-nitrogen pools in the interstitial water are thus buffered by smaller pools than P, and the sediments are depleted of nitrogen much more rapidly than phosphorus (Barko et al. 1991). Because of low sediment nitrogen, studies have indicated that nitrogen limitation may be significant in macrophyte communities (Clarke 2002).

1.2.5 Spatial heterogeneity of nutrient pools

Water current velocity varies across the width and length of a river, and is the predominant factor that drives river nutrient spatial heterogeneity. Scour and fill processes lead to depletion and accumulation of sediment-associated nutrients in highly localised areas, and results in greater habitat and nutrient pool heterogeneity in rivers than in lakes (Clarke et al. 2006). Spatial variation in nutrient concentrations within a river has often been observed in the water column (Dent and Grimm 1999, Francoeur 2001) and in the sediments (Clarke and Wharton 2001, Clarke 2002). Studies also suggest sediment nutrients vary spatially and temporally because flow differences induce differing exchange rates between overlying water and the hyporheic zone in the sediment layer (Clarke 2002). Fine textured sediments have been shown to retain more P and N than coarse, rocky substrate (Chambers and Prepas 1989). While nutrient pools in the water column and sediment are interconnected, the chemistry of the bottom sediments has been found to only partially depend on the chemistry of the overlying water (Chambers et al. 1992).

The nutrient that limits periphyton and macrophyte growth may be highly variable across space in a river because nutrient availability is patchy in space. Dent and Grimm (1999) observed that nutrient concentrations varied spatially, and found that N, which limited periphyton growth, was consistently more spatially heterogeneous than P. They concluded that the variation in nutrient concentration likely affects the spatial distribution of organisms and rates of primary production. Nutrients may decline to limiting levels in highly localised regions. Hoellein (2011) simultaneously measured spatial variation in nutrient concentrations and nutrient limitation of periphyton growth, and observed high spatial variability of the limiting nutrient, P, but low

spatial variability of N, suggesting saturation of biological N demand. The spatial variance of nutrient limitation over a set of mountain headwater streams was found to vary on small scales and is thought to be driven by local processes (Irvine and Jackson 2006). Periphyton response to nutrient additions has been shown to vary spatially within rivers (Scrimgeour and Chambers 2000, Irvine and Jackson 2006, Hoellein et al. 2011). Despite reports that show a high degree of sediment nutrient heterogeneity (Chambers et al. 1992, Clarke and Wharton 2001) there are few studies that examine subsequent growth limitation of macrophytes, and even fewer studies that examine how nutrient limitation may differ between periphyton and macrophytes. To better understand consumer-resource relationships between macrophytes and periphyton in streams, research is needed to establish the supply variability of sediment and water column nutrient concentrations relative to the growth demands of periphyton and macrophyte biomass.

1.3 Dissolved oxygen in rivers

Adequate dissolved oxygen concentrations are necessary to ensure the long-term sustainability of fish populations and aquatic organisms (Franklin 2014). Minimum guidelines are typically around 5 - 6 mg/L to support biologically relevant dissolved oxygen limits for the protection of fish communities (ESRD 2014a). Fish alter their behaviour and actively avoid areas of low dissolved oxygen concentrations (Anderson et al. 2006). Should oxygen depression become severe and widespread, fish die-offs may happen as has occurred in lakes (Carr 1962, Chartlton 1979). Understanding the role of macrophytes and periphyton in driving dissolved oxygen concentrations is needed if a management goal is to reduce nutrient loading to increase low dissolved oxygen concentrations.

River dissolved oxygen variability is influenced by various oxygen sources and sinks. The major oxygen sources are (1) reaeration from atmosphere, (2) enhanced aeration at weirs and other structures, (3) the introduction of dissolved oxygen from other sources such as tributaries, and (4) photosynthetic oxygen production (Chapra 1997). The main causes of oxygen depletion are (1) degassing of oxygen from supersaturated water to the atmosphere, (2) the oxidation of organic material in the water column, (3) oxygen demand from aerobic decomposition of organic matter by river bed sediments, and (4) respiration by aquatic plants (Chapra et al. 1992, Cox 2003).

The amount of oxygen that can be dissolved in water is a function of temperature, pressure and salinity (Chapra 1997). Oxygen crosses the air-water interface and enters or exits water as a function of reaeration (based on surface turbulence) multiplied by the deficit between the actual dissolved oxygen concentration of the water and the saturation concentration (Chapra et al. 1992). According to Henry's law, the exchange process will continue until an equilibrium is established between the partial pressure of oxygen in the atmosphere and the concentration of oxygen in the water in the absence of biological activity. The concentration of oxygen in water void of biological activity varies from 14.6 mg/L at 0°C to 7.6 mg/L at 30°C. Because reaeration continually drives oxygen concentrations towards the saturation concentration of water, in a turbulent river with large reaeration capacity, the dissolved oxygen concentration may not diverge much from 100% saturation. However, in a calm river with low reaeration, the dissolved oxygen concentration readily moves further from 100% saturation because of slow diffusion rates in the absence of turbulence between the surface and the atmosphere. Even rivers with scant

plant biomass exhibit diurnal dissolved oxygen fluctuations (Guasch et al. 1998) as influenced by natural temperature variation by the rising and setting of the sun on diel saturation concentration.

A dissolved oxygen sag typically occurs downstream of wastewater discharges (Sosiak 1990, Scrimgeour and Chambers 2000) due to organic matter and nitrogenous loading that elevates biochemical oxygen demand (BOD) and chemical oxygen demand (COD), respectively. As oxygen concentrations decline, atmospheric reaeration takes place due to differences in the concentration gradient. Initially, reaeration is dwarfed by oxidation of the BOD and COD as the organic matter is consumed and chemical compounds oxidized. At some point downstream, BOD and COD diminish as reaeration provides oxygen to supply the demand. Therefore, the lowest dissolved oxygen concentration is reached when these rates first reach equilibrium. Downstream of this point, reaeration dominates and so the oxygen concentrations begin to rise towards the saturation concentration.

Aquatic vegetation exerts a considerable impact on dissolved oxygen dynamics because photosynthesis produces oxygen and respiration depletes oxygen (Kaenel et al. 2000, Desmet et al. 2011). Oxygen is consumed as new biomass is synthesised and old biomass is maintained with metabolic activity (Wetzel 2001). As plants accrue biomass through the growing season, the proportion of non-photosynthetic plant tissue increases (due to self-shading) and the energy required for maintenance respiration increases (Carr et al. 1997). Net photosynthetic rates often decline with increasing temperature because respiration increases more rapidly than photosynthesis with temperature. During photosynthesis, plants use solar energy to convert carbon dioxide into carbohydrates and release oxygen as a by-product. Photosynthesis can occur

at rates that produce oxygen far faster than the rate at which oxygen can degas from the water to the atmosphere and result in supersaturated oxygen concentrations of 150 – 200% of saturation (Thomann and Mueller 1987).

Autotroph growth influences diel dissolved oxygen concentrations, yet the relative impact of periphyton and macrophytes is underexplored. During summer, when macrophytes were dominant in a Belgian river, diurnal fluctuations in dissolved oxygen concentrations coincided with solar irradiance levels (Desmet et al. 2011). Macrophytes were then planted in aquaria and were subjected to light variations simulating the natural day-night cycle. The aquaria showed similar oxygen dynamics as were observed in the river, which suggested that photosynthetic activity caused the observed oxygen fluctuations. In the same river during winter, dissolved oxygen concentrations showed little diel variation, except for slight fluctuations that corresponded to changes in water temperature. Kaenel et al. (2000) found that diel oxygen variation significantly decreased after the removal of dense macrophyte stands. In eutrophic rivers, periphyton blooms have been associated with supersaturated diurnal dissolved oxygen levels and hypoxic nocturnal dissolved oxygen concentrations (Sabater et al. 2000). Low dissolved oxygen concentrations are predicted during periods of high macrophyte growth in the Bow River in Calgary, Alberta based predictive modeling (Golder 2007). However, when dissolved oxygen concentrations were measured the year following a substantial flood in 2005, Robinson et al. (2009) found dissolved oxygen oscillations to be similar to pre-flood levels despite reductions in macrophyte abundance. The conflicting results were ascribed to the effects of periphyton photosynthesis and respiration on diel dissolved oxygen. Golder (2007) hypothesized that minimum dissolved oxygen concentrations in the Bow River, downstream of

wastewater treatment plants, is controlled primarily by macrophyte biomass, and that macrophyte biomass is controlled primarily through reductions in P loading. Further research is required to better understand (1) the relationships between nutrient loading and primary producers in the Bow River, and (2) the relationships between primary producers and diel dissolved oxygen concentrations.

1.4 Bow River

The Bow River originates in the Rocky Mountains with oligotrophic waters derived from mountain precipitation and snowmelt, and flows approximately 200 kilometers to The City of Calgary. The river flows through largely undeveloped foothills, which maintains low nutrient concentrations with little growth of periphyton and macrophytes upstream (Charlton et al. 1986). There is minimal canopy shading across the Bow River as it averages 100 m wide; therefore, growth is typically considered to be nutrient, not light, limited (Cross et al. 1986).

Population growth, increased wastewater discharge loading, and increased water abstraction could deteriorate The Bow's water quality. Approximately 1.2 million inhabitants, one third of the population of Alberta, live in the Bow Basin (ESRD 2014b). This population is expected to reach 2.28 million people by the year 2041(ESRD 2014b). There are currently six wastewater treatment plants (WWTP) that discharge effluent directly into the Bow River: Lake Louise, Banff, Canmore and three in Calgary (Bonnybrook WWTP, Fish Creek WWTP, and Pine Creek WWTP). The effluent from Calgary's WWTPs comprise 97% of the municipal effluent that enters the Bow River. Although Calgary has tertiary wastewater treatment, effluent total phosphorus concentrations (0.8 mg/L) are 30 – 40 times larger than the background total

phosphorus concentrations. Wastewater effluent adds up to 360 kg of phosphorus and 6500 kg of nitrogen daily to the Bow River. There are large bank-to-bank differences in nitrogen and phosphorus concentrations immediately downstream of Calgary's most upstream WWTP because Bonnybrook WWTP (BBWWTP) discharges effluent directly into the river from a right bank (looking downstream) point source discharge. The bank-to-bank mixing zone downstream of BBWWTP has been estimated to be approximately 15 km (Vandenberg et al. 2005). Despite phosphorus discharge from The City of Calgary today being reduced up to seven times compared to early 1980's, nuisance macrophyte and periphyton growth still occurs downstream of the WWTPs (Charlton et al. 1986, Hogberg 2004, Chung 2013). Instances of low dissolved oxygen concentrations still occur in the early morning hours (BRBC 2005, Chung 2013).

The principal nutrient responsible for primary producer growth in the Bow River is uncertain. Charlton (1986) found periphyton and macrophyte distribution to be influenced primarily by phosphorus, flow and temperature based on correlations. In the years following two phases of WWTP upgrades, periphyton declined in some areas in the river after enhanced phosphorus removal at WWTPs, yet periphyton biomass remained unchanged at other sites where water column phosphorus concentrations remained high (Sosiak 1990, 2002). Macrophyte biomass declined after enhanced phosphorus removal, but the greatest decrease followed a reduction in discharged nitrogen. Sosiak (2002) suggested the macrophyte decline was due to sediment N depletion that caused macrophyte nitrogen limitation. Golder 2007 advocated that P limits the growth of periphyton and macrophytes in the Bow River, which, in turn, controls the minimum dissolved oxygen levels. Other studies have provided evidence of macrophyte phosphorus limitation, but further downstream. For example, Carr and Chambers (1998)

concluded that macrophyte growth in the South Saskatchewan River was enhanced downstream of the City of Saskatoon's WWTP due to increased sediment P availability.

In June 2013, a severe flood, characterised as a 1 in 100-year event, occurred in the Bow River, which scoured away macrophytes and periphyton (Pomeroy et al. 2015, ESRD unpublished data). Heavy rain generated runoff at low and middle elevations in the Rocky Mountains, and was supplemented by rain-on-snow runoff at high elevations due to late lying snowpack (Pomeroy et al. 2015). As periphyton and macrophyte assemblages regrow and sediment nutrient stores are replenished following 2013, post-flood river changes present a unique opportunity to test hypotheses about how nutrients affect periphyton and macrophyte growth during recolonization, and how periphyton and macrophytes affect dissolved oxygen concentrations in the Bow River.

1.5 Thesis objectives and summary

The general objective of my thesis was to understand the role of N and P in diel dissolved oxygen fluctuations induced by periphyton and macrophytes in the Bow River. In Chapter 2, I tested periphyton and macrophyte nutrient limitation status directly using fertilization experiments at multiple locations within Calgary's WWTP affected urban footprint. Results show that immediately after the 2013 flooding, primary production was limited by P (periphyton) or N+P (macrophytes). In Chapter 3, I investigated empirical relationships between periphyton, macrophytes and diel dissolved oxygen concentrations before and after a 1 in 100-year flooding event. Results suggest that the near complete removal of macrophytes was associated with only a transient decrease in the magnitude of diel dissolved oxygen oscillations

at sites downstream of WWTPs. Macrophyte removal may have provided new habitat for periphyton by the following year as dissolved oxygen oscillations returned to pre-flood magnitudes despite the continued absence of macrophytes. Finally, Chapter 4 synthesizes the main findings and provides ideas for future research and nutrient discharge management.

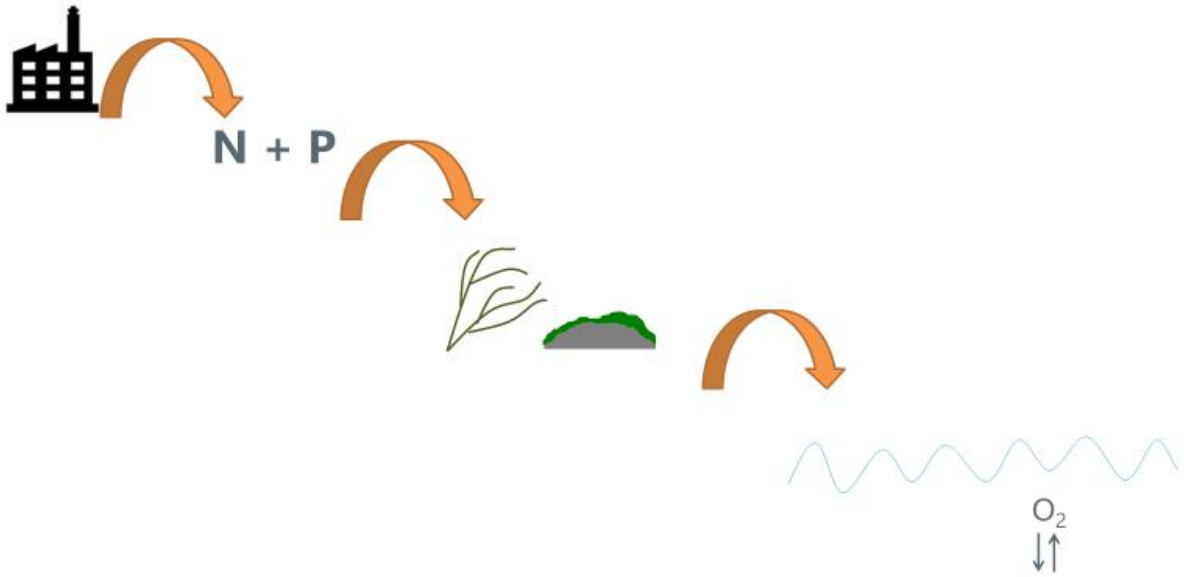

Figure 1.1. Conceptual model demonstrating the cascading effects of nitrogen (N) and phosphorus (P) discharge on dissolved oxygen (DO) concentrations. High nutrient loading increases macrophyte and periphyton biomass, resulting in higher rates of photosynthesis and respiration. Macrophyte and periphyton growth will have a diel impact on a river's oxygen concentrations because photosynthesis augments oxygen and respiration depletes oxygen. During daylight, oxygen concentrations rise; during darkness, oxygen concentrations fall. As primary producer biomass increases, the magnitude of the dissolved oxygen oscillation increases.

