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Lagoon Wastewater Effluent Impacts Stream Metabolism in Red River Tributaries

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Abstract and Keywords

Lagoons are the most common form of sewage treatment for rural Canadian communities and may therefore be a major source of pollution to local waterways. However, the environmental effects of pulse releases of lagoon effluent are largely unknown. This study reports on changes in physicochemical conditions and stream metabolism occurring as result of summer lagoon effluent releases into Red River tributaries, Manitoba, Canada. We calculated metrics of stream metabolism using the single-station, open water method. We found that an effluent release results in a significant short-term increase in physicochemical (i.e., water nutrients, stream discharge) conditions which had a subsidy effect on stream metabolism. We also found that stream metabolism was significantly greater in effluent exposed versus unexposed reaches; however, our results suggest the degree of effect depends on whether the release occurred early or late in the summer. The findings of this study have implications for lagoon management and future stream monitoring projects aimed at evaluating the effects of lagoon wastewater effluent.

Key Words: stream metabolism, primary production, respiration, lagoon, wastewater effluent, nutrients, streams, Red River Valley, Southern Manitoba

Co-Authorship Statement

This dissertation contains two manuscripts. Chris T. Chesworth will be the lead author for both as he played the lead role in defining the research problems, designing the research approach, analyzing the data and interpreting the results. Dr. Adam G. Yates will be the final author for both of these manuscripts as he advised on the study design, analysis and interpretation of data and provided the majority of the funding for this study. Joseph M. Culp, Patricia A. Chamber, and Robert B. Brua will be co-authors for both manuscripts as they provided equipment resources and advise on study design, analysis and interpretation of the data.

Dedication

I dedicate this thesis to my sister, Meaghan, who provided me with a tremendous source of inspiration through her courageous battle with cancer and of whom is now entering her sixth month of remission. I would also like to dedicate this thesis to the memory of my Nan, Simone Gilmore, who passed away in November, - “you will have more degrees than a thermometer!”.

Acknowledgments

I could not have completed this thesis without the unwavering support of family, friends, and colleagues. In particular I would like to thank my parents, Tom and Sandra, and my sister, Meaghan, of whom have provided me guidance, love, and support for my entire life. I would like to thank my girlfriend, Goldie, for her tremendous patience and motivation.

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General Introduction

1.1. Introduction

As the global human population continues to grow there are few ecosystems free from the effects of human influence. Globally, about one-third to one-half of all land-surface has been transformed as a result of human activity (Vitousek *et al.*, 1997). Land is often transformed to grow crops, raise animals, harvest resources, and to build cities; land transformation is thus one of the foundations of human society (Defries *et al.*, 2004). However, the cost of this development is often at the expense of the environment (Vitousek *et al.*, 1997; Allan, 2004). Today humans face the challenge of managing the trade-offs between immediate human needs and conserving the natural environment so it continues to provide goods and services (e.g., potable water) for the long term (Foley *et al.*, 2005). It is therefore important to understand the pathways by which human action leads to environmental degradation.

Urbanization is one type of land transformation (i.e., land-use) that continues to expand globally. Urbanization is the process by which populations concentrate into urban areas. From 1950 to 2014 the world population living in urban areas increased from 746 million to 3.9 billion (United Nations Population Division, 2014). The environmental costs of urbanization are often inherited by river ecosystems. The effects of urbanization on river systems include: elevated pollutant concentrations (e.g., nutrients); reduced biological diversity; disrupted hydrological pathways and patterns, and the modification of energy flow and nutrient cycling (Paul & Meyer, 2001; Walsh *et al.*, 2005).

In the United States more than 130,000 km^2 of streams and rivers are impacted as a result of urbanization (Paul & Meyer, 2001). A growing environmental concern regarding the effects of urbanization on streams and rivers is the increase in point-source pollution to local waterways, particularly as a result of wastewater effluent (Paul & Meyer, 2001). For example, a study conducted by Heaney & Huber (1984) found that 84% of the 248 urban centers they studied in the United States release wastewater effluent into river systems. Therefore, there is a high potential for wastewater effluent to pose a threat to freshwater ecosystems.

There are approximately 2800 municipal wastewater treatment facilities in Canada (Chambers *et al.*, 2001). These facilities range from large mechanical wastewater treatment plants that typically serve large cities to small wastewater treatment lagoons/stabilization ponds serving smaller communities (Chambers *et al.*, 2001). Both types of wastewater treatment rely on biological processes to treat wastewater (i.e., nutrient assimilation and organic matter processing) before releasing the effluent as a point source discharge into receiving waterways. Mechanical wastewater treatment plants (MWTPs) take an active approach to treating wastewater, whereas, wastewater treatment lagoons take a passive approach. MWTPs have an infrastructure design with different structures facilitating different stages of the treatment process that quickly treat the wastewater with mechanical and chemical interventions (Metcalf & Eddy, 2003). Lagoons (also known as stabilization ponds) are in-ground earthen basins that treat wastewater over extended periods of time with the use of aerobic, anaerobic, and facultative microorganisms that aid in the breakdown of organic matter, the assimilation of nutrients, and the conversion of nutrients into different forms (e.g., catalyzing the process of nitrification and denitrification) (NRC, 2004; Prince *et al.*, 1994).

Lagoons are the most common form of wastewater treatment in rural Canadian communities and may therefore be an important source of pollutants to rural waterways (Environment Canada, 1996). These rural communities generally release lagoon effluent once or twice annually for 2-4 week periods, and as such, act as a pulse of pollution to receiving waterways (NRC, 2004). Traditionally, studies assessing the effects of wastewater effluent on river ecosystem structure and function have focused on MWTPs with little attention given to lagoons (Aristi et al., 2015; Carey & Migliaccio, 2009; Gücker et al., 2006; Igbinosa & Okoh, 2009; Marti et al., 2004; Carlson et al., 2013). Little is known about how pulses of lagoon wastewater effluent effects downstream freshwater ecosystems (Yates *et al.,* 2013).

The release of wastewater effluent is often accompanied by increased downstream concentrations of nitrogen (N) and phosphorous (P) (Carey & Migliaccio, 2009; Carlson *et al.,* 2013; Aristi *et al.*, 2015). The concentrations of N and P entering freshwater systems will depend on the efficiency of the wastewater treatment facility in removing nutrients from wastewater. MWTPs are more likely to implement additional treatment technologies to achieve greater nutrient removal efficiencies than lagoons due to financial constraints (Mara *et al.*, 1992; OMEE, 1993; Graham *et al.*, 2014). Lagoons depend largely on biological activity to remove nutrients from wastewater effluent (Mbwele, 2006; OMEE, 1993). The pathways by which inorganic N compounds in lagoons are removed are volatilization through denitrification and algae/bacteria assimilation (Prince *et al,* 1994). Inorganic P compounds are treated via algae/bacteria assimilation and precipitation (Mbwele, 2006). Only N compounds can truly be expelled from the lagoon (diffusion from the water column to the atmosphere occurs via volatilization), whereas, P compounds become imbedded in the sludge layer. As a result lagoons are often characterized as having a low P removal capacity (Prince *et al.,* 1994; Mbwele, 2006). Furthermore, the biological treatment of wastewater in lagoons is often limited by climatic factors such as temperature. Temperature regulates the metabolism of the autotrophic and heterotrophic communities in the lagoons and thus the rate of wastewater treatment (Prince *et al.,* 1994). Lagoons in cold climates (e.g., Canada) will therefore be more susceptible to releasing undertreated effluent because effective treatment of nutrients can only take place during a few months of the year.

Poor lagoon management often results in the release of undertreated/low quality effluent (e.g., high in nutrient concentrations) into aquatic environments (Prince *et al.,* 1994). Poor lagoon management commonly occurs in rural communities leading to the release of effluent capable of adversely affecting aquatic life in the receiving streams (NRC, 2004). A survey by the OMEE (1993) found that 37% of 121 lagoons had no additional P removal treatments in place (e.g., adding aluminum and/or iron solutions during the treatment process). As such these lagoons were estimated to reduce effluent P levels by 66% through natural processes (i.e., biological assimilation and precipitation) compared to the 93% removal expected of lagoons with the additional P removal treatment. For nitrogen, lagoons with no additional treatment in place (e.g., aerators) reduce N levels by 10%, and those with additional treatment reduce N up to 99% (Chambers *et al.*, 2001). Lagoons thus represent a potentially substantial point source of nutrients to stream environments.

Nitrogen and phosphorous are a requirement for plant growth, these nutrients are often limiting in aquatic systems and when an abundance of these nutrients are provided to the system it can have undesirable ecological consequences (Smith *et al.*, 1999). One such effect of nutrient enrichment in freshwater systems is eutrophication. By definition eutrophication is the enrichment of bodies of freshwater by inorganic plant nutrients (e.g., nitrogen and phosphorus compounds) that may occur naturally or as the result of human activity (Lawrence *et al.*, 1998). When human activities lead to nutrient enrichment this is referred to as cultural eutrophication (Smith *et al.*, 1999). Eutrophication is one of the most widespread water quality problems on earth (Carpenter *et al.*, 1998). The effects of eutrophication on aquatic ecosystems include an increase in primary production through increased abundance and biomass of algae and other aquatic plants (Smith *et al.*, 1999). Associated effects of excessive primary production includes changes in species composition, oxygen depletion, higher incidence of fish kills, decreases in aesthetic value, and loss of ecosystem services (Carpenter *et al.*, 1998). Wastewater effluent often contributes a high concentration of bioavailable nutrients and is often an important cause of cultural eutrophication (Carlson et al., 2013; Aristi et al., 2015; Gücker et al., 2006; Andersen et al., 2004; Ekka et al., 2006; Dyer & Wang, 2002; Smith *et al.*, 1999). It is therefore important to understand how a release of wastewater lagoon effluent may affect nutrient dynamics in downstream environments.

1.1.1. Stream metabolism

Stream metabolism is the balance between gross primary production (GPP) and ecosystem respiration (ER) (Mulholland *et al.*, 2001). GPP is the amount of carbon produced via photosynthesis from autotrophic organisms whereas ER is the amount of carbon consumed by both autotrophic and heterotrophic organisms during cellular respiration (Allan & Castillo, 2007). Stream metabolism thus gives insight into the amount of energy being assimilated by stream communities allowing inferences regarding several key in-stream processes, such as nutrient cycling, organic matter processing, and stream trophic dynamics. Stream metabolism can be an indicator of nutrient cycling because plants require inorganic nutrients to form cellular structures (e.g., nucleotides containing a sugar-phosphate backbone and a nitrogenous base used to build and regulate protein activity) allowing nutrient uptake to be reflected by rates of GPP (Mulholland *et al.,* 2005; O'Brian *et al.,* 2014; Hall & Tank, 2003; Bernot *et al.*, 2010). Stream metabolism can also be an indicator of organic matter processing because ER is a measure of how much organic matter is being broken down (i.e., respired) by autotrophic and heterotrophic communities (Bernot *et al.,* 2010). The relationship between ER and GPP is an indicator of the overall trophic status of the stream because the calculated net ecosystem production (NEP) values and the production to respiration (P/R) ratio will suggest the primary source of energy to a stream (i.e., allochthonous (i.e., originating outside) or autochthonous (i.e., originating inside)). The state of the stream is considered net heterotrophic when ER rates are greater than GPP rates (i.e., traditionally resulting in a negative NEP value and a P/R ratio of less than 1) or net autotrophic when ER rates are less than GPP rates (i.e., traditionally resulting in a positive NEP value and a P/R ratio of greater than 1) (*sensu* Allan & Castillo, 2007). The ability of stream metabolism to infer instream processes allows it to be a valuable tool when distinguishing the effects of human activity on stream ecosystems.

Rates of stream metabolism are directly influenced by proximal drivers (i.e., hydrology, organic matter, light, and nutrients) which act at the stream reach scale (Fig.1; Bernot *et al.*, 2010). For example, the hydrology of the stream (i.e., flow rate and depth) regulates the presence and abundance of hydraulic habitat types (e.g., runs, riffles, and pools) available for autotrophic and heterotrophic communities (Konrad *et al.*, 2005). Organic matter is the primary energy source for heterotrophic organisms and therefore is a driver of ER (Allan & Castillo, 2007). Light is required for autotrophic organisms to perform photosynthesis and is therefore a driver of GPP (Allan & Castillo, 2007). Nutrients are also a driver of GPP, and to a lesser extent ER, as they are needed by autotrophs and sometimes heterotrophs (i.e., bacteria) for cellular growth (Bernot *et al.,* 2010; Allan & Castillo, 2007). Thus, it is clear how physicochemical environmental conditions directly affect metrics of stream metabolism, however, it is less clear how these proximal drivers are modified by landscape and regional characteristics.

Rates of stream metabolism are affected by distal drivers occurring at broad spatial scales (i.e., regional (e.g., climate) and catchment (e.g., land use)) (Fig.1). Thus, there is a hierarchical relationship where broader scale characteristics (e.g., regional and land use) regulate smaller scale (i.e., stream reach) proximal drivers of stream metabolism (Allan, 2004). For example, land use (e.g., agriculture and urban development) occurs at the landscape scale and acts as a distal driver of stream metabolism because it directly influences proximal drivers (e.g., nutrients and hydrology) at the stream reach scale. Land use characteristics can modify proximal drivers of stream metabolism through flow regime alterations (e.g., addition of impervious surfaces, upstream channelization, and subsurface drains) and/or with point source and non-point source inputs of nutrients, sediments, and organic material (Yates *et al.,* 2013; Bernot *et al.,* 2010). Regional characteristics, occurring at the broadest spatial scale, have direct effects on land-use and stream reach distal and proximal drivers of stream metabolism, respectively, through climate related features (e.g. temperature regimes and vegetation composition) (Bernot *et al.,* 2010). Furthermore, point source wastewater effluent occurs at the catchment scale which can influence stream metabolism through reach scale proximal drivers (Aristi et al., 2015; Carey & Migliaccio, 2009; Gücker et al., 2006; Igbinosa & Okoh, 2009; Marti et al., 2004; Carlson *et al.,* 2013). However, the connection of how wastewater effluent from lagoons affects proximal drivers of stream metabolism is largely unknown (Yates *et al.,* 2013).

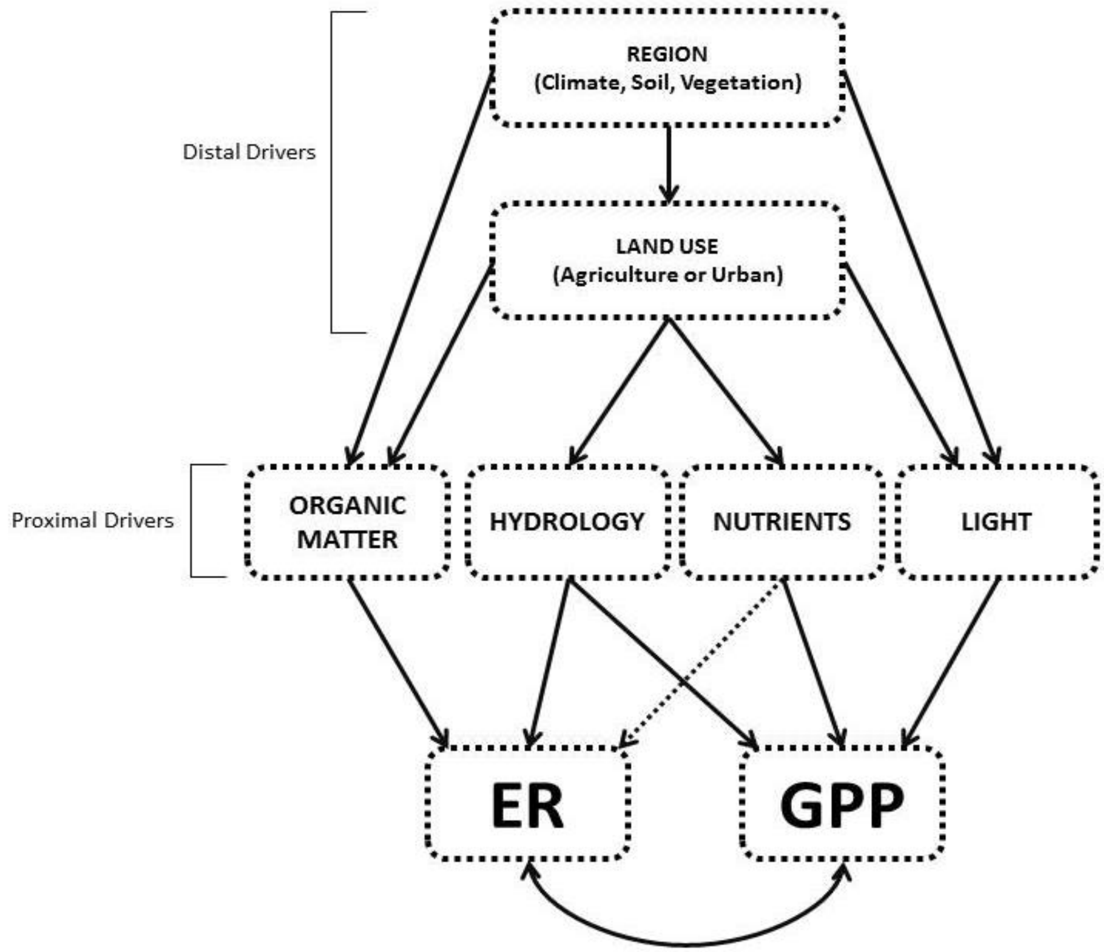

Figure 4.1**.** The hierarchical effects of distal and proximal drivers on Ecosystem Respiration (ER) and Gross Primary Production (GPP). Land use (catchment) and regional (physiography) scale characteristics are distal drivers that indirectly affect GPP and ER. Stream reach scale characteristics are proximal drivers (i.e., organic matter, hydrology, nutrients, and light) that directly affect GPP, ER or both (*Modified from:* Bernot *et al.*, 2010).

The pulse effects of lagoon wastewater effluent on stream metabolism have not yet been studied. However, there have been previous studies on the effects of continuous wastewater effluent releases from mechanical wastewater treatment plants on stream metabolism (e.g., Gücker et al., 2006; Aristi et al., 2015). These studies show increased ER downstream of the effluent outfall, relative to the upstream. Downstream increases in ER have been attributed to increased nutrient availability for autotrophs (i.e., Gücker et al., 2006), and increased organic matter concentrations for heterotrophs which can be used as an energy supply and substrate for bacteria (i.e., Artisti *et al.*, 2015; Young *et al.*, 2008). GPP has also been found to increase in the presence of wastewater effluent due to increased nutrient concentrations; however, this effect is dependent on light availability (Gücker et al., 2006; Aristi et al., 2015). For example, in stream reaches where light is limiting (e.g., dense riparian canopy cover), an increase in nutrient availability may have no effect on GPP (e.g., Aristi et al., 2015). Furthermore, the effects of a pulse release of lagoon wastewater effluent on stream metabolism can act as a subsidy. There have been studies describing the recovery of autotrophic communities following a disturbance event but there have not been any studies describing what happens to stream metabolism during a pulse-subsidy event. For example, a study by Murdock *et al.* (2004) in a watershed dominated by urban development and devoid of riparian stream canopy cover showed how quickly primary production can recover following a storm event in a stream receiving wastewater effluent. They found that chlorophyll A concentrations began to increase rapidly within a day of the storm event; the most rapid increases occurred directly downstream from the outfall pipes and declined progressively downstream as nutrient availability decreased due to upstream assimilation. Stream metabolism may therefore react similarly to a pulse-subsidy event (i.e., nutrient enrichment from lagoon wastewater effluent) as it does to a disturbance recovery event (i.e., a storm) in that GPP and ER will increase during the pulse event. Stream metabolism may thus be a suitable indicator of the ecological impacts of lagoon wastewater effluent pulses to stream ecosystems.

1.2. Research Goal, Objectives, and Hypotheses

Goal:

The goal of my research project was to assess the effects of lagoon wastewater effluent on stream metabolism and describe associated physicochemical changes in stream conditions within headwater Prairie stream reaches during the summer season.

Objectives:

1) Compare downstream physicochemical conditions before, during, and after a pulse release of lagoon effluent to conditions upstream.

