SOURCES AND CYCLING OF DISSOLVED ORGANIC CARBON ACROSS A LANDSCAPE OF ARCTIC DELTA LAKES

by

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ABSTRACT

Dissolved organic carbon (DOC) is a key regulator of aquatic ecosystems, and the primary substrate for aquatic bacteria. However, variations in function between different DOC sources are rarely studied. Arctic Mackenzie Delta lakes exhibit striking differences in DOC composition, with DOC sources ranging from C produced as a byproduct of aquatic plant (macrophyte) photosynthesis, to C derived from permafrost melting (thermokarst), and C delivered to lakes via river-water. This study assessed how variations in DOC source regulate the composition of the within-lake pool, drive bacterial metabolism and the contribution of bacterial biomass to higher trophic levels, and affect CO_2 flux from Delta lakes.

DOC-specific tracers showed macrophyte-derived DOC to comprise less than 15% of the DOC pool in macrophyte-rich lakes, despite macrophyte C levels 7-12-fold greater than total DOC. However, bacterial $\delta^{13}C$ indicated that bacteria preferentially incorporate DOC generated by macrophytes, while surveys and incubation experiments showed that bacterial metabolism is rapid on macrophytic DOC, with high rates of bacterial biomass production relative to respiratory loss as CO_2. Accordingly, $\delta^{13}C$, $\delta^{15}N$, and fatty acid biomarkers demonstrated that zooplankton from macrophyte-rich lakes receive a greater proportion of their biomass from bacterial organic matter than zooplankton from other lake types. At the same time, however, experiments indicated that the high pH resulting from rapid photosynthesis in macrophyte-rich lakes can decrease the rate of bacterial metabolism over the short-term, and increase CO_2 respiration at the expense of bacterial biomass production.

In contrast, DOC-specific tracers indicated that thermokarst-derived DOC accumulates in lakes. Incubation experiments and *in situ* surveys revealed thermokarst DOC to be a relatively poor bacterial substrate, which resulted in proportionately more CO_2 respiration, relative to bacterial biomass production, than observed for other Delta DOC sources. Moreover, multi-year surveys demonstrated that thermokarst lakes exhibit high levels of CO_2 emission, despite clear undersaturation in other lakes, presumably because permafrost-derived DOC was largely respired by bacteria. Understanding the divergent roles played by the contrasting sources of DOC to Delta lakes both adds insight to the functioning of other lake regions, globally, and helps clarify the effect of climate-induced changes in DOC on northern lakes.

Keywords: Mackenzie Delta, lakes, dissolved organic carbon, bacteria, carbon cycling, food web structure, carbon dioxide

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GLOSSARY

‰	Parts per thousand
λ	Wavelength, typically expressed in nm
$\delta^{13}C$	The ratio of ^{13}C to ^{12}C, in a sample, normalized to an external standard (PeeDee limestone, or standardized equivalent). Typically expressed in parts per thousand. Specifically: $\delta^{13}C = \{[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1\} \times 10^3$
$\delta^{15}N$	The ratio of ^{15}N to ^{14}N, in a sample, normalized to an external standard (atmospheric nitrogen gas). Typically expressed in parts per thousand. Specifically: $\delta^{15}N = \{[(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard}] - 1\} \times 10^3$
$\Delta\delta^{13}C$ / $\Delta\delta^{15}N$	The $\delta^{13}C$ / $\delta^{15}N$ of one substance normalized to another.
$a(\lambda)$	Absorbance at the specified wavelength
$a^*(\lambda)$	Absorbance at the specified wavelength, normalized to the dissolved organic carbon concentration of the sample
AIC	Aikake's Information Criterion
Allochthonous dissolved organic carbon	Dissolved organic carbon that originates externally to the lake
Autochthonous dissolved organic carbon	Dissolved organic carbon that is generated within the lake
BA	Bacterial abundance: number of bacteria per volume of lakewater
BP	Bacterial production: the production of new bacterial biomass (as carbon), assessed by measuring the incorporation of ^3H-Leucine
BR	Bacterial respiration: the conversion of organic carbon to CO_2 by bacteria, expressed as carbon equivalents
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
masl	Metres above sea level

PREFACE TO THE THESIS

The structure of my thesis is in paper format, and is presented as five manuscripts (Chapters 2 through 6). A general introductory chapter (Chapter 1) is intended to provide a brief background to the field of research and my research objectives and methods. A general conclusion (Chapter 7) is provided as a summary of conclusions drawn from across my thesis work, and suggestions for future research.

As with almost all scientific endeavours, this work could not have occurred without the contribution of several individuals. As such, I have written my manuscript chapters in the plural. Below are cited the manuscripts as they have been, or will be, submitted for publication in the scientific literature.

Chapter 2:
Tank, SE, LFW Lesack, and RH Hesslein. 2009. Northern delta lakes as summertime CO_2 absorbers within the arctic landscape. Ecosystems 12: 144-157.

Chapter 3:
Tank, SE, LFW Lesack, CL Osburn, and RH Hesslein. Multiple tracers demonstrate distinct sources of dissolved organic matter to lakes of the Mackenzie Delta, western Canadian Arctic. *In revision* for resubmission to Limnology and Oceanography.

Chapter 4:
Tank, SE, and LFW Lesack. Divergent bacterial metabolism on diverse DOM substrates in lakes of the Mackenzie Delta, western Canadian Arctic. *In preparation* for submission to Freshwater Biology.

Chapter 5:
Tank, SE, DJ McQueen, and LFW Lesack. *In press*. Elevated pH regulates bacterial carbon cycling in lakes with high photosynthetic activity. Ecology.

Chapter 6:
Tank, SE, and LFW Lesack. The contribution of bacteria to higher trophic levels in lakes of contrasting dissolved organic matter quality.

CHAPTER 1 GENERAL INTRODUCTION

1.1 Introduction

Northern Canadian freshwaters are particularly poorly understood (e.g., Schindler 2001, Schindler and Smol 2006). This lack of understanding is compounded by a shifting baseline resulting from the fact that northern ecosystems as a whole are in the midst of a period of rapid change. The effects of a changing climate, for example, are being seen with particular intensity across the Canadian arctic (ACIA 2004). Given this, it is apparent that studies to understand the current status of northern freshwaters are a necessity.

One factor that has a controlling influence on the functioning of all aquatic systems is dissolved organic carbon (DOC; organic carbon molecules derived from decomposed plant and animal material). Among other things, DOC governs the availability of nutrients, metals, and toxins in aquatic systems (Cotner and Heath 1990), and affects the penetration of sunlight through the water column (Williamson et al. 1996). In particular, however, DOC is one of the major C pools in lakes (Prairie 2008), and thus a key component of C cycling and flow in freshwaters. Because DOC comprises the primary substrate for bacterial metabolism, the speed, and manner in which bacteria process DOC plays a critical role in determining its fate. For example, while bacterial biomass production (BP) can provide an alternate energy source to higher trophic levels (Koshikawa et al. 1996, Havens et al. 2001), supplementing the upward flow of biomass provided by traditional algal-based food webs, bacterial respiration (BR) of metabolized DOC causes its 'loss' from the ecosystem as CO_2.

The composition of DOC can vary markedly between aquatic systems. Dissolved organic carbon that flows into a lake from the surrounding catchment (allochthonous DOC) tends to be more coloured, and less available as a bacterial substrate within lakes because it has been substantially metabolized on land. In contrast, DOC that is produced within a lake (autochthonous DOC) is more labile, and absorbs visible light much less strongly (Moran and Hodson 1990, McKnight et al. 1994). To date, the vast majority of studies examining the importance of DOC have occurred in deep, temperate lakes where allochthonous, coloured DOC from the surrounding watershed dominates, and any autochthonous DOC is overwhelmingly derived from phytoplankton (e.g., Kritzberg et al. 2004). Despite this, many waterbodies do not conform to this archetypal structure. For example, of the

numerous small, shallow lakes that are abundant globally (Downing et al. 2006), many are dominated by high rates of macrophyte productivity (Scheffer et al. 1993), while arctic lakes may receive significant inputs of allochthonous C from aged permafrost C, rather than contemporary sources (Gorham 1991, Walter et al. 2006). Across all lake regions, the variable effect of distinct pools of DOC on the functioning of aquatic ecosystems is generally poorly understood, particularly with respect to C cycling and energy flow. This lack of understanding is compounded by the fact that DOC from sources other than watershed- and algal-derived C remains poorly explored. In particular, however, examining the prevalence, and consequences of variable DOC sources to lakes is especially appropriate in the north, where climate-change induced changes in surrounding soils and vegetation, permafrost, and length of the ice free season are expected (e.g., Pientz and Vincent 2000, ACIA 2004, Smith et al. 2005).

1.2 Study site and thesis rationale

The Mackenzie Delta provides a unique site to study DOC in aquatic systems. This lake-rich region floods each spring, when ice-jam flooding causes a dramatic rise in the water level of the Mackenzie River. Because of small differences in elevation between Delta lakes, a flooding gradient exists that ranges from lakes that are connected to the river throughout the ice free season, to lakes that flood briefly each year, to lakes that flood less than annually (Fig. 1.1, Marsh and Hey 1989). Previous work has shown that the composition and concentration of DOC varies substantially along this gradient (e.g. Spears and Lesack 2006, Gareis 2007), whereby high elevation, isolated lakes show increased concentrations of total DOC (but less coloured, allochthonous DOC) when compared to lakes that are continually connected to the river system. Lakes with substantial thermokarst activity – permafrost melting around the lake margin – might also be expected to have unique DOC signatures, because of the large quantities of old C stored in these frozen soils (Gorham 1991). However, the effect of thermokarst activity on DOC composition has not been explored in the Delta, and is poorly known elsewhere.

The Mackenzie watershed drains 20% of the Canadian landmass, while the Delta itself is on the order of 200 km in length (Fig. 1.2). Furthermore, and like other deltas, the Mackenzie supports higher productivity and diversity than nearby freshwaters, including species that range beyond the Delta, such as birds, mammals, and fish (Mackay 1963). Thus, the C dynamics within Delta lakes both integrate processes occurring over a wide geographic area, and directly affect surrounding ecosystems.

One vital role of DOC is its influence on the CO_2 balance of lakes. In most world lakes examined to date, subsidies of watershed-derived DOC drive BR of organic C to outweigh primary production (Cole et al. 1994, Hanson et al. 2004), resulting in CO_2 supersaturation relative to the atmosphere, and an efflux of CO_2 from the lake. As such, the partial pressure of CO_2 in lakes (pCO_2) is thought to rise in step with the concentration of lakewater DOC (Hanson et al. 2003, Sobek et al. 2005), and lakes are often described as conduits of C from the landscape to the atmosphere (Kling et al. 1991). Not all lakes, however, derive most of their DOC from the surrounding watershed. For example, in the Delta, all but connected lakes appear to be largely cut off from external C inputs once floodwaters subside, while DOC concentrations are greatest in the highest elevation lakes, which presumably receive the lowest input of watershed-derived C but contain dense mats of submerged macrophytes (Lesack et al. 1991, Spears and Lesack 2006). In fact, previous studies in the Delta have shown that, in contrast to the predictions of the current model, these lakes tend towards CO_2 undersaturation as the summer progresses (Hesslein et al. 1991). However, the mechanism(s) driving the pattern observed in Delta lakes remains unexplored, while more generally, there have been no studies examining how lake-landscape connectivity, the presence of macrophyte productivity, or subsidies of thermokarst C to lakes affects the CO_2 balance of freshwaters.

The pattern of increased DOC concentrations in high elevation, isolated Delta lakes has been well documented (e.g., Spears and Lesack 2006, Gareis 2007), and has been proposed to occur because of the high levels of macrophyte productivity in infrequently flooded lakes. However, studies specifically designed to explore the origin of this increase, or DOC sources in general across Delta lakes, have not been conducted. Quantifying the

relative contribution of different DOC sources across the gradient of Delta lakes is important for several reasons. First, this understanding is fundamental to examinations of how DOC variation among Delta lakes affects within-lake processes such as C cycling and food web structure (described below). In addition, however, examining the DOC composition of Delta waterbodies provides insight into how sources of DOC that have been poorly examined to date, such as macrophyte and thermokarst C, might be incorporated into the within-lake DOC pool of freshwaters generally.

The degree to which DOC is able to provide energy for higher trophic levels is in part dependent upon its quality as a food substrate. Across aquatic systems, the ratio of BR to BP varies widely (del Giorgio and Cole 1998). When this ratio is high – and conversely, bacterial growth efficiency [BGE; (BP)/(BP+BR)] is low – bacterial metabolism of organic matter releases much more C as CO_2 than is incorporated as biomass available to higher trophic levels. Across ecosystems, BGE has been shown to range from levels as low as a few percent, to as high as 70 (del Giorgio and Cole 1998). Work in temperate lakes has shown that BGE is much higher on autochthonous, algal-derived DOC, than allochthonous, watershed derived C (Kritzberg et al. 2004). However, examinations of how BGE varies seasonally, or within a given landscape, have been scant. Previous work in the Delta suggests that during most of the ice-free season, BP and bacterial numbers are highest in infrequently flooded, macrophyte dominated lakes (Spears and Lesack 2006, but see Teichreb 1999), suggesting that BGE will also tend to increase in these macrophyte-rich systems. The substrate quality of other potential DOC sources to Delta lakes, such as floodwater and DOC derived from thermokarst processes is unknown. Elucidating how variations in bacterial metabolism are driven by the presence of diverse DOC sources is critical to understanding both how bacteria might contribute to aquatic foodwebs, and the potential residence time of DOC in lakes.

While variations in substrate quality are often investigated for their effect on bacterial metabolism (e.g., Kritzberg et al. 2004, Pérez and Sommaruga 2006), the importance of top-down and stressor-mediated effects on bacterial C cycling have been much less thoroughly explored. One factor that may be of particular importance in Delta

lakes is pH. In these macrophyte-rich systems, high productivity has been documented to increase pH to levels as great as 10.5 (Fee et al. 1988), which could certainly have direct, deleterious effects on at least some species in these communities (Beklioğlu and Moss 1995). Although the effects of acidification on aquatic ecosystem function have been extremely well studied, and documented to be greatest at higher trophic levels (Vinebrooke et al. 2003), research on high pH systems has been scant. However, elevated pH might affect bacterially-mediated C cycling through several mechanisms, including direct effects on the ability of bacteria to process organic matter, or indirect effects at higher trophic levels that may change grazing pressure on bacteria or the rate of nutrient recycling. As such, photosynthetically-induced increases in pH could markedly affect the ability of bacteria to metabolize the DOC produced as a byproduct of this photosynthesis, a phenomenon that could occur across high productivity systems that experience elevated pH.

Within the larger aquatic food web, factors that drive the speed and manner in which bacteria process DOC should also affect the importance of bacterial C for higher trophic levels. Past studies have shown both low levels of bacterial contribution to food webs where substrate quality, and resultant BGE, is low (Cole et al. 2002), and high levels of bacterial support for higher trophic levels, particularly when the bacterial substrate is very labile (e.g., glucose, Koshikawa et al. 1996, Havens et al. 2001). However, the relationship between substrate quality and bacterial contribution to foodwebs could be confounded by the fact that food chains with bacteria at their base may be longer, causing bacterial biomass to travels less efficiently to higher trophic levels (Berglund et al. 2007). However, the manner in which naturally-occurring variations in the DOC pool might affect bacterially-mediated energy flow to higher trophic levels has been poorly explored. The importance of bacterial transfer of DOC to higher trophic levels may be particularly relevant in macrophyte-dominated systems, because this source of primary production is generally thought to be unavailable to lower trophic level pelagic consumers (e.g., Kalff 2002).

1.3 Research questions and approach

In this study, I endeavoured to clarify the sources of DOC to Mackenzie Delta lakes, and assess how the presence of DOC of variable origin affects several specific aspects of C cycling and energy flow in these waterbodies. My work is based around 5 data chapters, which broadly address the following questions:

(1) Does the connection of lakes to their surrounding landscape, and thus the degree of watershed-derived DOC that a lake receives, affect pCO_2 in lakes? How do variations in lake-landscape connectivity act in concert with macrophyte productivity and the incidence of permafrost slumping to regulate pCO_2?

(2) What is the relative contribution of riverwater, macrophyte, and permafrost C to the lakewater DOC pool in Delta lakes? How does this contribution change across the gradient of Mackenzie Delta lakes?

(3) How are bacterially-mediated C cycling, and the relative magnitude of BR and BP, regulated by the different sources of DOC common to Delta lakes?

(4) How is bacterially-mediated C cycling affected by the incidence of elevated pH in Mackenzie Delta lakes, both over the short- and long-term, and in the presence and absence of other food web components?

(5) Does substrate-induced variation in BP and BGE lead to variable transfer of bacterial C to higher trophic levels?

To address these questions, I worked in a series of lakes in the east-central section of the Delta (Table 1.1, Figs. 1.2 and 1.3), using techniques that ranged from detailed laboratory incubations and experiments, to larger mesocosm experiments in select lakes, to broad-based surveys of a diverse series of waterbodies conducted several times over multiple years. I collected data that included intensive samples of pCO_2 in Delta lakes, specific measures of DOC absorbance, fluorescence, and isotopic signatures, bacterial abundance, respiration, and production, the isotopic signatures of bacteria and the isotopic and fatty acid signatures of zooplankton, and count-based measures of the response of various trophic levels to experimental variations in DOC composition and pH. Using this

suite of methods and approaches allowed me to gain a comprehensive understanding of the relative importance of variable DOC sources to Mackenzie Delta lakes, and the effects of this variability on the ultimate fate of DOC in this system. This work both greatly increases our understanding of the functioning of this important northern ecosystem, and helps elucidate how previously poorly explored sources of lakewater DOC may drive aquatic ecosystem function in lake regions globally.

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1.5 Tables

Table 1.1: Summary of studied lakes. Indicated are lakes visited during the 42-lake surveys (42LS; Chapters 2 and 3), 9-lake surveys (9LS; Chapters 4 and 6), and 6-lake surveys (6LS; Chapters 2, 3, 4, and 6). Concentrations of dissolved organic and inorganic carbon (DOC, DIC) measured during a series of 42-lake surveys undertaken in 2005 (see Chapter 2 for details) are given.

Lake	Sill				DOC (mg L^{-1})			DIC (mM)		
number	(masl)	42 LS	9 LS	6 LS	June	July	August	June	July	August
4	2.363	X			6.469	6.705	6.461	1.56	1.82	1.80
11	3.838	X			5.172	5.726	5.923	2.02	2.28	2.16
56	4.623	X	X	X	7.385	8.000	8.285	1.91	2.13	0.88
58	3.389	X			5.606	5.765	5.874	1.22	1.81	1.21
80	2.631	X	X	X	6.035	6.756	6.206	1.59	1.97	2.10
87	3.389	X		X	7.665	7.662	7.520	1.53	1.92	1.00
107	2.990	X			8.401	7.934	7.650	1.68	1.93	1.65
111	3.671	X			7.621	8.684	8.445	1.66	2.10	1.54
115	4.623	X			8.384	8.297	8.076	1.54	1.89	1.32
129	2.363	X	X	X	8.393	8.416	7.608	1.55	1.65	2.11
131	4.077	X			9.833	11.085	11.521	1.87	2.57	1.58
134	4.623	X			7.420	8.174	8.191	1.80	2.31	1.83
141	3.389	X			9.462	10.310	9.762	1.86	2.24	1.82
143	5.169	X	X		9.742	11.005	11.330	2.38	2.87	2.82
181	5.169	X	X		10.515	15.510	16.640	2.11	3.57	3.86
184	3.671	X			7.372	7.610	7.418	1.93	1.98	1.70
186	5.169	X			11.265	7.631	8.351	1.75	2.38	2.23
261	4.623	X			8.791	nd	7.486	1.59	1.74	0.89
272	2.990	X			8.606	8.519	8.243	1.29	2.08	1.75
275	4.768	X			6.790	7.430	7.141	1.31	2.14	1.36
278	4.077		X		nd	nd	nd	nd	nd	nd
280	3.838	X	X	X	7.666	8.047	7.943	1.58	1.83	1.71
287	4.077	X			6.510	6.630	6.366	1.54	1.47	0.78
300	2.990	X			5.892	6.298	6.938	1.96	2.11	1.41
501	2.631	X			7.061	6.753	5.842	1.62	1.89	1.88
511	3.389	X			8.122	8.322	8.356	1.85	2.09	0.93
517	4.077	X			7.021	7.025	7.006	1.71	1.70	0.75
520	4.913	X	X	X	10.725	14.045	15.373	2.28	3.35	3.54
521	5.169	X			10.090	11.175	11.313	2.68	3.13	1.85
522	4.913	X			7.864	7.891	7.170	1.63	1.97	1.64
538	3.838	X			8.870	8.774	8.555	1.59	2.03	1.10
148A	2.631	X			6.222	6.787	8.248	1.63	2.45	1.50
148B	3.389	X			7.845	7.863	7.783	1.55	2.00	1.74
148F	4.077	X			8.000	8.729	8.770	1.79	2.40	1.92
15A	2.177	X			5.989	6.2225	7.670	1.53	2.66	2.80
272B	3.671	X			9.003	9.381	10.180	1.60	2.61	2.33
301A	2.990	X			nd	7.074	7.219	1.75	2.02	1.51
302A	1.500	X			6.577	5.687	4.503	1.55	1.50	1.86
302B	2.631	X			6.096	6.429	6.764	1.79	2.24	1.44
527A	5.169	X			8.939	9.613	9.979	2.12	2.08	1.11
79A	2.631	X	X		7.742	7.535	6.633	1.58	1.77	2.12
85A	2.363	X			7.860	7.498	6.289	1.63	1.92	1.99
85B	2.990	X			7.938	7.829	7.136	1.81	1.78	1.97

1.6 Figures

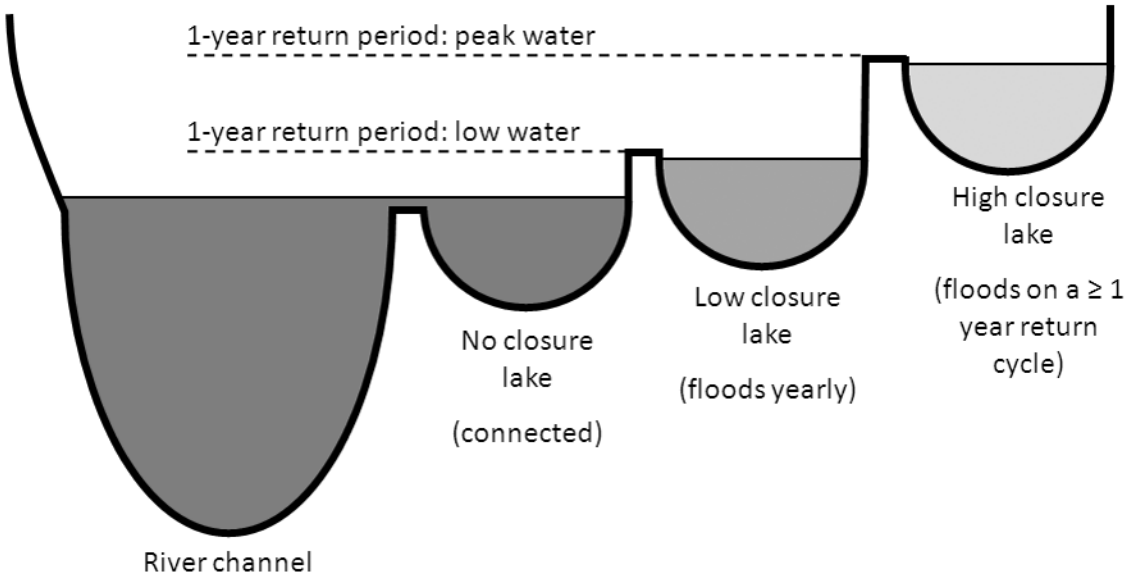

Figure 1.1: A schematic displaying differences in lake elevation for Mackenzie Delta lakes (after Emmerton et al. 2007). Lake classification types (no, low, and high closure) are described in Mackay (1963) and Marsh and Hey (1989). The density of submergent macrophytes within Delta lakes tends to increase with increasing lake elevation (Squires et al. 2002). Lakes that are severely affected by thermokarst (not directly illustrated in this schematic) occur at the highest lake elevations (Kokelj and Burn 2005).

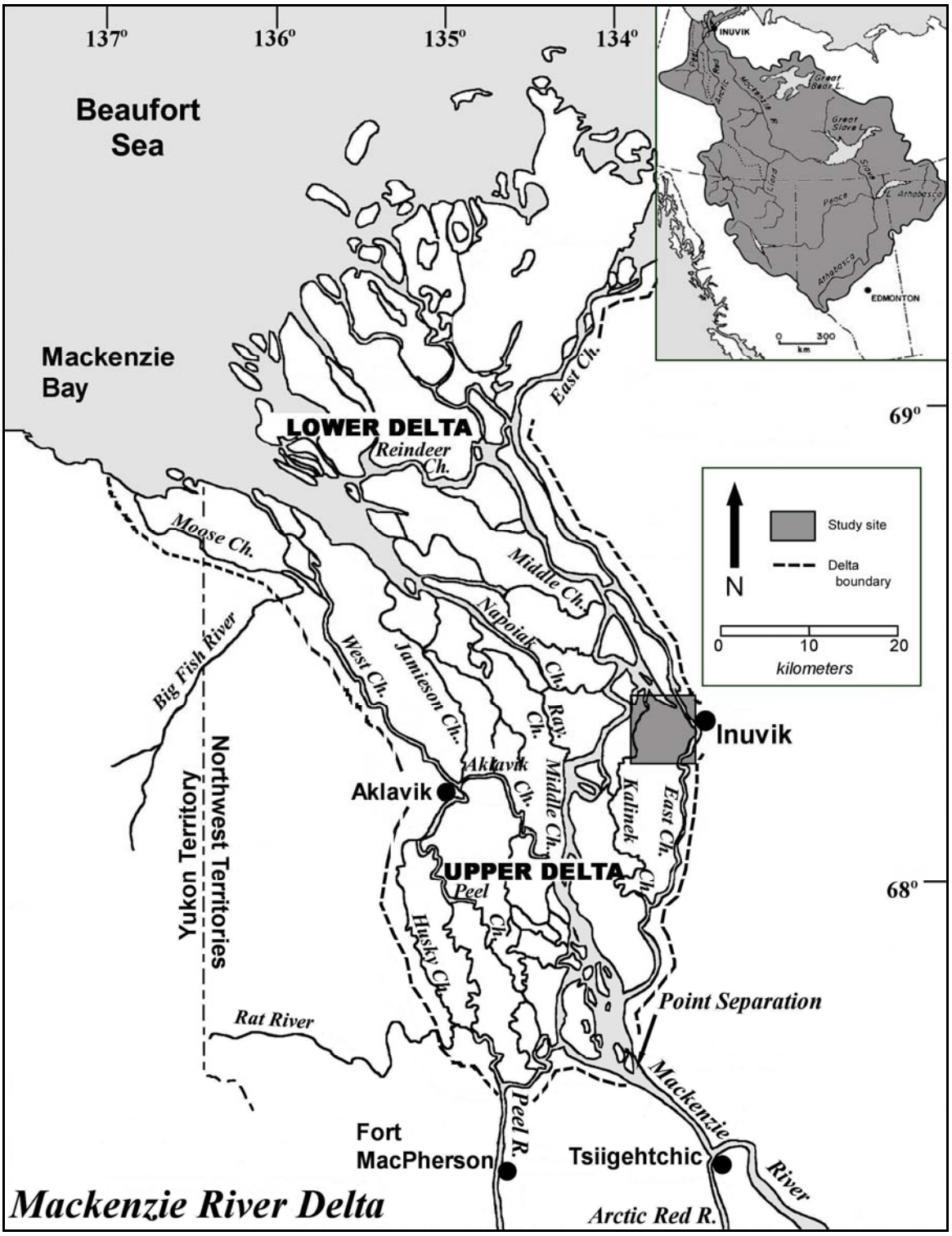

Figure 1.2: The Mackenzie River Delta, western Canadian Arctic. The general study area is highlighted in grey. The areal extent of the Mackenzie River basin is shown in the inset.

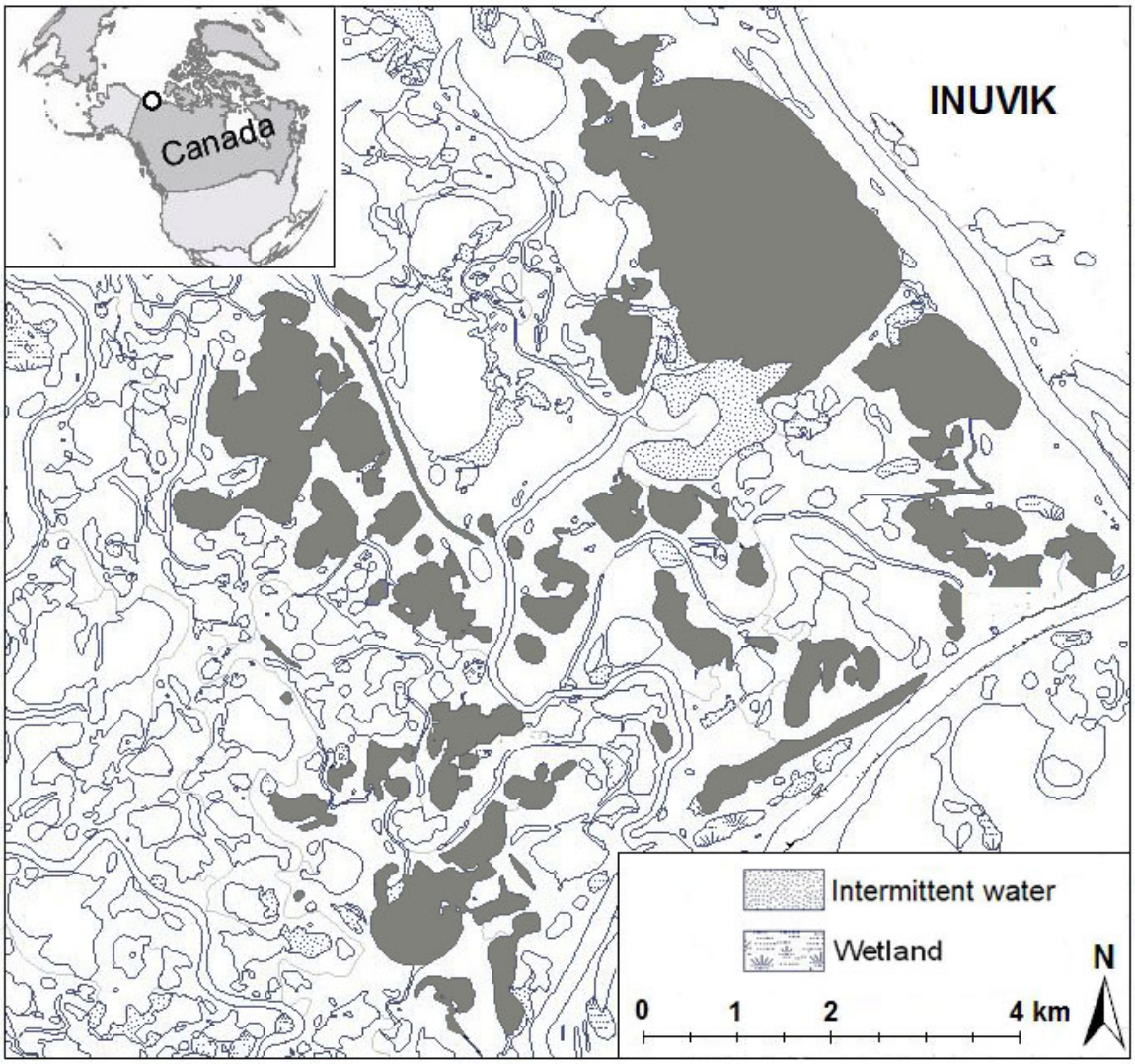

Figure 1.3: Study lakes examined during the undertaking of this thesis are highlighted in grey. The indicated lakes span the lake elevation gradient, and are located in the east-central section of the Delta, as indicated in Figure 1.2.

CHAPTER 2 NORTHERN DELTA LAKES AS SUMMERTIME CO_2 ABSORBERS WITHIN THE ARCTIC LANDSCAPE[1]

[1] With kind permission from Springer Science+Business Media:
<Ecosystems, Northern delta lakes as summertime CO_2 absorbers within the arctic landscape. volume 12, pages 144-157, Tank, SE, LFW Lesack and RH Hesslein>.