Chapter 2: Nutrient limitation of macrophytes and periphyton in the Bow River

2.1 Introduction

It has still not been unequivocally established whether nitrogen or phosphorus, if either, generally limits primary production in rivers (Hilton et al. 2006, Keck and Lepori 2012, Jarvie et al. 2013, Dodds and Smith 2016). Phosphorus is regarded as the primary limiting nutrient for nuisance algal growth in lakes (Schindler 1977, Smith and Schindler 2009), and has also been assumed to limit primary production in rivers (USEPA 2000, Hilton et al. 2006). Efforts to mitigate water quality degradation typically adopt strategies to reduce phosphorus discharged from agricultural lands and municipal wastewater treatment plants at substantial economic cost (Smith and Schindler 2009). Knowledge about the nutrients that limit primary production in the aquatic environment underpins the management of eutrophication and its negative consequences, and is fundamental to understanding the constraints that limit ecosystem productivity.

Mid-order, nutrient enriched rivers are dominated by periphyton and macrophytes, as opposed to high-order, long retention-time, nutrient enriched rivers, which are dominated by phytoplankton (Hilton et al. 2006). A common relationship between nutrients and periphyton and macrophytes has been difficult to establish because highly variable and confounding environmental factors also influence primary producer productivity, as reviewed in Chapter 1. Empirical evidence suggests that the principal nutrient responsible for primary production in a mixed community may depend on location. Sosiak (2002), in a long-term (16 year) study on the Bow River that examined the effects of lower nitrogen and phosphorus discharge from municipal wastewater treatment plant effluent on primary producer biomass, found periphyton biomass to

decline at sites with decreased phosphorus concentrations, but macrophyte biomass to decline at sites following reduced WWTP nitrogen loading. However, Carr and Chambers (1998) concluded that macrophyte growth in the South Saskatchewan River, much further downstream of Calgary, increases as a result of phosphorus discharged from the City of Saskatoon's wastewater treatment plant.

Nutrient limitation may vary with plant type (e.g. macrophyte, periphyton, and phytoplankton) because of organisms' different nutrient requirements and different access to nutrient pools. Kahlert (1998) showed the optimal atomic ratio of carbon to nitrogen to phosphorus for periphyton is 158:18:1, but there is large variation between species. Macrophytes appear to have a higher N:P, as the optimal ratio is 106:30:1 (Brown et al. 2006), with similarly large variation between species. Periphyton and macrophytes have different access to available nutrient pools. It is well known that rooted submerged macrophytes can derive much of their nutrient (Carignan 1982) and trace element (Jackson et al. 1994) requirements from the sediments. Periphyton uptake their nutrients directly from the water column (Bothwell 1988, Wetzel 2001).

The spatially heterogeneous nature of rivers, including flow rate, temperature, light and substrate (Clarke 2002), the amount of nitrogen and phosphorus available to organisms for uptake from the water column and sediment can vary significantly over distances as little as hundreds of meters (Dent and Grimm 1999, Irvine and Jackson 2006, Clarke et al. 2006). The spatial variability of the limiting nutrient is infrequently studied despite the likelihood that variation at these scales affects the local abundance and distribution of organisms and the rates of primary production (Tilman et al. 1982, Dent and Grimm 1999). The nutrient that limits

periphyton growth has been shown to vary within rivers (Scrimgeour and Chambers 2000, Irvine and Jackson 2006, Hoellein et al. 2011). Despite reports that show a high degree of sediment nutrient heterogeneity (Chambers et al. 1992, Clarke and Wharton 2001), there are few studies that examine how nutrients limit the growth of macrophytes among locations, and even fewer studies that examine how nutrient limitation may differ between periphyton and macrophytes.

Given the sediment and water column nutrient pools are differently available to macrophytes and periphyton, and the spatial variability of available nutrients, I asked (a) whether the same nutrients limit periphyton and macrophytes growing in the same river at the same location, and (b) whether the limiting nutrient changes with distance from WWTP effluent inputs. Relatively few studies have examined the effect of nutrients on periphyton and macrophytes in the same stream at the same time in close physical proximity. Moreover, no simultaneous tests for nutrient limitation of periphyton and macrophytes have apparently been published.

I investigated periphyton and macrophyte nutrient limitation status using fertilization experiments at locations upstream and downstream of WWTP effluent sources along the Bow River in Calgary, Alberta. I conducted experiments during the summer of 2014, which provided a unique opportunity to test nutrient limitation during periphyton and macrophyte regrowth and as sediment nutrient stores were replenished following a 1 in 100-year frequency flood in the Bow River in 2013. I predicted that periphyton and macrophytes would be limited by nutrient availability because their biomass had decreased over the last few decades at my experimental sites in conjunction with decreased nitrogen and phosphorus loading from WWTP upgrades (Sosiak 1990, 2002). My objectives were to (*i*) assess *in situ* nutrient limitation of benthic algae

and macrophytes at the same sites, (*ii*) compare changes in nutrient limitation status among multiple WWTP effluent point sources, and (*iii*) determine distance downstream of the WWTP outfalls where nutrient conditions saturated growth, if at all.

2.2 Methods

2.2.1 Experimental design and site selection

If macrophytes and periphyton are nutrient limited, then their biomass should significantly increase with the addition of the growth limiting nutrient. I used *in situ* nutrient diffusing substrata (NDS) to supplement nutrients for periphyton and *in situ* sediment fertilization to supplement nutrients for macrophytes. These techniques yielded a "snapshot" of N and P limitation. Water chemistry was measured to assess the extent of nutrient enrichment in the Bow River.

I conducted NDS experiments for periphyton at ten sites upstream and downstream of three WWTP effluent discharges along the Bow River (Fig. 2.1). Macrophyte areal coverage was sufficient at four of the periphyton experimental sites to conduct sediment fertilization experiments. Data from macrophyte site Bow 6 was discarded because plants were trampled by trespassers during the experiment. I chose experimental sites based on the locations of historical Alberta Environment and Parks and The City of Calgary water quality sampling sites and locations that would account for the spatial footprint of the Bonnybrook (BBWWTP), Fish Creek (FCWWTP), and Pine Creek (PCWWT) wastewater treatment plants. Water chemistry and nutrient concentrations were measured at the left and right banks of 13 locations in the Bow River to characterize any spatial variation of nutrients (Fig. 2.1). The ten NDS experimental sites

were interposed among the banks of the 13 water chemistry measurement locations. The most upstream NDS site was located at Bow 2 (Fig. 2.1). Bow 3 was located upstream of the first WWTP discharge. Between BBWWTP and FCWWTP, the NDS experiments were conducted near the right and left banks to account for the 12 – 18 km bank-to-bank mixing zone (Hogberg, 2004, Vandenberg et al. 2005, ESRD 2011) caused by the right bank discharge (looking downstream) of BBWWTP. FCWWTP and PCWWTP discharge effluent through mid-stream diffusers, and are not associated with differences in water column nutrient concentrations between right and left banks (Chung 2013). Bow 8 was located between FCWWTP and PCWWTP. Bow 10 was downstream of the Calgary's final wastewater effluent input, PCWWTP, and Bow 12 was well downstream of Calgary's estimated wastewater effluent spatial footprint (Hogberg 2004, ESRD 2011, Chung 2013). The macrophyte experimental sites overlapped the periphyton sites such that Bow 3 included a macrophyte experiment site that was upstream of any WWTP input. The damaged macrophyte site, Bow 6, was situated between BBWWTP and FCWWTP. One macrophyte site was between FCWWTP and PCWWTP at Bow 8. The most downstream macrophyte site was located downstream of PCWWTP at Bow 10.

Experiments were conducted in mid-to-late summer 2014, just as periphyton and macrophytes typically reach peak biomass (Charlton et al. 1986, Sosiak 1990, 2002, Chung 2013). After sediment patches were fertilized, macrophytes grew for five weeks before harvest. The NDS experiments occurred for three weeks mid-macrophyte experiment. The NDS experiment time duration was shorter than the macrophyte experiment to minimize periphyton sloughing that may occur as biomass accumulates on the NDS growth surface and to avoid the use of insecticides, like Malathion, which often contain nutrients.

At the end of the experiment, periphyton and macrophyte biomass that had accrued was collected, processed and analysed. Chlorophyll *a* was used as a proxy for the algal component of the periphyton matrix (ESRD 2011, Chung 2013). Macrophyte growth was quantified as the change in fresh biomass over the duration of the experiment. Macrophyte patches contained *Stuckenia pectinata* with trace *Potamogeton crispus*.

2.2.2 Nutrient diffusing substrata - periphyton

At each site, a total of 40 NDS devices were deployed, consisting of 10 replicates of 4 treatments (N, P, N+P, control). The NDS arrays consisted of 27 mm diameter pressed porous silica disks attached to polystyrene vials that contained 2% agar solution with either N (0.5 M $NaNO_3$), P (0.5 M KH_2PO_4), N and P (0.5 M $NaNO_3$ and 0.5 M KH_2PO_4), or a control (agar only). The silica disks perfused with the different agar treatments acted as a growth surface for the periphyton (Gibeau and Miller 1989). The resulting 40 NDS vials were arbitrarily distributed and affixed to five test tube racks. At each site, the five racks were then secured to the riverbed with rebar. All racks were placed in a run of the river to reduce site variance based on visual assessment of water depth, flow velocity and substrate characteristics. Racks were secured 10 cm below the water surface to standardize irradiation.

Each week, the NDS devices were checked 2 – 3 times to ensure they remained 10 cm below the water surface and that algal growth was not covered by any drift material. Sloughing and grazers were never observed on the disks. At the end of the 21-day incubation period the disks were removed and placed in 50 mL polyethylene centrifuge tubes. The tubes were kept in the dark on ice while in the field and were frozen within 24 hours. Chlorophyll *a* was extracted

from samples in the same centrifuge tubes that were prepared in the field to minimized chlorophyll *a* loss.

Chlorophyll *a* extracts were analysed spectrophotometrically with correction for phaeophytin by standard methods (Arar and Collins 1997, Nusch 1980). The disks were placed in 95% ethanol, sonicated for 60 seconds and then left in the ethanol for 24 h at 4 °C in the dark to extract chlorophyll *a*. The samples were then centrifuged and the absorbance of the supernatant was measured using a Genesys 20 spectrophotometer. Background readings were taken at 750 nm to correct for turbidity. Spectrophotometric readings for chlorophyll *a* were then taken at 665 nm before and after acidification to correct for phaeopigments. I then calculated periphyton chlorophyll *a* as mg/m^2 using an absorbance corrected equation (Arar and Collins 1997).

2.2.3 Sediment fertilization – macrophytes

At each site, a total of 24 sediment plots within the macrophyte bed were fertilized with either N, P, N+P, or a control (agar only); making six replicates of each nutrient treatment. Each experimental unit consisted of a 10 by 10 cm quadrat fertilized with the agar spikes containing the same nutrient treatment. Nine frozen agar spikes containing either N (0.5 M $NaNO_3$), P (0.5 M KH_2PO_4), N and P (0.5 M $NaNO_3$ and 0.5 M KH_2PO_4), or a control (agar only) were inserted into to the sediment in the macrophyte rooting zone. Treatments were made of 2% agar and were formed in 10 mL pipette tips. Plant fresh biomass was measured in six plots at the beginning of the experiment to give starting masses. At the end of the five weeks, plants from the plots were harvested, macrophytes identified to species, and growth was quantified as the change in plant

fresh biomass over the duration of the experiment. Harvested plants were placed into a centrifuge dryer ensuring that any rocks, sticks and detritus were removed, and were spun for approximately one minute at a moderate speed (approximately one revolution per second) until all surface moisture was removed. The plants were then weighed on an electronic balance to give fresh biomass values.

2.2.4 Physiochemical measurements

Water depth (cm), current velocity (cm s^{-1}), light (µmol m^{-2} s^{-1}), and temperature (°C) were measured three times throughout the experiment. Current velocity was assessed at 40% of the depth from the stream bottom next to each rack with a Model 2000, Marsh-McBirney Flo-Mate®. I measured depth with an aluminum meter stick at each current measurement location. I measured photosynthetically active radiation immediately above each rack's growth surface in full daylight conditions each week with a Protomatic light meter. The light measurements were averaged over the experimental period to estimate an overall irradiance measure for each experimental array. Temperature was recorded every 15 minutes throughout each experiment with an IBcod probe deployed alongside the experiment. River water nutrient analyses were conducted on water samples taken weekly during the NDS incubations. Water column nutrient concentrations were collected in acid-washed 1 L Nalgene bottles and analyzed at the City of Calgary Water Quality laboratory following standard procedures (APHA, 1997).

2.2.5 Statistical analyses

I used a two-factor analysis of variance (ANOVA) with equal replication to test whether periphyton and macrophyte biomass significantly increased with N enrichment (presence or

absence of $NaNO_3$ in agar) or P enrichment (presence or absence of KH_2PO_4 in agar) at each site (*in sensu* Tank and Dodds 2003). If the ANOVA interaction term was significant ($P<0.05$), indicating colimitation by N and P, p*ost-hoc* Bonferroni pairwise comparisons were performed to differentiate between biomass means. If the ANOVA interaction term was not significant, but either of the main effects were significant ($P<0.05$), indicating single nutrient limitation, *post-hoc* Bonferroni pairwise comparisons were performed. Possible outcomes from the ANOVA on the NDS bioassays are summarized in Table 1. Normality was confirmed using a one-sample Kolmogorov-Smirnov test, and homogeneity of variance was confirmed using Levene's test. If the biomass data were not normally distributed or the variances were unequal, the data were log-transformed and retested prior to analysis. All analyses were performed using R version 3.4.3 (R Core Team, 2016).

2.3 Results

2.3.1 Spatial patterns in nutrient limitation for periphyton and macrophytes

Phosphorus additions significantly increased periphyton chlorophyll *a* at all sites (*ANOVA*, $P < 0.05$, Table 2.2) except those at the right and left bank of Bow 4, the right bank of Bow 5, the right bank of Bow 6, and Bow 1 which significantly increased with nitrogen addition (Table 2.2). There was a marginally significant P effect at Bow 10 (ANOVA, $P = 0.045$, Table 2.2). Nitrogen additions did not significantly increase periphyton growth at any site tested except Bow 1 (*ANOVA*, $P < 0.01$, Table 2.2), and no significant interaction effects were detected between N and P for periphyton chlorophyll *a* accrual. Estimates of chlorophyll *a* ranged from a

minimum of 30 mg/m^2 for the control treatment at Bow 3 to 769 mg/m^2 at Bow 5 RB (Fig. 2). At sites where periphyton was limited by nutrients, P alone limited periphyton biomass.

Nitrogen and phosphorus additions significantly increased macrophyte biomass accrual at the three sites tested (*ANOVA*, $P < 0.05$, Table 2). There were marginally significant interactions between N and P for macrophyte biomass at Bow 3 (*ANOVA*, $P = 0.054$, Fig. 2.3) and Bow 10 (*ANOVA*, $P = 0.050$, Fig. 2.3). At these two sites, the effect of either nutrient on macrophyte biomass accrual depended on the availability of the other nutrient. There was no significant interaction effect detected between N and P for macrophytes at Bow 8 although N and P treatments independently, and significantly, increased macrophyte biomass (Table 2.3). Based on the definitions of N and P limitation (Table 2.1), macrophyte biomass was co-limited by N+P at the three experimental sites upstream and downstream of Calgary's WWTPs (Fig. 2.3). Increases in macrophyte biomass after five weeks of nutrient additions ranged from a minimum of 12 g/m^2 for control treatment at Bow 8 to 150 g/m^2 for the N+P treatment at Bow 3 (Fig. 2.3).

2.3.2 Water chemistry and abiotic measurements

Water temperature measured over the entire period of the experiments ranged from 13.7 to 20.4 °C. pH was slightly alkaline, ranging from 7.4 to 8.7. Irradiance reaching the river bottom at depths of 75 cm exceeded 80% of surface irradiance.

Nutrient concentrations were highest just downstream of Bonnybrook WWTP on the right bank (Fig. 2.3). Dissolved inorganic nitrogen (DIN) concentrations ranged from 50 to 120 μg/l upstream of Bow 4, but was typically ten times higher at sites downstream (Fig. 2.3b). Soluble reactive phosphorus (SRP) ranged from 10 to 30 μg/l upstream of point sources but was

typically four times higher downstream of BBWWTP (Fig. 2.3d). Nutrient concentrations between right and left banks tended to equalize downstream of Fish Creek WWTP at Bow 7 (Fig. 2.c).