2) Compare downstream biological conditions (i.e., stream metabolism) before, during, and after a pulse release of lagoon effluent.

3) Identify key physicochemical drivers stemming from the lagoon effluent associated with variation in stream metabolism metrics (i.e., GPP, ER, NEP, P/R).

4) Compare rates of stream metabolism across a range of reaches exposed and unexposed to lagoon wastewater effluent.

5) Measure daily rates of stream metabolism in stream reaches when lagoon wastewater effluent is present versus absent.

6) Compare stream metabolism before, during, and after an early and late summer lagoon effluent release.

Predictions:

a) Physicochemical parameters (e.g., nutrients and stream discharge) at the downstream reach will be significantly greater during a release of lagoon wastewater effluent versus the periods before and after the release.

b) Physicochemical parameters (e.g., nutrients and stream discharge) will be significantly greater at the downstream reach than at the upstream reach.

c) Metrics of stream metabolism (GPP, ER, P/R, and NEP) will increase significantly during a release of lagoon wastewater effluent.

d) In stream nutrient concentrations will be positively associated with stream metabolism metrics (Figure 1.2).

e) GPP, ER, and P/R will be significantly greater in reaches exposed to lagoon wastewater effluent than in unexposed reaches (Figure 1.3).

f) Stream metabolism values during days when lagoon wastewater effluent is absent will be more similar to each other than days when effluent is present (Figure 1.4).

g) GPP and ER will be significantly greater to that of the periods before and after the release and to that of control reaches not exposed to effluent (Figure 1.5).

h) There will be no significant difference in GPP or ER during a late summer release of lagoon wastewater effluent to that of the periods before and after the release and to that of control reaches not exposed to effluent (Figure 1.6).

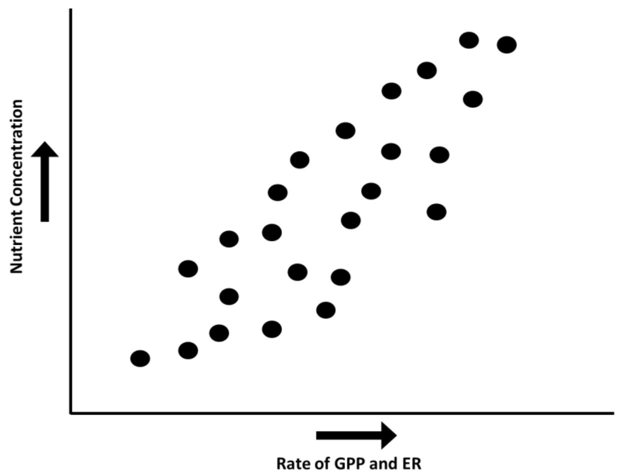

Figure 1.2. Predicted positive association of GPP and ER rates with increasing water nutrients concentrations stemming from a release of lagoon wastewater effluent (prediction: d).

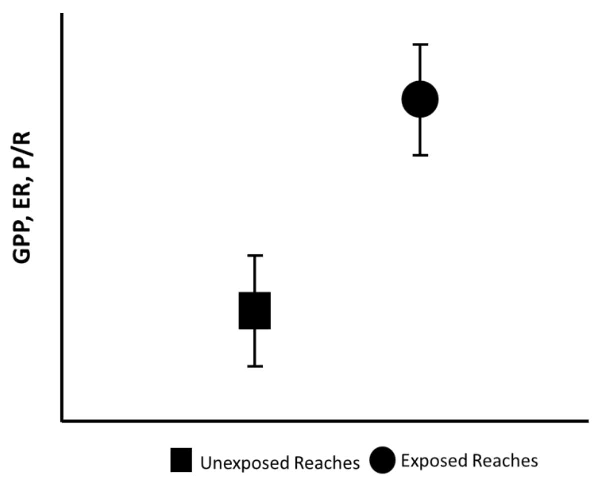

Figure 1.3. Predicted increase in average GPP, ER, and P/R for stream reaches exposed and unexposed to lagoon wastewater effluent from late-May until mid-September (prediction: e).

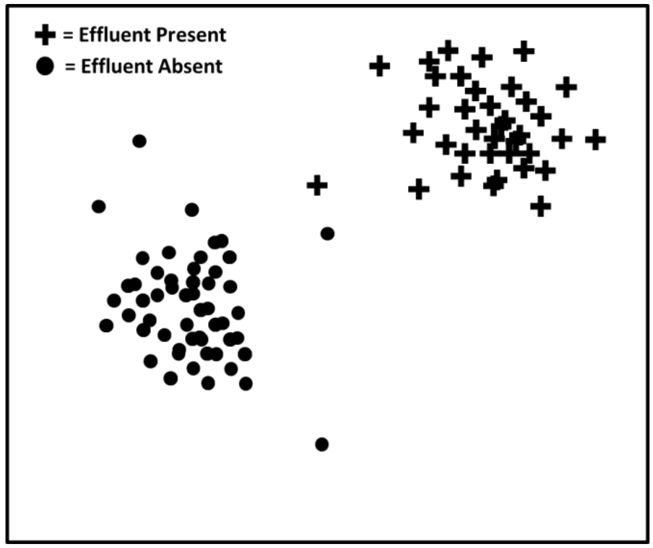

Figure 1.4. Predicted ordination of similarity for daily stream metabolism values (GPP and ER) when lagoon wastewater effluent is present versus absent (prediction: f).

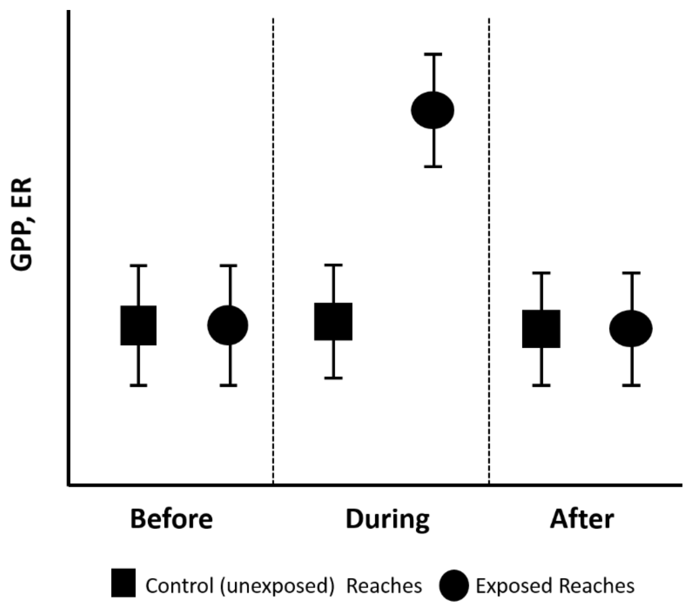

Figure 1.5. Predicted increase in GPP and ER for an effluent exposed reach during a release of lagoon wastewater versus a control reach unexposed to effluent (prediction: g).

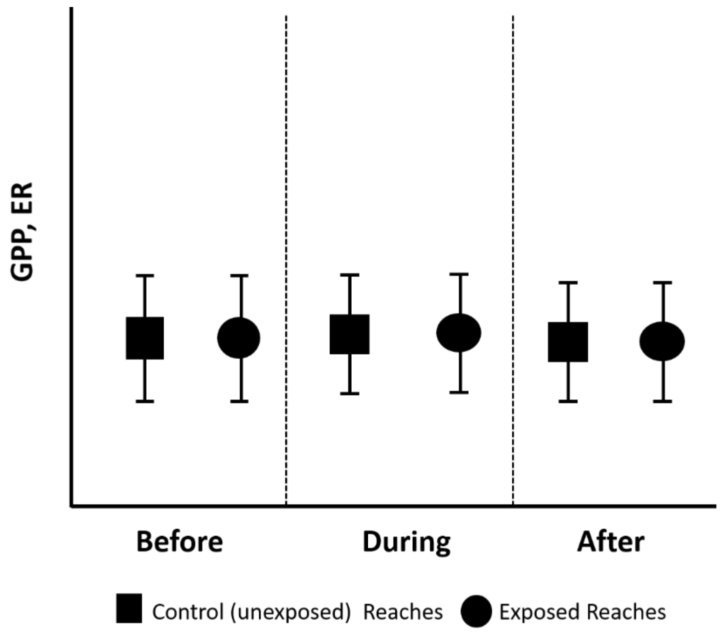

Figure 1.6. Predicted increase in GPP and ER for an effluent exposed reach during a late summer release of lagoon wastewater versus a control reach unexposed to effluent (prediction: h).

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Chapter 2

Changes in stream conditions during a release of lagoon wastewater effluent and associated effects on stream metabolism

2.1. Introduction

Municipal wastewater effluent from sewage treatment facilities is a common point source pollutant that impacts freshwater ecosystems worldwide (Grant *et al.*, 2012; Smith *et al.*, 1999). In Canada, there are over 2800 wastewater treatment facilities that release over 150 billion liters of effluent to aquatic ecosystems each year, making municipal wastewater the largest point source contributor of pollution, by volume, to this nation's surface waters (NRC, 2004; Chambers *et al.*, 2001; Government of Canada, 2010). Despite sometimes significant treatment efforts municipal wastewater contains many contaminants that can result in physical, chemical, and biological changes to receiving freshwater environments (Haggard *et al.*, 2005; Aristi *et al.*, 2015; Gros *et al.*, 2007). Common constituents of treated wastewater are bioavailable forms of nitrogen and phosphorus, hereafter referred to as nutrients. Nutrients can impact in-stream processes (i.e., nutrient cycling) by subsidizing biological activity (Hall & Tank, 2003; Bernot *et al.*, 2010). Enrichment effects have been reported by studies on stream metabolism where significant increases in gross primary production (GPP) and ecosystem respiration (ER) downstream of wastewater effluent have been measured (Aristi *et al.*, 2015; Gücker *et al.*, 2006; Graham *et al.*, 2014).

The forms and concentrations of nutrients entering freshwater environments and associated impacts on the ecosystem (i.e., stream metabolism) depend on the type and degree of wastewater treatment in place (Prince *et al.*, 1994; Metcalf & Eddy, 2003). Developed countries typically treat wastewater with either mechanical wastewater treatment plants (MWTPs) or with wastewater treatment lagoons (hereafter referred to as lagoons) (NRC, 2004; Environment Canada, 1996). Lagoons and MWTPs vary in both design and operation. Lagoons (also known as stabilization ponds) are in-ground earthen basins that treat wastewater via natural processes (i.e. organic matter breakdown and nutrient assimilation) (NRC, 2004). In contrast, MWTPs have a series of structures that facilitate different aspects of the treatment process catalyzed by chemical and mechanical influences (Metcalf & Eddy, 2003). Thus, lagoons take a more passive approach to treating wastewater whereas MWTPs take a more active approach. The active facilitation of wastewater for MWTPs allows for the implementation of advanced treatment stages to remove larger proportions of nutrients from wastewater effluent compared to lagoons (Mara *et al.*, 1992; Rockne & Brezonik, 2006; Graham *et al.*, 2014). Furthermore, the structures and processes involved when treating wastewater with MWTPs are costly in comparison to lagoons and therefore smaller, rural communities of populations of between a few hundred to several thousand typically rely on lagoons to treat their sewage waste (NRC, 2004; Environment Canada, 1996). In addition, MWTPs continuously release wastewater effluent into waterways, whereas, lagoons release wastewater effluent episodically into smaller streams. Timing of effluent release is important because the volume of effluent entering a stream relative to stream discharge often controls downstream pollutant concentrations, thus effluent from lagoons entering smaller streams may subject downstream biota to higher concentrations of contaminants due to less dilution (Carey & Migliaccio, 2009). Pulses of wastewater effluent associated with lagoon releases may also have a different effect on downstream environmental conditions than MWTPs, because the receiving ecosystem will be changing from a potential background state to one where excessive nutrients may be present over relative short periods of time. The effects these pulses of effluent have on environmental conditions, and in-stream processes in particular, are relatively unknown (but see Carlson *et al.*, 2013).

The goal of this study was to assess and compare variation in physical, chemical, and biological conditions before, during, and after a pulse release of lagoon effluent in stream reaches up and downstream of a lagoon outfall. Our specific objectives were to: 1) measure nutrient, turbidity, temperature, stream discharge, and stream metabolism conditions during a pulse release of lagoon effluent; 2) compare downstream physical and chemical conditions before, during, and after the effluent release to upstream conditions, and; 3) identify key physicochemical drivers stemming from the lagoon effluent associated with variation in stream metabolism metrics (i.e., GPP, ER, Net Ecosystem

Production (NEP), and Production to Respiration ratio (P/R)). Results of this study will generate critical knowledge regarding the effects of pulse releases of lagoon effluent on downstream ecosystems and inform lagoon management strategies aimed at mitigating effluent effects.

2.2. Methods

2.2.1. Study Site

This study took place in summer of 2014 in Devil's Creek. Devil's Creek is a 3rd order, prairie stream located approximately 30 km northeast of Winnipeg, Manitoba, Canada (Figure 1). Devil's Creek drains a catchment of the lower Red River basin. Most of the Devil's Creek catchment has been developed for agriculture with some small patches of urban land comprising the towns of Garson and Tyndall (Figure 1). Devil's Creek receives point source effluent from the Garson/Tyndall municipal facultative wastewater treatment lagoon, located 3.75 km downstream of the town of Tyndall (Figure 1C). This lagoon serves the municipalities of Garson and Tyndall, which have a combined population of 1,313 inhabitants (Statistics Canada, 2012). The lagoon discharges treated effluent episodically between the middle of June and middle of October of each year. This is a three-celled facultative lagoon (contains facultative bacteria which can break down organic matter in aerobic and anaerobic conditions) that released effluent twice in 2014, once in the early summer (i.e., mid-June) and once in the late summer (i.e., early September).

This study used a before, during, after, control-impact design to compare downstream conditions during an effluent release to conditions before and after the release to that of a control reach located upstream of the lagoon outfall. Monitoring took place 2.7 km upstream and 2.7 km downstream from the effluent outfall pipe. The downstream distance was based on the distance required to ensure even mixing of wastewater effluent with stream water (Figure 2.1C). This study monitored the 2014 late summer wastewater lagoon effluent release. The "before" period lasted from the 19th - 28th of August (n = 9), the "during" time period lasted from August 28th – September 15th (n = 19), and the "after" time period lasted from the 15th – 18th of September (n = 3).

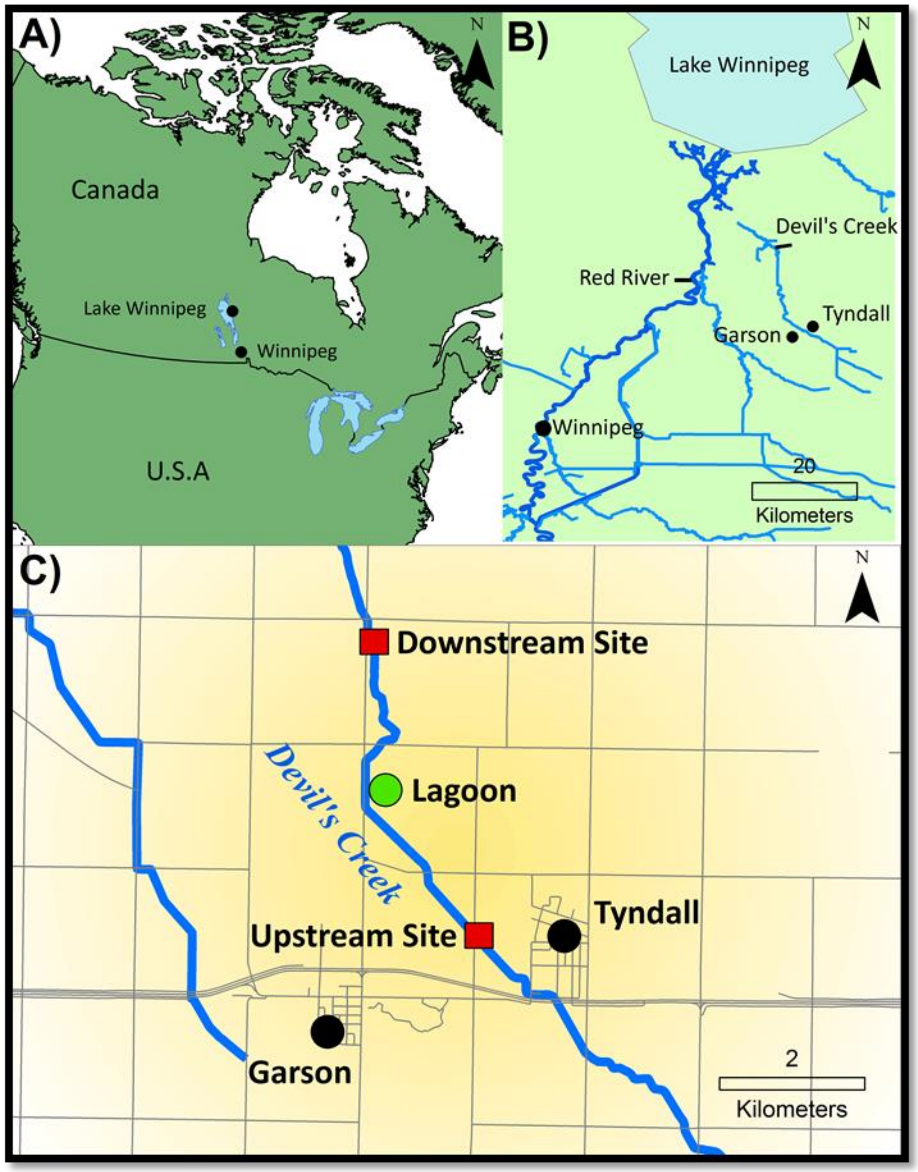

Figure 2.1. Map showing location of study area near Winnipeg in Southern Manitoba, Canada (A). Study site was a 3rd order stream (Devil's Creek) located within the lower Red River Valley and approximately 40 km northeast of Winnipeg (B). Monitoring reaches (red squares) were located 2.7 km upstream and 2.7 km downstream from the Garson/Tyndall municipal wastewater lagoon (yellow circle) on Devil's creek (C).

2.2.2. Data Collection

A data logging sonde (YSI sonde model 6600) was deployed at both the upstream and downstream reaches. Each sonde was strapped to a 20 cm cinder block and anchored to the stream bank before being placed on the stream bed in a well-mixed section of the reach. Sondes recorded temperature, dissolved oxygen (DO), depth, and turbidity every 15 minutes for the duration of the study period (August 19 to September 18). Due to instrument failure at the downstream reach temperature measurements from the upstream reach were used for the purpose of calculating % DO saturation at the downstream reach. The upstream temperature record was deemed an appropriate surrogate based on: 1) no differences between reach canopy cover; 2) no incoming tributaries between the reaches; 3) temperature measurements during a prior lagoon effluent release in June of 2014 indicated no deviations in temperature between the upstream and downstream reaches during the release period (Appendix 1), and; 4) temperature patterns at the upstream reach were consistent with other streams monitored across the region over the same time period.

Each sonde recorded water depth every 15 minutes using a strain gauge pressure transducer, these measurements were corrected for variation in atmospheric pressure using biweekly measurements of stream reach depth taken with a wading rod across five transects in each reach. Transects were spaced ten meters apart ascending upstream from the sonde. Depth was measured at ten evenly spaced locations along each transect for a total of 50 depth measurements per reach. Depth measurements from each sampling event were averaged and regressed against corresponding average pressure-depth estimates (averaged over the same time transects were measured) to determine their linear relationship. The plot was then used to calculate a line of best fit, the equation of the line of best fit (i.e., the relationship between the two types of depth measurements) was then used to calculate the transect corrected depth for each 15 minute interval for that individual sonde throughout the entire study period.

A photosynthetically active radiation (PAR) logger (Odyssey PAR Light Logger model Z412) was deployed on the stream bank on top of a 1.5 m piece of rebar; the logger recorded PAR measurements every 15 minutes to indicate day length. A densiometer was used to estimate reach canopy cover in order to decide where the PAR logger should be placed. The logger was deployed in an area with shade (canopy cover) conditions representative of the stream reach.