2.1 Abstract

The vast majority of lakes examined worldwide emit CO_2 to the overlying atmosphere, through a process by which catchment-derived subsidies of terrigenous C, often in the form of dissolved organic carbon (DOC), augment within-lake CO_2 production above the level consumed via photosynthesis. We show that shallow, macrophyte-rich lakes of the Mackenzie Delta, western Canadian Arctic, do not follow this pattern. These lakes are strong summertime CO_2 absorbers, despite DOC concentrations at or above levels commonly shown to produce CO_2 emission. Paradoxically, CO_2 levels were lowest where DOC was greatest, in lakes which appear to be annual net CO_2 absorbers, and have poor hydrologic connection to the terrestrial landscape. CO_2 in these lakes is depleted by high macrophyte productivity, and although catchment-derived C subsidies are low, within-lake DOC generation appears to occur as a byproduct of evapoconcentration and macrophyte photosynthesis. Additionally, after accounting for DOC and macrophytes, lakes that were least connected to the larger terrestrial landscape remained weaker CO_2 absorbers, suggesting that CO_2 balance may also be affected by DOC quality, foodweb structure, or inputs of pCO_2-rich riverwater to connected lakes. In contrast, a small subset of Delta lakes that were strongly affected by permafrost melting were CO_2 emitters, indicating that future permafrost degradation could engender a change in the overall CO_2 balance of these lakes from near-CO_2 neutral over the ice-free season, to clear CO_2 emission. Our work suggests that the current paradigm of lakewater CO_2 regulation may need to specifically incorporate shallow, productive lakes, and those that are poorly connected to their surrounding landscape.

2.2 Introduction

Because of its unmistakable importance to C cycling at the landscape level (Cole et al. 2007), understanding the factors that regulate the magnitude and direction of CO_2 fluxes within the world's lakes has become a focal point for research in aquatic environments. Worldwide, the vast majority of lakes examined to date are supersaturated in CO_2 relative to the overlying atmosphere (Kling et al. 1991, Cole et al. 1994, Sobek et al. 2005). This occurs because all lakes, to a greater or lesser extent, receive subsidies of terrigenous C from their catchments, which then becomes available for within-lake mineralization to CO_2 (Kling et al. 1991). When external C subsidies allow within-lake CO_2 production to outweigh its consumption by processes such as photosynthesis and C sedimentation, CO_2 supersaturation relative to the overlying atmosphere results (Hanson et al. 2004). Consequently, lakes function in varying degrees as landscape CO_2 conduits to the atmosphere (Kling et al. 1991).

Several studies, conducted in lake regions around the world, have shown dissolved organic carbon (DOC) to be the primary regulator of partial pressures of CO_2 (pCO_2) in lakewater (Sobek et al. 2005, Hanson et al. 2003). In the relatively deep, large catchment lakes that are the common focus of limnological study, DOC is principally derived from organic matter produced externally to the lake (e.g., Kritzberg et al. 2004). Thus, pCO_2 increases with increasing DOC; concentrations in excess of 5 mg L^{-1} have been shown to lead to pCO_2 levels supersaturated relative to the atmosphere (Jansson et al. 2000, Prairie et al. 2002). Factors that regulate algal photosynthesis (such as total phosphorus; TP) have similarly been shown to be secondarily important in regulating pCO_2 (Sobek et al. 2005, Hanson et al. 2003). Photosynthesis in the 'typical' steep-sided lake is largely limited to production by phytoplankton, and increases in factors that drive algal photosynthesis thus reduce pCO_2.

One of the key benefits provided by the measurement of lakewater pCO_2 is that it provides an integrated measure of all metabolic components within the ecosystem (the sum of pelagic and benthic components, and the balance between production and respiration; sensu Prairie 2008). Thus, pCO_2 measurement allows for an overall assessment of the

metabolic functioning of lakes within a larger landscape context, while at the same time providing a means to compare diverse groups of lakes in terms of their C balance. Here, we examine pCO_2 in a set of shallow lakes that show marked differences in their degree of hydrologic connection to the surrounding landscape, and thus, the subsidies of terrigenous C that they receive. We do this in lakes that can also exhibit extremely high macrophyte productivity.

The Mackenzie Delta, western Canadian Arctic, presents an ideal site to explore the relationship between pCO_2 and lake-landscape connectivity. Each spring, this lake-rich Delta floods, as meltwater from southern tributaries flows north to meet ice still present in this northern Delta. Most Delta lakes are inundated during the resultant rapid water rise. However, because of slight differences in elevation between Delta lakes, not all receive the same input of flood and river water: some lakes flood less than annually (defined as high closure; Marsh and Hey 1989), or for days to weeks (low closure), while other lakes remain connected to the river channel (no closure) throughout the ice-free season. Delta lakes are shallow, with average depths on the order of 1.5 m (Emmerton et al. 2007), and have extremely small, gradually sloped catchments (average catchment: lake area of 2.4; Emmerton et al. 2007). Permafrost surrounding these lakes is over 80 m thick and extends to bedrock (Johnston and Brown 1966), which renders sub-permafrost groundwater flow negligible (Marsh 1986). Because the region is relatively arid (Bigras 1990), subsurface and supra-permafrost groundwater flow are also insignificant, except during snowmelt and immediately following the spring flood (Marsh 1986). Thus, once lakes become cut off from their proximate river channel, they largely cease to receive inputs of terrigenous organic matter, and the variation in lake elevations among Delta lakes creates a strong gradient in hydrologic connectivity to the larger Mackenzie River watershed. Following this, water renewal time also varies along the lake elevation gradient, with higher elevation lakes being flushed on a yearly to multi-year timescale, and lower elevation, connected lakes frequently exchanging water with the system of nearby river channels.

Because almost all the terrigenous DOC delivered to Delta lakes comes through connection to the river, inter-lake differences in connection time lead to a strong gradient of

terrigenous DOC input among lakes. However, DOC also appears to be internally generated in these lakes as a byproduct of photosynthesis: many high elevation lakes possess extremely dense summertime stands of submergent macrophytes (largely *Potamogeton richardsonii*; Squires et al. 2002), which are able to take advantage of the sediment-associated nutrients delivered during the flood, and proliferate during the clearwater conditions that occur once lakes cease connection with the river. Submergent macrophytes are much less extensive in low elevation lakes, where continued inputs of suspended sediment-rich riverwater results in low water clarity. Emergent macrophytes (*Equisetum fluviatile*) are also common in many Delta lakes, occurring across the elevation gradient in lakes that possess significant areas of shallow littoral shelf. Phytoplankton productivity, in contrast, is extremely low in Delta lakes (Squires and Lesack 2002). In addition, thermokarst (permafrost melting) occurs to a substantial extent in soils adjacent to some Delta lakes, deepening the water column by several meters and causing slumps at the lake edge where it occurs (Mackay 1963), while potentially also contributing aged C to lakewater (Zimov et al. 2006). The distribution of the 3 major factors influencing lakes in this system; riverwater, macrophyte productivity, and thermokarst, are further detailed in Fig. 2.1, which provides a conceptual diagram outlining the major hydrologic and biotic differences across the lake elevation gradient. In this study, we show that Delta lakes are strong summertime absorbers of CO_2, and thus clear outliers to the current paradigm for pCO_2 regulation in lakes. Our results suggest that productive shallow lakes and waterbodies with limited hydrological connection to their surrounding landscape may follow unique patterns of pCO_2 regulation. We further show that thermokarst significantly alters CO_2 balance, pushing lakes from a state of net CO_2 absorption, to net CO_2 emission.

2.3 Methods

2.3.1 Field sampling

We chose a set of 42 study lakes in the east-central section of the Delta which spanned the flooding gradient (see Squires et al. 2002 and Fig. 1.3 for a detailed study map). We surveyed these lakes 3 times during 2005, and surveyed a 16-lake subset again 3 times

during 2006. We further chose a subset of 6 representative lakes to sample 2-3 times weekly during 2005 (intensively-studied). Both the intensively-studied set of 6 lakes, and surveyed set of 42 lakes, were chosen to maximize distribution over the flooding gradient (2005 connection times of 11-204 d, and 7-365 d, respectively). Sampling of the 6-lake set began as soon as travel on the Delta was possible post-flood. 2005 surveys were on June 10, July 2, and August 8, while 2006 surveys were on June 17, July 12, and August 15.

pCO_2 measurements were obtained as in Hesslein et al. (1991), with some modification. We sampled using a 1L polyethylene carboy (Nalgene EW-06080-10, Nalge Nunc International, Rochester, NY). The bottle mouth was fitted with a rubber stopper that was pierced with a luer-lok sampling needle, fitted with a 3-way luer stopcock. The lower opening of the carboy was attached to a 60 mL syringe using plastic tubing, to create an airtight seal. To collect lakewater CO_2 samples, the carboy was filled to overflowing with water from just below the lake surface and capped with the rubber stopper. A 60 mL headspace was created in the bottle by withdrawing the syringe plunger, allowing air to flow into the carbuoy through the sampling needle, with the stopcock in the open position. The carbuoy was sealed by closing the stopcock, and shaken vigorously for 3 min. The headspace was then sampled by attaching a syringe fitted with a second stopcock to the needle-attached stopcock, opening both valves, and depressing the initial syringe to force headspace air into the sampling syringe. Both valves were then closed, and the syringe was removed. Duplicate samples were taken from each sampling bottle. For the set of 6 lakes, 2 duplicate bottles (i.e., 4 syringes total) were sampled at each lake. For the larger lake surveys 1 sample (2 syringes) was collected from each lake. On each sampling occasion, an atmospheric sample was collected in a syringe, sealed with a 3-way stopcock, and analysed as for headspace samples. Gas samples were kept cool and in the dark until processing, within 3-8 h of collection. Tests using CO_2 gas standards showed concentration changes caused by syringe leakage to be less than 5% over 8 h. Lakewater samples for DOC, TP, and chlorophyll *a* (Chl *a*) were collected for each lake during each of the larger lake surveys (2005 and 2006, except TP), and weekly for each intensively sampled lake (2005). Samples

were collected immediately below the water surface, and kept cool and in the dark until laboratory processing (within 12 h).

2.3.2 Laboratory analyses

CO_2 concentrations were measured using gas chromatography (Carle 100 AGC, EG&G Chandler Engineering, Broken Arrow, OK), on a Hayesep A 60/80 column (VICI Valco Instruments, Houston TX), using a thermal conductivity detector and helium as the carrier gas (30 mL min^{-1}). Column temperature was maintained at 70°C, and samples were injected using a 1 mL sample loop. Sample peaks were integrated using a ChromJet SP4400 integrator (Spectra Physics Analytical Inc., San Jose CA). A 4-point calibration curve was constructed using CO_2 gas standards (Scott Specialty Gases, Plumsteadville, PA) for each sample run. Replicate samples from each lake were averaged to give a single pCO_2 estimate.

Samples for DOC were filtered (0.22 µm pore size, Millipore GSWP, Millipore Corp., Billerica, MA) and kept refrigerated until analysis as non-purgeable organic carbon (Shimadzu TOC-V, Shimadzu Corporation, Kyoto, Japan). Samples for TP were frozen until analysis, within 6 months, and analysed after digestion in potassium persulfate, following the molybdate-ascorbic acid method (APHA 1989). Samples for Chl *a* were filtered onto Whatman GF/F filters, immediately frozen, and kept frozen until analysis (Nusch 1980, Welschmeyer 1994).

2.3.3 Calculations and data acquisition

Headspace pCO_2 values were corrected to give lakewater pCO_2 using Henry's constant corrected for water temperature (Weiss 1974), and taking into account atmospheric CO_2 introduced into the sampling bottle. CO_2 fluxes (F, µmol hr^{-1} m^{-2}) across the air-water interface were calculated as:

$$F = 10\ kCO_2\ \alpha\ (C_W - C_A), \qquad [1]$$

with negative values of F indicating a CO_2 influx, and positive values indicating a CO_2 efflux. kCO_2 is the gas exchange coefficient (cm hr^{-1}), α is the chemical enhancement factor (dimensionless; Wanninkhof and Knox 1996) that defines the enhanced diffusion across the

boundary layer resulting from the hydration of CO_2 to HCO_3^- at high pH, and C_W and C_A are the concentrations of CO_2 in water and in equilibrium with the overlying atmosphere (μmol L^{-1}), respectively. Eq. 1 was used without α (i.e., no enhancement) when ($C_W - C_A$) was greater than zero. pH values for calculating α were derived from measured dissolved inorganic carbon and pCO_2 (Stumm and Morgan 1996) in 2005, and measured directly in the field in 2006. To obtain kCO_2, we calculated k_{600} (Cole and Caraco 1998), using windspeeds measured at the time of sampling at an Environment Canada meteorological station that lies within 15 km of all study lakes. k_{600} was converted to kCO_2 by multiplying by $(600/Sc)^n$, where n=0.67 for windspeeds <3 m s^{-1}, 0.5 for windspeeds ≥3 m s^{-1}, and Sc is the temperature-dependant Schmidt number for CO_2 (MacIntyre et al. 1995). Fluxes were extrapolated to a daily value by assuming constant flux levels across the day of pCO_2 measurement. Because of 24-hour summer sunlight, both wind and photosynthesis are much more stable in Delta lakes than in a more southerly, diurnal, climate. pCO_2 measured at different times during the day suggests that pressures do not show strong diurnal variation. To assess the rigor of our flux estimates, we undertook a sensitivity analysis that assessed how reasonable variations in windspeed (the 24-h average and 10^{th} and 90^{th} percentile windspeeds from the day of sampling) and an alternate model for calculating k_{600} (MacIntyre et al. 1995) affected our estimates. The highest and lowest fluxes obtained were used as confidence bounds on our estimates.

Submergent macrophyte densities (g m^{-2}) for a subset of our survey lakes (Squires et al. 2002), and sill heights for all study lakes (Marsh and Hey 1988), were obtained from the literature. The sill height of a given lake determines its connection to the river system: when river water levels fall below the sill elevation, the lake is cut off from the river. Connection time (d) to the river for each study lake was estimated by comparing sill elevations to daily 2005 and 2006 river water levels (Water Survey of Canada online data for hydrometric station 10LC002, Mackenzie River [East Channel] at Inuvik. http://scitech.pyr.ec.gc.ca/waterweb). Thermokarst around lakes was assessed visually via helicopter during our surveys, and confirmed using Google Earth. Thermokarst extent was

scored on a scale of 0 to 100 as the percentage of each lake's shoreline that had collapsed into the lake, assessed as trees sloping towards or falling onto the lake surface.

2.3.4 *Statistical analyses*

We explored the relative importance of predetermined physicochemical parameters for driving Delta pCO_2 with multiple linear regression, using Akaike's Information Criterion (AIC) to assess model fit (Burnham and Anderson 1998). Connection time, sill height, DOC, TP, Chl *a*, macrophyte density and thermokarst were considered as predictor variables for our models, which used data from the 3 2005 42-lake surveys. Predictor variables were chosen either because of their prominence in the literature as important determinants of pCO_2, or their relevance to our study site. Because its distribution was highly skewed, thermokarst was transformed to a categorical variable, as the presence of thermokarst (totally absent, or some to extensive thermokarst present around lake), and the extent of thermokarst (no or limited thermokarst, or extensive [>75%] thermokarst surrounding lake). All other variables were Box-Cox transformed when necessary to achieve normality.

We undertook a preliminary screening of each of the predictor variables from our candidate set, excluding those variables that showed no significant correlation with pCO_2 from further analysis. We report adjusted r^2 values for all correlations. We assessed the relative fit of all linear combinations of the remaining predictor variables to our pCO_2 data with AIC. We undertook several different AIC analyses: First, because pCO_2 changed dramatically throughout the summer, we separated analyses for the 3 survey periods. Second, because submergent macrophyte densities were available for only a 21-lake subset of our surveyed lakes, we undertook each analysis with and without this data to ensure we both captured the available macrophyte information, and included all surveyed lakes in our analysis. Finally, we undertook each analysis with and without thermokarst data, to ensure that these outlying points (see Results) did not skew our analyses. Because our sample size was relatively small compared to the number of models we assessed (ratio <40), we used the corrected AIC_c as our model selection parameter (Burnham and Anderson 1998). Following convention, we considered all models with an AIC_c difference (ΔAIC_c; the

difference between the lowest AIC model and the model under consideration) ≤ 4.0 to be plausible fits to the data (Burnham and Anderson 1998). For each analysis, we retained all models with $\Delta AIC_c \leq 4$ and a variance inflation factor ≤ 10, and calculated the Akaike weight for each retained model (Burnham and Anderson 1998). Akaike weights sum to 1.0 across all models, with models with the lowest AIC_c (best model fit) having the greatest weight. The model-averaged importance of each predictor variable was then calculated by summing the Akaike weights for each model in which that variable was present. Model-averaged regression coefficients for each variable were calculated by multiplying the coefficients from each model by the model weight, and summing across all models (Burnham and Anderson 1998). Finally, we determined the statistical significance of coefficients by calculating their model-averaged variance (Burnham and Anderson 1998), and using this to calculate confidence intervals.

2.4 Results

pCO_2 levels in the intensively-studied lakes were well above atmospheric equilibrium directly after ice-out (Fig. 2.2), similar to typically-reported values (Cole et al. 1994, Sobek et al. 2005). As the summer progressed, however, pCO_2 fell rapidly, to levels much lower than those reported elsewhere (Fig. 2.2). The extent of this decline varied with connection time: lakes that remained connected to the river throughout the ice-free season transitioned to pCO_2 levels at or near atmospheric equilibrium (Fig. 2.2a,b), while lakes that became cut off from the river system had extremely low summertime pCO_2. In lakes that became cut off, longer connection times were associated with a later switch from CO_2 emission to CO_2 absorption (Fig. 2.2c,d,e). The intensively-studied lake with the briefest connection time (Fig. 2.2f) is a clear outlier to this trend, with pCO_2 well above equilibrium throughout the study season. This lake has experienced substantial thermokarst, such that fully submerged dead, mature trees rise vertically through the water column.

Trends for the 42-lake set reinforce those of the intensively-studied lakes (Figs. 2.3 and 2.4). Springtime pCO_2 is above equilibrium in all study lakes in 2005 (Fig. 2.3a), but declines substantially throughout the season, and is well below atmospheric levels by late-

summer (Fig. 2.3c). Particularly towards the end of the summer, 4 clear outlier points emerge in the data (illustrated in grey in Fig. 2.3, and excluded from correlation analyses below). Of these 4 lakes, 3 are unique in our dataset as being clearly affected by strong and significant thermokarst, with slumps along at least 75% of their shoreline. The fourth lake, although a clear outlier to the observed trends, does not exhibit obvious signs of thermokarst. The trend in 2006 is similar, although with summertime pCO_2 even lower than for 2005 (Fig. 2.4a-c). The 4 outlier points to the observed 2006 trends are identical to the 2005 outliers (Fig. 2.4c).

We used our measured pCO_2 values to estimate CO_2 fluxes from Mackenzie Delta lakes, using results from our sensitivity analysis to set bounds on our estimates. Of the intensively studied lakes, the 3 lakes unaffected by thermokarst that had connection times <50 d were strong summertime CO_2 absorbers, with August fluxes ranging between -3.4 and -18.0 mg C hr^{-1} m^{-2}. As a result, 2 of these 3 lakes were net CO_2 absorbers over the 2005 sampling season, while a third had net fluxes indistinguishable from 0 (Fig. 2.5a). In contrast, the intensively sampled thermokarst lake and waterbodies with longer connection times were CO_2 emitters over the 2005 sampling season (Fig. 2.5a). Lakes in the 42-lake set were also strong summertime CO_2 absorbers in both survey years, with the exception of the previously noted outliers (Fig. 2.5b).

Extrapolating from the calculated Delta survey fluxes to estimate fluxes summed across all Delta lakes (detailed calculations for delta-wide lake flux outlined in Table 2.1) suggests that as a whole, Delta lakes are clear CO_2 absorbers by the time of our late summer survey, with estimated total across-lake CO_2 fluxes of -0.4 Gg C d^{-1} in 2005, and -0.6 Gg C d^{-1} in 2006 (Fig. 2.5c; -0.13 and -0.19 g C m^{-2} d^{-1}, respectively). Lakes that had the shortest connection time to the river system (high closure) were the first to transition to CO_2 absorption. However, by the summer's end all lake types, with the exception of the thermokarst outliers, were, on average, CO_2 absorbers (Fig. 2.5c).

We took a 2-pronged approach to examine the potential drivers of the pCO_2 patterns we observed. We began with a screening analysis to assess the correlation between pCO_2 and a suite of pre-selected physicochemical descriptor variables. Connection time, DOC, and

macrophyte density were all correlated with pCO_2 in both study years (Figs. 2.3 and 2.4), but displayed a relationship with pCO_2 that was not seasonally consistent. Springtime pCO_2 was either highest in the least connected lakes (2005; Fig. 2.3a), or unrelated to connection time (2006; Fig. 2.4a). However, by late summer pCO_2 was consistently greatest in lakes with the longest connection time to the river channel (Figs. 2.3c and 2.4c). Similarly, although spring pCO_2 either rose with, or was uncorrelated to, DOC (Figs. 2.3d and 2.4d), late summer pCO_2 levels tended to be lowest in high DOC lakes (Fig. 2.3f, 2005; Fig. 2.4f, marginal negative relationship, 2006). Macrophyte density was unrelated to pCO_2 in spring and early summer, but was strongly correlated to pCO_2 by late summer, when lakes with the highest macrophyte densities had the lowest pCO_2 (Figs. 2.3i and 2.4i). In contrast, predictors of pelagic productivity (Chl *a* and TP) were uncorrelated with pCO_2 across all survey dates (Fig. 2.6).

Following our screening analysis, we used Akaike's Information Criteria (AIC) to assess the relative importance of each of the significant descriptor variables for driving pCO_2 trends during our 2005 surveys (Table 2.2). Variables that have been identified in the literature as important drivers of pCO_2, or were significantly correlated with pCO_2 in our preliminary screening, did not always factor prominently in our AIC-selected models. In spring, DOC largely stood alone as a strong, positive driver of pCO_2. DOC continued to have a positive influence on pCO_2 in early-summer, but by this time river connectivity (sill) was also emerging as an important, positive, regulator of pCO_2 (Table 2.2). By late summer, the presence of thermokarst was the most important driver of pCO_2, followed closely by macrophyte density (Table 2.2). However, there was also evidence for a residual, positive influence of connectivity on late summer pCO_2, even when all other factors were accounted for (Table 2.2).

2.5 Discussion

On both regional and global scales, DOC is thought to be the fundamental determinant of lakewater pCO_2, and show a strong positive correlation with partial pressures (Sobek et al. 2005, Prairie et al. 2002). In Delta lakes, this was true only in the

early to mid summer of 2005: by late summer in both of our study years, pCO_2 tended to be lowest in lakes with the highest DOC concentrations. This paradoxical union of high DOC and low pCO_2 occurred in lakes with the lowest degree of hydrologic connectivity to the Mackenzie River watershed. DOC in Delta lakes increases with increasing lake elevation (Lesack et al. 1991; Fig. 2.7), despite the fact that the conventional source of lakewater DOC – inputs of terrigenous C – is delivered only sporadically to these poorly connected lakes. Instead, DOC in these lakes is likely augmented through internal generation, caused in part by high macrophyte productivity (Squires et al. 2002), and in part by evapoconcentration (Lesack et al 1998). Evaporation is an important component of the water budget of low connectivity, high elevation lakes, accounting for the only source of water removal once these lakes become cut off from their proximate river channel (Marsh 1986, Marsh and Lesack 1996). A detailed hydrologic study of one high elevation lake (z_{mean}=0.88 m) estimated a daily evaporation rate of 2.15 mm d^{-1} over the 28 d period immediately following loss of river connection, equivalent to a 7% decline in that lake's volume (Marsh, 1986). Another, modeled water balance for a Delta lake receiving no inputs of floodwater estimated a total water level decline of 0.4 m over a 10 yr period, with snowmelt and evaporation being the major sources of water input and removal, respectively (Marsh and Lesack 1996). Despite its clear importance, however, this degree of evaporation is not substantial enough to fully account for the augmented DOC concentrations observed in high elevation Delta lakes (Fig. 2.7, Lesack et al. 1998), underscoring the potential for macrophyte C to also contribute DOC to these waterbodies.

The combination of a low proportion of DOC derived from external subsidies, and rapid photosynthesis by submergent macrophytes, renders high elevation Delta lakes strong summertime CO_2 absorbers. However, in the spring a more traditional relationship between DOC and pCO_2 can occur (Fig. 2.3d), reflecting the fact that springtime DOC is much more dominantly derived from external C subsidies. Particularly in high-elevation lakes, temporal DOC subsidies occur, as C fixed by both submergent and emergent macrophytes during the previous growing season begins to decompose over the winter and spring. In addition, floodwater inputs create spatial DOC subsidies across all lake types in the spring.

Early season DOC in arctic rivers can be relatively labile (Holmes et al. 2008), while Mackenzie River DOC is high during the flood peak, with concentrations dropping rapidly post flood (Emmerton et al. 2008). Mackenzie River pCO_2 tracks the DOC trend, with moderate levels of springtime pCO_2 similar to those contemporaneously observed in low elevation lakes relaxing to more modest, but continually supersaturated, levels as the summer progresses (archived dataset described in McClelland et al. 2008; data shown in Fig. 2.8). The brief pulse of floodwater delivered to high elevation Delta lakes is therefore rich with labile, concentrated DOC. This may add both DOC and pCO_2 to these high elevation lakes, particularly in years when ice remains on the lake surface throughout much of the flooding period (as in 2005), allowing these waterbodies to be incompletely flushed, and accumulated internally-generated C to therefore remain in the lake. Lower elevation lakes, in contrast, flush more fully in all years, and their longer connection time means that the DOC-rich, moderately high pCO_2 floodwater that they initially receive is replenished with riverwater of declining DOC lability and pCO_2 levels as the season progresses.

Continued flushing of low elevation lakes with riverwater that is consistently saturated in pCO_2 is one mechanism to explain why connection time emerged as a significant driver of pCO_2 in our multivariate analysis in the summer. Even after DOC and macrophyte density had been accounted for, lakes least connected to the surrounding landscape had lower pCO_2 once water levels had subsided and high elevation lakes became hydrologically cut off. However, several additional factors related to connection time in and of itself could also facilitate higher pCO_2 in lower elevation lakes. These include the greater input of non-dissolved organic C that is continually delivered to connected lakes via riverwater (Squires and Lesack 2002), differences in DOC quality across the connectivity gradient that affect the relative amount of organic matter respired by bacteria (Chapters 4 and 6), and changes in food web structure that occur across the connectivity gradient (Schindler et al. 1997, Riedel 2002). In coming years, trends across this connectivity gradient may become more disparate as a result of global change: in the Mackenzie Delta, high elevation lakes are becoming increasingly cut off from the river because of decreasing

peak flood elevations, while low elevation lakes exhibit lengthening connection times due to rising sea levels and potentially more severe storm surges (Lesack and Marsh 2007).

Our work suggests that, in stark contrast to other lake regions examined to date, a significant proportion of Delta lakes may be net CO_2 absorbers over a full annual cycle. To calculate annual flux rates for the intensively studied lakes, we added the large outgassing of under-ice CO_2 that would occur during the ice-off period to our estimates (calculated from Pipke 1996), and assumed that pCO_2 remained stable between our last sampling day (Aug 23) and the month's end (calculations outlined in Table 2.3). Even with this large outgassing, the lowest connectivity, non-thermokarst lake in this set was a weak CO_2 absorber (-2.4 to -1.0 g C m^{-2}; Table 2.3). pCO_2 for this lake was well within average values observed for other high closure, low connectivity lakes in this system, suggesting that these high elevation Delta lakes (~15% of total lake aerial extent) may be CO_2 absorbers on an annual scale. Although less reliable, extrapolations from our survey measurements suggest that Delta lakes as a whole are near-neutral for CO_2 during the ice-free season (Table 2.4; total flux range for 2005 and 2006 equivalent to -1.1 to +3.4 g C m^{-2}) and weak CO_2 emitters on an annual basis, with CO_2 outgassing during ice-off incorporated (estimated as ~33 Gg C from Pipke 1996).

These estimates for the overall CO_2 balance of Delta lakes are remarkable, given that all lakes do receive at least some input of terrigenous C, while the emergent macrophytes common in the nearshore littoral zone of many Delta waterbodies add C to the system without depleting CO_2. A near-neutral to slightly negative CO_2 balance implies that C is either stored in these lakes, or lost in a form other than CO_2 efflux. Sediment organic matter content in Delta lakes increases with increasing within-lake macrophyte biomass (Squires and Lesack 2003), indicating that macrophyte biomass stored in lake sediments does act as a C sink in this system. However, this does not imply that Delta lakes are near-neutral from an overall greenhouse gas perspective. Buildup of CH_4, which is 23 times as potent a greenhouse gas as CO_2, is substantial under ice in this system (Pipke 1996), and simple dissolved CH_4 measurements taken during our summertime surveys suggest that open-water pCH_4 ranges from 100 to 5,000x saturated with respect to the atmosphere (Fig. 2.9).

Given that up to 95% of CH_4 efflux from lakes can occur as ebullition (Walter et al. 2006), it appears that Delta lakes follow the circumpolar pattern of being strong CH_4 emitters (Walter et al. 2007), and that a portion of the C fixed in these lakes is later lost from the system as CH_4, rather than CO_2.

All lakes in our dataset that had experienced substantial thermokarst were clear outliers to the observed pCO_2 trends, and strong CO_2 emitters. Thermokarst can be an important instigator of methanogenesis in arctic lakes (Zimov et al. 2006, Walter et al. 2007), as aged organic matter becomes available for heterotrophic decomposition. Although only a small aerial percentage (~0.2%) of our survey lakes are currently affected by severe thermokarst, permafrost melting is expected to increase dramatically with climate warming (ACIA 2004). In the Mackenzie Delta, terrain underlain with the ice-rich permafrost necessary for thermokarst is typically found surrounding high closure, low connectivity, Delta lakes (Kokelj and Burn 2005); approximately 15% of total Delta area (Lesack and Marsh 2007). If all high closure lakes in this system became thermokarst lakes similar to those that we currently observe, late summer CO_2 absorption would be reduced by 1.6-3.8 fold (Table 2.4), and Delta lakes would become clear CO_2 emitters when considered over the entire ice-free season (Table 2.4). In short, future changes in permafrost dynamics could engender a basic switch in the CO_2 dynamics of Delta lakes.

These arctic delta lakes are clear exceptions to the current paradigm for CO_2 balance in the world's waterbodies. Despite having DOC concentrations at or above the level commonly found to result in CO_2 supersaturation relative to the atmosphere (~5 mg L^{-1}; Jansson et al. 2000, Prairie et al. 2002), Delta lakes are often strong CO_2 absorbers. This occurs in a system where phytoplankton productivity – the second factor typically found to drive pCO_2 in lakes – is extremely low (Fig. 2.6), as a result of limitation by low water column nutrient levels across all lakes, and low water clarity in lakes with high connectivity to the river. Instead, shallow, high elevation Delta lakes are dominated by macrophyte and associated epiphyte productivity, which draws down CO_2 because the dominant genus (*Potamogeton* sp.) is submergent, and like many macrophytes, able to actively use bicarbonate for photosynthesis (Prins et al. 1982). At the same time, these lakes have DOC

concentrations that are not reflective of the subsidies of terrigenous organic carbon that they receive. Small, shallow waterbodies, which are relatively poorly studied, account for almost half of global lake area (Downing et al. 2006), but function quite differently from their large, deep, counterparts (Scheffer 1998). In the case of pCO_2 dynamics, although many shallow lakes certainly behave similarly to larger systems (Kling et al. 1991), many others are macrophyte-rich (Scheffer et al. 1993), and productive (Scheffer 1998). Likewise, significant inter-regional variation in the relative amount of within-lake DOC that is derived from terrigenous subsidies occurs as a result of differences in climate and hydrology (Curtis 1998). Specific exploration of pCO_2 regulation in a wide range of productive, shallow lakes, and those with poor hydrologic connectivity to their surrounding catchment, will greatly refine our understanding of how lakes act within landscapes to regulate CO_2 dynamics.

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2.7 Tables

Table 2.1: Calculated CO_2 fluxes from surveyed lakes, and extrapolated fluxes for all Delta lakes, for the spring, early-, and late-summer surveys of 2005 and 2006. Fluxes (top section) calculated as outlined in Methods; error bounds on these fluxes (bottom section) were calculated using reasonable variations in windspeed, and an alternate method for calculating piston velocity. The maximum and minimum values obtained were used as confidence limits on the estimate.