2.4 Discussion

My results show that immediately after the 2013 flooding primary production in the Bow River within the city's urban footprint was limited by P (periphyton) or N + P (macrophytes). The significant effect of phosphorus on increased periphyton chlorophyll *a* accrual and phosphorus and nitrogen on increased macrophyte yield at the majority of sites indicates that phosphorus is the common nutrient that limits both autotrophs in the Bow River. Nitrogen addition affected macrophytes differently from periphyton. Macrophyte growth at all sediment fertilization sites was co-limited by nitrogen and phosphorus, whereas periphyton growth did not significantly increase with nitrogen treatments at any NDS site except Bow 1, which may have been the result of the release of sediment P from Bearspaw reservoir immediately upstream. My results demonstrate that in 2014, periphyton and macrophytes responded differently to nutrient enrichment even though they experience the same ambient water column nutrient conditions.

Few studies have explored patterns of macrophyte and periphyton nutrient limitation where they co-occur; however, two hypotheses may help explain why macrophytes were unable to obtain sufficient nitrogen to satisfy their growth requirements at sites where nitrogen-limited growth was not detected for periphyton. First, macrophytes and periphyton have different nutrient requirements for optimal growth due to variation in their tissue stoichiometry. In both autotrophs, N is mostly found in nucleic acids, amino acids and proteins and P in nucleic acids

and adenosine triphosphate. The larger amount of structural carbon tied up in macrophyte vascular tissue compared to periphyton tissue has been shown to be independent of N and P concentrations and suggests that C for macrophyte structure, and N and P for metabolism are not strongly coupled (Demars and Edwards 2007). Yet empirically, significant differences in tissue nutrient concentrations have been found to exist between macrophytes and periphyton (Duarte 1992, Demars and Edwards 2007) and even among macrophyte species (Anderson and Kalff 1986) and periphyton species (Kahlert 1998). It has been suggested that the C:N:P ratio is affected by many additional factors such as taxonomy, nutrient availability, and light availability (Su et al. 2016), but the use of nutrient concentrations in autotroph tissue as indicators of nutrient availability and limitation is ineffective (Demars and Edwards 2007) because it has been difficult to decouple the seeming difference in nutrient requirements for optimal growth from inherent variation. In natural systems, tissue nutrient pools may be less variable than environmental supply rates, leading to weak correlations.

Second, water column and sediment nutrient pools are differently available to macrophytes and periphyton, and high nitrogen concentrations in the water column do not necessarily translate to high available sediment nitrogen concentrations. Periphyton uptakes nutrients directly from the water column ((Bothwell 1988, Wetzel 2001). Although several studies have emphasized the importance of macrophyte shoot uptake of nutrients from the water column (Robach et al. 1995, Madsen and Cedergreen 2002), others argue the importance of root uptake from the sediment nutrient compartment (Carignan 1982, Barko and James 1998). Nutrient absorption from sediment or water may depend on the relative availability of N and P in each compartment (Carignan and Kalff 1980) and on the ability of the species in question (Barko

and Smart 1986). Sediment nutrient concentrations were not measured in this study and a literature search indicates that sediment nutrient concentrations are not always predictable from water column nutrient concentrations (Barko et al. 1991, Chambers and Prepas 1994, Clarke 2002). Nitrogen is not particle reactive, thus coarse river beds that have abundant flow through large particles likely have smaller N pools. Barko (1991) and Chambers and Prepas (1994) found that pools of exchangeable N in sediments are smaller, more rapidly depleted, and more likely to limit production of macrophytes than pools of P. However, in contrast, Carr and Chambers (1998) found that macrophyte biomass below Saskatoon WWTP was related to sediment P but not N concentration. The fine textured sediment in the South Saskatchewan River (Carr and Chambers 1998) may retain an adequate supply of nitrogen more readily than the rocky substrate typical in the post-flood Bow River, although in the same basin. If sediments have accumulated organic matter, ammonification may produce an alternate N source to NO_3^-.

When the spatial trend of nutrient limitation status was examined, the nutrient(s) that limited periphyton and macrophyte biomass did not change upstream to downstream of WWTP discharges along the Bow River. Where biomass was nutrient limited, P consistently limited periphyton biomass, and N and P consistently co-limited macrophyte biomass. I used *in situ* nutrient add-back experiments to examine the spatial effect of WWTP nutrient pollution on periphyton and macrophyte nutrient limitation status upstream-to-downstream of WWTP discharges and transversally from bank-to-bank in the BBWWTP effluent mixing zone. I then identified the extent to which upstream additions of nutrients move downstream and interact with other point and non-point sources to affect nutrient limitation. I did not find significant variability in nutrient limitation in the Bow River. This contrasts with Scrimgeour and Chambers

(2000), Hoellein et al. (2011) and Irvine and Jackson (2006) who demonstrated that periphyton responses to nutrient diffusing substratum experiments can vary spatially on small and medium scales (10s to 100s m). This may be due to the three WWTP effluents having similar N:P ratios of approximately 50 to 1 (Table A.1, Table A.2; Appendix A), which would explain the increased water column nitrogen concentrations relative to phosphorus concentrations observed downstream of wastewater point sources in the Bow River.

Large volumes of nutrient-rich wastewater effluent may remove nutrient limitation downstream until the river returns to upstream conditions (Vannote et al. 1980). Nutrient concentrations should progressively decrease through dilution with waters having lower nutrient concentrations and nutrient removal from advective transport through assimilation, transformation, and sorption (Stream Solute Workshop 1990). Based on NDS results, the distance downstream of BBWWTP where periphyton growth was nutrient saturated was approximately 15 km as periphyton biomass did not respond to experimental nutrient fertilization downstream of BBWWTP until just upstream of PCWWTP. FCWWTP and PCWWTP effluent discharges were not associated with downstream nutrient replete conditions, which may be due to lesser loading from these smaller volume-producing municipal plants (Appendix A). Within the estimated BBWWTP effluent bank-to-bank mixing zone of 12 - 18 km (Hogberg 2004, Vandenberg et al. 2005, ESRD 2011), nutrient concentrations were different at the right bank compared to the left bank, yet NDS experiments show that the left bank was deplete and right bank replete until Bow 8, just upstream of PCWWTP. This suggests that either some other factor may have locally limited growth or a lack of statistical power failed to detect an effect (Francoeur 2001). The loss of the macrophyte experimental site due to damage between

BBWWTP and FCWWTP prevented a conclusion whether macrophytes were also nutrient replete downstream of BBWWTP. The closest macrophyte site to BBWWTP was located downstream of FCWWTP, which at this location in the Bow, N and P co-limited macrophyte growth.

My findings of the effects of N and P on periphyton and macrophytes are consistent with Sosiak (2002), who, in a long-term study, concluded periphyton in the Bow River to be phosphorus limited because biomass decreased at sites where total dissolved phosphorus declined following WWTP effluent improvements. Sosiak (2002) also found that although nitrogen and phosphorus influenced macrophyte growth, the greatest decrease in macrophyte biomass occurred at sites during declining nitrogen but stable phosphorus levels. Similarly, Chung (2013) found DIN and macrophyte biomass to be highly correlated. The N and P co-limitation for macrophytes found in my study, that is, an increase of either nutrient increases biomass, is consistent with N and P's relationship with macrophytes found in Sosiak's 2002 pre-flood study.

My conclusion that macrophytes are limited by N and P are inconsistent with the conventional view that plants are generally limited by one nutrient at a time as predicted by Liebig's law of the minimum. Nitrogen and phosphorus independently and significantly increased macrophyte biomass in my assessment of nutrient limitation, and there was a marginally significant interaction between N and P at two of the fertilization sites. Co-limitation is found at the community level when different primary producers are limited by different nutrients (Elser et al. 2007, Harpole et al. 2011). However, individual plant species may compensatory responses; for example, as N becomes more available, marine kelp have been

shown to up-regulate synthesis of phosphatase enzymes, enhancing their ability to take up P (Bracken et al. 2015). The addition of the primary limiting nutrient to low-nutrient sediments in the Bow River may deplete the supply of the secondary limiting nutrient such that both nutrients become limiting to growth of macrophytes. Bracken (2015) proposes that synergistic effects of N and P on plant production could arise from alternation between N limitation and P limitation as one nutrient, then the other, is incorporated into a plant's biochemical pathways. Evaluating the effects of N and P addition on internal N and P concentrations may provide insights into nutrient limitation and co-limitation and particularly as to whether sequential nutrient limitation or true multiple nutrient limitation controls biomass responses to simultaneous addition of N and P.

2.5 Conclusion

Knowledge about the nutrients that limit primary production in aquatic environments underpins the management of eutrophication, and is fundamental to understanding the constraints that limit ecosystem productivity. Wastewater effluent from the City of Calgary currently adds up to 360 kg of P and 6500 kg of N to the Bow River each day. Although correlative studies may suggest the nutrient that appears to limit production, experimental fertilization experiments provide direct evidence. The experimental results from my study indicate that phosphorus is the main nutrient limiting periphyton and macrophyte productivity in the Bow River, while nitrogen only limited macrophyte growth. Therefore, P is the best nutrient to manage to control autotroph production in the Bow River today. While nutrient control will likely result in reduced periphyton and macrophyte biomass, the extent of the reduction is difficult to quantify because the response of specific aquatic plant communities to reduced nutrient loading will depend on

species composition of those communities, substrate characteristics and nutrient pools stored therein, and whether nutrients in sediments and water still exceed levels required for optimal growth. The timing of the response is also not predictable because of the dependence of aquatic macrophytes on sediment nutrient pools and the likelihood that sediments will act as a reserve of nutrients for some time after reduction in water column nutrients. The results of this study suggest that nuisance growth of aquatic plants caused by municipal loading can be managed through control of nutrient discharge to some rivers.

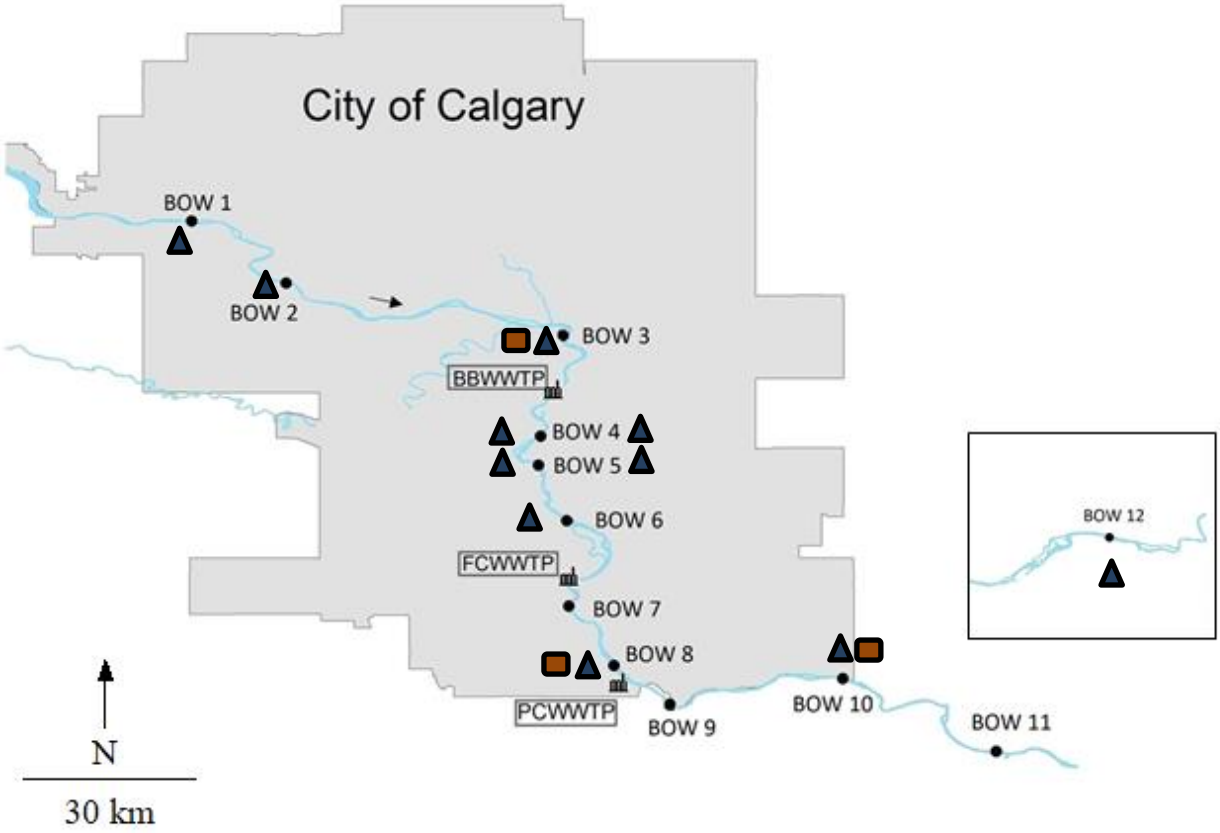

Figure 2.1. Map of sampling locations along the Bow River, Alberta. Inset box shows location of Bow 12, which was 25 km downstream of Bow 11. Black circles indicate locations where periphyton and macrophytes were sampled and dissolved oxygen loggers deployed near both banks. Blue triangles indicate locations of periphyton nutrient diffusion devices, and orange squares indicate locations of macrophyte sediment fertilization experiments. Triangle and square positions relative to the river on the map indicate left bank and right bank positions in the field. Arrow indicates direction of flow.

Table 2.1. Interpretation of responses to N and P addition. A bullet point in N or P treatment indicates a significant N or P effect in the two-way *ANOVA* ($p < 0.05$) and a bullet point in the NxP treatment indicates a significant interaction between the two treatments. (Adapted from Tank et al., 2003)

Interpretation	N effect	P effect	Interaction NxP
N limited	•	-	-
P limited	-	•	-
N and P colimited	-	-	•
N and P colimited	•	•	-
N and P colimited	•	•	•
1°N limited, 2°P limited	•	-	•
1°P limited, 2°N limited	-	•	•
Not limited by N or P	-	-	-

Table 2.2. Periphyton nutrient limitation status using ANOVA at each of 10 study sites. Relevant statistics from ANOVA are given only for the statistically significant positive responses to nutrient addition.

Site	Bank	N Effect	P Effect	NxP Effect	Interpretation
Bow 1	R	F = 13.12, *P*<0.01	-	-	N-limited
Bow 2	R	-	F = 54.65, *P*<0.0001	-	P-limited
Bow 3	R	-	F = 380.12, *P*<0.0001	-	P-limited
Bow 4	R	-	-	-	No detectable limitation
Bow 4	L	-	-	-	No detectable limitation
Bow 5	R	-	-	-	No detectable limitation
Bow 5	L	-	F = 7.81, *P*<0.01	-	P-limited
Bow 6	R	-	-	-	No detectable limitation
Bow 8	R	-	F = 41.38, *P*<0.0001	-	P-limited
Bow 10	L	-	F = 4.13, M (*P*=0.045)	-	(Marginal)
Bow 12	R	-	F = 7.62, *P*<0.01	-	P-limited

M: marginal significant p-value (p<0.05).

Table 2.3. Macrophyte nutrient limitation status using ANOVA at each of 3 study sites. Data from macrophyte sediment fertilization plots at Bow 6 were lost as a result of trespasser trampling (Na). Relevant statistics from ANOVA are given only for the statistically significant positive responses to nutrient addition.

Site	Bank	N Effect	P Effect	NxP Effect	Interpretation
Bow 3	R	F=13.03, *P*<0.01	F=15.75, *P*<0.001	F = 4.17, M (*P*=0.054)	N+P co-limited
Bow 6	L	Na	Na	Na	Na
Bow 8	R	F = 30.71, *P*<0.0001	F=6.72, *P*<0.05	-	N+P co-limited
Bow 10	L	F=31.44, *P*<0.0001	F =64.53, *P*<0.0001	F = 4.33, M (*P*=0.050)	N+P co-limited

N+P: colimitation of N and P. M: marginal significant p-value ($p<0.05$).

