WINTER LIMNOLOGY IN FLOODPLAIN LAKES OF THE SASKATCHEWAN RIVER DELTA, SK

A Thesis Submitted to the College of Graduate
Studies and Research in Partial Fulfillment of the
Requirements for the Degree of Master of
Environment and Sustainability in the School of
Environment and Sustainability

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By Brett D. MacKinnon

PERMISSION TO USE STATEMENT

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work; or, in their absence, permission may be granted by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other uses of materials in this thesis in whole or part should be addressed to:

Dr. Toddi Steelman, PhD

Executive Director, School of Environment and Sustainability
University of Saskatchewan
Room 323, Kirk Hall, 117 Science Place
Saskatoon, SK S7N 5C8
Canada

ABSTRACT

Floodplains are among the most productive and biologically diverse freshwater ecosystems on earth. The exchange of nutrients and biota that occurs within these systems during seasonal inundation is essential in maintaining floodplain and river health. Anthropogenic structures, such as weirs, channels, and dams, have altered the natural flood hydrology of floodplain systems minimizing the frequency, strength and duration of flood events. This reduction ultimately leads to the isolation of important floodplain habitat, such as off-channel lakes, from the main channel, decreasing connectivity. Although some studies have examined the productivity of off-channel floodplain lakes in relation to connectivity, most are limited to tropical or highly degraded systems. Northern floodplains are not as well understood, with most of the research limited to the spring, summer, and fall seasons, when waterbodies are free of ice. With research limited to ice free seasons, there is not a full understanding of the year-round processes that occur within these off-channel lake habitats. This knowledge is crucial as the winter season is often when conditions within these habitats are at their most extreme. Such conditions prevent many fish species from permanent settlement; however, no research has been attempted to understand fish presence within these habitats during the winter season. In tropical systems, hypoxia-tolerant species and juveniles utilize these habitats as refuge from intolerant predators, so such habitat may be used similarly in more northern systems.

The purpose of this research was to understand the connectivity, limnology and suitability as fish habitat of off-channel floodplain lakes in the Saskatchewan River Delta (SRD), SK, during winter months. I determined the degree of connectivity to the main channel for 26 individual lakes within the SRD by two modern methods: remote sensing imagery, and stable isotopes ($\delta^{18}O$, $\delta^{2}H$). Both of these techniques proved effective at determining connectivity of individual lakes and showed good agreement, with lakes arranged into five connectivity categories using remote sensing imagery. Winter limnological conditions within these lakes were significantly influenced by their degree of connectivity, with lakes that were more connected having characteristics similar to that of the river, with higher levels of dissolved oxygen (DO), nitrates (NO_3-NO_2), pH, and lower levels of nutrients (TN,TP). Lakes that were less connected were characterized by low levels of DO and nutrients, and high levels of ammonia/ammonium

(NH$_3$-NH$_4$), conditions that are not favourable for the survival of many fish species. Some of the more hypoxia-tolerant species found within the SRD appear, however, to use these habitats in the winter. This was supported by detection of fish presence using environmental DNA; five fish species were detected in many of the 26 lakes sampled, but only in lakes with NH$_3$-NH$_4$ levels below 1.77 mg/L and volumes greater than 178000 m^3.

Together, these analyses suggest the influence of a spring/summer flood pulse on limnology is not limited to the months following a flood event, but rather extends well into the ice-cover season. This knowledge is critical as it points to controls on key processes (e.g. nutrient cycling, provision of fish habitat) during the period when lake conditions are most severe. As a result of human induced climate change, and from increased water demands for agriculture and hydropower, the natural flood pulse is expected to further decrease in size and frequency in large river-wetlands such as the SRD. This will reduce the connection between the floodplain and the main channel, with profound impacts on the SRD ecosystem as a whole. Lakes that currently experience frequent inundation will likely have conditions characteristic of infrequently flooded lakes, with low DO and nutrients and high NH$_3$-NH$_4$. Lakes which currently experience infrequent inundation will likely dry up completely due to decreased water renewal.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

NH_3-NH_4	ammonia-ammonium
chl-*a*	chlorophyll-a
Cytb	cytochrome oxidase b
DO	dissolved oxygen
DOC	dissolved organic carbon
eDNA	environmental DNA
δ^2H	hydrogen-2 isotopic concentration
LEL	local evaporation line
LMWL	local meteoric water line
MANOVA	multivariate analysis of variance
NO_3-NO_2	nitrate-nitrite
$\delta^{18}O$	oxygen-18 isotopic concentration
PAD	Peace Athabasca Delta
PCA	principal component analysis
SO_4	sulfate
SRD	Saskatchewan River Delta
SWCA	surface water coverage area
TN	total nitrogen
TP	total phosphorus

CHAPTER 1: GENERAL INTRODUCTION

Floodplains are some of most productive and threatened freshwater ecosystems in the world (Tockner et al. 2009). While inundation of floodplains increases the availability of feeding and nursery habitat for local biota (Junk et al. 1989, Tockner et al. 2000; Gorski et al. 2012), anthropogenic changes have altered natural hydrology and reduced connection between the floodplain habitat and river, causing many floodplains to be classified as functionally extinct (Tockner and Stanford 2002). Dams in particular have a large impact on downstream floodplains, altering natural flow regimes by decreasing intra-annual variation, reducing peak floods and the inundation of off-channel habitats (Amoros and Bornette 2002; Miranda 2005; Thomaz et al. 2007). Northern floodplain ecosystems are also at risk from climate change (Natural Resources Canada, 2004), with forecasts predicting a reduction in the peak and total discharge entering these systems with potentially profound impacts on hydro-ecology (Wolfe et al. 2008). Floodplains contain a wide array of habitats, including permanently and temporarily flooded wetlands and lakes that provide critical nursing and feeding habitat for many aquatic and terrestrial species. Because the exchange of nutrients and biota in floodplains during seasonal inundation is essential for the maintenance of these functions, the impact of past and future changes to the hydrology of the system are likely to be significant.

1.1 Wet Season Floodplain Productivity

Inundation of floodplains increases spawning, feeding, and nursery habitat of fishes (Junk et al. 1989, Lindholm et al. 2007, Gorski et al. 2012) and the extent, timing and duration of inundation is key to these increases (Welcomme 1979; Bayley 1991). Extent of inundation describes the spatial increase in the amount of habitat area available for animals to exploit for foraging or spawning (Barko et al. 2006; Lindholm et al. 2007; Gorski et al. 2011a; Van de Wolfshaar et al. 2011; Paul 2012). For example, in the Mitchell River, Australia, large, seasonally available floodplains contributed 33% of the annual diet of a commercially important fish, barramundi (*Lates calcarifer*, Jardine et al. 2012). Timing refers to synchrony of inundation with significant life stages (Junk et al. 1989; Bayley 1991). Cañas and Waylen (2012) described the importance of flood timing for larval catfish which use the pulse for downstream movement. Longer

inundation duration also provides an adequate period for exploitation (Tockner et al. 2000; Gorski et al. 2011b). Prolonged periods of floodplain inundation trigger spawning of local fish species (Galat et al. 1998; Sparks et al. 1998) and temperature is also a key factor, providing optimal thermal conditions for growth and development (Tockner et al. 2000; Gorski et al. 2011a, b). The benefits of flood pulse events for floodplain fish biomass largely accrue to smaller fishes, including young-of–year (YOY) of larger species (Barko et al. 2006; Lindholm et al. 2007; Gorski et al. 2011b).

Ecosystem function of floodplain lakes depends on inundation from the main channel (Junk et al. 1989; Tockner et al. 2000; Amoros and Bornette 2002; Pringle 2003) to develop a wide array of habitats, including permanently and temporarily flooded wetlands and lakes. Habitat complexity is important for floodplain diversity and productivity, and fish species can be found in different areas of a floodplain depending on hydrological and geomorphic characteristics (Siziba et al. 2011; Gorski et al. 2012). Hotspots for overall species richness and biomass include temporarily flooded terrestrial habitats (White et al. 2012; Wu et al. 2013), and for fishes and invertebrates include floodplain lakes and wetlands (Gorski et al. 2012; Wu et al. 2013). Characteristics such as lake depth, turbidity, primary productivity, temperature, and flow velocity can impact the patterns at which fish assemble within off-channel floodplain habitats (Petry et al. 2003; Barko et al. 2006; Gorksi et al. 2011b; Alfermann and Miranda 2013).

The temporary or permanent exploitation of floodplain habitats by fishes is driven by an increase in river discharge and resulting inundation (Junk et al. 1989; Molls et al. 1999; Gorski et al. 2014). The connection between the river and its floodplain during the wet season is essential for the maintenance of fish populations, and is a significant determinate of community structure throughout the floodplain (Tockner et al. 1999; Siziba et al. 2011; White et al. 2012). However, once water levels drop and connections become severed, river water no longer has a direct influence on floodplain characteristics. During the dry season, floodplain habitats become disconnected from the main channel, and internal variables become the main determinates of fish community structure within lakes (Rodríguez and Lewis 1997; Tejerina-Garro et al. 1998; Petry 2003).

1.2 Dry Season Floodplain Productivity

As water recedes from the floodplain, many fish species return from off-channel floodplain water bodies to the river, due to their inability to survive the conditions in these lakes during the period of low water level. Once isolated, there is a hydrological drying of off-channel water bodies within tropical and more temperate floodplains, which leads to severe environmental conditions. To sustain a given species during a dry/winter season in arid or cold regions, permanent water bodies must retain favourable conditions. In tropical floodplains, off-channel lakes are important refugia for fish during dry seasons (Merron and Bruton, 1988; Henderson and Crampton, 1997; Welcomme, 2005). The significance of these off-channel lakes as potential habitat during the dry season is less understood within northern floodplain systems, where substantial ice-cover occurs in winter months. As water levels drop, water quality can decline, with oxygen depletion being of greatest concern for the viability of fishes. Temperature, pH and nutrient content can also be affected (Magoulick 2003). Drops in water level may also affect intra-species and inter-species interactions, by inducing crowding (Matthews and Marsh-Matthews 2003), resulting in increased competition (Lowe-McConnell 1975) and predation (Power 1984; Harvey and Stewart 1991). In addition to the potential anoxic conditions during the dry season, floodplain lakes can also experience anoxia at the start of the wet season. As flood waters initially enter floodplain lakes, due to the high biological oxygen demand, oxygen concentrations can drop drastically causing fish kills (Henderson and Crampton 1997; Mosepele et al. 2011). Even though conditions in some lakes during the dry season can be unforgiving, some fish species are still capable of feeding and even reproducing during this time (Winemiller and Jepsen 1998). Therefore, fish make trade-offs between staying and leaving floodplain lakes prior to the dry season (Matthews 1998).

In winter, in addition to the drying of off-channel water bodies that occurs in tropical and more temperate floodplains, northern floodplain lakes are subject to severe winter weather conditions that include periods of extensive ice-cover. These conditions exacerbate the severe hydrological conditions of floodplain water bodies often resulting in significant fish mortalities, hereafter referred to as winterkills. Ice- and snow-cover can greatly reduce the amount of light reaching the aquatic environment, reducing primary productivity. The reduction of primary productivity during severe ice cover limits photosynthetic oxygen production. In northern lakes, ice cover also prevents the exchange of atmospheric oxygen, further limiting any potential influx

of oxygen into the water column. During ice-cover, oxygen is depleted due to the decomposition of detritus and other matter within the water column, and by various biological and chemical processes within the sediment. In Canadian lakes, the depletion rate of oxygen within the sediment has been measured as 0.08 g O_2 m^2 d^{-1} in oligotrophic lakes, and 0.23 g O_2 m^2 d^{-1} in eutrophic lakes; decomposition within the water column (WOD) is typically ~0.01 g O_2 m^3 d^{-1} (Mathias and Barica 1980). The oxygen levels of lakes decline during ice cover until breakup, or until ice and snow thins enough to allow sufficient penetration of light to induce primary productivity, resulting in rapid recovery of oxygen to normal and even supersaturated levels (Barica and Mathias 1979).

The gradual decrease in oxygen levels in floodplain lakes during ice-cover to potentially anoxic conditions can greatly impact the seasonal and year-to-year assemblage patterns of fish in these habitats. Tolerance to hypoxia varies to a large degree both among (Moore 1942; Gee et al. 1978; Smale and Rabeni 1995) and within (Fox and Keast 1990; Robb and Abrahams 2003) species. Species such as brook stickleback (*Culaea inconstans*) and fathead minnow (*Pimephales promelas*) are well adapted to tolerate hypoxic conditions, even when lake oxygen levels drop below 0.30 mg/L (Klinger et al. 1982). Other fish species, such as walleye (*Sander vitreum*), and rainbow trout (*Salmo gairdneri*), have much higher minimum oxygen tolerance at 2.0-5.5 mg/L (Scherer 1971) and 4.34-9.74 mg/L (Downing 1954; Jones 1971b), respectively. Low oxygen levels within backwaters of floodplains often lead to the emigration of intolerant species (Magnuson et al. 1985). Generally, larger species have a higher critical oxygen concentration, [O_2 critical], and are unable to survive hypoxic or anoxic conditions; this often leads to the use of these habitats by smaller fish species as refuge from larger predators (Chapman et al. 1996; Robb and Abrahams 2002; 2003). Smaller hypoxia- and anoxia-tolerant species survive winterkills by various physiological adaptations, such as the lowering of metabolic and ventilation rates (Klinger et al. 1982); behavioural adaptations, such as reducing activity, migrating to high oxygen microzones such as springs and just beneath the ice (Klinger et al. 1982); and biological adaptations, including the adjustment of haematocrit and haemoglobin concentrations within the blood (Nikinmaa et al. 1984; Robb and Abrahams 2003). In northern floodplains, the significant ice cover that forms during the dry/winter season and the resulting harsh conditions likely promotes differential mortality and emigration of species.

Shallow backwater lakes are also at risk for elevated levels of ammonia, which can be toxic to fish at high concentrations. A by-product of bacterial metabolism and decomposition, at greater concentrations ammonia interrupts cerebral energy metabolism which can lead to convulsions and death (EPA 1989; Randall et al. 2002). At lower concentrations, ammonia can still impact fish survival and fecundity, reducing hatching success, growth rate and development (EPA 1989). The degree of toxicity for ammonia is dependent on numerous factors, including which form of ammonia is present (driven by the pH and temperature of the water), exposure time, and the age of an exposed fish. Ammonia occurs in two forms, un-ionized (NH_3) and ionized (Ammonium, NH_4^+), with the un-ionized being the more toxic of the two. Toxicity of un-ionized ammonia can be attributed to it being a neutral molecule, allowing it to more easily diffuse across the fish gill epithelium (EPA 1998). Hypoxic winter conditions, like those experienced during winter months in northern lakes, elevates ammonia/ammonium concentration in aquatic environments (Johnson 1965; Cong et al. 2009). Because the conversion of ammonium to nitrate (nitrification) is an aerobic process, in low oxygen environments this conversion is inhibited, resulting in elevated ammonia levels. The relative concentrations of the two forms of ammonia are highly temperature and pH dependent, with the ratio of un-ionized ammonia to ammonium ion increasing 10-fold for each unit rise in pH, and 2-fold for each 10°C rise in temperature between 0 and 30°C (Erickson 1985). The effects of higher pH occur because of a reduction in the concentration of hydrogen ions in a given solution, while higher temperatures increase the pKa of a solution, causing a reduction in the strength of the acid. Ammonia and oxygen concentrations together will determine the survival fish species, and ultimately dictate the suitability of floodplain lakes as fish habitat in winter

1.3 – Floodplain Lake Connectivity

Nutrient levels, primary productivity and biotic assemblage patterns are all impacted by connectivity and flood frequency between off-channel aquatic habitats and the parent river. During inundation, floodwaters from the river inundate floodplain habitat and homogenize the limnological characteristics of all habitats (Thomaz et al. 2007). River water typically consists of higher levels of sediment, and greater concentrations of total phosphorus (TP, consisting largely of particle bound P) and various dissolved ions, whereas lake and wetland water has greater concentrations of dissolved phosphorus (dP), and a lower concentration of ions (Sokal et al.

2010). Particle bound P is less bioavailable for uptake by aquatic biota compared to dP, but sediment- and TP-rich flood waters enter the floodplain during inundation and mix with off-channel aquatic habitats. Immediately post-flood, water column TP concentrations of off-channel lakes and wetlands drop as TP and suspended sediment begin to settle. As flood-waters recede, limnological conditions of the recently disconnected floodplains begin to diverge (Junk and Wantzen 2004; Thomaz et al. 2007; Wantzen et al. 2008; Wiklund et al. 2012). During isolation from river water, local processes within individual floodplain habitats lead to increased variation among lakes. These local processes are described by Thomas et al. (2007) as " i) water inputs from lateral tributaries and/or seepage leading to localized physical and chemical characteristics that are basin specific; ii) wind and animal induced sediment re-suspension, which affects water bodies according to their morphometry, and iii) differences in ecological succession." During significant isolation from flood-waters, these local processes leads to reduced turbidity, and higher levels of dissolved organic carbon (DOC), total nitrogen (TN), and bio-available nutrients (Sokal et al. 2010; Wiklund et al. 2012). As inundation rates for aquatic floodplain habitats differ annually and seasonally for each individual habitat, limnological conditions can vary greatly within a floodplain. Consequently, aquatic floodplain habitats that regularly connect to the main channel during floods are the most variable limnologically year-to-year (Sokal et al. 2008; 2010).

The frequency of inundation for an individual lake is dependent on its connectivity to the main channel, and this in turn affects overall lake productivity and limnology (Squires and Lesack 2003; Sokal et al. 2008; 2010; Reid et al. 2012). Connectivity is typically measured by the physical assessment and monitoring of water balances (Peters, 2003), and by surveying sill elevation (Marsh and Hey 1989); however such methods can be field intensive and can be limited in some floodplain systems due to extensive macrophyte growth (Wolfe et al. 2007). In northern floodplains, lakes of high connectivity possess limnological characteristics more similar to that of the main channel, with lower levels of several major nutrients, ions, and chl-*a* compared to more isolated lakes (Table 1.1; Wolfe et al. 2007; Sokal et al. 2008; 2010). Lakes with little to no connectivity to the main channel contain higher levels of dissolved nutrients (TKN, TP, dP, DOC) and higher pH and potassium compared with lakes of higher connectivity (Squires and Lesack 2002; Sokal et al. 2008; 2010). Nutrients and ion concentrations in isolated aquatic waterbodies tend to increase as a result of internal recycling and evaporative enrichment (Junk et al. 1989; Sokal et al. 2008). During significant periods of isolation, with water levels

receding and nutrients continually entering the system through processes such as local run-off, prolonged evaporation begins to concentrate nutrients and ions within these waterbodies.

Table 1.1 Summary of the effects of flooding on limnological variables from research conducted within the Mackenzie, Peace-Athabasca, and Slave River Delta.

System	Variable	Pattern	Reference
Mackenzie River Delta	TSS	↑ in highly connected lakes	Lesack and Marsh 2010
	Inorganic nutrients	↑ in highly connected lakes	
	DOC	↓ in highly connected lakes	
Peace-Athabasca Delta	Alkalinity	↑ in isolated lakes	Wolfe et al. 2007
	Conductivity	↑ in isolated lakes	
	dN	↑ in isolated lakes	
	Total nitrogen (TN)	↑ in isolated lakes	
	dP	↑ in isolated lakes	
	Chl a	↑ in isolated lakes	
	DOC	↑ in isolated lakes	
Peace-Athabasca Delta	TSS	↓ in isolated lakes	Wiklund et al. 2012
	SO_4	↑ in isolated lakes	
	pH	↑ in isolated lakes	
	TP	↑ in river	
	Total nitrogen (TKN)	↑ in isolated lakes	
	dP	↑ in isolated lakes	
	Chl a	↑ in connected lakes	
	DOC	↑ in isolated lakes	
Slave River Delta	Alkalinity	↑ in isolated and flood-dominated lakes	Sokal et al. 2008; 2010
	Conductivity	↑ in isolated and flood-dominated lakes	
	SO_4	↑ in river	
	Total nitrogen (TKN)	↑ in isolated lakes	
	dP	↑ in isolated lakes	
	TP	↑ in river	
	Chl a	↑ in isolated lakes	
	DOC	↑ in isolated lakes	
Slave River Delta	TSS	↑ in flooded lakes	Brock et al. 2007

1.4 Use of Environmental DNA (eDNA) in Floodplains

To examine the presence of fish in lake and wetland habitats, traditional sampling methods typically involved either one, or a combination of, electrofishing, seining, angling, trapping and netting (Nielsen and Johnson 1985; Adamus and Brandt 1990; Knight and Bain 1996). However, these techniques can become problematic when used in certain environments such as wetlands. Wetlands have characteristically muddy/spongy bottoms, and have a significant amount of submerged substrate (e.g. woody debris) and macrophyte growth; making

the implementation of some of the aforementioned techniques difficult. Some sampling techniques, including electrofishing and seining, involve a fair amount of heavy equipment. Wetland sites can often be isolated, and therefore trekking heavy equipment may cause logistical and safety concerns. Additionally, in order to get thorough and comprehensive data on a fish community within a given wetland site, a combination of techniques is often necessary (Knight and Bain 1996). Using multiple traditional techniques effectively within these habitats can be challenging; therefore, it is worth evaluating the ability of novel techniques to provide similar information as traditional methods but in a more logistically realistic manner.

Environmental DNA (eDNA) is the genetic material that can be detected by sampling the non-living environment (e.g., soil, water; Wilson and Wright 2013). DNA that is found within an environment consists of a range of genetic material including chromosomes and plasmids and can be found either free or adsorbed to particles (Siuda et al. 2000). While it has formerly been used to detect the presence of plants, animals and microbes within ancient sediment and ice (Hofreiter et al. 2003; Willerslev et al. 2003; 2007), recently it has become an effective sampling tool in aquatic systems for the detection of particular species (Ficetola et al. 2008; Takahara et al. 2013; Sigsaard et al. 2015) and determining assemblage patterns (Thomsen et al. 2012). If DNA of a particular species is found within a system, one can assume that that species is or has been present. Aquatic species expel DNA into their surroundings through excretion of feces, urine, gametes, and mucous or by the shedding of skin, hair and dead tissue, allowing this DNA to be measured from the environment by collecting water samples. The sample can then be extracted and amplified using Polymerase Chain Reaction (PCR) to enable detection of even very low concentrations of eDNA (Ficetola et al. 2008). The quantitative PCR (qPCR) approach is most commonly used in eDNA analysis and is considered superior to traditional PCR due to its lower detection threshold and greater sensitivity (Goldberg et al. 2013).

The use of eDNA within aquatic environments to detect the presence or absence of species was first evaluated by Ficetola et al. (2008) to detect the invasive American Bullfrog (*Rana catesbeiana*) within ponds in France. They sampled ponds with known invasion by the bullfrog and ponds without invasion. The technique was able to discriminate between absence and presence of frogs, even at low densities, and is now being used to determine the presence of invasive and endangered species within aquatic ecosystems (Jerde et al. 2011; Takahara et al. 2012; Thomsen et al. 2012).

In natural environments DNA rapidly decays due to endogenous nucleases, UV radiation, bacteria and fungi (Shapiro 2008). This rapid decay means that detection of a species' eDNA provides relative certainty that the species was recently present within a given body of water (Ficetola et al. 2008; Thomsen et al. 2012). Dejean et al. (2011) observed this rapid degradation of eDNA within tanks and ponds containing sturgeon and amphibians. The eDNA within the tanks gradually decreased after the removal of the individuals, with no eDNA being present after ~30 days. The persistence of eDNA within a given environment is dependent on numerous factors associated with the environment and the physical structure of the DNA. Environmental factors such as lower temperatures (Kreader 1998), low light exposure (Dick et al. 2010; Green et al. 2011) and anoxia (Borin et al. 2008) preserve DNA. Thus, the winter conditions in northern floodplain lakes may be beneficial for eDNA analysis, as conditions found in these lakes favour DNA preservation, ultimately increasing the chance for eDNA detection.

1.5 Thesis Objective

The overall goal of this thesis was to advance understanding of the ecology of northern floodplains by investigating how winter limnology and fish habitat in floodplain lakes are influenced by connectivity to the main channel, and to improve our understanding of how inundation frequency influences these features by extending sampling into the winter season, when lakes experience significant ice cover. It was conducted in the Saskatchewan River Delta (SRD), SK. The SRD is located at the Saskatchewan-Manitoba border. At an area of 10,000 km², the SRD is the largest active inland delta in North America. It is designated as a Canadian Important Bird Area, and contains numerous economically valuable fish and mammal species. It is located downstream of three large hydroelectric dams, the Francois Finley Dam, the E.B. Campbell Dam and the Gardiner Dam, which have altered the natural flow regime downstream. The SRD also supports local commercial fisheries based in Cumberland House and The Pas. These towns consist of primarily First Nations People (Metis, Cree and Non-status First Nations) that still retain a tight economic, social, and spiritual connection to the river and delta landscape.

The objectives for this study were to:
  i)   determine the connectivity of individual floodplain lakes within the SRD using two modern methods, remote satellite imagery and stable isotopes ($\delta^{18}O$ and δ^2H),

and compare results of the different methods to determine the significance of their agreement,

ii)   ii) use the degree of connectivity for individual lakes to determine the influence that flood frequency has on winter limnological conditions, and

iii)   iii) use the eDNA method to detect fish presence within the lakes during winter to test its effectiveness for sampling under-ice fish presence.

To accomplish this, water chemistry, stable isotopes ($\delta^{18}O$ and δ^2H), and eDNA samples were taken during the winter of 2014 in 26 shallow lakes along a hydrological gradient in the SRD. A total of five lake connectivity categories were determined by combining the stable isotope data with optical remote-sensing images of surface water coverage area from years of varying flood intensities. Based on these classifications, I first compared limnological conditions among the five connectivity categories to understand how conditions related to the connectivity of a lake. Then, I focused on whether, in this northern floodplain, fish presence could be detected using the eDNA method. Fish presence was compared to various lake characteristics, including connectivity and chemistry, to understand how these factors influence fish eDNA detection. I determined the winter limnology of these off-channel lakes in this large and ecologically important northern floodplain system to provide a first look at the winter conditions experienced in these systems, and their potential to act as habitat during a season that has been little studied. Understanding the year-round seasonal variation within these systems can lead to better decision making in the future to mitigate the potential impacts of climate change and increased water retention by upstream impoundments that affect this delta. Additionally, the use of eDNA during the winter season in aquatic ecosystems could more broadly provide us with a tool for the detection and understanding of under-ice fish community structure.