	2005					2006				
	No closure	Low closure	High closure	Outlier	**Total**	No closure	Low closure	High closure	Outlier	**Total**
n	5	20	13	4		2	5	5	4	
Surveyed lake area (km^2)[a]	17.095	6.447	1.896	0.055		0.884	0.557	0.759	0.055	
Delta-wide lake area (km^2)[b]	1999	830.3	490.2	11.5		1999	830.3	490.2	11.5	
Flux, surveyed lakes (g C m^{-2} d^{-1})[c]										
Spring	0.274	0.443	0.700	0.730		0.386	0.285	0.417	0.535	
Early-summer	0.074	0.119	-0.002	0.779		0.153	0.114	-0.161	0.553	
Late summer	-0.034	-0.222	-0.387	0.274		-0.112	-0.256	-0.376	0.076	
Delta-wide flux (Gg C d^{-1})[d]										
Spring	0.547	0.368	0.343	0.008	**1.267**	0.772	0.236	0.204	0.006	**1.219**
Early-summer	0.149	0.099	-0.001	0.009	**0.256**	0.306	0.095	-0.079	0.006	**0.328**
Late summer	-0.069	-0.185	-0.190	0.003	**-0.440**	-0.224	-0.212	-0.182	0.001	**-0.618**
Error bounds on Delta-wide flux (Gg C d^{-1})[e]										
Daily averaged winds										
Spring	0.484	0.325	0.304	0.007	**1.120**	0.722	0.221	0.191	0.006	**1.139**
Early-summer	0.143	0.095	-0.001	0.009	**0.246**	0.307	0.095	-0.079	0.006	**0.329**
Late summer	-0.061	-0.150	-0.169	0.002	**-0.377**	-0.204	-0.207	-0.181	0.001	**-0.591**
10^{th} percentile winds										
Spring	0.349	0.240	0.222	0.005	**0.817**	0.546	0.167	0.144	0.004	**0.862**
Early-summer	0.128	0.085	-0.002	0.008	**0.220**	0.233	0.072	-0.071	0.005	**0.239**
Late summer	-0.055	-0.101	-0.148	0.001	**-0.303**	-0.187	-0.202	-0.180	0.000	**-0.568**
90^{th} percentile winds										
Spring	0.689	0.463	0.432	0.011	**1.596**	0.924	0.283	0.245	0.007	**1.460**
Early-summer	0.166	0.110	0.000	0.010	**0.286**	0.403	0.125	-0.091	0.008	**0.445**
Late summer	-0.081	-0.232	-0.222	0.004	**-0.531**	-0.252	-0.222	-0.186	0.001	**-0.659**
Alternate piston velocity										
Spring	0.554	0.373	0.348	0.009	**1.283**	0.866	0.265	0.229	0.007	**1.367**
Early-summer	0.074	0.049	-0.005	0.004	**0.122**	0.230	0.071	-0.071	0.005	**0.235**
Late summer	-0.079	-0.224	-0.216	0.004	**-0.516**	-0.198	-0.205	-0.180	0.001	**-0.583**

[a] Areas of all surveyed lakes are given in Marsh and Hey (1988).

[b] From Lesack and Marsh (2007). The delta-wide area of outlier lakes was calculated based on the proportion of these lakes within the surveyed lakes.

[c] Calculated for each lake class as {∑[individual lake flux (g C m^{-2} d^{-1})]*[lake area (m^2)]}/(surveyed lake area). Calculations used to obtain individual lake fluxes outlined in Methods.

[d] Calculated as [surveyed lake flux (g C m^{-2} d^{-1})]*[delta-wide lake area (m^2)].

[e] These delta-wide fluxes calculated as for ([d]), but using surveyed lake fluxes obtained by modifying windspeeds or using an alternate method of calculating k_{600}, as outlined in Methods.

Table 2.2: Results of Akaike's Information Criteria (AIC) model selection analysis, showing AIC_c weights, from 0 to 1, of each predictor variable for explaining pCO_2 trends. Variables with regression coefficients significant at α=0.05 (α=0.10) are given in bold (italics) with sign indicated in brackets. n=number of lakes in each analysis, with and without outlier points removed. nd=not determined (variables omitted). The analysis was conducted with and without data for macrophyte density and the four outlier points, to ensure the full variability of the dataset was captured.

	Spring		Early summer		Late summer	
	all data	outliers removed	all data	outliers removed	all data	outliers removed
All study lakes (n = 42, 38)						
Connection time	0.197	0.228	0.266	0.293	**0.635 (+)**	**0.631 (+)**
Sill height	0.210	0.246	*0.454 (-)*	*0.523 (-)*	**0.365 (-)**	**0.369 (-)**
DOC	**1.000 (+)**	**1.000 (+)**	**1.000 (+)**	*1.000 (+)*	0.186	0.229
Thermokarst (presence)	0.140	nd	0.323	nd	0.175	nd
Thermokarst (extent)	**1.000 (-)**	nd	0.379	nd	**1.000 (+)**	nd
Subset of lakes with macrophyte data (n = 21, 18)						
Macrophyte	0.148	0.130	0.126	0.168	*0.568 (-)*	**0.685 (-)**
Connection time	0.278	0.128	0.232	*0.256 (+)*	**0.443 (+)**	*0.367 (+)*
Sill height	0.258	0.130	*0.525 (-)*	*0.744 (-)*	**0.302 (-)**	0.160
DOC	0.381	**1.000 (+)**	**1.000 (+)**	**1.000 (+)**	0.228	*0.448 (-)*
Thermokarst (presence)	0.165	nd	0.325	nd	0.151	nd
Thermokarst (extent)	0.189	nd	0.445	nd	**1.000 (+)**	nd

Table 2.3: Extrapolation of fluxes from the open-water season to the full annual cycle in 6 intensively-studied lakes. Open water flux was calculated using measured pCO_2 values taken between June 3 and August 23, 2005 to estimate total flux from June 3 to August 31. The magnitude of CO_2 outgassing at ice off was calculated as the difference between measured under-ice pCO_2 in each of these lakes (Pipke 1996), and the June 3 pCO_2 values, and assumed that all excess CO_2 was lost to the atmosphere. Numbers in brackets give error bounds on the flux estimates, as described in Methods.

Connection time	Lake type	Open water flux g C m^{-2}	Ice-off outgassing g C m^{-2}	Annual flux g C m^{-2}
204 d	No closure	14.6 (9.9, 20.4)	3.1	17.6 (12.9, 23.4)
157 d	Low closure	6.4 (4.4, 9.8)	9.5	15.9 (13.9, 19.3)
45 d	Low closure	-0.4 (-1.9, 1.6)	8.4	8.0 (6.5, 10.1)
36 d	Low closure	-2.4 (-2.4, -3.5)	10.7	8.3 (8.3, 7.2)
15 d	High closure	-9.0 (-10.4, -9.0)	8.0	-1.0 (-2.4, -1.0)
11 d	High closure, thermokarst	34.5 (24.0, 49.3)	17.8	52.2 (41.8, 67.1)

Table 2.4: Estimated CO_2 fluxes over the 2005 and 2006 ice-free seasons, and potential future 'thermokarst' fluxes. Current-day fluxes were estimated by assuming that the measured survey flux represented an average value for the time window shown. Values shown in parentheses following totals represent error bounds on our estimate, from the range of fluxes calculated in Table 2.1. 'Thermokarst' fluxes were calculated by assuming that pCO_2 levels in all high closure lakes transition to mirror those currently observed for present-day thermokarst lakes, and were calculated separately based on 2005 and 2006 survey results.

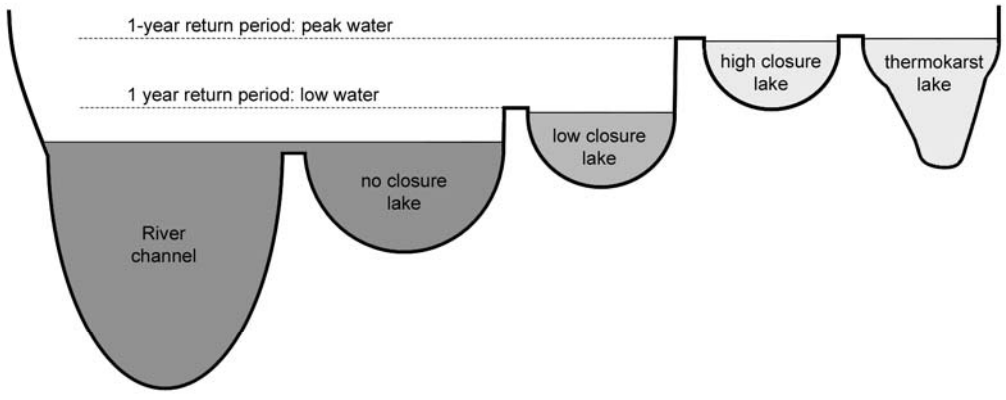
	2005				2006			
	Spring	Early summer	Late summer	Total Ice-free season	Spring	Early summer	Late summer	Total Ice-free season
Current-day flux								
Flux (Gg C d^{-1})[a]	1.267	0.256	-0.440		1.219	0.328	-0.618	
Days[b]	20	25	55		25	25	55	
Total flux (Gg C)	25.339	6.395	-24.206	7.528 (0.321, 9.865)	30.474	8.208	-33.966	4.716 (-3.732, 11.388)
Thermokarst flux								
Flux (Gg C d^{-1})	1.282	0.639	-0.116		1.277	0.679	-0.398	
Days[c]	20	25	55		25	25	55	
Total flux (Gg C)	25.644	15.981	-6.367	35.258 (24.778, 42.697)	31.930	16.967	-21.874	27.022 (14.853, 37.460)
Relative change	1.01	2.50	3.80	4.68	1.05	2.07	1.55	5.73

[a] As given in Table 2.1.

[b] Time window estimated using time of peak water levels (spring breakup), and detailed pCO_2 measurements taken from the intensively sampled 6 lakes. Time windows were 01-20 June, 21 June-15 July, and 16 July-08 September in 2005, and 01-25 June, 26 June-20 July, and 21 July-08 September in 2006.

[c] Estimated as in current day flux.

2.8 Figures



Average z_{mean} (range)	1.66 m (0.9-4.6 m)	1.37 m (0.7-2.0 m)	1.84 m (0.65-4.1 m)
Relative macrophyte biomass (submergent)	low	moderate	high
2005 connection time (d)	> 204 d	16-203 d	< 15 d
2006 connection time (d)	> 167 d	17-166 d	< 16 d

Figure 2.1: A conceptual diagram detailing the major hydrologic and biologic gradients among Delta lakes relevant to this study, as modified from Emmerton et al. (2007). Information on mean depths (z_{mean}) gives both average values and ranges for a group of surveyed lakes within each lake class. Because the ice-rich permafrost necessary for substantial thermokarst activity is typically limited to the terrain surrounding high closure lakes (Kokelj and Burn 2005), thermokarst lakes have been represented as a subset of high closure lakes in this schematic.

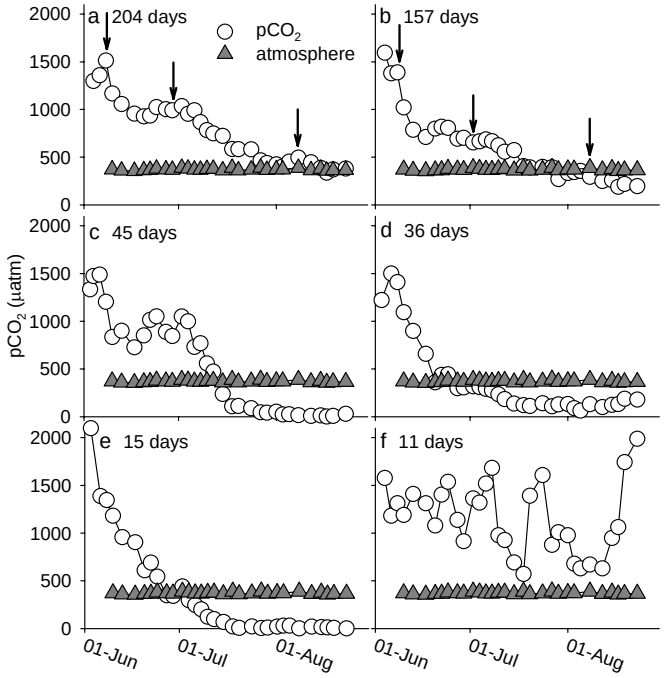


Figure 2.2: pCO_2 in 6 intensively studied Mackenzie Delta lakes. Length of connection to the river system is given for each lake. Open circles: lakewater pCO_2. Shaded triangles: atmospheric CO_2. The observed fluctuations in pCO_2 in panel (f) show a strong dependence on water temperature (rising water temperatures are accompanied by falling pCO_2, data not shown). Black arrows indicate dates of the 3 2005 surveys.

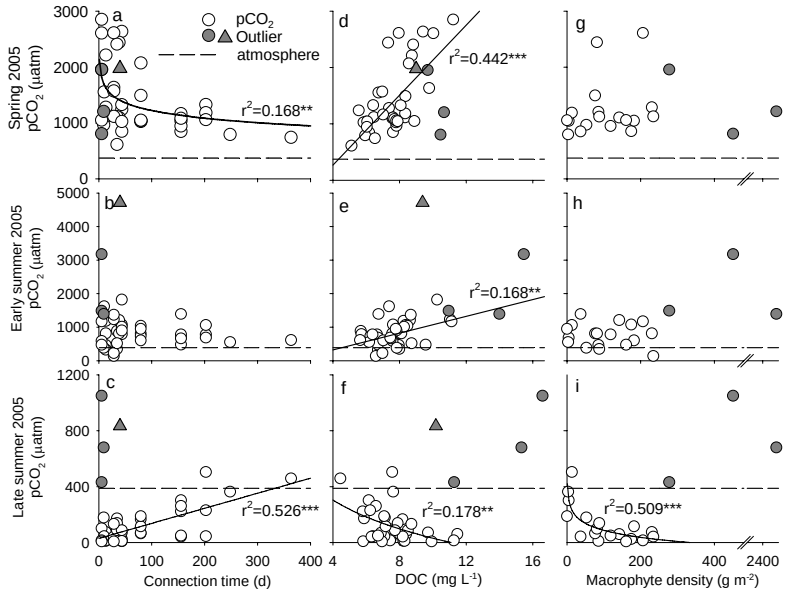


Figure 2.3: Log and linear correlations between pCO_2 and connection time to the river, dissolved organic carbon (DOC) and macrophyte density for the 3 42-lake surveys conducted during 2005. Where relationships are significant, regression lines and adjusted r^2 values are shown. Dashed lines indicate atmospheric CO_2 on the day of sampling. Shaded circles indicate outlier (thermokarst) lakes that were excluded from the analyses. The shaded triangle in each panel indicates the outlier lake for which thermokarst was not confirmed. **=p<0.01, ***=p<0.001.

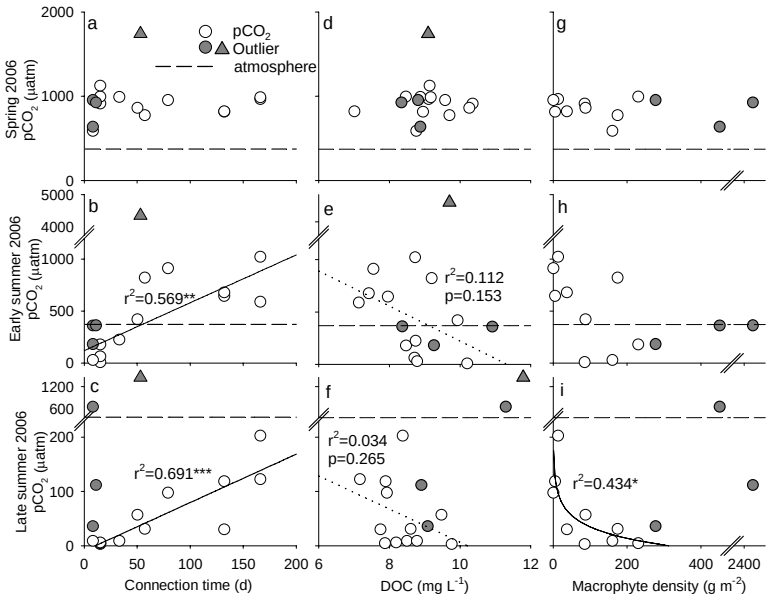


Figure 2.4: Log and linear correlations between pCO_2 and connection time to the river, dissolved organic carbon (DOC) and macrophyte density for the 3 16-lake surveys conducted during 2006. Where relationships are significant, regression lines and adjusted r^2 values are shown. Dotted regression lines are given for marginal relationships between pCO_2 and DOC. Dashed lines indicate atmospheric CO_2 on the day of sampling. Shaded circles indicate outlier (thermokarst) lakes that were excluded from the analyses. The shaded triangle in each panel indicates the outlier lake for which thermokarst was not confirmed. *=p<0.05, **=p<0.01, ***=p<0.001.

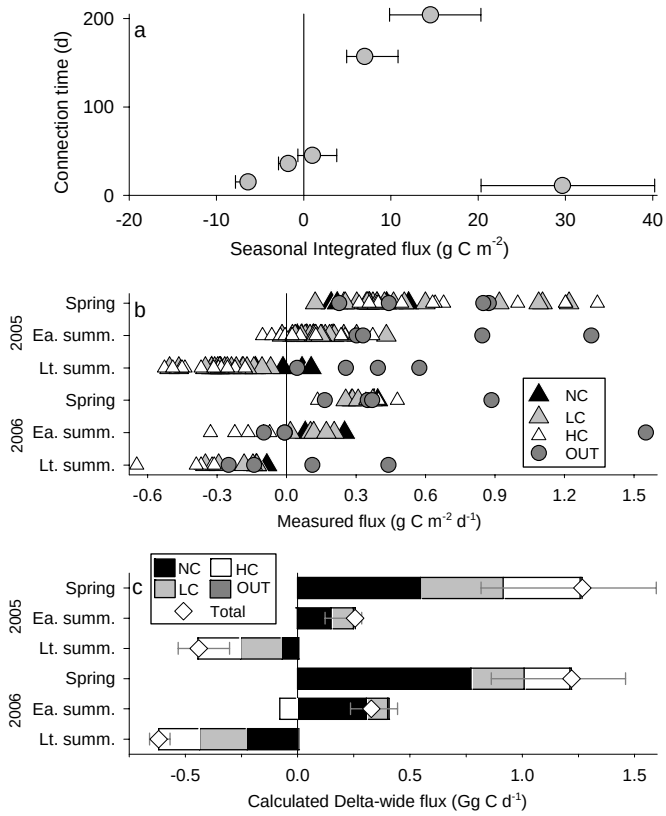


Figure 2.5: Calculated CO_2 fluxes from Mackenzie Delta lakes. Panel (a) shows fluxes integrated over the sampling season for the 6 intensively sampled lakes. Panel (b) shows fluxes calculated for individual lakes for each of the larger lake surveys, classified as no closure (NC; black triangles), low closure (LC; light grey triangles), high closure (HC; white triangles), and outlier lakes (OUT: dark grey circles). Panel (c) shows extrapolated fluxes for all delta lakes at the time of each lake survey, broken down as NC (black bars), LC, (light grey bars), HC (white bars) and OUT (dark grey bars). White diamonds indicate the sum across the Delta as a whole. In all panels, negative values indicate CO_2 influx, while positive values indicate CO_2 efflux. Confidence intervals in panels (a) and (c) indicate minimum and maximum values obtained through sensitivity analysis.

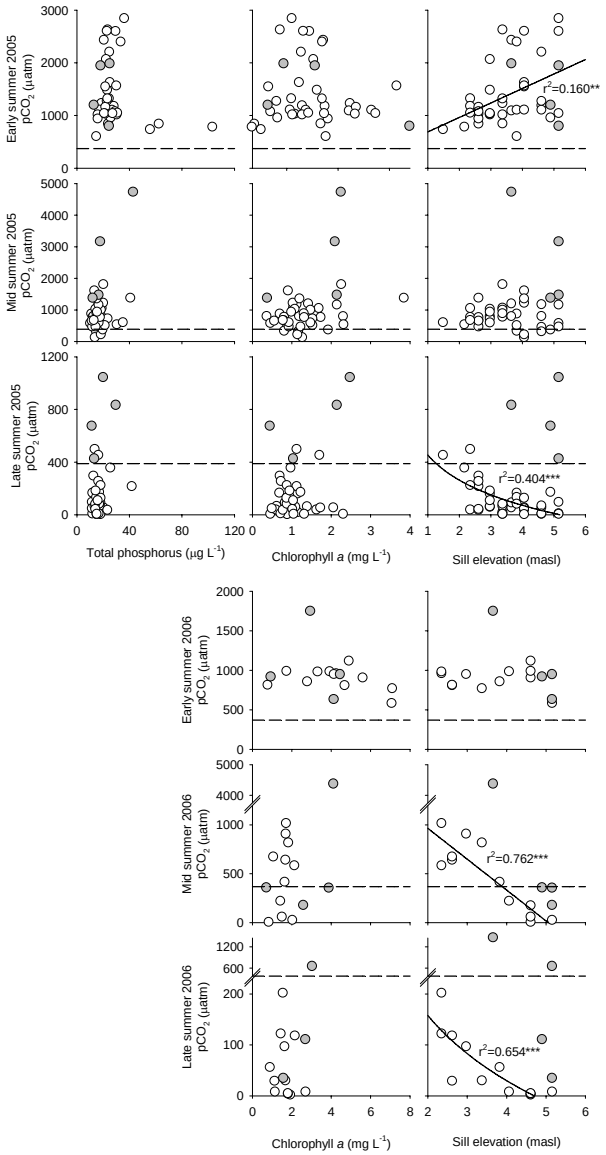


Figure 2.6: Log and linear correlations between partial pressures of CO_2 (pCO_2) and total phosphorus (2005), chlorophyll *a* (2005 and 2006), and sill elevation (2005 and 2006). Where relationships are significant, solid regression lines and adjusted r^2 values are shown. Dashed lines indicate atmospheric CO_2 concentrations on the day of sampling. Shaded circles indicate outlier (thermokarst) lakes that were excluded from the analyses. ** = $p<0.01$, *** = $p<0.001$.

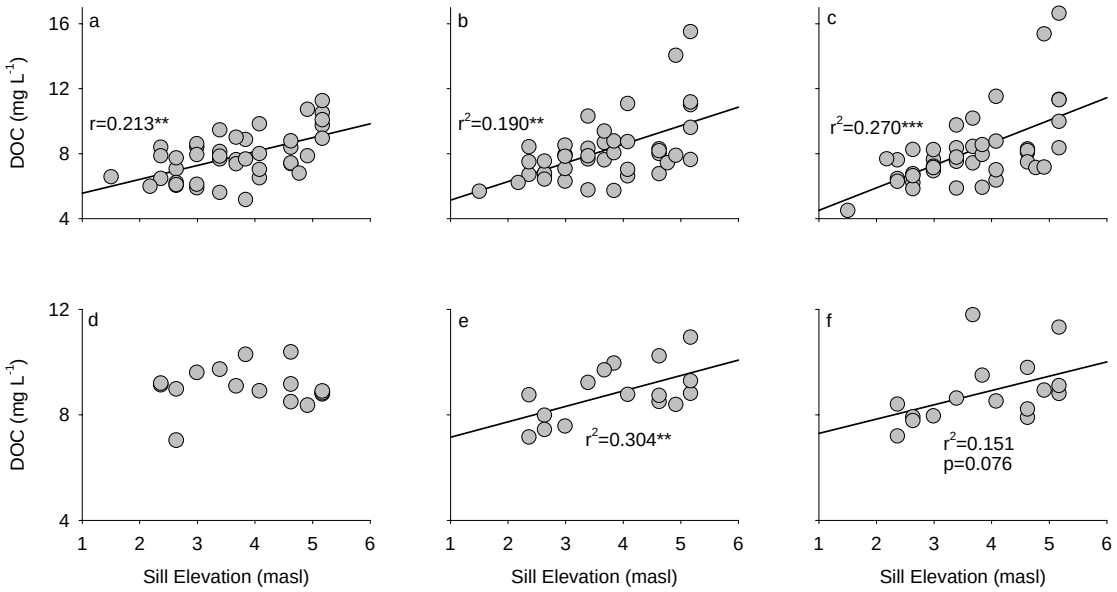


Figure 2.7: Relationship between sill elevation and dissolved organic carbon (DOC) for (a) early, (b) mid, and (c) late summer in 2005, and (d) early, (e) mid, and (f) late summer in 2006. Adjusted r^2 values are shown. **p<0.01, ***p<0.001

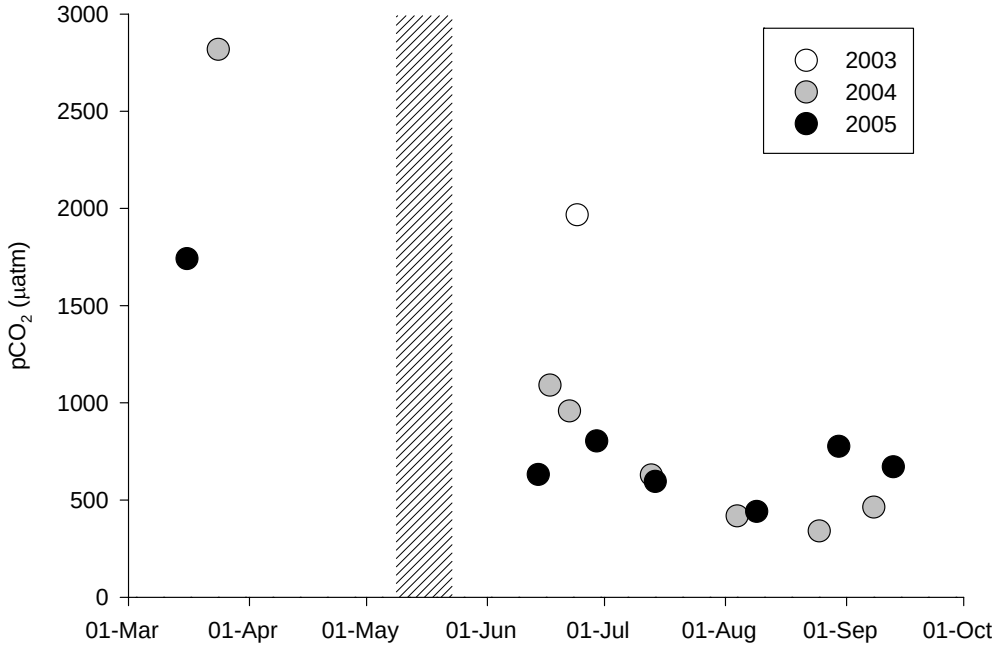


Figure 2.8: Mackenzie River pCO_2, calculated from alkalinity, pH and temperature data for riverwater collected immediately upstream from the entry point of the Mackenzie River into the Delta, during 2003, 2004, and 2005. Obtained from publicly available data, archived at http://www.eol.ucar.edu/projects/aon-cadis (McClelland et al. 2008). The cross-hatched area indicates the time period during which our study lakes re-connected with the river channel during the rising limb of the spring hydrograph in 2005. Lakes re-connect with the river channel based on their sill elevations, with the lowest elevation lakes being the first to flood.

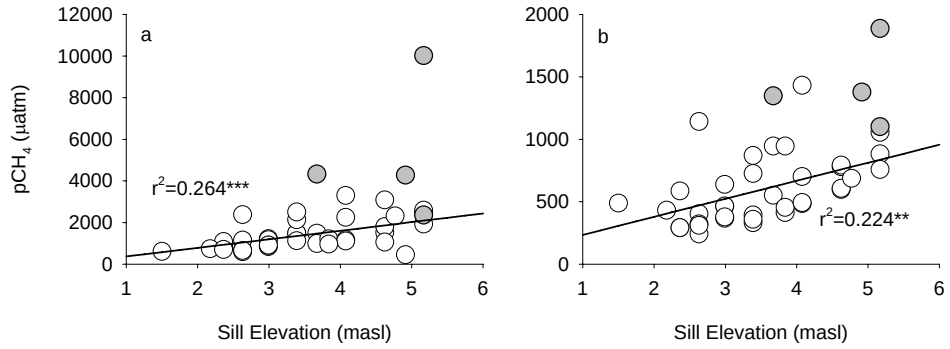


Figure 2.9: pCH_4 values for Mackenzie Delta lakes obtained during the (a) early summer, and (b) late summer surveys of 2005. Samples were collected using the headspace method described in the text for pCO_2, and values corrected using the Henry's Law constant for CH_4, taking into account temperature at the time of sampling, and atmospheric CH_4 introduced into the sampling vial as headspace. The outlier lakes identified in the text are noted as grey circles, and have been excluded from the regression.

CHAPTER 3 MULTIPLE TRACERS DEMONSTRATE DISTINCT SOURCES OF DISSOLVED ORGANIC MATTER TO LAKES OF THE MACKENZIE DELTA, WESTERN CANADIAN ARCTIC[1]

[1] A version of this chapter is in revision for resubmission as:
Tank, SE, LFW Lesack, CL Osburn, and RH Hesslein. Multiple tracers demonstrate distinct sources of dissolved organic matter to lakes of the Mackenzie Delta, western Canadian Arctic. *In revision* for Limnology and Oceanography.

3.1 Abstract

Research quantifying the origin of DOM in lakes has largely focused on modern-day terrigenous and algal sources. Here, we examine the role of macrophytes and permafrost melting (thermokarst) as sources of dissolved organic matter (DOM) to a series of northern Delta waterbodies. Lakes of the Mackenzie Delta occur across a gradient that contains 3 clear endmembers: some lakes remain connected to riverwater channels throughout the summer; others receive only brief inputs of riverwater during an annual spring flood but contain dense macrophyte stands; still others experience significant permafrost melting along their margins. We measured dissolved organic carbon (DOC) concentration, DOM absorptivity and fluorescence, and stable isotopes of DOM, DOM precursor materials, and bacteria to elucidate the importance of riverwater, macrophytes, and thermokarst as DOM sources to these lakes. DOC increased with increasing macrophyte density in Delta lakes. However, despite macrophyte C levels 7 - 12-fold greater than total DOC, stable isotope signatures indicated that autochthonous sources contributed less than 10% to the overall DOM pool in macrophyte-rich lakes, with remaining DOM accumulation potentially resulting from the effects of infrequent flushing in these lakes. Instead, bacterial $\delta^{13}C$ suggests bacteria preferentially incorporate autochthonous C. In thermokarst lakes, summertime DOC accumulation was substantial, and stable isotopes indicate that this accumulation occurred as result of thermokarst. Collectively, our findings suggest that rapidly-assimilated macrophyte DOM could be an important contributor to microbial foodwebs, while more recalcitrant thermokarst-origin DOM could be more important to the physico-chemical processes that DOM is known to regulate.

3.2 Introduction

Resolving the relative importance of dissolved organic matter (DOM) from watershed-derived allochthonous and algal-derived autochthonous sources continues to be a focus of limnological study (Pace et al. 2007). Among other things, differences in DOM source, and lability, can influence its effect on aquatic C cycling and food web structure. For example, while allochthonous DOM typically constitutes the bulk of the within-lake pool (Kritzberg et al. 2006), autochthonous, algal-derived DOM tends to be more microbially labile, and rapidly incorporated into bacterial biomass (Kritzberg et al. 2006, McCallister et al. 2006).

Despite this, the significance of other potential sources of DOM to the within-lake pool has received much less attention. One possible non-algal source of autochthonous DOM is that derived from macrophyte productivity. Particularly in shallow lakes, macrophytes can occur in extremely dense stands, with productivity rates often exceeding those of algal sources (Wetzel 1992). Both the exudates that occur as a result of unbalanced growth (e.g. Wetzel and Manny 1972), and the leachates that result from the breakdown of senesced vegetation, could render macrophytic organic matter an important source of lakewater DOM. Like autochthonous DOM of algal origin, macrophytic DOM appears to be extremely labile, as assessed by studies that have measured bacterial production on laboratory-extracted macrophyte leachates (Findlay et al. 1986, Mann and Wetzel 1996). However, like all other forms of DOM, this macrophytic organic matter likely requires a bacterial shunt to be available to higher trophic levels, because macrophyte tissue tends to be an inaccessible food source for planktonic organisms (Lewis et al. 2000).

An alternate source of allochthonous-like DOM in northern lakes results from the effects of thermokarst (landscape slumping resulting from permafrost melting), which is currently increasing at lake margins, and across the northern landscape (Smith et al. 2005, Walter et al. 2006). Substantial stores of organic matter exist within the world's permanently frozen soils (Gorham 1991, Zimov et al. 2006). In freshwater systems, thermokarst exposes aged, previously frozen soils to greatly increased biological activity, which can result in substantial generation of both CH_4 (Walter et al. 2006) and CO_2 (Chapter

2). At the same time, surface soils and vegetation are exposed to within-lake processes as these become covered by water. Given the importance of thermokarst for facilitating the release of previously stored organic matter in a gaseous form, it seems likely that this process could also result in substantial DOM additions to the lakes in which it occurs.