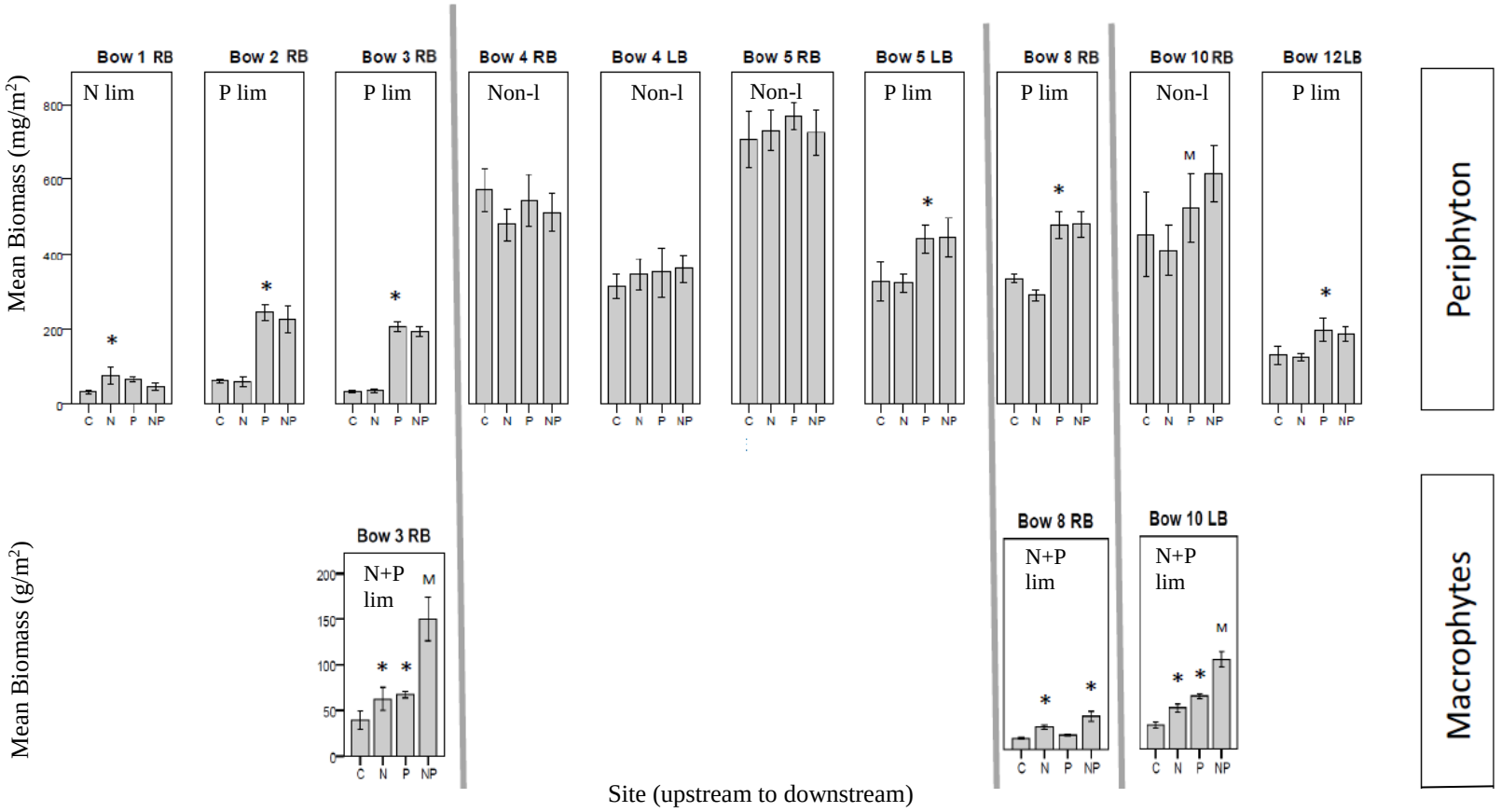

Figure 2.2. Mean (±1 SE) concentrations of Chl *a* on NDS at 10 sites and mean (±1 SE) macrophyte fresh biomass in sediment fertilization plots at 3 sites in the Bow River, summer 2014. * Significant (p<0.05) ANOVA models. M is marginally significant. Non-l, non-limited. Conclusions of N and P limitation were based on pairwise comparisons of treatments using Bonferroni adjusted p values. Grey vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP, and Pine Creek WWTP from upstream to downstream respectively.

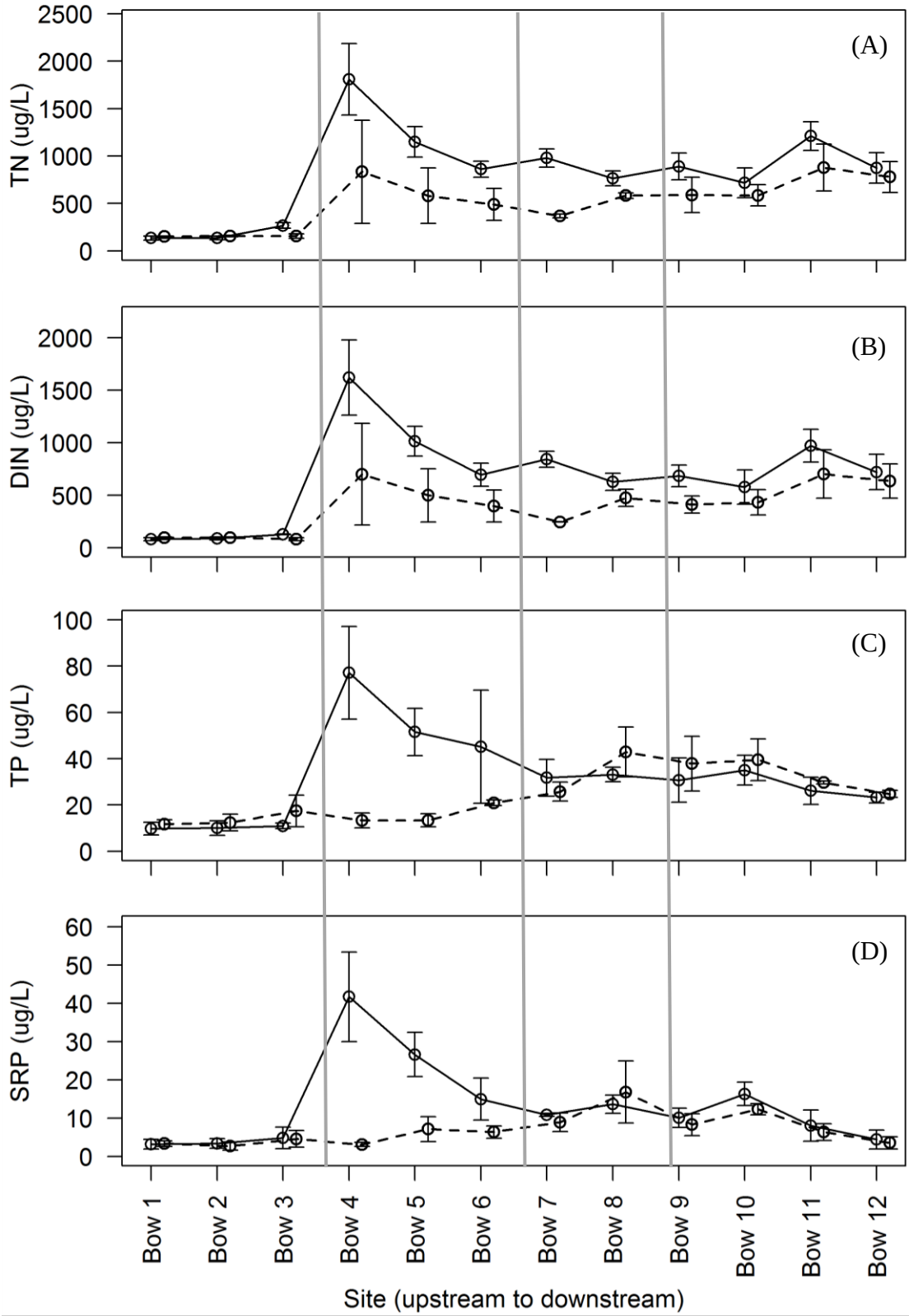
(A)
TN (ug/L)
2500
2000
1500
1000
500
0
(B)
DIN (ug/L)
2000
1500
1000
500
0
(C)
TP (ug/L)
100
80
60
40
20
0
(D)
SRP (ug/L)
60
50
40
30
20
10
0
Bow 1
Bow 2
Bow 3
Bow 4
Bow 5
Bow 6
Bow 7
Bow 8
Bow 9
Bow 10
Bow 11
Bow 12
Site (upstream to downstream)

Figure 2.3. Longitudinal pattern of total nitrogen (A), dissolved inorganic nitrogen (B), total phosphorus (C), and soluble reactive phosphorus (D) along 80 km of the Bow River on the right and left banks (facing downstream) in mid-late summer 2014. Right bank is indicated by solid black line; left bank is indicated by dashed line. Plotted values are averages of all measured values with 95% confidence interval bars. Grey vertical lines indicate locations of Calgary's WWTPs – Bonnybrook WWTP, Fish Creek WWTP, and Pine Creek WWTP from upstream to downstream respectively.

Chapter 3: Empirical relationships between periphyton, macrophytes and diel dissolved oxygen concentrations in the Bow River: a pre-flood, post-flood dissolved oxygen contrast

3.1 Introduction

Streams exhibit diel swings in dissolved oxygen (DO) concentrations that result from a dynamic equilibrium between biological, chemical and physical components of the ambient environment (Chapra et al. 1992, Cox 2003). In oligotrophic streams, dissolved oxygen concentrations remain close to saturation and are predominantly driven by oxygen's temperature-dependent solubility. Conversely, in streams that receive substantial nutrient loading and have high rates of biological metabolism, diel DO oscillations have higher diurnal highs and lower nocturnal lows driven by photosynthesis and respiration rates (Chapra 1997). As water flows downstream and traverses plant beds, periphyton and macrophyte biomass have a cumulative impact on a stream's oxygen concentrations (Chung 2013) and may cause severe depletion just before dawn at sites downstream of nutrient point sources, such as wastewater treatment plants (Golder 2007).

Water quality modeling studies have described the factors that influence dissolved oxygen concentrations (Cox 2003, Franklin 2014). Re-aeration regulates the concentration of oxygen in water and is affected by temperature, wind mixing, water depth and velocity, and the presence of morphological features such as waterfalls, dams and rapids (Chapra et al. 1992) that increase exchange across the air-water interface. Water temperature and salinity control dissolved oxygen concentrations through their influence on the saturation capacity of water (Chapra 1997). As temperature and salinity increase, saturation capacity is reduced. Biological

oxygen demand (BOD) is a measure of the amount of oxygen required by microorganisms as they consume organic matter, and thus reduces oxygen concentration in water. Photosynthesis and respiration by aquatic vegetation can exert a considerable impact on diel dissolved oxygen concentrations (Kaenel et al. 2000, Desmet et al. 2011). However, there are few studies that differentiate macrophyte from periphyton influence on dissolved oxygen dynamics in rivers. Depending on depth, two types of autotrophs dominate. In deep rivers, suspended phytoplankton dominate. In shallower streams where light can reach the sediments, fixed macrophytes and periphyton make up most of the primary producers (Charlton et al. 1986). Macrophytes and periphyton have a greater impact on stream oxygen than suspended phytoplankton because attached producers are concentrated longitudinally, fixed in space and usually situated in shallower water (Chapra 1997). Kaenel (2000) observed daily oxygen variation in unshaded, nutrient rich rivers to decrease slightly, but significantly, after dense macrophyte stands were harvested. In eutrophic rivers, periphyton blooms occur during dry, hot periods in summer when dissolved oxygen concentrations are already low because water temperature is high and water flow is low (Charlton et al. 1986, Sabater et al. 2000). Periphyton blooms are associated with oversaturated and hypoxic dissolved oxygen concentrations (Sabater et al. 2000). Although the independent effects of periphyton and macrophytes have received some attention, the combined influence of periphyton and macrophytes on dissolved oxygen concentrations in river systems is not well understood.

Low DO concentrations in the Bow River downstream of Calgary, Alberta, have long been a concern (Sosiak 1990, 2002, ESRD 2014b), and are thought to be largely governed by nocturnal macrophyte respiration (Sosiak 2002, Golder 2007, Chung 2013). The Alberta Surface

Water Quality Guideline for dissolved oxygen outlines a one-day minimum acute value of 5 mg/L and a seven-day mean chronic value of 6.5 mg/L (ESRD 2014a). Minimum recorded DO concentrations increased from 3.6 mg/L to 6.3 mg/L in hourly monitoring when macrophyte biomass declined following nutrient removal improvements at Calgary's wastewater treatment plants (WWTPs) in the 1990s (Sosiak 2002). Yet, despite nutrient reductions, the Bow River still contains periphyton chlorophyll *a* of 158mg/m^2 ± 17 (Robinson et al. 2009) and macrophyte dry weight of 241g/m^2 ± 29 (Sosiak 2002) downstream WWTP effluent outfalls. Instances of low dissolved oxygen still occur in local patches in the Bow River (Iwanyshyn 2008, Chung 2013) when macrophyte biomass is at its maximum in late summer and early fall (Charlton et al. 1986). Chung (2013) hypothesised that macrophytes, rather than periphyton, drive diel oxygen variation downstream of Calgary's WWTPs because sites with high macrophyte biomass exhibited larger dissolved oxygen swings than sites without macrophytes. However, when diel dissolved oxygen patterns were measured the year following a substantial flood in 2005, Robinson et al. (2009) found dissolved oxygen patterns to be like pre-flood variations despite reductions in macrophyte abundance. The conflicting results were ascribed to the influence of quickly regrown periphyton following flooding on dissolved oxygen concentrations.

Post-flood river conditions present a unique opportunity to better understand to what extent macrophytes drive low dissolved oxygen concentrations in the Bow River. In June 2013, macrophytes and periphyton were scoured from the Bow River when flows peaked to 1800 m^3/sec, eight times the regular seasonal flow, by the largest flood in the basin in the past 60 years (Pomeroy et al. 2015). Periphyton returned to pre-flood biomass quicker than macrophytes in the year following previous floods (Robinson 2009, ESRD 2010). In the summer of 2013,

periphyton and macrophyte biomass was expected to be negligible, whereas in the summer of 2014, periphyton biomass was expected to be near the historic average while macrophyte biomass was expected to remain minimal.

I tested the hypothesis that macrophyte biomass is the principle factor that governs large diel dissolved oxygen oscillations in the Bow River in Calgary's WWTP affected urban footprint. I measured diel dissolved oxygen concentrations, periphyton biomass and macrophyte biomass near the banks of 12 locations upstream and downstream of WWTP point sources during the summers of 2013 and 2014. I then compared 2013 and 2014 dissolved oxygen concentrations to 2010 and 2011 dissolved oxygen concentrations from the same 12 sites (City of Calgary unpublished data, Chung 2013). I predicted that if macrophytes primarily drove dissolved oxygen variation, then the magnitude of diel DO oscillations pre-flood would be significantly more than the magnitude of diel DO oscillations post-flood. I also asked whether the flood's scouring of macrophyte biomass was related to a lasting change in diel DO concentrations for the two years following the 2013 flood or merely associated with a temporary change that slowly returned to pre-flood concentrations.

3.2 Methods

3.2.1 Study sites

The Bow River originates at the continental divide on the eastern slopes of the Rocky Mountains. It flows roughly 200 km eastward through largely undeveloped and low intensity agricultural land before entering the city of Calgary. The Bow River has a mean annual discharge of 105 m^3/sec at Calgary (Water Survey of Canada gauging station 05BH004) and

receives approximately 5.1 m3/sec of effluent from three tertiary wastewater treatment plants (WWTP) (Wastewater Treatment Plants Historical Data 2014). Macrophyte biomass peaks downstream of the WWTPs during late summer and early fall (Charlton et al. 1986, Chung 2013). I selected 12 sites along the Bow River to capture a high spatial resolution of dissolved oxygen concentrations amongst Calgary's WWTP outfalls and to coincide with sites sampled by the City of Calgary in 2010, 2011, 2013, and 2014 and by Chung (2013) in 2010 and 2011 (Figure 3.1). Biomass measurements collected by Chung (2013) were pooled to represent pre-flood conditions, as there was no significant difference between biomass in 2010 and 2011. I measured DO concentration, water temperature, current velocity, depth, macrophyte biomass and periphyton biomass within 0.5 m-3.0 m from each shore and 0.2-1.0 m depth in August 2013 and 2014 to coincide with peak biomass and maximum DO fluctuations. Measurements were taken near both banks to account for any bank-to-bank differences induced by stormwater inputs and by Bonnybrook WWTP's right bank (looking downstream) discharge into the Bow River.

3.2.2 Study design

To evaluate the impact of macrophyte removal on diel DO amplitude (hereafter referred to as ΔDO), I used a Before-After-Control-Impact design (BACI) (Stewart-oaten et al. 1986, Underwood 1992, 1994), in which I estimated the mean difference-in-the-difference of DO concentration amplitudes between control and impact sites that occurred between pre-flood and post-flood years. The resulting BACI contrasts provided hypothesis test statistics and effect sizes with a measure of precision. A significant BACI contrast may then be considered evidence of an impact on ΔDO associated with macrophyte removal. I analysed DO measurements from two

years before and two years after the 2013 flood to assess ΔDO was reduced and if the reduction persisted for multiple years.