1.6 Literature Cited

Adamus, P. R. and Brandt, K. 1990. Impacts on quality of inland wetlands of the United States: a survey of indicators, techniques, and applications of community-level biomonitoring data. U. S. Environmental Protection Agency EPA/600/3-90/073.406 pp.

Alfermann, T.J. and Miranda, L.E. 2013. Centrarchid assemblages in floodplain lakes of the Mississippi Alluvial Valley. Transactions of the American Fisheries Society **142**: 323-332.

Amoros, C., and Bornette, G. 2002. Connectivity and bio-complexity in water bodies of riverine floodplains. Freshwater Biology **47**: 761-776.

Barica, J., and Mathias, J. A. 1979. Oxygen depletion and winterkill risks in small prairie lake under extended ice cover. Canadian Journal of Fisheries and Aquatic Science **36**: 980-986.

Barko, V. A., Herzog, D. P., and O'Connell, M. T. 2006. Response of fishes to floodplain connectivity during and following a 500-year flood event in the unimpounded upper Mississippi River. Wetlands **26**: 244-257.

Bayley, P. B. 1991. The flood pulse advantage and the restoration of river-floodplain systems. Regulated Rivers: Research & Management **6**: 75-86.

Borin, S., Crotti, E., Mapelli, F., Tamagnini, I., Corselli, C., and Daffonchio, D. 2008. DNA is preserved and maintains transforming potential after contact with brines of the deep anoxic hypersaline lakes of the Eastern Mediterranean Sea. Saline Systems **4**.

Brock, B.E., Wolfe, B.B., and Edwards, T.W.D. 2007. Characterizing the hydrology of shallow floodplain lakes in the Slave River Delta, NWT, using water isotope tracers. Arctic, Antarctic and Alpine Research **39**: 388–401.

Cañas, C.M., and Waylen, P.R. 2012. Modelling production of migratory catfish larvae (Pimelodidae) on the basis of regional hydroclimatology features of the Madre de Dios Basin in southeastern Peru. Hydrological Processes **26**: 996-1007.

Chapman, L. J., Chapman, C. A., and Chandler, M. 1996. Wetland ecotones as refugia for endangered fishes. Biological Conservation **78**: 263-270.

Cong, H., T. Huang, B. Chai, and Zhao, J. 2009. A new mixing–oxygenating technology for water quality improvement of urban water source and its implication in a reservoir. Renewable Energy **34**:2054-2060.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., and Miaud, C. 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS one **6**: e23398.

Dick, L. K., Stelzer, E. A., Bertke, E. E., Fong, D. L., and Stoeckel, D. M. 2010. Relative decay of *Bacteroidales* microbial source tracking markers and cultivated *Escherichia coli* in freshwater microcosms. Applied and environmental microbiology **76**: 3255-3262.

Downing, K. M. 1954. The influence of dissolved oxygen concentration on the toxicity of potassium cyanide to rainbow trout J. Exp. Biol. **3l**: 16l-164.

Erickson, R. J. 1985. An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms. Water Research **19**:1047-1058.

Fox, M.G., and Keast, A. 1990. Effects of winterkill on population structure, body size, and prey consumption patterns of pumpkinseed in isolated beaver ponds. Can. J. Zool. **68**: 2489.2498.

Ficetola, G. F., Miaud, C., Pompanon, F., and Taberlet, P. 2008. Species detection using environmental DNA from water samples. Biology Letters **4**: 423-425.

Galat, D.L. et al. 1998 Flooding to restore connectivity of regulated, large-river wetlands: natural and controlled flooding as complementary processes along the lower Missouri River. Bioscience **48**: 721–733.

Gee, J. H., Tallman, R. F., and Smart, H. J. 1978. Reactions of some great plains fishes to progressive hypoxia. *Can. J. Zool.* **56**: 1962–1966.

Goldberg, C. S., Laramie, M. B., and Waits, L. P. 2013. Application of Environmental DNA for Inventory and Monitoring of Aquatic Species. US Department of the Interior, US Geological Survey.

Gorski, K., L.V. Van Den Bosch, K.E. Van De Wolfshaar, H. Middelkoop, L.A.J. Nagelkerke, O.V. Filippov, D.V. Zolotarev, , J.J. De Leeuw, A.D. Buijse, and J.A.J. Verret. 2011a. Post-damming flow regime development in a large lowland river (Volga, Russian Federation): implications for floodplain inundation and fisheries. River Research and Applications DOI: 1-.1002/rra 1499

Gorski, K., De Leeuw, J. J., Winter, H. V., Vekhov, D. A., Minin, A. E., Buijse, A. D., and
Nagelkerke, L. A. 2011b. Fish recruitment in a large, temperate floodplain: the
importance of annual flooding, temperature and habitat complexity. Freshwater
Biology **56**: 2210-2225.

Górski, K., Collier, K. J., Hamilton, D. P., and Hicks, B. J. 2012. Effects of flow on lateral
interactions of fish and shrimps with off-channel habitats in a large river-floodplain
system. Hydrobiologia 1-14.

Górski, K., Collier, K. J., Hamilton, D. P., and Hicks, B. J. 2014. Effects of flow on lateral
interactions of fish and shrimps with off-channel habitats in a large river-floodplain
system. Hydrobiologia **729**: 161-174.

Green, H. C., Shanks, O. C., Sivaganesan, M., Haugland, R. A., and Field, K. G. 2011.
Differential decay of human faecal *Bacteroides* in marine and freshwater. Environmental
microbiology **13**: 3235-3249.

Harvey, B.C., and Stewart, A.J. 1991. Fish size and habitat depth relationships in headwater
streams. Oecologia **87**: 336-342.

Henderson, P.A., and Crampton, W. G. 1997. A comparison of fish diversity and abundance
between nutrient-rich and nutrient-poor lakes in the Upper Amazon. Journal of Tropical
Ecology **13**: 175-198.

Hofreiter M., Mead J.I., Martin P., and Poinar H.N. 2003. Molecular caving. Current Biology **13**:
693–695.

Jardine, T.D., Pusey, B.J., Hamilton, S.K., Pettit, N.E., Davies, P.M., Douglas, M.M., Sinnamon,
V., Halliday, I.A., and Bunn, S.E. 2012. Fish mediate high food web connectivity in the
lower reaches of a wet-dry tropical floodplain river. Oecologia. **168**: 829-838.

Jerde, C.L., Mahon, A.R., Chadderton, W.L., and Lodge, D.M. 2011. "Sight-unseen" detection
of rare aquatic species using environmental DNA. Conservation Letters **4**:150-157.

Johnson, M. G. 1965. Limnology of Ontario ponds in relation to winterkill of largemouth bass.
The Progressive Fish-Culturist **27**:193-198.

Jones, D. R. 1971a. Theoretical analysis of factors which may limit the maximum oxygen uptake
of fish: the oxygen cost of the cardiac and branchial pumps. J. Theor. Biol. **32**: 341-349.

Junk, W.J., Bayley, P.B., and Sparks, R.E. 1989. The flood pulse concept in river-floodplain
systems. In Proceedings of the International Large River Symposium (LARS), ed. by

D.P. Dodge, pp. 110-127. Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, Canada.

Junk, W. J. and Wantzen, K. M. 2004. The flood pulse concept: New aspects, approaches, and applications—an update. In Welcomme, R. and T. Petr (eds), Proceedings of the 2nd Large River Symposium (LARS), Pnom Penh, Cambodia. Bangkok. RAP Publication: 117–149.

Knight J.G., and Bain M.B. 1996. Sampling fish assemblages in forested floodplain wetlands. Ecology of Freshwater Fish **5**: 76-85.

Kreader, C. A. 1998. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water. Applied and environmental microbiology **64**: 4103-4105.

Klinger, S. A., Magnuson, J. J., and Gallepp, G. W. 1982. Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. Environmental Biology of Fishes **7**: 113-120.

Lesack, L. F. W., and P. Marsh. 2010. River-to-lake connectivities, water renewal, and aquatic habitat diversity in the Mackenzie River Delta, Water Resour. Res. **46**: W12504, doi:10.1029/2010WR009607.

Lindholm, M., Hessen, D.G., Mosepele, K., and Wolski, P. 2007. Food webs and energy fluxes on a seasonal floodplain: the influence of flood size. Wetlands **27**: 775-784.

Lowe-McConnell, R. H. 1975. Fish Communities in Tropical Freshwaters. Longman Inc., New York. 337 pp

Magnuson, J. J., Beckel, A. L., Mills, K., and Brandt, S. B. 1985. Surviving winter hypoxia: behavioral adaptations of fishes in a northern Wisconsin winterkill lake. Environmental Biology of Fishes **14**: 241-250

Magoulick, D. D., and Kobza, R. M. 2003. The role of refugia for fishes during drought: a review and synthesis. Freshwater biology **48**: 1186-1198.

Marsh, P. and M. Hey. 1989. The flooding hydrology of Mackenzie Delta lakes near Inuvik, N.W.T., Canada. Arctic **42**: 41-49.

Mathias, J. A., and Barica, J. 1980. Factors controlling oxygen depletion in ice-covered lakes. Canadian Journal of Fisheries and Aquatic Science **37**: 185-194.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. Springer.

Matthews, W. J., and Marsh-Matthews, E. 2003. Effects of drought on fish across axes of space, time and ecological complexity. Freshwater Biology **48**: 1232-1253.

Merron, G.S. and Bruton, M.N. 1988. The ecology and management of the fishes of the Okavango delta, Botswana, with special reference to the role of the seasonal floods. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa. Investigational Report **29**: 291.

Miranda, L.E. 2005. Fish assemblages in oxbow lakes relative to connectivity with the Mississippi River. Transactions of the American Fisheries Society **134**: 1480–1489.

Molls, F. 1999. New insights into the migration and habitat use by bream and white bream in the floodplain of the River Rhine. Journal of Fish Biology **55**: 1187–1200.

Moore, H. B. 1964. Marine Ecology, 3rd ed. John Wiley and Sons, Inc., London.493 P.

Mosepele K., Mosepele B., Bokhutlo T., and Amutenya K. 2011. Spatial variability in fish species assemblage and community structure in four subtropical lagoons of the Okavango delta, Botswana. Physics and Chemistry of the Earth **36**: 910–917.

Natural Resources Canada. 2004. Climate change impacts and adaptation: a Canadian perspective.

Nielsen, L.A. and Johnson, D.L. 1985. Fisheries Techniques. American Fisheries Society, Bethesda, MD.

Nikinmaa, M., Cech Jr, J. J., and McEnroe, M. 1984. Blood oxygen transport in stressed striped bass (*Morone saxatilis*): role of beta-adrenergic responses. Journal of Comparative Physiology **154**: 365-369.

Paul, A.J. 2012. Environmental flows and recruitment of walleye *(Sander vitreus)* in the Peace-Athabasca Delta. Canadian Journal of Fisheries and Aquatic Sciences **70**: 307-315.

Peters D.L. 2003. Controls on the persistence of water within perched basins of the Peace-Athabasca Delta, northern Canada. Ph.D. Thesis, Watershed Ecosystems Graduate Program, Trent University, Peterborough, Ontario, Canada, pp. 194.

Petry, P., Bayley, P. B, and Markle, D. F. 2003. Relationships between fish assemblages, macrophytes and environmental gradients in the Amazon River floodplain. Journal of Fish Biology **63**: 547-579.

Power, M. E. 1984. Depth distributions of annored catfish: predator-induced resource avoidance? Ecology **65**: 523-528.

Pringle, C. 2003. What is hydrologic connectivity and why is it ecologically important? Hydrological Processes **17**: 2685-2689.

Randall, D. J., and Tsui, T. K. N. 2002. Ammonia Toxicity In Fish. Mar Pollut Bull. **45**: 17-23.

Reid, M. A, Delong, M. D., and Thoms, M. C. 2012. The influence of hydrological connectivity on food web structure in floodplain lakes. River Research and Applications **28**: 827–844.

Robb, T., and Abrahams, M. V. 2002. The influence of hypoxia on risk of predation and habitat choice by the fathead minnow, Pimephales promelas. Behavioral Ecology and Sociobiology **52**: 25-30.

Robb, T., and Abrahams, M. V. 2003. Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? Journal of Fish Biology **62**: 1067–1081.

Rodríguez, M.A., and Lewis, W.M. Jr. 1997. Structure of fish assemblages along environmental gradients in floodplain lakes of the Orinoco River. *Ecological Monographs* **67**: 109-128.

Scherer, E. 1971. Effects of oxygen depletion and of carbon dioxide buildup on the photic behavior of the walleye (Stizostedion vitreum vitreum).J . Fish. Res. Board Can. **28**: 1303-130

Shapiro, B. 2008. Engineered polymerases amplify the potential of ancient DNA. Trends Biotechnol **26**: 285–287.

Sigsgaard, E.E., Carl, H., Møller, P.R., and Thomsen, P.F. 2015. Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. Biol. Cons. **183**: 46–52.

Siuda, W., and Chrost, R. J. 2000. Concentration and susceptibility of dissolved DNA for enzyme degradation in lake water-some methodological remarks. Aquatic Microbial Ecology **21**: 195-201.

Siziba, N., Chimbari, M. J., Masundire, H., and Mosepele, K. 2011. Spatial and temporal variations of micro-invertebrates across temporary floodplains of the lower Okavango Delta, Botswana. Physics and Chemistry of the Earth, Parts A/B/C **36**: 939-948.

Smale, M.A., and C.F. Rabeni. 1995a. Hypoxia and hypothermia tolerances of headwater stream fishes. Transactions of the American Fisheries Society **124**: 698-710.

Sokal, M. A., Hall, R. I., and Wolfe, B. B. 2008. Relationships between hydrological and limnological conditions in lakes of the Slave River Delta (NWT, Canada) and

quantification of their roles on sedimentary diatom assemblages. Journal of Paleolimnology **39**: 533-550.

Sokal, M. A., Hall, R. I., and Wolfe, B. B. 2010. The role of flooding on inter-annual and seasonal variability of lake water chemistry, phytoplankton diatom communities and macrophyte biomass in the Slave River Delta (Northwest Territories, Canada). Ecohydrology **3**: 41-54.

Sparks, R. E., Nelson, J. C., and Yin, Y. 1998. Naturalization of the Flood Regime in Regulated Rivers. Bioscience **48**: 706–720.

Squires, M. M., and Lesack, L. F. 2002. Water transparency and nutrients as controls on phytoplankton along a flood-frequency gradient among lakes of the Mackenzie Delta, western Canadian Arctic. Canadian Journal of Fisheries and Aquatic Sciences **59**: 1339-1349.

Squires, M. M., and Lesack, L. F. 2003. The relation between sediment nutrient content and macrophyte biomass and community structure along a water transparency gradient among lakes of the Mackenzie Delta. Canadian Journal of Fisheries and Aquatic Sciences **60**: 333-343.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z. I. 2012. Estimation of fish biomass using environmental DNA. PloS one **7**: e35868.

Takahara, T., Minamoto, T., and Doi, H. 2013. Using Environmental DNA to Estimate the Distribution of an Invasive Fish Species in Ponds. PloS one **8**: e56584.

Tejerina-Garro, F.L., Fortin, R., and Rodriguez, M.A. 1998. Fish community structure in relation to environmental variation in floodplain lakes of the Araguaia river, Amazon basin. Environmental Biology of Fishes **51**: 399-410.

Thomaz, S. M., Bini, L. M., and Bozelli, R. L. 2007. Floods increase similarity among aquatic habitats in river-floodplain systems. Hydrobiologia **579**: 1-13.

Thomsen, P., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P. and Willerslev, E. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology **21**: 2565-2573.

Tockner, K., Schiemer, F., Baumgartner, C., Kum, G., Weigand, E., Zweimüller, I., ... and Layzer, J. B. 1999. The Danube restoration project: species diversity patterns across

connectivity gradients in the floodplain system. Regulated Rivers: Research & Management **15**, 245-258.

Tockner, K, Malard, F., and Ward, J.V. 2000. An extension of the flood pulse concept. Hydrological Processes **14**: 2861-2883.

Tockner K., Uehlinger, U., and Robinson, C.T. 2009. Rivers of Europe. Academic Press, San Diego.

Tockner K., and Stanford, J.A. 2002. Riverine flood plains: present state and future trends. Environmental Conservation **3**: 308-330.

US Environmental Protection Agency, 1989. Summary review of health effects associated with ammonia. Washington, DC (EPA/600/8-89/052F).

EPA (U.S. Environmental Protection Agency). 1998. 1998 Update of Ambient Water Quality Criteria for Ammonia. Office of Water. EPA 822-R-98-008. Washington, D.C. 148 p.

Van de Wolfshaar, Middelkoop, K. E., H., Addink, E., Winter, H. V., and Nagelkerke, L. A. J. 2011. Linking flow regime, floodplain lake connectivity and fish catch in a large river-floodplain system, the Volga-Akhtuba floodplain (Russian Federation). Ecosystems **14**: 920–934.

Wantzen, K, Junk, W, and Rothhaupt, K. 2008. An extension of the flood pulse concept (FPC) for lakes. Hydrobiologia **613**: 151-170.

Welcomme, R.L. 1979. Fisheries Ecology of Floodplain Rivers. Longman Group Limited, New York.

Welcomme, R. L. 2005. Impacts of fish on inland fish populations in Africa, p. 81–84. In M. L. Thieme, R. Abell, M. L. J Stiassny, P. Skelton, B. Lehner, G. G. Teugels, E. Dinerstein, A. K. Toham, N. Burgess and D. Olson [eds.], Freshwater ecoregions of African and Madagascar: A conservation assessment. Island Press, Washington.

White, S.M., Ondrackova, M., and Reichard, M. 2012. Hydrologic connectivity affects fish assemblage structure, diversity, and ecological traits in the unregulated Gambia River, West Africa. Biotropica **4**: 521-530.

Wiklund, J. A., Hall, R. I., and Wolfe, B. B. 2012. Timescales of hydrolimnological change in floodplain lakes of the Peace-Athabasca Delta, northern Alberta, Canada. Ecohydrology **5**: 351-367.

Willerslev E., Hansen A.J., Binladen J., Brand T.B., and Gilbert M.T.P. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science **300**: 791–795.

Willerslev E., Cappellini E., Boomsma W., Nielsen R., and Hebsgaard M.B., 2007. Ancient biomolecules from deep ice cores reveal a forested Southern Greenland. Science **317**: 111–114.

Wilson, C. and Wright, E. 2013. Using Environment DNA (eDNA) as a Tool in Risk-Based Decision-Making. Ontario Ministry of Natural Resources, Aquatic Research and Development Section, Aquatic Research Series 2013- 01

Winemiller, K. O., and Jepsen, D. B. 1998. Effects of seasonality and fish movement on tropical river food webs. Journal of Fish Biology **53**: 267-296.

Wolfe, B. B., Karst-Riddoch, T. L., Hall, R. I., Edwards, T. W. D., English, M. C., Palmini, R., McGowan, S., Leavitt, P. R., and Vardy, S. R. 2007. Classification of hydrological regimes of northern floodplain basins (Peace–Athabasca Delta, Canada) from analysis of stable isotopes (δ^{18}O, δ^2H) and water chemistry. Hydrological Processes **21**: 151–168.

Wolfe, B. B., Hall, R. I., Edwards, T. W., Vardy, S. R., Falcone, M. D., Sjunneskog, C., ... and van Driel, P. 2008. Hydroecological responses of the Athabasca Delta, Canada, to changes in river flow and climate during the 20th century. Ecohydrology **1**: 131-148.

Wu, N., Górski, K., and Daniel, A. J. 2013. Abundance of larval native and non-native fishes in floodplain habitats of the lower Waikato River, New Zealand. Inland Waters **3**: 359-368.

CHAPTER 2: INFLUENCE OF HYDROLOGICAL CONNECTIVITY ON WINTER LIMNOLOGY IN FLOODPLAIN LAKES OF THE SASKATCHEWAN RIVER DELTA, SASKATCHEWAN[1]

Globally, hydrological connectivity between rivers and their floodplains has been reduced by river flow management and land transformation. The Saskatchewan River Delta is North America's largest inland delta and a hub for fish and fur production. To determine the influence of connectivity on limnology within this northern floodplain, water chemistry and stable isotopes ($\delta^{18}O$ and δ^2H) were analyzed during the winter of 2014 in 26 shallow lakes along a hydrological gradient. A total of five lake connectivity categories were determined by optical remote-sensing images of surface water coverage area from years of varying flood intensities. Accuracy of categories were verified by degree of ^{18}O and ^2H enrichment within lakes. Both isotopes showed marked successional enrichment between connectivity categories with more isolated lakes exhibiting greater enrichment. Water chemistry in lakes with greater connectivity to the main channel were characterized by higher pH, dissolved oxygen, nitrates and sulfates, and lower total nitrogen, total phosphorus, and ammonia/ammonium, compared to more isolated lakes. These findings illustrate how connectivity influences water chemistry in northern floodplain lakes and how it might determine the suitability of these lakes as winter refuge for fishes. Additionally, our study provides supporting evidence for the effective use of optical remote sensing imagery, an inexpensive and accessible source of data for researchers, when determining connectivity characteristics of large northern floodplain systems. Additionally, this study provides further evidence that the inundation of floodplain lakes by river water during peak discharge has an impact on the conditions within the lakes long into the winter ice-cover season. Understanding the year-round influence of river-floodplain connection is imperative for assessing potential impacts of climate change and future water regulation on such ecosystems.

2.1 Introduction

    Floodplains are among the most productive and threatened ecosystems on earth. As a result of anthropogenic river flow management and land transformation, 90% of floodplains within North America have become functionally extinct (Tockner and Stanford 2002). The most characteristic process within a river-floodplain system, the flood pulse, is a key driver of the high biodiversity and seasonal productivity observed in these disturbance-dominated environments (Welcomme 1979; Junk et al. 1989; Tocker and Stanford 2002). Yearly and seasonal variability in river-discharge creates a mosaic of limnological conditions throughout the floodplain (Tockner et al. 2000; Amoros and Bornette 2002). The properties of a pulse event, including amplitude, duration, frequency and magnitude, combines with the degree of lateral connection a site has to the main channel to ultimately shape the biotic and abiotic properties within off-channel habitat (Junk et al. 1989; Wolfe et al. 2007; Sokal et al. 2008; 2010). Due to the spatial heterogeneity and sheer breadth of floodplain valleys, connectivity classes are often created for water bodies within a river-floodplain system, with each class possessing a characteristic set of limnological and ecological conditions (Tockner et al. 2000; Wolfe et al. 2007; Sokal et al. 2008; 2010; Brock et al. 2009). Within-class variation also exists even in the absence of overbank flows as a result of subsurface connection by hyporheic exchange (Mertes 1997; Tockner et al. 2000) and surface connection through levee breaks and small channels (Brock et al. 2007; Sokal et al. 2008) that maintain some degree of influence from the main channel on limnological conditions.

    During inundation from a pulse event, floodwaters from the river overflow the banks, inundating floodplain habitat and homogenizing the limnological features of floodplain water bodies to conditions more characteristic of the main channel (Thomaz et al. 2007). River water that typically carries higher levels of sediment and greater concentrations of most nutrients, mixes with flood-connected lake water that often has high concentrations of organic detritus and algal biomass, ultimately introducing nutrients into the usually autogenic system of a floodplain lake. After floodwaters begin to recede and connection to the main channel is severed, floodplain lakes begin to take on local characteristics (Junk and Wantzen 2004; Pithart et al. 2007; Thomaz et al. 2007; Wantzen et al. 2008; Wiklund et al. 2012). Local processes within individual water bodies, such as overland flow from rainfall or snowmelt, seepage from local aquifers or other subsurface water sources, and sedimentation begin to impact the physical and chemical

conditions of a disconnected lake (Thomaz et al. 2007). If off-channel waterbodies become isolated from flood-waters for a significant amount of time, the local processes mentioned above, along with evaporative enrichment, create isolated water bodies that have less turbid water, and higher dissolved organic carbon (DOC), total nitrogen (TN), and bio-available nutrients (Sokal et al. 2010; Wiklund et al. 2012).

Accurately assessing the hydrological connectivity gradient of a river-floodplain system is imperative in order to determine its impact on off-channel limnology. More conventional methods for determining connectivity of off-channel habitats include physical assessment of floodplain topography (Gibson et al. 1996; Peters 2003) and monitoring of water balances (Mackay 1963; Marsh and Hey 1989), both heavily field-intensive methods. An alternative method, optical remote sensing, has proven successful in monitoring floodplain inundation in many tropical (Hess et al. 2003; Ward et al. 2013; 2014) and temperate (Pavelsky and Smith 2009; van de Wolfshaar et al. 2011; Long and Pavelsky 2013) systems. Optical remote sensing, however, can be limited by dense vegetation, smoke, and cloud cover, as they can obscure image clarity. This limitation, along with the high cost of accessing microwave remote sensing images that can penetrate many obstructions, calls for combined approaches. On-ground spot measurements of stable isotopes of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) have been shown to be a cost-effective and accurate method for assessing connectivity within a floodplain system because evaporative enrichment occurs in lakes less frequently inundated, leading to greater concentrations of the heavy isotopes. This method proved to be effective for two large Canadian deltaic systems (Slave River Delta and Peace-Athabasca Delta, PAD) in classifying basin-wide off-channel lake hydrology (Brock et al. 2007; Wolfe et al. 2007). Studies applying both remote sensing and stable isotope methods to assess connectivity classes of off-channel lakes have not been conducted within river-floodplain systems, and could prove effective in evaluating the accuracy of optical remote sensing in determining river-floodplain hydrology.

In this study, we characterised connectivity and determined its influence on winter limnology within off-channel lakes and wetlands (hereafter referred to as lakes) of the Saskatchewan River Delta (SRD), a large and productive inland delta with a flood regime that has been altered by upstream river flow management (Sagin et al. 2015). Our overall aim was to evaluate the use of combined optical remote sensing and stable isotope methods to determine hydrological connectivity of large river floodplains, and better understand the influence of river

flooding on limnology within these systems. First, we determined connectivity classes for SRD lakes using a series of optical remote-sensing images representing different flood stages for the SRD (Sagin et al. 2015). Next, we compared these classes with stable isotope composition measured in each of the lakes during winter. Finally, we tested for differences in the winter biogeochemistry of lakes in the different classes, including measurements of dissolved oxygen, nutrients, and algal biomass. We hypothesized that less connected lakes, as indicated by optical remote sensing images, would exhibit greater stable isotope enrichment within site water samples. Additionally, we hypothesized that lakes within the same connectivity class would possess similar limnological characteristics, with classes of higher connectivity having characteristics more similar to the main channel.