Here, we use a suite of tracers to quantify the importance of macrophytes and thermokarst to within-lake DOM in the Mackenzie Delta, western Canadian arctic. The Delta contains numerous lakes that are both macrophyte rich, and severely affected by thermokarst (see *Study Site*). Previous work has shown that dissolved organic carbon concentrations ([DOC]) are elevated in macrophyte-rich and thermokarst-affected lakes, but that DOM absorption is lowest in these waterbodies (Spears and Lesack 2006, Gareis 2007). This suggests that macrophyte and thermokarst DOC may augment the within-lake pool, while a higher proportion of autochthonous (macrophytic) organic matter in these lakes, or the effects of photodegradation, may decrease DOM absorption (Gareis 2007). We coupled standard measures of DOC concentration ([DOC]) and DOM absorption with the assessment of DOM fluorescence (as Excitation Emission Matrices (EEMs) and synchronous fluorescence) and the $\delta^{13}C$ and $\delta^{15}N$ signatures of DOM and DOM precursor materials to trace the origin of DOM in Delta lakes. We specifically chose these metrics to allow a comprehensive assessment of Delta lakewater DOM: higher DOM absorption is indicative of terrestrial-origin DOC, while lower absorption per [DOC] can indicate autochthonous-origin DOM, or the presence of photobleaching (Osburn et al. 2001). Analysis of DOM fluorescence can resolve the relative importance of autochthonous (as protein-like) and allochthonous (humic and fulvic) components within the DOM pool (Lu et al. 2003, Stedmon and Markager 2005). Stable isotope signatures can further be used to resolve the relative importance of source materials (here, DOM precursors) to an overall mixture (lakewater DOM; Peterson and Fry 1987). In addition to our assessment of the composition of the DOM pool in Delta lakes, we examined bacterioplankton $\delta^{13}C$ signatures in macrophyte-rich and thermokarst affected lakes, to assess the relative contribution of these different DOM sources to bacterial biomass.

3.3 Methods

3.3.1 Study site

The Mackenzie Delta is a lake-rich region situated where the Mackenzie River enters the Beaufort Sea of the Arctic Ocean. Each spring, meltwater from southern tributaries flows north to meet ice still present in this northern delta; the resultant ice jam causes a rapid rise in water levels that allows most Delta lakes to flood. However, because of slight differences in elevation between lake inflows, some lakes flood for days to weeks, while others remain connected to the river channel throughout the ice-free season (Marsh and Hey 1989; Fig. 3.1a). This flooding regime plays a key role in regulating these lakes. Delta lakes have extremely small catchments, are surrounded by permafrost that inhibits subsurface and groundwater flow, and the region receives little precipitation (Marsh 1986). Thus, riverine inputs and the yearly pulse of snowmelt runoff appear to be the only notable sources of terrigenous DOM to Delta lakes. Once lakes loose connection with the river, sediments rapidly fall out of suspension and the water column clears. This clarity, coupled with flood-delivered nutrients and the shallow depths of Delta lakes ($z_{mean} \approx 1.5$ m, Emmerton et al. 2007) create conditions ideal for submergent macrophyte (s-macrophyte) growth in higher elevation lakes (Fig. 3.1b), with summer biomasses reaching 350 g m^{-2} (dry weight; Squires et al. 2002). In contrast, suspended sediments in lower elevation, connected lakes remain high throughout the summer, resulting in much lower s-macrophyte densities. Thus, Delta lakes vary in a predictable manner along the lake elevation gradient: as lake elevation increases, the length (in days) of connection to the system of deltaic river channels decreases, while s-macrophyte densities increase (Fig. 3.1a-b). Emergent macrophytes (e-macrophytes; predominantly *Equisetum* sp.) can also occur across the elevation gradient, in lakes that possess significant areas of shallow littoral shelf. In addition, a small subset of high-elevation lakes has been considerably deepened by the action of thermokarst, and do not fit the above-described, regular pattern. These lakes are characterized by visible slumps at the lake's edge, and mature, dead, trees rising vertically through the water column.

3.3.2 Sample collection

We collected samples from a series of lakes in the east-central section of the Delta chosen to span the lake elevation gradient, with 2005 connection times ranging from 7 to 365 d (Fig. 3.1a,c,d). Samples were collected from 3 lake sets that were nested within one-another (Fig. 3.1c), which were surveyed during the spring and summer of 2005. A set of 42 lakes (the largest lake set) was sampled in the spring (June 10), early summer (July 2) and mid-summer (August 8) for DOC concentration ([DOC]) and chromophoric DOM (CDOM) absorption. A set of 6 lakes (the smallest lake set) was further sampled once weekly between early June and late August for [DOC] and CDOM. This 6-lake set was also sampled in the spring (June 14-17), mid-summer (July 23-27), and late summer (August 22-26) for stable isotopes ($\delta^{13}C$, $\delta^{15}N$) of DOM precursor materials, and $\delta^{15}N$ of DOM. Samples for $\delta^{13}C$ of DOM were collected from the 6-lake set at the same time as other isotopic samples, and also in the early summer (July 4-6). A further set of 9 lakes was sampled for $\delta^{13}C$ of DOM during the 42-lake surveys, such that overall, $\delta^{13}C$ samples were collected from a series of 15 lakes (Fig. 3.1). Samples for CDOM fluorescence were collected from the 6-lake set during the spring and late summer sampling periods, and from the 15-lake set during the mid-summer sampling period. On each sampling occasion, riverwater samples were collected from a nearby river channel (Fig. 3.1). Samples of Mackenzie River floodwater were collected in late May to early June. We refer specifically to "riverwater" and "floodwater" to acknowledge that floodwater likely accumulates a greater proportion of Delta-origin terrigenous DOM, after being subject to the significant mechanical mixing that occurs during the flood.

Lake, river, and floodwater samples were collected in clean, acid rinsed bottles directly beneath the water surface and immediately transported to the laboratory for processing. Samples were filtered within 8 h of collection (0.22 μm, Millipore GSWP, Millipore Corp., Billerica, MA). Samples for [DOC], CDOM, and $\delta^{13}C$ of DOM were stored in darkness at 4°C until analysis (4 months, 2-4 weeks, and 3 weeks, respectively); $\delta^{13}C$ samples were specifically stored in gas-tight glass EPA vials with no headspace. Samples for $\delta^{15}N$ of DOM were dried down to ~10 mL at 60°C, and stored frozen until later processing

and analysis.

DOM precursor materials for analysis included s- and e-macrophytes, seston (for the estimation of algal isotopic signatures, described below), and epiphytic algae. Common macrophyte species were collected from each of the 6 survey lakes and cleaned by repeated rinses in distilled water. Samples for seston were collected by filtering 80 μm-filtered water samples through a pre-combusted Whatman GF/F filter (nominal pore size 0.7 μm). Epiphytic algae were collected by vigorously shaking macrophytes in distilled water, using repeated rinses until no algae were visibly dislodged. Samples for bacterioplankton $\delta^{13}C$ were also collected following Kritzberg et al. (2006): bacteria were cultured in dialysis tubing (SpectraPor 2, 12,000-14,000 Da molecular cutoff, 45mm flat width) by filling duplicate tubes with a 900 mL of 0.22 μm filtered lakewater (Millipore GSWP) combined with 100 mL of grazer-free inoculum (lakewater filtered with Whatman GF/A filters, 1.6 μm nominal pore size), and incubating sealed tubes ~20 cm below the water surface. Cultures were harvested after 48 h by filtering replicates through separate pre-combusted GF/F filters. A 10 mL subsample of each culture was also preserved with glutaraldehyde (2.5%) to evaluate whether samples were contaminated with phytoplankton or heterotrophic flagellates. Microscopic counts of DAPI-stained samples confirmed the absence of these organisms, or other types of contamination. All stable isotope precursor samples were immediately dried at 60°C, at which time they were stored frozen until later processing and analysis.

3.3.3 Lake characterization

Sill heights for all sampled lakes (Marsh and Hey 1988), and s-macrophyte densities (g dry weight m^{-2}) for a subset of lakes (Squires et al. 2002), were obtained from the literature. The height of a lake's sill determines its connection to the proximate deltaic river channel: when river water levels fall below the sill elevation, the lake becomes cut off from the river. Connection time (d) to the river for each study lake was estimated by comparing sill elevations to daily river water levels (Water Survey of Canada online data for hydrometric station 10LC002, Mackenzie River [East Channel] at Inuvik;

http://scitech.pyr.ec.gc.ca/waterweb). The extent of thermokarst around lakes was assessed visually via helicopter during our surveys, and confirmed using Google Earth. Thermokarst extent was scored as the percentage of each lake's shoreline that had collapsed into the lake, assessed as trees sloping towards or falling onto the lake surface. Lakes with greater than 75% thermokarst along their shoreline were considered to be significantly affected by thermokarst for the purposes of this study. Of lakes classed as not significantly affected by thermokarst, 77% had no visible slumping along their shoreline, and the remaining 23% had an average of 23±9% (95% confidence interval) slumping.

3.3.4 Sample analysis

Samples for [DOC] were analysed as non-purgeable organic carbon (Shimadzu TOC-V_{csh}, Shimadzu Corporation, Kyoto, Japan) after sparging for 5 min with 20 mM HCl (final concentration). CDOM absorbance was measured using a Genesys 5 scanning spectrophotometer (Milton Roy, Ivyland, PA) using a 5 cm quartz cuvette. Measurements were corrected using a MilliQ water blank run every 12 scans. Duplicate scans from 250 to 750 nm (1 nm interval) were performed. Absorption coefficients ($a(\lambda)$, m^{-1}) were calculated by multiplying measured absorbances by 2.303, and dividing by the path length of 0.05 m. We present results for absorbance at 440 nm ($a(440)$) as an overall measure of water colour, specific absorptivity at 440 nm ($a^*(440) = a(440)/[DOC]$) as a measure of colour per [DOC], and the $a(250):a(365)$ ratio as a proxy for DOM molecular weight, where higher ratios are indicative of a lower molecular weight DOM pool, and thus either autochthonous or degraded DOM (Strome and Miller 1978). CDOM fluorescence was measured using a Shimadzu RF-5301 spectrofluorometer with excitation wavelengths ranging from 250 to 450 nm (every 5 nm), and emission wavelengths ranging from 300 to 600 nm. DOM fluorescence values were corrected for inner filter effects and normalized to the water Raman fluorescence as described in Boyd and Osburn (2004). Excitation data for synchronous fluorescence scans were extracted from the EEM datasets, using a constant offset ($\delta\lambda$) of 14 nm.

Samples for $\delta^{13}C$ of DOM were analysed following St-Jean (2003), using a method

that allows for the quantification of $DI^{13}C$ and $DO^{13}C$ in concert. Samples were injected into a OI Analytical model 1010 wet oxidation TOC analyser (College Station, Texas), which was modified to allow vented CO_2 to be tapped and redirected to a ThermoFinnigan DeltaPlusXP continuous flow isotope ratio mass spectrometer, via an electrolytic copper scrubber, water trap, and a packed NCS GC column to focus the CO_2 (Osburn and St-Jean, 2007).

Samples for $\delta^{15}N$ of DOM were acidified to pH 2 to remove carbonates, dialyzed using a 100 Da membrane (SpectraPor CE membrane, Spectrum Laboratories Inc., Rancho Dominguez CA) in a continuous flow system to remove dissolved inorganic nitrogen from the sample (Lee and Westerhoff 2005), and lyophilized. Samples for $\delta^{13}C$ and $\delta^{15}N$ of macrophytes and epiphytic algae were ground to a fine powder using a mortar and pestle, and powdered samples were acidified using 2N HCl to remove carbonates. Acidified, dried samples of DOM, macrophytes, and epiphytic algae were then weighed into tin capsules (Elemental Microanalysis Limited). Filtered seston samples were folded into tin capsules after the top layer of the filtered sample had been separated from the remaining filter, and analysed for $\delta^{13}C$ and $\delta^{15}N$. Filtered bacterioplankton samples were folded into tin capsules, and analysed for $\delta^{13}C$ only, because ^{15}N fractionation by bacteria can be substantial and variable (Peterson and Fry 1987), and thus bacterial $\delta^{15}N$ is a poor indicator of food source. Samples were analysed by the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Canada) using a Vario III elemental analyser (Elementar, Germany) coupled to a DeltaPlus XP IRMS (ThermoFinnigan, Germany) via a ConFlo II interface. The $\delta^{13}C$ values obtained in concert with the DOM $\delta^{15}N$ results were always in good agreement with $\delta^{13}C$ values from the wet oxidation method, with an average absolute deviance of 0.67‰ between methods.

3.3.5 *Data analysis*

Fluorescence modeling:

The collected EEMs were analysed using parallel factor analysis (PARAFAC) following Stedmon et al. (2003). PARAFAC resolves the fluorescence signal of the complex DOM pool into separate fluorophores with characteristic excitation emission curves, and

can be used to extract fluorophores characteristic of both allochthonous and autochthonous DOM (Stedmon and Markager 2005). The PARAFAC analysis was performed using the N-way toolbox for MATLAB 3.10 (Anderson and Bro 2002) in MATLAB 7.5. The model was initialized with randomized orthagonalized values, and non-negativity constraints were applied to each dimension. The appropriate number of components of the model was determined using split-half analysis (Stedmon et al 2003). Results of the PARAFAC model are expressed as F_{max}, the maximum fluorescence of each component in a given sample (Stedmon and Markager 2005). Within components, variation in F_{max} across samples corresponds to variations in concentration. However, because different components have different concentration-specific fluorescent intensities, the absolute difference in F_{max} between components is not equivalent to their absolute differences in concentration.

Synchronous fluorescence spectra were analysed by calculating the relative abundance of Peak I, as outlined in Lu et al. (2003). Our spectra contained 4 distinct peaks, as has been reported previously (e.g. Lu et al. 2003). Peak I (in our samples at ~280 nm) has been assigned to the presence of protein-like (autochthonous) materials, while peaks II-IV (~345, 380, 450 nm) reflect the presence of humic and fulvic (allochthonous) materials in the sample.

Stable isotope mixing models:

Stable isotope analyses are particularly useful when source materials differ markedly in their isotopic composition. We expected this to be true for DOM precursor materials in Delta lakes, given known differences in fractionation between these endmembers. In the case of $\delta^{13}C$, allochthonous DOM displays a characteristically $\delta^{13}C$-depleted terrestrial signature (~-27‰), while increasing boundary layer thicknesses in attached algae and s-macrophytes reduces isotopic discrimination during photosynthesis, and enriches $\delta^{13}C$ (discussed in Hecky and Hesslein 1995). In s-macrophyte-rich lakes, the striking depletion in CO_2 that occur progressively through the growing season as a result of rapid photosynthesis (Chapter 2), should in turn increase phytoplankton CO_2 limitation, and again decrease photosynthetic ^{13}C discrimination.

We used IsoSource (Phillips and Gregg 2003) to estimate the relative contribution of

floodwater, riverwater (for connected lakes) and within-lake primary production (s-macrophytes, phytoplankton, epilithon) to lakewater DOM signatures. Because of their almost complete overlap with terrestrial signatures (see *Results*) we chose not to include e-macrophytes in our models, but discuss this potential source to the DOM pool qualitatively. Traditional mixing models allow the determination of one, unique, solution for the contribution of n+1 sources to a mixture, for an n-isotope model. For example, in a 1-isotope model, the percent contribution of 2 sources to the mixture can be uniquely resolved, as:

$$\%_a(sig_a) + (100-\%_a)(sig_b) = sig_{mix}, \text{ and}$$

$$\%_b = 100 - \%_a,$$

Where $\%_a$ and $\%_b$ are the contribution of sources a and b, and sig_a, sig_b, and sig_{mix} are the isotopic signatures of the 2 sources and the mixture. IsoSource allows quantitative determination of the range possible contributions (i.e., between 0 and 100%) of sources to a mixture for situations in which a unique mixing model solution is not possible because more than n+1 different sources signatures exist (Phillips and Gregg 2003). The model was run separately for each of the 6 survey lakes. We chose to use late summer DOM $\delta^{13}C$ and $\delta^{15}N$ in the model, because autotrophic contribution to the DOM pool is likely to be highest near the end of the growing season. Source signatures from each of the early, mid-, and late summer surveys were used as model inputs. The signature for phytoplankton was estimated in two ways, and the model run separately for the 2 resulting estimates. First, we assumed that seston was composed entirely of phytoplankton, and used the seston signature as a phytoplankton proxy. Given that many of these lakes have low inputs of allochthonous particulate organic C (POC; Squires and Lesack 2003), and sestonic C:N molar ratios across all lakes (ranging from 5.5 and 7.0) were close to ratios expected for phytoplankton, this seems reasonable for Mackenzie Delta lakes. However, we additionally used the mixing model of Bade et al. (2006) to calculate adjusted phytoplankton $\delta^{13}C$ values, based on measured chlorophyll *a* (Chl *a*) concentrations, published C:Chl *a* ratios (Ramlal et al. 1991), and POC concentrations (Anema et al. 1990) for Delta lakes, and assuming a terrestrial endmember $\delta^{13}C$ of -28‰. This approach adjusts phytoplankton $\delta^{13}C$, based on

the estimated proportion of algal C in the overall sestonic (POC) pool, assuming that non-algal sestonic organic C is terrestrially derived. Although phytoplankton $\delta^{13}C$ is also commonly calculated using $DI^{13}C$ and laboratory-measured fractionation factors for algal C uptake during photosynthesis, we did not use this approach. Recent work has shown that *in situ* rates of algal photosynthetic fractionation can be highly variable in lakes, likely as a result of active uptake of bicarbonate for photosynthesis by some species, and inter-specific variations in the rate of CO_2 diffusion into cells in mixed-species assemblages (Bade et al. 2006, Marty and Planas 2008). However, we include $DI^{13}C$ values in Table 3.1 for comparison purposes. A further discussion of phytoplankton $\delta^{13}C$ estimates in Delta lakes is given in Chapter 6.

Two approaches were used to accommodate variations within $\delta^{13}C$ and $\delta^{15}N$ source signatures. In cases where variations in source signatures were distributed amongst several discrete, tightly grouped clusters, each grouping was entered into the model as a separate source. Alternatively, when variations in source signatures were distributed along a gradient of values, maximum and minimum $\delta^{13}C$ and $\delta^{15}N$ values for the source were determined, and the model was run multiple times, such that all possible combinations of maximum/minimum isotope signatures were assessed (McCallister et al. 2004). Because phytoplankton productivity is extremely low in these lakes (mean 2005 summer Chl *a* ≈ 1.0 μg L^{-1}) and phytoplankton C tends to be rapidly depleted from the DOC pool (Kritzberg et al. 2006), we constrained the maximum contribution of phytoplankton to the DOM pool to be equal to the maximum ratio of ([phytoplankton C]/[DOC]) in any of our surveyed lakes, or 6%.

We further used mixing models to assess the relative contribution of DOM source materials (flood and riverwater, s-macrophytes, epiphytes and phytoplankton) to overall bacterial biomass. Mixing models were run using the $\delta^{13}C$ alone, because of the problems related to using $\delta^{15}N$ to assess bacterial diet (Peterson and Fry 1987). Methods were identical to those outlined for the DOM mixing models, except that the relative contribution of phytoplankton to overall bacterial biomass was left unconstrained, to reflect the fact that bacteria may preferentially take up this autochthonous C source (e.g., Kritzberg et al. 2006,

McCallister et al. 2006).

3.4 Results

3.4.1 [DOC] across a gradient of Delta lakes

[DOC] consistently increased with increasing lake elevation in each of the spring, early summer, and late summer surveys (Fig. 3.2 a-c). Increasing s-macrophyte densities were also associated with higher [DOC] in the early summer (Fig. 3.3a, but note outlier point), and mid-summer (outlier point again removed; $p=0.031$, $r^2=0.225$, relationship not shown), although this relationship was weaker than for with lake elevation. In thermokarst lakes, [DOC] was similar to concentrations in non-thermokarst lakes in the spring, but rose rapidly in the early season (Fig. 3.2d), such that summertime [DOC] was considerably elevated in lakes experiencing thermokarst activity (Fig. 3.2a-c). [DOC] in other high-elevation lakes rose slightly as the summer progressed, while concentrations in lower elevation lakes remained steady, or dropped slightly through the summer (Fig. 3.2d,e). Floodwater [DOC] was moderate in comparison to values for lake water, while river [DOC] tended to be at, or below the level found in the lowest elevation Delta lakes (Fig. 3.2a-c).

3.4.2 CDOM absorbance

In the spring survey, $a(440)$ increased proportionately with [DOC], such that $a^*(440)$ (specific absorptivity) did not vary with [DOC] ($r^2=-0.007$, $p=0.400$). Later in the season, however, $a^*(440)$ declined with increasing [DOC], and thus absorbance per unit DOC was lowest in the highest [DOC] lakes in both the early summer ($r^2=0.121$, $p=0.015$) and late summer ($r^2=0.092$, $p=0.028$) surveys. This trend was mirrored across the lake elevation gradient; $a^*(440)$ was uncorrelated with lake elevation in the spring (Fig. 3.2f), but declined significantly with increasing lake elevation in the early- and late summer (Fig. 3.2g,h). $a^*(440)$ also decreased as within-lake s-macrophyte density increased (Fig. 3.3b). Within lakes, specific absorptivities declined consistently as the summer progressed, with a sharp drop occurring in mid- to late-June (Fig. 3.2i,j). In contrast to the [DOC] results, $a^*(440)$ from thermokarst lakes did not differ substantially from the pattern seen for non-

thermokarst lakes (Fig. 3.2f-h). Throughout the summer, $a^*(440)$ values for flood and riverwater were similar to, or greater than, those in the lowest elevation Delta lakes.

DOM molecular weight, as inferred from $a(250):a(365)$ ratios, decreased in all lakes as the summer progressed (Fig. 3.2k-o). Similar to the trends for $a^*(440)$, springtime DOM molecular weights did not differ across the lake elevation gradient (Fig. 3.2k). However, by summertime, DOM molecular weight decreased significantly with increasing lake elevation (Fig. 3.2l,m). Again, DOM from thermokarst lakes did not differ significantly from this overall pattern (Fig. 3.2f-h). Molecular weights of flood and river DOM were consistently similar to, or greater than, those found in lower elevation surveyed lakes. Summertime lake DOM molecular weights decreased with increasing s-macrophyte density (Fig. 3.3c).

3.4.3 CDOM fluorescence

We were reliably able to extract 3 components from our EEM dataset using PARAFAC. Beyond this, split-half analysis gave inconsistent results across the 2 random sample groups, while repeated runs of the model initialized with random values gave results that differed between replicate models. Fig. 3.4a-c compares measured and modeled EEMs for a sample of floodwater DOM, and the residuals from this comparison. Residual values were well below those of modeled and measured spectra (maximum values were 17-fold lower in the comparison shown in Fig. 3.4). The PARAFAC model explained 99.7 % of the variation in our measured EEM spectra.

Of the 3 components extracted (Fig. 3.4d-f), component 1 was characteristic of a humic peak, component 2 was characteristic of a fulvic peak , and component 3 was characteristic of a protein-like peak (Stedmon and Markager 2005). F_{max} values for the extracted components did not differ significantly across the gradient of lake elevations (data not shown). When the F_{max} of each component was normalized to [DOC], however, several significant trends emerged. Within lakes, F_{max}:[DOC] declined as the summer progressed for all 3 components (Fig. 3.5a-c). F_{max}:[DOC] also decreased with increasing lake elevation, in the early- and mid- summer for component 1, and throughout the season for component 2 (Fig. 3.5a,b). Although F_{max}:[DOC] for component 3 appeared to be highest in mid-elevation

lakes (Fig. 3.5c), unimodal (quadratic) regressions were not significant. However, [DOC]-normalized component 3 did decline significantly with increasing lake elevation in the mid-summer (Fig. 3.5c). As was the case for measures of CDOM absorbance, the thermokarst lake within this sampled set did not differ from the fluorescence trends for non-thermokarst lakes. For components 1 and 2, [DOC]-normalized F_{max} values for flood and riverwater were greater than those observed within lakes, while for component 3, flood and riverwater F_{max} tended to be lower than for lakewater (Fig. 3.5a-c).

Clear differences in the relative contribution of each component to the overall CDOM fluorescent signature were also evident between sampling periods, and across the lake elevation gradient (Fig. 3.5d-f). The percent contribution of component 2 to the CDOM fluorescent pool tended to be highest in the early summer, while the percent contribution of component 3 tended to be lowest in the early summer. The importance of component 2 declined significantly with increasing lake elevation in the early and mid-summer, and declined marginally with increasing lake elevation in the late summer (Fig. 3.5e). This decline across the lake elevation gradient was compensated for by increases in the relative importance of component 3, which increased significantly with increasing lake elevation in the early and mid-summer (Fig. 3.5f).

The relative proportion of peak I in our samples, indicative of an autochthonous, protein-like DOM source, increased marginally with lake elevation in the early summer, and significantly with lake elevation in the mid-summer (Fig. 3.6). There was no relationship between the proportion of Peak I and lake elevation in the spring.

3.4.4 Stable isotope signatures and mixing models

$\delta^{13}C$ for river and floodwater DOM were close to values typical for terrestrially-derived C, ranging from -25.5‰ to -27.1‰. The isotopic $\delta^{13}C$ signature for within-lake DOM closely overlapped these values. In the spring and early summer DOM $\delta^{13}C$ ranged from -25.3‰ to -27.6‰ in non-thermokarst lakes, and did not vary across the lake elevation gradient (data not shown). This was similar to the range in DOM $\delta^{13}C$ values observed during the mid-summer survey (Fig. 3.7), while in the late-summer there was a

slight tendency for lakewater DOM to be somewhat enriched in comparison to floodwater, in higher elevation non-thermokarst lakes (Fig. 3.8a-f). DOM $\delta^{13}C$ for the thermokarst lake included in this survey was slightly $\delta^{13}C$ depleted in comparison to riverwater, ranging from -27.2‰ to -28.3‰ through the summer.

Generally, there was good separation between the $\delta^{13}C$ and $\delta^{15}N$ signatures of the different end member types for different DOM source materials (Fig. 3.8a-f). Signatures for s-macrophytes and epiphytic algae were the most ^{13}C enriched, while in s-macrophyte rich lakes (shown in Fig. 3.8 c,d,e) sestonic $\delta^{13}C$ became increasingly enriched as the summer progressed. In contrast, values for e-macrophytes, which use atmospheric CO_2 for photosynthesis, were terrestrial-like in nature (Fig. 3.8a,d,e). Across all lakes, signatures for river and floodwater DOM, and lakewater DOM, were in very close proximity to one another on our isotopic biplots, while autotrophic end members lay much more distant from lakewater DOM in biplot space. Although the mixing model results were generally poorly constrained, they strongly suggest that a majority of the DOM pool is derived from terrestrial or terrestrial-like sources. Across all lake types, no more than 10% of the DOM pool could be ascribed, with certainty, to autochthonous sources (Fig. 3.9a). There was no consistent trend in the relative importance of riverwater to the DOM pool when compared either to lake elevation, or within-lake productivity.

In contrast to the DOM results, bacterial $\delta^{13}C$ in non-thermokarst lakes was consistently ^{13}C enriched compared to both the riverwater and within-lake DOM signatures (Fig. 3.9b). Consequently, mixing models used to assess the origin of bacterial biomass from within the DOM pool showed that bacteria were preferentially taking up autochthonous C (Fig. 3.9a). The minimum proportion of autochthonous C with bacterial biomass was 3-32% greater than the proportion of autochthonous C in lakewater DOM, across the gradient of non-thermokarst lakes. In thermokarst lakes, both DOM and bacterial biomass were predominantly comprised of allochthonous C.

3.5 Discussion

3.5.1 Macrophyte organic matter is strongly underrepresented in the DOM pool

In macrophyte-dominated shallow lakes, it has been proposed that autochthonous C should comprise a significant proportion of the DOM pool (e.g., Wetzel 2001, Bertilsson and Jones 2003). Photosynthetic exudates from macrophytes have been conservatively estimated at 4% of net macrophyte productivity (Wetzel and Manny 1972), while leaching from senesced vegetation could also add significant DOM to the water column (Findlay et al. 1986, Mann and Wetzel 1996). In high elevation Delta lakes, the volume-adjusted standing stock C load of s-macrophytes is 7-12 fold that of [DOC] (calculations based on Squires et al. 2002, Squires and Lesack 2003), while photosynthesis by s-macrophytes and their associated epiphytes is rapid enough to drive water column pCO_2 to levels near zero (Hesslein et al. 1991, Chapter 2). Thus, it seems reasonable that this autochthonous C would form an important component of the DOM pool.

When examined in isolation from one another, several of our DOM tracers are consistent with autochthonous C forming an important component of the DOM pool in macrophyte-rich lakes. Elevated [DOC], as we observe in these lakes, has previously been attributed to autochthonous DOM augmenting the within-lake pool where macrophyte productivity is high (Briggs et al. 1993, Mann and Wetzel 1995). Additionally, macrophyte photosynthetic exudates have been suggested to be relatively clear, and comprised of simple, low molecular weight molecules (Wetzel and Manny 1972, Bertilsson and Jones 2003; however, DOM from the breakdown of senesced macrophytes could be larger in size and much more coloured). Thus, the decreases in DOM molecular weight, specific absorptivity, and humic and fulvic content seen in macrophyte-rich lakes could again all be consistent with autochthonous DOM augmenting the within-lake pool.

However, when examined as a comprehensive package, our suite of tracers suggests macrophyte DOM forms at most, a modest component of the bulk DOM pool. Stable isotopes, in particular, indicate that terrestrial or terrestrial-like DOM dominates across the

lake elevation gradient. Even in the most macrophyte-rich lakes, DOM from autochthonous sources could only be confirmed to account for 10%, or less, of the within-lake pool. DOM values were slightly enriched relative to riverine DOM in macrophyte-rich Delta lakes, which is consistent with the incorporation of some macrophyte or epiphyte organic matter into the bulk pool (Fig. 3.8). However, a portion of this isotopic enrichment could also be a result of photobleaching, which can enrich DOM $\delta^{13}C$ as a result of selective photolytic decomposition (Osburn et al. 2001, Vähätalo and Wetzel 2008). These macrophyte-rich lakes, which are hydrologically isolated from the surrounding landscape, provide an ideal site for DOM photobleaching (Gareis 2007). Reduced CDOM specific absorptivity (Moran et al. 2000), molecular weight (Moran and Zepp 1997), and DOM humic and fulvic content (Moran et al. 2000), are all well described consequences of DOM photolysis. The declines in specific absorptivity and molecular weight observed in macrophyte-rich Delta lakes were most rapid in June, when 24-hour arctic sunlight and high solar angle would allow for strong photochemical activity. Conversely, rapid macrophyte photosynthesis does not begin until late June, and continues throughout the summer (Chapter 2). Previous work examining DOM quality in Delta lakes using specific ultraviolet absorbance and spectral slope also concluded that photobleaching is an important determinant of the composition of Delta lakewater DOM (Gareis 2007).

In part, the high [DOC] observed in macrophyte-rich Delta lakes could result from their brief connection to the river. These high elevation lakes have a negative water balance between flooding periods (Marsh and Lesack 1996) that can decrease water levels by ~2 mm d^{-1} after the loss of river connection (Marsh 1986) in lakes with z_{mean} ~1.5 m. Hydrologically isolated lakes have been shown to have remarkably high [DOC] in other lake regions (e.g., Curtis and Adams 1995). Given that the [DOC] trend correlates more strongly with lake elevation (and thus hydrologic isolation) than within-lake s-macrophyte biomass, it seems likely that evaporative concentration is as or more important than macrophyte productivity for determining the makeup of the bulk DOM pool. In addition, floodwater [DOC] is relatively high, but concentrations drop rapidly post flood (Fig. 3.2, Emmerton et al. 2008). While the highest elevation lakes receive a pulse of this relatively concentrated DOC

before becoming isolated from the river, the lowest elevation lakes are flushed periodically with riverwater throughout the season. Inputs of other, potentially DOC-rich material that exhibit a terrestrial-like $\delta^{13}C$ signature, including post-flood runoff (rillwater; Lesack, unpublished data) and the decomposition products of e-macrophytes, could also be important DOM sources to high elevation lakes. While there is no reason to expect the importance of these DOC sources to vary systematically across the elevation gradient, the lack of flushing in high elevation systems may increase their relative permanence.