Because macrophytes mainly proliferate downstream of Calgary's WWTPs (Charlton et al. 1986, Sosiak 2002), I considered sites upstream of Bonnybrook WWTP as control sites and sites downstream of Bonnybrook WWTP as impact sites. For BACI analysis, I selected two upstream sites as control sites (Bow 1 and 3; Figure 3.1) and two downstream sites as impact sites (Bow 9 and 11; Figure 3.1) from the 12 DO monitoring sites to control for spatial autocorrelation. Bow 9 and Bow 11 were chosen for downstream analysis because they represent sites that are exposed to the highest cumulative respiration rates over an entire night (Golder 2007) and consequently capture the largest oxygen difference possible. My two selected impact sites have also been the locations of The City of Calgary's long term DO monitoring probes.

3.2.3 Biomass sample collection

Periphyton was sampled from three cobbles chosen randomly from the riverbed near each bank. Periphyton biomass was scraped within a 4 cm^2 internal square template from two locations on each cobble. The material was collected in a small plastic container and used for ash-free dry mass (AFDM) determination, which was processed following Biggs & Kilroy (2000). I use AFDM as a surrogate for organic matter to distinguish this mass from the total dry mass, which would also include any trapped inorganic particles. The samples were dried to constant mass at 65 °C, weighed for dry weight, then combusted at 550 °C before being reweighed to determine ash-free dry mass by difference.

Macrophyte biomass was negligible during sampling in 2013 and 2014. In 2010 and 2011, macrophytes were harvested from establishment to end of season to capture maximum seasonal biomass. Methods have previously been detailed in Chung (2013).

3.2.4 Dissolved oxygen measurements

Dissolved oxygen loggers (RBR DO-1050 and D-Opto Logger) were calibrated to 100% O_2 saturation using either Ruskin 1.5.25 (RBR) or D-OptoLog software. The DO loggers were deployed at each site with a temperature logger (Alpha Mach iBCod Type Z) for 72 hours to capture diel oxygen concentration amplitudes. The City of Calgary's DO loggers were permanently deployed throughout August to continuously monitor DO concentrations at Bow 1 and Bow 11. DO loggers sampled oxygen concentrations once every 15 minutes. I determined the change in dissolved oxygen concentrations ([DO]) within a 24-hour period by standardizing [DO] with temperature and pressure to adjust for differences in oxygen's temperature and pressure-dependent solubility. I calculated expected [O_2] (mg/L) at 100% saturation based on Mortimer's (1981) temperature (°C) and atmospheric pressure (mmHg) relationship:

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

where, O_2 saturated = [O_2] at 100% saturation (mg/L); T = water temperature (°C); and P = atmospheric pressure (mmHg). The resulting change in DO over 24 hours was an average of the measurement from the deployment periods.

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). I calculated ΔDO as the difference between the temperature-adjusted maximum and minimum [DO].

3.2.6 Statistical analysis

To determine whether there were significant differences in ΔDO concentrations between pre- and post-flood, I followed a BACI-procedure (Underwood 1992, 1994) in which I created the following linear mixed model (LMM) to account for fixed and random effects:

$$MeanLn\Delta DO = SiteClass \;\; Site(R) \;\; Period \;\; Year(R) \;\; SiteClass*Period$$

Where *SiteClass* is the classification of sites as upstream or downstream of BBWWTP; *Site(R)* is the random site effect within *SiteClass*, *Period* is the classification of time as before or after flood, *Year(R)* represents the multiple years within each period, and the *SiteClass*Period* term represents the BACI contrast. I used a restricted maximum likelihood estimation (REML) fit to my LMM, which is considered more robust for unbalanced designs (Fletcher and Underwood 2002). To avoid pseudo-replication of the day-to-day ΔDO measurements, I calculated mean monthly ΔDO for use in the model. The response variable, ΔDO, was natural log transformed to meet assumptions of normality and adjust variance to be equal for all values of the mean. Interest lies in the BACI interaction term (*SiteClass*Period*), which, if significant, would indicate there was an impact on ΔDO at downstream locations before and after flooding. The statistical significance of the interactions was tested with an analysis of variance (ANOVA) on the fitted models using Kenward-Rogers approximations to the degrees of freedom for unbalanced data. I then estimated the marginal means of the four combinations of upstream and

downstream sites and before and after periods. These values were then used to estimate the BACI effect as the difference of the differences

$$BACI = \mu_{UB} - \mu_{UA} - (\mu_{DA} - \mu_{DB})$$

Where U is upstream, D is downstream, A is after flood and B is before flood. The estimated BACI effect was back-transformed because I analysed ΔDO on a natural logarithmic scale. I then estimated the variance components for site-to-site effects, the year-to-year effects and the residual variation. I completed the above procedure with 2014 data removed and again with it included to account for immediate and next year changes in the river.

To ascribe any detected differences in diel DO concentrations to changes in instream biology between pre- and post-flood, I analysed differences in macrophyte and periphyton biomass with a one-way repeated measures ANOVA with post-hoc Bonferroni adjusted contrasts. I checked data for normality using the Shapiro-Wilk test for normality and sphericity using Mauchly's test for sphericity and used the Greenhouse-Geisser correction if the assumption of sphericity was violated.

All statistical analyses were conducted in R version 3.4.3 (R Core Team 2016). A mixed-effects model was used with the package lme4 (Bates et al. 2015). Multiple comparisons were conducted using the lmerTest package (Kuznetsova et al 2016).

3.3 Results

3.3.1 BACI results

In 2013 there was a significant BACI interaction effect of SiteClass and Period on ΔDO ($F_{(1,5)}$ =16.90, p = 0.009; Fig. 3.2). Delta DO significantly decreased by a factor of 1.50 at

downstream sites relative to upstream sites between pre-flood and 2013 (BACI contrast estimate= 1.50, SE = 0.0987, p = 0.006, 95% CI[0.52, 0.85]). In 2013, ΔDO was significantly greater downstream than upstream ($F_{(1,8)}$ =27.33, p = 0.0347; Fig. 3.2). In 2014 there was no significant BACI interaction effect detected between SiteClass and Period on ΔDO ($F_{(1,8)}$ =16.90, P = 0.468, BACI contrast estimate = 0.923, 95% CI[-3.72, 1.87.]). Delta DO was significantly greater downstream than upstream ($F_{(1,8)}$ =27.33, p = 0.0347; Fig. 3.2) in 2014.

3.3.2 Spatial patterns of biomass and delta dissolved oxygen

From upstream to downstream, ΔDO increased steadily in all years (Fig. 3.3A). DO oscillations were generally higher at sites during 2010/2011 than 2013 and 2014 (Fig. 3.3A). There were instances of dissolved oxygen concentrations below 5 mg/L at many sites along the Bow River before the flood (Table 3.1). DO concentrations were not detected below 5 mg/L at any site in either year following the 2013 flood (Table 3.1).

3.3.3 Biomass results

Periphyton biomass changed significantly between pre-flood and post-flood ($F_{(2,22)}$ =15.509, p = 0.000063; Fig. 3.4). Post hoc analysis with a Bonferroni adjustment revealed that periphyton biomass had significantly decreased from pre-flood to 2013-post-flood (*M* = 0.254 g/100cm^2, *SE* = 0.038, *p* < 0.001; Fig. 3.4) and significantly increased from 2013-post-flood to 2014-post-flood (*M* = 0.354 g/100cm^2, *SE* = 0.058, *p* < 0.001; Fig. 3.4). There was no significant change in periphyton biomass between pre-flood and 2014-post-flood (*M* = 0.100 g/100cm^2, *SE* = 0.090, *p* = 0.865; Fig. 3.4).

Macrophyte biomass decreased from pre-flood (M = 25.77, SD = 24.48 g/100cm^2; Fig. 3.3) to negligible in 2013-post-flood and negligible in 2014-post-flood flood ($F_{(2,22)}$ =13.296, p = 0.000164; Fig. 3.4). Post hoc analysis with Bonferroni adjustment indicates a significant decrease pre-flood to post-flood (M = 25.77 g/100cm^2, SE = 7.07, p = 0.012). Macrophyte biomass showed a different pattern than periphyton AFDM before and after the flood in 2013 (Fig. 3.3B; Fig. 3.3C).

3.4 Discussion

The near complete removal of macrophytes, except for very small, highly localized patches, by the largest bed moving force in the Bow River basin in half a century was associated with only a decrease in ΔDO at sites downstream of wastewater treatment plants (WWTPs) for one year following the flood. Although ΔDO significantly decreased in August 2013 when macrophytes were absent and periphyton biomass was significantly lower than pre-flooding, ΔDO returned to pre-flood levels by August 2014 despite the continued absence of macrophytes. A significant difference in ΔDO was not detected between pre-flood and 2014-post-flood, which is likely due to periphyton's quick regrowth and consequentially greater biomass at downstream sites by 2014. The Bow River has shown a quick recovery in ΔDO after past floods. Robinson et al. (2009) observed a return in ΔDO in the following season after a flood in 2005. Despite the large difference in scouring flows between 2005 flood (790 $m^3 \cdot sec^{-1}$) and the 2013 flood (1800 $m^3 \cdot sec^{-1}$), ΔDO returned to pre-flood levels the following season in both study conditions. However, a significant decrease in ΔDO was detected in August proceeding the June 2013 flood.

The magnitude of the ΔDO decrease between pre-flood and 2013-post-flood was interpreted from the BACI contrast estimate, or significant Site-Class*Period interaction. The interaction represents the inconsistency in the response between the upstream and downstream sites to the pre-flood versus post-flood period, which answers the question: if the scouring of macrophytes and periphyton were removed from the data, by what proportion would a typical data point change in value in the downstream reaches? The BACI effect size was estimated to be 1.50 in 2013, which implies that the change in ΔDO between pre-flood and post-flood was 1.5 times as large in the downstream reach as in the upstream reach, and in absolute terms was an average decrease in ΔDO of 3.25 mg/l downstream of WWTPs. The biological meaningfulness of this decrease in ΔDO was most apparent in the upwards shift of minimum DO concentrations detected within dense plant beds before the flood to much higher minimum DO concentrations detected in the absence of macrophytes post flood (Table 3.1). Although low DO concentrations were detected only in local patches of dense plant beds, hypoxia has been shown to increase susceptibility of fish to contaminants (Pollock et al. 2007) and may exacerbate other sublethal interactions (Anderson et al. 2006).

The lack of a significantly discernable BACI interaction in 2014 was surprising. I presumed that if macrophytes predominantly drove ΔDO, then their slow accumulation to pre-flood biomass amounts over many years would result in a proportionally slow return to pre-flood DO oscillations, but the resulting return to pre-flood ΔDO by 2014 and the quick regrowth of periphyton suggests the relative contribution of communities to ecosystem metabolism shifts rapidly from macrophytes to periphyton following a flood (Vis et al. 2007, Vilches and Giorgi 2010), especially at downstream sites. The Bow is an unshaded, oligotrophic river upstream of

Calgary, but downstream, WWTPs discharge a P-load up to 360 kg/day and an N-load up to 6400 kg/d. Nutrient availability has been shown to strongly influence rates of periphyton accrual following flooding (Lohman et al. 1992, Marti and Sabater 1996, Martí et al. 1997). My BACI results support the hypothesis that the nutrients discharged downstream affect the rate of return to pre-flood ΔDO. There was a significant difference in ΔDO between upstream and downstream reaches during pre-flood and 2014-post-flood, as indicated by the significant Site-Class main effect. However, this upstream-downstream difference was not significantly different (BACI interaction) between pre-flood and 2014-post-flood, which indicates a quick rebound to pre-flood DO fluctuations. Other research has indicated that harvesting dense macrophyte stands resulted in a relatively small but significant reduction of daily oxygen variations in unshaded, nutrient-rich rivers (Kaenel et al. 2000). However, the effect appeared to be only transient because plant removal opened space for rapid algae growth. Vilches et al. (2010) also found benthic algae was the most productive biological metabolism compartment following a flood in the Argentinian, Las Flores stream. Similarly, lack of competition from macrophytes (Vis et al. 2007), exposed substrate suitable for periphyton to colonize (Bourassa and Cattaneo 1998), sufficient nutrients downstream of WWTPs (chapter 2, Lohman et al., 1992), periphyton's faster carbon fixation rate (Vis et al. 2007), and the flood's removal of grazers for top down control (Welch et al. 1992) may have contributed to periphyton's significantly higher biomass than pre-flood years and its greater potential influence on ΔDO.

The balance between primary producers may skew towards macrophytes with sufficient time post-flood because the development of benthic algae has been shown to correlate negatively with macrophyte biomass due to decreased light availability (Sand-Jensen and Borum 1991,

Sand-Jensen et al. 1999). Shifts from macrophyte dominance to periphyton dominance can substantially influence aquatic ecosystems (Scheffer et al. 2001, Hilton et al. 2006). If macrophytes predominantly drove ΔDO, then even with a periphyton community recovery, the overall ecosystem biomass should be reduced enough to change metabolism, but it does not appear to offset metabolism enough to make ΔDO different by the second year. It may take longer to observe conditions where reportable DO concentrations fall below regulatory limits However, Kaenel (2000) found stream metabolism was not dominated by macrophytes even when macrophyte biomass was very high. She found that removal of plants in a stream coincided with only a moderate increase in nocturnal oxygen concentration, and that a rapid, partial recovery in stream metabolism suggested that in unshaded, nutrient-rich streams improvements in oxygen situation after plant cutting were transient. It was perplexing that ΔDO was not significantly different pre-flood compared to 2014-post-flood yet there were many exceedances of the DO guideline below 5 mg/L in 2010/2011 but not 2014. The low DO concentrations measured pre-flood occurred within dense macrophyte beds where water movement was slowed. During 2014, in the absence of dense plant beds, low DO concentrations were not detected. Oxygen concentrations may be far more dynamic within dense vegetation than adjacent open waters (Goodwin et al. 2008). A better understanding of the spatial and temporal relationships of hypoxic DO concentrations within dense plant beds to adjacent open water would help integrate the relevance of local DO measurements over sites and reaches of the Bow River.

Climate change and human activities are expected to exacerbate the conditions that lead to low DO concentrations in the many rivers (Schindler 2001, Scheffer et al. 2001, Kerkhoven and Gan 2011). My study compared post-flood ΔDO to pre-flood ΔDO in 2010 and 2011, and

did not detect a significant difference one year following the 2013 flood. One possible explanation to remediate this seemingly surprising lack of detecting an effect so soon after the flood is that ΔDO during 2010-2011 comparative years was not particularly extreme. DO oscillations have been greater in the past (Sosiak 1990). The City of Calgary has reduced nutrient loading from its WWTPs over the last few decades, which has decreased macrophyte and periphyton biomass in the downstream reaches (Sosiak 2002). Over the last number of years, macrophyte dry biomass has averaged 240 ± 29 g/m^2 and periphyton chlorophyll *a* has averaged of 158 ± 17 mg/m^2 (ESRD, unpublished data). Dissolved oxygen concentrations within the main channel are currently and consistently reported above 5.0 mg/l by The City of Calgary's monitoring station at the Bow 11 site (City of Calgary unpublished data). However, low DO concentrations have been measured within The Bow's dense plant beds (Chung 2013). The combined effect of lower flows and higher water temperatures from climate warming will aggravate low oxygen in these systems (Garvey et al. 2007). The Prairie Provinces are already a region with the highest water use to streamflow in the country (Statistics Canada 2009). Human activities that reduce flow, like the construction of barriers for flood control and diversions for freshwater consumption and irrigation will further compromise oxygen availability. If no refuges are present in adjacent areas, fragmentation among sites within river reaches will likely limit diversity when oxygen becomes occasionally low (Garvey et al. 2007). Explicit predictions about the interaction among these factors with emerging contaminants and multiple stressors are not yet possible give a lack of robust observational and experimental data.