2.2 Methods

2.2.1 Study Area

The SRD is located at the Saskatchewan-Manitoba border (approx. 53°29'N; 100°37'W). The delta covers an area of 10,000 km² and is the largest active inland delta in North America, draining the North Saskatchewan River, the South Saskatchewan River, and their tributaries, an area of approximately 405,864 km². The SRD consists of two areas that are separated by The Pas Moraine: the upper delta, located primarily in Saskatchewan; and the lower delta, located in Manitoba. The delta is characterized by a mosaic of large and small river channels, fens, bogs, forests and numerous shallow wetlands and lakes (<3m depth). The SRD is located downstream of three large hydroelectric dams, the Gardiner Dam, Francois Finley Dam and E.B. Campbell Dam, that impact the natural flow regime downstream (Wheater and Gober 2013). Though flood peaks are smaller than those observed prior to dam construction in the 1960s, there is still sufficient flow in many years to cause inundation and connect off-channel lakes (Smith and Perez-Arlucea 2008).

The SRD is highly seasonal in temperature, precipitation, and discharge. Temperatures reach as low as – 49.4°C in the winter (e.g. mean temperature December 2013 = -25.1°C) and as high as 37.6°C in the summer (e.g. mean temperature July 2013 = 17.8°C) (WMO ID: 71867; Environment Canada 2014). It receives an average of 450mm of precipitation annually with most rainfall occurring between June and August (peaking in July), and snowfall occurring between November and March (peaking in December) (WMO ID: 71867; Environment Canada 2014).

Due to contributions from snowmelt and later runoff from the Rocky Mountain headwaters, the SRD typically experiences both a spring and a summer flood event. River discharge within the SRD increases in mid-April during spring melt with a peak in late-April/early-May (historical mean spring peak discharge at station 05KD003 South Saskatchewan River below Tobin Lake = ~650 m^3/s; Environment Canada 2014). After spring peak, water levels continue to drop until mid-June when rain on snow events in the Rocky Mountains trigger runoff that soon reaches the SRD. Summer river discharge is often greater than spring discharge (historical mean summer peak discharge at station 05KD003 = ~870 m^3/s; Environment Canada 2014) causing more extensive flooding in the delta with a peak in late-June/early-July. Prior to our sampling in winter 2014, a large flood event occurred within the SRD in 2013, with spring and summer river discharge much greater compared to the historical average (spring peak discharge at station 05KD003 = 1690m^3/s; summer peak discharge at station 05KD003 = 3640m^3/s; Environment Canada 2014). As a result of such large flood events, an extensive amount of the historically connected floodplain within the SRD was inundated (Sagin et al. 2015).

2.2.2 Remote sensing

Water coverage data for the SRD were obtained using optical remote sensing images as described in Sagin et al. (2015). A combination of Landsat, Spot, and RapidEye images were used to determine surface water coverage area (SWCA) during flood events of varying magnitudes. Landsat data were obtained from the United States Geological Survey (USGS) Earth Resources Observation and Science Center's (EROS) Global Visualization Viewer (GLOVIS, http://glovis.usgs.gov/), SPOT data were obtained from the Alberta Terrestrial Imaging Center (ATIC), and RapidEye data were purchased from BlackBridge Geomatics. For greater resolution, datasets during days with minimal cloud cover were targeted for map production. SWCA maps were created using a surface water extraction coverage area tool (SWECAT: Sagin et al. 2015). SWECAT was developed by extracting SWCA for flood events from Landsat, and comparing them to Canadian National Hydro Network, SPOT and RapidEye SWCA datasets during a similar timeframe to verify results. Comparison of SWCA derived from Landsat images for three flood events (moderate flood, high flood, extreme flood) to those obtained from RapidEye and SPOT showed good agreement, with less than 7% difference in SWCA (Sagin et al. 2015).

SWCA maps of flood events of varying flood frequencies were layered to produce a system-wide map displaying the connectivity gradient for a 1315 km^2 study area within the upper delta (Figure 2.1). This map was then used to manually select connectivity categories for lakes within the delta. Only when a connection pathway of a lake to a main channel or side channel was apparent was it classified as connected. An increase in size of a lake without a clear connection pathway was insufficient to classify it as connected due to the potential influence of local precipitation, over-land runoff, and groundwater infiltration. SRD lakes were classified into five categories based on their connection during different river discharges (drought = <350 m^3/sec; low flood = 350-500 m^3/sec: moderate flood 500-1000 m^3/sec, high flood = 1000-2000 m^3/sec; extreme flood >2000 m^3/sec). The remote sensing satellite maps obtained for different flood frequencies showed marked differences in SWCA and therefore degree of floodplain inundation and connectivity. All lakes that were connected to the river in an image from 6 August 2001 when river discharge and SWCA was lowest (discharge= 327m^3/sec; SWCA = 56 km^2) were classified as drought-connected. Low flood-connected lakes were based on an image from 13 September 1990 (discharge= 422 m^3/sec; SWCA = 89 km^2) while moderate flood-connected lakes were from 8 June 2005 (discharge= 1110 m^3/sec; SWCA = 151 km^2). The map for high flood-connected lakes was obtained using an image from 29 July 2011 (discharge= 1050 m^3/sec; SWCA = 178 km^2). The image corresponding to the largest available flood was from 8 July 2005 (discharge= 1810 m^3/sec; SWCA = 289 km^2) and used to categorize extreme flood-connected lakes.

Figure 2.1 Location of the Saskatchewan River Delta, Canada and sampling sites with an image of surface water coverage area for different flood categories, including drought-connected (discharge= 327m^3/sec; SWCA = 56 km^2), low flood-connected (discharge= 422 m^3/sec; SWCA = 89 km^2), moderate flood-connected (discharge= 1110 m^3/sec; SWCA = 151 km^2), high flood-connected (discharge= 1050 m^3/sec; SWCA = 178 km^2), extreme flood-connected (discharge= 1810 m^3/sec; SWCA = 289 km^2)
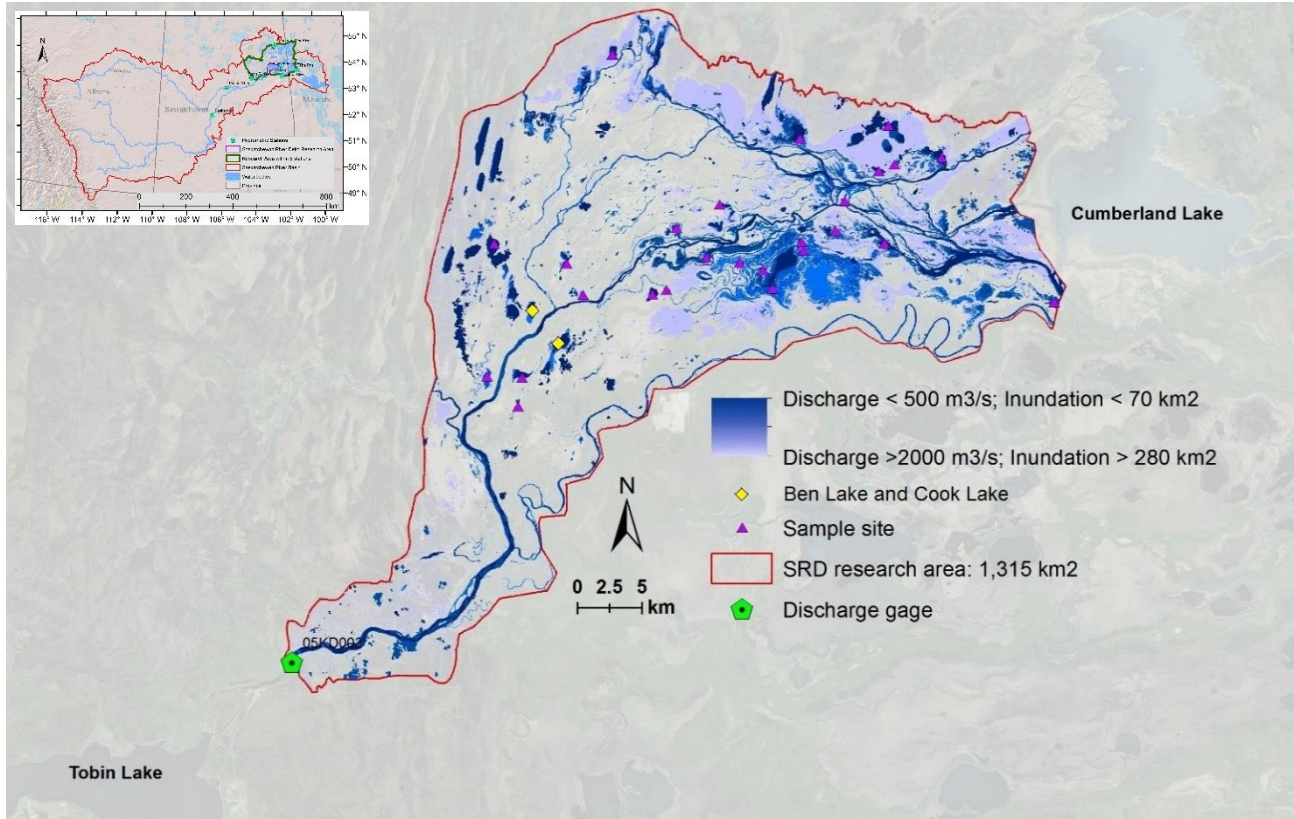
2.2.3 Stable isotope hydrology

Stable isotope compositions within local water bodies are generally dependent on two factors: source waters and evaporation. The local meteoric water line (LMWL) and the local evaporation line (LEL) ultimately constrain δ^2H and δ^{18}O. LMWL is dependent on summer and winter isotopic composition of precipitation, whereas LEL is dependent on the LMWL and local atmospheric conditions. For the SRD, a LMWL of δ^2H = 7.7 x δ^{18}O – 1.2 was used based on regional isotope composition of precipitation from 1990-2005 (Pham et al. 2009).

To develop a LEL for the SRD, we analyzed δ^2H and δ^{18}O composition of floodwaters and wetlands in a spillway channel downstream of E.B. Campbell Dam from June to August 2013 (Figure 2.2). During summer, when water levels in Tobin Lake reservoir (formed by E.B.

Campbell Dam) begin to rise and discharge reaches the maximum capacity of the hydroelectric station, the spillway gates are opened to release excess water, inundating wetlands in the spillway channel. When the spillway is closed these wetlands immediately drain and disconnect, leaving shallow residual pools that slowly evaporate. The spillway wetlands were sampled monthly for isotopic composition in June (disconnected), July (connected) and August (disconnected) 2013 to envelop the inundation and isolation/evaporation phases and benchmark our isotope data for lakes in the SRD that were filled by the same floodwaters. δ^2H and $\delta^{18}O$ values for spillway sites were plotted and a best-fit line determined to obtain a LEL for the SRD, using ordinary least squares regression (Figure 2.3a).

2.2.4 Field sampling

From early February to late March, 2014, a total of 26 SRD lakes of varying connectivity to the main channel were sampled (Figure 2.1). The 26 lakes were selected to ensure wide coverage within a 1315 km^2 study area in the upper delta and included all five lake hydrological categories (drought-connected, n = 3; low-flood-connected, n = 6; moderate flood-connected, n = 3; high flood-connected, n = 7; extreme flood-connected, n = 7). As much as possible, lakes were selected to ensure an approximately equal lake-surface area distribution among the five lake categories.

At each site, holes were augured through the ice and water quality measurements were taken, including dissolved oxygen (DO), pH, turbidity, and conductivity, at mid depth using a YSI EXO2 Sonde at the perceived deepest part of each lake. Unfiltered surface-water samples were collected for water column chlorophyll (chl-a), total nitrogen (TN) and total phosphorus (TP) at each site in sterile 500ml Nalgene bottles. Filtered surface-water samples (0.45µm filter) for dissolved organic carbon (DOC), hydrogen and oxygen stable isotopes (δ^2H, $\delta^{18}O$), nitrate-nitrite (NO_3-NO_2), ammonia-ammonium (NH_3-NH_4), and sulfate (SO_4) were also collected at each site. All water samples were collected from 10cm below the water surface. Filtered samples for DOC were stored in amber polyethylene bottles, while samples for δ^2H/$\delta^{18}O$, NO_3, NH_3-NH_4, and SO_4 were stored in 50ml Falcon tubes. All water samples, excluding δ^2H/$\delta^{18}O$ samples, were frozen at -20°C until further laboratory analysis. In addition to water samples, physical measurements including ice thickness, snow depth, and lake depth were taken.

To provide temporal information on water chemistry to complement our spatial study in winter, two of the lakes from the high flood-connected category (BMO5; Ben's Lake; 53°56'N; 103° 0'W; and BMO6; Cook Lake; 53°55'N; 102°58'W) and the main channel were also sampled monthly from May to September 2014 (Figure 2.2). One of these lakes (Ben's Lake) and the main channel were also sampled prior to the winter sampling in August 2013, to provide a pre-winter sampling baseline immediately after the flood peak. Sampling methods for water column chl-*a*, TN, TP, DOC, NO_3-NO_2 and SO_4, and pH, conductivity, and turbidity were as previously described.

Figure 2.2 Daily discharge for the study area (station 05KD003, Saskatchewan River below Tobin Lake, Water Survey of Canada) from June 2013- November 2014 with markers indicating winter and spring/summer SRD sampling dates, and the spillway sample dates collected below E.B. Campbell Dam that were used to determine the LEL
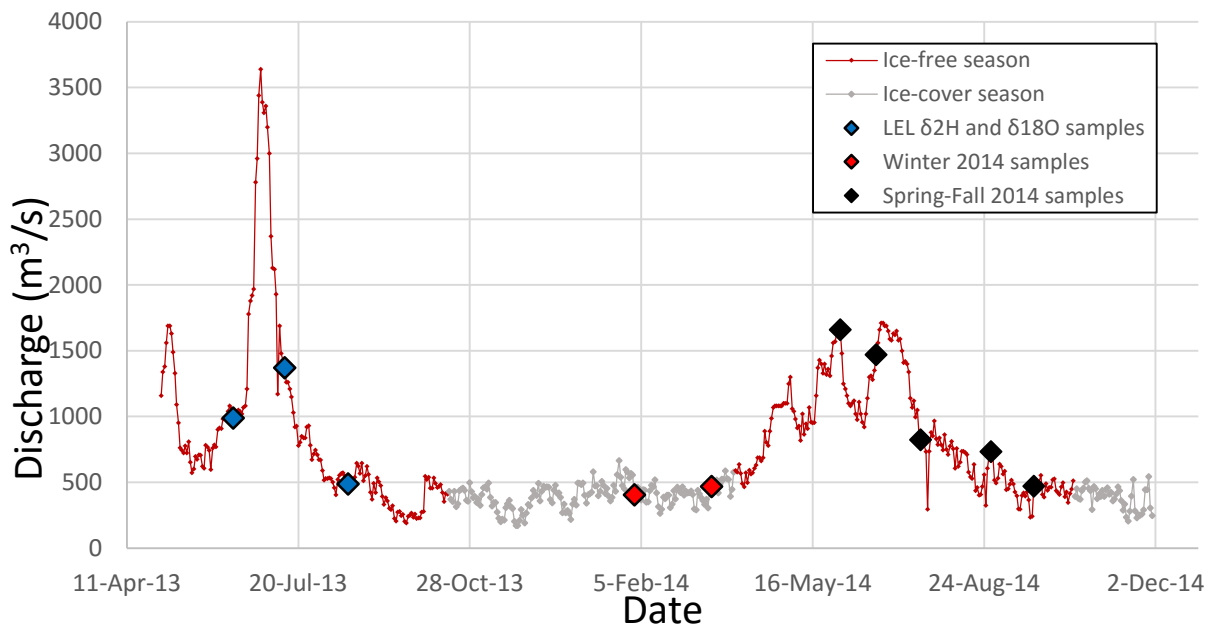
2.2.5 Laboratory Analysis

Samples for δ^2H and $\delta^{18}O$ were stored at room temperature in the dark until they were analysed at Environment Canada's National Hydrology Research Centre. Isotope ratios were

analysed with a LGR DLT-100 OA-ICOS liquid water isotope analyzer coupled to a LC-PAL autosampler. Each sample was injected six times; the results of the first three injections were discarded to eliminate memory effect between samples. Two reference waters that isotopically bracket the sample values were included in each sample run. These references were previously calibrated with Standard Light Antarctic Precipitation (SLAP) and Vienna Standard Mean Ocean Water (VSMOW). Results are calculated based on a rolling calibration so that each sample is calibrated by the three standards run closest in time to that of the sample.

Water samples were analyzed for TN, TP, DOC, chl-a, SO_4, NO_3-NO_2, and NH_3-NH_4, using conventional techniques. TN and TP samples were analyzed using techniques outlined by Parson et al. (1984), Crumpton et al. (1992) and Bachmann and Canfield (1996). Following persulfate digestion, TN was measured by second-derivative spectroscopy analyses. TP samples were analyzed following treatment with a reagent containing molybic acid, ascorbic acid and trivalent antimony; the resulting blue TP solution was measured at 885 nm. DOC was analyzed using an automated Shimadzu TOC-V C, P and N analyzer. Water column chl-a samples were analysed using a Turner Trilogy fluorometer following a 7 minute digestion in 90% EtOH at 80°C. Sulfate was analyzed by Method SUL-001-A (based on ASTM method D516-90, 02 and standard methods 426C 16th Ed), a turbidometric analysis where sulfate is converted to a barium sulfate suspension and turbidity determined at 420nm (minimum detectable limit = ~1 mg/L). Nitrate-nitrite were analysed colorometrically following reduction of nitrate to nitrite (cadmium reduction) using Smartchem method NO3-001-A (based on EPA method 353.2, rev. 2 and standard. methods 4500 NO3F), with a range of 0.02-2mg N/L. NH_3-NH_4 was analysed colorometrically by the phenol-hypochlorite method (EPA 350.1) with a range of 0.01-2mg/L. All SO_4, NO_3-NO_2, and NH_3-NH_4 samples below the detectable limit of their associated analyzer were reported as half the value of the minimum detection limit.

2.2.6 Data analysis

To assess the utility of remote-sensing based-classifications of connectivity, we tested for differences in δ^2H and $\delta^{18}O$ values within different lake categories. We used a Multivariate Analysis of Variance (MANOVA) with δ^2H and $\delta^{18}O$ values as the dependent variables and connectivity class as the independent variable. To elucidate potential conditions that may be impacted by the degree of connectivity to the river, a MANOVA was used to compare multiple

independent variables (DO, TN, TP, DOC, chl-*a*, SO_4, NO_3-NO_2, NH_3-NH_4, turbidity, pH, and conductivity) of the sampled lakes among connectivity categories as the independent variable. In addition to the MANOVA, a principal component analysis (PCA) was used to assess differences in limnological conditions among the five lake connectivity categories and to determine which variables were correlated. PCA was performed using the statistical program R. Prior to statistical analysis, all variables were assessed visually for normality using histograms and Q-Q plots with the computer program SPSS Statistics 22 (IBM Ireland); equal variance for variables between connectivity was assessed using Levene's test. Appropriate transformations were applied to the dataset when necessary to create normality and to equalize variance. Normality and homogeneity of variance were achieved for all variables except NO_3-NO_2, and NH_3-NH_4. This included 26 sites (drought-connected, n = 3; low flood-connected, n = 6; moderate flood-connected, n = 3; high flood-connected, n = 7; extreme flood-connected, n = 7) for TN, TP, DOC, chl-*a*, SO_4, NO_3-NO_2, and NH_3-NH_4; and 24 sites (drought-connected, n = 3; low flood-connected, n = 6; moderate flood-connected, n = 3; high flood-connected, n = 5; extreme flood-connected, n = 7) for DO, turbidity, pH and conductivity. Differences among categories in the MANOVA were compared post-hoc with a Tukey's HSD test. All statistical analyses were conducted using SPSS.

2.3 Results

2.3.1 Stable isotope hydrology

Hydrogen and oxygen stable isotope values of floodwaters from sites upstream of the SRD ranged from -17.8 to -16.5‰ for $\delta^{18}O$ and -144.8 to -133.3‰ for δ^2H, while those for the isolated spillway channel wetlands ranged from -17.5 to -13.9‰ for $\delta^{18}O$ and -139.3 to -125.0‰ for δ^2H (Figure 2.3a). Combining these values created the LEL (equation1; $r^2 = 0.83$, p <0.001; Figure 2.3a).

$$\delta^2H = 3.97 \text{ x } \delta^{18}O - 68.87 \tag{2.1}$$

Figure 2.3 Hydrogen and oxygen stable isotope ratios of river and wetland water (A) in a spillway downstream of E.B. Campbell Dam in the Saskatchewan River, from June- August 2013, and (B) from lakes of drought-connected (+), low flood-connected (◇), moderate flood-connected (□), high flood-connected (▲), and extreme flood-connected categories (✱) sampled in the Saskatchewan River Delta from February-March 2014. The corresponding Local Meteoric Water Line (LMWL) is based on regional isotope composition of precipitation from 1990-2005 (Pham et al. 2009) and the Local Evaporation Line (LEL) is a best fit line based on the samples collected at river and wetland sites in panel A.

a)
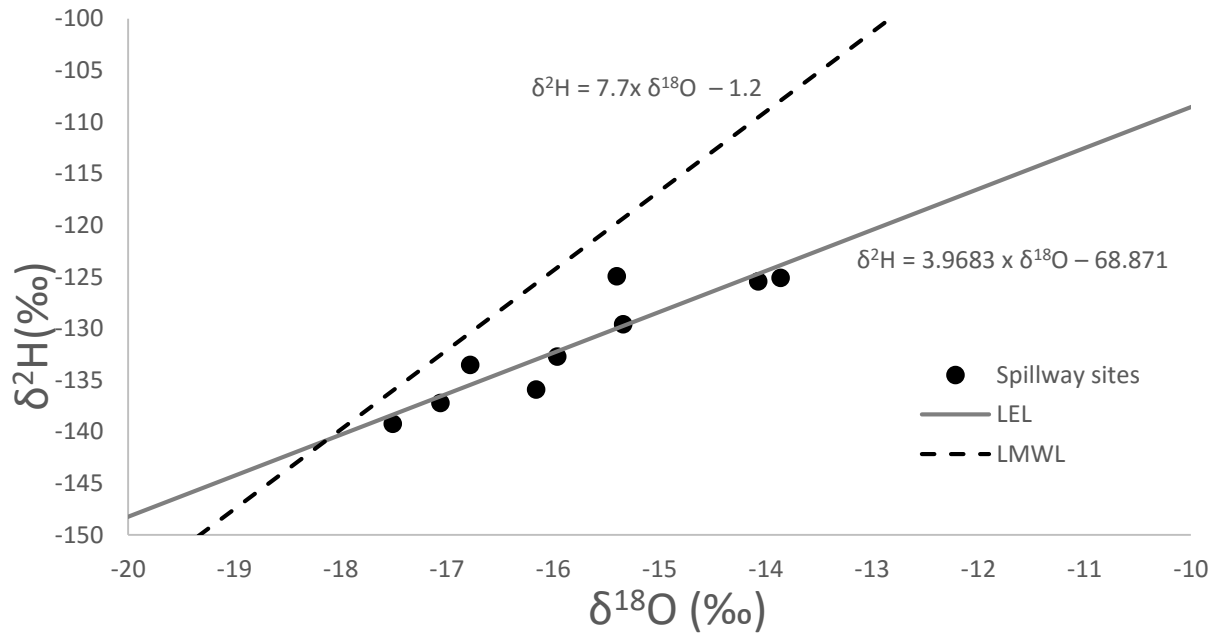
b)
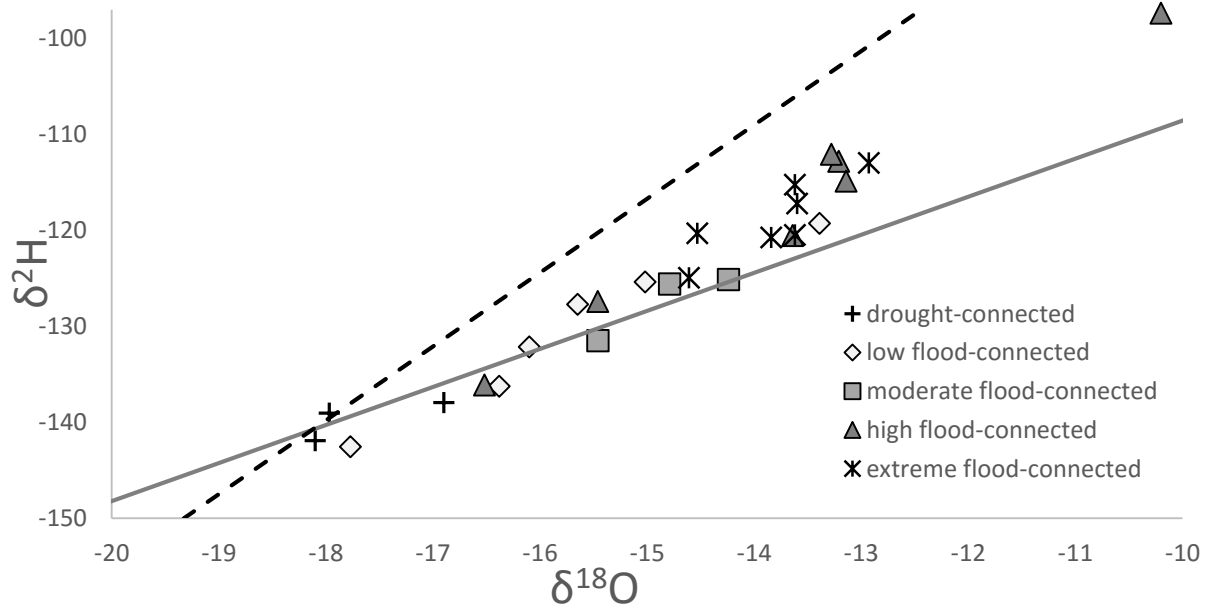
Isotopic values for the SRD samples collected between February and March of 2014 during the ice-covered season differed among connectivity categories (Figure 2.3b). These data followed a trend line of $\delta^2H = 5.76 \times \delta^{18}O - 39.46$ ($r^2 = 0.96$, p <0.001, Figure 2.3b), which generally followed that of the expected LEL, confirming a common water source (Saskatchewan River floodwaters) for these lakes. Lakes in the more isolated connectivity categories (high flood-connected, extreme flood-connected) were typically located further along the LEL compared to lakes that were more often connected (drought-connected, low flood-connected), suggesting greater evaporative enrichment in more isolated lakes. Comparatively, waters from drought-connected lakes were in close proximity to the LMWL, with minimal evaporative enrichment and isotopic composition more similar to that of the source water. Isotopic composition of water varied significantly between connectivity categories for both δ^2H (p = 0.003), and $\delta^{18}O$ (p = 0.002). As shown in Table 2.2, δ^2H values for drought-connected lakes had significantly lower isotopic signatures compared with high flood-connected (p = 0.005) and extreme flood-connected lakes (p = 0.010), and $\delta^{18}O$ showed similar patterns, with drought-connected lakes having significantly lower values compared to high flood-connected (p = 0.003) and extreme flood-connected lakes (p = 0.004).