3.5.2 The fate of autotrophic organic matter in Mackenzie Delta lakes

Given their striking biomass levels and overall C load, it seems likely that s-macrophytes play some, significant role in the C balance of Delta lakes. Our CDOM fluorescence data suggests that the DOM pool becomes proportionately more labile with increasing lake elevation and macrophyte density, based on increasing proportions of protein-like DOM within the overall pool. Protein-like DOM fluorescence has been attributed both to recent bacterial activity (Cammack et al. 2004), and the presence of autochthonous DOM (Stedmon and Markager 2005). Thus, although autotrophic DOM is not an obvious component of the bulk pool in macrophyte-rich Delta lakes, continued inputs of fresh DOM may be occurring in these waterbodies.

Accordingly, $\delta^{13}C$ mixing models indicate that preferential assimilation of autotrophic DOM by bacteria acts as a sink for macrophyte DOM in these lakes, as has been shown in systems where primary productivity is dominated by phytoplankton (e.g. Kritzberg et al. 2006, McAllister et al. 2006). Like that of phytoplankton origin, DOM derived from macrophyte leachates is an extremely labile bacterial substrate, supporting high rates of bacterial production and growth efficiency (Findlay et al. 1986, Mann and Wetzel 1996). Another potential sink for macrophyte-derived DOM is sequestration before it reaches the pelagic zone. The rapid cycling that we observe between autochthonous DOM and bacterioplankton in the water column likely also occurs on macrophyte and sediment surfaces: bacteria colonizing the epiphytic and epipelic mats that occur on standing, and senesced, macrophytes may be able to consume a large proportion of macrophyte exudates

and leachates before this DOM enters the water column.

3.5.3 The importance of thermokarst to DOM in Delta lakes

In contrast to our results for macrophytic DOM, several lines of evidence suggest that thermokarst allows previously sequestered organic matter to form an important component of the within-lake DOM pool. The remarkably high [DOC] observed in thermokarst lakes, relative to other Delta lakes, was terrestrial in origin, as indicated by the $\delta^{13}C$ signatures of both DOM and bacteria. At the same time, the factors proposed to be augmenting terrestrial or terrestrial-like DOM in macrophyte-rich lakes are not proportionately greater in lakes experiencing thermokarst. In the Mackenzie Delta, thermokarst tends to occur in high elevation waterbodies, and thermokarst lakes are therefore poorly connected to the river. Thus, evaporation is certainly an important component of the water budget of these lakes. However, thermokarst, by definition, deepens lakes, and the thermokarst lakes in our study had an average lake depth of ~2.0 m (compared to an overall average close to 1.5 m for Delta lakes), presumably decreasing the relative importance of evaporation as a result of increased volume: surface area ratios. Furthermore, flushing rates and the input of floodwater and post-flood runoff to thermokarst lakes should be similar to other non-thermokarst, high elevation lakes. Finally, e-macrophytes occur only sparsely in these waterbodies, because lake margins fall off quickly as a result of increased lake depth.

DOM $\delta^{13}C$ from the intensively-studied thermokarst lake was also closer to the standard terrestrial endpoint (-28 to -29‰) than was the riverwater DOM signature, suggesting some input of autochthonous DOM to the riverwater pool, and an enhanced proportion of terrestrial DOM in the thermokarst lakewater pool. Although algal $\delta^{13}C$ is also depleted in this lake, algal biomass is extremely low (<1% of the DOM pool, per unit C), and thus this autochthonous C source is unlikely to account for the shift towards a terrestrial-like $\delta^{13}C$ signature that we observe. Thus, the contribution of previously sequestered organic matter to the water column seems the most likely explanation for the enhanced [DOC] observed in Delta thermokarst lakes. Because of logistical constraints, we were

unable to collect DO^{14}C data from these waterbodies, which would add significant additional insight into the origin of DOM in these lakes. In particular, information about DO^{14}C in Delta thermokarst lakes would help to resolve the relative importance of aged, permafrost-derived organic matter, newer vegetation that has slumped into these lakes as a result of thermokarst processes, and other, terrestrial or terrestrial-like organic matter sources.

Unlike macrophytic DOM, the DOM released as a result of thermokarst processes appears to accumulate in the water column of Delta lakes. There is some evidence to suggest that thermokarst-liberated organic matter is relatively labile (Zimov et al. 2006). However, in general, terrestrially-derived DOM is not expected to support the rapid cycling characteristic of autochthonous organic matter (Kritzberg et al. 2006). A DOM pool that continues to accumulate over the summer may explain the high rates of photobleaching that appear to occur in Delta thermokarst lakes, as demonstrated by $a^*(440)$, DOM molecular weight, and the relatively low humic and fulvic content of the DOM pool. In previous studies, experimental exposure of Delta lakewater to natural sunlight resulted in DOM absorbency losses on the order of 6% over an 8 hr incubation period (Gareis 2007). Photobleaching rates of this magnitude are certainly consistent with the relatively low molecular weight, transparent DOM observed in these hydrologically isolated waterbodies.

3.5.4 The consequence of diverse DOM sources to Delta lakes

The divergent fates of different DOM sources in Mackenzie Delta lakes suggests that these distinct DOM pools have very different effects on within-lake biological, chemical, and physical processes. For example, preferential uptake of macrophytic DOM has clear implications for microbial food web structure, particularly given the high growth efficiency of bacteria on this substrate (Findlay et al. 1986, Mann and Wetzel 1996, Chapter 4): once it enters the DOM pool, macrophytic organic matter has the potential to provide an important subsidy to higher trophic levels via a bacterial shunt (see also Waichman 1996). In Delta lakes, this subsidy could be particularly important, because algal photosynthesis is extremely low (Squires and Lesack 2002), but macrophyte production is high. At the same time, because it is consumed rapidly, macrophytic DOM should play a modest role in the

many physico-chemical processes that DOM is known to influence, such as light penetration and water chemistry (reviewed in Prairie 2008). In contrast, the accumulation of DOM derived from thermokarst processes suggests that it is less biologically labile, and thus may provide little energy to higher trophic levels, similar to the role described for temperate, watershed-derived allochthonous DOM (Cole et al. 2006). However, its longer residence time likely allows thermokarst-derived DOM to have a greater influence on the physico-chemistry of Delta lakes.

Future changes expected across the Mackenzie Delta landscape could have clear repercussions for the composition of DOM in these lakes. Although only a small proportion of Delta lakes currently experience strong thermokarst effects, permafrost melting is increasing (ACIA 2004). The high-elevation lakes surrounded by the ice-rich permafrost necessary for thermokarst (Kokelj and Burn 2005) account for approximately 15% of total Delta lake area (Lesack and Marsh 2007). Thus, future permafrost degradation could markedly alter the DOM composition of many high elevation Delta lakes. At the same time, springtime peak flood heights and the connection time of higher elevation lakes to the river appears to be decreasing, because of declining effects of river-ice breakup (Lesack and Marsh 2007). The resulting increased water clarity – particularly in mid-elevation lakes – could act to increase macrophyte densities in these waterbodies, while inputs of riverwater DOM decline. Increased autochthonous, macrophytic DOM in some lakes, and allochthonous, thermokarst-derived DOM in others, could accentuate the differentiation that we currently observe between Delta lake types, creating clear classes of lakes that function very differently from one another as a result of their DOM composition. This increasing importance of macrophytic and thermokarst-derived DOM should also be expected for other lake regions globally, as the prevalence of permafrost melting increases in the north (ACIA 2004), and shallow lakes recover from eutrophication, allowing them to revert to a previously stable, macrophyte-rich state (Scheffer et al. 1993).

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3.7 Tables

Table 3.1: $DI^{13}C$ values for the set of 6 surveyed lakes, and nearby river channel. Samples for the determination of $DI^{13}C$ were collected on the same day as those for stable isotopes of DOM and DOM precursor materials. The thermokarst lake within this dataset is indicated as (TK). Depleted $DI^{13}C$ values in the late summer occur in macrophyte-rich lakes, where CO_2 depletion can be severe.

Lake sill (masl)	$DI^{13}C$ (‰)			
	Flood	Spring	Mid-summer	Late summer
River	-7.4	-6.4	-5.9	-6.5
2.4		-7.7	-8.1	-7.4
2.6		-6.6	-7.3	-7.1
3.4		-7.4	-4.8	-13.6
3.8		-7.4	-5.3	-7.7
4.6		-6.4	-6.5	-15.5
4.9 (TK)		-2.5	-2.2	-3.3

3.8 Figures

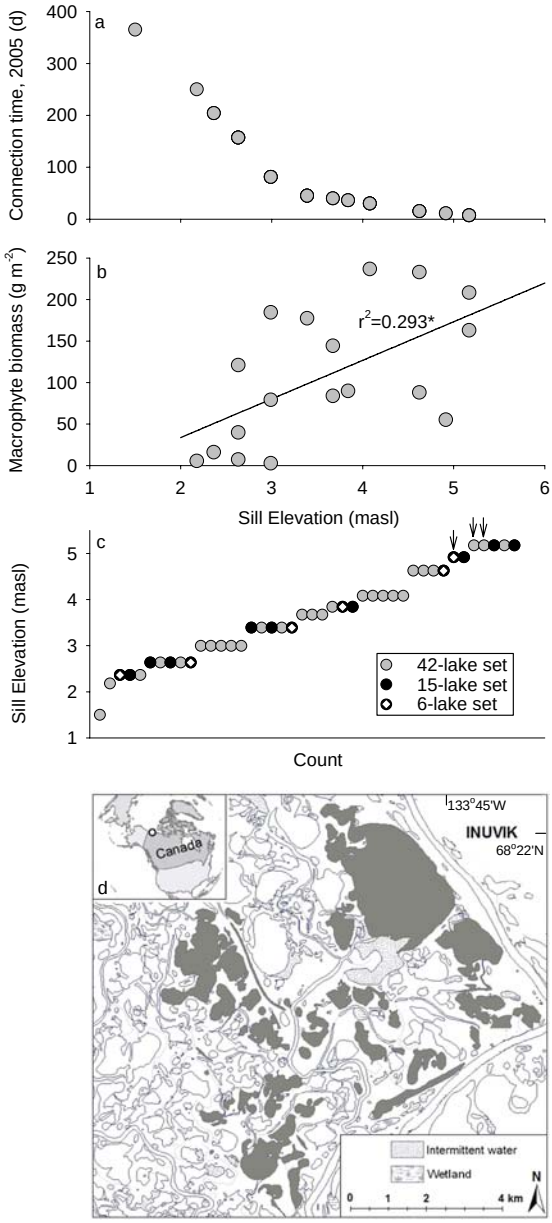

Figure 3.1: A general overview of surveyed Mackenzie Delta lakes. Panel (a) gives connection time, and (b) gives macrophyte biomass across the sill elevation gradient. Data in panel (b) are from Squires et al. (2002). Panel (c) gives the distribution of surveyed lakes across the sill elevation gradient; the smaller surveyed lake sets are subsets of the larger surveyed sets. Arrows in panel (c) indicate thermokarst lakes in the dataset. Shaded areas in panel (d) indicate all surveyed lakes.

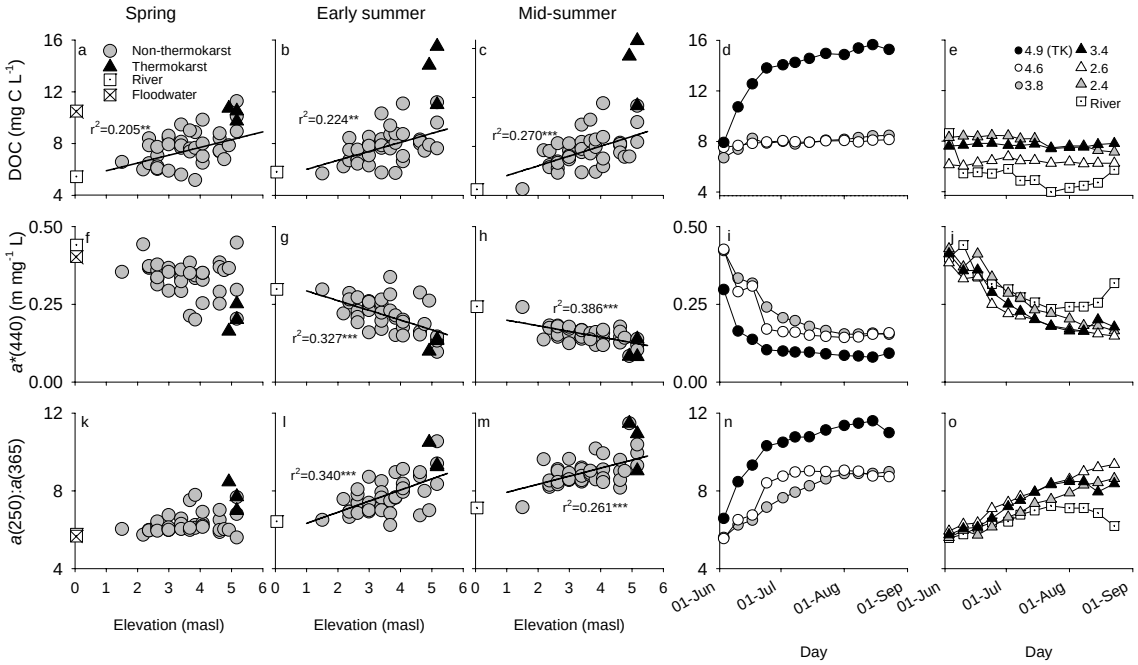

Figure 3.2: DOC concentration, specific absorptivity [$a^*(440)$], and inferred molecular weight [$a(250):a(365)$] for dissolved organic matter from Mackenzie Delta lakes, as related to lake elevation. Note that increasing $a(250):a(365)$ indicates decreasing molecular weight. Values for a set of 42 lakes surveyed in spring, early summer, and mid-summer are shown in the first three columns. Weekly values for riverwater and the set of 6 lakes are shown in the last 2 columns; lake sill elevations are indicated in the figure legend. Lines indicate a significant correlation between DOC concentration, specific absorptivity, or molecular weight and sill elevation. *p<0.05; **p<0.01; ***p<0.001.

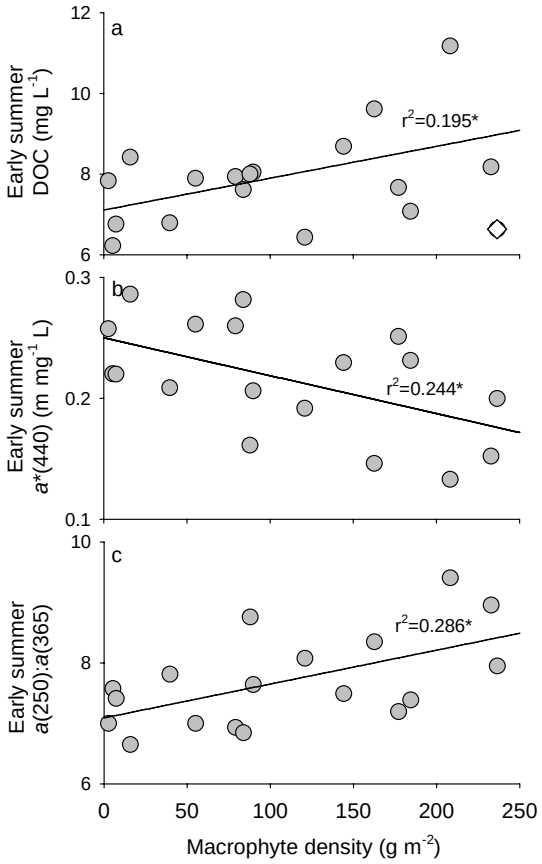

Figure 3.3: DOC concentration, specific absorptivity [$a^*(440)$], and inferred molecular weight [$a(250):a(365)$] for dissolved organic matter from Mackenzie Delta lakes, as related to macrophyte density. Note that increasing $a(250):a(365)$ indicates decreasing molecular weight. The outlier point removed from panel (a) is represented as a white diamond. Significance levels for correlations are as in Figure 3.2.

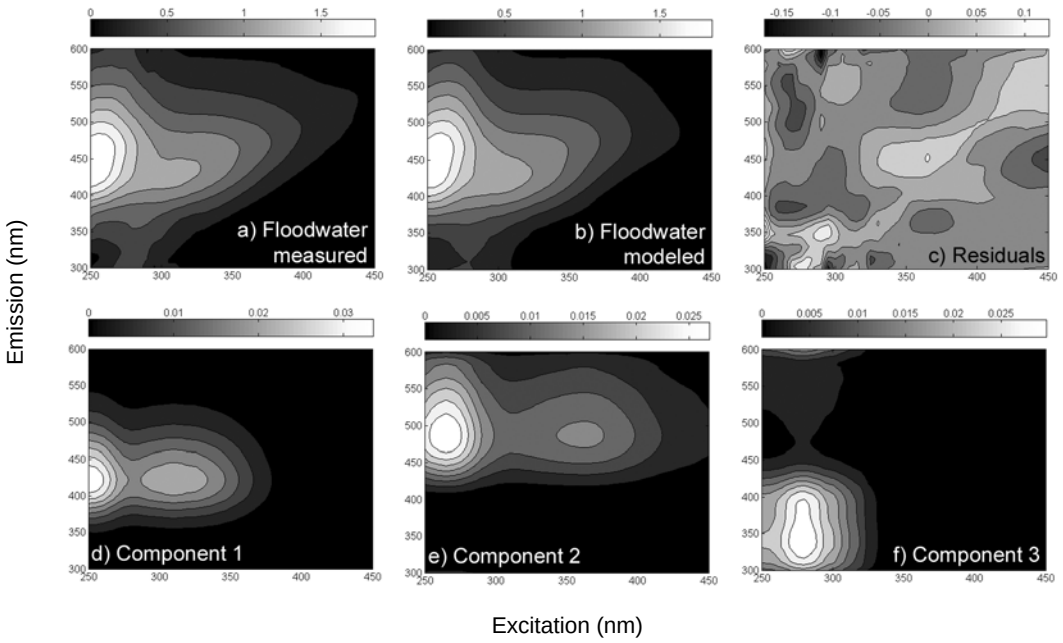

Figure 3.4: Results of the PARAFAC analysis for measured Excitation-Emission Matrices from Delta lake- and riverwater. Panels (a) through (c) show a measured and modeled floodwater sample, and the residuals of this model. Note the differences in scale across these panels. The 3 components extracted from the PARAFAC model are shown in panels (d) – (f).

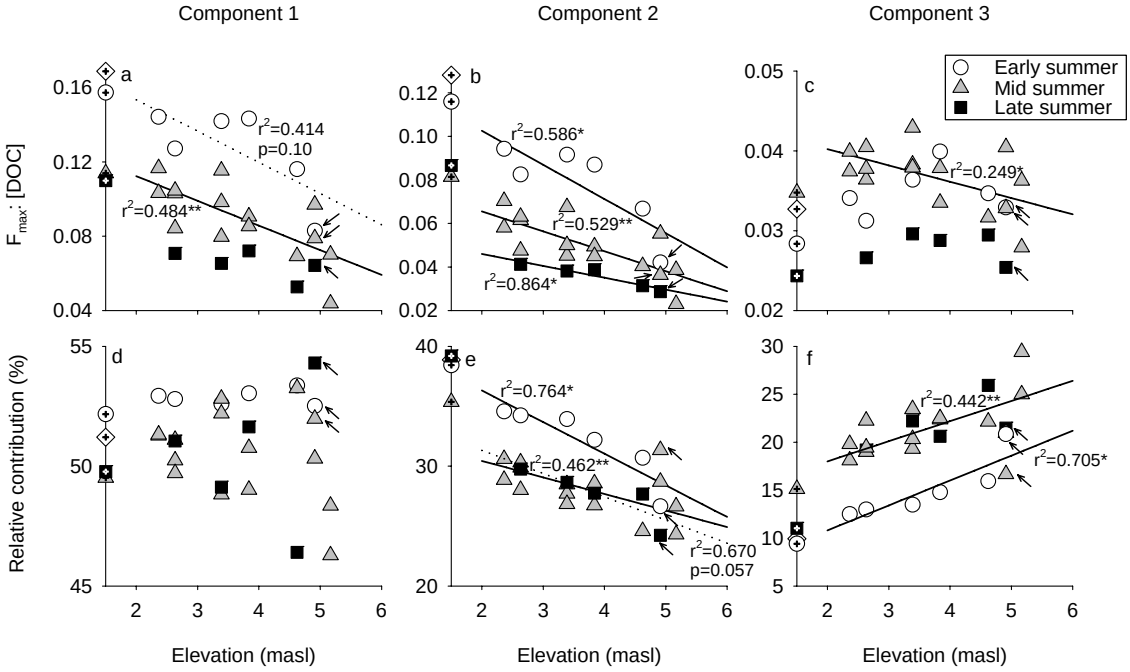

Figure 3.5: F_{max}:[DOC], and the relative contribution to the overall fluorescent pool for the 3 components extracted from our PARAFAC model: component 1 (left column), component 2 (middle column), component 3 (right column). Samples for early summer (open circles) mid-summer (grey triangles) and late summer (black squares) are shown in each panel. Significant relationships with sill elevation are designated using solid black regression lines, with significance levels as in Figure 3.2. Marginal relationships are designated with dotted regression lines, and the corresponding p-value is indicated. Arrows indicate samples taken from a thermokarst lake. Riverwater samples for early, mid- and late summer are shown in each panel with hatched symbols that correspond to the lakewater samples from that time period. Floodwater samples are shown in each panel as open hatched diamonds.

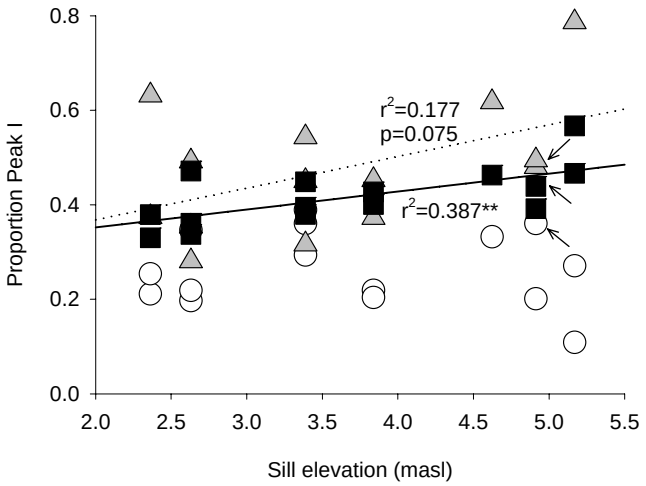

Figure 3.6: The proportion of Peak I from synchronous fluorescence scans of lakewater. Samples for early summer (open circles), mid-summer (grey triangles), and late summer (black squares) are shown. Significant relationships with sill elevation are designated using solid black regression lines, with significance levels as in Figure 3.2. Marginal relationships are designated with a dotted regression line, and the corresponding p-value for the relationship is indicated. Arrows indicate samples taken from a thermokarst lake.

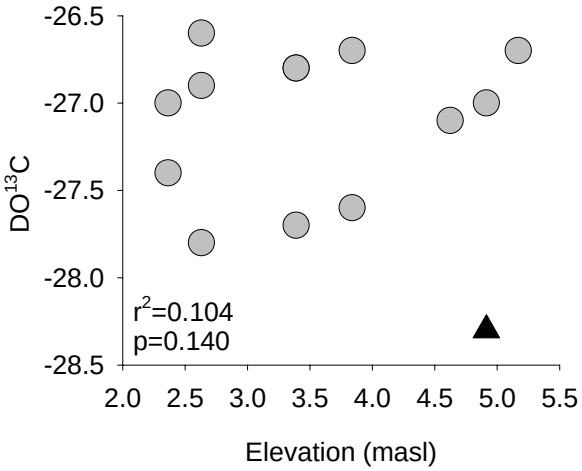

Figure 3.7: Mid-summer $\delta^{13}C$ values for dissolved organic matter (DOM) from Mackenzie Delta lakes. Non-thermokarst (grey circles) and thermokarst (black diamond) lakes are indicated. The r^2 and p-value for the linear relationship between DOM $\delta^{13}C$ in non-thermokarst lakes and lake elevation is shown.

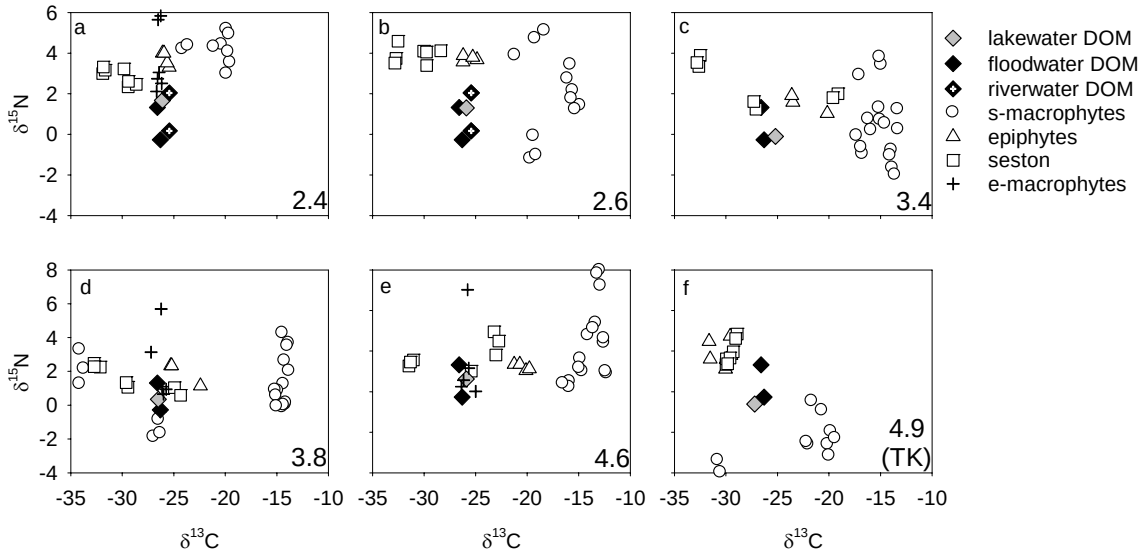

Figure 3.8: Biplots of $\delta^{13}C$ and $\delta^{15}N$ for DOM and DOM source materials across a gradient of Delta lakes. Lakes are shown in separate panels, with lake sill elevation (in m) given in each panel. The thermokarst lake is indicated as (TK). Shown are isotopic values for late summer lakewater DOM (grey diamonds) floodwater DOM (black diamonds), submergent macrophytes (open circles), epiphytes (open triangles) and seston (open squares). In lakes that remained connected to the river channel post flood (panels a and b), isotopic values for riverwater DOM are shown (black hatched diamonds). Isotopic values for emergent macrophytes are shown for the lakes in which they occur (crosses; panels a, d, e), but were not included in our mixing models.

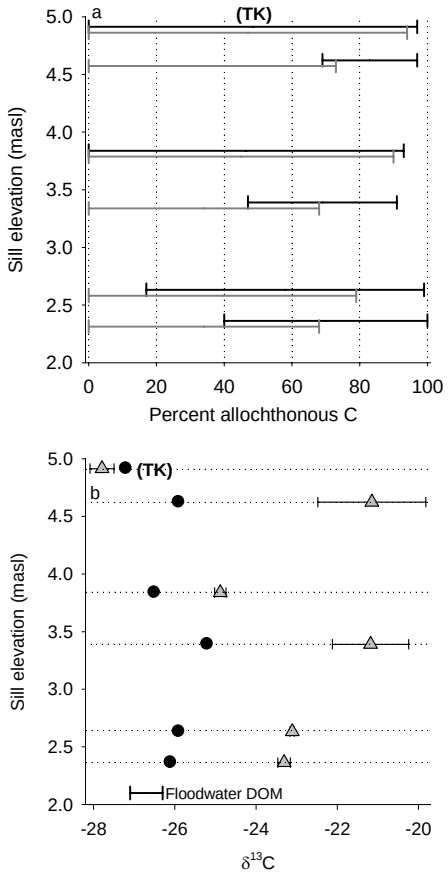

Figure 3.9: Results of mixing models to determine the percent contribution of allochthonous C to lakewater DOM (black), and bacterial biomass (grey), in a series of Mackenzie Delta lakes. Panel (a) shows the range of possible solutions to the mixing models. Panel (b) compares measured $\delta^{13}C$ values for DOM (black circles) and bacterial biomass (grey diamonds) in each lake. The thermokarst lake is labeled in both panels as (TK). The range of measured floodwater $\delta^{13}C$ values is indicated with a solid bar in panel (b).

CHAPTER 4 DIVERGENT BACTERIAL METABOLISM ON DIVERSE DOM SUBSTRATES IN LAKES OF THE MACKENZIE DELTA, WESTERN CANADIAN ARCTIC[1]

[1] A version of this chapter is in preparation for submission as:
Tank, SE, and LFW Lesack. Divergent bacterial metabolism on diverse DOM substrates in lakes of the Mackenzie Delta, western Canadian Arctic. *In preparation* for submission to Freshwater Biology.

4.1 Abstract

Dissolved organic matter (DOM) from different sources is known to differ in quality as a substrate for bacterial metabolism. We investigated bacterial production (BP), respiration (BR) and growth efficiency (BGE; the proportion of C assimilated by bacteria that is apportioned to growth) on 3 DOM sources that are prevalent in Mackenzie Delta lakes: DOM derived from (1) riverwater, (2) macrophytes, and (3) permafrost melting at the margins of lakes (thermokarst). BP was rapid on macrophyte-derived substrates, while BR was proportionately low, leading to extremely high BGE on macrophytic DOM. In contrast, BP and BGE on riverwater DOM, and particularly on permafrost-derived substrates were low. These results suggest that macrophyte-derived DOM becomes available for consumption by higher trophic levels as a result of bacterial growth, but participates little in overall lake respiration. In contrast, DOM derived from thermokarst appears largely to be respired, and therefore play an important role in pushing thermokarst-affected lakes towards the emission of CO_2 to the atmosphere. As such, the functioning of bacterial communities in Delta lakes may switch between functioning as a biomass link between DOM and higher trophic levels, and a respiratory sink, depending on the origin of the DOM substrate. Although previous studies have extensively studied the dichotomy between watershed- and algal-derived DOM in temperate lakes, the importance of macrophytic DOM is less well understood, and the consequence of thermokarst-induced subsidies of DOM to aquatic ecosystems is largely unknown. Understanding the fate of the divergent sources DOM to Mackenzie Delta lakes provides critical insight into the future functioning of these northern waterbodies, where climate-induced changes in DOM quality may be great.

4.2 Introduction

Dissolved organic carbon (DOC) typically comprises the largest pool of organic C in the water column of lakes (Prairie 2008), and forms the primary substrate for bacterial metabolism. Bacterial uptake of DOC and its associated organic constituents (i.e., dissolved organic matter; DOM) results in one of two outcomes: on the one hand, bacterial respiration (BR) of the ingested DOC as CO_2; or on the other, the production of new biomass (bacterial production; BP). Because the CO_2 produced by BR is either lost from the system or recycled back into new photosynthesis, while the biomass created through BP becomes available for consumption by higher trophic levels within the microbial web, the balance between these 2 processes could critically affect energy flow and the CO_2 balance of aquatic ecosystems.

The relative proportion of C assimilated by bacteria that is apportioned to growth is known as bacterial growth efficiency, or yield (BGE = BP/(BP+BR); del Giorgio and Cole 1998). One of the factors known to regulate BGE is substrate quality, which is often determined by the source of DOC. The majority of DOC in most temperate lakes is derived from external (allochthonous) sources, and flows in to lakes from the surrounding catchment (e.g., Kritzberg et al. 2004). Although allochthonous DOC is known to support BP in temperate regions (e.g., Coffin and Cifuentes 1999, Kritzberg et al. 2006), recent work suggests that the majority of this C is respired by bacteria (Kritzberg et al. 2005, Cole et al. 2006). In contrast, growth efficiencies, BP and bacterial abundance (BA) are all known to increase with increasing algal abundance (as inferred from Chl *a*; Bird and Kalff 1984, del Giorgio and Cole 1998, Biddanda et al. 2001), presumably because the DOC that results as a byproduct of within-lake (autochthonous) photosynthesis supports greater bacterial growth. At the same time, algal exudates have directly been shown to be a high-quality substrate for both overall bacterial metabolism and BP (Chen and Wangersky 1996, Pérez and Sommaruga 2006).