Research on how interactions among flow, physical habitat, and nutrients affect patterns of oxygen in rivers across multiple spatiotemporal scales is important. This study and Chung

(2013) not only indicates that DO concentrations are spatially variable, but also supports the hypothesis that the site just upstream of the confluence of the Highwood River and the Bow River represents the largest swing in DO concentrations under most primary producer conditions in the Bow. It was expected that just before sunrise, DO levels would be at a minimum, and the lowest concentration would occur within the parcel of water that has experienced the highest cumulative respiration rates over the entire night. Based on travel times through the Bow River, the low point was predicted to occur just upstream of the Highwood River in the parcel of water that has passed through the areas of the river containing the highest levels of aquatic vegetation (Golder 2007). As this study's spatial DO measurements indicated, oxygen can vary markedly among measurement sites within reaches and between years. Continuous information about oxygen concentrations should be used by policy-makers in a diagnostic as well as regulatory capacity when rivers are assessed for compliance with aquatic life expectations. Dissolved oxygen concentrations are the result of the underlying interactions among hierarchical levels. These underlying causes should also be of regulatory focus rather than attempting to adhere to some acute or chronic value for oxygen concentration.

3.5 Conclusion

The results of this study indicated that significant decreases in ΔDO are transient following substantial floods in the Bow River basin. Macrophytes and any associated epiphytic community may substantially contribute to the metabolism of this nutrient-rich, unshaded river. However, my results also indicated that floods in the Bow River can change the relative contribution of periphyton to river DO dynamics. A significant decrease in macrophyte biomass

does not necessarily result in a lasting significant decrease in ΔDO when the new opened space results in significant increase to periphyton biomass. Management of low DO concentrations resulting from large ΔDO values should not rely on flood scouring to reset primary producer influence on DO dynamics for any substantial period. Management of low DO would more likely be influenced by reduced nutrient loading that would impact slow and fast growing primary producers.

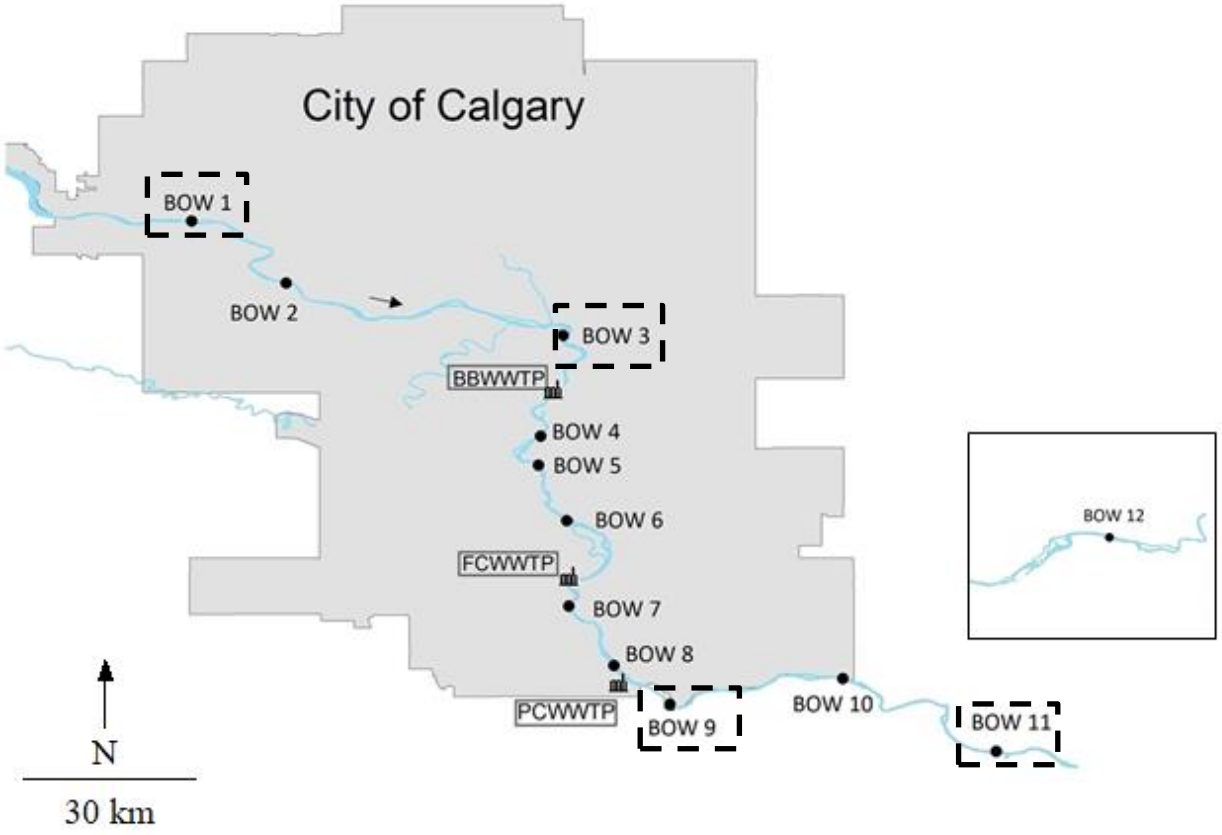

Figure 3.1. Map of sampling locations along the Bow River, Alberta. Inset box shows location of Bow 12, which was 25 km downstream of Bow 11. Black circles indicate locations where periphyton and macrophytes were sampled and dissolved oxygen loggers deployed near both banks. Dashed boxes indicate sites included in Before-After-Control-Impact statistical analysis.

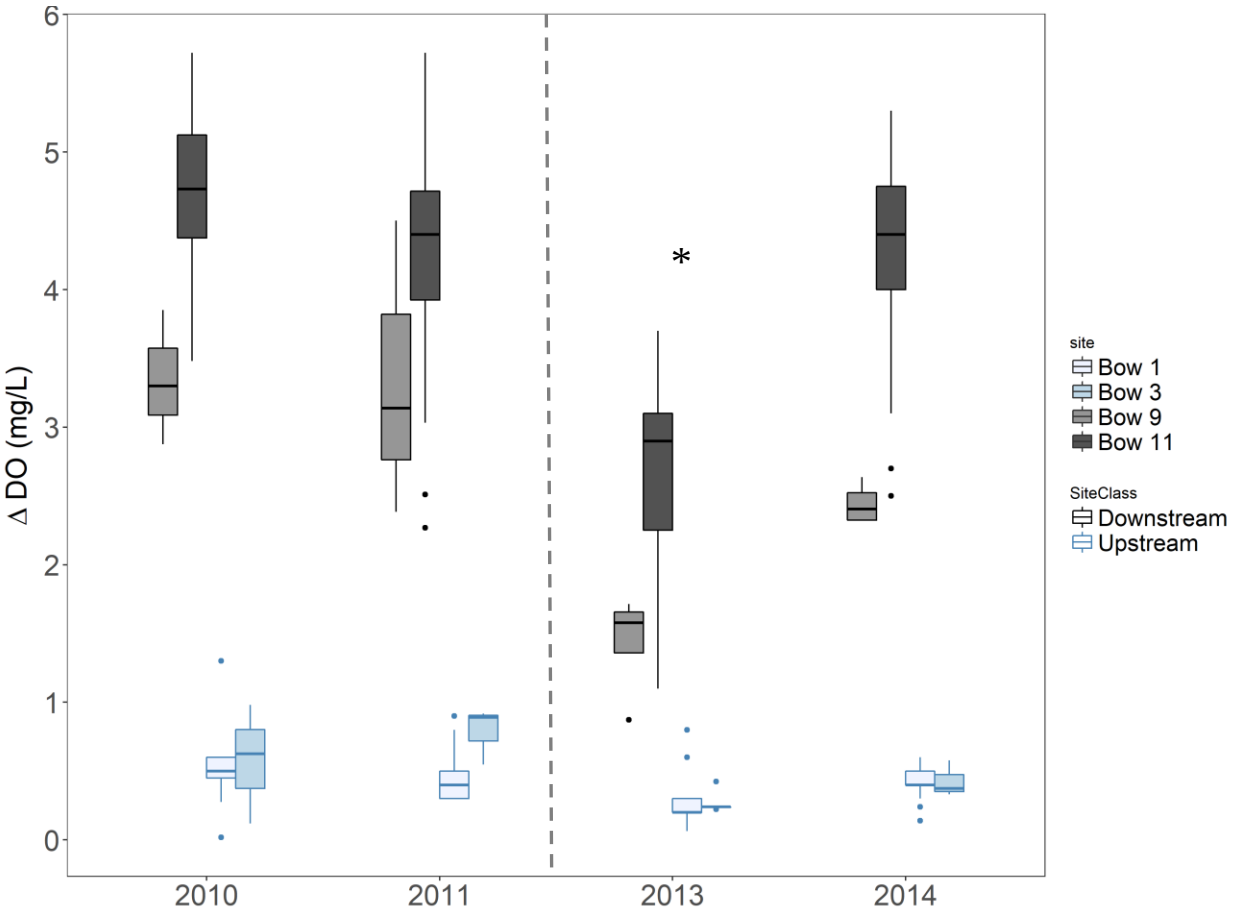

Figure 3.2. Delta dissolved oxygen concentrations in the Bow River, Alberta during August from 2010 to 2014. Dashed, grey line demarks the 2013-flood. Asterisk denotes a significant Before-After-Control-Impact interaction between SiteClass and Period (two-way mixed-effects ANOVA; $p<0.05$). For each boxplot, the line within the box represents the median ΔDO concentration. The box ranges from the first to the third quartile (interquartile range), the whiskers extend to ±1.5 times the interquartile range, and values beyond this are denoted as black dots.

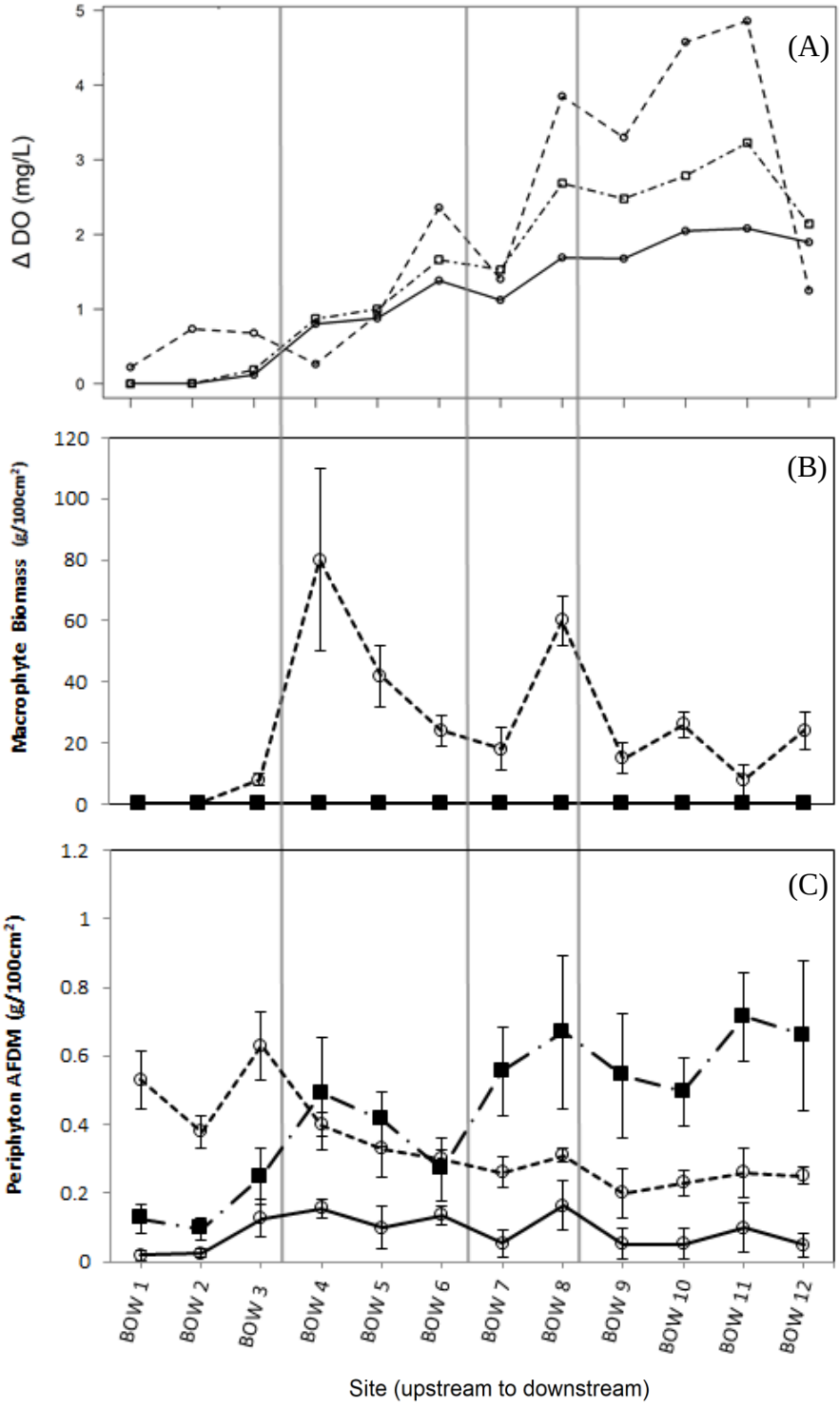
(A)
Δ DO (mg/L)
0
1
2
3
4
5
(B)
Macrophyte Biomass (g/100cm²)
0
20
40
60
80
100
120
(C)
Periphyton AFDM (g/100cm²)
0
0.2
0.4
0.6
0.8
1
1.2
BOW 1
BOW 2
BOW 3
BOW 4
BOW 5
BOW 6
BOW 7
BOW 8
BOW 9
BOW 10
BOW 11
BOW 12
Site (upstream to downstream)

Figure 3.3. Longitudinal pattern of delta dissolved oxygen concentrations (A), macrophyte fresh biomass (B), and periphyton ash-free dry mass (C) along 80 km of the Bow River. 2010/2011 data is indicated by the dashed line, 2013 data is indicated by the solid line, and 2014 data is indicated by the dashed-dot line. Macrophyte biomass was negligible at all sites in 2013 and 2014. 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 August 2010/2011, 2013 and 2014.

Table 3.1. Minimum dissolved oxygen concentration detected in August by site. Values were obtained from dissolved oxygen loggers deployed at each site for up to 72-hours in August of each year.

Site	2010/2011 (mg/L)	2013 (mg/L)	2014 (mg/L)
Bow 1	6.81	8.40	7.52
Bow 2	6.73	8.23	7.50
Bow 3	6.65	8.21	7.65
Bow 4	5.72	7.75	7.10
Bow 5	4.93	7.50	7.15
Bow 6	3.43	7.86	7.16
Bow 7	3.51	9.80	7.15
Bow 8	2.25	6.96	6.64
Bow 9	2.48	7.00	6.92
Bow 10	2.55	6.99	6.90
Bow 11	1.79	6.86	6.64
Bow12	4.18	9.51	6.83

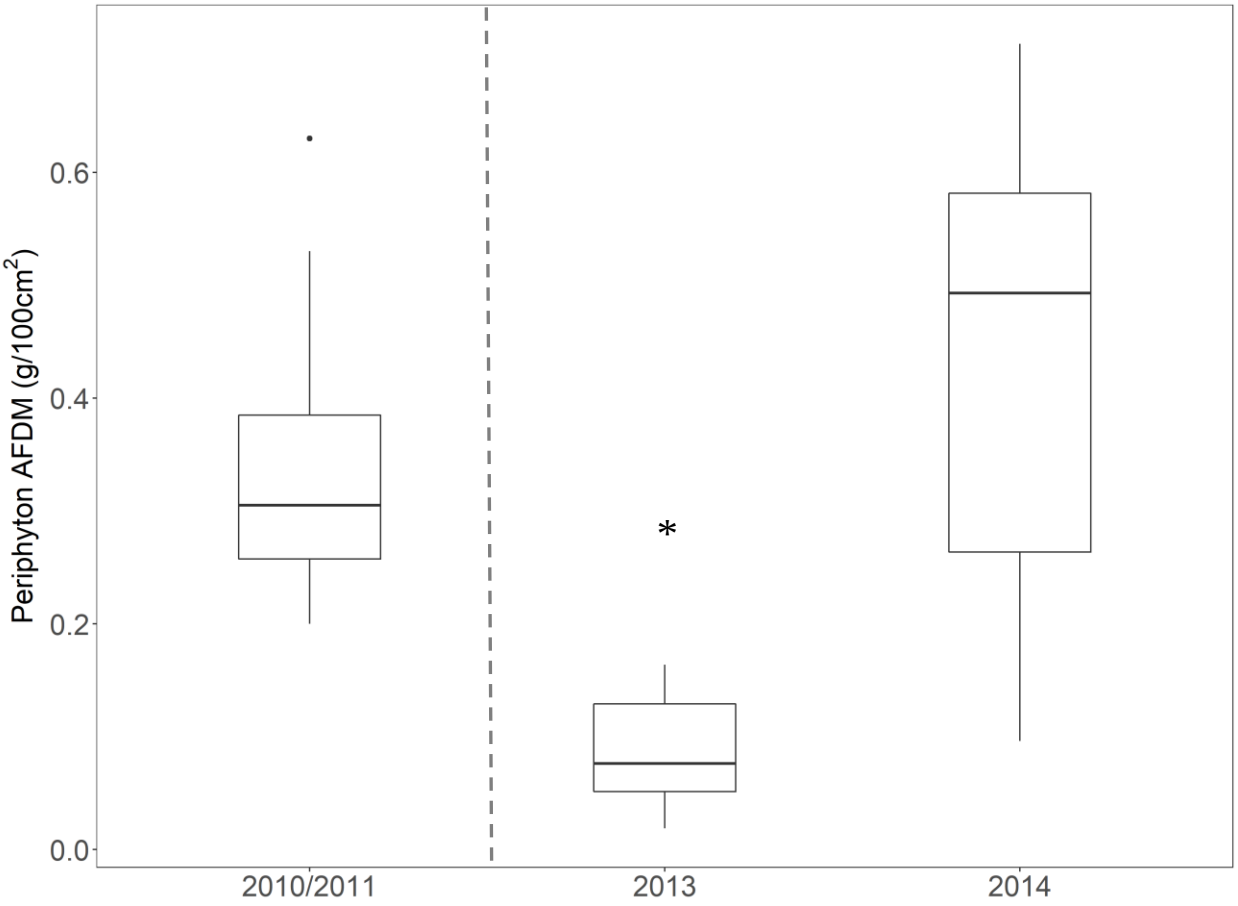

Figure 3.4. Dashed, grey line demarks the 2013-flood. Asterisk denotes a significant difference between biomasses (ANOVA; $p<0.05$). For each boxplot, the line within the box represents the median periphyton ash-free dry weight. The box ranges from the first to the third quartile (interquartile range), the whiskers extend to ±1.5 times the interquartile range, and values beyond this are denoted as black dots.