Stream discharge at the downstream site was estimated using measurements calculated every five minutes from data collected at a gauging station 9.2 km downstream from the downstream reach (Water Survey Canada, 2014). There were no incoming tributaries or water abstraction between the gauging station and the downstream reach.

Water nutrient samples were collected daily at both sites in 250 ml Nalgene™ HDPE sterile sampling bottles throughout the entire sampling period. Sampling bottles were rinsed three times with stream water, the rinsate was removed and the bottles were filled with stream water at 60% stream depth. The following nutrient parameters were analyzed from each sample: ammonia (NH_3), nitrate-nitrate (NO_2^{-}+NO_3^{-}), total-nitrogen (TN), soluble reactive phosphorous (SRP), total dissolved phosphorous (TDP), and total phosphorous (TP). Nutrients were measured using a Lachat QuickChem QC8500 FIA Automated Ion Analyzer. TN and NO_2^{-}+NO_3^{-} concentrations were analyzed using the US EPA protocol (United States Environmental Protection Agency (USEPA), 1993a,b) and the rest were measured using the APHA protocol (American Public Health Associated (APHA), 2012a,b,c).

2.2.3. Stream Metabolism

Stream metabolism was calculated by modeling the diel fluctuations of DO. Due to failure of the oxygen sensor at the upstream reach, stream metabolism was only calculated for the downstream reach. Stream metabolism was estimated using the single station open-system method (Grace & Imberger, 2006).

The reaeration coefficient (K) for each day was calculated using the Delta method (Chapra & Di Toro, 1991). Where the length in time between the minimum deficit in DO, relative to solar noon, was used to estimate K (Chapra & Di Toro, 1991). This method is effectively used in streams that are slow moving with no canopy cover (Grace & Imberger, 2006), and is therefore well suited for this study site. K could not be reasonably estimated for three days during the study period and these days were excluded from further analysis. For days where K could be estimated accurately, the change in DO over 15 minute intervals was used to calculate estimates of Ecosystem Respiration (ER) and Gross Primary Production (GPP) based on the following equation (Grace & Imberger, 2006):

$$\mathbf{(1)} \qquad \mathbf{\Delta DO = GPP - ER \pm K(D)}$$

Where ΔDO is the change in DO concentration during the 15min intervals, GPP is the volume of DO produced via photosynthesis, ER is the volume of DO consumed by cellular respiration, K is the reaeration coefficient, and D is the DO deficit (based on 100% DO saturation). Daily %DO saturation was corrected for temperature and data from the PAR light sensor was used to estimate photosynthetically productive hours. ER was first estimated during the night-time by setting GPP=0, and inserting the ΔDO, K, and D into equation 1 for each time interval; an average of these values was then taken and used to calculate daytime values by interpolating between night-time ER averaged over each daylight interval. Now with ER values, equation 1 was rearranged to solve for GPP. Night-time and day-time ER and GPP values were summed and divided by the number of time intervals to give daily volumetric rates (g O_2 m^{-3} day^{-1}) for both metrics. The daily volumetric rates of GPP and ER were converted into areal rates by multiplying by daily average reach depth (i.e., the transect corrected depth). Daily Net Ecosystem Productivity (NEP) values were then calculated by taking the difference between daily GPP and ER rates; the daily production/respiration ratio was calculated by dividing GPP by ER rates.

2.2.4. Data Analysis

Summary statistics were generated for parameters measured at the upstream and downstream reaches based on the entire study period. Daily averages of turbidity (n = 31) and daily water nutrients measurements for the chemical parameters (water nutrients) (n = 30) were analyzed for both reaches. Daily averages of temperature (n = 31) was only analyzed at the upstream reach, whereas, daily averages of stream discharge (n = 31) and the stream metabolism metrics (ER, GPP, NEP, P/R) (n = 28) were only analyzed for the downstream reach.

ANOVAs and Tukeys post hoc tests were used to determine the statistical significance ($\alpha = 0.10$) of observed differences between reaches (upstream vs downstream) and among time periods (before, during, and after effluent release) for both physicochemical parameters and stream metabolism metrics. Averages of water nutrient measurements from both the upstream and downstream sites were taken from the before period (n = 8), during the release period (n = 19), and the after period (n = 3). Turbidity averages from both sites were taken from the before period (n = 9), during the release period (n = 19), and the after period (n = 3). Stream discharge averages from the downstream site were taken from the before period (n = 9), during the release period (n = 19), and the after period (n = 3). Stream metabolism averages from the downstream site were taken from the before period (n = 8), and during the release period (n = 18). However, before any of the data was run through an ANOVA a Shapiro-Wilks test for normality was first conducted. If parameters failed the normality test they were transformed logarithmically and the test repeated. If the normality test failed again the data were analyzed using a non-parametric Kruskal-Wallis one way analysis of variance. The following parameters/metrics were analyzed non-parametrically at the downstream site: stream discharge, P/R, TN, NO_2^-+NO_3^-, NH_3, TP, TDP, and SRP. The following parameters were analyzed non-parametrically at the upstream site: turbidity, TN, NO_2^- +NO_3^-, NH_3, TP, TDP, and SRP. Significant non-parametric models were assessed using a Dunn’s post hoc test to determine significance between pairs. Data was analyzed using Systat statistical software (Systat Software Inc, 2015).

A backwards stepwise linear regression was used, with a confidence interval of 0.9, a tolerance of 1^{-012}, and probability of 0.15, to establish the relationship between measured environmental parameters and the observed variability in stream metabolism metrics (i.e., GPP and ER). Predictor variables included in the stepwise regression were first tested for collinearity to ensure their accuracy in predicting the response variables. A variance inflation factor (VIF) > 5 was used to determine if variables were collinear ($VIF_x = 1/1\text{-}R^2$). SRP,TP and TDP were deemed to be collinear (VIF > 5) and as a result only SRP was retained for further analysis. The remaining predictors (turbidity, SRP, TN, NH_3, and NO_2^-+NO_3^-) were run as independent variables within a multiple linear regression model against each metric of stream metabolism (GPP, ER, NEP and P/R) as the dependent variables using Systat statistical software (Systat Software Inc, 2015). If the regression model was significant and the standard errors were normally distributed (based on a Shapiro-Wilks test) than those models were put through the backwards stepwise linear regression analysis, if the data were not normal and/or not linear than it would be logarithmically transformed.

2.3. Results

Average daily stream temperature was 16.9 (±3.7) ^{o}C and average daily discharge was 0.1 (±0.03) m^3 sec^{-1} (Table 2.1). Daily stream temperature and discharge exhibited low variability (CV ≤ 0.3) over the period of study. Average daily turbidity at the upstream site was 81.72 (±15.9) ntu and 348.99 (±341.9) ntu at the downstream site. Turbidity at the downstream site was on average about 4 times greater than at the upstream site. Variation in turbidity was small throughout the entire study period at the upstream site (CV = 0.19), but larger at the downstream site (CV = 0.98).

Nutrient concentrations (i.e., NH_3, NO_2+NO_3, TN, SRP, TDP, TP) from the downstream site were all larger, on average, than those at the upstream site throughout the study period (Table 2.1). All nutrient parameters were at least five fold larger, on average, at the downstream site and as much as 20 times larger (NH_3). Daily variation in nutrient concentrations varied the most at the upstream site with TN (CV = 0.63) showing the least variability and NO_2^-+NO_3^- (CV = 3.45) showing the most. At the downstream site, TN (CV = 0.27) was the least variable parameter and NH_3 (CV = 1.84) was the most variable.

Proportional changes in nitrogen compounds comprising total nitrogen (e.g. NH_3 and NO_2^-+NO_3^-) were variable throughout the study period. NH_3 comprised 0.8% of the TN (organic + NO_2^-+NO_3^- + NH_3) at the upstream site before and during the effluent release and 1.1% after the release (Figure 2.2). At the downstream site NH_3 comprised 0.7% of the TN before, 12.7% during, and 0.2% after the release. NO_2^-+NO_3^- at the upstream site comprised 1.5% of the TN before, 21.8% during, and 0.1% after the release (Figure 2.2). During the release NO_2^-+NO_3^- at the downstream site was 52.8% of the TN before release, 43.1% during, and 50.8% after.

Proportional changes in phosphorous compounds comprising total phosphorous (e.g. TDP) were variable throughout the study period. TDP at the upstream site comprised 70.2% of the TP (particulate + dissolved P) before, 62.2% during the release and 23.4% after the effluent release (Figure 2.3). TDP at the downstream site comprised 76.8% before and after the effluent release and 94.6% during the release.

Table 2.1. Descriptive statistics for physical (n = 31) and nutrient parameters (n = 30) sampled at an upstream and downstream reach from a lagoon outfall on Devil's Creek, Manitoba, Canada.

		Mean	Standard Deviation	Median	Max	Min	Coefficient of Variation
Temperature (ºC)	Upstream	16.9	3.7	17.1	23.4	11.1	0.22
	Downstream	N/A	N/A	N/A	N/A	N/A	N/A
Discharge (m^3 sec^{-1})	Upstream	N/A	N/A	N/A	N/A	N/A	N/A
	Downstream	0.1	0.03	0.1	0.2	0.1	0.30
Turbidity (ntu)	Upstream	81.7	15.9	78.8	132.1	11.0	0.19
	Downstream	348.7	341.9	213.5	1366.2	88.0	0.98
NO_2^- + NO_3^- (µg L^{-1})	Upstream	173.9	599.9	<DL	3210	<DL	3.45
	Downstream	1052.5	483.2	1040.0	2660	<DL	0.46
NH_3 (µg L^{-1})	Upstream	9.8	7.9	7.0	24	<DL	0.81
	Downstream	199.8	367.4	9.0	1270	<DL	1.84
TN (µg L^{-1})	Upstream	1158.5	725.1	981.0	4940	906	0.63
	Downstream	2286.7	625.5	2085.0	3720	1180	0.27
TP (µg L^{-1})	Upstream	77.9	64.3	59.5	376	38	0.83
	Downstream	423.5	450.1	239.5	1507	45	1.06
TDP (µg L^{-1})	Upstream	47.8	62.9	35.0	355	15	1.32
	Downstream	395.7	442.7	216.0	1460	22	1.12
SRP (µg L^{-1})	Upstream	37.6	64.8	21.5	354	8	1.72
	Downstream	386.8	439.3	204.5	1462	22	1.14

*<DL = sample was below a detectable limit

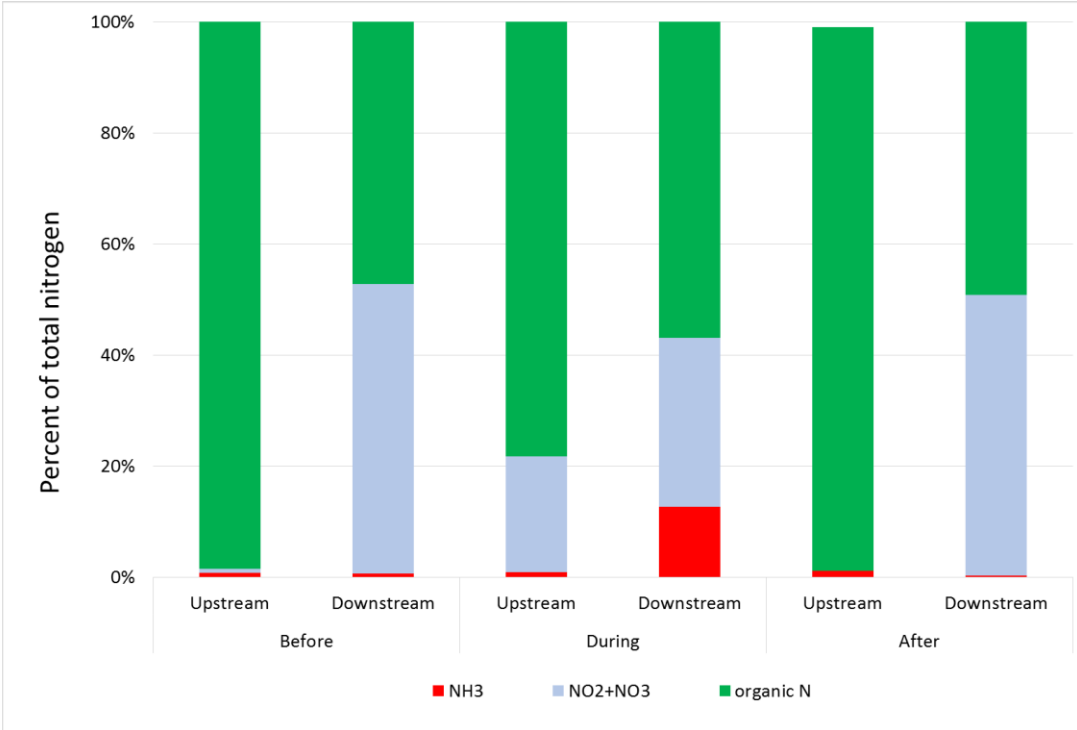

Figure 2.2. Percent of nitrogen compounds in the form of NH_3 (red), NO_2^-+NO_3^- (blue), and organic N (green) comprising total N. Percentages were calculated from daily average nutrient concentrations during the period before the effluent release (n = 8), the period during the release (n = 19), and the period after the release (n = 3) from both upstream and downstream reaches located on Devil's Creek in the lower Red River Valley, Manitoba, Canada.

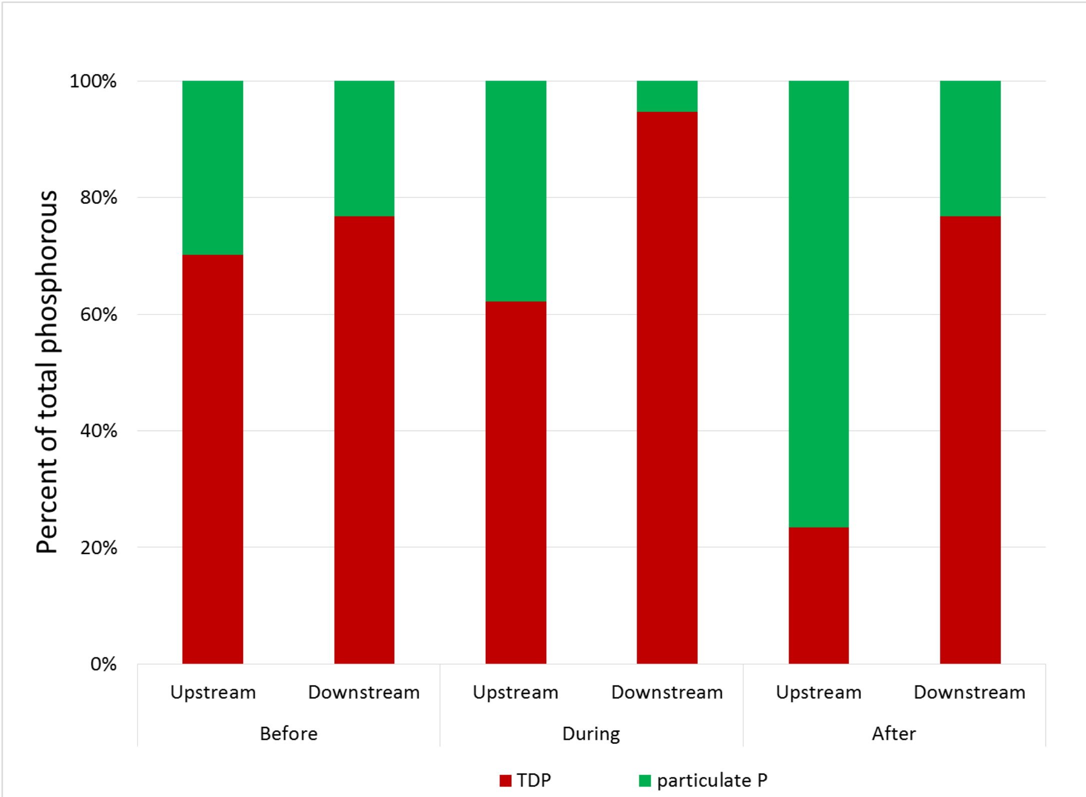

Figure 2.3. Percent total of phosphorous in the form of total dissolved phosphorus (TDP, red) and particulate phosphorous (green) taken before an effluent release (n = 8), during the release (n = 19), and after the release (n = 3) from an upstream and downstream reach located in Devil’s Creek, Manitoba, Canada.

Variation in the concentrations of N and P water nutrients were greatest during the effluent release. Concentrations of NH_3 at the downstream site ranged from <3 to 1270 µg L^{-1} during the effluent release (Figure 2.4). The largest concentrations of NH_3 occurred during the first three days and the last three days of the release. Concentrations of NH_3 at the upstream site remained consistent throughout the study with a range of < 3 - 24 µg L^{-1}. Concentrations of NO_2^-+NO_3^- at the upstream site ranged from < 2 - 3210 µg L^{-1} during the effluent release, with the largest peak occuring on the first of September following a substantial rain event (Figure 2.4). NO_2^-+NO_3^- concentrations at the downstream site ranged from 84 – 1790 µg L^{-1} during the effluent release. The largest NO_2+NO_3 concentration (2660 µg L^{-1}) was recorded on September 16th, the first day after the release ended (Figure 2.4). TN concentrations remained fairly consistent over the course of the study at both the upstream and downstream sites. An exception was a large concentration (4949 µg L^{-1}) measured on the first of September at the upstream site (Figure 2.4). Concentrations of SRP at the downstream site ranged from 68 - 1462 µg L^{-1} during the effluent release with the largest peaks occuring within the first four days and within the last four days of the release (Figure 2.4). Concentrations of SRP at the upstream site varied throughout the study with a range of 8 - 354 µg L^{-1}. TDP and TP followed the same trend in concentration fluxuations as SRP for the upstream and downstream sites.

Over the study period the average daily ER was 15.42 (±7.21) g O_2 m^{-2} day^{-1}, whereas average daily GPP was 11.49 (±4.39) g O_2 m^{-2} day^{-1} (Table 2.2). The average daily NEP was -3.92 (±3.67) g O_2 m^{-2} day^{-1} and the average daily P/R value was 0.82 (±0.25). Estimated daily rates of ER and GPP at the downstream site showed that ER was 21% more variable over the study period than was GPP. ER and GPP both showed moderate variability with CV’s of 0.47 and 0.38, respectively. NEP showed the largest amount of variability (CV = 0.93) and P/R showed the smallest (CV = 0.31).