2.3.2 Water chemistry and nutrients

Limnological conditions varied greatly among the floodplain lakes of the SRD. Turbidity (range = 1.0-39.0 NTU), pH (range = 7.04-8.77), and conductivity (range = 288-1840 µS) all had large among-site variation. Lake DO levels varied from anoxic (0.4 mg/L O_2) to near saturation (12.0 mg/L O_2) depending on the lake sampled. Concentrations of all nutrients ranged from oligotrophic to eutrophic conditions, with TN (range = 446-6480 µg/L), TP (range = 7-874 µg/L), DOC (range = 0-49.1 mg/L), NO_3-NO_2 (range = 0-0.40 mg/L), NH_3-NH_4 (range = 0.01-3.69 mg/L), and SO_4 (range = 6.88-72.81 mg/L) all showing large among-lake variation. Corresponding chl-a levels also ranged widely from very low (0.3 µg/L) to very high (28.6 µg/L).

Limnological conditions of floodplain lakes significantly differed among the five connectivity categories (Figure 2.4). DO, pH, NO_3-NO_2, and SO_4 were all significantly influenced by connectivity to the river (p < 0.05). As connectivity to the main channel decreased, we observed decreasing DO (p = 0.019), pH (p = 0.030), NO_3-NO_2 (p < 0.001), and

Table 2.1 Summary of MANOVA results (p-values for post-hoc comparisons) for differences in limnological variables among connectivity categories. Asterisks indicate significant differences at a = 0.05.

		Drought	Low flood	Moderate flood	High flood	Extreme flood
$\delta^2 H$	Drought	-	0.525	0.376	0.005*	0.010*
	Low flood		-	0.981	0.560	0.109
	Moderate flood			-	0.400	0.558
	High flood				-	0.996
	Extreme flood					-
$\delta^{18} O$	Drought	-	0.288	0.115	0.003*	0.004*
	Low flood		-	0.882	0.770	0.123
	Moderate flood			-	0.706	0.814
	High flood				-	0.999
	Extreme flood					-
DO	Drought	-	0.169	0.04*	0.067	0.013*
	Low flood		-	0.731	0.957	0.547
	Moderate flood			-	0.969	1.000
	High flood				-	0.940
	Extreme flood					-
pH	Drought	-	0.077	0.032*	0.077	0.028*
	Low flood		-	0.880	1.000	0.982
	Moderate flood			-	0.922	0.984
	High flood				-	0.994
	Extreme flood					-
NO_3-NO_2	Drought	-	0.005*	0.001*	<0.001*	<0.001*
	Low flood		-	0.447	0.237	0.292
	Moderate flood			-	1.000	1.000
	High flood				-	1.000
	Extreme flood					-
SO_4	Drought	-	0.028*	<0.001*	<0.001*	0.001*
	Low flood		-	0.109	0.202	0.279
	Moderate flood			-	0.915	0.852
	High flood				-	1.000
	Extreme flood					-
TN	Drought	-	0.416	0.037*	0.157	0.488
	Low flood		-	0.383	0.950	1.000
	Moderate flood			-	0.695	0.279
	High flood				-	0.867
	Extreme flood					-
TP	Drought	-	0.853	0.251	0.172	0.939
	Low flood		-	0.608	0.493	0.997
	Moderate flood			-	1.000	0.431
	High flood				-	0.282
	Extreme flood					-
NH_3-NH_4	Drought	-	0.645	0.091	0.206	0.733
	Low flood		-	0.448	0.846	0.999
	Moderate flood			-	0.880	0.329
	High flood				-	0.699
	Extreme flood					-

Figure 2.4 Boxplots of physical and chemical variables for drought-connected lakes (connectivity category 1; n = 3), low flood-connected lakes (connectivity category 2; n = 6), moderate flood-connected lakes (connectivity category; n = 3), high flood-connected lakes (connectivity category 4; n = 7), and extreme flood-connected lakes (connectivity category 5; n = 7) .The boxplots present the median, the 25th and 75th quartiles, and the minimum and maximum values for the data.
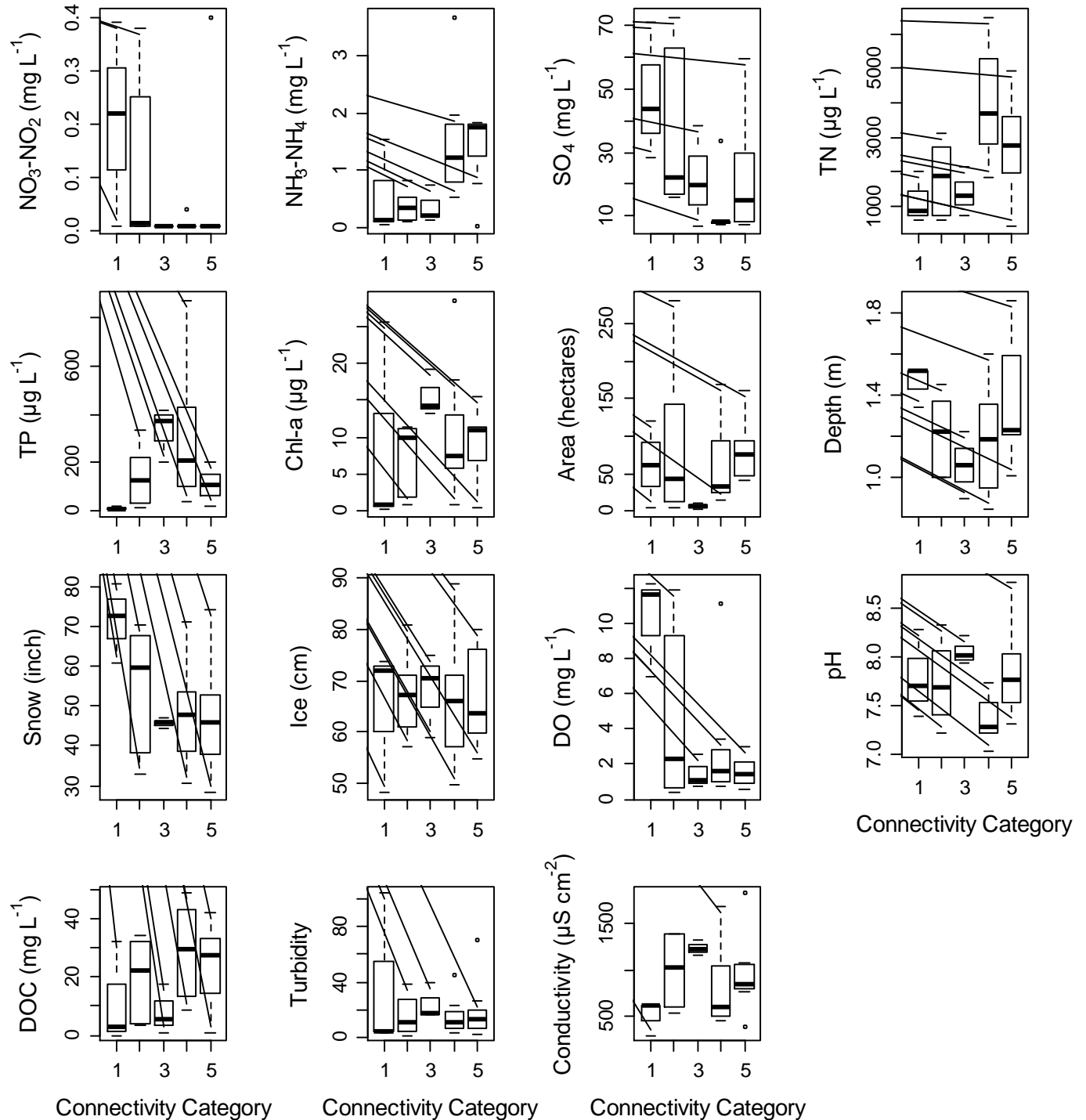
SO$_4$ (p < 0.001). DO levels were highest in drought-connected lakes (mean = 10.2±2.8 mg/L) and declined to minimal levels in all other categories, with the only exception being SRD13 which was a high flood-connected lake and possessed oxygen levels more characteristic of drought-connected lakes. As shown in Table 2.1, there were significantly higher DO levels within drought-connected compared to moderate flood-connected (p = 0.040) and extreme flood-connected lakes (p = 0.013). The pH was highest in drought-connected lakes (mean = 8.39±0.36) with a gradual decrease in pH as connectivity declined, with drought-connected lakes having significantly higher pH than moderate flood-connected (p = 0.032) and extreme flood-connected (p = 0.028) lakes. NO$_3$-NO$_2$ concentrations were also highest in drought-connected lakes (mean = 0.35±0.08 mg/L) then quickly decreased in the low flood-connected category and above. For this variable, there was significant separation between drought-connected and all other connectivity categories (p < 0.005 for all comparisons), with low flood-connected being the only other category with mean values (0.11±0.16 mg/L) not bordering the minimum detection limit of 0.02 mg/L. SO$_4$ concentrations were highest in drought-connected lakes (mean = 66.37±5.63 mg/L) and decreased as connectivity declined. Similar to NO$_3$-NO$_2$ concentrations, there was significant separation between drought-connected lakes and all other connectivity categories for SO$_4$ (low flood-connected, mean = 33.97±22.99 mg/L; p = 0.028; moderate flood-connected, mean = 8.20±0.67 mg/L, p < 0.001; high flood-connected, mean = 16.36±12.52 mg/L, p < 0.001; extreme flood-connected, mean = 17.92±9.33 mg/L, p = 0.001). TN, TP, and NH$_3$-NH$_4$ tended to be higher in less-connected lakes but these differences were not significant (TN, p = 0.059; TP, p = 0.092; NH$_3$-NH$_4$, p = 0.096). There were no differences among connectivity categories for turbidity (p = 0.164), conductivity (p = 0.300), chl-*a* (p = 0.616), DOC (p = 0.277), snow depth (p = 0.123), ice thickness (p = 0.992), and lake depth (p = 0.191).

PCA for the winter limnological data indicated that water chemistry and isotopes for the lakes of the SRD differed among lake connectivity categories (Figure 2.5). Eigenvalues were 51.9% for the first axis and 18.5% for the second axis, and explained a large amount of variation within the dataset (70.4%). Dissolved oxygen, pH, NO$_3$, and SO$_4$ were positively correlated to the first axis, while nutrients (TN, TP, NH$_3$-NH$_4$), chl-a, conductivity, and turbidity were negatively correlated to the first axis. DOC was negatively correlated with axis 2, while δ^{18}O and δ^{2}H was negatively associated with both axes 2 and 1. All drought-connected lakes and two of

the low flood-connected lakes plotted high on axis 1 characterized by high DO, pH, NO_3, and SO_4 (Figure 2.5). The remaining low flood-connected lakes and moderate flood-connected lakes plotted low on axis 1 characterized by high nutrients, chl-a, conductivity, and turbidity. High flood and extreme flood-connected lakes had a wide range along axis 1 and were relatively low compared to the other lake categories along axis 2, indicative of greater $\delta^{18}O$, δ^2H, and DOC concentrations.

Figure 2.5 Principal component analysis (PCA) displaying the vectors of the 13 physical and chemical variables sampled from lakes of the SRD during the winter of 2014, and the distribution of lakes from the five connectivity categories with respect to the 13 variables based on individual lake limnological conditions
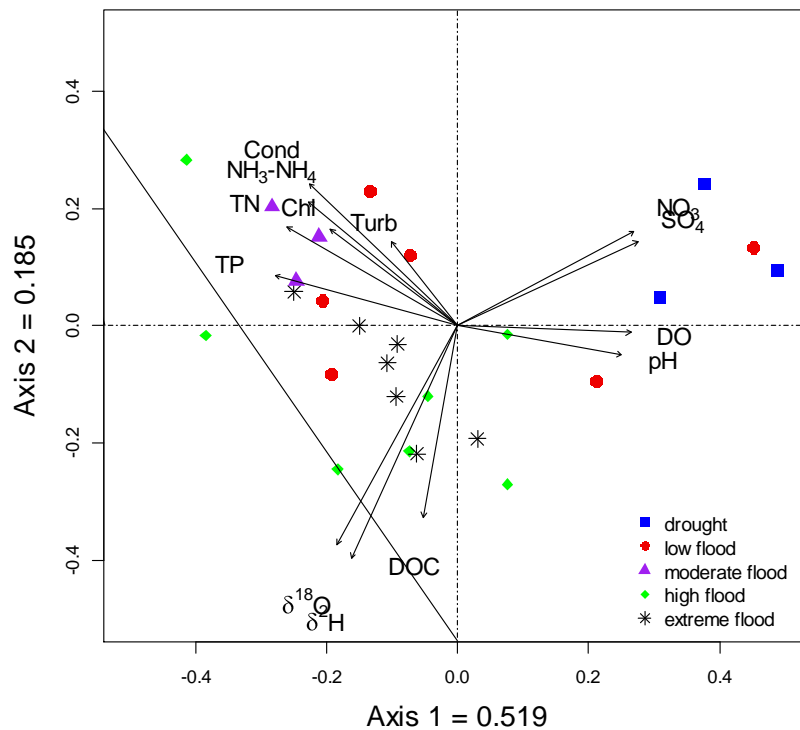
TN, TP, SO_4, chl-a, DOC, turbidity, and conductivity all showed variation among seasons for the two high flood-connected lakes (Ben's and Cook Lake, Table 2.1). Highest values were observed during the winter sampling event for TN, TP, SO_4, chl-a, DOC, turbidity, and conductivity. The lowest levels were observed during the 2014 summer months for TN, TP, SO_4, chl-a, DOC, turbidity, and conductivity. pH was variable over the sampling period, with no consistent seasonal differences.

Table 2.2 Summary of limnological data for two floodplain lakes from the high flood-connected category found within the Saskatchewan River Delta (Ben's Lake (SRD05) and Cook Lake (SRD06)), and the Saskatchewan River. Sites were sampled intermittently from August 2013 to September 2014.

Lake site	Variable	Date						
		24-Aug-13	1-Feb-14	1-Jun-14	22-Jun-14	19-Jul-14	28-Aug-14	22-Sep-14
Ben's Lake	Turbidity (NTU)	4.96	12.52	1.93	2.37	2.47	2.38	4.87
	chl-a (µg/L)	9.61	5.78	3.78	4.24	3.00	11.0	-
	TP (mg/L)	0.13	0.11	0.04	0.04	0.04	0.07	0.04
	TN (mg/L)	1.00	2.00	0.91	0.73	0.94	0.77	1.60
	Conductivity	480	1069	383	262	313	363	421
	pH	7.70	7.40	8.04	8.24	8.14	7.34	8.15
	DOC (mg/L)	9.2	11.8	8.1	9.4	-	13.4	-
	SO$_4$ (mg/L)	37.0	28.2	15.0	-	6.9	-	9.1
		24-Aug-13	1-Feb-14	2-Jun-14	23-Jun-14	20-Jul-14	28-Aug-14	23-Sep-14
Cook Lake	Turbidity (NTU)	-	70.00	2.39	1.43	1.43	1.75	4.63
	chl-a (µg/L)	-	7.93	15.14	3.55	7.90	5.1	-
	TP (mg/L)	-	0.15	0.04	0.04	0.02	0.03	0.03
	TN (mg/L)	-	2.74	1.00	0.73	0.90	0.85	1.20
	Conductivity	-	762	398	218	283	500	454
	pH	-	7.88	8.09	9.24	8.19	7.62	8.03
	DOC (mg/L)	-	42.2	-	9.5	11.5	14.6	-
	SO$_4$ (mg/L)	-	16.8	13.0	-	8.1	-	6.2
		22-Aug-13	1-Feb-14	01-Jun-14	23-Jun-14	20-Jul-14	20-Aug-14	20-Sep-14
Saskatchewan River	Turbidity (NTU)	3.02	-	4.48	2.13	3.95	3.38	2.75
	chl-a (µg/L)	4.04	-	4.80	7.45	1.76	2.7	-
	TP (mg/L)	0.03	-	0.01	0.02	0.02	0.02	0.02
	TN (mg/L)	0.73	-	0.72	0.49	0.62	0.19	0.42
	Conductivity	471	-	473	323	495	461	499
	pH	8.36	-	8.10	8.93	8.66	8.75	8.60
	DOC (mg/L)	6.51	-	4.39	4.42	5.04	5.37	-
	SO$_4$ (mg/L)	94	-	81	-	80	-	78

2.4 Discussion

The degree of connection to the main channel for floodplain lakes within the SRD was associated with distinct limnological conditions within lakes. Connectivity to the main channel influenced the degree of isotope enrichment as well as pH, DO and the concentrations of many nutrients. Our findings are in agreement with similar studies done in large northern floodplain systems (Wolfe et al. 2007; Sokal et al. 2008; 2010; Wiklund et al. 2012) that show highly

connected lakes possess similar characteristics as the parent river. Connected floodplain lakes are greatly influenced by the existing conditions in the main channel, whereas isolated lakes are more impacted by local precipitation, evaporation, and other environmental processes. As a result, this gradient of limnological conditions for lakes within the SRD floodplain forms the foundation of biogeochemical diversity in this important northern delta.

Determining connectivity can often involve a substantial amount of field research physically analyzing local topography and water balances. As a result, many researchers have begun to use a combination of desktop and on-ground methods in order to accurately determine the connectivity of floodplain lakes (e.g. Wolfe et al. 2007; van de Wolfshaar et al. 2011, Ward et al. 2013). Stable isotope composition of water samples from lakes within the SRD during the winter following a large summer flood event (2013-2014), provided an effective validation of classifications based on remote sensing. Lakes with greater connection to the main channel showed minimal ^{2}H and ^{18}O enrichment, whereas more isolated lakes exhibited marked ^{2}H and ^{18}O enrichment. This pattern was also observed within the PAD (Wolfe et al. 2007), and the Slave River Delta (Brock et al. 2007; 2009). The five connectivity classes determined by optical remote sensing in our study generally showed good agreement with the isotope data (Figure 2.3b), providing evidence for the effectiveness of remote sensing as a cost-effective tool for making initial classifications.

Although there was considerable agreement between isotopic enrichment and remotely-sensed connectivity, not all lake categories showed clear separation isotopically because of considerable variation within categories. Lakes of the high flood-connected category ranged widely in isotopic composition compared to other lake categories, with low values neighbouring low flood-connected lakes and high values neighbouring extreme flood-connected lakes. Isotopic values outside of those expected based on their connectivity categories derived from remote sensing data could be attributed to many factors; these include overhanging vegetation that can obscure lake-river connections in remote sensing images leading to incorrect classification, variation in the influence of subsurface lake-river connection leading to unexpected replenishment of isotopically depleted waters, and/or degree of macrophyte/physical cover reducing evaporation within lakes. As previously reported by Brock et al. (2009), isotopic composition of floodplain lakes is driven by hydrology more than lake size; therefore, the deviation of isotopic values from expected values in the aforementioned sites is not likely a

result of variation in lake size. Snow melt can be a significant isotopic input, with the degree of snowmelt input being driven by lake catchment size and snowpack density (Brock et al. 2007). However, since our site sampling was done during winter prior to snowmelt, the impact of snowmelt on isotopic composition of lakes would be minimal. Additionally, the majority of isotope data points for the SRD plotted above the LEL. This occurs as a result of greater precipitation input, whereas data points below the LEL result from greater snowmelt input (Wolfe et al. 2007). This further reinforces our expectation that the main water input into the lakes of the SRD was floodwaters derived largely from precipitation in the basin's headwaters in 2013 (Wheater and Gober 2013).

The limnological conditions within floodplain lakes of the SRD depended on their degree of connectivity, as has been observed in both the PAD (Wolfe et al. 2007; Wiklund et al. 2012) and the Slave River Delta (Brock et al. 2007; Sokal et al. 2008; 2010). Lakes of the SRD with greater connectivity to the main channel possessed characteristics similar to that of the main channel (higher levels of dissolved oxygen, pH, NO_3-NO_2, and SO_4). The higher DO levels in lakes of greater connectivity may be attributable to permanent direct exchange with the river during the winter months. This exchange assists in maintaining oxygen levels near saturation at levels suitable for fish (Mathias and Barica 1980) despite the potentially high respiration rates within these lakes due to decomposition of organic matter (Molles et al. 1998). As less connected lakes are not replenished by oxygen-rich river water, oxygen levels within such lakes become depleted during ice cover. Rates of under-ice oxygen consumption in northern lakes during winter months are a function of mean depth and nutrient levels (Barica and Mathias 1979; Mathias and Barica 1980; Babin and Prepas 1985). Our SRD lakes did not differ in depth across connectivity categories, but lakes of mid-range connectivity did have higher water column nutrient levels compared to highly connected lakes. With eutrophic lakes experiencing O_2 consumption rates that are 3 times higher than oligotrophic lakes (Mathias and Barica 1980), the low oxygen levels within less connected lakes could be attributed to higher nutrient concentrations. It could also be explained by their initial dissolved oxygen storage. Lakes of higher connectivity maintain direct exchange with oxygen-rich river water longer into the ice-free season than lakes of less connectivity, potentially resulting in greater DO concentrations at the time when ice forms on the lakes. Assuming a constant rate of DO depletion across lakes, lakes with greater oxygen concentrations prior to ice cover will maintain higher concentrations

throughout the winter (Barica and Mathias 1979). Similarly, timing of ice cover formation will also influence DO concentrations into the winter. If lakes of higher connectivity remain ice-free later into the season because direct connection with the main channel slows ice formation, atmospheric oxygen exchange will also be maintained longer.

TN, TP, and NH_3-NH_4 also appeared to be influenced by connectivity, but this was not statistically significant. Highly connected lakes, with close association to river water, remained consistently low in TN and TP throughout the study period (Figure 2.4), indicative of oligotrophic conditions (Smith et al. 1999). The higher levels of nutrients in less connected lakes suggests nutrient flux into these lakes is not solely derived from the parent river, and that flooding may not be required in order to maintain high nutrient levels. This is consistent with findings from the Slave River Delta and the PAD (Sokal et al. 2008; Wiklund et al. 2012), though these conclusions were based on findings from concentrations of bio-available nutrients, not TN and TP. The floodplain itself may be a source of nutrients for the lakes. In river floodplains, leaf litter, vegetation, and sediment are capable of providing significant nutrients and organic matter to adjacent aquatic systems (Fisher and Likens 1973; Cuffney 1988; Ostojić et al. 2013), and are an essential part of nutrient cycling in river floodplain systems (Baldwin 1999; Inglett et al. 2008). Although highly connected lakes inundate their surrounding terrestrial zone during times of peak river flow, nutrients that do enter the lakes have greater potential to be diluted or flushed out of the lakes by nutrient-poor river water. Additionally, as the surrounding terrestrial zones of infrequently flooded lakes have been exposed to the atmosphere for a greater amount of time and are highly organic (Molles et al. 1998; Sokal et al. 2010), we postulate that inundation of these areas releases a greater amount of nutrients compared to the terrestrial zone of more connected lakes. The peaks in TP and chlorophyll in intermediate connectivity lakes appear to imitate patterns expected for floodplain biodiversity. In riverine systems, high species diversity is expected for habitats of intermediate disturbance (Amoros and Bornette, 1999; Ward et al. 1999). Additionally, the high variation in nutrient concentrations, and the range of other limnological variables measured among lake connectivity categories may also contribute to the high biodiversity found within this delta as biota become adapted to exploit the various conditions found throughout the floodplain (Welcomme 1979; Junk et al. 1989; Ward et al. 1999).

Characteristics of the parent river water, as influenced by erosion and deposition occurring upstream, dictates its role in supplying sediment and associated nutrients to floodplain lakes. Relatively low TN within the river water of the SRD (420-730 mg/L) was also observed for the PAD (240-820 µg/L; Wolfe et al. 2007) but our TP levels (mean TP May-Sept 2014 = 20 µg/L) were much lower compared to that delta (mean TP Oct 2000 = 84 µg/L; Wolfe et al. 2007). High TP levels within the rivers of the PAD are likely a result of the associated high suspended sediment load (mean TSS Oct 2000 > 150 mg/L; Wiklund et al. 2012), while the Saskatchewan River delivers less sediment to the SRD (mean TSS May-Sept 2014 = 6 mg/L). Phosphorus adsorbs to sediment particles to a larger degree compared to nitrogen (50-70% vs. 2-3%, Olde Venterink et al. 2006), and it is sediment-bound phosphorus that makes up the major pathway of supplementation to the floodplain for deltaic systems (Forsberg et. al 1988; Wolfe et al. 2007). Retention of river sediment by Tobin Lake reservoir upstream of the SRD has been recorded as significant, reducing the sediment load from 9×10^6 t/year to less than 0.1×10^6 t/year (Ashmore and Day 1988), and may explain P-depletion in downstream river water feeding the SRD. Phosphorus retention by reservoirs can be large (up to 90%), with higher retention of P than N (Kunz et al. 2011a; 2011b). Though the PAD has a large dam (Bennett Dam) in its headwaters, suspended sediments are largely derived from the lower reaches of these large continental rivers (Ashmore and Day 1988); thus, waters contained within upland reservoirs are likely sediment- and nutrient-poor, leading to limited impacts on nutrient levels downstream. Conversely, the SRD has the potential to be significantly impacted by the influence of reservoirs (Lake Diefenbaker, Codette Lake and Tobin Lake) as they are located more immediately upstream of the delta (Ashmore and Day 1988). Low levels of phosphorus and comparatively higher levels of nitrogen in the SRD suggest potential disproportionate retention of nutrients by these reservoirs, with likely consequences for wetlands located downstream (Bosch 2008; Bosch and Allen 2008; Kunz et al. 2011a; 2011b).