Chapter 4: Conclusion

4.1 Synthesis

Eutrophication remains a major global problem for freshwaters and marine systems (Smith et al. 1999). Urban wastewater can be a substantial contributor to nutrient inputs, and cities continuously pursue wastewater treatment upgrades to minimize eutrophication. My results present a unique perspective of the effects of an initial major urban nutrient footprint on an oligotrophic river that substantially alters dissolved oxygen concentrations. My primary thesis objective was to assess whether there was evidence to suggest how a reduction in N or P loading would result in a decrease of macrophyte or periphyton biomass, and whether any decrease in biomass would render a decrease in the magnitude of dissolved oxygen concentration ([DO]) fluctuations. In Chapter 2, I presented evidence, through nutrient add-back experiments, that P limited the growth of periphyton and macrophytes at multiple locations in the Bow River, whereas N, in conjunction with P, was associated more so with macrophyte biomass limitation. My results demonstrated that periphyton and macrophytes can respond differently to nutrient enrichment even though they experience the same ambient water column nutrient conditions. In Chapter 3, I provided evidence of a significant decrease in [DO] oscillations downstream of Calgary's wastewater treatment plants following a major river basin scouring event. However, the reduced [DO] oscillations lasted for only one season and returned to pre-flood magnitudes the following year despite the continued absence of macrophyte biomass. Periphyton biomass was observed to quickly grow at downstream sites to be higher within one-year post-flood than it was pre-flood. My results suggest that decreases in macrophyte biomass after major floods in the Bow River do not produce long lasting reductions to the magnitude of diel [DO].

My thesis presents a snapshot of conditions after a large scale 'reset' to a river system, when nutrient concentrations were expected to be low and nutrient spiral lengths long. After a scouring event, my results revealed multiple locations where primary productivity was limited by lack of nitrogen and phosphorus. I demonstrated a spatial component to nutrient limitation status, but similarly, I expect nutrient limitation to have a temporal component as nutrients accumulate in sediment over years following a flood (Noe and Hupp 2005). Annual competition between periphyton and macrophytes are likely to influence future measurements of nutrient limitation status (Sand-Jensen and Borum 1991) and the relative impact on DO dynamics (Chapra 1997). Conceptual models of community succession predict periphyton to accumulate quickly following a disturbance, but if flood return period (frequency), flood timing (through the growing season), flood magnitude and flood duration remained conducive to slower growing colonizers, macrophytes would eventually dominate in stable rivers (Biggs et al. 1996, Riis and Biggs 2003) of a trophic status (Hilton et al. 2006).

Ecosystems may exhibit a range of functionally unique, stable states. A major current issue in ecosystem ecology involves how communities shift from one alternative stable state to another (Scheffer et al. 2001, Beisner et al. 2003). These properties have important implications for management of such systems because hysteresis implies that ecosystems may be pushed into configurations that may persist until a major perturbation forces the system into a new stable configuration. My thesis raises some important questions as I observed a change in structure in the Bow River from macrophytes to periphyton after a large perturbation. How stable is this new regime? When and how does it revert to a macrophyte dominated river? Is a periphyton regime preferable to a macrophyte regime? And, how might nutrients be managed to foster a preferred

state? I provided evidence that ΔDO returned to pre-flood levels within one season in conjunction with a community shift from macrophytes to periphyton, but in the absence of dense plant beds, DO concentrations below 5 mg/L were not detected, so one may disproportionately assume a focus on macrophytes. However, in terms of nutrient management, phosphorus most consistently influenced macrophyte and periphyton growth. From a practical cost-benefit approach, the conclusions of my thesis predict reductions in phosphorus loading to have the greatest influence to reduce nuisance plant growth, as it would affect both macrophytes and periphyton thus requiring a choice of which primary producer to target somewhat irrelevant. However, differentiating between the response to nutrient addition of periphyton and macrophytes is critical to understanding how nutrients may differentially influence or control stream ecosystem function. My thesis represents a baseline to which future comparison can be made.

4.2 Future research

The ability to modify surface water management practices and mitigate effects from potential changes in climate will be enhanced by a better understanding of river systems and processes. Do the relationships between nutrients and primary producers identified in this thesis change over the multiple growing seasons that occur between major river scouring events as nutrients are deposited in the riverbed? Previous studies have indicated that riverbed sediments can represent either a sink of nutrients, under depositional conditions, or a source of nutrients under conditions conducive to internal loading (Chambers and Prepas 1994). Macrophyte nutrient limited growth can be affected depending on when plants rely upon sediments rather

than water-column nutrients (Carignan 1982). Macrophytes can also invoke positive feedback cycles which lead to increased sedimentation and nutrient retention (Sand-Jensen and Borum 1991, Schulz and Gücker 2005). As sediments are recharged, the nutrients which limited macrophyte growth in my study may also change. It is unlikely that the Bow River will exist in a static condition between flood events.

Further research should compare a detailed record of DO concentrations within dense plant beds to the adjacent main channel in the Bow River. Garvey (2007) predicts that even the best habitat may experience occasional localized periods of hypoxia. Goodwin (2008) also identified oxygen concentrations to be more dynamic and more frequently hypoxic in shallow vegetated areas than in adjacent open water. As my results demonstrated, minimum detected DO concentrations increased in the absence of dense macrophyte beds, but oscillations varied markedly between sites and years. If no refuges are present in adjacent areas, then local populations may suffer. Fragmentation among sites within river reaches will likely limit diversity when oxygen becomes occasionally limiting. A better understanding of how interactions among flow, physical habitat, and nutrients affect patterns of oxygen in rivers across multiple spatiotemporal scales is important. While periphyton and macrophytes may be patchily distributed in space, flowing water effectively connects such patches in an upstream to downstream direction. Biological impacts frequently result from the additive, synergistic and antagonistic interplay between multiple stressors. Ongoing management efforts may need to emphasize mitigation of low dissolved oxygen within plant beds as well as the main channel in the Bow River.

4.3 Implications for watershed and wastewater treatment nutrient management

Dissolved oxygen concentrations have reached minimum regulated values of 5 mg/L in the Bow River in the past (Iwanyshyn 2008, Chung 2013), but have yet to reach catastrophic events (i.e. fish kills). The risk of such an occurrence should be lessened should natural events like high flow and low temperatures fail to mitigate low concentrations. Climate warming is predicted to lead to the accumulation of heat and lower flows in rivers (Schindler 2001, Garvey et al. 2007). Future construction of flow barriers such as dams for flood control and diversions for irrigation will further compromise oxygen availability. Calgary's population will grow over the upcoming decades, and nutrient loading concerns will continue to drive costly WWTP upgrades to further reduce the nutrient concentration of effluents. My results suggest that proactively managing nutrient discharge, specifically phosphorus, is a more effective approach to reduce nuisance periphyton and macrophyte biomass than reliance on periodic scouring of macrophytes by floods in the Bow River Basin to mitigate low dissolved oxygen concentrations.

References

Anderson, K. E., A. J. Paul, E. McCauley, L. J. Jackson, J. R. Post, and R. M. Nisbet. 2006. Instream flow needs in streams and rivers: The importance of understanding ecological dynamics. Frontiers in Ecology and the Environment 4:309–318.

Anderson, M. R., and J. Kalff. 1986. Nutrient limitation of Myriophyllum spicatum growth in situ. Freshwater Biology 16:735–743.

APHA (American Public Health Association). 1995. Standard methods for the examination of water and wastewater. 19th edition. Washington, DC.

Arar, E. J., and G. B. Collins. 1997. Method 445.0 In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence. Page National Exposure Research Laboratory USEPA.

Barko, J., W., and W. James. 1998. Effects of submerged aquatic macrophytes on nutrient dynamics, sedimentation and resuspension, p. 197–214. In E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christofferson [eds.], The structuring role of submerged macrophytes in lakes. Ecological Studies, V. 131. Springer

Barko, J. W., D. Gunnison, and S. R. Carpenter. 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquatic Botany 41:41–65.

Barko, J. W., and R. M. Smart. 1986. Sediment-related mechanisms of growth limitation in submersed macrophytes. Ecology 67:1328–1340.

Bates, D., Maechler, M., Bolder, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. stat. Softw. 67(1), 1-48.

Beisner, B. E., Haydon, DT., Cuddington, D. 2003. Alternative stable states in ecology. Frontiers

in Ecology and the Environment 1:376-382.

Biggs, B. J. F. 1996. Hydraulic habitat of plants in streams. Regulated Rivers: Research & Management 12:131–144.

Biggs, B. J. F., and M. E. Close. 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biology 22:209–231.

Biggs, J. F. B., and C. Kilroy. 2000. Stream Periphyton Monitoring Manual. First edition. NIWA, Christchurch, New Zealand.

Bornette, G., and S. Puijalon. 2010. Response of aquatic plants to abiotic factors: a review. Aquatic Sciences 73:1–14.

Bothwell, M. L. 1988. Growth Rate Responses of Lotic Periphytic Diatoms to Experimental Phosphorus Enrichment: The Influence of Temperature and Light. Canadian Journal of Fisheries and Aquatic Sciences 45:261–270.

Bourassa, N., and A. Cattaneo. 1998. Control of Periphyton Biomass in Laurentian Streams (Québec). Journal of North American Bentological Society 17:420–429.

Bracken, M. E. S., H. Hillebrand, E. T. Borer, E. W. Seabloom, J. Cebrian, E. E. Cleland, J. J. Elser, D. S. Gruner, W. S. Harpole, J. T. Ngai, and J. E. Smith. 2015. Signatures of nutrient limitation and co-limitation: Responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. Oikos 124:113–121.

Brown, C. M., J. E. Lawrence, and D. a. Campbell. 2006. Are phytoplankton population density maxima predictable through analysis of host and viral genomic DNA content? Journal of the Marine Biological Association of the UK 86:491.

Carignan, R. 1982. An empirical model to estimate the relative importance of roots in

phosphorus uptake by aquatic macrophytes. Canadian Journal of Fisheries and Aquatic Sciences 39:243–247.

Carignan, R., and J. Kalff. 1980. Phosphorus sources for aquatic weeds : water or sediments? Science 207:987–989.

Carr, G. M., and P. A. Chambers. 1998. Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. Freshwater Biology 39:525–536.

Carr, G. M., H. C. Duthie, and W. D. Taylor. 1997. Models of aquatic plant productivity: a review of the factors that influence growth. Aquatic Botany 59:195–215.

Carr, J. F. 1962. Dissolved oxygen in Lake Erie, past and present. In Proc. Fifth Conf. Great Lakes Res., pp. 1-4. Great Lakes Res. Div., Univ. of Michigan

Cattaneo, A., T. Kerimian, M. Roberge, and J. Marty. 1997. Periphyton distribution and abundance on substrata of different size along a gradient of stream trophy. Hydrobiologia 354:101–110.

Chambers, P. A., and E. E. Prepas. 1994. Nutrient dynamics in riverbeds: the impact of sewage effluent and aquatic macrophytes. Water Research 28:453–464.

Chambers, P. A., E. E. Prepas, and K. Gibson. 1992. Spatial dynamics in riverbed chemistry: Influence of flow and sediment composition. Canadian Journal of Fisheries and Aquatic Sciences 49:2128–2140.

Chambers, P. A., E. E. Prepas, H. R. Hamilton, and M. L. Bothwell. 1991. Current velocity and its effect on aquatic macrophytes in flowing waters. Ecological Applications 1:249–257.

Chambers, P., and E. Prepas. 1989. Roots versus shoots in nutrient uptake by aquatic macrophytes in flowing waters. Canadian Journal of Fisheries and Aquatic Sciences

46:435–439.

Chapra, B. S. C., A. Member, and D. M. Di Toro. 1992. Delta method for estimating primary production, respirtation, and reaeration in streams. Journal of Environmental Engineering 117:640–655.

Chapra, S.C. 1997. Surface Water Quality Modeling. McGraw-Hill, New York.

Charlton, M. N. 1979. Hypolimnion oxygen depletion in central Lake Erie: Has there been any change? I. W. D. Scientific series, #110, Ottawa.

Charlton, S., H. Hamilton, and P. Cross. 1986. The limnological characteristics of the Bow, Oldman and South Saskatchewan Rivers (1979-82) - Part II - Primary producers. Alberta Environment.

Chung, C. W. Y. 2013. Diel oxygen cycles in the Bow River: Relationships to Calgary's nutrient footprint and periphyton and macrophyte biomass. University of Calgary.

Clarke, S. J. 2002. Vegetation growth in rivers: influences upon sediment and nutrient dynamics. Progress in Physical Geography 26:159–172.

Clarke, S. J., and G. Wharton. 2001. Sediment nutrient characteristics and aquatic macrophytes in lowland English rivers. The Science of the total environment 266:103–112.

Clarke, S. J., G. Wharton, and J. a. Cotton. 2006. Spatial and Temporal Variations in the Sediment Habitat of Ranunculus spp. in Lowland Chalk Streams – Implications for Ecological Status? Water, Air, & Soil Pollution: Focus 6:393–401.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Ecology. Controlling eutrophication: nitrogen and phosphorus. Science (New York, N.Y.) 323:1014–5.

Cox, B. A. 2003. A review of dissolved oxygen modelling techniques for lowland rivers. Science of the Total Environment 314–316:303–334.

Cross, P.M., Hamilton, H.R., Charlton, S. E. D. 1986. The limnological characteristics of the Bow, Oldman and South Saskatchewan Rivers Part I .pdf.

Danger, M., T. Daufresne, F. Lucas, S. Pissard, and G. Lacroix. 2008. Does Liebig's law of the minimum scale up from species to communities? Oikos 117:1741–1751.

Demars, B. O. L., and a. C. Edwards. 2007. Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. Freshwater Biology 52:2073–2086.

Dent, C. L., and N. B. Grimm. 1999. Spatial Heterogeneity of Stream Water Nutrient Concentrations over Successional Time. Ecology 80:2283–2298.

Desmet, N. J. S., S. Van Belleghem, P. Seuntjens, T. J. Bouma, K. Buis, and P. Meire. 2011. Quantification of the impact of macrophytes on oxygen dynamics and nitrogen retention in a vegetated lowland river. Physics and Chemistry of the Earth 36:479–489.

Dodds, W. K. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. Journal of the North American Benthological Society 22:171–181.