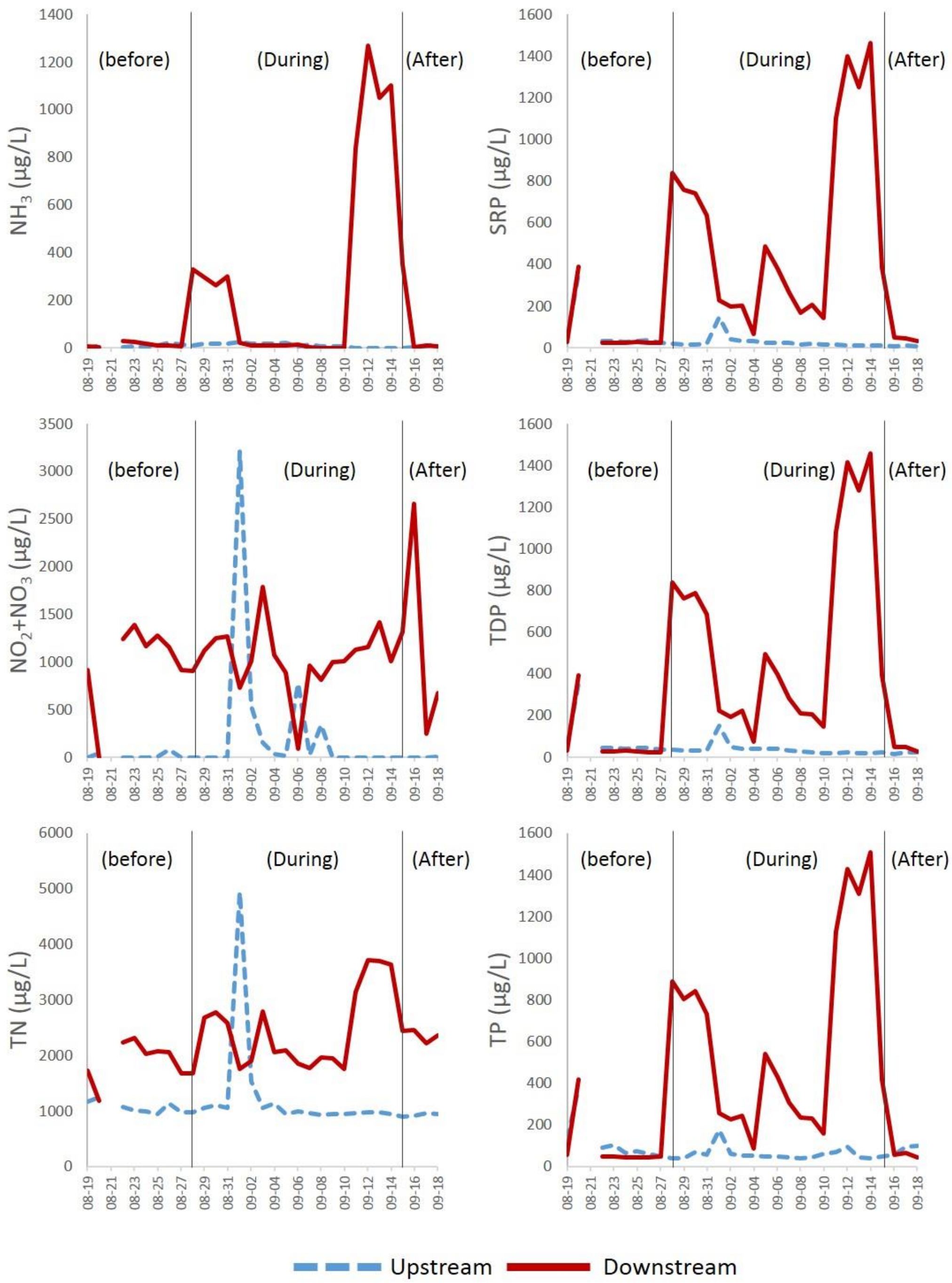

Figure 2.4. Time-series of water nutrient concentrations (µg L^{-1}) measured before (August 19-28), during (August 28- September 15), and after (September 15-18) a lagoon wastewater effluent release at an upstream and downstream site in Devil's Creek, Manitoba, Canada.

Table 2.2. Descriptive statistics for metrics of stream metabolism (n = 28) estimated for the downstream reach of Devil's creek in the lower Red River Valley, Manitoba, Canada.

	Mean	Standard Deviation	Median	Max	Min	Coefficient of Variation
ER ($g\ O_2\ m^{-2}\ day^{-1}$)	-15.42	7.21	-16.37	-27.88	-1.45	0.47
GPP ($g\ O_2\ m^{-2}\ day^{-1}$)	11.49	4.39	11.83	17.92	2.64	0.38
NEP ($g\ O_2\ m^{-2}\ day^{-1}$)	-3.92	3.67	-3.15	2.30	-10.30	0.93
P/R	0.82	0.25	0.79	1.82	0.47	0.31

Comparison of physical parameters between sites and among time periods showed significant differences in discharge and turbidity. Stream discharge during the effluent release (average 0.12 (±0.02) m^3 sec^{-1}) was significantly greater than before ($p < 0.001$) and after the release ($p = 0.016$) when discharge averaged 0.07 (±0.01) m^3 sec^{-1} and 0.08 (±0.002) m^3 sec^{-1}, respectively (Figure 2.5). Turbidity at the downstream reach was significantly greater ($p < 0.001$) than turbidity upstream within each time period (Figure 2.6). Turbidity at the downstream site averaged 196 (± 123) ntu during the release and was significantly less than the average turbidity recorded before ($p = 0.002$) and after ($p < 0.001$) the release. There was no significant differences in turbidity ($p > 0.1$) among the before, during and after periods at the upstream site (Figure 2.6).

All measured nitrogen forms were significantly greater in concentration at the downstream reach relative to the upstream reach during the effluent release (p = <0.001 for NO_2^-+NO_3^-; p = 0.012 for NH_3; p = <0.001 for TN) (Figure 2.7). Average increases in nitrogen concentrations were at minimum a two-fold difference (TN) but up to a 30 fold increase (NH_3).

All the measured forms of phosphorus were significantly greater during the effluent release at the downstream site in comparison to the upstream site (p = <0.001 for SRP, TDP, and TP; Figure 2.8). SRP at the downstream site was on average eight fold greater during the wastewater release than before (p = <0.001) and 14 times greater during the release than after (p = <0.001). TDP and TP showed the same trends as SRP. The upstream site did not show a significant difference ($p > 0.1$) between any of the time periods for any of the phosphorus parameters except for SRP, which was eight times greater before the wastewater release than after the release ($p = 0.052$).

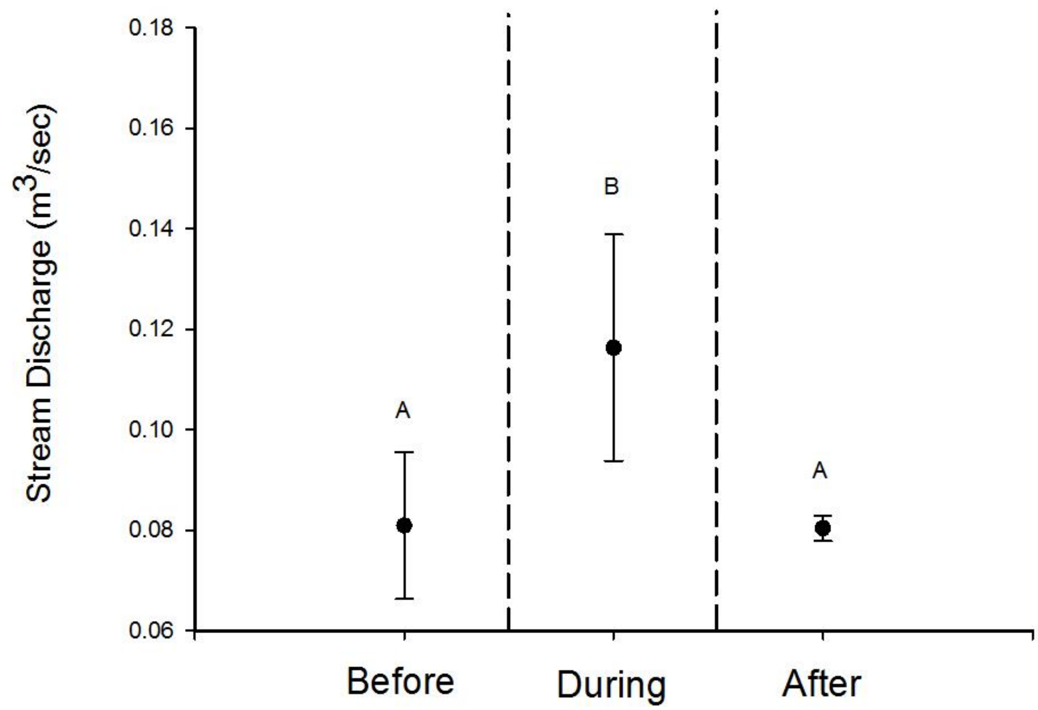

Figure 2.5. Average stream discharge (± standard deviation) and significance difference ($p < 0.1$; indicated by lettering) before (n=9), during (n=19), and after (n=3) a wastewater release to Devil's Creek, Manitoba, Canada.

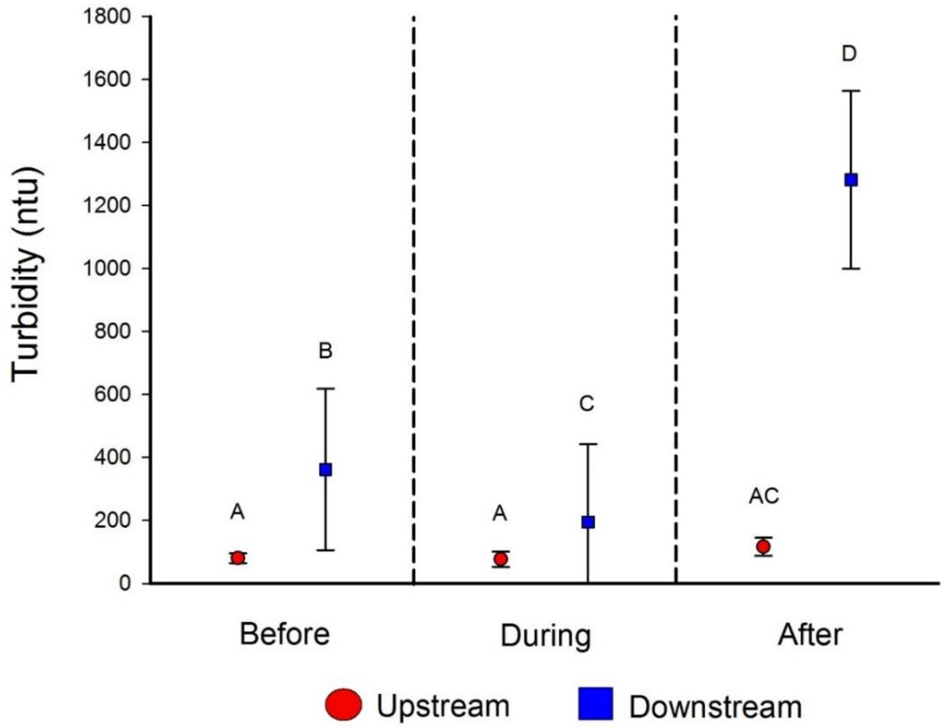

Figure 2.6. Average upstream and downstream reach turbidity (± standard deviation) and significance difference ($p < 0.1$; indicated by lettering) before (n=9), during (n=19) and after (n=3) a wastewater release to Devil's Creek, Manitoba, Canada.

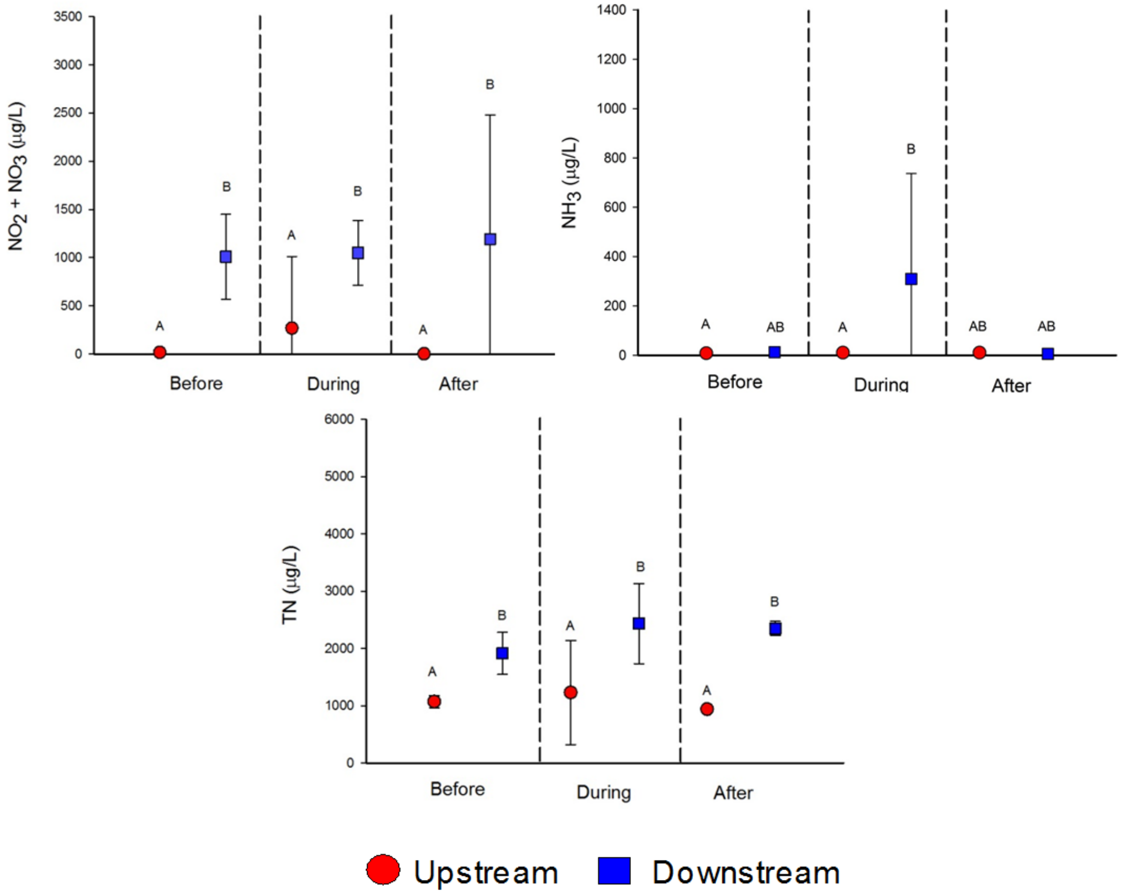

Figure 2.7. Average upstream and downstream reach NO_2^-+NO_3^-, NH_3, and TN concentrations (± standard deviation) and significance difference ($p < 0.1$; indicated by lettering) before (n=8), during (n=19), and after (n=3) a wastewater release to Devil's Creek, Manitoba, Canada.

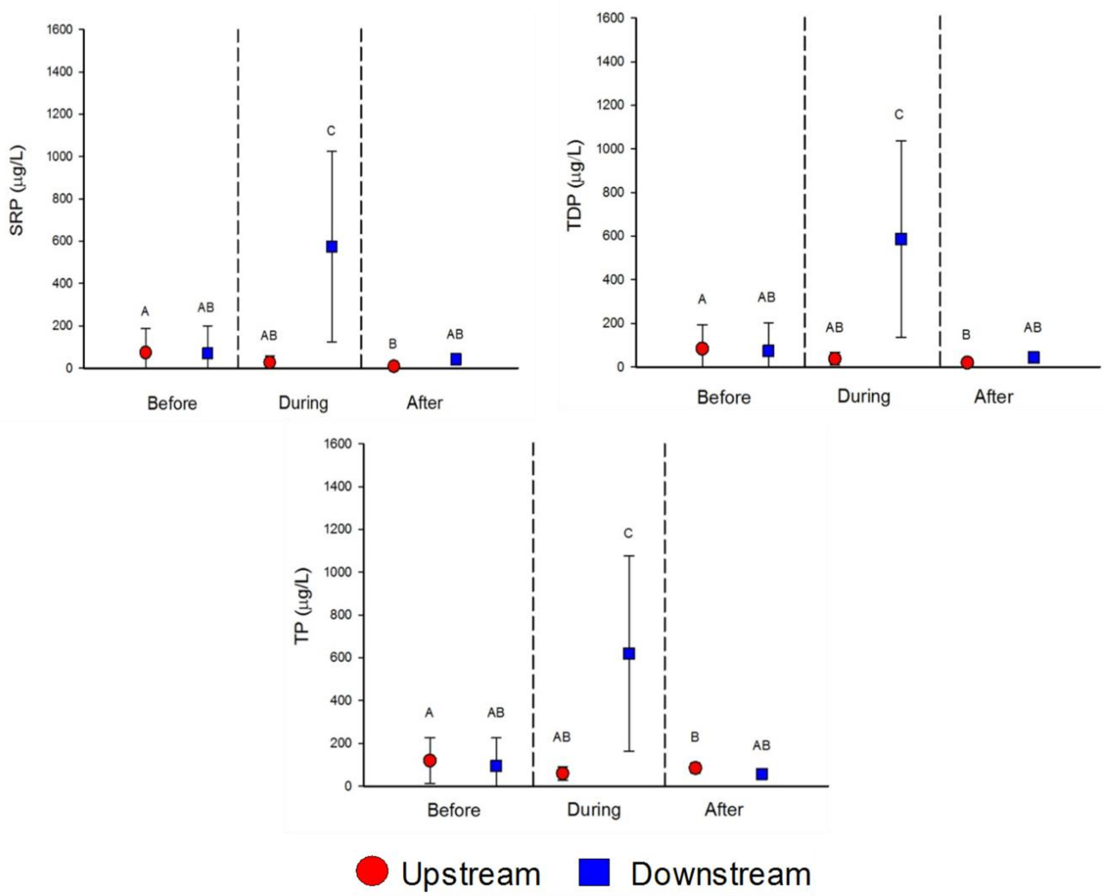

Figure 2.8. Average upstream and downstream reach SRP, TDP, and TP concentrations (± standard deviation) and significance difference ($p < 0.1$; indicated by lettering) before (n=8), during (n=19), and after (n=3) a wastewater release to Devil's Creek, Manitoba, Canada.

All metrics of stream metabolism showed a significant difference at the downstream site from the period before to during the effluent release ($p = <0.001$ for ER; $p = <0.001$ for GPP; $p = 0.008$ for NEP; $p = 0.008$ for P/R) (Figure 8). Average daily ER and GPP both doubled in magnitude during the effluent release whereas NEP decreased by nearly 10 g O_2 m^{-3} day^{-1} (i.e., ER exceeded GPP) (Figure 2.9), and P/R decreased by 25% (Figure 8).

Stepwise regression identified significant drivers for both ER and GPP (Table 2.3). Turbidity, NH3, NO_2+NO_3, and SRP were the significant predictors retained in the model for ER and together explained 37% of the variation in ER throughout the study period. NH3 was negatively associated with ER whereas the remaining 3 parameters were positively associated. SRP was the only environmental parameter significantly associated with GPP. GPP increased with increased SRP concentrations. SRP explained 29% of the variation in GPP. In contrast, NEP and P/R were not significantly associated with any of the measured environmental parameters.

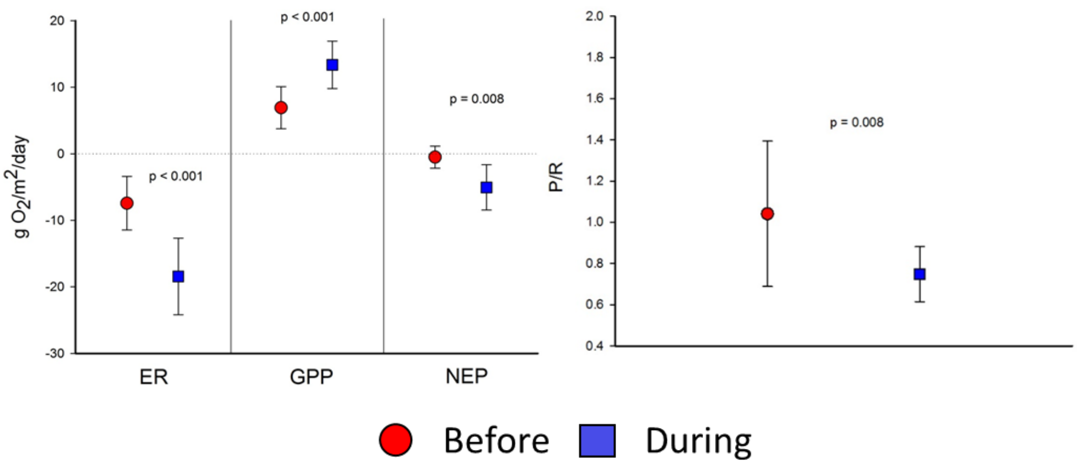

Figure 2.9. Average downstream ER, GPP, NEP, and P/R (± standard deviation) before and during a wastewater release to Devil's Creek, Manitoba, Canada.

Table 2.3. Results of backward stepwise regression analysis showing significant predictors of metrics of stream metabolism and associated model R^2 values for an effluent release in Devil's Creek in the lower Red River Valley, Manitoba, Canada.