Given the clear disparity between the effects of watershed-derived allochthonous, and algal-derived autochthonous, DOC on C cycling in lakes, it seems likely that alternate forms of allochthonous and autochthonous C could similarly play an important role in lake metabolism where they occur. For example, macrophytes have been proposed to be a major contributor to aquatic DOC pools, particularly when they occur in dense stands (Wetzel 2001, Bertilsson and Jones 2003). Previous work in macrophyte-rich Delta lakes (Chapter 3) has shown that bacteria preferentially incorporate DOC from macrophytes, suggesting that this C source is quickly cycled in these lakes. Although several studies have determined that BP can be rapid on macrophyte leachates (Findlay et al. 1986, Mann and Wetzel 1996), the manner in which naturally-occurring macrophytic DOC drives BP, BR, and BGE is lakes is poorly understood. Additionally, in northern ecosystems, permafrost melting (thermokarst) slumps at the edge of lakes expose long-frozen soils to within-lake processes which could also provide a significant, alternate source of DOC to aquatic environments (Chapters 2 and 3). Despite the increasing importance of thermokarst in arctic regions, the role of this allochthonous DOC source in lakes has not been considered.

In this study, we endeavoured to determine how DOC derived from macrophytes and thermokarst drive pelagic BP, BR, and BGE, and thus the functioning of this organic matter in lake ecosystems. We undertook this work in lakes of the Mackenzie Delta, western Canadian Arctic, which occur in distinct classes that are clearly demarcated between (1) lakes with dense macrophyte stands, but typically little connection to their surrounding watershed; (2) lakes that are frequently flushed by riverwater from the Mackenzie River, and (3) lakes that are strongly affected by thermokarst, but receive little contemporary allochthonous DOC (i.e., riverwater-derived). We hypothesized that older sources of organic C, such as that from thermokarst, and also riverwater, are poorer substrates for bacterial metabolism than DOC derived from newer autochthonous (macrophytic) production. We undertook this work by conducting surveys of bacterial

metabolism across the gradient of Delta lake types, in concert with a series of experimental incubations that measured bacterial metabolism on DOM substrates common to Delta lakes. We show a clear divergence in the role of macrophytic and thermokarst-derived DOM in Mackenzie Delta lakes, with the former being quickly depleted from the overall DOM pool as a result of rapid bacterial metabolism, and the latter tending towards recalcitrance and bacterial processing that results in CO_2 emission. Throughout the remainder of this work, we refer to the combined, inseparable pool of DOM, which includes DOC, nutrients (i.e., dissolved organic nitrogen [DON] and phosphorus [DOP]), and other organic constituents.

4.3 Methods

4.3.1 Study site

The Mackenzie Delta contains approximately 45,000 lakes (Emmerton et al. 2007), and the pattern of flooding across lakes of this northern delta has created a complex freshwater landscape. Peak flooding occurs at river breakup, when ice-jam flood levels far surpass those that occur under open water flow. Lakes occur along a gradient that ranges from infrequently flooded (once every several years) perched lakes, to those that are connected to the river throughout the ice-free season via a narrow channel (Marsh and Hey 1989). Among other physico-chemical parameters, DOM concentration and composition changes dramatically across this lake elevation gradient (Gareis 2007, Chapter 3).

Low elevation, connected (CON) lakes are dominated by inputs of terrestrially-derived riverwater DOM (Chapter 3). Because suspended sediment loads remain high throughout the ice-free season in CON lakes, light penetration is low, limiting phytoplankton and macrophyte primary production (Squires et al. 2002, Squires and Lesack 2002). At the other extreme of the lake elevation gradient, higher-elevation, perched Delta lakes are often extremely macrophyte-rich (MAC). The short burst of floodwater provides one of the few sources of terrestrially-derived DOM to high elevation Delta lakes, because they have

extremely small catchments, are surrounded by permafrost which inhibits subsurface and groundwater flow (Marsh 1986), and receive little precipitation (Bigras 1986). The water column of these lakes quickly clears once they lose connection to the river, and this clarity, coupled with flood-delivered nutrients and the shallow depths of Delta lakes create conditions ideal for submergent macrophyte growth. Summer macrophyte biomasses can reach 350 g m^{-2} (dry weight; Squires et al. 2002), with volume-adjusted standing stock C loads 7-12 fold greater than the DOC pool (Chapter 3). In addition, a small subset of high-elevation lakes has been considerably deepened by the action of thermokarst (TK; slumping as a result of permafrost melting). TK lakes are characterized by visible slumps at the lake's edge, and mature, dead, trees rising vertically through the water column. Deep thermokarst lakes also have dense multi-year mats of the macroalgae *Chara* sp. along the lake bottom. Previous work characterizing the DOM composition of Delta lakes suggests that the DOM pool is overwhelmingly terrestrial in CON lakes, and is augmented by additional inputs of terrestrially-derived DOM in TK lakes. While the standing DOM pool in MAC lakes also appears to also be largely terrestrial in nature, bacteria from these lakes have a stable isotope signature that indicates preferential bacterial consumption of autochthonous DOM, suggesting that macrophytic DOM may largely be consumed before it accumulates in the overall pool (Chapter 3). We differentiate between DOM from CON lakes and riverwater (RIV), to acknowledge that within-lake processes may cause DOM from CON and RIV sources to differ in their characteristics, despite the fact that DOM from CON lakes is largely riverine in origin. We also differentiate between DOM from RIV sources, collected during the open water season, and that collected during the spring flood (FLOOD), because floodwater likely accumulates a greater proportion of Delta-origin terrigenous DOM, after being subject to the significant mechanical mixing that occurs during the flood.

4.3.2 Data collection and experimental design

In-situ *surveys*

To assess *in-situ* bacterial growth and metabolism in CON, MAC, and TK lakes, we surveyed 3 lakes of each class during the early (10 July) and mid- (30 July) summer of 2007 (Table 4.1). Lakewater samples were collected from directly below the lake surface and immediately returned to the laboratory and processed for BA, BP, and BR. Samples from each lake were filtered through a Whatman GF/D filter to remove bacterial grazers (2.7 μm nominal pore size; following Roland and Cole 1999, Maranger et al. 2005, Kritzberg et al. 2006). From each filtered sample, triplicate aliquots were removed for BA, and immediately preserved using glutaraldehyde (2.5%). Aliquots were also removed for analysis of BP, both immediately and after a 24 h incubation. The remaining water was distributed among 10 darkened glass biological oxygen demand (BOD) bottles for determination of BR, measured as decrease in dissolved oxygen (DO). Water was added to the bottles using a carbuoy with tubing attached to the bottom outflow to avoid turbulence while filling. Five BOD bottles were measured for DO immediately, and 5 were measured after a 24 h incubation. Incubated BP and BR samples were submerged to the neck in a darkened, covered water bath that was regulated to the average *in situ* temperature for the sampled lakes, using a circulating chiller pump. Duplicate, unfiltered samples from each lake were also preserved with glutaraldehyde (2.5%) for the assessment of raw BA. A portion of the unfiltered water was processed for later analysis of dissolved organic carbon (DOC), chlorophyll *a* (Chl *a*), total dissolved phosphorus and nitrogen (TDP, TDN), soluble reactive phosphorus (SRP), and dissolved inorganic nitrogen (i.e., NH_4^+ and NO_3^-), as described below. For our surveys, filtered BA samples were used for the calculation of per cell (specific) BP and BR (SBP, SBR), while raw samples were used to assess actual *in situ* abundances. BP samples from 0 h were used to assess *in situ* BP, while samples from both 0

and 24 h were used in the calculation of BGE. Methodological details for the analysis of BA, BP and BR are presented below.

Incubation experiments: overall setup

To further examine the effect of CON, MAC, and TK DOM on bacterial growth and metabolism, bacteria from Delta lakes were isolated and incubated with either (a) DOM obtained directly from CON, MAC, and TK lakes (CON_{DOM}, MAC_{DOM}, TK_{DOM}), or (b) DOM leached from macrophytes, or extracted from permafrost or near-surface soils, as described below (MAC_{leach}, $PERM_{leach}$, $SOIL_{leach}$). DOM obtained directly from floodwater ($FLOOD_{DOM}$) and riverwater (RIV_{DOM}) were also used as substrates during our incubations. Results of leachate incubations undertaken in 2007 and 2008, and lakewater DOM incubations undertaken in 2008 are presented below. We conducted our incubations using a modified version of del Giorgio et al. (2006), and illustrated in Fig. 4.1. Replicate experimental incubations were prepared as described below, and the resulting water was used to fill one acid washed amber glass bottle, and one acid washed cubitainer. The cubitainer was sealed with a silicone stopper pierced with a single line of PVC tubing that flowed to the glass bottle. The glass bottle was also sealed with a silicone stopper, which was pierced with 2 lines of tubing: one connected to the cubitainer, and one which was sealed with a tubing clamp and used as a sampling port. The glass bottle and cubitainer were sealed to exclude all air. Sampling from the port caused the cubitainer to collapse, such that repeated samples could be taken over time without exposing the incubated sample to outside air, allowing for repeated measurement of BR from the same experimental replicate. For all incubations, BR was calculated as loss of DO over the entire incubation period, and BGE was calculated using BP values that had been integrated across the various incubation periods. SBP and SBR were calculated as BP and BR per cell, respectively, using BA obtained from counts of the filtered bacterial inocula. SBP was calculated for each sampling point, using time-specific measures of BP and BA, while SBR was calculated using the overall measure of BR, and an

integrated measure of BA from across the duration of the experiment. Methodological details for the analysis of BA, BP and BR are presented below.

Incubation experiments: leachate DOC

Leachates from submerged macrophytes (*Potamogeton* sp.), near-surface soils, and permafrost soils were extracted in the laboratory. Macrophyte leachates were prepared following Findlay et al. (1992): *Potamogeton* sp. was collected from the MAC lake (Table 4.1) shortly after macrophyte emergence in early July, washed thoroughly to remove all epiphytes, and dried completely at 60°C. Dried macrophyte tissue was ground to a coarse powder, and allowed to leach into distilled, deionized water for 72 h. Soil and permafrost leachates were prepared following Teichreb (1999), using a soil core that was collected ~20 m from the shoreline of the TK lake (Table 4.1), and partitioned into active layer and permafrost horizons. DOM was extracted from soil by the addition of 0.1N NaOH. Extraction proceeded for 2 days, at which time the liquid was decanted, and pH normalized to lake levels by adding HCl. After extraction, all leachates were filtered (0.2 μm; Pall maxi culture capsule) and stored frozen until use.

For the 2007 incubation (19-21 July), all leachates were dialysed in distilled water (48 h) and lakewater (a further 24 h) directly preceding the incubation (SpectraPor cellulose ester membrane, 100 Da cutoff), to remove the salts that resulted from our soil extraction techniques, and to equalize inorganic nutrient concentrations to those found *in situ.* On the morning of the incubation, lakewater was collected from one of each of the lake types, and filtered (Whatman GF/D filter, 2.7 μm) to create a bacterial inoculum. Leachates were re-filtered (0.2 μm) immediately prior to the experimental set-up, and diluted with distilled water to mimic *in situ* DOC concentrations. 2L of bacterial inocula (CON_{bact}, MAC_{bact}, TK_{bact}) was added to 2L of leachate (MAC_{leach}, $PERM_{leach}$, $SOIL_{leach}$) or, for reference purposes, 2L of filtered riverwater (RIV_{DOM}). This resulted in 12 different inocula: DOM combinations. Each of the 12 treatments was mixed well, distributed between 2-1L glass bottles and 2-1L

cubitainers, and sealed to make 2 experimental replicates (Fig. 4.1). Replicates were incubated at the average *in situ* temperature for the sampled lakes, by submerging glass bottles and cubitainers to the neck in a darkened recirculating water bath regulated with a chiller pump. Initial samples were taken for BA and BP, which were also sampled after 1 and 2 days. DO (for respiration) was sampled at 0 and 2 days, at which time the experiment was dismantled. In addition, a portion of the water from each of the processed DOM sources, and each bacterial inocula, were processed for later analysis of DOC, Chl *a*, TDN and TDP.

Post-experimental counts to confirm that our filtration treatment removed all bacterial grazers revealed the presence of small heterotrophic nanoflagellates in our incubations, particularly towards the end of the experiments. Because of the resulting potential confounding effects on our measurements of bacterial metabolism, leachate incubations were repeated in 2008. The previously collected MAC and SOIL leachates, which had been stored continuously frozen, were thawed and dialyzed. SOIL leachates were dialyzed using a 1,000 Da membrane (SpectraPor cellulose ester), while MAC leachates were dialyzed using both 100 (SpectraPor cellulose ester) and 1,000 Da membranes (SpectraPor 7, regenerated cellulose), to account for the fact that the 100 Da treatment did not completely remove soluble reactive phosphorus (SRP) from the 2007 MAC_{leach} samples (see Results). The 2008 macrophyte leachates are referred to as $MAC+P_{leach}$ (100 Da dialysed), and $MAC-P_{leach}$ (1,000 Da dialysed). In all cases, leachates were dialyzed for 96 h in distilled, deionized water, 0.2 µm-filtered and diluted to mimic *in situ* DOC concentrations. Raw water was collected from the MAC and CON lake (Table 4.1) in early Aug., and this raw water was immediately shipped in chilled, darkened coolers to Simon Fraser University for processing and experimental setup. Bacterial inocula were created by filtering raw water through a Whatman GF/F (0.7 µm nominal pore size) filter, to ensure complete removal of all bacterial grazers. The two bacterial inocula (MAC_{bact} and

CON_{bact}) were combined with each of the 3 dialyzed DOM leachates ($MAC+P_{leach}$, $MAC-P_{leach}$, $SOIL_{leach}$). These 6 treatments were set up in duplicate, by combining 1.2 L of bacterial inocula with 2.8 L of DOC (a 30:70 dilution) and splitting the combined water equally between 2 1L glass bottles and 2 1L cubitainers (Fig. 4.1). Experimental replicates were incubated in an environmental chamber at *in-situ* temperatures, and sampled for BA, BP, and BR at day 0, 1, 2, and 5, at which time the incubation was dismantled. For each incubation, a portion of the water used for the leachate DOM substrates and bacterial inocula were processed for later analysis of DOC, Chl *a*, TDN and TDP.

Incubation experiments: lakewater DOM

Incubations with floodwater and lakewater DOM were undertaken in 2008, in concert with the leachate incubations. Samples for floodwater DOM ($FLOOD_{DOM}$) were collected from the river during the 2008 freshet, and samples for lakewater DOM were collected from CON (CON_{DOM}), MAC (MAC_{DOM}) and TK (TK_{DOM}) lakes in late July (Table 4.1). Both were immediately filtered (0.2 μm; Pall maxi culture capsule), stored at 4°C, and shipped to Simon Fraser University with the raw water samples described above. Raw water from the TK lake (Table 4.1) was additionally collected in early Aug. for this incubation. Bacterial inocula were prepared as described for the 2008 leachate experiments, and each of the 4 pre-filtered DOM substrates (CON_{DOM}, MAC_{DOM}, TK_{DOM}, $FLOOD_{DOM}$) were combined with CON_{bact} and MAC_{bact} inocula. For reference purposes, TK_{bact} was also combined with TK_{DOM}. These 9 treatments were set up in triplicate, by combining 1.8 L of bacterial inocula with 4.2 L of DOC (a 30:70 dilution) and splitting the combined water equally between 3 1L glass bottles and 3 1L cubitainers. Experimental replicates (Fig. 4.1) were incubated in an environmental chamber at *in-situ* temperatures, and sampled as described for the 2008 leachate experiments.

4.3.3 Laboratory analyses

Assessment of bacterial abundance and metabolism, and flagellate abundance

Preserved BA samples were stored in the dark at 4°C until counting, within 6 months of collection. To provide even sample dispersal once filtered, samples were amended with TWEEN-80 (10 ppm) and sonicated following Bae Yoon and Rosson (1990). Sonicated, DAPI-stained (10 mg L^{-1}) samples were filtered onto 0.22 µm black polycarbonate filters (GE Osmonics), and counted at 1000 x using a Zeiss Axioplan microscope fitted with a 50 W bulb (Osram HBO) and filters specific for the DAPI stain (Zeiss filter set 02). For the 2007 incubation experiments, DAPI-stained flagellates were also counted at 1000x on the above slides by scanning one complete length of the filtered area, and enumerating all observed flagellates. Slides prepared for the 2007 lake surveys, and 2008 incubations, were assessed for the presence of flagellates, but not counted.

BP was assessed by measuring ^{3}H leucine (Leu) incorporation, following Smith and Azam (1992). 20 µL of Leu (73.1-74.5 nM; saturating concentration) was added to sterile microcentrifuge tubes at ratios of hot (Amersham TRK 636, 158-161 Ci $mmol^{-1}$): cold (Sigma L8000) Leu ranging between 1:0.5 (2008) and 1:4 (2007). For the *in-situ* surveys, incubations were conducted with 4 replicates plus 1 blank from each lake. For the incubation experiments, incubations were conducted with 3 (leachate DOM) or 2 (lakewater DOM) replicates from each experimental replicate, plus 1 blank per DOM-bacteria combination. In 2007, samples and blanks were read on a Triathler portable scintillation counter (Hidex Oy, Finland), and corrected for quench by comparing select samples and known standards measured on the Triathler and on a Beckman Coulter LS6500 (Beckman Coulter Inc., Fullerton, CA). In 2008, samples and blanks were read on the Beckman Coulter LS6500, using internal quench corrections. In both years, samples were read within 24 h of incubation, using Amersham BCS scintillation cocktail. Dilution experiments were conducted to confirm the isotope dilution factor (Roland and Cole 1999), and BP (as g C L^{-1}

h^{-1}) was calculated by multiplying the Leu incorporation rate by the standard correction factors (Simon and Azam 1989). BR was measured as loss of DO in samples incubated in stoppered glass BOD bottles. 150 mL BOD bottles were used for the lake surveys, and 60 mL bottles were used for all incubation experiments. DO concentration was measured following Roland et al. (1999) and APHA (1989), and converted into CO_2 assuming a respiratory quotient of 1.

Assessment of Chl a, DOC, and dissolved nutrients

Water samples were analysed for DOC after filtration (0.22 µm Millipore GSWP), using a Shimadzu TOC-V_{csh} total organic carbon analyzer. Samples for Chl *a* were collected by filtration onto Whatman GF/C filters, and immediately frozen until the time of extraction. Filters were immersed in 90% ethanol following Nusch (1980), and allowed to extract for 24 h at 4°C. Chl *a* was determined spectrofluorometrically (Welschmeyer 1994). Samples for TDN and TDP were filtered through pre-combusted (4 h at 475°C) GF/F filters, stored at 4°C, and sent to the laboratory at the Freshwater Institute (Fisheries and Oceans Canada, Winnipeg, MB) for analyses.

4.3.4 Statistical analyses

In-situ *surveys*

2-way analysis of variance (ANOVA) was used to determine differences in bacterial abundances, growth, and metabolic activity (BA, BP, BR, SBP, SBR, and BGE), and water chemistry (DOC, Chl *a*, TDN and TDP) between the 3 lake types, with lake type (CON, TK, MAC) and sampling date (July 10, July 30) as factors. Data were checked for normality before our analysis was run. Because of the lack of independence between early- and mid-summer data, the lake type effect alone is presented in the results. When significant differences between lake type occurred, a post-hoc Tukey's HSD test was used to determine

where significant differences lay. Both ANOVA and post-hoc tests were performed using SYSTAT 12 (2007, SYSTAT Software, Inc.).

To explore how the multiple factors that differed between the 3 lake types might have affected bacterial populations in these lakes, we explored the relative importance of DOC, dissolved nutrients (TDN and TDP), and algal (as Chl *a*), macrophyte (as relative importance of submerged macrophytes among lakes) and thermokarst extent (as no or significant thermokarst; Chapter 2) as drivers of BA, BP, and BR. We did this with multiple linear regression, using Akaike's Information Criterion (AIC) to assess model fit (Burnham and Anderson 1998). We examined the relative fit of all linear combinations of these predictor variables to our bacterial data with AIC. Briefly, we used the corrected AIC_c as our model selection parameter, considered all models with an AIC_c difference (ΔAIC_c; the difference between the lowest AIC model and the model under consideration) ≤ 4.0 to be plausible fits to the data, and calculated the Akaike weight for each retained model. Using these weights, we calculated the model-averaged importance of each of our predictor variables, model-averaged regression coefficients, and used model-averaged variances to calculate confidence intervals for each regression coefficient (Burnham and Anderson 1998). The importance of each predictor variable ranges from 0 to 1, with 1 denoting inclusion in all plausible models; predictor variables with regression coefficients whose confidence intervals do not include 0 are considered to be significant. Data were checked for normality before inclusion in the model.

Incubation experiments

The results of our incubation experiments were analysed using ANOVA. For all experiments, 2-way ANOVAs were conducted, with bacterial inocula (CON_{bact}, MAC_{bact}, and/or TK_{bact}) and DOM source (lakewater and leachate DOM) as factors. Response variables with multiple measurements (BP, BA, SBP) were entered into the ANOVA analysis using values that were averaged across the experimental period, taking into account any

differences in period between samplings. Response variables for which an experiment-long response was calculated (BR, SBR, BGE) were entered into the ANOVA analysis without modification. For the 2007 leachate experiment, flagellate counts from the experiment's termination point, which represented peak flagellate numbers, were used as the response variable. When significant interaction effects were detected, the 2-way ANOVA analysis was followed by an analysis of the simple effects of DOM source at each level of bacterial inocula (Keppel 1982). When the test of simple effects was significant, simple contrasts were employed to determine where significant differences lay (Keppel 1982). Data were transformed using a Box-Cox transformation where necessary to achieve normality and heteroscedasticity.

4.4 Results

4.4.1 In-situ rates of bacterial metabolism across 3 classes of Delta lakes

Our 2007 survey measures of BA, BP, and BR showed clear, consistent differences between the 3 lake types, and were lowest in CON lakes, when compared to either MAC or TK lakes (Table 4.2, Fig. 4.2). Although BP was significantly greater in MAC lakes than in TK lakes, there was no difference between these 2 lake types in terms of BR or BA (Table 4.2, Fig. 4.2). Cell-specific measures of both BP and BR (SBP and SBR) showed trends that were identical to those for the community-wide measurements. Both of these measures were lower in CON lakes than in TK or MAC lakes, which did not differ from one another (Table 4.2, Fig. 4.2). In contrast BGE did not differ significantly across lake types.

At the time of our surveys, inorganic P in these lakes ranged from the detection limit to 2 μg L^{-1}, while inorganic N (NO_3 + NH_4) was always ≤ 10 μg L^{-1} (data not shown). Thus we used TDN and TDP (Fig. 4.3) as proxies for DON and DOP. Concentrations of TDN, TDP, and DOC across the 3 lake types largely followed the trends for bacterial metabolism (Table 4.2, Fig. 4.3). CON lakes consistently showed the lowest concentration of each of these water

chemistry parameters. TDN and DOC did not differ significantly between MAC and TK lakes, while TDP was significantly greater in MAC lakes than in TK lakes (Table 4.2, Fig. 4.3). In contrast, Chl *a* did not differ significantly among any of the lake types at the time of our surveys (Table 4.2, Fig. 4.3).

The results of our AIC analysis showed that TDP was consistently the most important factor for explaining patterns in bacterial metabolism and abundance across lake types. TDP was the only significant descriptor variable for BA (Table 4.3). Macrophyte density was also a significant, but secondary, descriptor variable for BP, while TDN was secondarily important for explaining patterns in BR (Table 4.3).

4.4.2 Bacterial response to incubation with DOM leachates

Bacteria incubated on leachate and riverwater DOM showed results that differed significantly among DOM type (Tables 4.4 and 4.5, Figs. 4.4 and 4.5). Similar to the survey results, BP, BA, SBP from the 2007 incubations were generally greatest on MAC_{leach}, compared to substrates associated with terrestrial-origin DOM ($SOIL_{leach}$, $PERM_{leach}$ and RIV_{DOM}). However, in contrast to the surveys, these bacterial attributes were almost always lowest on the substrate associated with thermokarst ($PERM_{leach}$). Specifically, BP and BA differed significantly between each of the substrate types, and were greatest on MAC_{leach}, followed by $SOIL_{leach}$, $PERM_{leach}$, and RIV_{DOM} (Table 4.4, Fig. 4.4). Results for SBP were similar, but varied slightly depending on the source of the inoculum: with all 3 inoculum types, SBP was higher on MAC_{leach} than on $SOIL_{leach}$, and SBP on both of these substrates was greater than for $PERM_{leach}$ or RIV_{DOM}. For bacteria from MAC lakes, there was no difference in SBP between $PERM_{leach}$ and RIV_{DOM}; for inocula from TK and CON lakes, SBP on $PERM_{leach}$ was greater than on RIV_{DOM} (Table 4.4, Fig. 4.4).

As a result of the inclusion of flagellates in our 2007 incubations, we report community respiration rates for these samples, and have not calculated BGE because of the

requirement for bacteria-specific measures of respiration. Similar to the bacterial attributed discussed above, community respiration was greater on MAC_{leach} than on any of the other DOM substrates (Table 4.4, Fig. 4.4). Amongst substrates of terrestrial origin, respiration was generally highest for $SOIL_{leach}$, and lowest for $PERM_{leach}$, although there were slight differences in this pattern depending on inocula origin (Table 4.4, Fig. 4.4). In contrast, flagellate abundances did not follow the above trends. Incubations conducted with CON_{bact} did not differ between substrates. For incubations with TK_{bact} and MAC_{bact}, flagellate numbers were lowest on MAC_{leach}. For MAC_{bact}, flagellate abundances did not differ between incubations using substrates of terrestrial origin, while for TK_{bact}, abundances were greatest on $SOIL_{leach}$ (Table 4.4, Fig. 4.4).

Nutrient analyses showed that the 2007 leachates had elevated nutrient levels in comparison with lakewater sources of DOM (Table 4.6). In particular, TDP was strikingly elevated in MAC_{leach}, and modestly elevated in $SOIL_{leach}$ and $PERM_{leach}$. Analysis of MAC_{leach} SRP concentrations showed that 54% of this TDP was reactive. Levels of TDN in our leachate substrates were about 2-fold those found in lakewater DOM.

The results of the 2008 leachate incubations were similar to those for 2007. Generally, bacterial abundance and metabolism was greatest on $MAC+P_{leach}$, secondary on $MAC-P_{leach}$, and lowest on $SOIL_{leach}$ (Table 4.5, Fig. 4.5). BGE did not differ between the $MAC+P_{leach}$ and $MAC-P_{leach}$ substrates, while for incubations using MAC_{bact}, BA, BR, and SBR did not differ between the $MAC-P_{leach}$ and $SOIL_{leach}$. For incubations with CON_{bact}, BA did not differ significantly between treatments. In all other cases (BP and SBP for all bacteria inocula, and BR and SBR for CON_{bact}) measurements on $MAC+P_{leach}$ were greater than for $MAC-P_{leach}$, which were greater than for $SOIL_{leach}$ (Table 4.5, Fig. 4.5).

Nutrient levels for 2008 leachates were again higher than those for lakewater sources of DOM (Table 4.6). The $MAC+P_{leach}$ substrate showed strikingly elevated TDP concentrations, which SRP analyses showed to be 34% reactive. While TDP was also clearly

elevated in both MAC-P_{leach} (~15-fold lakewater DOM levels) and $SOIL_{leach}$ (~5-fold) substrates, in both cases ≤1% of this TDP was reactive. Levels of TDN in our 2008 leachate substrates were either similar to ($SOIL_{leach}$) or approximately 2-fold those found in lakewater DOM (MAC+P_{leach}, MAC-P_{leach}; Table 4.6).

4.4.3 Bacterial response to incubation with lakewater DOM

Although more subtle, results from the 2008 lakewater incubations were similar to those for the leachate incubations. Generally, bacteria incubated on MAC_{DOM} showed the strongest metabolic response, followed by an intermediate response by bacteria incubated on CON_{DOM} and $FLOOD_{DOM}$. Similar to our leachate results, the weakest response was typically observed for bacteria incubated on TK_{DOM} (Table 4.7, Fig. 4.6). Specifically, incubations using CON_{bact} had BP rates that were highest on MAC_{DOM}, and greater on CON_{DOM} than on TK_{DOM}. For incubations using MAC_{bact}, BP was lowest on TK_{DOM}, and greater on MAC_{DOM} than $FLOOD_{DOM}$ (Table 4.7, Fig. 4.6). The response for BA was also dependant the source of the bacterial inocula: For incubations using MAC_{bact}, BA was significantly greater when incubated on CON_{DOM} than on any of the other DOM substrates. For incubations using CON_{bact}, BA was greatest when incubated on MAC_{DOM} and CON_{DOM}, intermediate for incubations with $FLOOD_{DOM}$, and lowest for incubations with TK_{DOM} (Table 4.7, Fig. 4.6). BR did not differ between the DOM substrate types (Table 4.7, Fig. 4.6). Measures of BGE largely reflected the trend seen for BP: for incubations with MAC_{bact}, BGE was greatest on MAC_{DOM} and CON_{DOM}, intermediate on $FLOOD_{DOM}$, and lowest on TK_{DOM}. For incubations with CON_{bact}, BGE was greater on MAC_{DOM} than any of the other substrates (Table 4.7, Fig. 4.6).

Cell-specific measures of BP and BR (SBP and SBR) largely mirrored the community-wide findings (Table 4.7, Fig. 4.6). SBP was greatest on MAC_{DOM} and lowest on TK_{DOM} for incubations with MAC_{bact}; for incubations with CON_{bact}, SBP was greater on MAC_{DOM} than the other 3 DOM substrates. Differences among treatments were muted for SBR. SBR was

greater on $FLOOD_{DOM}$ than CON_{DOM} for incubations with MAC_{bact}, and greater on TK_{DOM} than the other DOM substrates for incubations with MAC_{bact} (Table 4.7, Fig. 4.6). Dissolved nutrient concentrations were similar across the DOM sources.

4.5 Discussion

4.5.1 Effect of filtration treatments on bacteria and their grazers

GF/D filters (2.7 μm nominal pore size) have commonly been used separate bacteria from their grazers for incubation-type experiments (e.g., Roland and Cole 1999, Maranger et al. 2005, Kritzberg et al. 2006). In our 2007 experiments, flagellate abundances directly after our GF/D filtration treatment were extremely low (Fig. 4.4), and always <5% of typical *in situ* abundances. However, flagellate densities increased quickly while incubated with relatively labile bacterial substrates (leachates) in the absence of predators, and abundances increased substantially by the end of our 2-day incubation (Fig 4.4).

Although we also used a GF/D filter treatment for 24-h incubations carried out for BR and BGE during our 2007 *in situ* surveys, we are confident that the potential, low density presence of small flagellates in 24 h incubations that relied on GF/D filtration did not affect our measures of bacterial metabolism. For these experiments, scans of slides made with 0 h samples showed negligible densities of bacterial grazers. Although we did not assess bacterial grazer abundance after 24 h for the *in situ* incubations, flagellates counts after 24 h of incubation in our 2007 leachate incubations showed that densities were ~10% of *in situ* abundances (data not shown). In addition, flagellate densities observed for a 2007 incubation experiment where GF/D-based bacterial inocula were incubated with lakewater DOM were also low after 24 h, and always less than 20% of typical *in situ* values (experimental data not reported). We note that although flagellates typically range to 20 μm, only the smallest organisms were included in our filtered inocula. Additionally, even at natural abundances and size distributions BR is the dominant component of pelagic

respiration in oligotrophic lakes, and thus typically overwhelms respiration from other sources (e.g., Biddanda et al. 2001).

4.5.2 Diverse DOM substrates have contrasting effects on bacterial metabolism

Across the *in situ* surveys and incubation experiments, DOM derived from macrophyte tissue, and obtained from macrophyte-rich lakes, was consistently a highly labile bacterial substrate. This was particularly true for BP, which was always greatest in macrophyte-rich lakes, on macrophyte leachates, and on DOM collected from lakes with dense macrophyte stands. Although the response was more muted, BR was also elevated on macrophyte-derived DOM, and particularly on macrophyte leachates. While several other authors have shown both macrophyte leachates (Findlay et al. 1986, Mann and Wetzel 1996) and the presence of dense macrophyte beds (Rooney and Kalff 2003) to lead to high rates of BP, the effect of this DOM substrate on BR has been poorly examined.