Dodds, W. K., and V. H. Smith. 2016. Nitrogen, phosphorus, and eutrophication in streams. Inland Waters 6:155–164.

Dodds, W. K., and E. B. Welch. 2000. Establishing Nutrient Criteria in Streams Establishing. Journal of the North American Benthological Society 19:186–196.

Duarte, C. M. 1992. Nutrient concentration of aquatic plants : across species patterns. Limnology

and Oceanography 37:882–889.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology letters 10:1135–42.

Elwood, J. W., J. D. Newbold, A. N. N. F. Trimble, and R. W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: Effects of P enrichment on leaf decomposition and primary producers. Ecology 62:146–158.

ESRD. 2011. Bow River BioSonics pilot survey with water quality ground-truth monitoring.

ESRD. 2014a. Environmental quality guidelines for Alberta surface Waters. Page Water Policy Branch, Policy Division.

ESRD. 2014b. Bow River Phosphorus Management Plan.

Fletcher, D. J., and A. J. Underwood. 2002. How to cope with negative estimates of components of variance in ecological field studies. Journal of Experimental Marine Biology and Ecology 273:89–95.

Francoeur, S. N. 2001. Meta-Analysis of Lotic Nutrient Amendment Experiments : Detecting and Quantifying Subtle Responses Meta-analysis of lotic nutrient amendment experiments : detecting and quantifying subtle responses. North American Benthological Society 20:358–368.

Franklin, P. 2014. Dissolved oxygen criteria for freshwater fish in New Zealand: a revised approach. New Zealand Journal of Marine and Freshwater Research preprint:1–15.

Garvey, J. E., M. R. Whiles, and D. Streicher. 2007. A hierarchical model for oxygen dynamics

in streams. Canadian Journal of Fisheries and Aquatic Sciences 64:1816–1827.

Gibeau, G. G., and M. C. Miller. 1989. A Micro-Bioassay for Epilithon using Nutrient-Diffusing Artificial Substrata. Journal of Freshwater Ecology 5:171–176.

Golder. 2007. Bow river impact study - phase 2: Development of total loading management targets for The City of Calgary.

Goodwin, K., N. Caraco, and J. Cole. 2008. Temporal dynamics of dissolved oxygen in a floating-leaved macrophyte bed. Freshwater Biology 53:1632–1641.

Gorban, A. N., L. I. Pokidysheva, E. V. Smirnova, and T. A. Tyukina. 2011. Law of the Minimum Paradoxes. Bulletin of Mathematical Biology 73:2013–2044.

Guasch, H., J. Armengol, E. Mart??, and S. Sabater. 1998. Diurnal variation in dissolved oxygen and carbon dioxide in two low-order streams. Water Research 32:1067–1074.

Harpole, W. S., J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E. S. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin, and J. E. Smith. 2011. Nutrient co-limitation of primary producer communities. Ecology letters 14:852–62.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments : A review of recent evidence on the enrichment 33:796–822.

Hilton, J., M. O'Hare, M. J. Bowes, and J. I. Jones. 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment 365:66–83.

Hoellein, T. J., C. P. Arango, and Y. Zak. 2011. Spatial variability in nutrient concentration and biofilm nutrient limitation in an urban watershed. Biogeochemistry 106:265–280.

Hogberg, L. K. 2004. A chemical, biological, and isotopic analysis of the spatial extent of wastewater effluent on rivers in Southern Alberta, Canada. University of Calgary.

Holeton, C., P. A. Chambers, L. Grace, and K. Kidd. 2011. Wastewater release and its impacts on Canadian waters. Canadian Journal of Fisheries and Aquatic Sciences 68:1836–1859.

Irvine, R. L., and L. J. Jackson. 2006. Spatial variance of nutrient limitation of periphyton in montane, headwater streams (McLeod River, Alberta, Canada). Aquatic Ecology 40:337–348.

Iwanyshyn, M. 2008. River Water Quality and Total Loading Management in an urban River Reach. University of Calgary.

Jackson, L., D. Rowan, R. Cornett, and J. Kalff. 1994. Myriophyllum spicatum pumps essential and nonessential trace elements from sediments to epiphytes. Canadian Journal of Fisheries and Aquatic Sciences 51:1764–1773.

James, W. F., J. W. Barko, and H. L. Eakin. 2004. Impacts of sediment dewatering and rehydration on sediment nitrogen concentration and macrophyte growth. Canadian Journal of Fisheries and Aquatic Sciences 61:538–546.

Jarvie, H. P., A. N. Sharpley, P. J. a. Withers, J. T. Scott, B. E. Haggard, and C. Neal. 2013. Phosphorus Mitigation to Control River Eutrophication: Murky Waters, Inconvenient Truths, and "Postnormal" Science. Journal of Environment Quality 42:295.

Kaenel, B. R., H. Buehrer, and U. R. S. Uehlinger. 2000. Effects of aquatic plant management on stream. Freshwater Biology:85–95.

Kahlert M. (1998) C:N:P ratios of freshwater benthic algae. Arch. Hydrobiol. Spec Issue Adv. Limnol. 51, 105–14.

Kalff, J. 2002. Limnology: Inland water ecosystems. Prentice-Hall Inc. Upper Saddle River, NJ, USA.

Keck, F., and F. Lepori. 2012. Can we predict nutrient limitation in streams and rivers? Freshwater Biology 57:1410–1421.

Kerkhoven, E., and T. Y. Gan. 2011. Differences and sensitivities in potential hydrologic impact of climate change to regional-scale Athabasca and Fraser River basins of the leeward and windward sides of the Canadian Rocky Mountains respectively. Climatic Change 106:583–607.

Kuznetsova, A., Brockhoff, A.P.B., Christensen, R.H.B., 2016. lmerTest: Tests in linear mixed effects models (lmer objects of lme4 packages). R package version 2.0-33. Available via https://CRAN.R-project.org/package=lmerTest

Lewis, W. M., and W. A. Wurtsbaugh. 2008. Control of Lacustrine Phytoplankton by Nutrients: Erosion of the Phosphorus Paradigm. International Review of Hydrobiology 93:446–465.

Lewis, W. M., W. A. Wurtsbaugh, and H. W. Paerl. 2011. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. Environmental Science & Technology 45:10300–5.

Lohman, K., J. R. Jones, and B. D. Perkins. 1992. Effects of Nutrient Enrichment and Flood Frequency on Periphyton Biomass in Northern Ozark Streams. Canadian Journal of Fisheries and Aquatic Sciences 49:1198–1205.

Madsen, T. V., and N. Cedergreen. 2002. Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. Freshwater Biology 47:283–291.

Martí, E., N. Grimm, and S. Fisher. 1997. Pre-and post-flood retention efficiency of nitrogen in a Sonoran Desert stream. Journal of the North American Benthological Society 16:805–819.

Marti, E., and F. Sabater. 1996. High variability in temporal and spatial nutrient retention in

Mediterranean streams. Ecology 77:854–869.

Mebane, C. A., N. S. Simon, and T. R. Maret. 2014. Linking nutrient enrichment and streamflow to macrophytes in agricultural streams. Hydrobiologia 722:143–158.

Miller, A. J., and M. D. Cramer. 2005. Root nitrogen acquisition and assimilation. Plant and Soil 274:1–36.

Mortimer, C. H., 1981. The oxygen content of air-saturated fresh waters over ranges of temperature and atmospheric pressure of limnological interest. Mitt. Inertat. Verein. Limnol. 22:1-23.

Moss, B., E. Jeppesen, M. Søndergaard, T. L. Lauridsen, and Z. Liu. 2012. Nitrogen, macrophytes, shallow lakes and nutrient limitation: resolution of a current controversy? Hydrobiologia 710:3–21.

Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring Nutrient Spiralling in Streams. Canadian Journal of Fisheries and Aquatic Sciences 38:860–863.

Newbold, J., and J. Elwood. 1984. Phosphorus dynamics in a woodland stream ecosystem. BioScience 34:43–44.

Noe, G. B., and C. R. Hupp. 2005. Carbon, nitrogen, and phosphorus accumulation in floodplains of Atlantic Coastal Plain rivers, USA. Ecological Applications 15:1178–1190.

Nusch, E. A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Archiv für Hydrobiologie 14:14-36.

Paerl, H. W. 2009. Controlling Eutrophication along the Freshwater–Marine Continuum: Dual Nutrient (N and P) Reductions are Essential. Estuaries and Coasts 32:593–601.

Paerl, H. W., W. S. Gardner, M. J. Mccarthy, B. L. Peierls, and S. W. Wilhelm. 2014. Algal

blooms : Noteworthy nitrogen Algal blooms : Proactive strategy Ocean acidification foils chemical signals. Science 346:2014–2016.

Pollock, M. S., L. M. J. Clarke, and M. G. Dubé. 2007. The effects of hypoxia on fishes: from ecological relevance to physiological effects. Environmental Reviews 15:1–14.

Pomeroy, J. W., R. E. Stewart, and P. H. Whitfield. 2015. The 2013 flood event in the South Saskatchewan and Elk River basins: Causes, assessment and damages. Canadian Water Resources Journal / Revue canadienne des ressources hydriques 41:105–117.

R Core Team. 2016. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. URL http://www.R-project.org/.

Redfield A.C., On the proportions of organic derivations in sea water and their relation to the composition of plankton. In James Johnstone Memorial Volume. (ed. R.J. Daniel). University Press of Liverpool, pp. 176–192, 1934.

Riis, T., and B. J. F. Biggs. 2003. Hydrologic and hydraulic control of macrophyte establishment and performance in streams. Limnology and Oceanography 48:1488–1497.

Robach, F., I. Hajnsek, I. Eglin, and M. Trémolières. 1995. Phosphorus sources for aquatic macrophytes in running waters: Water or sediment? Acta Botanica Gallica 142:719–731.

Robin L Vannote, G Wayne Minshall, Kenneth W Cummins, James R Sedell, C. E. C. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137.

Robinson, K. L., C. Valeo, M. C. Ryan, a. Chu, and M. Iwanyshyn. 2009. Modelling aquatic vegetation and dissolved oxygen after a flood event in the Bow River, Alberta, Canada. Canadian Journal of Civil Engineering 36:492–503.

Sabater, S., J. Armengol, E. Comas, F. Sabater, I. Urrizalqui, and I. Urrutia. 2000. Algal biomass

in a disturbed Atlantic river: water quality relationships and environmental implications. The Science of the total environment 263:185–95.

Salisbury, F. (1992). Plant physiology (4th ed.). Wadsworth Publishing Company. Belmont, California.

Sand-Jensen, K., K. Andersen, and T. Andersen. 1999. Dynamic Properties of Recruitment, Expansion and Mortality of Macrophyte Patches in Streams. International Review of Hydrobiology 84:497–508.

Sand-Jensen, K., and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquatic Botany 41:137–175.

Scheffer, M., S. Carpenter, J. a Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature 413:591–596.

Schindler, D. 1977. Evolution of Phosphorus Limitation in Lakes. Science 195:260–262.

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

Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. Limnology and Oceanography 51:356–363.

Schindler, D. W. 2012. The dilemma of controlling cultural eutrophication of lakes. Proceedings. Biological sciences / The Royal Society 279:4322–33.

Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng, and S. E. M. Kasian. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proceedings of

the National Academy of Sciences of the United States of America 105:11254–8.

Schulz, M., and B. Gücker. 2005. Macrophytes increase spatial patchiness of Fluvial Sedimentary Records and Effect Temporal Particulate Nutrient Storage. Aquatic Geochemistry 11:89–107.

Scrimgeour, G. J., and P. A. Chambers. 2000. Cumulative effects of pulp mill and municipal effluents on epilithic biomass and nutrient limitation in a large northern river ecosystem. Canadian Journal of Fisheries and Aquatic Sciences 57:1342–1354.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environmental science and pollution research international 10:126–139.

Smith, V. H., S. B. Joye, and R. W. Howarth. 2006. Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography 51:351–355.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends in Ecology and Evolution 24:201–207.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental pollution (Barking, Essex : 1987) 100:179–96.

Sosiak, A. 1990. An evaluation of nutrients and biological conditions in the Bow River, 1986 to 1988. Environmental Quality Monitoring Branch Environmental Assessment Division, Calgary, Alberta.

Sosiak, A. 2002. Long-term response of periphyton and macrophytes to reduced municipal nutrient loading to the Bow River (Alberta, Canada). Canadian Journal of Fisheries and Aquatic 59:987–1001.

Stewart-oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental Impact Assessment : "Pseudoreplication" in Time ? Ecology 67:929–940.

Stream Solute Workshop. 1990. Concepts and Methods for Assessing Solute Dynamics in Stream Ecosystems Concepts and methods for assessing solute dynamics in stream ecosystems. Journal of North American Bentological Society 9:95–119.

Tank, J., and W. Dodds. 2003. Nutrient limitation of epilithic and epixylic bio lms in ten North American streams. Freshwater Biology 48:1031–1049.

Thomann, R. V. and Mueller, J. A. (1987). Principles of surface water quality modeling and control. Harper Collins Publishers Inc. New York, NY 10022-5299, 301.

Thomaz, S. M., P. a. Chambers, S. A. Pierini, and G. Pereira. 2007. Effects of phosphorus and nitrogen amendments on the growth of Egeria najas. Aquatic Botany 86:191–196.

Tilman, D., S. S. Kilham, and P. Kilham. 1982. Phytoplankton Community Ecology: The Role of Limiting Nutrients. Annual Review of Ecology and Systematics 13:349–372.

Townsend, A. R., G. P. Asner, and C. C. Cleveland. 2008. The biogeochemical heterogeneity of tropical forests. Trends in Ecology and Evolution 23:424–431.

Underwood, A. J. 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. Journal of Experimental Marine Biology and Ecology 161:145–178.

Underwood, A. J. 1994. On Beyond BACI : Sampling Designs that Might Reliably Detect Environmental Disturbances. Ecological Applications 4:3–15.

USEPA. 2000. Nutrient criteria technical guidance manual: rivers and streams.

Vandenberg, J. a, M. C. Ryan, D. D. Nuell, and A. Chu. 2005. Field evaluation of mixing length

and attenuation of nutrients and fecal coliform in a wastewater effluent plume. Environmental monitoring and assessment 107:45–57.

Vilches, C., and A. Giorgi. 2010. Metabolism in a macrophyte-rich stream exposed to flooding. Hydrobiologia 654:57–65.

Vis, C., C. Hudon, R. Carignan, and P. Gagnon. 2007. Spatial Analysis of Production by Macrophytes, Phytoplankton and Epiphyton in a Large River System under Different Water-Level Conditions. Ecosystems 10:293–310.

Water Environment Federation. 2005. Biological nutrient removal (BNR) operation in wastewater treatment plants: WEF manual of practice, Issue 30. McGraw Hill Professional. New York, NY

Welch, E., R. Horner, and C. Patmont. 1989. Prediction of nuisance periphytic biomass: A management approach. Water Research 23:401–405.

Welch, E., J. Quinn, and C. Hickey. 1992. Periphyton biomass related to point-source nutrient enrichment in seven New Zealand streams. Water Research 26:669–675.

Wetzel, R.G., 2001. Limnology: lake and river ecosystems. (Third Edition) Academic Press. New York, NY.

Appendix A: Wastewater treatment plant effluent summary

Table A.1. Mean daily flow and nutrient loading from each of Calgary's wastewater treatment plants in August 2014.

	Bonnybrook WWTP	**Fish Creek WWTP**	**Pine Creek WWTP**
Flow ($1000 m^3 \cdot day^{-1}$)	350	26	87
TP ($kg \cdot day^{-1}$)	128	5	12
TN ($kg \cdot day^{-1}$)	6580	838	713
NH_3-N ($kg \cdot day^{-1}$	88	748	23
NO_3-N ($kg \cdot day^{-1}$)	5950	15	551

Legend

TP = Total phosphorus
TN = Total nitrogen
NH_3-N = Ammonia-Nitrogen
NO_3-N = Nitrate-Nitrogen

Table A.2. Mean daily flow from each of Calgary's wastewater treatment plants, and the Bow River, and estimated effluent flow contribution into the Bow River (% effluent) in August 2014.

Plant	**WWTP flow ($m^3 \cdot sec^{-1}$)**	**Bow River flow ($m^3 \cdot sec^{-1}$)**	**Effluent contribution (%)**
Bonnybrook	4.05	55.2	7
Fish Creek	0.30	59.7	0.5
Pine Creek	1.01	59.7	1.6