Parameter	R^2	P – Value	Significant Predictor(s)	Standard Coefficient
ER	0.373	0.006	Turbidity	0.497
			NH_3	-0.531
			NO_2+NO_3	0.492
			SRP	1.125
GPP	0.263	0.004	SRP	0.540
NEP	n/a	0.200	n/a	n/a
P/R	n/a	0.100	n/a	n/a

2.4. Discussion

2.4.1. *Physicochemical changes in stream conditions during an effluent release*

Our study found significantly greater concentrations of nitrogen (i.e., 30X in ammonia; 4X in nitrate-nitrite) and phosphorous (i.e., 21X in SRP) at our site downstream of the effluent outfall in comparison to our upstream site; an expected findings given nutrient removal by lagoon wastewater treatment is often limited (Prince *et al.,* 1994; NRC, 2004). The downstream increases we observed in nutrient concentrations are comparable to increases reported by past studies on mechanical treatment plants, which ranged from an 8-15 fold increase in SRP, a 4-18 fold increase in nitrate, and a 2-160 fold increase in ammonia (Aristi *et al.,* 2015; Gücker *et al.,* 2006; Andersen *et al.,* 2004; Ekka *et al.,* 2006; Dyer & Wang, 2002). Our results are consistent with a previous study in Southern Manitoba that also measured upstream and downstream nutrient concentrations from the periods before, during, and after a lagoon wastewater effluent release (Carlson *et al.,* 2013). Carlson *et al.* (2013) found a significant increase in phosphorous and ammonia and a non-significant increase in nitrate-nitrite concentrations from their upstream to downstream site, they also found that the maximum summer concentrations of phosphorous and ammonia occurred during the twice-annual release of lagoon wastewater effluent. However, unlike Carlson *et al.* (2013) we did not find a significant increase in ammonia during the effluent release compared to the before or after periods at the downstream site. We did observe elevated concentrations of ammonia during the first and last three days of the effluent release but concentrations returned to pre- and post- release levels in-between. It is unclear why we observed this pattern in ammonia but we speculate that it was due to nutrient stratification in the lagoon water column. Ammonia can become stratified in lagoons as a result of oxygen availability (oxygen is required for ammonia to undergo nitrification resulting in it being transformed into nitrite), deeper portions of the water column with less oxygen will therefore tend to have higher concentrations of ammonia (Ruiz *et al.,* 2003). This same effect has been shown to occur in lakes (Edmond *et al.,* 1993). We therefore likely saw the two distinct increases in downstream ammonia when a new lagoon cell was beginning to be released. However, further work is required to gain a better understanding of the nutrient profile of a lagoon water column.

The proportion of stream discharge in Devil's Creek attributable to wastewater effluent was small relative to other studies. Past studies have showed that effluent from mechanical wastewater treatment plants have contributed 70-100% of stream/river discharge (Ekka *et al.,* 2006; Murdock *et al.,* 2004; Dennehy *et al.,* 1998; Andersen *et al.,* 2004). In contrast, we found that lagoon effluent comprised approximately 33% of flow in Devil's Creek during the release. The volume of wastewater effluent entering a stream relative to stream discharge is important because it often controls downstream nutrient concentrations (Carey & Migliaccio, 2009). The previously mentioned studies show that stream discharge was predominately effluent suggesting minimal dilution of effluent took place when released into the streams. In comparison, stream flow in Devil's Creek would have diluted the wastewater effluent by approximately a factor of two. Yet, despite this comparatively large dilution ratio, we observed 30 and 20 fold increases in ammonia and SRP, respectively, which is comparable or greater than past studies where little to no effluent dilution occurred (e.g., Ekka *et al.*, 2006; Andersen *et al.*, 2004). Thus, it appears lagoon wastewater effluent may have larger concentrations of nutrients than effluent treated by larger mechanical treatment plants; which is plausible given lagoons generally are less effective at removing nutrients than modern mechanical plants (NRC, 2004).

The release of lagoon wastewater effluent into Devil's Creek shifted the streams nutrient composition to higher proportions of more biologically available species (i.e., ammonia and SRP). We found that our downstream ammonia concentrations consisted of a larger proportion of the total dissolved inorganic nitrogen (DIN) during a release of wastewater effluent; a finding similar to Huggard *et al*. (2005). We also found that the proportion of SRP to total phosphorous (TP) increased substantially in association with a release of wastewater effluent at our downstream site; however, this finding was in contrast to Graham *et al.,* (2014). Graham *et al.,* (2014) may not have seen the same proportional increase in SRP from their upstream to downstream site because their mechanical wastewater treatment plant had recently implemented a chemically modified primary treatment phase to enhance phosphorous removal. Lagoon treatment has largely been considered ineffective at phosphorous removal (Mbwele, 2006). Furthermore, unlike ammonia and SRP, the proportion of nitrite-nitrate to total DIN did not increase in association with lagoon wastewater effluent; a finding opposite to Graham *et al.,* (2014) who found that nitrite-nitrate increased from 15-20% of total N upstream to 80-90% of total N downstream of a wastewater outfall. The proportion of DIN that consisted of nitrite-nitrate at our downstream site was greater before than during the effluent release suggesting nitrite-nitrate concentrations were already high in our stream reach. A similar study that was conducted in the same region as ours also found that nitrite-nitrate concentrations did not change significantly with the presence of effluent since concentrations were already high (Carlson *et al.,* (2013). Therefore, due to high pre-existing nitrite-nitrate concentrations within our downstream reach, lagoon wastewater effluent was incapable of causing a noticeable change in the proportion of DIN that consisted of nitrite-nitrate during an effluent release. Our findings not only suggest that lagoon wastewater effluent contains large concentrations of nutrients, in comparison to mechanical treatment plants, but also that the effluent is shifting the composition of stream nutrients into more biologically favourable forms.

2.4.2. Changes in stream metabolism during an effluent release

We found that a release of lagoon wastewater effluent into Devil’s Creek significantly increased rates of GPP and ER; a finding similar to previous studies on larger mechanical treatment plants (Gücker *et al.,* 2006; Aristi *et al.,* 2015). For example, Gücker *et al.* (2006) saw a significant increase in ER and GPP downstream of a wastewater effluent release compared to an upstream site. Likewise, Aristi *et al.,* (2015) saw nearly a 3 fold increase in ER, but only a slight increase in GPP from upstream to downstream. Aristi *et al.* (2015) did not see a similar increase in GPP as our study because their stream reaches had dense canopy cover, whereas ours had none, and therefore light was likely limiting their primary production. Overall, we found that the effluent from our lagoon and the effluent from larger mechanical treatment plants had similar outcomes on stream metabolism, this may suggest that the mechanics in how wastewater effluent effects aquatic ecology remains unvaried across treatment types.

By monitoring stream conditions before, during, and after the lagoon wastewater effluent release we were able to determine how quickly stream metabolism responds to a pulse of nutrient rich wastewater effluent. We found that ER rates increased to a higher state within a day and GPP increased within a period of a few days to a pulse release of lagoon wastewater effluent. To our knowledge, there has not been any other studies that closely monitored the effects of a pulse release of wastewater effluent on stream metabolism but there have been studies describing the recovery rate of autotrophic communities following a short-term disturbance event in which some reaches were exposed to effluent and others were not. For example, a study by Murdock *et al.* (2004) in a catchment dominated by urban land-use and within stream reaches devoid of any riparian canopy cover compared how quickly primary production recovered from a physical disturbance (i.e., storm events) between reaches with and without effluent additions. They found that chlorophyll *a* concentrations can respond immediately following the disturbance event (i.e., within a day). Furthermore, Murdock *et al.* (2004) and that chlorophyll *a* concentrations increased more rapidly in their reach immediately downstream from the effluent outfall to that of their upstream reach. Therefore, our findings suggest that a pulse subsidy event (*sensu* Aristi *et al.,* 2015) may influence ecological processes (e.g, nutrient cycling) just as quickly as nutrient additions can influence primary production following a disturbance event (e.g., Murdoch *et al*., 2004). Our study therefore allowed us to determine the time-frame in which wastewater effluent may impact downstream ecology which until now has not been known.

Our results showed that P/R and NEP decreased significantly during the release of lagoon wastewater effluent, suggesting that Devil's Creek becomes more heterotrophic in the presence of effluent; a finding similar to Aristi *et al.* (2015). We speculate that the disproportional increase in ER to GPP, along with the rapid increase in ER during the effluent release period (i.e., almost 3-fold increase within two days), was mostly caused by heterotrophic bacteria. These bacteria could have originated from the stream, the lagoon, or a combination of the two. It has been demonstrated that dissolved and particulate organic matter will increase in streams receiving wastewater effluent, which could promote bacterial growth due to the increase in substrate availability to colonize and organic carbon to consume (Paul & Meyer *et al.,* 2001). Likewise, the release of lagoon wastewater effluent could have provided stream reach bacteria with substrate and organic carbon resulting in bacterial growth and the increase we observed in ER. However, it is not clear because we did not measure organic matter. Furthermore, because wastewater treatment relies so heavily on heterotrophic bacteria to treat sewage, previous studies have shown that an effluent release can contribute immense counts of bacteria into receiving reaches (Brion & Billen, 2000; Servais *et al.*, 1999; Young & Thackston, 1999). We therefore hypothesize that the observed heterotrophic shift that occurred in our study could also have been as a result of the effluent flushing a substantial community of bacteria from the lagoon into our downstream reach. However, further studies are required to better understand the dynamics of bacterial communities in effluent exposed versus unexposed reaches.

Our study showed that out of all our measured environmental conditions, in-stream nutrients were the primary driver of GPP and ER. SRP was the only parameter significantly associated with both GPP and ER; a finding similar to a study on broad-scale controlling factors of stream metabolism (Mulholland *et al.*, 2001). Furthermore, a study by Bothwell (1989) found that SRP in concentrations of less than 50 µg/L can limit plant growth within lotic systems. SRP concentrations at our downstream site averaged 30 µg/L before and after the release and 574 µg/L during the release. As such, limiting SRP concentrations could have been alleviated with the release of lagoon wastewater effluent resulting in the positive association we observed in GPP to increasing SRP concentrations. The nutrients selected by our regression analysis as predictors of ER were SRP, nitrite-nitrate, and ammonia; since we believe that variation in ER was largely due to bacteria we expect these nutrients were driving rates of bacterial production. Similar to primary production, bacterial production requires inorganic N and P nutrients to construct cellular structures (e.g., phospholipid membranes and proteins) but only when dissolved organic matter (DOM) is readily available (Kirchman, 1994). Bacterial production in lotic systems is not likely limited by nutrients but rather by how quickly CO_2 is converted into organic carbon by primary producers because bacteria typically acquire N and P compounds though grazing of autotrophs (Kirchman, 1994). Therefore, we speculate that the release of lagoon wastewater effluent was accompanied by an increase in DOM concentrations which likely promoted bacterial production and resulted in the uptake of inorganic nutrients explains why we found SRP, nitrite-nitrate, and ammonia to be predictors of ER. However, further work is required to gain a better understanding of what exactly is driving rates of ER in a wastewater stream. Results from our study show that a release of lagoon wastewater effluent contributes drivers of stream metabolism that subsidizes GPP and ER.

2.4.3 Conclusions

To our knowledge there has been no previous studies on the effects of a pulse release of lagoon wastewater effluent on stream metabolism. Our results show that a pulse release of lagoon wastewater effluent into Devil's Creek has a significant impact on physicochemical conditions and stream metabolism. We found evidence suggesting that the release of lagoon wastewater effluent alleviated nutrient limitations in the downstream reach which rapidly subsidized (i.e., within a day or two) ecosystem production (i.e., GPP and ER). Findings of our study compliment past wastewater studies (on how continuous effluent releases effect stream metabolism) by describing at a daily temporal resolution the initial effect wastewater effluent has on downstream function (i.e., stream metabolism) and stream reach physicochemical conditions.

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Chapter 3

Difference in stream metabolism between stream reaches exposed and unexposed to summer releases of lagoon wastewater effluent

3.1. Introduction

Point source pollution to local riverscapes, particularly resulting from the release of treated sewage wastewater effluent, is a key source of pollutants to river systems (Grimm *et al.*, 2008). Nutrients and organic matter are two pollutants commonly associated with the release of wastewater effluent into freshwater ecosystems (Carey *et al.*, 2008; Shon *et al.*, 2006). Thus, wastewater effluent has the potential to impact in-stream processes, such as nutrient cycling and organic matter processing. Past studies on stream/river reaches receiving a continuous supply of wastewater effluent from mechanical wastewater treatment plants have consistently reported increases in downstream nutrient concentrations, nutrient uptake lengths, and the supply of allochthonous carbon (Haggard *et al.*, 2005; Marti *et al.*, 2004; Shon & Vigneswaran, 2006; Gucker *et al.*, 2006; Aristi *et al.*, 2015). Mechanical wastewater treatment plants can reduce their ecological impact by improving effluent quality with the implementation of recently developed treatment technologies (Graham *et al.*, 2014; Shon & Vigneswaran, 2006). However, many smaller urban centres lack the capital resources to employ mechanical treatment plants but rather rely on lower tech approaches to treatment of municipal wastewater such as wastewater treatment lagoons.

In Canada, rural areas with populations of between a few hundred to several thousand rely on wastewater lagoons (also known as stabilization ponds) to treat their sewage (Environment Canada, 1996). As a result, about 50% of the wastewater treatment facilities in Canada are wastewater lagoons (Smith & Finch, 1985). Lagoons differ from mechanical treatment plants in both design and operation. Lagoons are in-ground earthen basins that passively treat wastewater via biological processes (i.e. organic matter breakdown and nutrient cycling) whereas mechanical treatment plants have a series of structures that actively facilitate treatment processes with chemical and mechanical interventions (NRC, 2004; Metcalf & Eddy, 2003). Lagoons and mechanical treatment plants both release effluent as a point source of pollution into receiving aquatic environments. However, lagoons often release wastewater effluent seasonally (e.g. during the open water period) into small streams in short 2-4 week pulses, whereas larger mechanical treatment plants release effluent continuously throughout the year into larger waterways (Prince *et al.,* 1994; Carlson *et al.,* 2013; Gucker *et al.,* 2006; Aristi *et al.,* 2015). Previous studies on the effects of mechanical wastewater treatment plants have given insight into the capacity of downstream environments to biologically mitigate the effects of long-term increases in nutrient and organic carbon concentrations (Aristi *et al.,* 2015; Gucker *et al.,* 2006). However, less is known about how short-term pulses of lagoon wastewater effluent effect environmental conditions of downstream ecosystems.

Stream metabolism is the balance between gross primary production (GPP) and ecosystem respiration (ER) (Grace & Imberger, 2006). Stream metabolism can serve as an indicator of nutrient cycling because nutrients are a requirement for plant growth and rates of plant growth are reflected through GPP (Hall & Tank, 2003). Stream metabolism can also be an indicator of organic matter processing because it directly relates the breakdown of organic carbon into inorganic carbon through ER (Young *et al.,* 2008). Because stream metabolism is mechanistically connected to nutrient uptake/cycling and organic matter processing it makes an effective functional indicator of many human activities that result in ecological disturbances, such as the release of wastewater effluent. Past studies in agriculturally dominated catchments and in streams and rivers receiving wastewater effluent have shown increased rates of GPP and ER to be associated with increasing concentrations of nutrients and organic carbon (Frankforter *et al.,* 2010; Bernot *et al.,* 2006; Aristi *et al.,* 2015; Gucker *et al.,* 2006). These studies provide evidence that sustained nutrient and organic carbon enrichment of streams as a result of human activities are detectable through measures of stream metabolism. However, the sensitivity of stream metabolism to short-term pulses of nutrients and organic matter is less well established.

The goal of this study was to assess the effects of summer releases of lagoon wastewater effluent on stream metabolism within the Prairie biome. This study assessed and compared stream metabolism in reaches exposed and unexposed to lagoon wastewater effluent across Southern Manitoba, from early-May to mid-September, to fulfill the following objectives: 1) compare rates of stream metabolism between reaches exposed and unexposed to lagoon wastewater effluent; 2) compare daily stream metabolism values from reaches when lagoon wastewater effluent was present versus absent; and 3) compare stream metabolism before, during, and after an early and late summer lagoon effluent release. Results of this study will inform point source pollution management strategies aimed at mitigating the downstream environmental impacts of lagoon wastewater effluent.

3.2. Methods

3.2.1. Study Design

This study took place during the spring and summer (late-May to mid-September) of 2014 in 6 low order (i.e., 2^{nd} or 3^{rd}) prairie streams located within the lower Red River valley in Southern Manitoba, Canada (Figure 1). Study sites were located within a distance of 60 km of the city of Winnipeg. Stream conditions were measured in 10 wadable stream reaches that flowed continuously throughout the study period. Stream catchments drained predominately agricultural lands (with effluent exposed reaches draining catchments with 14-68% agricultural land-cover and unexposed reaches draining catchments with 20-87% agricultural land-cover). The studied stream channels were strongly modified through past straightening and entrenchment and generally had minimal riparian vegetation; all stream reaches were characterized as having a stream bed composed of fine particles (i.e., < 2 mm).

Study sites consisted of four treatment reaches receiving treated lagoon effluent whereas the remaining six sites were located on control reaches unexposed to lagoon effluent. Control reaches were located either upstream of the treatment reaches or on streams without a lagoon outfall. Monitoring took place between 2.6 and 6 km downstream from the wastewater lagoon effluent pipe in wastewater reaches. Distances were based on: 1) travel distances required for complete mixing of effluent with stream water; and 2) site accessibility from the local road network. Treatment reaches received effluent from one or two release events over the course of the study period. Effluent release durations were between 2 to 5 weeks with the earliest release starting June 15^{th} and the last release finishing September 15^{th}. Wastewater effluent entering treatment reaches originated from lagoons serving the communities of Oakville, Garson/Tyndall, Niverville, and Steinbach with populations that range from 400 to 14000 individuals (Figure 1) (Statistics Canada, 2012).

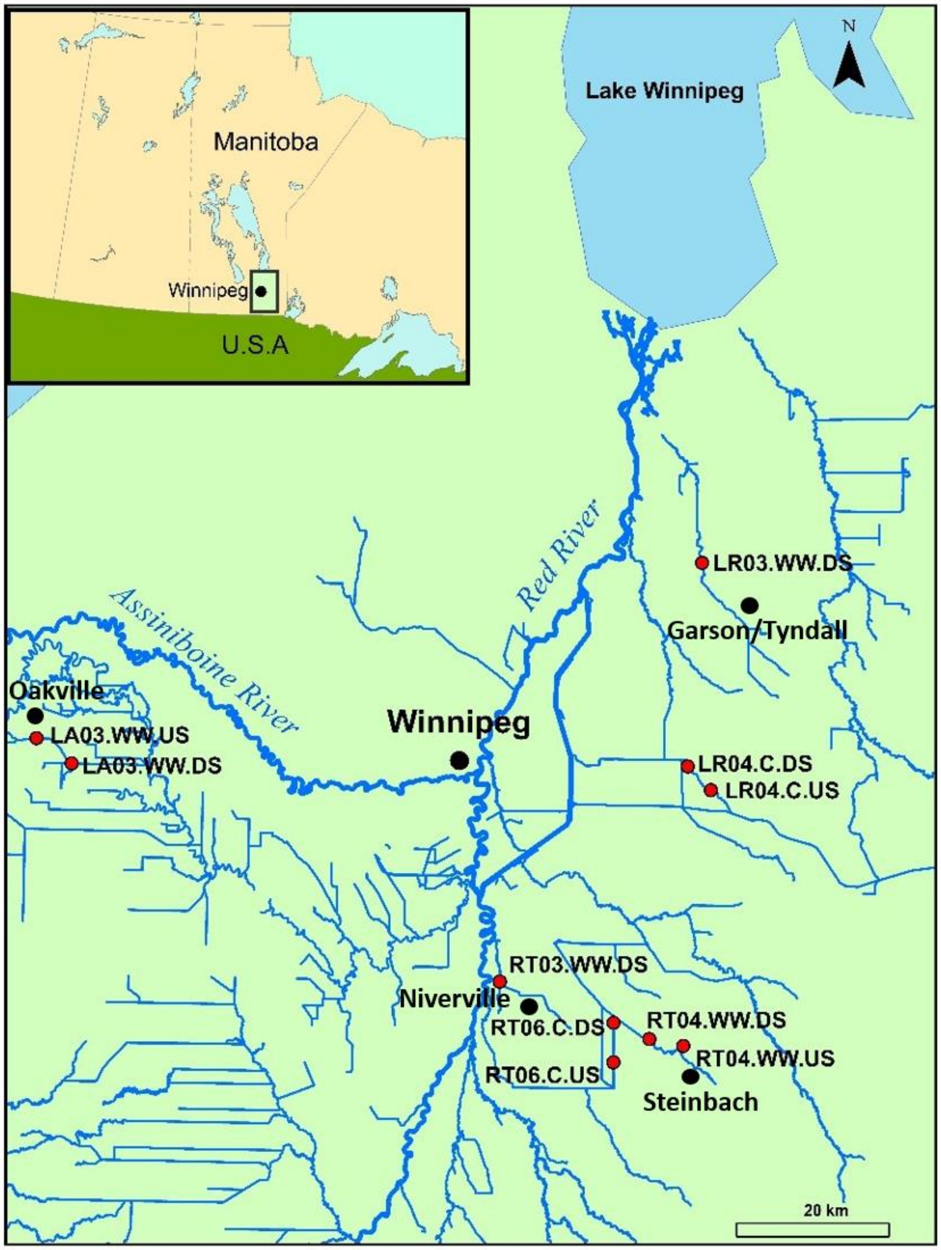

Figure 3.1. Map of ten study sites (red circles) located on stream reaches in the lower Red River Valley in Southern Manitoba that were monitored from late-May until mid-September of 2014. The stream reach code corresponds to the site id code (e.g., LA03), the type of stream segment (either wastewater (WW) or a control (C)), and if it is upstream (US) or downstream (DS) from a lagoon wastewater effluent outfall.