Time series data for the two rarely connected lakes (Ben's Lake and Cook Lake) provided insight on how limnological conditions vary from mid-winter to the ice free season, and within the ice free season (~May-Sept). These lakes had high concentrations of TN and TP during the winter, and low levels during the spring and summer. During winter, when decomposition exceeds production, particularly for submerged macrophytes, there is little uptake of available nutrients; however, during the summer season, when productivity is very high within these

disconnected lakes, there is a rapid uptake of available nutrients. High macrophyte cover is associated with decreased levels of nutrients and phytoplankton growth (Søndergaard and Moss 1998; Rooney and Klaff 2003; Norlin et al. 2005) due to increased metabolic activity of macrophytes and their inhibition of phytoplankton through competition for space and light (Søndergaard and Moss 1998; Wiklund et al. 2012). Less connected lakes also experience greater macrophyte growth compared to highly connected lakes (Sokal et al. 2010), and we observed extensive macrophyte beds in both Ben's and Cook Lakes during the summer of 2014 (B. MacKinnon, personal observation). Lake-ice formation within floodplain lakes may also contribute to greater concentrations of nutrients and ions during winter months through cryoconcentration, or freeze-out. As ice forms, dissolved substances are excluded with efficiencies of up to 97% for some major ions, however exclusion is less efficient for nutrients, with TN and TP efficiencies around 53% and 60% respectively (Welch and Legault 1986). Although less efficient compared to ions, the excluded nutrients from ice can still lead to a significant increase in water column concentrations compared to levels prior to ice formation (Belzile et al. 2002). High chl-*a* concentrations during the low water stage for the SRD is consistent with findings within other floodplain systems (Knowlton and Jones 1997; Persic and Horvatic 2011; Mayora et al. 2013). However, high winter chl-*a* concentrations are not necessarily indicative of increased phytoplankton biomass, but instead could be driven by an increase in phytoplankton cellular chlorophyll content to maximize photosynthesis under significant ice-cover and low-light conditions (Hunter and Law 1981; Prézelin and Matlick 1983). Low light conditions in the lakes of the SRD are likely the limiting factor for phytoplankton during the winter months, as chlorophyll did not differ across lake connectivity categories and all lakes were underneath 50-100 cm of ice and an additional 50-100 cm of snow.

The winter DO and NH_3-NH_4 levels experienced within floodplain lakes of the SRD, although nearing toxicity for many species, maintain levels capable of supporting some tolerant fish species. Since large, intolerant species are unlikely to inhabit these off-channel waterbodies during the ice-cover season, these habitats may be used as winter refuge by tolerant species, similar to what has been observed in tropical floodplain systems (Chapman et al. 1996; Robb and Abrahams 2002; 2003). Additionally, future isolation of these off-channel lakes by reduction in river discharge could ultimately lead to intermittent, or even permanent, desiccation due to lack

of hydrological recharge (Brock et al. 2007), and eliminating the potential for these lakes to act as winter habitat for aquatic species.

Floodplain ecosystems provide essential habitat for a diverse array of biota, and can provide critical ecological and cultural services for local peoples. Northern floodplains are known to be greatly affected by upstream river impoundments that alter the natural flow regime of the river and disrupt the connection between the river and its floodplain (Prowse et al. 2002). For the SRD, in addition to alteration of the flow regime, the close proximity of the upstream impoundments to the delta also appears to affect nutrient concentrations in downstream river water. The retention of phosphorus-rich sediment has potentially decreased phosphorus levels downstream, lowering levels entering the floodplain lakes of the SRD. In addition to river impoundment, climate change is also projected to cause a reduction in both peak and total discharge within these systems (Wolfe et al. 2008), potentially leading to further disconnection between the floodplain and the main channel. Since our findings show the large effect inundation by river water has on the limnological conditions of floodplain lakes, further reduction in the connectivity of these lakes will ultimately impact nutrient and water quality dynamics within these ecosystems.

2.5 Literature Cited

Amoros, C. and Bornette, G. 1999. Antagonistic and cumulative effects of connectivity: a predictive model based on aquatic vegetation in riverine wetlands. Large Rivers **11**: 311–327.

Amoros, C., and Bornette, G. 2002. Connectivity and bio-complexity in water bodies of riverine floodplains. Freshwater Biology **47**: 761-776.

Ashmore, P.E. and Day, T.J. 1988. Spatial and temporal patterns of suspended sediment yield in the Saskatchewan River basin. Canadian Journal of Earth Sciences **25**: 1450-1463.

Babin, J. and Prepas, E.E. 1985. Modelling Winter Oxygen Depletion Rates in Ice-Covered Temperate Zone Lakes in Canada. Canadian Journal of Fisheries and Aquatic Sciences **42**: 239-249.

Bachmann, R. W., and Canfield Jr, D. E. 1996. Use of an alternative method for monitoring total nitrogen concentrations in Florida lakes. Hydrobiologia **323**: 1−8.

Baldwin, D.S. 1999. Dissolved organic matter and phosphorus leached from fresh and "terrestrial" aged river gum leaves: implications for assessing river-floodplain interactions. Freshwater Biology 41: 675–685.

Barica, J. and Mathias, J.A. 1979. Oxygen depletion and winterkill risk in small prairie lakes under extended ice cover. Journal of Fisheries Research Board of Canada **36**: 980-986.

Belzile, C., Gibson, J.A.E., Vincent, W.F. 2002. Colored dissolved organic matter and dissolved organic carbon exclusion from lake ice: Implications for irradiance transmission and carbon cycling. Limnology and Oceanography **5**: 1283–1293.

Bosch, N. S. 2008. The influence of impoundments on riverine nutrient transport: An evaluation using the Soil and Water Assessment Tool. Journal of Hydrology **355**: 131–147. doi:10.1016/j.jhydrol.2008.03.012.

Bosch, N. S., and Allan, J. D. 2008. The influence of impoundments on nutrient budgets in two catchments of Southeastern Michigan. Biogeochemistry **87**: 325–338. doi:10.1007/s10533-008-9187-6.

Brock, B.E., Wolfe, B.B., and Edwards, T.W.D. 2007. Characterizing the hydrology of shallow floodplain lakes in the Slave River Delta, NWT, using water isotope tracers. Arctic, Antarctic and Alpine Research **39**: 388–401.

Brock, B.E., Yi, Y., Clogg-Wright, K.P., Edwards, T.W.D., and Wolfe, B.B. 2009. Multi-year landscape-scale assessment of lakewater balances in the Slave River Delta, NWT, using water isotope tracers. Journal of Hydrology **379**: 81–91.

Chapman, L.J., Chapman, C.A., and Chandler, M. 1996. Wetland ecotones as refugia for endangered fishes. Biol. Conserv. **78**: 263-270. doi:10.1016/S0006-3207(96)00030-4.

Crumpton, W. G., Isenhart, T. M., and Mitchell, P. D. 1992. Nitrate and organic N analyses with second derivative spectroscopy. Limnology and Oceanography **37**: 907−913.

Cuffney, T.F. 1988. Input, movement and exchange of organic matter within a subtropical coastal blackwater river-floodplain system. Freshwater Biology **19**: 305–320.

Environment Canada. 2015. Climate Data Online (http://climate.weather.gc.ca/). Accessed 3 February 2015.

Fisher, S.G., and Likens, G.E. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs **43**: 421–439.

Forsberg, B. R., Devol, A.H., Richey, J.E., Martinelli, L.A., and H. Des Santos, H. 1988. Factors controlling nutrient concentrations in Amazon floodplain lakes. Limnology and Oceanography **33**: 41-56.

Gibson, G.R., M.T. Barbour, J.B. Stribling, J. Gerritsen, and Karr, J.R. 1996. *Biological criteria: Technical guidance for streams and small rivers (revised edition).* U.S. Environmental Protection Agency, Office of Water, Washington, D. C. EPA 822-B-96-001.

Hess, L.L., Melack, J.M., Novo, E.M.L.M., Barbosa, C.C.F., and Gastil, M. 2003. Dual-season mapping of wetland inundation and vegetation for the central Amazon basin. Remote Sensing of Environment **87**: 404-428.

Hunter, B.L. and Laws, E.A. 1981. ATP and chlorophyll-a as estimators of phytoplankton carbon biomass. Limnology and Oceanography **26**: 944-956.

Inglett, P.W., Inglett, K.S., and Reddy, K.R. 2008. Biogeochemical processes and implications for nutrient cycling. Report in report titled 'Summary and synthesis of the available literature on the effects of nutrients on spring organisms and systems' submitted to FDEP Springs Initiative.

Junk, W.J., Bayley, P.B., and Sparks, R.E. 1989. The flood pulse concept in river-floodplain systems. In Proceedings of the International Large River Symposium (LARS), ed. by

D.P. Dodge, pp. 110-127. Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, Canada.

Junk, W. J. and Wantzen, K. M. 2004. The flood pulse concept: New aspects, approaches, and applications—an update. In Welcomme, R. and T. Petr (eds), Proceedings of the 2nd Large River Symposium (LARS), Pnom Penh, Cambodia. Bangkok. RAP Publication: 117–149.

Knowlton, M.F. and Jones, J.R. 1997. Trophic status of the Missouri River floodplain lakes in relation to basin type and connectivity. Wetlands **17**: 468-475.

Kunz, M. J., Anselmetti, F. S., Wüest, A., Wehrli, B., Vollenweider, A., Thüring, S. and Senn, D. B. 2011a. Sediment accumulation and carbon, nitrogen, and phosphorus deposition in the large tropical reservoir Lake Kariba (Zambia/Zimbabwe). Journal of Geophysical Research **116**: G03003. doi:10.1029/2010JG001538.

Kunz, M. J., Wüest, A., Wehrli, B., Landert, J., and Senn, D. B. 2011b. Impact of a large tropical reservoir on riverine transport of sediment, carbon, and nutrients to downstream wetlands, Water Resources Research **47**: W12531. doi:10.1029/2011WR010996.

Long, C.M., and Pavelsky, T.M. 2013. Remote sensing of suspended sediment concentration and hydrologic connectivity in a complex wetland environment. Remote Sensing of Environment **129**: 197-209.

Mackay, J.R. 1963. The Mackenzie Delta area, N.W.T. Miscellaneous Report 23. Geological Survey of Canada.

Marsh, P., and Hey, M. 1989. The flooding hydrology of Mackenzie Delta lakes near Inuvik, N.W.T., Canada. Arctic **42**: 41-49.

Mathias, J.A. and Barica, J. 1980. Factors controlling oxygen depletion in ice-covered lakes. Canadian Journal of Fisheries and Aquatic Sciences **37**: 185-194.

Mayora, G., Devercelli, M., and Giri, F. 2013. Spatial variability of chlorophyll-a and abiotic variables in a river–floodplain system during different hydrological phases. Hydrobiologia **717**: 51–63.

Mertes, L.A.K. 1997. Documentation and significance of the perirheic zone on inundated floodplains. Water Resour. Res. **33**: 1749-1762. doi:10.1029/97WR00658.

Molles, M.C., Jr., Crawford, C.S., Ellis, L.M., Valett, H.M., and Dahm, C.N. 1998. Managed flooding for riparian ecosystem restoration: Managed flooding reorganizes riparian forest ecosystems along the middle Rio Grande in New Mexico. BioScience **48**:749–756.

Norlin, J. I., Bayley, S. E. and Ross, L. C. M. 2005. Submerged macrophytes, zooplankton and the predominance of low- over high-chlorophyll states in western boreal, shallow-water wetlands. Freshwater Biology **50**: 868–881. doi: 10.1111/j.1365-2427.2005.01366.x.

Olde Venterink, H., Vermaat, J. E., Pronk, M., Wiegman, F., van der Lee, G. E. M., van den Hoorn, M. W., Higler, L., and Verhoeven, J. T. A. 2006. Importance of sediment deposition and denitrification for nutrient retention in floodplain wetlands. Applied Vegetation Science **9**: 163–174.

Ostojić, A., Rosado, J., Miliša, M., Morais, M., and Tockner, K. 2013. Release of Nutrients and Organic Matter from River Floodplain Habitats: Simulating Seasonal Inundation Dynamics. Wetlands **33**: 847-859.

Parsons, T. R., Maita, Y., and Lalli, C. M. 1984. Manual of chemical and biological methods for seawater analysis; Pergamon Press: Oxford, New York.

Pavelsky, T. M., and Smith, L. C. 2009. Remote sensing of suspended sediment concentration, flow velocity, and lake recharge in the Peace-Athabasca Delta, Canada. Water Resources Research **45**, W11417. doi:10.1029/2008WR007424.

Persic, V. and Horvatic, J. 2011. Spatial distribution of nutrient limitation in the Danube River floodplain in relation to hydrological connectivity. Wetlands **31**: 933-944.

Peters D.L. 2003. Controls on the persistence of water within perched basins of the Peace-Athabasca Delta, northern Canada. Ph.D. Thesis, Watershed Ecosystems Graduate Program, Trent University, Peterborough, Ontario, Canada, pp. 194.

Pham, S. V., Leavitt, P. R., McGowan, S., Wissel, B., and Wassenaar, L.I. 2009. Spatial and temporal variability of prairie lake hydrology as revealed using stable isotopes of hydrogen and oxygen. Limnology and Oceanography **54**: 101–118.

Pithart D., Pichlová R., Bílý M., Hrbáèek J., Novitná K., and Pechar L. 2007. Spatial and temporal diversity of small shallow waters in river Lunice floodplain. Hydrobiologia **584**: 265-275.

Prézelin, B.B. and Matlick, H.A. 1983. Nutrient-dependent low-light adaptation in the dinoflagellate Gonyaulax polyedra. Marine Biology **74**: 141-150.

Prowse, T. D., Conly, F. M., Church, M. and English, M. C. 2002. A review of hydroecological results of the Northern River Basins Study, Canada. Part 1. Peace and Slave rivers. River Research Applications **18**: 429–446. doi: 10.1002/rra.681.

Robb, T., and Abrahams, M.V. 2002. The influence of hypoxia on risk of predation and habitat choice by the fathead minnow, *Pimephales promelas*. Behav. Ecol. Sociobiol. **52**: 25-30. doi:10.1007/s00265-002-0474-2.

Robb, T., and Abrahams, M.V. 2003. Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? J. Fish Biol. **62**: 1067-1081. doi:10.1046/j.1095-8649.2003.00097.x.

Rooney, N., and Kalff, J. 2003. Interactions among epilimnetic phosphorus, phytoplankton biomass and bacterioplankton metabolism in lakes of varying submerged macrophyte cover. Hydrobiologia **501**, 75–81.

Sagin, J., Sizo, A., Wheater, H., Jardine, T.D., and Lindenschmidt, K. 2015. A water coverage extraction approach to track inundation in the Saskatchewan River Delta, Canada. International Journal of Remote Sensing 36: 764-781.

Smith, V. H., Tilman, G. D. and Nekola, J. C. 1999. Eutrophication: impacts of excess nutrient input on freshwater, marine, and terrestrial ecosystems. Environmental Pollution **100**: 179–196.

Smith, N., and Perez-Arlucea, M. 2008. Natural levee deposition during the 2005 flood of the Saskatchewan River. Geomorphology **101**: 583-594.

Sokal, M.A., Hall, and Wolfe, B.B. 2008. Relationships between hydrological and limnological conditions in lakes of the Slave River Delta (NWT, Canada) and quantification of their roles on sedimentary diatom assemblages. Journal of Paleolimnology **39**: 533-550.

Sokal, M.A., Hall, and Wolfe, B.B. 2010. The role of flooding on inter-annual and seasonal variability of lake water chemistry, phytoplankton diatom communities and macrophyte biomass in the Slave River Delta (Northwest Territories, Canada). Ecohydrology **3**: 41-54.

Søndergaard, M., and Moss, B. 1998. Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes. In Jeppesen, E. M., M. Søndergaard, M. Søndergaard

and K. Christoffersen (eds), The Structuring Role of Submerged Macrophytes in Lakes. Ecological Studies Vol. 131. Springer-Verlag, New York: 115–132.

Thomaz, S.M., Bini, L.M., and Bozelli, R.L. 2007. Floods increase similarity among aquatic habitats in river-floodplain systems. Hydrobiologia **579**: 1-13.

Tockner, K., Malard, F., and Ward, J.V. 2000. An extension of the flood pulse concept. Hydrological Processes **14**: 2861-2883.

Tockner K., and Stanford, J.A. 2002. Riverine flood plains: present state and future trends. Environmental Conservation **3**: 308-330.

Van de Wolfshaar, K. E., Middelkoop, H., Addink, E., Winter, H. V., and Nagelkerke, L. A. J. 2011. Linking flow regime, floodplain lake connectivity and fish catch in a large river-floodplain system, the Volga-Akhtuba floodplain (Russian Federation). Ecosystems **14**: 920–934.

Wantzen, K., Junk, W., and Rothhaupt, K. 2008. An extension of the flood pulse concept (FPC) for lakes. Hydrobiologia **613**: 151-170.

Ward, J.V., Tockner, K. and Schiemer F.1999. Biodiversity of floodplain river ecosystems: ecotones and connectivity. Regulated Rivers: Research and Management **15**: 125–139.

Ward, D. P., Hamilton, S. K., Jardine, T. D., Pettit, N. E., Tews, E. K., Olley, J. M. and Bunn, S. E. 2013. Assessing the seasonal dynamics of inundation, turbidity, and aquatic vegetation in the Australian wet–dry tropics using optical remote sensing. Ecohydrology **6**: 312–323.

Ward, D.P., Petty, A., Setterfield, S.A., Douglas, M.M., Ferdinands, K., Hamilton, S.K., and Phinn, S. 2014. Floodplain inundation and vegetation dynamics in the Alligator Rivers region (Kakadu) of northern Australia assessed using optical and radar remote sensing. Remote Sensing of Environment **147**: 43–55.

Welch, H.E., and Legault, J.A. 1986. Precipitation chemistry and chemical limnology of fertilized and natural lakes at Saqvaqjuac, N.W.T. Canadian Journal of Fisheries and Aquatic Science **43**: 1104-1134.

Welcomme, R.L. 1979. Fisheries Ecology of Floodplain Rivers. Longman Group Limited, New York.

Wheater, H. and Gober, B. 2013. Water security in the Canadian Prairies: science and management challenges. Philosophical Transactions of the Royal Society A. doi:10.1098/rsta.2012.0409

Wiklund, J.A., Hall, R.I., and Wolfe, B.B. 2012. Timescale of hydrolimnological change in floodplain lakes of the Peace-Althabasca Delta, northern Alberta, Canada. Ecohydrology **5**: 351-367.

Wolfe, B. B., Karst-Riddoch, T. L., Hall, R. I., Edwards, T. W. D., English, M. C., Palmini, R., McGowan, S., Leavitt, P. R. and Vardy, S. R. 2007. Classification of hydrological regimes of northern floodplain basins (Peace–Athabasca Delta, Canada) from analysis of stable isotopes (δ^{18}O, δ^2H) and water chemistry. Hydrological Processes **21**: 151–168.

Wolfe, B. B., Hall, R.I., Edwards, T. W. D., Vardy, S. R., Falcone, M. D., Sjunneskog, C., Sylvestre, F., McGowan, S., Leavitt, P. R., and van Driel, P. 2008. Hydroecological responses of the Athabasca Delta, Canada, to changes in river flow and climate during the 20th century, Ecohydrology **1**: 131-148.

CHAPTER 3: DETECTING WINTER FISH PRESENCE IN FLOODPLAIN LAKES OF THE SASKATCHEWAN RIVER DELTA, SK, USING ENVIRONMENTAL DNA

Environmental DNA (eDNA) has emerged as a valuable tool for detecting fish in a wide range of habitats. . As a new tool, the full spectrum of environments in which eDNA can be applied is still unknown. Northern ecosystems, where under-ice sampling for fish can sometime require a substantial amount of fieldwork to assess fish community composition, may be one such environment. The under-ice conditions experienced within these northern water bodies may favor DNA preservation, and potentially eDNA detectability as well. The Saskatchewan River Delta, Canada, is a large northern deltaic system with numerous floodplain lakes, which are ideal sites to examine if eDNA can be used to detect under-ice fish presence. Environmental DNA samples, along with numerous water quality samples, were collected during February and March of 2014 at 26 lakes with a varying degree of hydrological connectivity to the main channel in this delta. Of the 26 lakes sampled, 16 tested positive for eDNA from at least one of the five target fish species. The number of species detected in a given lake ranged from zero to three. Fathead minnow eDNA was most prevalent, testing positive in 11 of the 26 sites. Of the limnological variables sampled, eDNA detection was only constrained by ammonia (NH_3-NH_4) concentrations and lake volume, with detections limited at high ammonia concentrations and low lake volume. Hypoxia tolerant and intolerant species were both detected in lakes of low DO, likely resulting from either fish stranding from the previous year's large flood, primer non-specificity, sediment/DNA resuspension, or contamination. The positive detection of a variety of fish species by eDNA analysis suggests that eDNA could be valuable as a method for under-ice detection of fish. These findings demonstrate the potential for eDNA analysis to detect aquatic organisms in lakes with significant ice cover; however, in order to apply this method in future research a greater understanding of how winter limnological conditions impact eDNA detection is imperative.

3.1 Introduction

Floodplain ecosystems are renowned for their high aquatic and terrestrial productivity. Containing a wide array of habitats, including permanently and temporarily flooded wetlands and lakes, the inundation of floodplain habitat is critical for the maintenance of local species diversity (Junk et al. 1989; Tockner et al. 2000). Limnological conditions within individual lakes and wetlands can vary widely, with conditions dependent on local processes and lake location (Thomaz et al. 2007, Chapter 2). Main river channels can have a significant influence on lake condition when river discharge is high and lakes become inundated (Wolfe et al. 2007; Sokal et al. 2008; 2010). Additionally, the inundation of a floodplain lake by the main channel provides the opportunity for aquatic biota from the river, or from more connected lakes, to immigrate to the newly available habitat for either temporary or permanent settlement (Junk et al. 1989; Molls et al. 1999; Gorski et al. 2014). Thus, hydrological connectivity has a substantial influence on fish community structure during the high water season (Tockner et al. 1999; Siziba et al. 2011; White et al. 2012). However, as river discharge decreases and connections are severed, internal variables become the main determinates of fish community structure within lakes (Rodríguez and Lewis 1997; Tejerina-Garro et al. 1998; Petry 2003).

To sustain a given species during the low water season within floodplains, permanent water bodies must retain favourable conditions. As water levels within off-channel lakes drop, water quality can decline with oxygen, pH, and ammonia being of concern for the viability of fish species. Shallow, productive lakes found within floodplains are more prone to dry/winter season hypoxia and fish kills (Barica and Mathias 1979; Albert and Reis 2011). Even though conditions experienced within some lakes during the dry season can be unforgiving, some fish species are still capable of feeding and reproducing during this time (Winemiller & Jepsen 1998). In addition to the drying of off-channel water bodies witnessed in tropical and warm temperate floodplains, northern floodplain lakes are subject to severe winter weather conditions, often experiencing periods of extensive ice-cover. Because ice cover reduces atmospheric oxygen exchange, lakes can become hypoxic, affecting fish assemblage structure (Magnuson et al. 1989; Tonn 1990; Danylchuk and Tonn 2003). Generally, larger species are unable to survive the hypoxic conditions in such habitats, often leading to their use by smaller fish species as refuge from predators (Chapman et al. 1996; Robb and Abrahams 2002; 2003). Within tropical floodplains, off-channel lakes have the potential to act as important refuge for fish during dry seasons (Merron and Bruton,

1988; Henderson and Crampton, 1997; Welcomme, 2005). The significance of these off-channel lakes as potential refuge, or habitat, during the dry/winter season is less understood within northern floodplain systems, where substantial ice-cover occurs. They are known to provide a hypoxic refuge for small fish species during the ice-free season (Hedges and Abrahams 2015).

Investigating fish presence in shallow ice-covered lakes during winter can be difficult using traditional sampling methods, particularly when dealing with small individuals or rare species. Trapping and netting, and the use of underwater cameras, are some examples of traditional techniques that have been used effectively to determine under-ice fish presence in the past (Tonn and Magnuson 1982; Mueller 2006; Hubert et al. 2012). The use of these traditional methods are not applicable to all habitats. The effectiveness of traps and nets are often limited to periods when fish activity and feeding is relatively high, and areas of high turbidity reduces the usefulness of underwater cameras. Environmental DNA (eDNA) is a relatively recent tool used within aquatic ecosystems to detect presence/absence of species (Ficetola et al. 2008; Takahara et al. 2013) and community assemblage patterns (Thomsen et al. 2012b). Aquatic species expel DNA into their surrounding waterbody through excretion of feces, urine, gametes, and mucous. This genetic material can then be detected by sampling the aquatic environment (Wilson and Wright 2013). The unique ability of eDNA to detect the presence of species even at very low concentrations (Ficetola et al. 2008) has resulted in this tool quickly becoming a standard detection method within aquatic ecosystems (Jerde et al. 2011; Takahara et al. 2012; Thomsen et al. 2012b). Using eDNA to detect fish presence in ice-covered waterbodies could prove to be particularly effective because cold temperatures (Kreader 1998), low light conditions (Dick et al 2010; Green et al. 2011) and low oxygen (Borin et al. 2008) are all favourable for DNA preservation (Shapiro 2008). Therefore, the conditions experienced in northern waterbodies may be beneficial for eDNA analysis.

In this study I determined whether eDNA could be used effectively in determining under-ice fish presence within lakes of varying degrees of connectivity within the Saskatchewan River Delta (SRD) during the winter season. Lakes within the SRD experience significant ice-cover and internal conditions that are potentially favourable for the preservation of eDNA during the winter season; an ideal field environment to test the under-ice fish presence using the eDNA method. I tested for the presence of five fish species with different tolerances to hypoxia: brook stickleback (*C. inconstans*), fathead minnow (*P. promelas*), northern pike (*Esox lucius*), brown trout (*Salmo trutta*), and lake sturgeon (*Acipenser fulvescens*). Next, I compared species presence

53

to the winter biogeochemistry of these lakes and lake connectivity categories (Chapter 2) to determine any potential environmental predictors. I hypothesized that fish species presence within lakes would be detectable using eDNA, and that their presence would be influenced by connectivity, with smaller, hypoxia-tolerant species being present in less connected lakes, and larger, hypoxia-sensitive species being present in connected lakes. My goal for this study was to understand whether eDNA could be used as a tool to detect the under-ice presence of fish species and to better understand which conditions may control fish assemblage patterns and eDNA detectability, during the winter season.

3.2 Methods
3.2.1 Study Area

The sampling sites were located in the Saskatchewan River Delta (SRD; approx. 53°29'N; 100°37'W), located at the Saskatchewan-Manitoba border. At an area of 10,000 km², the SRD is the largest active inland delta in North America and drains approximately 347,000 km². It consists of the upper delta, located primarily in Saskatchewan; and the lower delta, located in Manitoba. It is comprised of numerous meandering river channels, bogs, and permanently and temporary flooded lakes and wetlands, providing important ecological services for many species of wildlife and for people. The SRD is located downstream of three large hydroelectric dams, the Gardiner Dam, Francois Finley Dam and E.B. Campbell Dam, that impact the natural flow regime downstream (Wheater and Gober 2013).