Rapid rates of BP and, to a lesser extent, BR on macrophyte-derived substrates have 2 important implications with respect to C cycling and energy flow in these macrophyte-rich lakes. First, the overall speed of organic matter processing (i.e., BP + BR) is typically more rapid on macrophytic DOM than on other DOM substrates. At the rates of carbon uptake (BP + BR) that we observed, the MAC+P substrate used in our incubations would have been fully metabolized after just 9 d, while the MAC-P substrate would have been metabolized after 25. The rates of BP + BR measured on macrophyte-derived substrates could metabolize the equivalent of 15-80% of the macrophyte standing stock that occurs in macrophyte-rich Delta lakes (Table 4.1, Squires et al. 2002), over a typical 2-month arctic summer, based on rates of C uptake ranging from those observed for bacteria incubated on MAC_{DOM}, to those incubated on $MAC+P_{leach}$. In practice, the *in situ* rate of bacterial C uptake on macrophyte-derived substrates is likely to lie somewhere between these two extremes. The MAC_{DOM} substrate represents a naturally occurring DOM pool composed of both

macrophytic and terrestrial-origin substrates (Chapter 3), while the lag time between MAC_{DOM} collection and filtration likely resulted in the loss of the most highly labile substrates in the DOM pool, which are generally broken down on the time scale of minutes to days (Carlson 2002). At the other extreme, our MAC+P substrate is composed of DOM released within the first 96 h of leaching, and thus represents the most labile fraction of DOM that might result from macrophyte productivity. This potential range of assimilation of macrophytic C by bacterioplankton suggests that while pelagic bacteria are quickly able to take up substrates of macrophytic origin, uptake of macrophytic C by epipelic and epiphytic bacteria, and burial in the sediments, are also likely to play an important role in the fate of this C in Delta lakes.

Secondly, the modest increase in BR relative to BP led, generally, to significantly higher growth efficiencies on macrophyte-derived DOM. This occurred in lakewater and leachate incubations, but was not observed during our *in situ* surveys. BGE measured on macrophyte leachates was extremely high (65-75%), indicating that the vast majority of C processed was funneled towards biomass production, rather than respiration. This level of BGE is at the high end of the range observed in nature, but within good range of BGE predicted from the concurrently high levels of BP on macrophyte leachates, particularly given the large degree of scatter observed in the BP: BGE relationship (del Giorgio and Cole 1998). Such high growth efficiencies indicate that macrophyte-derived DOM may play a very different role in lakes than that currently understood for the more commonly studied terrestrially-derived DOM: while allochthonous C is largely respired and generally acts to push freshwater ecosystems towards net heterotrophy (Hanson et al. 2004, Cole et al. 2006), little macrophytic DOM appears to be mineralized to CO_2. At the same time, high rates of BP allow this organic matter to be available for higher trophic levels within the microbial web. The role of bacteria in facilitating the transfer of DOM through the microbial

food web may be particularly critical for macrophytes, because intact macrophyte tissue is generally considered to be unavailable to lower food web consumers (e.g., Kalff 2002).

In contrast to the striking results observed for BGE and the overall rate of organic matter processing, the effect of macrophytic DOM on BA was relatively muted. This result may reflect changes in bacterial cell size, rather than number, in response to this higher quality substrate. Although we did not take specific measurements, bacterial cells were visibly larger when incubated on macrophyte leachates. This increased cell size may also account for the poor growth of flagellate population numbers on bacteria reared on macrophyte leachates (Fig. 4.4): the small flagellates able to pass through our filter treatment may not have been able to consume the large cells that proliferated on the MAC_{leach} substrate. As such, the low flagellate numbers observed in our MAC_{leach} treatments do not necessarily imply that these bacteria are a poor food source to the microbial foodweb. The larger flagellates and ciliates that would co-occur with bacteria under natural conditions could likely consume these cells.

Although we cannot specifically attribute the elevated TDP observed in macrophyte-rich lakes to the presence of macrophytes, the relatively high P concentrations in these lakes was a key driver of BP, BR, and BGE during our *in situ* surveys. Macrophyte leachates also had extremely high levels of TDP, and C:P ratios well below those normally observed *in situ* for bacterial cells (<175; Hochstädter 2000). Although a significant portion of the P in the 2007 MAC_{leach} and 2008 $MAC+P_{leach}$ substrates was reactive, this was not the case for $MAC\text{-}P_{leach}$ DOM. BP and BGE were also extremely high on $MAC\text{-}P_{leach}$, suggesting that the composition of the organic fraction of macrophyte leachates plays an important role in bacterially-mediated C cycling. In contrast, the C:P ratios of non-macrophytic DOM substrates indicate that P was universally limiting. Other authors have shown that P, rather than DOC, limits bacterial growth and growth efficiency in natural waters (Smith and Prairie 2004). Because macrophytes preferentially obtain nutrients from the sediments in

nutrient-poor waters (Carignan 1982), the dense macrophyte stands in Delta lakes may allow limiting nutrients sequestered in the sediments to be released into the pelagic zone.

Results from our measures of bacterial metabolism on DOM from thermokarst-associated sources were conflicting, both across years, and between survey and experimental results. For lakewater results, bacterial metabolism on DOM from TK lakes showed striking differences between the 2007 *in situ* surveys, and the 2008 lakewater DOM incubation experiments. While the 2007 *in situ* surveys showed rates of bacterial metabolism in TK lakes equivalent, or slightly less than, those seen in macrophyte-rich lakes, measures of bacterial metabolism on TK_{DOM} in the 2008 incubations were universally amongst the lowest of the DOM substrates that we measured. It seems unlikely that the methodological differences inherent in these 2 techniques could account for this clear disparity. Measurements of BP on the raw TK water collected to create the 2008 bacterial inocula were also extremely low: 36% of that measured for the MAC lake, and 39% of that measured for the CON lake (data not shown). During our incubation, TK bacterial inocula incubated with TK_{DOM} also resulted in extremely low bacterial metabolism (Fig. 4.6). Thus, it seems more likely that substrate quality differed between the dates sampled. This may have been caused by differences in water column P concentration, the effects of Charophytes on the DOM pool (both described below), or episodic thermokarst slumping events that cause the release or thermokarst-associated DOM into the water column.

In 2007, P was high in TK lakes compared to other lake types, and was an important determinant of rates of bacterial metabolism. The high concentrations of CH_4 known to occur in these lakes (Chapter 2) suggest anoxic sediments, and thus the potential for substantial within-lake P regeneration. Temporal differences in mixing or the extent of anoxia might lead to differences in water column P levels, and thus the substrate quality available for bacteria. The presence of dense beds of the macroalgae *Chara* sp. in TK lakes may also affect DOM quality. Unlike vascular plants, Charophytes do not posses true roots

or transport tissues. However, Charophyte rhizoids can take up nutrients from the sediments, which can be further transported to above-ground biomass through mechanisms involving cell-to-cell contact (Kufel and Kufel 2002). The degree to which this relatively energy intensive nutrient uptake and transport occurs is unclear (Box 1986, Andrews 1987). Because Charophytes undertake multi-year growth (clearly evident in Delta lakes) and break down slowly, they have been suggested to act as a nutrient sink in freshwater systems (Kufel and Kufel 2002, Rodrigo et al. 2007). However, *Chara* sp. tissue is relatively nutrient rich (Kufel and Kufel 2002), and lacks the structural tissue characteristic of vascular plants, suggesting that *Chara*-derived DOM could be relatively labile. If the Charophytes common to Delta TK lakes obtain a significant portion of their nutrients from the sediments they may, like the vascular macrophytes common to other Delta lakes, act as a nutrient shunt between sediments and the water column.

In contrast to our results for lakewater DOM, bacterial metabolism on leachates associated with thermokarst activity was consistently low. Thermokarst causes both permafrost and active layer soils to become exposed to within-lake and below lakebed bacterial activity, due to thawing for the former, and slumping into the lake for the latter. Other sources of organic material that we did not quantify, such as terrestrial vegetation, also become submerged below the water surface as a result of thermokarst-associated slumping. As might be expected given the greater age of these soils, $PERM_{leach}$ was consistently a poorer bacterial substrate than $SOIL_{leach}$ across all of the bacterial attributes that we measured. This result carried through to flagellate populations, with $SOIL_{leach}$, in general, supporting greater numbers of flagellates in our 2007 incubations. Although the organic C contained in permafrost soils has been characterized as 'labile' because it does decompose once thawed (Zimov et al. 2006), it seems clear that within lakes, permafrost-derived DOM will likely turn over slowly compared to other DOM pools. Additionally, despite promoting greater rates of bacterial metabolism than $PERM_{leach}$, bacteria incubated

on $SOIL_{leach}$ displayed relatively low levels of BP, BR, and BGE when compared to known ranges of bacterial metabolic rates (del Giorgio and Cole 1999). This is consistent with previous studies that have also shown low BP on DOM leached from soil (Pérez and Sommaruga 2006). DOC is known to accumulate in Delta TK lakes between flushing events (Chapter 3), suggesting that DOM in these lakes is decomposed relatively slowly. Thus, despite the results of our 2007 surveys, the balance of evidence suggests that the DOM resulting from thermokarst processes is a relatively poor bacterial substrate. The DOM substrates that we examined which represent contemporary sources of terrigenous DOM for aquatic bacteria in addition to $SOIL_{leach}$ – RIV_{DOM} and $FLOOD_{DOM}$ – also displayed the established hallmarks of allochthonous DOM: a low rate of BP overall, and relative to BR (i.e., BGE). This suggests that, in general, DOM of terrigenous origin plays a similar role in these northern lakes as it does in more commonly studied, southern systems.

The Mackenzie Delta contains tens of thousands of lakes tightly packed across the Delta landscape (Emmerton et al. 2007). This work indicates that despite their close proximity, the functioning of neighbouring lakes can vary significantly, and that this variation is driven by differences in DOM source to the within-lake pool. At one extreme, DOM released as a result of macrophyte productivity is quickly taken up by bacteria, and thus appears to have an extremely short residence time in Delta lakes (see also Chapter 3). As such, this organic matter is likely to play an insignificant role in many of the physico-chemical processes in which DOM is known to participate, such as binding metals and nutrients, or contributing to photochemical reactions in the water column (e.g., Prairie 2008). However, because growth efficiencies are high on this substrate, bacteria consuming macrophytic DOM may be able to act as a biomass shunt, allowing otherwise unavailable DOM to be passed to higher trophic levels. In the phytoplankton-poor, macrophyte-rich lakes of the Mackenzie Delta, the importance of bacteria as a biomass shunt may be particularly notable. Finally, because macrophytic DOM is generated within the lake and

supports low rates of respiration, its production will not cause the CO_2 efflux commonly observed in the world's lakes (e.g., Prairie 2008).

At the other extreme, DOM entering Delta lakes as a result of permafrost slumping is slowly broken down, which appears to lead to a relatively long residence time of this organic matter in thermokarst-affected lakes (Chapter 3). At the same time, low growth efficiencies on this substrate indicate that only a small proportion of this DOM can become available for further consumption within the microbial web, and thus the role of bacteria in thermokarst lakes switches to become one of largely carbon and nutrient remineralizers. Because respiration rates are high on thermokarst-derived substrates, permafrost melting can augment within-lake CO_2 production, and bacteria in thermokarst lakes become key players in the transformation of previously fixed organic matter to CO_2 that is released to the atmosphere (Chapter 2). As such, the increase in permafrost melting predicted across the circumpolar north could lead to significant changes in lakes that become affected by thermokarst, increasing both their efflux of CO_2 to the overlying atmosphere, and concentrations of biologically recalcitrant DOM in the within-lake pool.

4.6 References

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4.7 Tables

Table 4.1: Select physico-chemical characteristics of the macrophyte-rich (MAC), thermokarst affected (TK), and connected (CON) lakes surveyed in 2007. Asterisks indicate those lakes that were sampled for the 2007 and 2008 incubation experiments. In TK lakes, macrophyte biomass is almost entirely composed of dense, multi-year mats of the macroalgae *Chara* sp., which were classified as macrophytes at the time of data collection[a]. Macrophyte biomass in other (CON, MAC) lakes is almost entirely composed of *Potamogeton* sp. nd=not determined.

Lake number	Lake type	Closure day (2007)[b]	Macrophyte biomass (g m^{-2})[a]	Thermokarst extent (%)[c]
129	CON *	01 Dec	15.9	0
80	CON	09 Sept	7.3	0
79a	CON	09 Sept	Nd	0
520	TK *	03 June	2446 (multi-year)	100
181	TK	02 June	453.4 (multi-year)	100
143	TK	02 June	280.2 (multi-year)	75
56	MAC *	04 June	87.9	0
278	MAC	09 June	359.0	0
280	MAC	12 June	89.0	0

[a]Macrophyte biomass data from Squires et al. (2002).
[b]Closure day represents the day on which the lake first became isolated from its proximate river channel. For CON lakes, minor fluctuations in water levels may have caused subsequent loss of closure.
[c]As calculated in Chapter 2.

Table 4.2: Results of 2-way ANOVA comparing measures of bacterial growth and metabolism (top panel) and physico-chemical characteristics (bottom panel) between season (early- or mid-summer) and across the 3 lake types (connected [CON], thermokarst [TK], and macrophyte-rich [MAC]) during the in-situ lake surveys. Significant results are highlighted in bold. Post-hoc contrasts using the Tukey's HSD test are shown for comparisons between different lake types. Contrast results are shown with increasing average values from left to right. Because of the lack of independence between early- and mid-summer data, the main effect of lake type along is presented.

Parameter	df	*F*	p	Contrasts
BP	2	32.656	**<.0001**	CON < TK < MAC
BR		17.154	**0.0003**	CON < TK = MAC
BA		11.693	**0.0015**	CON < TK = MAC
SBP		9.528	**0.0033**	CON < TK = MAC
SBR		9.645	**0.0032**	CON < MAC = TK
BGE		1.759	0.2327	
TDN		29.512	**<.0001**	CON < MAC = TK
TDP		24.636	**<.0001**	CON < TK < MAC
DOC		18.776	**0.0002**	CON < MAC = TK
Chl a		0.905	0.4305	

Table 4.3: Results of Akaike's Information Criteria (AIC) model selection analysis, showing model averaged importance, from 0 to 1, of each predictor variable for explaining trends in bacterial metabolism. Variables with regression coefficients significant at α=0.05 (α=0.10) are given in bold (italics). The sign of significant regression coefficients is given in brackets.

Predictor variable	Parameter		
	BP	BR	BN
TDN	0.257	*0.230 (+)*	0.077
TDP	**0.593 (+)**	**0.761 (+)**	**0.915 (+)**
DOC	0.087	0.153	0.077
Chl *a*	0.071	0.051	0.164
Thermokarst	0.318	0.276	0.209
Macrophyte biomass	**0.586 (+)**	0.206	0.160

Table 4.4: Results of 2-way ANOVAs for the 2007 leachate experiments, with source of bacterial inocula and DOM as factors. Shown are results for bacterial production (BP), abundance (BA) and specific bacterial production (SBP), and community respiration rates and flagellate abundances. Response variables with multiple measures across time (BP, BA, SBP) were averaged before analysis, as outlined in Methods. Significant results are highlighted in bold. Where the interaction effect was not significant contrasts for the overall 2-way ANOVA were conducted using the Tukey's HSD test. When the interaction effect was significant, simple effects were tested, and significant simple effects were followed by simple contrasts. Contrast results are shown with increasing average values from left to right, and indicate riverwater DOM (RV), permafrost leachate (PR), soil leachate (SL), and macrophyte leachates (MC). Substrate types that share a common underscore are not significantly different from one another. Shaded areas indicate inappropriate effect tests and contrasts.

Source	*df*	BP		BA		Community respiration		SBP		Flagellates	
Main effects		*F*	p	*F*	p	*F*	P	*F*	p	*F*	p
Bacteria	2	9.649	0.003	192.165	**<.001**	174.971	**<.001**	13.997	**<.001**	245.180	**<.001**
DOM	3	2651.092	**<.001**	573.261	**<.001**	1670.199	**<.001**	955.860	**<.001**	40.097	**<.001**
Bacteria x DOM	6	49.180	**<.001**[a]	2.034	0.139	23.473	**<.001**	26.343	**<.001**	10.656	**<.001**
Error	12										
Contrasts		RV PR SL MC		RV PR SL MC							
Simple effects											
CON_{bact}	3					780.328	**<.001**	454.057	**<.001**	3.295	0.058
TK_{bact}	3					476.354	**<.001**	297.392	**<.001**	40.220	**<.001**
MAC_{bact}	3					461.684	**<.001**	257.708	**<.001**	17.898	**<.001**
Error	12										
Contrasts											
CON_{bact}						PR RV SL MC		RV PR SL MC		NSD	
TK_{bact}						PR RV SL MC		RV PR SL MC		MC RV PR SL	
MAC_{bact}						PR RV SL MC		RV PR SL MC		MC RV PR SL	

[a]Simple effects and contrasts produced identical results to contrasts for the main effects.

Table 4.5: Results of 2-way ANOVAs for the 2008 leachate experiments, with source of bacterial inocula and DOM leachate type as factors. Shown are results for bacterial production (BP), abundance (BA) and respiration (BR), specific bacterial production (SBP) and respiration (SBR), and bacterial growth efficiency (BGE). Response variables with multiple measures across time (BP, BA, SBP) were averaged before analysis, as outlined in Methods. Significant results are highlighted in bold. Where the interaction effect was not significant contrasts for the overall 2-way ANOVA were conducted using the Tukey's HSD test. When the interaction effect was significant, simple effects were tested, and significant simple effects were followed by simple contrasts. Contrast results are shown with increasing average values from left to right, and indicate soil leachates (SL), macrophyte-P leachates (M-P), and macrophyte+P leachates (M+P). Substrate types that share a common underscore are not significantly different from one another. Shaded areas indicate inappropriate effect tests and contrasts.

Source	*df*	BP		BA		BR		SBP		SBR		BGE	
Main effects		*F*	P	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p
Bacteria	1	3.608	0.106	0.037	0.854	1.226	0.319	3.350	0.117	2.752	0.158	1.688	0.251
DOM	2	1138.734	**<.001**	17.722	**0.003**	360.673	**<.001**	1447.297	**<.001**	232.053	**<.001**	27.317	**0.002**
Bacteria x DOM	2	7.387	0.024[a]	6.477	**0.032**	17.350	**0.006**	4.499	0.064	6.096	**0.046**	3.798	0.099
Error	6												
Contrasts		SL M-P M+P						SL M-P M+P				SL M-P M+P	
Simple effects													
CON_{bact}	2			4.567	0.062	14.584	**0.008**			108.333	**<.001**		
MAC_{bact}	2			19.620	**0.002**	238.380	**<.001**			142.083	**<.001**		
Error	6												
Contrasts													
CON_{bact}				NSD		SL M-P M+P				SL M-P M+P			
MAC_{bact}				SL M-P M+P		SL M-P M+P				SL M-P M+P			

[a]Simple effects and contrasts produced identical results to contrasts for the main effects.

Table 4.6: Dissolved nutrient and chlorophyll *a* (Chl *a*) concentrations for DOM substrates (top panel) and bacterial inocula (bottom panel) used in the 2007 and 2008 incubation experiments. Samples for nutrient concentrations in inocula water were GF/F filtered before analysis; samples for Chl *a* in inocula water (2007) were collected by filtering raw water using a GF/C filter.

	TDN ($\mu g\ L^{-1}$)	TDP ($\mu g\ L^{-1}$)	DOC ($\mu g\ L^{-1}$)	C:N (molar)	C:P (molar)	Chl *a*[a] ($\mu g\ L^{-1}$)
2007 Incubations (DOM source)						
Riverwater DOM	256	5	6662	30.35	3436	--
Permafrost leachate	555	31	6300	13.24	524.0	--
Soil leachate	768	24	7881	11.97	846.8	--
Macrophyte leachate	879	1855	3548	4.71	4.93	--
2008 Incubations (DOM source)						
Soil leachate	396	111	9224	27.16	214.30	--
Macrophyte leachate (+P)	874	3378	6529	8.71	4.98	--
Macrophyte leachate (-P)	860	378	9884	13.40	67.43	--
Thermokarst DOM	466	20	9338	23.37	1204.04	--
Floodwater DOM	425	28	9510	26.10	875.87	--
Connected DOM	277	20	7597	31.98	979.56	--
Macrophyte-rich DOM	393	26	9213	27.34	913.79	--
2007 Incubations (bacterial inocula)						
Thermokarst	465	6	8994	22.55	3865	1.05
Connected	427	8	8336	22.77	2687	1.77
Macrophyte-rich	362	6	7667	24.70	3295	0.80
2008 Incubations (bacterial inocula)						
Thermokarst	503	31	9423	21.85	783.87	--
Connected	290	24	7514	30.22	807.38	--
Macrophyte-rich	423	32	9040	24.92	728.51	--

[a] Chlorophyll *a* concentrations for lakewater collected to prepare bacterial inocula were analysed for 2007 samples only.

Table 4.7: Results of 2-way ANOVAs for the 2008 lakewater DOM experiments, with source of bacterial inocula and DOM source as factors. Shown are results for bacterial production (BP), abundance (BA) and respiration (BR), specific bacterial production (SBP) and respiration (SBR), and bacterial growth efficiency (BGE). Response variables with multiple measures across time (BP, BA, SBP) were averaged before analysis, as outlined in Methods. Significant results are highlighted in bold. Where the interaction effect was not significant contrasts for the overall 2-way ANOVA were conducted using the Tukey's HSD test. When the interaction effect was significant, simple effects were tested, and significant simple effects were followed by simple contrasts. Contrast results are shown with increasing average values from left to right, and indicate DOM from thermokarst (TK), connected (CN) and macrophyte-rich (MC) lakes, and floodwater (FL). Substrate types that share a common underscore are not significantly different from one another. Shaded areas indicate inappropriate effect tests and contrasts.

Source	*df*	BP	BA	BR	SBP	SBR	BGE
Main effects							
Bacteria	1	25.962 **<.001**	23.981 **<.001**	0.367 0.554	32.420 **<.001**	0.086 0.773	39.492 **<.001**
DOM	3	89.619 **<.001**	125.417 **<.001**	1.202 0.345	75.406 **<.001**	3.961 **0.031**	82.205 **<.001**
Bacteria x DOM	3	10.701 **0.001**	83.528 **<.001**	2.503 0.102	15.280 **<.001**	5.104 **0.014**	14.304 **<.001**
Error	14						
Contrasts				NSD			
Simple effects							
CON_{bact}	3	34.400 **<.001**	9.709 **0.001**		27.607 **<.001**	4.348 **0.023**	33.253 **<.001**
MAC_{bact}	3	65.937 **<.001**	199.055 **<.001**		64.417 **<.001**	4.726 **0.018**	63.259 **<.001**
Error	14						
Contrasts							
CON_{bact}		TK FL CN MC	TK FL CN MC		TK FL CN MC	FL MC CN TK	TK FL CN MC
MAC_{bact}		TK FL CN MC	FL TK MC CN		TK FL CN MC	CN TK MC FL	TK FL CN MC

4.8 Figures

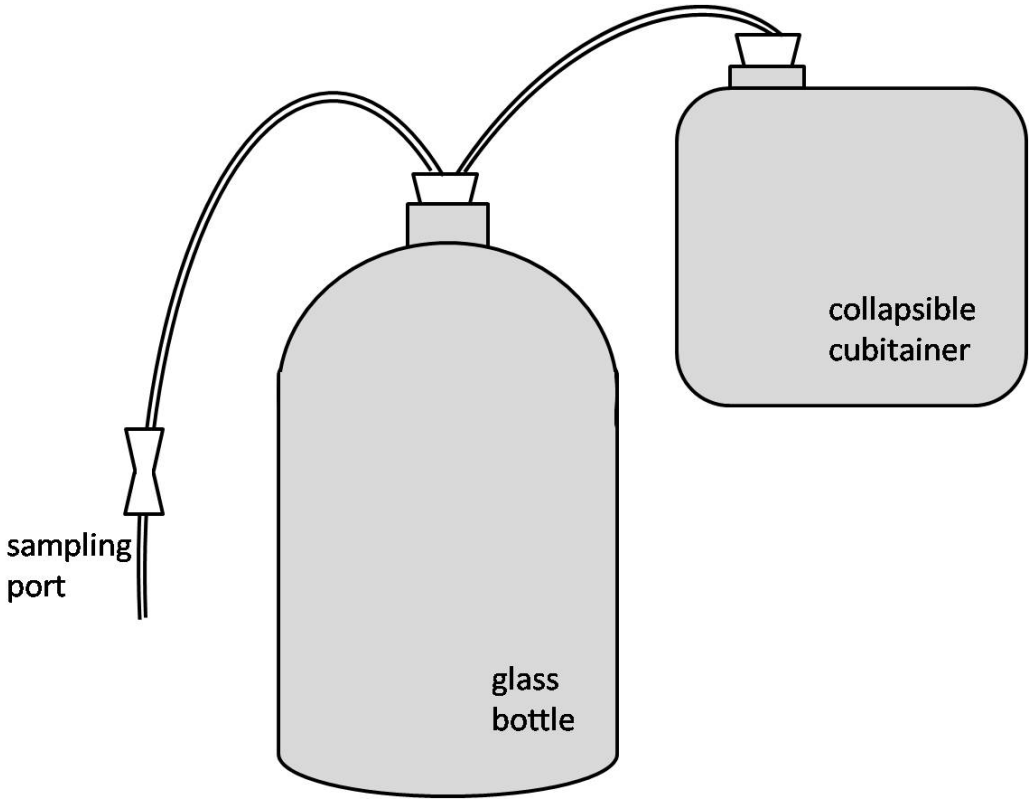

Figure 4.1: Sampling design of experimental replicates for the incubation experiments, as modified from del Giorgio et al. (2006). Both the glass bottle and cubitainer were sealed using a teflon stopper pierced to allow PVC tubing to fit through. The cubitainer was elevated above the glass bottle, and the sampling port remained clamped, except for during sampling. Removing water from the sampling port caused the cubitainer to collapse, allowing the system to remain air-tight during sampling.

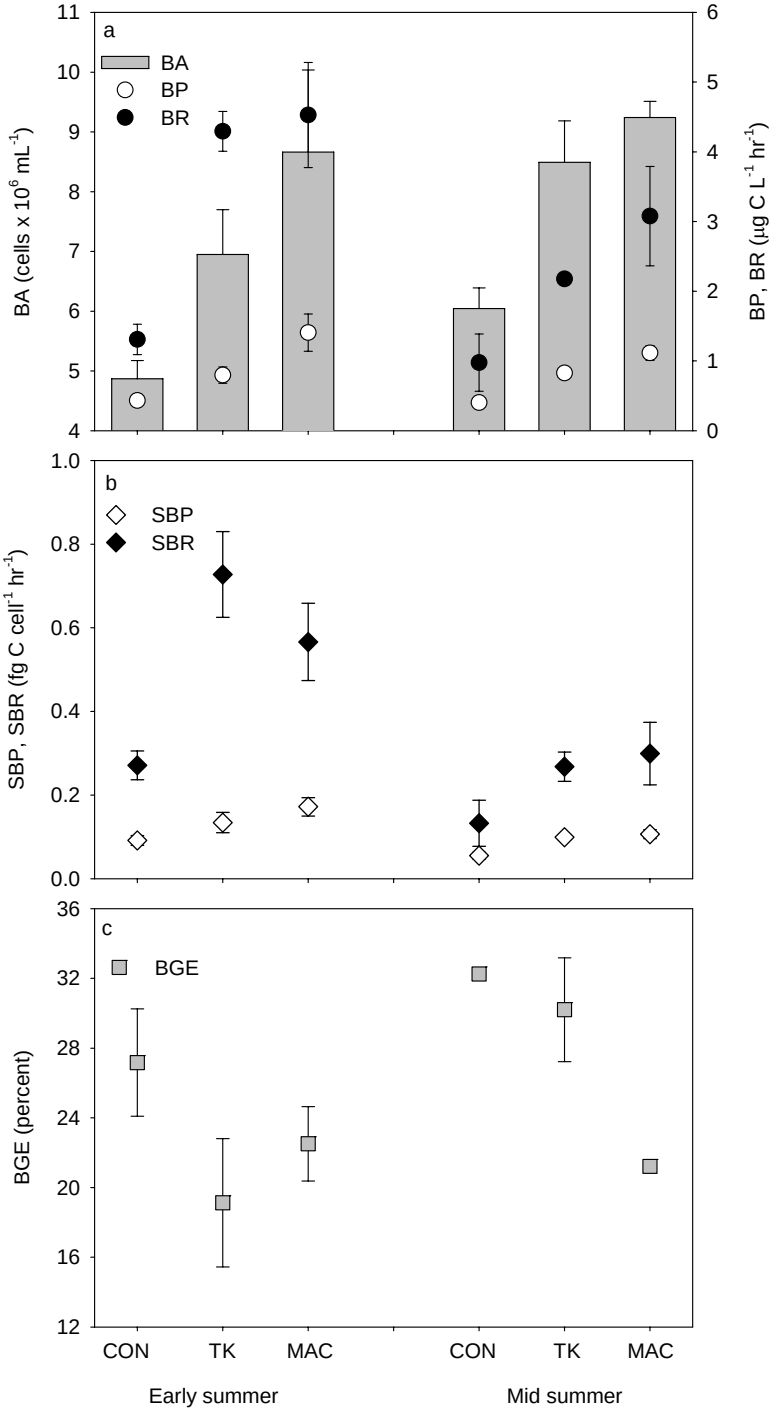

Figure 4.2: *In situ* measurements of bacterial metabolism and abundance measured in connected (CON), thermokarst (TK) and macrophyte-rich (MAC) Delta lakes in the early- and mid-summer surveys. Panel (a) shows bacterial abundance (BA), production (BP) and respiration; panel (b) shows specific bacterial production (SBP) and respiration (SBR); panel (c) shows bacterial growth efficiency (BGE). Error bars show standard errors for the 3 lakes within each category.

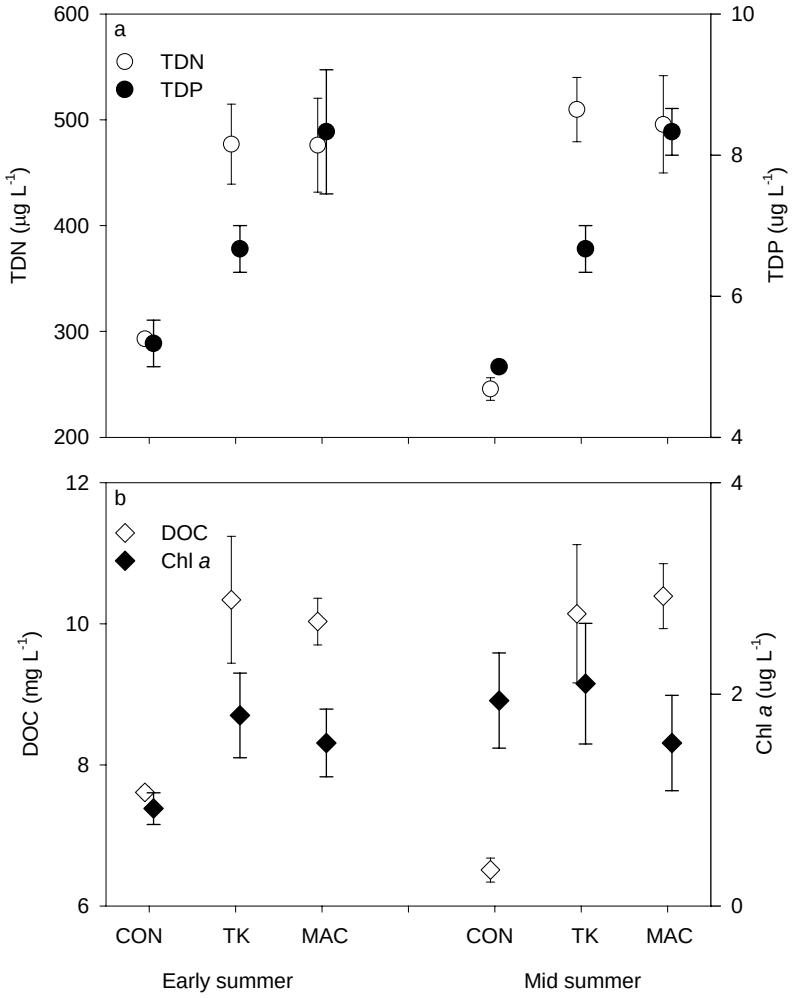

Figure 4.3: Total dissolved nitrogen and phosphorus (TDN, TDP), dissolved organic carbon (DOC), and chlorophyll *a* (Chl *a*) concentrations measured in connected (CON), thermokarst (TK) and macrophyte-rich (MAC) Delta lakes in the early- and mid-summer surveys. Panel (a) shows TDN and TDP; panel (b) shows DOC and Chl *a*. Error bars show standard errors for the 3 lakes within each category.