This study compared rates of stream metabolism in stream reaches that received lagoon wastewater effluent (exposure reaches) to reaches that do not (control reaches). Control reaches included any reaches that do not receive wastewater effluent (reach codes containing: “WW.US” or “C”). Exposure reaches must have received effluent at some point during the study period (reach codes containing: “WW.DS”). Two groupings of sites were used to compare wastewater reaches to non-wastewater reaches:

1.) A control-exposure design was used to compare rates of stream metabolism over the entire study period (late-May – mid-September) between a group of reaches that receive wastewater effluent (n=4) and a group of control reaches (n=4) over the entire study period.

2.) A before, during, after, control-exposure design was used to compare rates of stream metabolism surrounding an early and late summer wastewater effluent release. This was done with two groups of sites (LA03-LR04, and RT04-RT06-LR04) each group had an upstream-downstream impact and upstream-downstream control stream reach (the RT04 group had two upstream-downstream control stream reaches). The LA03-LR04 sites were used to compare an early summer wastewater effluent release (June 15- 30) whereas the RT04-RT06-LR04 sites were used to compare a late summer effluent release (August 1 – September 2).

3.2.2. Data Collection

A data logging sonde (YSI sonde model 6600) was deployed in each stream reach. Each sonde was strapped to a 20 cm cinder block and anchored to the stream bank before being placed on the stream bed in a well-mixed section of the reach. Sondes recorded temperature, dissolved oxygen (DO), and depth every 15 minutes for the duration of the study period (late-May to mid-September). Sondes were removed for a day in the middle of July to have their data files uploaded, batteries replaced, and probes cleaned and re-calibrated.

Each sonde recorded water depth every 15 minutes using a differential strain gauge pressure transducer, these measurements were corrected to give average reach depth by taking biweekly empirical measurements of reach depth from late-May until August. Biweekly depth measurements were taken with a wading rod along five evenly spaced transects ascending in ten meter intervals upstream from the sonde. Wetted width was recorded before taking ten evenly spaced depth measurements along each transect (from bank to bank). Distance between intervals along transects were determined by dividing the wetted width by 11. A total of 50 measurements were recorded for each reach during each biweekly sampling period. Depth measurements from each biweekly sampling period were averaged and plotted against corresponding average pressure-depth estimates (averaged over the same time transects were measured) to give a linear regression. The equation of the line of best fit from the regression was used to correct daily sonde depth to give predictions of daily average reach depth. The average R^2 value from all linear models (i.e., all 10 sites) was 0.91(±0.07) with a range of 0.77-0.98. Therefore, based on the strength of these relationships the models were used to estimate average reach depth. Daily average reach depth values were then multiplied by volumetric rates of stream metabolism to give area based rates of stream metabolism.

Photosynthetically active radiation (PAR) loggers (Odyssey PAR Light Logger model Z412) were deployed on top of a 1.5m piece of rebar located on the stream bank in an area with shade (canopy cover) representative of the stream reach (as determined by desiometer measurements). PAR measurements were recorded every 15 minutes and used to indicate the daily duration of PAR to the stream surface.

A densiometer was used to take biweekly estimates of percent canopy cover for each stream reach. Measurements were taken at three stream transects starting at the sonde and ascending in 20m intervals upstream. Four measurements were taken at the middle of each transect facing upstream, downstream, the right bank, and the left bank. Percent canopy cover was estimated for each transect based on the sum of the four measurements divided out of a total of 384. An average of the three transects was taken to give a percent canopy cover estimate of the stream reach for each biweekly sampling period.

Flow velocity and discharge measurements were taken biweekly at each stream at either the upstream or downstream reach. A SonTec Flowtracker was used to take ten measurements of flow velocity along each transect; the transect was chosen based on it being representative of the stream reach and it not having any obscurities (e.g., flow eddies). The Flowtracker calculated stream discharge based on the velocity and depth measurements.

Water samples for analysis of nutrients (ammonia (NH3), nitrate-nitrate (NO2+NO3), total-nitrogen (TN), soluble reactive phosphorous (SRP), total dissolved phosphorous (TDP), and total phosphorous (TP)), total suspended solids (TSS) and turbidity were collected biweekly at each stream. The sampling bottles were rinsed three times with stream water, the rinsate was removed, and the bottles were filled with stream water at 60% stream depth. Samples were shipped to a biogeochemical analytical service laboratory (within three days of being collected) to be analyzed for water nutrient parameters. Nutrients were measured using a Lachat QuickChem QC8500 FIA Automated Ion Analyzer. TN and NO2 + NO3 concentrations were analyzed in accordance to the guidelines outlined by the US EPA (United States Environmental Protection Agency (USEPA), 1993a,b), and NH_3, TP, TDP, and SRP were analyzed in accordance to the guidelines outlined by the APHA (American Public Health Associated (APHA), 2012a,b,c). TSS and turbidity samples were placed in cold storage to be later analyzed for TSS and turbidity. TSS was analyzed in accordance to the guidelines outlined by the APHA, (2012d), and Turbidity was analyzed in accordance to the guidelines outlined by APHA, (2012e).

3.2.3. Stream Metabolism

Stream metabolism was estimated using the single station open-system method (Grace & Imberger, 2006). The reaeration coefficient (K) for each day was calculated using either the night-time regression method or the Delta method (Owens, 1974; Chapra & Di Toro, 1991). The night-time regression method was used for each site except for LR03 where the Delta method was used. The night-time regression method takes into account that photosynthesis does not occur during the night so the only changes observed in the dissolved oxygen (DO) measurements at night are as a result of ER and K (*sensu* Grace & Imberger, 2006). Based on this premise, the rate of change in the DO measurements for each time interval in the nighttime period are regressed against the oxygen deficit (D).

(1) $$\mathbf{D = DO_m - DO_{100\%}}$$

Where D is the oxygen deficit, DO_m is the measured DO, and $DO_{100\%}$ is the DO concentration at 100% saturation. The resultant slope and intercept of DO_m and D are equal to K and ER, respectively (Young & Huryn, 1996; Grace & Imberger, 2006). The night-time regression method was suitable to calculate K for reaches where shading from the bank, riparian vegetation, and clouds was present. Furthermore, the night-time regression method was only used on days where there was no substantial precipitation or changes in flow (as this would interfere with being able to accurately quantify the amount of DO produced from autotrophic communities and the amount of DO dissolving into the stream from the atmosphere). Or when there was a good night-time regression fit ($R = >0.6$) which would give confidence that estimates of stream metabolism will be accurate (*sensu* Grace & Imberger, 2006). A spreadsheet macro developed by Young & Collier (2009) was used to calculate K. The Delta method can only be used for stream reaches with no canopy or during days with minimal cloud cover. The Delta method was used in one stream (LR03) because it was typically slow moving, there was no shade from the bank or riparian vegetation, and the diel changes in DO resulted in a poor night-time regression fit ($R = <0.6$). This method estimates K based on the time lag between solar noon and the daily maximum DO concentration (*sensu* Chapra & Di Toro, 1991).

With an accurate estimate of K, estimates of ecosystem respiration (ER) and gross primary production (GPP) could then be calculated using the following equation (Grace & Imberger, 2006):

(2) $$\mathbf{\Delta DO = GPP - ER \pm K(D)}$$

Where ΔDO is the change in DO concentration during the 15min intervals, GPP is the volume of DO produced via photosynthesis, ER is the volume of DO consumed by cellular respiration, K is the reaeration coefficient, and D is the DO deficit. ER was first estimated during the night-time by setting GPP=0, and inserting the ΔDO, K, and D into equation 1 for each time interval; an average of these values was then taken and used to calculate daytime values by interpolating between night-time ER averaged over each daylight interval. Now with ER values, equation 1 was then rearranged to solve for GPP. Night-time and day-time ER and GPP values were summed and divided by the diel number of time intervals to give daily volumetric rates (g O_2 m^{-3} day^{-1}) of which was multiplied by average reach depth values to give daily areal rates (g O_2 m^{-2} day^{-1}) for both ER and GPP. Daily Net Ecosystem Productivity (NEP) values were then calculated based on the difference in daily GPP and ER rates. The daily production/respiration ratio was calculated by dividing daily GPP by daily ER.

3.2.4. Data Analysis

Control-exposure analysis:

Descriptive statistics were generated from biweekly measurements of physical and chemical parameters (except temperature which was measured daily) for the group of reaches exposed to wastewater effluent (n=4) and the control reaches unexposed to effluent (n=4).

A repeated measures ANOVA was used with an alpha value of 0.1 to test the hypothesis that metrics of stream metabolism of the exposed group significantly differed to the control group. This analysis used daily values of stream metabolism from exposed (n=4) and control (n=4) reaches for the entire study duration. Averages from days where stream metabolism could accurately be calculated from at least 3 of the 4 reaches were used to compose the control and the exposed group. The data for each analysis was first tested for normality using the Shapiro-Wilks method. If parameters failed the normality test they were transformed logarithmically. Data was analyzed using SigmaPlot statistical software (Systat Software Inc, 2015).

A Euclidean distance similarity matrix was generated using daily GPP and ER values for: 1) days when effluent was released in wastewater exposed reaches (hereafter called: exposed reaches-effluent present), 2) days when wastewater was absent in wastewater exposed reaches (hereafter called: exposed reaches-effluent absent), and 3) days in reaches that never receive wastewater effluent (hereafter called: control reaches). The Euclidean distance between each data point was then converted to rankings using nonmetric multidimensional scaling (NMDS). The ordination of this data was then plotted to determine the 2D stress value. If the 2D stress value was less than 0.3 then we were confident that the ordination accurately depicted where the data points fit relative to each other. An analysis of similarities (ANOSIM) was then used to determine the statistical significance between GPP and ER in the three types of reach days. Data was analyzed using Primer statistical software (Primer-E Ltd, 2015).

Before, during, after, control-impact analysis:

An analysis of variance (one-way ANOVA) was conducted on GPP and ER values from the group pairings (LA03-LR04, and RT04-RT06-LR04) after each data set was tested for normality using the Shapiro- Wilks method. If the data set passed the normality test then an ANOVA and Tukey's post-hoc tests were used to determine the statistical significance between comparisons. Data that failed the test was logarithmically transformed, if it still failed then a nonparametric Krustal-Wallis one-way ANOVA and Dunn's post-hoc test was used to determine the statistical significance between comparisons (only used for: LA03-ER). Comparisons were made between (i.e., US vs DS) and within (before vs during vs after) stream reaches. The before period comprised of daily stream metabolism values measured within two weeks before the effluent release, the during period comprised of daily values taken during the effluent release, and the after period comprised of values taken in a period extending two weeks following the termination of the release (RT04-RT06 sondes were pulled 9 days into the after period due to monitoring time constraints). Data was analyzed using SigmaPlot statistical software (Systat Software Inc, 2015).

3.3. Results

3.3.1. Control - Exposure comparison

All measured environmental parameters were larger, on average, for effluent exposed reaches than control reaches (Table 3.1). However, average temperature was similar for exposed and control reaches; both types of reaches also exhibited low variability (i.e., coefficient of variation (CV) = 0.16). Stream discharge, depth, and wetted width for exposed reaches were on average 31%, 23%, and 1% greater than control reaches, respectively. Average turbidity was 4% greater and total suspended sediment (TSS) was 21% greater for exposed than control reaches. However, control reaches showed greater variability in turbidity and TSS (CV = 1.6 and 1.4, respectively) than exposed reaches (CV = 0.77 and 0.89, respectively). Water nutrient concentrations showed the greatest difference between the exposed and control reaches, effluent exposed reaches ranged from a 47% (i.e. NH_3) to a 91% (i.e. NO_2^-+NO_3^{-2}) increase from the control reaches. Average NO_2^-+NO_3^{-2} concentrations for the exposed reaches showed the greatest variation of all descriptive parameters with a CV of 1.84.

All metrics of stream metabolism were larger, on average, in reaches exposed to lagoon wastewater effluent (Table 3.2). Exposed reaches generated averages 63%, 26%, and 34% greater in GPP, ER, and P/R, respectively, than control reaches. Out of these three metrics, the coefficient of variation (CV) was greater at exposed versus control reaches except for GPP, which generated a CV of 0.36 in the exposed and 0.42 in the control reaches. NEP generated similar mean and median values for exposed and control reaches but the exposed reaches exhibited a greater range in values. NEP at exposed reaches ranged from a minimum of -42.2 g O_2 m^{-2} day^{-1} to a maximum of 5.92 g O_2 m^{-2} day^{-1} whereas the control reaches ranged from a minimum of -21.05 g O_2 m^{-2} day^{-1} to a maximum of -1.65 g O_2 m^{-2} day^{-1}.

Table 3.1. Descriptive statistics for environmental parameters in effluent exposed (n=4) and control (n=4) reaches. Reach descriptive statistics were generated from biweekly measurements of environmental parameter taken over the course of the study (late May – mid September) except temperature, which was measured daily. Stream reaches were located within the Red River Valley in Southern Manitoba, Canada.

Parameter	Group	n	Mean	Standard Deviation	Median	Max	Min	C.V.
Temperature (^{o}C)	Exposed	460	19.54	3.09	20.01	25.32	10.20	0.16
	Control	460	19.41	3.06	20.02	24.78	9.60	0.16
Stream Discharge (m^3/se	Exposed	24	0.41	0.41	0.29	1.32	0.00	1.01
	Control	24	0.30	0.23	0.31	0.85	0.00	0.78
Depth (m)	Exposed	19	60.95	18.75	65.84	85.28	25.84	0.31
	Control	21	48.50	19.21	53.48	79.54	7.42	0.40
Wetted Width (m)	Exposed	20	5.84	1.53	5.49	9.40	3.42	0.26
	Control	21	5.79	2.70	4.64	9.46	2.26	0.47
Turbidity (ntu)	Exposed	19	10.19	7.84	10.18	29.25	0.83	0.77
	Control	23	9.78	13.65	5.08	60.87	0.47	1.40
TSS (mg L^{-1})	Exposed	25	9.83	8.75	7.07	34.06	0.40	0.89
	Control	26	7.93	12.65	3.80	64.67	0.40	1.60
NH_3 (μg L^{-1})	Exposed	25	21	24.80	8	95	0	1.17
	Control	28	13	10.64	10	38	0	0.82
NO_2^-+NO_3^-(μg L^{-1})	Exposed	25	171	314.49	40	1,280	0	1.84
	Control	28	64	109.99	6	352	0	1.73
SRP (μg L^{-1})	Exposed	25	579	527.04	391	1,796	13	0.91
	Control	28	224	157.91	206	542	9	0.70

Table 3.2. Descriptive statistics for metrics of stream metabolism that were generated daily from stream reaches exposed and not exposed (i.e. control) to lagoon wastewater effluent; metrics were estimated over the entire study period (late May – mid September) from streams within the Red River Valley in Southern Manitoba, Canada.

Metric	Group	n	Mean	Standard Deviation	Median	Max	Min	C.V.
GPP ($g\ O_2\ m^{-2}\ day^{-1}$)	Exposed	81	12.20	4.28	11.99	21.78	3.18	0.35
	Control	81	6.58	2.66	6.68	13.77	1.00	0.40
ER ($g\ O_2\ m^{-2}\ day^{-1}$)	Exposed	81	-18.74	6.67	-16.77	-47.29	-8.58	0.36
	Control	81	-13.79	4.64	-13.50	-30.86	-2.22	0.34
NEP ($g\ O_2\ m^{-2}\ day^{-1}$)	Exposed	81	-8.30	7.57	-7.67	5.92	-42.30	0.91
	Control	81	-6.47	3.59	-5.43	-1.65	-21.05	0.56
P/R	Exposed	81	0.72	0.27	0.71	1.72	0.17	0.37
	Control	81	0.51	0.12	0.51	0.79	0.15	0.24

Repeated measures ANOVA indicated that GPP, ER, and P/R were significantly greater ($p < 0.001$) in the effluent exposed reaches than in the control reaches (Figure 3.2). NEP was not significantly different between exposed and control reaches with a p-value of 0.351.

NMDS ordination of daily values of GPP and ER revealed substantial similarity between measured values at control and exposed reaches both when effluent was present and absent (Figure 3.3). Separation of sites and periods was largely due to daily values measured at a single site (i.e., LR03.W.DS). However, ANOSIM revealed a significant difference in stream metabolism between control reaches and exposed reaches-effluent present ($P = 0.001$, $R = 0.19$) and effluent exposed reaches-effluent absent ($P = 0.001$, $R = 0.11$). An ANOSIM also revealed a difference between exposed reaches-effluent present and exposed reaches-effluent absent ($P =0.011$, $R = 0.05$).

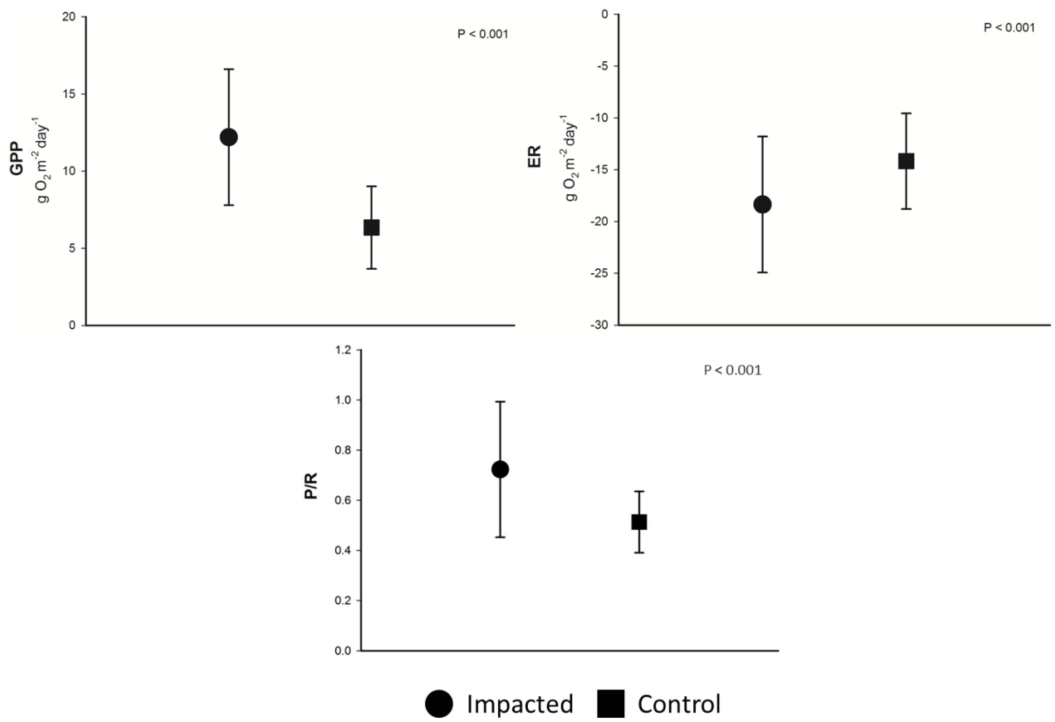

Figure 3.2. Average daily values of stream metabolism (mean ± standard deviation) generated from exposed and control reaches taken over the entire study period (n= 81) (late May – mid September) from streams within the Red River Valley in Southern Manitoba, Canada.