Though flood peaks are smaller than those observed prior to dam construction in the 1960s, there is still sufficient flow in many years to cause inundation and connect wetlands (Smith and Perez-Arlucea 2008). The SRD is characterized by both spring and summer flood events; the latter that results from runoff from the Rocky Mountains is often greater in size than spring discharge, and can cause extensive flooding in the delta. This was the case in 2013 (the year preceding sampling) and resulted in much of the historically connected floodplain within the SRD being inundated (Sagin et al. 2015, Chapter 2). Maximum daily discharge in 2013 was the 14th highest in the 100-year record (2213 m³/s, station 05KJ001, Saskatchewan River at The Pas, Water Survey of Canada).

3.2.2 Water sample collection and filtration

Sampling took place in February and March of 2014 within an area of the Upper SRD at 30 lake sites with varying degrees of connectivity to the main channel (Figure 3.1). The connectivity of lakes were categorized using satellite imagery from five flood events of varying sizes (detailed methods described in Chapter 2). The 30 lakes were separated into five connectivity categories (drought-connected, n = 3; low-flood-connected, n = 6; moderate flood-connected, n = 3; high flood-connected, n = 7; extreme flood-connected, n = 7) based on thresholds for connection to the main channel.

Figure 3.1 Location of the Saskatchewan River Delta, Canada and sampling sites, with circles indicating the number of eDNA detections at each site. Dry lake site indicates a wetland that was sampled but had no water under ice.
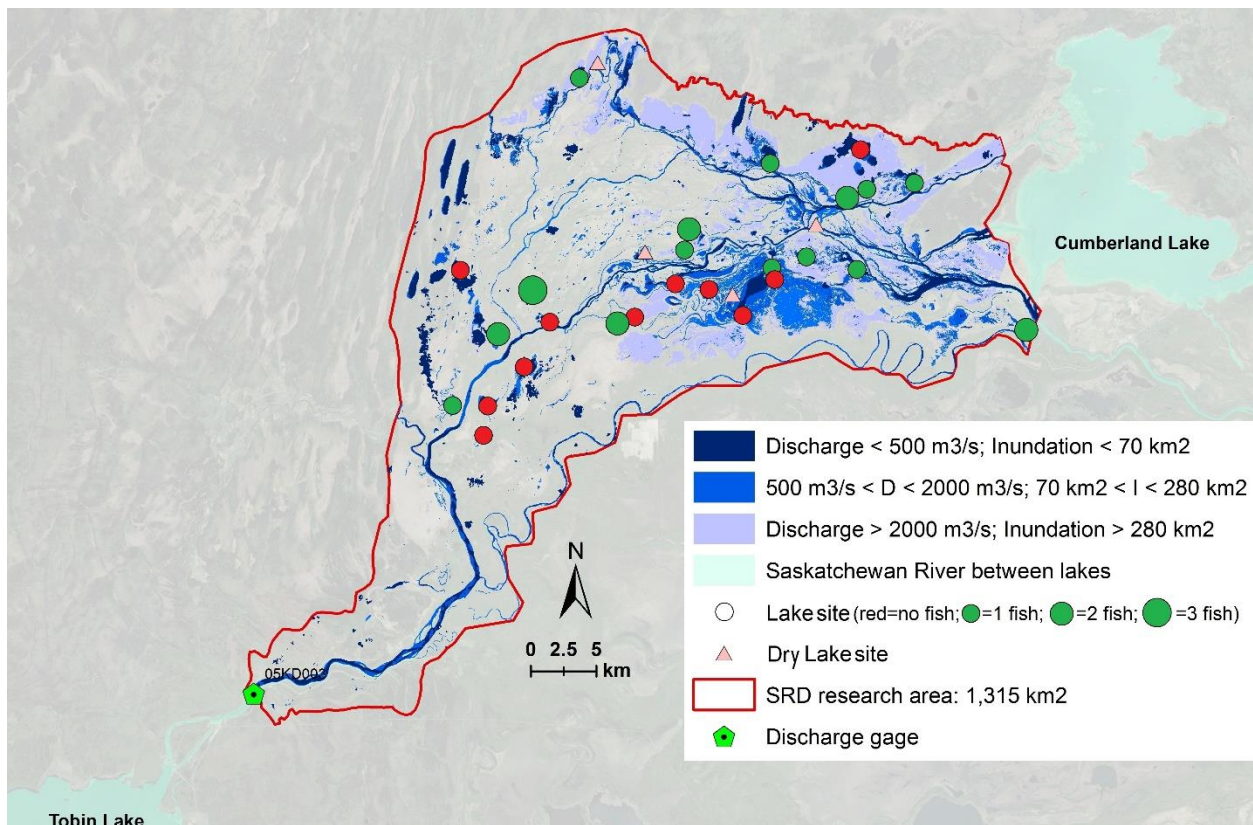
At each site a minimum of two holes were augured near the deepest part of each lake. Environmental DNA samples were collected first to minimize possible contamination with other sample bottles, and for the 30 lakes sampled, only 26 eDNA water samples were obtained

because four sites had insufficient liquid water under the ice. Water samples for eDNA were taken at the surface of each hole, for a minimum of two eDNA water samples for each lake site. Within-site locations were spread out to ensure sufficient coverage of a lake site. These samples were not immediately filtered due to complications associated with filtering in such low temperatures, and instead were collected in sterile 500ml Nalgene bottles inside a sterile bag to reduce possible cross-contamination, left outdoors for freezing ($< -30°C$), and kept frozen until they were returned to the laboratory. Water samples for other limnological endpoints that could influence fish habitat such as NH_3-NH_4 were also collected and stored as described in Chapter 2, and spot measurements of dissolved oxygen (DO) and pH were measured at each site at mid depth using a YSI EXO2 Sonde at the deepest part of each lake. Upon return to the lab, I immediately transferred the eDNA samples to a -20°C freezer.

3.2.3 DNA extraction

Frozen water samples were thawed at room temperature and then filtered using sterile Nalgene Filter Units with 0.45 µm cellulose nitrate filters (Thermo Scientific). In addition to the 26 SRD lakes, a method blank (distilled water) and field control (water from a shallow, fishless pond) were also filtered after collection and storage using the same techniques. For all samples and controls, immediately after filtration, filters were removed from filter housing, rolled up, and placed in a 5mL tube to begin the extraction process. DNA was extracted from water samples using the Powerwater DNA Isolation Kit (MoBio laboratories, CA, USA) using the manufacturer's protocol, with modifications outlined by Wilson et al. (2015). DNA extraction consisted of a minimum 30 minute bead beating step with a lysing reagent to ensure sufficient breaking of cells and DNA recovery. DNA was eluted in a final volume of 100µL and stored at -20°C until PCR.

3.2.4 PCR primer design

For PCR, five species-specific primer sets targeting the mitochondrial sequences for cytochrome oxidase b (Cytb) gene, were developed for five native taxa found within the SRD. These included brook stickleback, fathead minnow, northern pike, lake sturgeon and brown trout

(Table 3.1). This latter species was chosen as a representative of the family Salmonidae, of which lake herring (*Coregonus artedi*), lake whitefish (*C. clupeaformis*), brown trout (*S. trutta*), brook trout (*S. fontinalis*), and rainbow trout (*Oncorhynchus mykiss*) are species known to occur either in the SRD or its tributaries, as primer non-specificity is common within a given family (Bronnenhuber and Wilson 2013). Primer design followed procedures described by Wilson et al. (2015) and fragment size for the primers ranged from 95-260 bp (Table 3.1). Target species Cytb sequences were obtained from the GenBank nucleotide database (http://www.ncbi.nlm.nih.gov) and aligned using MEGA 6.06 software. Potential species-specific PCR markers were designed by the Primer-Blast function available in Genbank (http://www.ncbi.nlm.nih.gov/tools/primer-blast). Specificity checking was limited to the "nr" database, with the remaining parameters following those used by Bronnenhuber and Wilson (2013). Primer specificity was optimized by aligning potential non-target species in MEGA Alignment and identifying mismatches. A minimum of three or more mismatches with non-target species at the 3' end was used to increase specificity.

Table 3.1. Primers used for PCR analysis from five different species/families

Assay	Target	Locus	Fragment (bp)	Primer	Sequence (5' to 3')
NorthernPikeCyt*b*	Northern Pike	Cytochrome *b*	172	NP-CYTB-F1	CCGCCCCCTTACCCAATTAC
				NP-CYTB-R1	GTTCTCTAATCAGCCGGCCA
BrookSticklebackCyt*b*	Brook Stickleback	Cytochrome *b*	156	BS-CYTB-F1	TCATTGCTGGAGCCACCTTAG
				BS-CYTB-R1	TCAGAGCAATGAGAAGGGTTGC
FatheadMinnowCyt*b*	Fathead Minnow	Cytochrome *b*	260	FM-CYTB-F1	TTGAGGGGGCTTTTCAGTGG
				FM-CYTB-R1	CAAGAAGAGTGGGGGCGAAT
BrownTroutCyt*b*	Brown Trout	Cytochrome *b*	164	BT-CYTB-F1	GCACTAGTCGATCTCCCAGC
				BT-CYTB-R1	CGGCAAATGTGGCAAACAGA
LakeSturgeonCyt*b*	Lake Sturgeon	Cytochrome *b*	95	LS-CYTB-F1	CCGTCATCACCAACCTCCTC
				LS-CYTB-R1	AAGGGTGGCGTTGTCTACTG

Isolated genomic DNA or water samples from tanks containing target species or closely related species were used as positive controls and to test primer specificity. I filtered water from tanks containing northern pike, rainbow trout and white sturgeon (*A. transmontanus*) in the Aquatic Toxicology Research Facility at the University of Saskatchewan as positive controls for pike, Salmonidae and sturgeon, respectively. Samples were extracted using the same methods as described above for the eDNA samples. I used cDNA from brook stickleback and genomic DNA isolated from fathead minnow as positive controls for those two species. In these latter

cases, DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, USA), following the manufacturer's protocols and eluted in a final volume of 100µL. All positive control samples were also stored at -20°C. Primer specificity was tested among the five species to assess cross-species amplification by reacting primers against positive controls in a pairwise fashion.

3.2.5 Real-time PCR

The presence of the five species was assessed using real-time quantitative polymerase chain reaction (qPCR) for each sample that was collected from lakes, method blanks and the field controls and positive controls. These qPCR reactions were performed using an Applied Biosystems 7300 Real Time PCR System (Applied Biosystems). Reactions consisted of 10µL of Quantifast 2x SYBR Green Master Mix (Qiagen, ON, Canada), 2 µL of primer mix (1:1 of forward and reverse primer), 5µL of DNA extract from samples, and 3µL of nucleotide-free water, for a total reaction volume of 20µL. PCR conditions consisted of an initial 50°C stage for two minutes, followed by an incubation stage at 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 60 seconds. A dissociation stage was added with an additional 95°C for 15 seconds, 60°C for 60 seconds, 95°C for 15 seconds, and a final 60°C for 15 seconds. Using a dissociation curve analysis, the melting temperature (Tm) of the PCR product from positive results was compared to the Tm of products using positive controls in order to determine product specificity. In addition to running PCR on method blanks and field controls, PCR analysis was run with nucleotide-free water to further test for any potential contamination that could influence PCR results. A site was considered to have a positive detection for the species of interest if PCR resulted in a cycle threshold (Ct) representing nucleotide amplification above the baseline readings; and, a product melting temperature (Tm) that was similar to that of the product from the positive control. Melting temperatures are determined by the size and composition of a given product and allow an assessment of the similarity of multiple products; products with similar Tm's are more likely to have similar composition (nucleotide sequence).

Positive hits were tabulated for each of the five species and compared to likely drivers of fish presence, including TN, TP, NH_3-NH_4, NO_3, SO_4, DOC, Chl, DO, pH, turbidity, conductivity, $\delta^{18}O$, and δ^2H, and to determine which variables were correlated. A principal component analysis (PCA) was used to visualize the relationship between limnological

conditions and eDNA detectability. The PCA was performed using the statistical program R with the aforementioned environmental factors as the explanatory variables and lake connectivity categories as the response variable. The numbers of species detected per site using the eDNA method were displayed in the PCA by editing the size of individual site data points.

3.3 Results

3.3.1 Primer reactivity and specificity

Melt temperatures of PCR products for each species, based on positive controls (tissue and tank water), were 81.0°C for Brook Stickleback, 77.4°C for Northern Pike, 81.1°C for Lake Sturgeon, 79.4 °C for Fathead Minnow, and 78.8°C for Salmonidae. Primers had reasonable specificity when tested against non-specific positive controls, with only occasional PCR products (Table 3.2). In two of these latter cases the products had very different Tm compared with the specific control (>2°C difference, Table 3.2), while greater non-specificity, as indicated by similar Tm, occurred between the *C. inconstans* primer and *A. fulvescens* tissue (1.6°C difference), and the *A. fulvescens* primer and *E. lucius* tissue (1.8°C difference). PCR analysis also exhibited no amplification from all method blanks and field controls tested (pre- and post-filtration step).

Table 3.2 The results of primer specificity testing among primers and the tissues or tank water from the five fish species used in this study. Controls are for distilled water (D.W.) and a pond on the U of S campus with no fish present (Pond). Numbers indicate the melting temperature (Tm) of a product that occurred from qPCR. Blank cells indicate that no products occurred during qPCR.

Species	Primers				
	Brook Stickleback *C. inconstans*	Northern Pike *E. lucius*	Fathead Minnow *P. promelas*	Lake Sturgeon *A. fulvescens*	Brown trout *S. trutta*
Brook Stickleback	81				
Northern Pike		77.4		82.9	
Fathead Minnow		81	79.4		
Lake Sturgeon	82.6			81.1	82.6
Rainbow trout					78.8
Control (D.W.)					
Control (Pond)			76.3	77	

59

3.3.2 Detection of species in SRD lakes

Of the 130 reactions conducted (five species x 26 lakes), 37 samples had amplified products with Ct above the baseline. Of these, 13 amplified products were determined to have an incorrect melting temperature, either too high or too low compared to the control product, and therefore were not recorded as a positive eDNA detection (Figure 3.2). Of these 13 products that were not recorded, two occurred with the primer for Brook Stickleback, three occurred with Northern Pike, three occurred with Salmonidae, and four occurred with Fathead Minnow.

Of the 26 lakes sampled, 16 tested positive for eDNA from at least one of the five taxa (Table 3.3). The number of fish detected in a given lake ranged from zero to three per lake for the five species/families tested, with 10 lakes having no positive hits for any species, nine lakes having one hit for a single species, five lakes having two hits from two separate species, and one lake having three hits from three separate species (Figure 3.1). Fathead Minnow eDNA was most prevalent, testing positive in 11 out of the 26 sites, followed by Northern Pike with four out of the 26 lakes testing positive. The salmonid and Lake Sturgeon eDNA both were positive in three of the 26 lakes. Brook Stickleback eDNA was the least prevalent with only two of the 26 lakes testing positive.

3.3.3 Relationship with environmental variables

Water quality data displayed a wide range of values among the lakes sampled (Chapter 2). Fish presence, as indicated by positive eDNA detection, did appear to be constrained by NH_3-NH_4 and volume, with no species detected in lakes with high NH_3-NH_4 concentrations (>1.74 mg/L) or low water volumes (<$2.0x10^5$ m^3; with the exception of SRD28 for northern pike, Table 3.2). Additional water quality variables sampled, including dissolved oxygen, did not appear to constrain species detectability, nor did connectivity.

The PCA for eDNA detection based on lake characteristics and water quality indicated some degree of influence on fish DNA presence. Eigenvalues were 40.6% for the first axis and 36.0% for the second axis, and explained a large amount of variation within the dataset (76.6%). Variables influencing the axes, and the position of the lakes in different connectivity categories, were as reported in Chapter 2. From the PCA, lake connectivity category did not appear to

Figure 3.2 Dissociation curves and melting temperatures from PCR analysis of sites for Brook Stickleback. The positive control melting temperature (Tm) for Brook Stickleback was 81.0°C. The dissociation curve shows five products, with two products (blue and green curve) that had Tm within close proximity to that of the positive control Tm. Other products with dissimilar Tm (purple curves) or an insufficient product peak (not visible) were not classified as detections.

Detector = 1, Tm = 60.0 °C
Well(s): E9-E10,F1-F10,G1-G4
Document: BM Pike Brook.sds (Standard Curve)
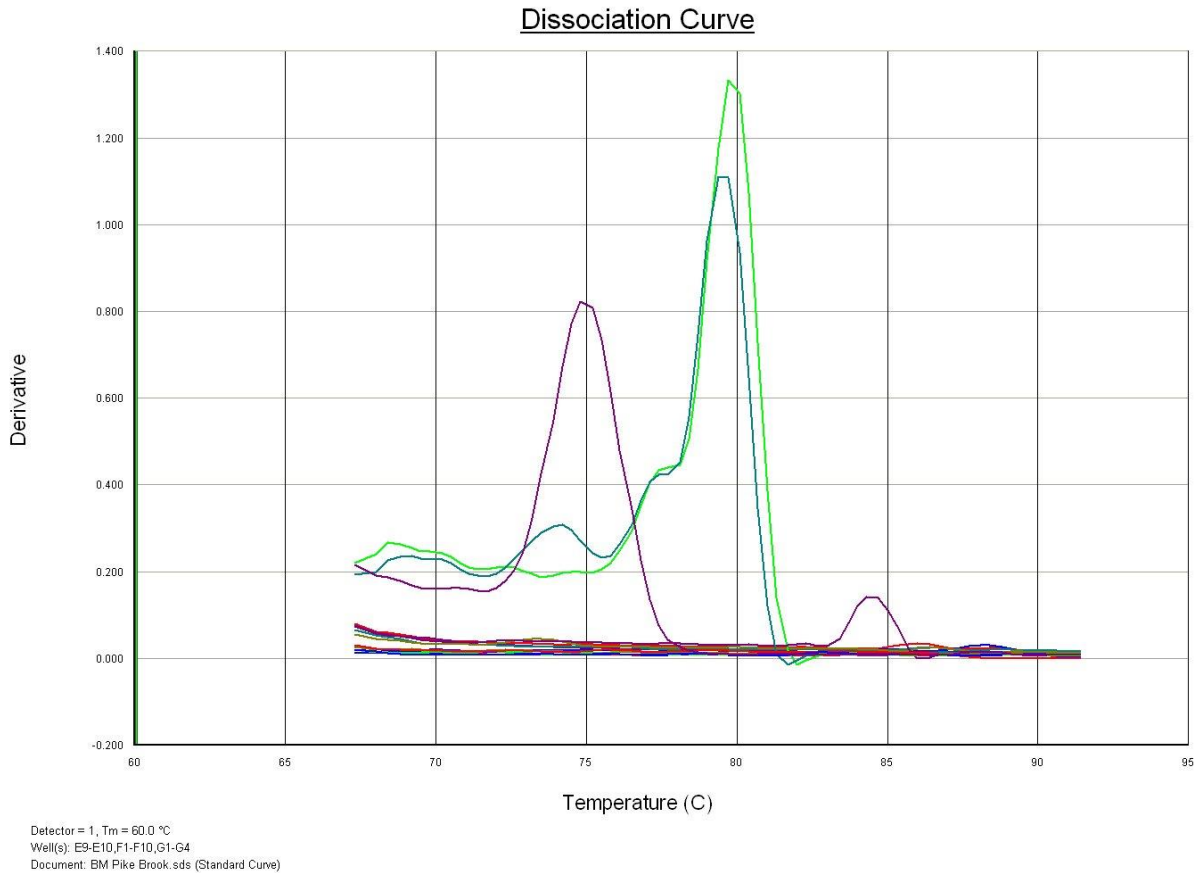
exhibit any dramatic influence on detection frequency, with detection occurring in a range of lake categories. Additionally, the other variables included in the PCA had little influence on eDNA detection rate. Detections were not constrained along Axis 1, occurring at both extremes. Detections were not constrained on the positive end of the Axis 2, but appeared to be on the negative end of this axis, with no detections in the three sites below an axis value of -0.2.

Figure 3.3 Principal component analysis (PCA) displaying the vectors of the 13 physical and chemical variables sampled from lakes of the SRD, the distribution of lakes in the five connectivity categories (drought to extreme flood) and the number of eDNA detections (size of bubble).
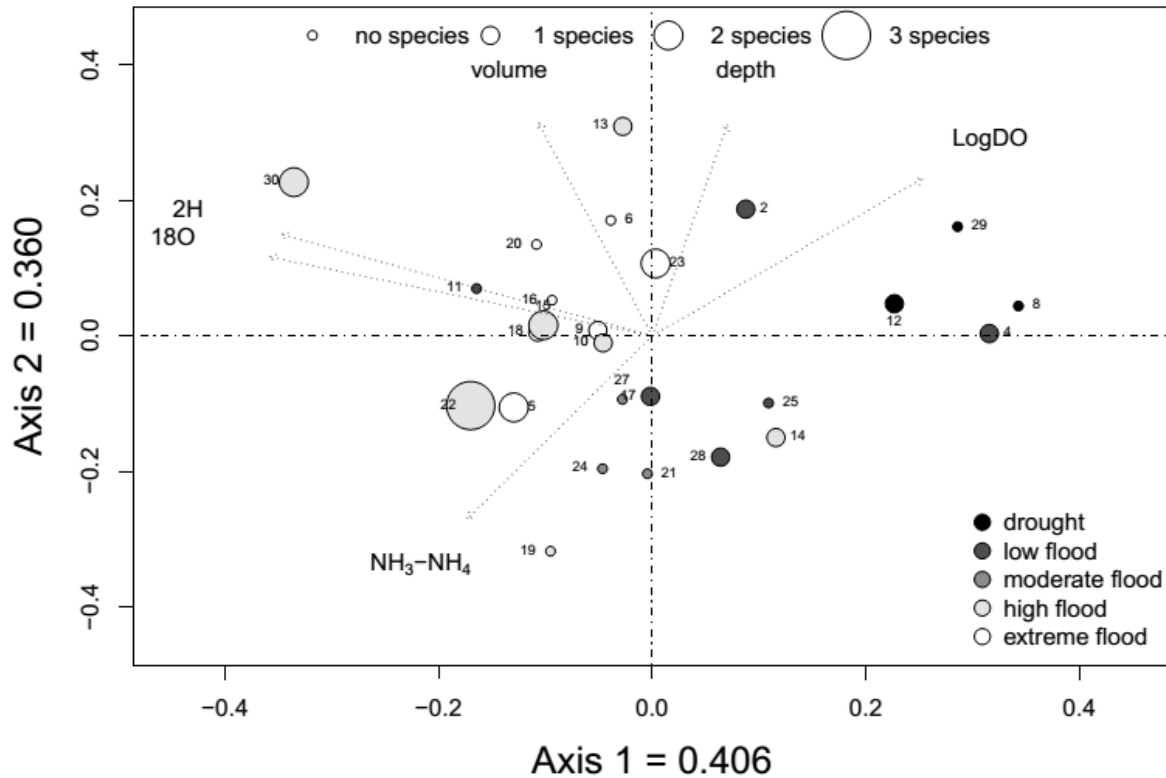
With species-specific detection at individual sites arranged according to the variables that could influence fish presence and eDNA detection, some trends emerged (Table 3.3). Overall, DO and lake connectivity did not appear to constrain species detection; however, individual species were affected. Brook stickleback, northern pike, and lake sturgeon DNA was detected in lakes that were at the lower end of oxygen concentrations (<2.33 mg/L). Salmonid DNA was detected at moderate oxygen concentrations (1.23-3.45 mg/L), and fathead minnow DNA was detected at the widest range of oxygen concentrations (0.74-11.9 mg/L). Similar to the overall species detection, lake connectivity did not appear to have any obvious influence on DNA detection for individual species, while NH_3-NH_4 and lake volume constrained detection of all individual species. Brook stickleback, northern pike, salmonid, and lake sturgeon DNA detections were in the mid-range of both lake volume (excluding SRD028) and NH_3-NH_4 concentrations (Table 3.3). Fathead minnow DNA detections ranged more broadly, occurring in lakes of medium to high volume, and medium to low NH_3-NH_4 concentrations.

Table 3.3 Results of eDNA detection at each site ordered by dissolved oxygen, connectivity, NH₃-NH₄, and volume.

Table 1. eDNA detection from PCR analysis by DO

Species	Common name														Site												
		SR011	SR005	SR017	SR019	SR038	SR021	SR022	SR023	SR024	SR014	SR020	SR009	SR010	SR015	SR025	SR028	SR027	SR023	SR005	SR010	SR006	SR016	SR002	SR013	SR029	SR004	SR008
C. inconstans	Brook Stickleback																											
P. promelas	Fathead Minnow																											
E. lucius	Northern Pike																											
Salmonidae	Salmonid																											
A. fulvescens	Lake Sturgeon																											
Dissolved Oxygen Level (mg/L)		0.42	0.53	0.65	0.71	0.74	0.75	0.81	1.11	1.12	1.23	1.44	1.54	1.63	2.23	2.26	2.33	2.56	2.78	2.98	3.45	7.01	9.32	11.10	11.63	11.94	12.30

eDNA detection from PCR analysis by Connectivity (Low to High)

Species	Common name														Site												
		SR004	SR008	SR029	SR002	SR025	SR014	SR028	SR021	SR009	SR010	SR018	SR011	SR020	SR015	SR023	SR025	SR005	SR028	SR009	SR006	SR016	SR017	SR022	SR023	SR028	SR030	SR024
C. inconstans	Brook Stickleback																											
P. promelas	Fathead Minnow																											
E. lucius	Northern Pike																											
Salmonidae	Salmonid																											
A. fulvescens	Lake Sturgeon																											
Deuterium (δ2H)		-142.6	-141.9	-139.1	-138.0	-136.3	-136.1	-132.2	-131.5	-127.7	-127.4	-125.6	-125.4	-125.1	-125.0	-120.7	-120.5	-120.5	-120.3	-119.3	-117.2	-115.2	-114.9	-113.0	-112.8	-112.1	-97.4	

eDNA detection from PCR analysis by NH3-NH4

Species	Common name														Site												
		SR029	SR004	SR008	SR002	SR019	SR027	SR014	SR009	SR010	SR018	SR011	SR020	SR015	SR022	SR023	SR025	SR006	SR016	SR010	SR009	SR005	SR023	SR018	SR016	SR029	SR020	SR025
C. inconstans	Brook Stickleback																											
P. promelas	Fathead Minnow																											
E. lucius	Northern Pike																											
Salmonidae	Salmonid																											
A. fulvescens	Lake Sturgeon																											
Ammonium Levels (mg/L)		0.03	0.06	0.09	0.12	0.13	0.13	0.21	0.32	0.36	0.53	0.53	0.56	0.75	0.75	0.81	1.02	1.21	1.53	1.66	1.74	1.74	1.77	1.84	1.84	1.97	3.69

eDNA detection from PCR analysis by volume

Species	Common name														Site											
		SR024	SR028	SR021	SR008	SR019	SR027	SR025	SR014	SR015	SR004	SR030	SR022	SR009	SR017	SR020	SR012	SR005	SR023	SR002	SR013	SR006	SR011			
C. inconstans	Brook Stickleback																									
P. promelas	Fathead Minnow																									
E. lucius	Northern Pike																									
Salmonidae	Salmonid																									
A. fulvescens	Lake Sturgeon																									
Lake volume (m3)		31170	54372	76895	78683	1E+05	1E+05	2E+05	3E+05	3E+05	3E+05	3E+05	4E+05	5E+05	5E+05	7E+05	8E+05	8E+05	9E+05	9E+05	9E+05	1E+06	2E+06	2E+06	2E+06	2E+06	3E+06

63

3.4 Discussion

In this study, I demonstrated that a relatively new analytical tool, eDNA analysis, could be used in the detection of fish under ice. Positive detections of fish DNA within the water column of ice covered lakes show the effectiveness of this tool for the Saskatchewan River Delta, as all five species selected for this study were detected in at least two lakes. Additionally, by including chemical and physical data with eDNA sampling, I have demonstrated the potential for some environmental factors to influence fish habitat in shallow water bodies with significant ice coverage. To the best of my knowledge, the use of eDNA to detect fish in a waterbody with significant ice coverage has not previously been attempted. The relative success of eDNA in this study, and the potential for the use of eDNA in such environments in the future, could provide a better understanding of aquatic community structure in ecosystems with significant ice-coverage where traditional sampling methods are less effective.