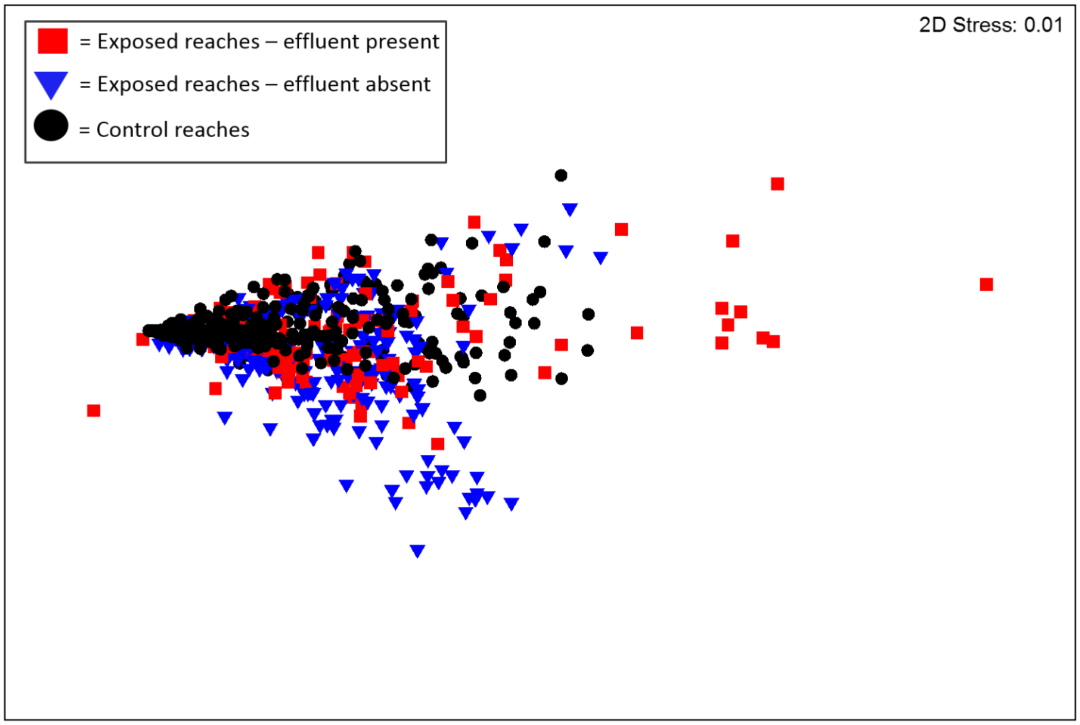

Figure 3.3: Non-metric Multidimensional scaling (NMDS) ordination plot indicating similarity in daily GPP and ER values among three types of stream reaches: 1) exposed reaches with effluent present; 2) exposed reaches with effluent absent, and; 3) control reaches. Days from the entire study period (May 28 – Sept 15) were included within this ordination from 8 stream reaches within the Red River Valley in Southern Manitoba, Canada.

3.3.2. *Before, During, After, Control-Exposure comparison*

During the periods surrounding the early summer effluent release (i.e., before (June 1-14), during (June 15-30), and after the release (July 1-13) the stream reach receiving the effluent exhibited the greatest average GPP and ER (i.e., downstream LA03; Table 3.3). However, an ANOVA indicated that there was no significant difference in GPP among sites between the upstream and downstream for any of the time periods (Before-During, $p = 0.172$; Before-After, $p = 0.106$; During-After, $p = 0.916$). Therefore, there was no significant change in GPP attributable to the effluent release. ER for the downstream LA03 reach increased significant during the effluent release from the before period ($p = 0.075$), however, it did not differ significantly to that of its upstream reach (i.e., upstream LA03) during the effluent release ($p = 0.584$) (Figure 3.4). Furthermore, ER for both the upstream and downstream LA03 reaches increased significantly from the period before the release to after the release ($p = 0.017$ for downstream; $p < 0.001$ for upstream). Therefore, there was no significant change in ER attributable to the effluent release.

During the periods surrounding the late summer effluent release (i.e., before (July 11-31), during (Aug. 1-Sept. 2), and after the release (Sept 3-14) the stream reach receiving the effluent exhibited the greatest average GPP and ER (i.e., downstream RT04; Table 3.4). GPP and ER for downstream RT04 were also less variable than the other reaches with C.V. values of 0.45 and 0.55, respectively. GPP was significantly greater at the downstream RT04 site during the lagoon wastewater effluent release than both the before ($p = 0.026$) and after ($p = <0.001$) periods (Figure 3.5). In contrast, GPP at the upstream RT04 reach did not differ significantly between any of the time periods (Before-During, $p = 0.679$; Before-After, $p = 0.729$; During-After, $p = 0.494$). Furthermore, GPP during the release period was significantly larger at the RT04 downstream site than the upstream site ($p = <0.001$). GPP in the control reaches (RT06 and LR04) was either significantly declining from the period before to during the effluent release or remained the same; thus, GPP was only increasing significantly in the effluent exposed reach during the release period. ER increased significantly at the downstream RT04 reach from the period before to the period during the effluent release but it did not differ significantly to that of its upstream reach and therefore the increase could not be attributed to the effluent release.

Table 3.3. Descriptive statistics for metrics of stream metabolism that were generated daily before, during, and after an early summer lagoon wastewater effluent release (i.e., June 1 – July 13). Metrics of stream metabolism were estimated at an upstream and downstream control and effluent exposed stream reaches within the Red River Valley in Southern Manitoba, Canada.

Metric	Site	Reach	n	Mean	Standard Deviation	Median	Max	Min	C.V.
GPP ($g\ O_2\ m^{-2}\ day^{-1}$)	LA03	US	30	8.51	5.39	6.39	21.59	3.13	0.63
		DS	36	14.25	4.74	13.54	26.73	4.57	0.33
	LR04	US	41	7.01	2.70	6.63	13.84	1.50	0.38
		DS	41	4.83	2.41	4.46	9.65	0.95	0.50
ER ($g\ O_2\ m^{-2}\ day^{-1}$)	LA03	US	30	-16.40	10.27	-12.59	-52.13	-3.95	0.63
		DS	36	-17.84	5.03	-18.27	-32.92	-9.95	0.28
	LR04	US	41	-11.92	5.17	-11.78	-21.37	-2.38	0.43
		DS	41	-8.65	4.51	-7.37	-19.95	-1.21	0.52

Table 3.4. Descriptive statistics for metrics of stream metabolism that were generated daily before, during, and after a late summer lagoon wastewater effluent release (i.e., July 11 – September 14). Metrics of stream metabolism were estimated at an upstream and downstream control and effluent exposed stream reaches within the Red River Valley in Southern Manitoba, Canada.

Metric	Site	Reach	n	Mean	Standard Deviation	Median	Max	Min	C.V.
GPP ($g\ O_2\ m^{-2}\ day^{-1}$)	RT04	US	41	2.59	2.89	1.93	18.79	0.32	1.12
		DS	48	7.14	3.24	7.04	15.52	0.98	0.45
	RT06	US	59	6.00	3.46	5.91	12.01	0.05	0.58
		DS	59	4.51	2.30	4.63	9.03	1.37	0.51
ER ($g\ O_2\ m^{-2}\ day^{-1}$)	RT04	US	41	-10.43	7.52	-9.74	-41.65	-1.88	0.72
		DS	48	-12.40	6.83	-11.94	-40.74	-4.94	0.55
	RT06	US	59	-12.62	7.79	-12.63	-28.67	-0.09	0.62
		DS	59	-8.08	4.49	-8.41	-21.49	-1.74	0.56

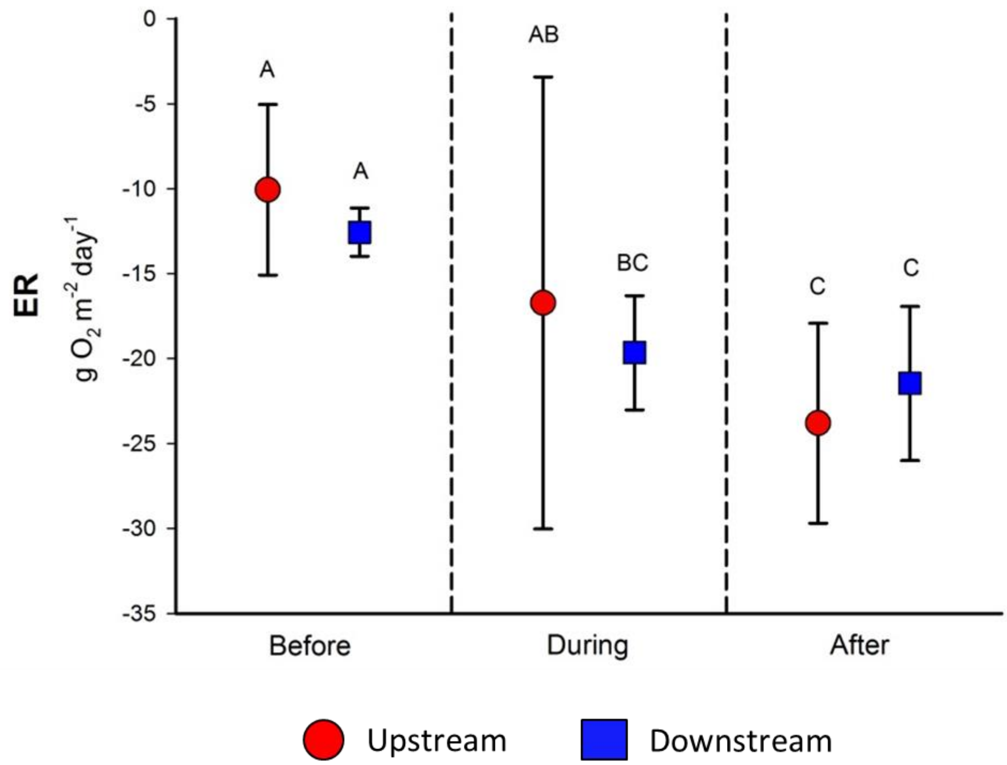

Figure 3.4. Comparison of daily values ER (mean ± standard deviation) at upstream and downstream reaches of LA03 before, during, and after the release of lagoon wastewater effluent during the early summer season (June 1 – July 13).

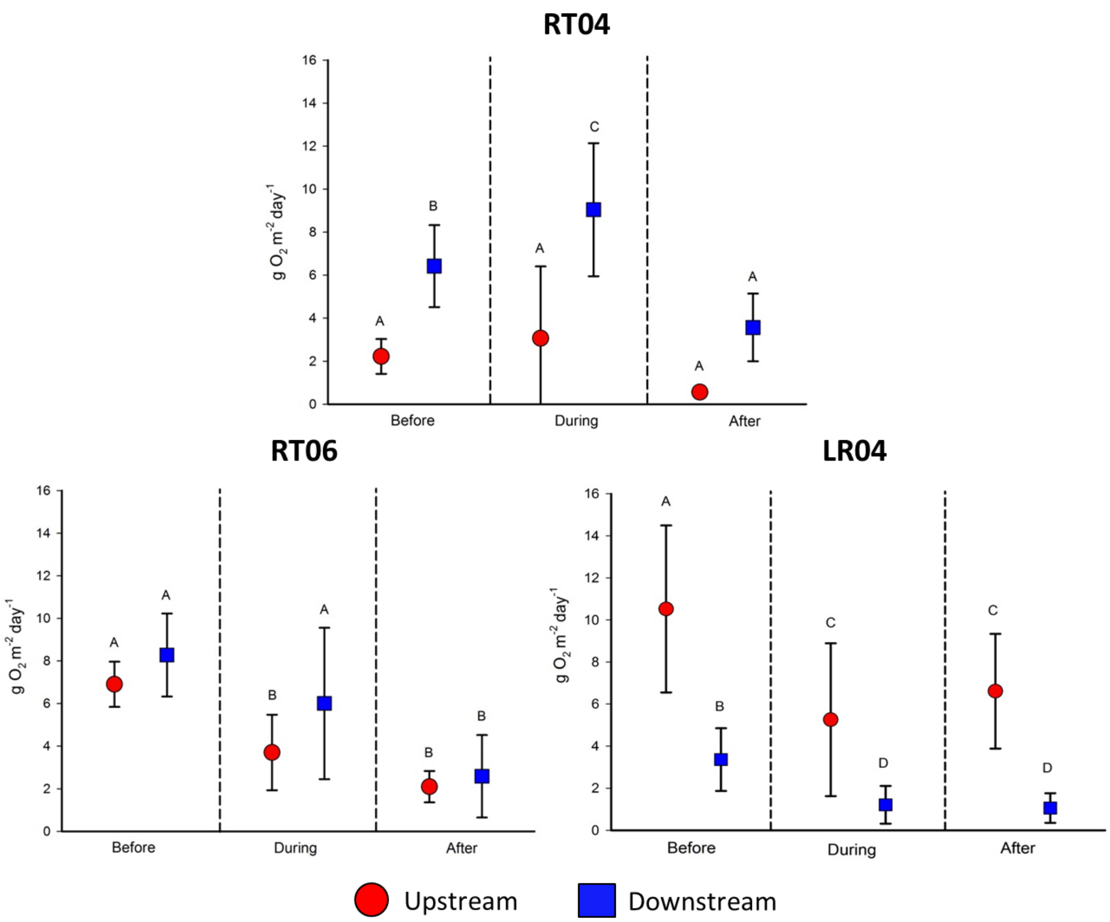

Figure 3.5. Comparison pf daily mean GPP (mean ± standard deviation) at upstream and downstream sites on RT04, RT06, and LR04 in southern Manitoba, Canada before, during, and after a late summer release (July 11 – September 14) of lagoon wastewater effluent.

3.4. Discussion

3.4.1. Control-Exposure comparison

Our study found that reaches exposed to lagoon wastewater effluent have, on average, greater rates of GPP and ER over the summer season than similar stream reaches unexposed to effluent. Previous studies on larger mechanical wastewater treatment plants show comparable results to ours in that rates of GPP and ER also increased in effluent exposed versus unexposed stream reaches (Gücker *et al.,* 2006; Aristi *et al.,* 2015). However, our study design allowed us to determine that stream metabolism rapidly (i.e., within days) returns to pre-exposure levels following the end of the release period. Indeed, our NMDS analysis indicated that outside of the release periods the effluent exposed reaches were comparable to the control reaches. Lagoon effluent releases therefore only have short term effects on stream metabolism, rather than causing a permanent regime shift (*sensu* Carpenter et al., 2008), as would have been suggested if exposure reaches were consistently dissimilar to control reaches for the entire summer season. Thus, we conclude that a small number of pulse releases of lagoon wastewater effluent annually is insufficient to permanently shift the ecosystem into a more productive regime.

Not all exposed reaches showed an equal response to effluent within release periods. For example, one stream reach (i.e., RT03) showed a greater than average increase in ER during the effluent release relative to that of the other exposed reaches. It is unclear why the response of stream metabolism varied among effluent releases. We hypothesize the differences we observed may be due to lagoon characteristics and/or the timing of the release. A study by Gücker *et al.,* (2006) found that large and small wastewater treatment plants have different physicochemical effects (i.e., nutrient and dissolved organic carbon concentrations) on downstream reaches resulting in variable responses of stream metabolism. Our lagoons served populations ranging from 400-14000 people, suggesting that we too could have seen an effect of lagoon size on stream metabolism. Furthermore, a study by Uehlinger (2006) found that rates of stream metabolism during the summer will vary depending on light and temperature regimes.

Our release dates ranged from early (i.e., mid-June) until late (i.e., early-September) summer and thus variation in light and temperature could have influenced the response we observed in stream metabolism to wastewater effluent. Thus, the timing of the release and the lagoon size, or in combination, may account for why we observed variable responses in stream metabolism during effluent release periods. However, further work is required to understand how the effluent release period and the lagoon size may affect stream metabolism.

We found that GPP increased disproportionately (i.e., a significantly larger P/R value) to ER in effluent exposed versus unexposed reaches, suggesting that effluent exposed reaches are more autotrophic than unexposed reaches. Effluent exposed stream reaches therefore enable stream communities to rely more heavily on autochthonous (i.e., instream carbon sources) opposed to allochthonous (i.e., external carbon sources); a finding similar to Gücker *et al.* (2006). They attributed the increase in autotrophic productivity to the increase in nutrient availability in their reach downstream from the wastewater effluent outfall, suggesting that the effluent has a subsidy effect on stream productivity. We also found increased nutrient concentrations (i.e., SRP, NH_3, NO_2+NO_3), on average, in our effluent exposed reaches to that of our control reaches. Effluent associated increases in downstream nutrient concentrations may therefore be an important driver of stream metabolism in our exposed reaches; a conclusion that is consistent with the findings of Chapter 2. Our study therefore shows that pulses of lagoon wastewater effluent may have a significant impact on the trophic dynamics of receiving stream reaches.

3.4.2. Early and late summer effluent release comparison

The late summer release of lagoon wastewater effluent (i.e., August 1-30) appeared to extend the plant growth season in the exposed reach while primary production was concurrently declining in all unexposed reaches. Previous studies have documented that the release of wastewater effluent can cause elevated levels of nutrients in receiving waterways (Carlson *et al.,* 2013; Aristi *et al.,* 2015; Gücker *et al.,* 2006; Andersen *et al.,* 2004; Ekka *et al.,* 2006; Dyer & Wang, 2002). The addition of nutrients into freshwater systems is often accompanied by an increase in plant growth, which is likely why GPP increased in the effluent exposed reach during the effluent release whereas our reaches with no effluent did not (Smith *et al.,* 1999; Gücker *et al.,* 2006). GPP in all the reaches should be declining during the late summer season because temperature directly effects metabolic activity in stream ecosystems, and thus the production of biomass (Phinney *et al.,* 1965). For example, decreasing stream temperature leads to senescence and reduced growth in macrophytes and algae (Uehlinger, 2006; Izagirre *et al.,* 2008). However, a study by (Gudmundsdottir *et al.,* 2011) on the effects of nutrient enrichment and temperature found that chlorophyll A biomass increased regardless of stream temperature during times of nutrient enrichment. Therefore, the addition of lagoon wastewater effluent this late in the summer could have extended the plant growth season when normally GPP would be declining with temperature. Furthermore, our study shows that stream reaches may have greater assimilative capacity for wastewater effluent in colder temperatures than what our unexposed reaches suggest.

Our early summer release (i.e., June 15-30) of lagoon wastewater effluent had no effect on primary production, whereas the late summer release did. It is unclear as to why we did not see a similar increase in GPP at the exposed reach during the early summer release as we did for the late summer release. However, we speculate the difference between the two releases could be due seasonal variation masking our ability to distinguish a GPP response to an effluent release and/or due to the relative sizes of the two lagoons releasing into these reaches. Since these lagoons only released once during our study period we could not compare an early and late summer release from one lagoon and thus control for lagoon size and seasonal variation as plausible causes to differences observed in GPP. We hypothesize that during the early summer effluent release GPP for exposed and unexposed reaches was already increasing due to favorable natural environmental conditions (i.e., increase in temperature and light availability) that may have masked a significant increase in GPP associated with the effluent release. A multiyear study of stream metabolism by Uehlinger (2006) that also took place in a temperate region in a catchment dominated by agriculture found that increases in GPP were strongly correlated with seasonal variation in temperature and light; with GPP tracking rapid increases in temperature and light from mid-June until early July and declines in August and September. We also found that rates of GPP were greater (i.e., 51%), on average, in reaches during periods surrounding the early summer release in June compared to the late summer release in August. Therefore, during the early summer effluent release variation in GPP could largely have been to the result of climactic drivers, such as temperature and light, masking the effect of lagoon effluent on GPP. Results of our study show that an early summer release on wastewater effluent has no impact on primary production, and thus more work is required to understand how temporal variation in stream conditions interferes with which drivers best predict variation in GPP.

The observed differences between GPP associated with the early and late summer effluent releases may also be partly attributable to differences in the sizes of the lagoons being assessed. Our late summer effluent release came from a lagoon that served a population 35 times larger than that of the early summer release leading us to consider that differences in nutrient loads from the two releases could account for the observed difference in the response pattern of stream metabolism. Similarly, a study by Gücker *et al.,* (2006) evaluated the downstream effects of a large and small wastewater treatment plant. They found that the release of effluent from their larger treatment plant resulted in at least a doubling of downstream nutrient concentrations (with an effluent to stream discharge ratio of 2:1) to that of the upstream, whereas nutrient concentrations downstream of the smaller treatment plant were not substantially different from concentrations upstream (with an effluent to stream discharge ratio of 1:110). Our late summer effluent exposed reach saw a 56% average increase in stream discharge at times during the release (in comparison to the month prior) whereas our early summer effluent exposed reach only saw a 17% increase in stream discharge. Due to the larger increase in stream discharge during the late summer effluent release to that of the early summer release it is likely that a larger portion of the stream discharge was attributable to wastewater effluent, whereas the early summer effluent release would have been greatly diluted after entering the stream. Furthermore, Gücker *et al.,* (2006) found higher nutrient uptake rates downstream of their larger treatment plant (relative to their upstream reach), whereas no upstream-downstream difference in nutrient uptake rates was observed for their smaller treatment plant. Therefore, nutrient availability could have been lower during our early summer release than that of the late summer release resulting in no discernable change in GPP. Future studies need to look closely at the operation and scale of lagoons to see how these aspects may influence the degree of impact on downstream GPP.

3.4.3. Conclusion

We found evidence that lagoon wastewater effluent can have a significant effect on stream metabolism. However, the effect appears to be short in duration, our results suggest that the impact of lagoon effluent on receiving reaches is confined to the release period. We know that lagoon wastewater effluent releases do not have a sustained impact on receiving stream reaches, however, we do not know what proportion of the effluent is assimilated in the receiving stream reach and thus its ability to mitigate downstream impacts. Therefore, more work is required to understand if lagoon wastewater effluent can impact downstream environments (e.g., larger river sections and lakes). Our results also show that early and late summer releases of effluent impact stream metabolism differently. However, since we were unable to control variation associated with lagoon size and seasonality we are unsure if the difference we observed in GPP corresponded to when the effluent release occurred or due to the magnitude of the pollutant loads entering the stream associated with large and smaller lagoons. We also found that colder stream reaches still have assimilative capacity for lagoon wastewater effluent. This may influence lagoon operation knowing that effluent can be held later in the productive summer months allowing better effluent treatment, and therefore a higher quality of effluent, while knowing receiving stream reaches still retain assimilative capacity.

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Chapter 4

General Discussion

4.1. Discussion

The goal of this study was to assess the effects of lagoon wastewater effluent on stream metabolism and describe associated physicochemical changes in stream conditions within headwater Prairie stream reaches during the summer season. Previous studies have assessed the effects of continuous effluent releases from larger mechanical wastewater treatment plants on stream metabolism (Gücker et al., 2006; Aristi et al., 2015); however, little attention has been given to how wastewater treatment lagoons affect stream metabolism. I accomplished my goal by describing lagoon wastewater effluent related physicochemical changes in stream conditions and their associated effects on stream metabolism during a release (Chapter 2), and by comparing stream metabolism in reaches exposed to the effects of lagoon wastewater effluent releases with unexposed reaches (Chapter 3). I found that a release of lagoon wastewater effluent has a significant effect on stream metabolism and physiochemical stream reach conditions, and that stream reaches respond and recover quickly following the termination of an effluent release. I also found that effluent exposed reaches, on average, have significantly greater stream metabolism production (i.e., GPP and ER) than unexposed reaches; however, the effect of effluent on stream metabolism may depend on lagoon characteristics and when in the summer season the release occurred. Results of my study have implications for lagoon management strategies and monitoring projects aimed at collected data describing the effects of effluent on stream ecology.

I confirmed my prediction that lagoon wastewater effluent would significantly affect stream metabolism during a release event. It has been known that pollutants associated with the wastewater effluent (e.g., nutrients) from large mechanical treatment plants influence rates of stream metabolism (e.g., Graham *et al.*, 2014; Gücker et al., 2006; Aristi et al., 2015), however, until now it was not known if pulses of effluent from smaller scale wastewater lagoons would have a similar effect. Indeed, in chapter 2 I found that variation in stream metabolism is driven by changes in reach physicochemical conditions associated with wastewater effluent and in chapter 3 I found that GPP and ER responds and recovers quickly (i.e., within a day) to wastewater effluent. My study therefore shows that the presence of pollutants associated with lagoon wastewater effluent can quickly impact stream reach function (i.e., GPP and ER) and equally quickly disappear in the absence of these pollutants.

I predicted that the release of lagoon wastewater effluent would lead to significant changes in reach physicochemical (e.g., nutrients and stream discharge) conditions. In particular, I expected to see increased concentrations of inorganic nutrients because these pollutants are common constituents of wastewater effluent (Carlson *et al.,* 2013; Aristi *et al.,* 2015; Andersen *et al.,* 2004; Ekka *et al.,* 2006; Dyer & Wang, 2002). Indeed, both of my data chapters suggest reaches exposed to lagoon wastewater have greater concentrations of nutrients relative to control reaches. In Chapter 3 I found that effluent exposed reaches were more nutrient enriched than unexposed reaches, but it was not clear if this was due to lagoon wastewater effluent because the systematic biweekly water nutrient sampling did not adequately capture the effluent events due to their short duration (i.e., 2-4 week effluent pulses). However, I partially confirmed our prediction that the release of lagoon wastewater effluent would result in increased concentrations of inorganic nutrients in Chapter 2 where we found a distinct and significant increase in inorganic phosphorous, but not nitrogen, associated with the release of effluent. My results suggest that lagoon effluent may be a significant source of inorganic phosphorous to local waterways. Phosphorous enrichment of aquatic systems is known to cause eutrophication, which often leads to undesirable ecological conditions (*sensu* Smith *et al.,* 1999). It may therefore be beneficial for our studied lagoon to achieve better wastewater phosphorous removal to decrease potential ecological impacts. A study by Cameron *et al.* (2003) showed that it is possible for rural communities with a small tax bracket to implement cost effective phosphorous treatment. They found that constructing a flow-through wetland system, in series to their lagoon, and adding slag filters (a reactive substrate that absorbs phosphorous) removed up to 99% of total phosphorous in the wastewater. Further assessment of ecological effects of lagoon effluent on streams should be conducted to better inform cost-analyses evaluating the environmental trade-offs of financing additional phosphorous removal techniques to allow municipalities to take appropriate action regarding lagoon treatment.

I predicted that nutrients stemming from lagoon wastewater effluent would be the largest driver of stream metabolism during an effluent release period. I confirmed my prediction in Chapter 2, which showed that effluent associated nutrients explained a statistically significant amount of variation in stream metabolism during the release period. In Chapter 3 we observed a significant increase in autotrophic activity over heterotrophic activity (i.e., increase in P/R) in effluent exposed versus unexposed reaches which, based on the results of Chapter 2, may have been caused by effluent associated nutrients. Nutrients are a requirement for plant growth and are often the limiting factor in aquatic systems, and therefore when these systems become nutrient enriched (i.e., eutrophication) it can lead to excessive plant growth and undesirable downstream ecological consequences (Smith *et al.*, 1999). Based on how quickly stream reach ecosystems appear to respond to the presence and absence of lagoon wastewater effluent (Chapter 3) and that effluent associated nutrients appear to be driving these ecological processes (Chapter 2) I speculate that pulse releases of effluent may result in short-term environmental degradation through increases in productivity. However, it is unclear if this type of environmental degradation is confined to the reach immediately downstream from the effluent outfall or if the effects of these effluent releases are further reaching. Previous studies on temperate stream reaches found that the effects of wastewater effluent on stream metabolism were seen as far downstream as monitoring took place (e.g. 4.5km, 50km, and all the way to the river mouth for Aristi *et al.*, 2015, Wasseneer *et al.*, 2010, and Venkiteswaran *et al.*, 2015, respectively). I therefore believe that my headwater streams may not be able to mitigate all the environmental effects associated with a pulse release of lagoon wastewater effluent and that downstream environments are also at risk of nutrient enrichment. For example, our study took place in Red River tributaries, which eventually drain into Lake Winnipeg. Lake Winnipeg is notorious for having and receiving high nutrient loads from a variety of river networks, particularly the Red River, resulting in algal blooms and water quality degradation (Schindler *et al.*, 2012; Jones & Armstrong, 2001). Thus, my study may provide insight on a potential nutrient source that may be contributing to undesirable environmental consequences in Lake Winnipeg.

Future studies should evaluate the assimilative capacity of headwater stream reaches to take up nutrients and thereby mitigate undesirable downstream environmental impacts.

The ability of headwater streams to assimilate lagoon wastewater effluent may depend on when the release occurs. I predicted that a late summer effluent release would have no effect on stream metabolism whereas an early summer release would. Our results suggested the opposite. Late summer effluent releases described in Chapters 2 and 3 showed a significant increase in GPP, whereas the early summer release described in Chapter 3 showed no effluent associated change in GPP or ER. It is unclear why our early summer release did not show a significant increase in GPP, similar to the late summer release, especially given climatic conditions (i.e., longer days and warmer temperatures) would have been more favorable to autotrophs in early summer than in late summer. However, my study was limited in the sense that I was unable to compare a late and early effluent release from the same lagoon because most lagoons in the study region only release effluent once annually. I therefore speculate that I did not see a significant response in reach GPP during the early summer release because climatic seasonal variation could have been masking our ability to distinguish a GPP response to the effluent release (i.e., GPP was already increasing rapidly due to increasing temperature and light which masked the effects of the effluent release; (*sensu* Uehlinger, 2006) and/or due to the relative sizes of the two lagoons releasing into these reaches (e.g., Gücker et al., 2006). However, the addition of nutrients in the late summer appeared to extend the plant growth season as other reaches unexposed to effluent either declined in GPP or remained the same. Therefore, my study shows that headwater streams still have assimilative capacity for lagoon wastewater effluent even when climactic conditions are likely less favorable for metabolism late in the summer season. As a result lagoon operations should consider withholding effluent until late in the summer (if they are not already at holding capacity) which would allow for a longer residence time of the effluent during the warmer months and thus a higher quality of effluent.

4.2. Monitoring implications for lagoon wastewater effluent

The findings of my study have significant implications regarding the effectiveness of stream monitoring programs. My study specifically highlights the importance of choosing an appropriate sampling frequency capable of detecting short-term ecological subsidy events (i.e., pulses of lagoon wastewater effluent) and the importance of choosing a biological indicator that is sensitive to short term events while also being feasible. My study has shown that pulses of lagoon wastewater effluent are short in duration (i.e., 2-4 weeks); thus, the impact of lagoons on river ecosystems may be much greater than previously thought as traditional monitoring programs typically do not sample environmental conditions frequently enough to detect an effluent signal. For example, Manitoba Conservation, the provincial agency responsible for water monitoring in Manitoba, generally collects water nutrient samples on a monthly interval per stream site for their long-term nitrogen and phosphorous monitoring program. Based on my findings this interval is likely too long to detect a lagoon effluent release that may only last 2-4 weeks (Jones & Armstrong, 2001). Thus, current monitoring practices (e.g., Jones & Armstrong, 2001) may grossly underestimate the role lagoons may play in contributing pollutants (e.g., nutrients) to downstream ecosystems. My study illustrated how quickly environmental conditions may change with a pulse event; for example, in Chapter 2 I observed ammonia concentrations went from below detection levels to its highest measured concentration of the study within a period of one day. Therefore, short term pulses of effluent require a high frequency of samples to distinguish effluent associated variation in stream reach water nutrient concentrations. Thus, monitoring programs should integrate an event-based sampling procedure to register the effects of short term events, such as pulses of lagoon wastewater effluent, as they may be contributing to a significant portion of nutrients and other pollutants to local and downstream environments

In order to determine the associated ecological impacts of short term pulses of effluent a biological indicator that exhibits rapid response to stressors is required. Benthic macroinvertebrates (BMI's) are widely used in lotic system monitoring projects; however, they require too long of a time period to integrate environmental conditions into their community structure to produce a clear signal of a short term pulse of lagoon wastewater effluent (e.g., Metcalfe, 1989). Fish assemblages are also a widely used ecosystem indicator; however, because fish can migrate to and from an area of interest (i.e., the stream reach receiving effluent) they are not a suitable indicator of a reach scale disturbance (e.g., Oberdorff *et al.*, 2001). Furthermore, traditional endpoints (i.e., BMI's and fish) which describe ecological structures are not sufficient to capture short-term pulses of lagoon wastewater effluent, and therefore measures of ecosystem function (i.e., stream metabolism) that have the ability to rapidly respond are likely to be more robust. Therefore, based on the results of my study I conclude that stream metabolism seems to be a good indicator of short-term pulses of lagoon wastewater effluent because it responds quickly to environmental change and is directly linked to many key ecosystem services such as nutrient assimilation and carbon cycling.

4.3. Future studies

This thesis has demonstrated that short-term pulses of lagoon wastewater effluent have an effect on stream metabolism. However, my study was just a preliminary estimation of how pulses of lagoon wastewater effluent may impact downstream environments. For example, I found that headwater streams can assimilate effluent associated pollutants (e.g., nutrients). However, I was unable to determine how well these headwater streams can mitigate downstream impacts. Future studies should therefore determine how effective headwater streams are at minimizing downstream effects of lagoon wastewater effluent releases. For example, future studies could take a series of stream metabolism and nutrient uptake rate/length measurements downstream during and surrounding an effluent release event. I was also able to describe how stream metabolism responds to effluent associated physicochemical change in reach conditions late in the summer season, however, it is not clear if these changes can be generalized to different stream reaches (receiving effluent from different lagoons) and/or during different time periods. Future studies should therefore assess the effects of variability in lagoon characteristics (e.g., lagoons that range in the populations they serve), and how releases from the same lagoon during different seasonal time periods (e.g., early versus late summer) may influence stream metabolism. Developing a larger and more robust database to assess the influence of effluent pulses on downstream conditions will grant better predictive outcomes enabling lagoon managers to make informed decision as to when effluent releases should occur and what pollutants require further treatment in order to minimize either local or downstream environmental impacts.

4.4. Conclusion

My study found that effluent associated changes to stream conditions significantly impact downstream ecosystems. In Chapter 2 I determined that rates of stream metabolism are sensitive to the abundance of pollutants associated with the release of lagoon wastewater effluent. In Chapter 3 I determined that the effects of lagoon wastewater effluent on stream metabolism are widespread (e.g., across Southern Manitoba) and contained within the effluent release periods. I therefore conclude that lagoon wastewater effluent poses significant short term threat to receiving ecosystems. I also conclude that stream metabolism has a resolution great enough to distinguish short term changes in environmental conditions associated with anthropogenic activity; as suggested by Yates *et al.* (2013). Stream metabolism should therefore be considered in future stream monitoring projects where systems are exposed to short and unpredictable pulses of lagoon wastewater effluent.

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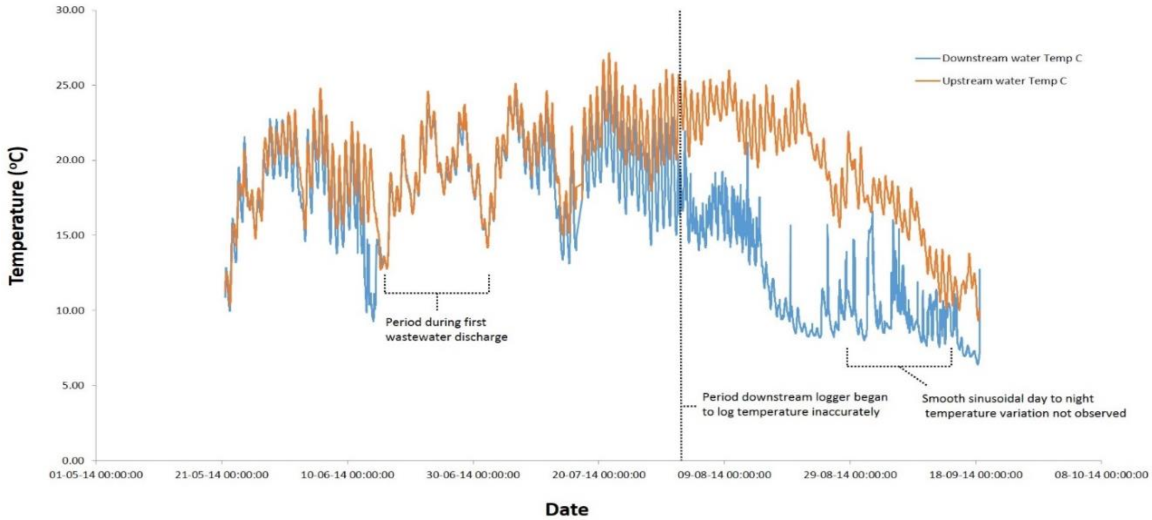
Appendix 1



Temperature measurements logged every 15 minutes from May 28 – September 18 at the upstream (red) and downstream (blue) sites on Devil's Creek. June effluent release took place from June 16 – July 4 when both upstream and downstream loggers were operational and exhibited no differences in temperature between sites. The downstream temperature logger malfunctions at the beginning of August.

Curriculum vitae

Chris. T. Chesworth, M.Sc. Candidate

Department of Geography

The University of Western Ontario

December, 2018

1. EDUCATION

Master of Science (2015) – University of Western Ontario (Geography)

- Thesis Research: The effects of wastewater effluent on stream metabolism within the Red River Valley in Southern Manitoba
- Awards: a) Recipient of NSERC's CREATE WATER scholarship; b) Recipient of a full tuition financial support package from the department of Geography

Bachelor of Science (2011) – University of New Brunswick (Environmental Biology)

- Research: Senior year thesis in topics relating to cellular and marine biology
- Awards: a) Recipient of the Governor Thomas Carleton scholarship; b) Recipient of the Credit Counselling Service scholarship of Atlantic Canada

2. RELEVANT WORK EXPERIENCE

Project Manager (May 2014 – Dec 2015), Tobacco Creek Model Watershed Consortium, Winnipeg, MB.

Research Assistant (May 2013 – Sept 2013), Great Lakes Nutrient Initiative, London, ON.

Teaching Assistant (Sept 2013 – Dec 2015), University of Western Ontario, London, ON.

Research Assistant (May- August 2010, 2011), Provincial Department of Agriculture, Fredericton. NB.

Veterinary Services (Sept 2011 – Feb 2012), Provincial Department of Agriculture, Fredericton, NB.

Research Internship (Feb 2012 – May 2012), Bimini Biology Field Station, Bimini, Bahamas.

3. SKILLS AND CERTIFICATION

- Competent at conducting environmental research/publishing results
- Extensive experience assessing, monitoring, and modelling aquatic environments
- Significant project management experience conducting environmental research in fresh and salt water environments
- Competent using a number of statistical programs (i.e., R, Excel, Sigmaplot, Systat, Primer)
- Knowledgeable in the policies and regulations governing wastewater management (i.e., Wastewater Systems Effluent Regulations in the Fisheries Act).
- Certified field technician in the Canadian Aquatic Biomonitoring Network (CABIN)
- Class 2 Backpack Electrofishing certified
- Certification from the Royal Ontario Museum's identification of Ontario fishes workshop
- Certified in Swift Water Rescue, Wilderness First Aid, and Workplace Standard First Aid with CPR

4. PRESENTATIONS

Chesworth, C.T., Culp, J.M., Chamber, P.A., Brua, R.B. & Yates, A.G. (2015). Changes in stream conditions during a municipal wastewater release and associated effects on stream metabolism. Society of Freshwater Science. May 17-21. Milwaukee, Wisconsin, US.

Chesworth, C.T., Culp, J.M., Chamber, P.A., Brua, R.B. & Yates, A.G. (2015). Comparison of stream metabolism upstream and downstream of a municipal wastewater lagoon discharge.

- University of Western Ontario Earth Day Colloquium. April 9. London, On.
- Canadian Rivers Institute Days. October 30-31. Charlottetown, PEI.