The winter-season conditions measured in the lakes of the SRD were as expected, with very low oxygen and high NH_3-NH_4 concentrations. The low oxygen concentrations are attributed to the decomposition of organic matter fueling microbial respiration within the water column and sediment. High ammonia/ammonium concentrations are also a result of the decomposition of organic matter and release from sediments, as well as from the discharge of ammonia by biota (Environment Canada 1997; Geadah 1985; Beutel 2006). As the removal of ammonia within lakes via nitrification is dependent on sufficient levels of DO (EPA, 2000), low oxygen can also maintain high ammonia. Such harsh environments can be lethal to many fish species. Oxygen concentrations varied widely among the lakes (0.42-11.9 mg/L), with species eDNA detection occurring at some of the highest oxygen levels (11.94 mg/L), as well as some of the lowest levels (0.52 mg/L). NH_3-NH_4 levels also varied widely among the lakes (0.03-3.69 mg/L) but species were not detected at the high concentrations of NH_3-NH_4 (>1.77mg/L; Table 3.3), suggesting constraints on fish survival. Total ammonia consists of two forms, un-ionized ammonia (NH_3) or ionized ammonium (NH_4), with the former being toxic to fish species. Although winter conditions would reduce the lethality of ammonia/ammonium levels, because low temperature and pH favors a higher proportion of NH_4, the lakes with highest NH_3-NH_4 concentrations border the limits for fish survival (1.54 mg/L NH_3 at 5ºC and pH of 8; Canadian Council of Ministers of the Environment 2010).

All five species were detected in the floodplain lakes of the SRD under a range of limnological conditions. This was expected for some of the species due to their life history characteristics, but not for others. Positive hits for brook stickleback and fathead minnow DNA were expected as these species are known to tolerate hypoxic winter conditions (Suthers et al. 1982) since they possess physiological, morphological and behavioural adaptations that allow them to inhabit such environments (Klinger et al. 1982). Additionally, brook stickleback and fathead minnow are known to regularly inhabit these isolated and backwater habitats (Baschuk et al. 2012; Hedges and Abrahams 2015). The detection of northern pike was also expected, but to a lesser degree than brook stickleback and fathead minnow, as pike are less tolerant to hypoxia (Siefert et al. 1973). Northern pike do, however, inhabit small lakes of the SRD regularly during the ice-free season (Baschuk et al. 2012; B. Mackinnon, personal observation), and potentially some lake sites during the ice-cover season as well (Lapointe 1986). The salmonidae and lake sturgeon detections were not expected within my sites, as they are not tolerant to low oxygen conditions (Downing 1954; Beamish 1964; Jenkins et al. 1993), are more commonly found in larger lakes and rivers, and are generally less abundant than the other three species. The detection of these improbable species in the floodplain lakes of the SRD could be due to the extreme summer flood that occurred prior to the winter sampling season (Sagin et al. 2015, Chapter 2). Such a large flood event could have allowed movement of these species into these backwater habitats. If these individuals then became stranded once flood waters receded, it is unlikely that they would survive the hypoxic winter conditions, potentially leaving behind enough of their DNA to be detected.

Fathead minnow had the highest positive detection rate (11/26 lake sites) out of the five species tested. Such a high detection rate may be attributed to the species being common within these habitats (Baschuk et al. 2012; Hedges and Abrahams 2015), but it may also be a symptom of the primers used being non-specific. Though my primer exhibited good specificity when tested against positive controls from other families, non-specific reactions within families are common (Bronnenhuber and Wilson 2013). There are eight species of Cyprinidae that are likely to occur in the SRD. Of the eight species, only the pearl dace (*Margariscus margarita*) occur in similar backwater habitats as fathead minnow, with the remaining cyprinid species found primarily in streams, rivers, or large lakes (Scott and Crossman 1973). Being close relatives, and inhabiting similar niches, it is possible some of the eDNA detection for fathead minnow may be

a result of non-specific reactions with pearl dace DNA. Additionally, some of the eDNA detection for fathead minnow in the more connected lakes may be a result of non-specific reactions with DNA from some of the more riverine cyprinid species, such as the spottail shiner (*Notropis hudsonius*) or emerald shiner (*N. atherinoides*). Achieving sufficient primer specificity can be difficult, particularly when sampling sites with closely related species, and the only way to confirm if a product is comprised of only the intended target sequence is to have it purified and sequenced (Laramie et al. 2015). While I did not complete this sequencing step for product confirmation, my use of extensive positive controls (Wilson et al. 2014) was critical in order to have confidence in the eDNA detection results at the family level.

Although the eDNA method was effective in this study, further research is needed to determine how the winter environmental conditions in these shallow floodplain lakes may impact the preservation of eDNA in the water column. In typical waterbodies, the detection of eDNA implies the recent presence of a species, with detectability after species removal determined by conditions in the water column, namely pH, solar radiation, and temperature (Barnes et al. 2014; Pilliod et al. 2014; Strickler et al. 2015). Genetic material persists in the water column up to 25 days, but most studies are done with ambient temperatures that range from 7-25 °C (Dejean et al. 2011; Goldberg et al. 2013; Barnes et al. 2014; Pilliod et al. 2014; Thomsen et al. 2012a; b). When analyzing frog eDNA, Strickler et al. (2015) reported its persistence within the water column for 58 days when water temperature was held at 5 °C. Environments that are cold, with low light and oxygen levels provide conditions capable of long-term DNA preservation (Kreader 1998; Shapiro 2008; Corinaldesi et al. 2011), which can impact the findings of eDNA analysis. The floodplain lakes of the SRD are characterized as shallow bodies of water that experience significant ice-cover and oxygen depletion during winter months, and water temperatures ranged from 0.02 to 1.56 °C during sampling. Since many northern waterbodies are characterized by these aforementioned conditions, significant ice cover limits the ability for external light and oxygen to penetrate the water column below and this, along with low temperatures, could potentially preserve genetic material longer than expected and thus overestimate the recent presence of species.

In the shallow floodplain lakes of the SRD, sediment resuspension may be an additional confounding factor that ultimately impacts the ability to use eDNA as an indicator of recent

species presence. Due to adsorption of DNA molecules, DNases, and chemicals to soils and sediments (Levy-Booth et al. 2007; Corinaldesi et al. 2008; Pietramellara et al. 2008), DNA can be preserved much longer than in the water column, persisting for decades after deposition (Andersen et al. 2012; Yoccoz et al. 2012); therefore, resuspension could act as a source of contamination by long-time preserved DNA from the soil. For example, Turner et al. (2015) reported eDNA concentrations in sediment that were 8-1000 times greater than those in water, and detection of a target species, bigheaded Asian carp (*Hypophthalmichthys* spp), occurred months after its removal. The resuspension of sediment and the associated genetic material therefore has the potential to lead to false interpretations about the recent history of a given site (Goldberg et al. 2015). As a result, there is increased interest in fully understanding the implications of a changing chemical and physical environment on eDNA preservation and persistence (Stickler et al. 2015; Turner et al. 2015).

In conclusion, I was able to effectively use eDNA to detect the presence of fish DNA in lakes of a northern floodplain during the winter with significant ice coverage. In recent years eDNA has become a powerful tool to detect species presence (Ficetola et al. 2008; Takahara et al. 2013) and community structure (Thomsen et al. 2012b) within aquatic ecosystems, and my research suggests eDNA could also enhance understanding of aquatic communities below ice. Using eDNA instead of, or in conjunction with, traditional techniques is advantageous as it can often detect more or different species compared to traditional methods (e.g. Dejean et al. 2012; Biggs et al. 2015). Additionally, eDNA could provide a way to understand community structure in remote ecosystems where extended monitoring studies are not possible, or where the understanding of community assemblage patterns may be limited. In northern ecosystems like the SRD, where effects of climate change have the potential to be most severe (Natural Resources Canada 2004), more research is necessary to determine a year-round understanding of aquatic community composition. This will allow predictions of the potential biological impacts of climate change that will combine with existing dams and diversions to further reduce and alter flows from upstream (Schindler and Donahue 2006, Wheater and Gober 2013). The reduction of annual precipitation, runoff and the disappearance of small northern waterbodies that are forecast as consequences of climate change in this region (Smith et al. 2005; Smol and Douglas 2007; Wolfe et al. 2008) would profoundly impact those species reliant on these waterbodies. Environmental DNA has the potential to be used effectively to not only gain a better

understanding of the species composition within these ecosystems within and between seasons, but also to provide a baseline to determine the impacts of climate change and other anthropogenic effects.

3.5 Literature Cited

Albert, J. S. and R. E. Reis, R. E.. 2011. Introduction to Neotropical Freshwaters. Pp. 3-19. In: Albert, J. S. & R. E. Reis (Eds.). Historical Biogeography of Neotropical Freshwater Fishes. Berkeley. University of California Press.

Andersen, K., Bird, K.L., Rasmussen, M., Haile, J., Breuning-Madsen, H., Kjaer, K.H., Orlando, L., Gilbert, M.T.P., Willerslev, E., 2012. Meta-barcoding of ''dirt'' DNA from soil reflects vertebrate biodiversity. Mol. Ecol. **21**: 1966–1979.

Barica, J. and Mathias, J.A. 1979. Oxygen depletion and winterkill risk in small prairie lakes under extended ice cover. Journal of Fisheries Research Board of Canada **36**: 980-986.

Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L., and Lodge, D.M. 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environmental Science and Technology **48**: 1819–1827.

Baschuk, M.S., Koper, N., Wrubleski, D.A., Goldsborough, G. 2012. Effects of water depth, cover and food resources on habitat use of marsh birds and waterfowl in boreal wetlands of Manitoba, Canada. Waterbirds **35**: 44–55.

Beamish, F. W. H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. Can. J. Zool. **42**: 355-36.

Beutel, M.W. 2006. Inhibition of ammonia release from anoxic profundal sediments in lakes using hypolimnetic oxygenation. Ecological Engineering **28**: 271-279.

Biggs J., Ewald, N., Valentini, A., Gaboriaud C., Dejean, T., Griffiths R.A., Foster, J., et al. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). Biological Conservation **183**: 19-28.

Borin, S., Crotti, E., Mapelli, F., Tamagnini, I., Corselli, C., and Daffonchio, D. 2008. DNA is preserved and maintains transforming potential after contact with brines of the deep anoxic hypersaline lakes of the Eastern Mediterranean Sea. Saline Systems 4.

Bronnenhuber, J.E. and Wilson, C.C. 2013. Combining species-specific COI primers with environmental DNA analysis for targeted detection of rare freshwater species. Conservation Genetics Resources **5**: 971-975.

Canadian Council of Ministers of the Environment. 2010. Canadian water quality guidelines for the protection of aquatic life: Ammonia. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Chapman, L. J., Chapman, C. A., and Chandler, M. 1996. Wetland ecotones as refugia for endangered fishes. Biological Conservation **78**: 263-270.

Corinaldesi, C., Beolchini, A., and Dell'Anno, A. 2008. Damage and degradation rates of extracellular DNA in marine sediments: implications for the preservation of gene sequences. Mol. Ecol. **17**: 3939-3951.

Corinaldesi, C., Barucca, M., Luna, G.M., Dell'anno, A. 2011. Preservation, origin and genetic imprint of extracellular DNA in permanently anoxic deep-sea sediments. Mol. Ecol. **20**: 642–654.

Danylchuk, A.J. and Tonn, W.M. 2003. Natural disturbance and fish: local and regional influences on winterkill of fathead minnows in boreal lakes. Trans. Am. Fish. Soc. **132**: 289-298.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., and Miaud, C. 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS one **6**: e23398.

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., Miaud, C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog Lithobates catesbeianus. J. Appl. Ecol. **49**: 953–959.

Dick, L. K., Stelzer, E. A., Bertke, E. E., Fong, D. L., and Stoeckel, D. M. 2010. Relative decay of *Bacteroidales* microbial source tracking markers and cultivated *Escherichia coli* in freshwater microcosms. Applied and environmental microbiology **76**: 3255-3262.

Downing, K. M. 1954. The influence of dissolved oxygen concentration on the toxicity of potassium cyanide to rainbow trout. J. Exp. Biol. **31**: 161-164.

Environment Canada. 1997. Problem formulation for Ammonia in the aquatic environment. Canadian Environmental Protection Act Priority Substances List 2. Version 5.0, November 4, 1997.

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. 2008. Species detection using environmental DNA from water samples. Biology Letters **4**: 423-425.

Geadah, M. 1985. National Inventory of Natural and Anthropogenic Sources and Emissions of Ammonia (1980). Environmental Protection Programs Directorate, Environmental Protection Service, Environment Canada Report EPS5/IC/1.

Green, H. C., Shanks, O. C., Sivaganesan, M., Haugland, R. A., and Field, K. G. 2011. Differential decay of human faecal *Bacteroides* in marine and freshwater. Environmental microbiology **13**: 3235-3249.

Goldberg, C. S., Laramie, M. B., and Waits, L. P. 2013. Application of Environmental DNA for Inventory and Monitoring of Aquatic Species. US Department of the Interior, US Geological Survey.

Goldberg, C.S., Strickler, K.M., and Pilliod, D.S. 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. Biological Conservation (special issue) **183**:1-3.

Górski, K., Collier, K. J., Hamilton, D. P., and Hicks, B. J. 2014. Effects of flow on lateral interactions of fish and shrimps with off-channel habitats in a large river-floodplain system. Hydrobiologia **729**: 161-174.

Henderson, P. A., and Crampton, W. G. 1997. A comparison of fish diversity and abundance between nutrient-rich and nutrient-poor lakes in the Upper Amazon. Journal of Tropical Ecology **13**: 175-198.

Hedges, K. J. and Abrahams, M. V. 2015. Hypoxic refuges, predator–prey interactions and habitat selection by fishes. J Fish Biol **86**: 288–303.

Hubert,W.A., Pope, K.L., Dettmers, J.M. 2012. Passive capture techniques. In. Zale AV, Parrish, D.L., Sutton, T.M. (eds) Fisheries techniques, 3rd edn. American Fisheries Society, Bethesda, pp 223-266.

Jenkins, W.E., Smith, T.I.J., Heyward,L.D., and Knott, D.M. 1993. Tolerance of shortnose sturgeon, *Acipenser brevirostrum*, juveniles to different salinity and dissolved oxygen

concentrations. Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies **47**: 476-484.

Jerde, C.L., Mahon, A.R., Chadderton, W.L., and Lodge, D.M. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters **4**:150-157.

Junk, W.J., Bayley, P.B., and Sparks, R.E. 1989. The flood pulse concept in river-floodplain systems. In Proceedings of the International Large River Symposium (LARS), ed. by D.P. Dodge, pp. 110-127. Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, Canada.

Klinger, S.A., Magnuson, J.J., and Gallepp, G.W. 1982. Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. Environmental Biology of Fishes **7**: 113-120.

Kreader, C. A. 1998. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water. Applied and environmental microbiology **64**: 4103-4105.

Lapointe, G.S. 1986. Fish movement and predation of macroinvertebrates in a lakeshore marsh. M.Sc. Thesis, University of Minnesota, St. Paul, Minnesota.

Laramie, M.B., Pilliod, D.S., Goldberg, C.S. 2015. Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biol. Conserv. **183**: 29–37.

Levy-Booth, D.J., Campbell, R.G., Gulden, R.H., Hart, M.M., Powell, J.R., Klironomos, J.N., Pauls, K.P., Swanton, C.J., Trevors, J.T., Dunfield, K.E. 2007. Cycling of extracellular DNA in the soil environment. Soil Biol. Biochem. **39**: 2977–2991.

Magnuson, J.J., Paszkowski, C.A., Rahel, F.J., and W.M. Tonn. 1989. Fish ecology in severe environments of small isolated lakes in northern Wisconsin. Pages 487-515 in R.R. Sharitz and J.W. Gibbons (eds), Freshwater Wetlands and Wildlife.

Merron, G.S. and Bruton, M.N. 1988. The ecology and management of the fishes of the Okavango delta, Botswana, with special reference to the role of the seasonal floods. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa. Investigational Report **29**: 291.

Molls, F. 1999. New insights into the migration and habitat use by bream and white bream in the floodplain of the River Rhine. Journal of Fish Biology **55**: 1187–1200.

Mueller, R.P., Brown, R.S., Hop, H., Moulton, L .2006. Video and acoustic camera techniques for studying fish under ice: a review and comparison. Rev Fish Biol Fish **16**: 213–226.

Natural Resources Canada. 2004. Climate change impacts and adaptation: a Canadian perspective.

Petry, P., Bayley, P. B, and Markle, D. F. 2003. Relationships between fish assemblages, macrophytes and environmental gradients in the Amazon River floodplain. Journal of Fish Biology **63**: 547-579.

Pietramellara, G., Ascher, J., Borgogni, F., Ceccherini, M.T., Guerri, G., Nannipieri, P., 2008. Extracellular DNA in soil and sediment: fate and ecological relevance. Biol. Fertil. Soils **45**: 219–235.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., Waits, L.P. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. Molecular Ecology Resources **14**: 109-116.

Robb, T., and Abrahams, M. V. 2002. The influence of hypoxia on risk of predation and habitat choice by the fathead minnow, Pimephales promelas. Behavioral Ecology and Sociobiology **52**: 25-30.

Robb, T., and Abrahams, M. V. 2003. Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? Journal of Fish Biology **62**: 1067–1081.

Rodríguez, M.A., and Lewis, W.M. Jr. 1997. Structure of fish assemblages along environmental gradients in floodplain lakes of the Orinoco River. *Ecological Monographs* **67**: 109-128.

Sagin, J., Sizo, A., Wheater, H., Jardine, T.D., and Lindenschmidt, K. 2015. A water coverage extraction approach to track inundation in the Saskatchewan River Delta, Canada. International Journal of Remote Sensing **36**: 764-781.

Schindler, D.W. and W.F. Donahue. 2006. An impending water crisis in Canada's western prairie provinces. Proceedings of the National Academy of Sciences USA **103**: 7210-7216.

Scott, W.B, and Crossman, E. J. 1973. Freshwater Fishes of Canada. Bulletin 184. Fisheries Research Board of Canada **184**: 1-966.

Siefert, R. E., Spoon, W.A., and Synrett, R.F. 1973. Effects of reduced oxygen concentrations on northern pike (Esox lucius) embryos and larvae. J. Fish. Res. Board Can. **30**: 849-852.

Shapiro, B. 2008. Engineered polymerases amplify the potential of ancient DNA. Trends Biotechnol **26**: 285–287.

Siziba, N., Chimbari, M. J., Masundire, H., and Mosepele, K. 2011. Spatial and temporal variations of micro-invertebrates across temporary floodplains of the lower Okavango Delta, Botswana. Physics and Chemistry of the Earth, Parts A/B/C, **36**: 939-948.

Smith, L.C., Sheng, Y., MacDonald, G.M., and Hinzman, L.D. 2005. Disappearing Arctic lakes. Science **308**: 1429.

Smith, N., and Perez-Arlucea, M. 2008. Natural levee deposition during the 2005 flood of the Saskatchewan River. Geomorphology **101**: 583-594.

Smol, J.P., and Douglas, M. 2007. From controversy to consensus: making the case for recent climate change in the Arctic using lake sediments. Frontiers in Ecology and the Environment **5**: 466–474.

Sokal, M.A., Hall, and Wolfe, B.B. 2008. Relationships between hydrological and limnological conditions in lakes of the Slave River Delta (NWT, Canada) and quantification of their roles on sedimentary diatom assemblages. Journal of Paleolimnology **39**: 533-550.

Sokal, M.A., Hall, and Wolfe, B.B. 2010. The role of flooding on inter-annual and seasonal variability of lake water chemistry, phytoplankton diatom communities and macrophyte biomass in the Slave River Delta (Northwest Territories, Canada). Ecohydrology **3**: 41-54.

Strickler, K.M., Fremier, A.K., Goldberg, C.S. 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biological Conservation **183**: 85-92.

Suthers, I.M. 1982. A preliminary study of the daily and seasonal distributions of fish in Blind Channel, Delta Marsh. University of Manitoba Field Stations (Delta Marsh) Annual Report **17**: 90-105.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z. I. 2012. Estimation of fish biomass using environmental DNA. PloS one 7: e35868.

Takahara, T., Minamoto, T., and Doi, H. 2013. Using Environmental DNA to Estimate the Distribution of an Invasive Fish Species in Ponds. PloS one **8**: e56584.

Tejerina-Garro, F.L., Fortin, R., and Rodriguez, M.A. 1998. Fish community structure in relation to environmental variation in floodplain lakes of the Araguaia river, Amazon basin. Environmental Biology of Fishes **51**: 399-410.

Thomaz, S.M., Bini, L.M., and Bozelli, R.L. 2007. Floods increase similarity among aquatic habitats in river-floodplain systems. Hydrobiologia **579**: 1-13.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M., Willerslev, E. 2012a. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS ONE **7**, e41732.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., Orlando, L., Willerslev, E. 2012b. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology **21**: 2565–2573.

Tockner, K., Schiemer, F., Baumgartner, C., Kum, G., Weigand, E., Zweimüller, I., ... and Layzer, J. B. 1999. The Danube restoration project: species diversity patterns across connectivity gradients in the floodplain system. Regulated Rivers: Research & Management **15**: 245-258.

Tockner, K., Malard, F., and Ward, J.V. 2000. An extension of the flood pulse concept. Hydrological Processes **14**: 2861-2883.

Tonn,W.M. 1990. Climate change and fish communities: a conceptual framework. Transactions of the American Fisheries Society **119**: 337-352.

Tonn, W.M., and Magnuson, J.J. 1982. Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. Ecology **63**: 1149–1160.

Turner, C.R., Uy, K.L., and Everhart, R.C. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biological Conservation **183**: 93-102.

Welcomme, R. L. 2005. Impacts of fish on inland fish populations in Africa, p. 81–84. In M. L.

Wheater, H. and Gober, B. 2013. Water security in the Canadian Prairies: science and management challenges. Philosophical Transactions of the Royal Society A. doi:10.1098/rsta.2012.0409.

White, S.M., Ondrackova, M., and Reichard, M. 2012. Hydrologic connectivity affects fish assemblage structure, diversity, and ecological traits in the unregulated Gambia River, West Africa. Biotropica **4**: 521-530.

Wilson, C.C., Bronnenhuber, J.E, Boothroyd, M., Smith, C., and Wozney, K. 2014. Environmental DNA (eDNA) monitoring and surveillance: field and laboratory standard operating procedures. OMNRF Aquatic Research Series 2014-05.

Wilson, C., Wright, E. , Bronnenhuber, J., MacDonald, F. , Belore, M., and Locke, B. 2015. Tracking ghosts: combined electrofishing and environmental DNA surveillance efforts for Asian carps in Ontario waters of Lake Erie. Management of Biological Invasions **5**: 225-231.

Wilson, C. and Wright, E. 2013. Using Environment DNA (eDNA) as a Tool in Risk-Based Decision-Making. Ontario Ministry of Natural Resources, Aquatic Research and Development Section, Aquatic Research Series 2013- 01.

Winemiller, K. O., and Jepsen, D. B. 1998. Effects of seasonality and fish movement on tropical river food webs. Journal of Fish Biology **53**: 267-296.

Wolfe, B. B., Karst-Riddoch, T. L., Hall, R. I., Edwards, T. W. D., English, M. C., Palmini, R., McGowan, S., Leavitt, P. R. and Vardy, S. R. 2007. Classification of hydrological regimes of northern floodplain basins (Peace–Athabasca Delta, Canada) from analysis of stable isotopes (δ^{18}O, δ^{2}H) and water chemistry. Hydrological Processes **21**: 151–168.

Wolfe, B. B., Hall, R. I., Edwards, T. W., Vardy, S. R., Falcone, M. D., Sjunneskog, C., ... and van Driel, P. 2008. Hydroecological responses of the Athabasca Delta, Canada, to changes in river flow and climate during the 20th century. Ecohydrology **1**: 131-148.

Yoccoz, N.G., Bråthen, K.A., Gielly, L., Haile, J., Edwards, M.E., Goslar, T., Von Stedingk, H., Brysting, A.K., Coissac, E., Pompanon, F., Sønstebø, J.H., et al., 2012. DNA from soil mirrors plant taxonomic and growth form diversity. Mol. Ecol. **21**: 3647–3655.

CHAPTER 4: GENERAL CONCLUSIONS

Floodplain lakes of the SRD exhibited a degree of limnological diversity that would be expected from the largest inland delta in North America, and with similar diversity to that which has been witnessed in similar large northern floodplain systems (Sokal et al. 2008; 2010; Wiklund et al. 2012). Such diversity was not limited to inter-lake comparisons, as individual lake limnology also exhibited seasonal variation. This study provides supporting evidence that river-lake connectivity, which has been lost in many parts of the world, influences lake condition, and demonstrates that the influence extends well after water-levels recede into the winter season. Additionally, remote sensing imagery and stable isotopes (δ^2H and δ^{18}O) were combined to analyze the connectivity of individual floodplain lakes. The combination of multiple techniques provides a framework that has the potential to more accurately assess the connectivity of off-channel water bodies, which provide important habitat to local fish species.

Winter sampling of the 26 lakes of the SRD provided an opportunity to assess the utility of eDNA in detecting the under-ice presence of fish. Such an application of eDNA has not previously been attempted prior to this study, and this ability could provide a greater understanding of ice-covered ecosystems that have previously been inaccessible. The positive detection of fish eDNA in floodplain lakes of the SRD gives some assurance to future applications in ice-covered environments; however, further research is needed to understand the potential impact of primer non-specificity and winter conditions on eDNA detection.

The number of studies employing eDNA has increased drastically in the last decade due to its many advantages compared to more traditional sampling techniques (Table 1.2). One of the most important benefits of eDNA is its overall effectiveness at detecting species. When compared to traditional sampling techniques, eDNA had greater probability of detection at low densities (Dejean et al., 2012; Jerde et al., 2011), which is valuable when researching invasive, endangered, or cryptic species. Environmental DNA is also a much more versatile sampling tool. It can be used in a variety of freshwater ecosystems, including streams, rivers, lakes, and wetlands; for researching a variety of aquatic animals, including frogs (Ficetola et al. 2008), salamanders (Goldberg et al. 2011), fish (Jerde et al. 2011), snails (Goldberg et al. 2013), and even mammals (Thomsen et al. 2012). The field sampling method for eDNA analysis, which consists of only an uncontaminated water sample, is another advantage, due to the its simplicity.

A minimal sampling method is beneficial as it reduces the number of people needed to sample a given site, the amount of time at each site, and the overall cost. Such a minimalist sampling method also reduces the likelihood of damaging a site, either physically, or by the introduction of invasive species, parasites or diseases.

Despite these advantages, like all sampling methods, eDNA does have its limits and drawbacks (Table 1.2). The information obtained from eDNA analysis is limited for the most part to presence/absence for a given species. With the exception of a few controlled aquarium experiments (Takahara et al., 2012; Thomsen et al., 2012a), in real world applications eDNA is unable to determine fish biomass or density due to numerous unaccounted-for variables. Positive species detection using eDNA also does not give any additional information about the species, such as age, life stage, health, or fecundity. The ability to detect a species within a body of water is valuable, but additional information is often needed before decisions are made regarding invasive or endangered species. Depending on the research question, a positive detection by eDNA analysis at a given site may also require more intensive sampling using more traditional techniques, due to the fear of false-positives resulting from either contamination during analysis, or the non-specificity of a primer (Darling and Mahon 2011). Strict experimental design and protocols can often minimize the potential of contamination, and prior testing can increase primer sensitivity and specificity; however, specificity for a single species is not always possible. DNA that is present in the water column is often highly degraded, with the fragment size rarely exceeding 150 bp (Deagle et al., 2006). Short fragment size can limit the probability of developing species-specific primers, making them unable to discriminate between species. The rate of degradation of genetic material within a water column is dependent on local environmental conditions, mainly pH, solar radiation, and temperature (Barnes et al. 2014; Pilliod et al. 2014; Strickler et al. 2015). These factors can vary greatly between sites and habitats, and as a result, the degradation rate of eDNA and time frame for the ability to detect a species can vary widely as well (Shapiro 2008; Dejean et al. 2011; Corinaldesi et al. 2011; Goldberg et al. 2013).
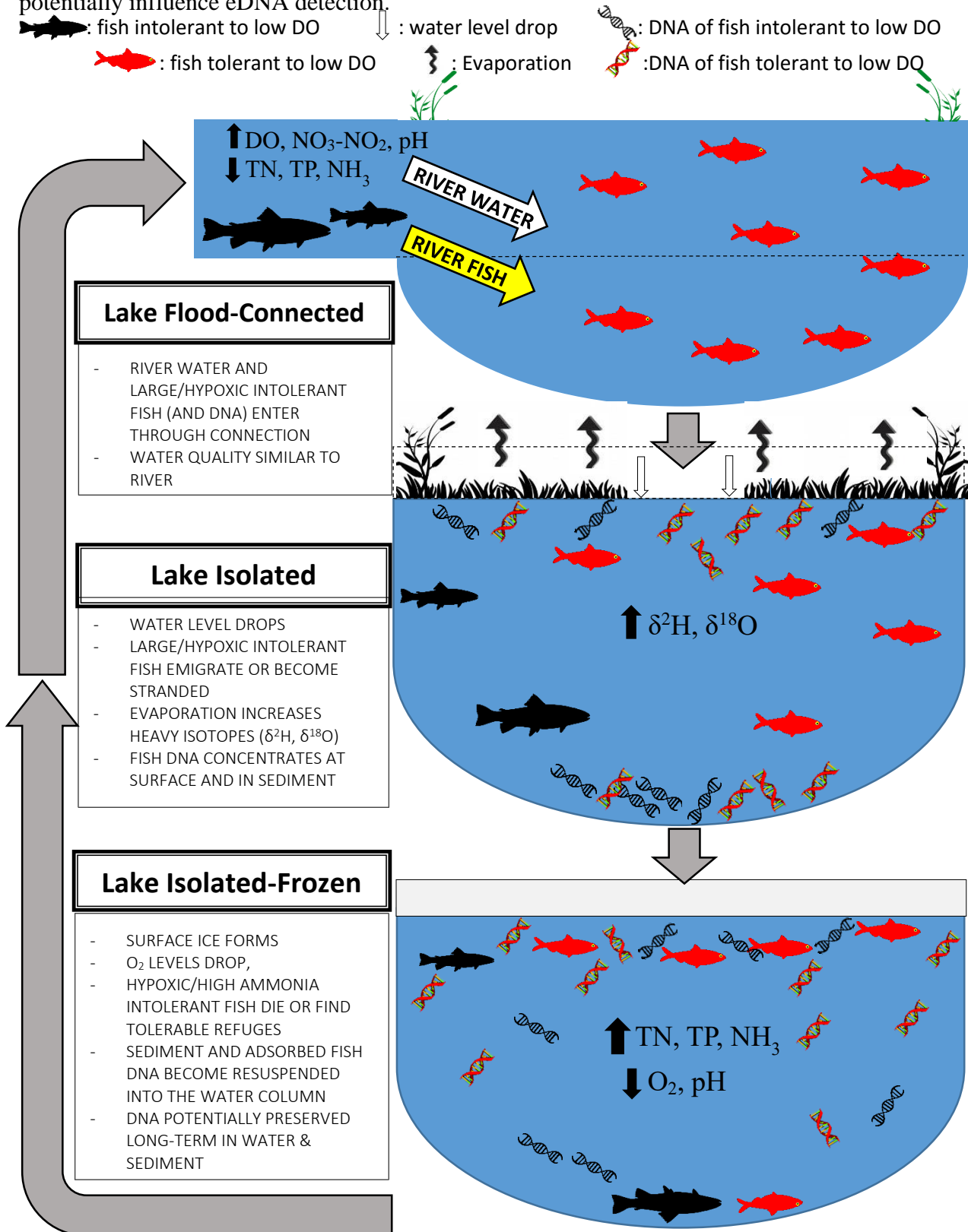
Table 4.1 Summary of some of the advantages and disadvantages for using the eDNA method in research for the detection of fish species in aquatic ecosystems (Huver et al. 2015; Herder et al.2014).

Advantages		Disadvantages	
Detection Probability	- eDNA greater species detection compared to traditional methods - high sensitivity allows detection at low species density when traditional methods can fail to detect	**Quantification**	- unable to determine biomass or density for many habitats - species density and amount of eDNA released into the environment has been researched, but highly influenced by field conditions
Cost Effective	- collection of water samples much less time consuming compared to traditional - sampling can be accomplished by a single person - the collection and analysis of water sample costs less than traditional surveying techniques	**Species Information**	- unable to determine any additional information beyond detection - life stages, population structure, fecundity and health of individuals cannot be obtained from eDNA analysis
Species Specificity/ Taxonomic Resolution	- with sufficient primer selection researchers are able to sample only the species of interest - eliminates identification error between species that are similar at any life stage by using species-specific primers	**Species Specificity**	- eDNA is highly degraded and the fragment size rarely exceeds 150 bp (Deagle et al., 2006) - short fragment may not give enough information for discrimination between individuals or species.

Versatility	- used in numerous freshwater (lakes, wetlands, river, streams) and marine habitats
	- used for frogs, fish, newts, salamanders, shrimp, mussels, and even mammals
		Reliability	- false positives can be of big concern for the eDNA method if experimental design is not sufficient or followed properly
			- minimize false positives by:
			    - confirmation of all positive eDNA product
			    - strict field and laboratory standards
			    - prior testing for sensitivity and specificity

Non-invasive	- disturbing habitat not necessary to establish species presence - sampling for eDNA involves the use of sterile materials which reduces the potential to transfer invasives, parasites, diseases	**Detection Variation**	- time frame for detection, and eDNA preservation, can vary greatly between sites, and even within a site, depending on local water conditions - factors such as temperature, UV radiation, pH influence eDNA degradation rates

In the SRD, the seasonal and spatial variation in lake limnology (Chapter 2) has the potential to influence interpretation of the eDNA results (Chapter 3). The inundation-isolation cycle, along with the freeze-thaw cycle, appear to have profound effects on fish habitat suitability and on eDNA preservation and detectability (Fig. 4.1). During the ice-free season, water level rises in the river to a point where floodplain lakes become inundated, and take on similar water quality characteristics to that of the river. In addition to similar water quality characteristics, depending on the degree of connection, fish community composition in lakes are likely to resemble those in the main channel, with fish species tolerant to a range of both ammonia and oxygen concentrations. During this connected phase which can last for two months in the summer (Sagin et al. 2015), it can be assumed that eDNA preservation and detectability

Figure 4.1 A conceptual diagram illustrating how the inundation-isolation cycle influences floodplain lake limnology of the SRD, and how it, along with the freeze-thaw cycle, could potentially influence eDNA detection.

: fish intolerant to low DO ⬇ : water level drop : DNA of fish intolerant to low DO

: fish tolerant to low DO : Evaporation :DNA of fish tolerant to low DO

⬆DO, NO$_3$-NO$_2$, pH
⬇TN, TP, NH$_3$

RIVER WATER

RIVER FISH

Lake Flood-Connected

- RIVER WATER AND LARGE/HYPOXIC INTOLERANT FISH (AND DNA) ENTER THROUGH CONNECTION
- WATER QUALITY SIMILAR TO RIVER

Lake Isolated

- WATER LEVEL DROPS
- LARGE/HYPOXIC INTOLERANT FISH EMIGRATE OR BECOME STRANDED
- EVAPORATION INCREASES HEAVY ISOTOPES (δ^2H, δ^{18}O)
- FISH DNA CONCENTRATES AT SURFACE AND IN SEDIMENT

⬆δ^2H, δ^{18}O

Lake Isolated-Frozen

- SURFACE ICE FORMS
- O$_2$ LEVELS DROP,
- HYPOXIC/HIGH AMMONIA INTOLERANT FISH DIE OR FIND TOLERABLE REFUGES
- SEDIMENT AND ADSORBED FISH DNA BECOME RESUSPENDED INTO THE WATER COLUMN
- DNA POTENTIALLY PRESERVED LONG-TERM IN WATER & SEDIMENT

⬆TN, TP, NH$_3$
⬇O$_2$, pH

preservation and detectability would be within the range observed in other river systems (<2 weeks following removal of the species; Dejean et al. 2011; Thomsen et al. 2012a).

When river discharge decreases and floodplain lakes disconnect from the main channel, local process lead to higher nutrients and NH_3-NH_4 levels (Table 4.1). The fish community also begins to diverge. Species that are unable to tolerate the conditions during the dry season have emigrated from the lakes to more habitable waters (Magnuson et al. 1985; Lucas and Baras 2002). Whether or not a fish emigrates from lake prior to the dry season will be determined by its sensitivity to oxygen and ammonia concentrations. Ammonia tolerance varies greatly between species, Arthur et al. (1987) noted a range of winter toxicity tolerance (96hr LC50) from 0.26 mg/L for rainbow trout to 1.83 mg/L for fathead minnow.

Table 4.2 Calculated percent un-ionized aqueous ammonia solutions for individual lake sites calculated using lakes NH_3-NH_4, pH and temperature measurements and equations from Emerson et al. 1975.

Site	NH3-NH4 (mg/L)	pH	Temperature (°C)	Temperature (K)	pKA	Percent of NH3
SRD02	0.126	7.7	0.209	273.359	10.074	0.421
SRD04	0.057	8.28	0.064	273.214	10.079	1.562
SRD05	1.528	7.4	0.930	274.080	10.048	0.224
SRD06	0.810	7.88	0.000	273.150	10.082	0.624
SRD08	0.088	8.33	0.020	273.170	10.081	1.743
SRD09	0.324	7.49	1.490	274.640	10.028	0.289
SRD10	0.362	7.41	0.118	273.268	10.077	0.215
SRD11	0.533	7.23	0.116	273.266	10.078	0.142
SRD12	0.124	8.06	0.051	273.201	10.080	0.946
SRD13	0.127	8.22	0.169	273.319	10.076	1.375
SRD14	0.212	7.94	0.000	273.150	10.082	0.716
SRD15	0.751	8.01	0.323	273.473	10.070	0.864
SRD16	1.023	7.73	1.033	274.183	10.044	0.483
SRD17	1.214	7.28	0.685	273.835	10.057	0.167
SRD18	0.530	7.04	0.195	273.345	10.075	0.092
SRD19	3.688	7.27	0.066	273.216	10.079	0.155
SRD20	0.562	7.7	0.617	273.767	10.059	0.435
SRD21	1.972	7.19	0.352	273.502	10.069	0.132
SRD22	1.664	7.36	0.225	273.375	10.074	0.193
SRD23	0.752	7.65	0.188	273.338	10.075	0.375
SRD24	1.770	7.31	0.106	273.256	10.078	0.170
SRD25	1.840	7.42	0.086	273.236	10.079	0.219
SRD27	1.840	7.77	0.055	273.205	10.080	0.488
SRD28	1.737	8.08	0.000	273.150	10.082	0.986
SRD29	0.026	8.77	0.000	273.150	10.082	4.651
SRD30	1.744	7.98	0.293	273.443	10.071	0.804

The detection and preservation of eDNA during the isolation phase is likely dependent on the substance sampled. Environmental DNA samples collected from sediment would have a high detection rate, even for fish species that may have been removed for an extended period of time, due to the large amount of DNA bearing particles being deposited in the sediment layer (Turner et al. 2014), and having conditions conducive to DNA preservation (Kreader 1998; Shapiro 2008; Corinaldesi et al. 2011). DNA degradation would be high at the water surface due to the high temperatures and UV radiation (Shapiro 2008), and eDNA detections from surface water samples would be limited to fish species currently inhabiting the lake. However, eDNA at the water surface has the potential to be replenished from wind or biotic action resuspending DNA-rich sediment from the benthic zone, potentially influencing detection (Turner et al. 2015). Additionally, the highly connected lakes that maintain connection to the main channel during winter have the potential to be replenished by an eDNA-rich water source if fish are more concentrated in the main channel.

Significant ice formation on isolated lakes can have a dramatic impact on lake condition (Fig. 4.1). Without atmospheric exchange, and with little or no light for photosynthesis, oxygen levels begin to drop. During the winter, the decrease in oxygen concentration also has a cascading effect on other limnological variables; reducing concentrations of sulfate and nitrate, and increasing concentrations of ammonia/ammonium. Low oxygen and high ammonia concentrations can be very detrimental to fish survival. During the ice-cover season only tolerant species, and individuals of non-tolerant species capable of migrating to high oxygen microzones (Klinger et al. 1982), are likely to survive. The eDNA of these species has the potential to become easily detectible during the winter. Significant ice-cover reduces the amount of UV radiation entering the water column and lowers oxygen levels, leading to prime conditions for DNA preservation within the water column (Shapiro 2008). With conditions highly conducive to DNA preservation, any eDNA that does become resuspended prior to ice-up or during ice-cover, will remain preserved and therefore easily detected once sampled.

Taken together, my results suggest there is a strong relationship between winter limnological conditions and the degree of connectivity of floodplain lakes. Large northern floodplain systems, similar to the SRD, have been well studied in the past (Squires and Lesack 2003; Brock et al. 2007; 2009; Wolfe et al. 2007; Sokal 2008; 2010; Lesack and Marsh 2010;

81

Wiklund 2012); however, much of the research conducted has been directed to the ice-free seasons. By extending research into the winter season, this research extends the understanding of how a flood pulse event influences off-channel floodplain lakes. High-latitude river floodplain systems are expected to be drastically impacted by the effects of climate change and further upstream water resource development, reducing the connection between the floodplain and the main channel (Prowse et al. 2006; Schindler and Smol 2006; Wolfe et al 2007; Wiklund et al. 2012). Lakes that currently experience frequent inundation will likely have conditions characteristic of infrequently flooded lakes, and lakes which currently experience infrequent inundation will likely dry up, converting to terrestrial habitat (Brock et al. 2007; Wolfe et al. 2007; Sokal 2010). With such changes expected, it is important to obtain a greater understanding of the year-round ecological services that northern floodplain ecosystems provide. Additionally, new scientific tools such as eDNA may allow researchers to efficiently detect and catalogue aquatic species within such at-risk ecosystems in order to map potential changes resulting from future hydrological change. Understanding the contribution of off-channel habitats to the functioning of river floodplains could potentially lead to improved management of water resources upstream by providing floodplains with necessary flow to inundate these crucial habitats.

Northern Canadian deltaic systems are hydrologically dynamic ecosystems, characterized by spring/summer flood events during the ice-free season, and low water levels and significant ice cover during the winter season. Conditions experienced within these systems can create a hostile environment for both terrestrial and aquatic species to survive. The conceptual diagram in Figure 4.1 illustrates how water quality, and fish species use, may vary seasonally; however, it is based on the results of my research which was spatially extensive but limited temporally to two winter sampling events. In order to get a greater understanding of changes in water quality and fish species use over the course of a winter in the SRD, more research with repeated winter sampling of individual lakes is essential.

Future research within the SRD should also focus on how fish use the different habitats that are available to them within the delta in relation to daily, seasonal, and yearly variation in river flow, concurrent with research on how limnological variables govern fish presence. The SRD is downstream of three large hydroelectric dams, the Gardiner Dam, Francois Finley Dam and E.B. Campbell Dam, which impact the natural flow regime downstream (Wheater and Gober 2013)

and reduce floodplain connectivity (Sagin et al. 2015). Elsewhere these changes are known to negatively impact fish populations reliant on floodplains (Welcomme 1979; Bailly et al. 2008; Balcombe and Arthington 2009). As a result, many floodplain ecosystems downstream of dams experience significant declines is fish production (Kingsford 2000; Gorski et al. 2011a, b). The SRD lake sturgeon (*Acipenser fulvescens*) population has experienced drastic declines over the past several decades, with estimates suggesting an 80-92% reduction in abundance during the past 40 years (Saskatchewan River Sturgeon Management Board 2002). Further understanding the diurnal and seasonal habits of important fish species could lead to improved water management strategies upstream of the SRD, and potentially healthier fish stocks. In addition to the use of eDNA in winter described here, this technique could also prove useful for some of the research associated with fish movement during the ice-free season as post-larval fish find suitable rearing habitats. Such research would help increase our understanding of how fish use the many and varied habitats of the SRD year-round.

4.1 Literature Cited

Balcombe, S.R. and Arthington, A.H. 2009. Temporal changes in fish abundance in response to hydrological variability in a dryland floodplain river. Marine and Freshwater Research 60: 146-159.

Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L., and Lodge, D.M. 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environmental Science and Technology **48**: 1819–1827.

Brock, B.E., Wolfe, B.B., and Edwards, T.W.D. 2007. Characterizing the hydrology of shallow floodplain lakes in the Slave River Delta, NWT, using water isotope tracers. Arctic, Antarctic and Alpine Research **39**: 388–401.

Brock, B.E., Yi, Y., Clogg-Wright, K.P., Edwards, T.W.D., and Wolfe, B.B. 2009. Multi-year landscape-scale assessment of lakewater balances in the Slave River Delta, NWT, using water isotope tracers. Journal of Hydrology **379**: 81–91.

Corinaldesi, C., Barucca, M., Luna, G.M., Dell'anno, A. 2011. Preservation, origin and genetic imprint of extracellular DNA in permanently anoxic deep-sea sediments. Mol. Ecol. **20**: 642–654.

Darling, J.A. and Mahon, A.R. 2011. From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments Environ. Res., **111**: 978–988.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., and Miaud, C. 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS one, 6: e23398.

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., Miaud, C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog Lithobates catesbeianus. J. Appl. Ecol. **49**: 953–959.

Emerson, K., R.E. Lund, R.V. Thurston and R.C. Russo. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32: 2379-2383.

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. 2008. Species detection using environmental DNA from water samples. Biology Letters **4**: 423-425.

Goldberg, C.S., Pilliod, D.S., Arkle, R.S., Waits, L.P., 2011. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. PloS One 6, e22746.

Goldberg, C. S., Laramie, M. B., and Waits, L. P. 2013. Application of Environmental DNA for Inventory and Monitoring of Aquatic Species. US Department of the Interior, US Geological Survey.

Gorski, K., L.V. Van Den Bosch, K.E. Van De Wolfshaar, H. Middelkoop, L.A.J. Nagelkerke, O.V. Filippov, D.V. Zolotarev, , J.J. De Leeuw, A.D. Buijse, and J.A.J. Verret. 2011a. Post-damming flow regime development in a large lowland river (Volga, Russian Federation): implications for floodplain inundation and fisheries. River Research and Applications DOI: 1-.1002/rra 1499

Gorski, K., De Leeuw, J. J., Winter, H. V., Vekhov, D. A., Minin, A. E., Buijse, A. D., and Nagelkerke, L. A. 2011b. Fish recruitment in a large, temperate floodplain: the importance of annual flooding, temperature and habitat complexity. Freshwater Biology **56**: 2210-2225.

Herder, J.E., A. Valentini, E. Bellemain, T. Dejean, J.J.C.W. van Delft, P.F. Thomsen and P. Taberlet, 2014. Environmental DNA - a review of the possible applications for the detection of (invasive) species. Stichting RAVON, Nijmegen. Report 2013-104.

Huver, J.R. et al. 2015. Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. Ecol. Appl. **25**: 991–1002

Jerde, C.L., Mahon, A.R., Chadderton, W.L., and Lodge, D.M. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters **4**:150-157.

Klinger, S. A., Magnuson, J. J., and Gallepp, G. W. 1982. Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. Environmental Biology of Fishes 7: 113-120.

Kreader, C. A. 1998. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water. Applied and environmental microbiology **64**: 4103-4105.

Lesack, L.F.W, and Marsh, P. 2010. River-to-lake connectivities, water renewal, and aquatic habitat diversity in the Mackenzie River Delta. Water Resources Research 46: W12504. doi: 10.1029/2010WR009607.

Lucas, M. C., Baras, E., Thom, T. J., Duncan, A., and Slavík, O. 2001. Migration of freshwater fishes.

Magnuson, J. J., Beckel, A. L., Mills, K., and Brandt, S. B. 1985. Surviving winter hypoxia: behavioral adaptations of fishes in a northern Wisconsin winterkill lake. Environmental Biology of Fishes **14**: 241-250.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., Waits, L.P. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. Molecular Ecology Resources **14**: 109-116.

Prowse, T.D., S. Beltaos, J.T. Gardner, J.J. Gibson, R.J. Granger, R. Leconte, D.L. Peters, A Pietroniro, L.A. Romolo and B. Toth. 2006. Climate change, flow regulation and land-use effects on the hydrology of the Peace-Athabasca-Slave system; findings from the Northern River Ecosystem Initiative. Environmental Monitoring and Assessment **113**: 167-197.

Sagin, J., Sizo, A., Wheater, H., Jardine, T.D., and Lindenschmidt, K. 2015. A water coverage extraction approach to track inundation in the Saskatchewan River Delta, Canada. International Journal of Remote Sensing **36:** 764-781.

Schindler, D.W., and Smol, J.P. 2006. Cumulative effects of climate warming and other human activities on freshwaters of arctic and subarctic North America. Ambio **35**: 160-168.

Shapiro, B. 2008. Engineered polymerases amplify the potential of ancient DNA. Trends Biotechnol **26**: 285–287.

Sokal, M.A., Hall, and Wolfe, B.B. 2008. Relationships between hydrological and limnological conditions in lakes of the Slave River Delta (NWT, Canada) and quantification of their roles on sedimentary diatom assemblages. Journal of Paleolimnology **39**: 533-550.

Sokal, M.A., Hall, and Wolfe, B.B. 2010. The role of flooding on inter-annual and seasonal variability of lake water chemistry, phytoplankton diatom communities and macrophyte biomass in the Slave River Delta (Northwest Territories, Canada). Ecohydrology **3**: 41-54.

Squires, M. M., and Lesack, L. F. 2003. The relation between sediment nutrient content and macrophyte biomass and community structure along a water transparency gradient among lakes of the Mackenzie Delta. Canadian Journal of Fisheries and Aquatic Sciences **60**: 333-343.

Strickler, K.M., Fremier, A.K., Goldberg, C.S., 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biol. Cons. **183**: 85–92.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z. I. 2012. Estimation of fish biomass using environmental DNA. PloS one 7: e35868.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M., Willerslev, E. 2012a. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS ONE **7**, e41732.

Turner, C.R., Barnes, M.A., Xu, C.C.Y., Jones, S.E., Jerde, C.L., and Lodge, D.M. 2014. Particle size distribution and optimal capture of aqueous microbial eDNA. Methods Ecol. Evol. **5**: 676–684.

Turner, C.R., Uy, K.L. & Everhart, R.C. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Conservation Biology **183**: 93–102.

Wiklund, J.A., Hall, R.I., and Wolfe, B.B. 2012. Timescale of hydrolimnological change in floodplain lakes of the Peace-Althabasca Delta, northern Alberta, Canada. Ecohydrology **5**: 351-367.

Wolfe, B. B., Karst-Riddoch, T. L., Hall, R. I., Edwards, T. W. D., English, M. C., Palmini, R., McGowan, S., Leavitt, P. R. and Vardy, S. R. 2007. Classification of hydrological regimes of northern floodplain basins (Peace–Athabasca Delta, Canada) from analysis of stable isotopes (δ^{18}O, δ^2H) and water chemistry. Hydrological Processes **21**: 151–168.