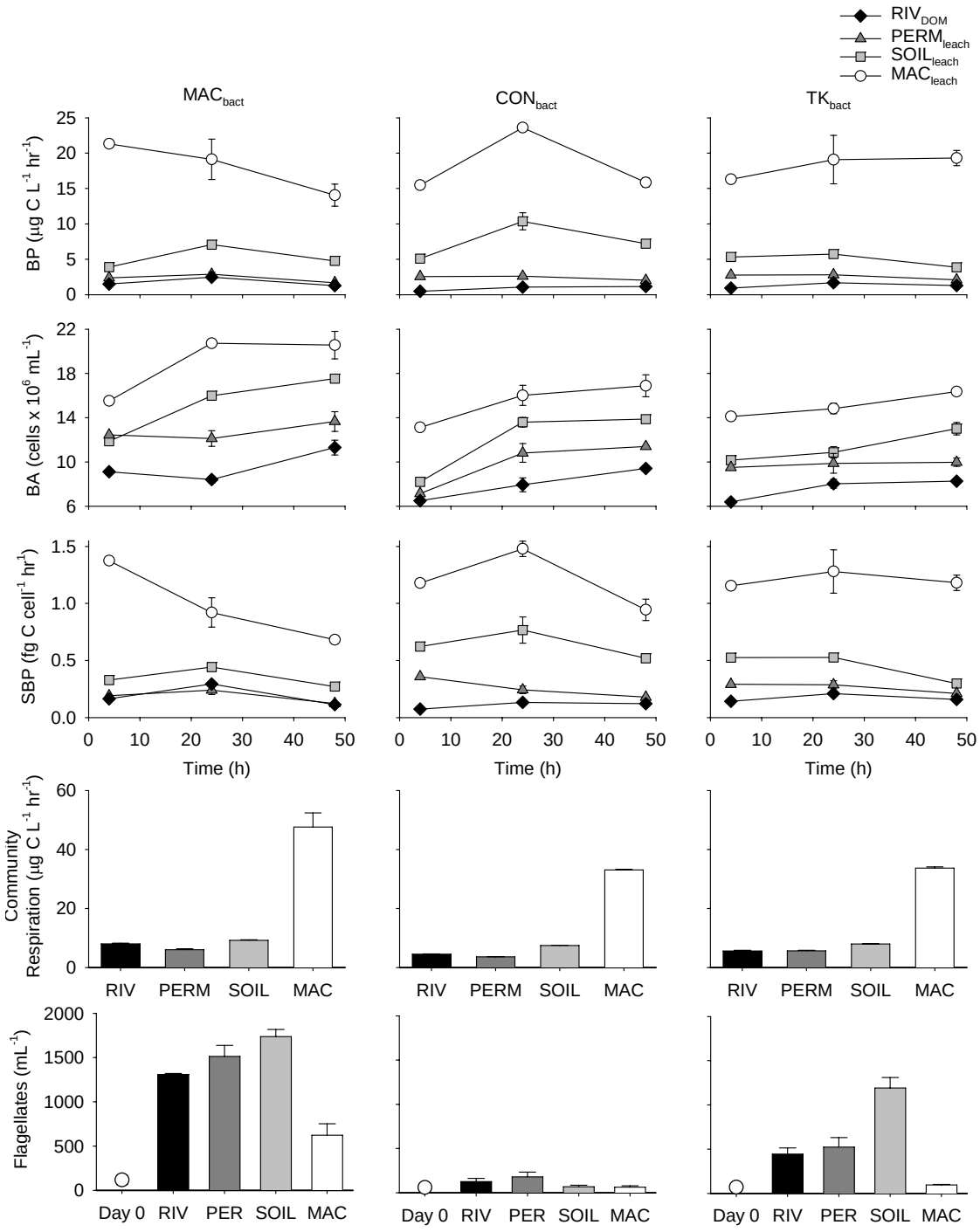

Figure 4.4: Results of the 2007 leachate experiment. Shown are bacterial production (BP), bacterial abundance (BA), specific bacterial production (SBP), community respiration and flagellate densities for incubations using bacterial inocula from connected (CON_{bact}), thermokarst (TK_{bact}) and macrophyte-rich (MAC_{bact}) lakes, and DOM from riverwater (RIV_{DOM}), permafrost leachates ($PERM_{leach}$), soil leachates ($SOIL_{leach}$), and macrophyte leachates (MAC_{leach}). Community respiration rates are integrated over the full experimental period. Flagellate densities are shown for day 0 (as indicated) and day 2 (bars). Error bars indicate standard error across experimental replicates.

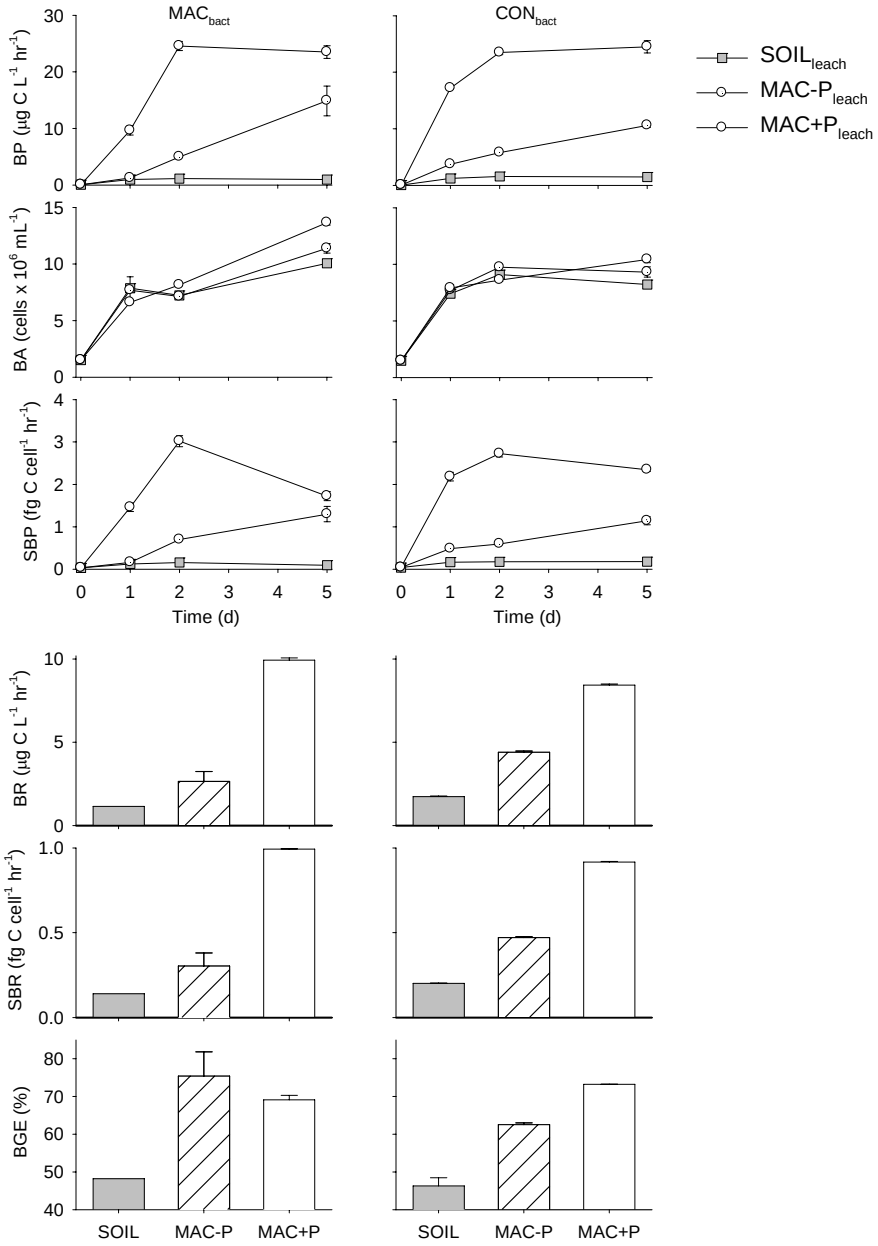

Figure 4.5: Results of the 2008 leachate experiment. Shown are bacterial production (BP), bacterial abundance (BA), specific bacterial production (SBP), bacterial respiration (BR), specific bacterial respiration (SBR) and bacterial growth efficiency (BGE) for incubations using bacterial inocula from macrophyte-rich (MAC_{bact}) and connected (CON_{bact}) lakes and DOM from soil leachates ($SOIL_{leach}$), macrophyte-P leachates ($MAC\text{-}P_{leach}$), and macrophyte+P leachates ($MAC+P_{leach}$). Rates of BR, SBR and BGE are calculated over the full experimental period. Error bars indicate standard error across experimental replicates.

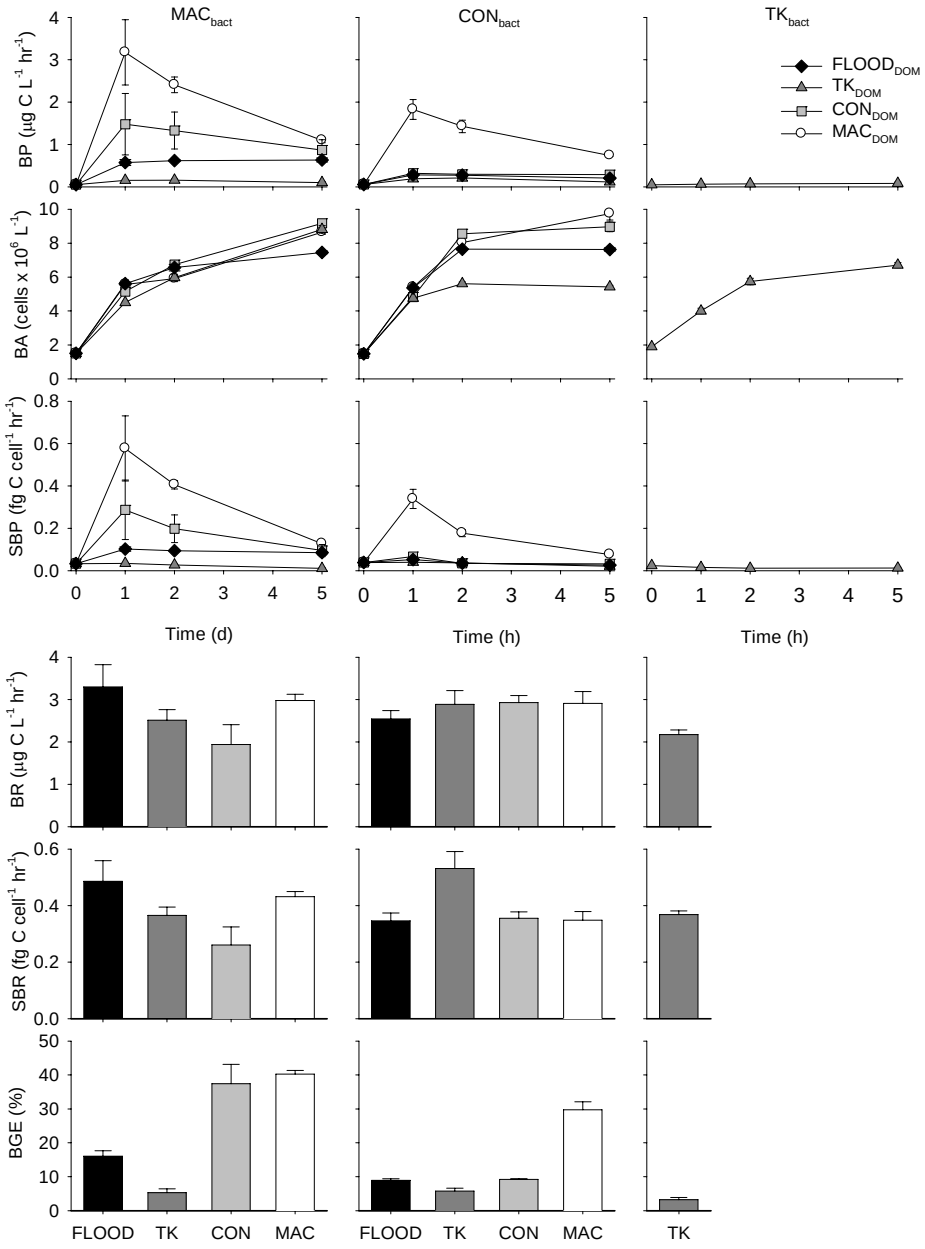

Figure 4.6: Results of the 2008 lakewater DOM experiment. Shown are bacterial production (BP), bacterial abundance (BA), specific bacterial production (SBP), bacterial respiration (BR), specific bacterial respiration (SBR) and bacterial growth efficiency (BGE) for incubations using bacterial inocula from macrophyte-rich (MAC_{bact}) and connected (CON_{bact}) lakes, and DOM from floodwater ($FLOOD_{DOM}$), thermokarst lakewater (TK_{DOM}), connected lakewater (CON_{DOM}), and macrophyte-rich lakewater (MAC_{DOM}). For comparison purposes, results from incubations in which inocula from thermokarst lakes (TK_{bact}) were grown on TK_{DOM} are also shown. Rates of BR, SBR and BGE are calculated over the full experimental period. Error bars indicate standard error across experimental replicates.

CHAPTER 5 ELEVATED pH REGULATES BACTERIAL CARBON CYCLING IN LAKES WITH HIGH PHOTOSYNTHETIC ACTIVITY[1]

[1] A version of this chapter has been accepted for publication as:
Tank, SE, DJ McQueen, and LFW Lesack. *In press.* Elevated pH regulates bacterial carbon cycling in lakes with high photosynthetic activity. Ecology.

5.1 Abstract

Bacteria are critically important for carbon (C) cycling and energy flow in aquatic environments. However, studies to date have largely focused on the role of substrate quality in the regulation of this important process. As such, we know little about the role of other ecological drivers in shaping bacterially-mediated C cycling. Here, we examine how planktonic bacterial abundance (BA), productivity (BP), respiration (BR), and growth efficiency (BGE), and thus C cycling are affected by elevated pH, an ecological factor that occurs commonly in highly productive aquatic systems. We undertook our study in lakes that routinely experience high pH caused by rapid macrophyte photosynthesis. Two different experiment types were employed: first, a series of short-term experiments was used to assess the direct effects of elevated pH on bacteria experiencing differing pH levels *in situ*. Second, long-term mesocosms were used to explore the effect of elevated pH on bacteria over longer time scales, and in the presence of other trophic levels. BP and BR slowed dramatically with elevated pH over the short term, potentially uncoupling bacterial processing of organic matter from its in-lake production, and causing a switch away from bacterial biomass creation, and towards C mineralization. With longer term exposure, bacterial communities adapted to the direct stress of elevated pH, but responses at higher trophic levels caused a cascade that mediated the effect of alkalization on bacteria, in a manner that could well vary among aquatic ecosystems. Our study establishes elevated pH as a key driver of bacterial C cycling and energy flow in aquatic systems with high autotrophic productivity.

5.2 Introduction

Bacteria are the most important biological component of carbon (C) cycling in the biosphere (del Giorgio and Cole 1998). In freshwaters the balance between rates of bacterial productivity (BP) and respiration (BR) controls the degree to which dissolved organic matter (DOM) is subsequently passed to higher trophic levels, or emitted from lakes as carbon dioxide (CO_2; Cotner and Biddanda 2002). Thus, elucidating the mechanisms that shape bacterial C processing is essential to understanding energy flow and C flux within aquatic ecosystems. While there has been much work examining the effects of bottom-up (i.e., substrate quality) processes towards this end, we know little about how other ecological drivers regulate bacterial C cycling.

Elevated pH has been clearly documented in freshwaters, often as the result of rapid photosynthesis sequentially depleting CO_2 and then HCO_3^- in the water column, which can lead to pH levels in excess of 10.0 (e.g., Schindler et al. 1972, Mitchell and Prepas 1990, Hesslein et al. 1991). Such high pH coupled with high autotrophic productivity could have unexpected implications for bacterial C cycling. The release of DOM that is a byproduct of algal and macrophytic photosynthesis provides a high quality substrate for bacterial growth (Findlay et al. 1992, Pérez and Sommaruga 2006) that can represent an important energy source to the microbial food web. The rapid use of this labile DOM by bacteria sets up a tight coupling between autotrophic DOM production and its subsequent bacterial processing (Kritzberg et al. 2005). However, when photosynthesis increases to the point where pH also rises, resultant impacts on the bacterial community could disrupt C cycling through several different, potentially co-occurring, effects. First, a pH-mediated reduction in the rate of bacterial DOM processing could uncouple the continued production of autotrophic DOM from its concomitant use by bacteria. Second, a change in the rate of BP relative to BR might cause a switch in microbial loop function, with a shift in the balance of C available to higher trophic levels, versus that which is respired as CO_2. Finally, any change in BR could alter the relative amount of CO_2 available for photosynthesis derived directly from atmospheric flux, versus that from BR of DOM. To date, the effect of elevated pH on bacterial C processing has not been considered.

Factors acting over both the short- and long-term may be expected to cause bacterial communities to respond to elevated pH. Because of their diversity, bacteria and other lower trophic levels are generally expected to exhibit relatively high resistance and functional compensation in the face of ecological stress, as resilient species counterbalance the loss of function of more susceptible taxa (Yachi and Loreau 1999, Vinebrooke et al. 2003). However, stressors that vary periodically or occur over short time scales can inhibit adaptation, thus effecting a response in even the most functionally redundant communities. In systems with high pH resulting from rapid photosynthesis, pH peaks often occur on a diel cycle that tracks photosynthesis, or on the scale of weeks when favorable atmospheric conditions prevail (e.g., low winds, high pressure; Maberly 1996). Over the long term, the effect of a stressor on any given trophic level will also depend on indirect, food-web mediated responses (Tank et al. 2003), with differential responses of distinct trophic levels often causing effects that are counter-intuitive in the face of single-species results (Abrams et al. 1996), and that vary across systems (e.g., Pace et al. 1999).

In this study, we experimentally examined how planktonic bacterial abundance (BA), BP, BR, bacterial growth efficiency (BGE; the efficiency of bacterial growth on the organic substrate), and thus the functioning of bacterial C cycling are affected by elevated pH. We worked in lakes of the Mackenzie Delta, western Canadian arctic, which naturally experience elevated pH caused by rapid macrophyte photosynthesis. We used short-term experiments to explore the direct effect of elevated pH in bacterial communities exposed to high and circumneutral pH levels *in situ*. We further used long-term experiments to examine how the response of higher trophic levels to elevated pH might cascade to indirectly mediate the bacterial response. Our results establish elevated pH as an important regulator of bacterial C cycling over both the short- and long-term when autotrophic production is high, and demonstrate a heretofore unexplored interaction between primary producers and the microbial loop.

5.3 Methods

5.3.1 Study site

The Mackenzie Delta is a lake-rich region formed where the Mackenzie River enters the Arctic Ocean. Every spring, meltwater flowing from southern tributaries meets ice still present in this northern Delta; the resulting rapid water level rise causes most Delta lakes to flood. However, because of slight differences in elevation between lake inflows, some lakes flood for days to weeks, while others remain connected to the river channel throughout the ice-free season (Marsh and Hey 1989). A detailed location map is given in Squires et al. (2002).

The flooding regime of Delta lakes plays a key role in regulating these waterbodies. High pH lake 56 (L56; 68°19.4'N, 133°50.8'W) is a high elevation lake that flooded for 16 days during spring 2006. In high elevation waterbodies, sediments rapidly fall out of suspension and the water column clears once floodwaters subside. Flood-delivered nutrients and the shallow depths of Delta lakes create conditions that facilitate rapid macrophyte growth: summer macrophyte biomass in L56 can reach 90 g m^{-2} (dry weight; Squires et al. 2002). Rapid photosynthesis in elevated lakes depletes CO_2, and substantially drives up pH (Hesslein et al. 1991). In the east-central section of the Delta in which our study lakes lie, more than 50% of lakes have a late summer pH greater than 9.5, and fully 25% have pHs greater than 10. pH values calculated from 2005 measurements of pCO_2 and dissolved inorganic carbon suggest that diurnal pH fluctuations over the course of our experiments in high pH Delta lakes were muted in comparison to those that can occur at temperate latitudes (e.g., Maberly 1996), as a result of daily sunlight durations ranging from 24 h (until July 17) to 17 h (Aug 21). The pH of samples taken from L56 on 3 consecutive days in July at 7, 3, and 7 h past solar midnight, respectively, did not differ from one another, while August samples taken at 7 and 12 h past solar midnight (5 h prior to, and at, solar noon) averaged 10.0 and 10.5, respectively. By the end of the summer, L56 lake water appears to contain a discernible component of macrophyte origin DOM (Chapter 3).

In contrast, nearby low pH lake 129 (L129; 68°18.2'N, 133°51.1'W) remained connected to the river throughout the 2006 ice-free season (connection time of 167 days).

Suspended sediments in connected lakes remain high throughout the summer, resulting in much lower macrophyte growth (Squires et al. 2002). Consequently, pH levels remain near the base pH for river water in this system. Thus, despite similar inocula to these lakes each spring, conditions quickly diverge with increasing time since isolation of high elevation, macrophyte-rich lakes.

5.3.2 Experimental incubations

To test the direct and indirect effects of elevated pH on bacterial communities, we employed 2 different experiments. First, short-term incubations were undertaken three times during the summer of 2006, to examine the immediate, direct effects of elevated pH at varying levels of *in situ* pH. Whole lake water was collected from both high pH L56 and low pH L129, and kept cool and in the dark until laboratory processing (within 3 h). Samples were filtered through a large pore size (Whatman GF/D) filter to remove algae and bacterial grazers. Visual inspection of all samples for algae and grazers during BA counts (see below) confirmed that this removal treatment was effective. Where necessary, the pH of filtered samples was adjusted to pH 8, 9.3, or 10.5 using 1 N solutions of HCl or NaOH. pH-adjusted samples were transferred to biological oxygen demand (BOD) bottles for incubation. Incubation temperatures were identical across all samples for each of the 3 incubations, and were set to mimic *in situ* conditions. BA and BP (at 24 h), and BR (as the difference between dissolved oxygen [DO] at 1 and 25 h), were measured as outlined below.

Second, long-term mesocosm experiments were used to test the interactive, food-web mediated effects of alkalization. 1500 L mesocosms (1.25 m circular diameter x 1.25 m depth), closed at the bottom, were suspended in the water column of low pH L129. A 20 cm foam collar was positioned around the outside top rim of each mesocosm, and the polycarbonate sheeting of the mesocosm was folded down over the collar and secured. The collars were attached to square 1.3 m x 1.3 m wooden frames for support, and the frames were firmly anchored in the lake sediments. 9 enclosures (to give 3 replicates of each of the 3 pH treatments below) were installed. Mesocosms were set up in early July of 2006 using a bucket to collect L129 subsurface water. Because of its shallow depth and northern

location, L129 is well mixed throughout the summer; temperature profiles confirmed L129 was not stratified during our set up. Fish, which occur only at low density in L129, were specifically excluded from our experiments. Mesocosms were set up on a cool, overcast day to minimize the effect of gradients in zooplankton distribution, and initial species composition and abundance in the mesocosms was in accordance with L129 abundances. Enclosures were left to acclimate for 2 d, at which time day zero samples were collected using an integrated tube sampler that allowed us to sample the full depth of the mesocosm. Immediately following initial sample collection, the pH was raised using NaOH to 9.3 in 3 of the enclosures, and 10.5 in an additional 3 enclosures. 3 enclosures were left unamended. Treatments were dispersed randomly among the 9 enclosures. Following the initial pH adjustment, pH levels were measured every 2-3 d, and when necessary NaOH was added to adjust the pH to the target level. Mesocosms were sampled as above after 9, 19, and 29 d. When pH was adjusted on sampling days, samples were collected prior to NaOH addition. Samples were kept cool and dark until laboratory processing (within 3 h), and immediately preserved or analysed. Samples were collected for GF/D filtered BA, BR, and BP, in addition to chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), absorbance, and counts of protozoans, zooplankton, and total (unfiltered) BA. Collection and processing methods are outlined below.

5.3.3 Validation of NaOH and HCl methodology

To confirm that the changes in ionic strength and alkalinity that accompanied our pH treatments (Table 5.1) had no confounding effect on our results, we undertook a laboratory manipulation of a cultured bacterial community reared on Mackenzie Delta lakewater. The bacterial culture was set up by collecting whole lake water from a productive lake nearby Simon Fraser University, from which algae and bacterial grazers were removed by filtering with a Whatman GF/C filter. Isolated bacteria were used to inoculate 0.22 µm-filtered water from L56, at a per volume ratio of 1:10. The bacterial culture was allowed to reach stable equilibrium, assessed as stabilized rates of BP, and experimental manipulations were performed on cultures at the equilibrium state, on 20 mL

aliquots from the original culture. The experimental manipulations described below were all undertaken in duplicate over the same 24-h period. The response of the bacterial community was assessed as change in BP relative to a duplicated unamended control.

Treatments were as follows: first, to confirm that the pH treatment effected by our NaOH additions replicated that which would occur as a result of photosynthesis, we increased pH by removing dissolved CO_2 from a set of bacterial cultures, and compared these to cultures in which pH was increased using NaOH. A 60-mL plastic syringe was filled with EMASorb CO_2 absorbant (Elemantal Microanalysis Limited**,** distributed by Isomass Scientific, Calgary, Canada), and sealed with a stopper that was fitted with a length of tubing. Pressurized air was forced through the sealed syringe to strip it of CO_2, and bubbled through duplicate bacterial cultures via a second length of tubing. Using this method, we were able to achieve a pH of 9.65 over a 24-hour treatment period. Because the rise in pH using this method was gradual, we also raised the pH in the NaOH-treated cultures gradually, in a matched fashion.

Second, we undertook manipulations to further verify that the changes in ionic strength and alkalinity effected by our long- and short-term experimental manipulations did not affect bacterial communities. We added (a) $NaHCO_3$ to mimic the stepped NaOH addition above, (b) CaCl at the maximum Cl^- molarity experienced in our short-term incubations, and (c) $NaHCO_3$ at the maximum Na^+ molarity experienced in our short-term incubations. The $NaHCO_3$ treatments were designed to mimic the concurrent increase in total alkalinity and Na^+ that occurs at the same time as an NaOH-mediated pH increase. The CaCl treatments were designed to mimic the increase in Cl^- brought about by HCl addition.

Changes in alkalinity and ionic balance had no significant effect on BP, while the two methods of pH elevation resulted in rates of BP that were not significantly different from one another ($p=0.47$; Fig. 5.1). We recognize, however, that these 2 methods of pH manipulation (CO_2 removal vs. NaOH addition) are not exactly equivalent to one another. Changes in CO_2 can be both a cause, and consequence, of fluctuations in pH. In the case of photosynthetically-driven pH changes, dissolved CO_2 depletion drives increased pH. In the case of experimental NaOH additions, increased pH results in CO_2 depletion. However, both

processes lead to equivalent CO_2 concentrations and pH levels, and the results of the above comparative pH manipulation experiment demonstrate that NaOH addition is a reasonable mimic of the pH shifts driven by photosynthetic demand in our hard water study lakes. We also recognize that manipulating pH by both of the above approaches may differ in as yet unknown ways from the manner in which high *in situ* pH is chemically created in lakes as a result of rapid photosynthesis.

5.3.4 Laboratory analyses

Assessment of BA, BP, BR, and BGE

Triplicate samples for BA were preserved with glutaraldehyde (final concentration 2.5%) in glass vials, and stored in the dark at 4°C until counting, within 6 months of collection. To ensure even sample dispersal once filtered, samples were prepared following Bae Yoon and Rosson (1990). TWEEN-80 (10 ppm) was added to samples, which were incubated for 2 h at 4°C and then sonicated (30 s at 10 W). After sonication, DAPI was added to samples (final concentration 10 mg L^{-1}), which were filtered onto 0.22 µm black polycarbonate filters (GE Osmonics). Filters were rinsed with 2 mL of phosphate buffered saline both before and directly after filtering to clear excess humic material. Filtered samples were counted at 1000 x using a Zeiss Axioplan microscope fitted with a 50 W bulb (Osram HBO) and filters specific for the DAPI stain (Zeiss filter set 02). GF/D filtered bacteria samples from the long-term mesocosm experiment were additionally sized to allow for calculation of cell-specific BR and BP. Cells were measured at 1000 x using a Zeiss Axioscop 2 microscope fitted with a 100 W bulb (Osram HBO) and Zeiss filter set 01. The scope was fitted with an Orca ER digital camera (Hamamatsu Corp., Bridgewater NJ), and images captured using Openlab 5.02 software (Improvision Inc., Waltham MA). Cell areas were measured using the open source software ImageJ (http://rsb.info.nih.gov/ij/), and their volumes calculated using standard formula. Total bacterial volume per L was calculated by relating average measured bacterial volume per cell to overall counts of BA.

BP was measured using ^{3}H leucine (Leu), following Smith and Azam (1992). 20 µL of Leu (71.3 nM; saturating concentration) was added to sterile microcentrifuge tubes at

ratios of hot (Amersham TRK 636, 165 Ci $mmol^{-1}$): cold (Sigma L8000) Leu ranging between 1:3 and 1:1.5. Incubations were conducted with 4 replicates plus 1 blank for each sample. Samples and blanks were read within 24 h on a Triathler portable scintillation counter (Hidex Oy, Finland) using Amersham BCS scintillation cocktail. Samples were corrected for quench by comparing select samples and known standards measured on the Triathler and on a Beckman Coulter LS6500. Dilution experiments were conducted (Roland and Cole 1999) to confirm an isotope dilution factor of approximately 2, and BP was calculated by multiplying the Leu incorporation rate by the standard correction factor of 3.1 kg C mol Leu^{-1}.

BR was measured as loss of oxygen over 24 h in samples incubated in stoppered glass BOD bottles. O_2 content was measured at the beginning and end of the 24 h period following Roland *et al.* (1999) and APHA (1989), and converted into CO_2 assuming a respiratory quotient of 1. BGE, a measure of bacterial biomass created per C assimilated, was calculated as BP/(BR+BP) (del Giorgio and Cole 1998), with BP values averaged over 24 h for the short-term incubations. For the short-term incubations, specific BP (SBP) and BR (SPR) were calculated as BP and BR per cell, respectively, using BA obtained from counts of GF/D filtered samples. For the long-term mesocosms, SBP and SBR were calculated as BP and BR per total cell volume, to account for possible changes in cell size over the duration of the mesocosm experiment.

Assessment of proto, micro, and macrozooplankton abundance

To analyse protozoan (ciliate and heterotrophic nanoflagellate, HNAN) abundance, 40-50 mL of unfiltered mesocosm water was immediately fixed with a mixture of Lugol's solution, formalin, and sodium thiosulfate (Sherr and Sherr 1993). Samples were filtered onto 0.8 µm black polycarbonate filters (GE Osmonics) after staining with DAPI (1 mg mL^{-1}), within 1 week of collection. Samples were kept frozen (-20°C) until counting with epifluorescence microscopy, within 6 months of filtration. Ciliates were counted at 400x, by scanning multiple lengths of the filtered area. For HNAN, 15 fields of view or a minimum of 50 cells were counted at 1000x. Micro- and macrozooplankton were preserved with

sugared formalin and counted at 50x. Entire samples were counted for all species except rotifers, which were subsampled from well-mixed samples.

Analysis of water chemistry parameters

Water samples were analysed for DOC and absorbance after filtration (0.22 μm Millipore GSWP). DOC was analysed using a Shimadzu TOC-V total organic carbon analyzer. Absorbance (m^{-1}) was measured using a scanning spectrophotometer. Chl *a* samples were collected by filtration onto Whatman GF/F filters, and immediately frozen until analysis following Nusch (1980) and Welschmeyer (1994).

5.3.5 Statistical analyses

Short-term incubations

The effect of pH on BA, BP, and BR was assessed using 3-way ANOVA, with pH (8, 9.3, and 10.5), month (June, July, and August), and lake (L129 and L56) as factors. When 3-way interaction effects were significant, the simple interaction (Keppel 1982) of pH x month was assessed at each lake level. When the 2-way simple interaction effect was significant the simple effect of pH was assessed at each level of lake and month (Keppel 1982). Significant simple effects were followed by simple contrasts.

Because matched replicates were not used for concurrent measurement of BP and BR in the short-term incubations, analysis of BGE using ANOVA was not possible. We therefore used linear regression to explore the relative effect of increased pH on BGE. BGEs from each experiment were normalized relative to the BGE of the low pH treatment in that experiment, such that relative BGEs from each of the 6 experiments were always 1 for the low pH treatment. This was then plotted against the difference between the manipulated experimental pH and the *in situ* pH at the time of the experiment. Adjusted r^2 values are reported.

Long-term mesocosms

The effect of pH on bacterial parameters and higher trophic level abundances was assessed using repeated measures ANOVA (RM-ANOVA). Significant interactions between pH and time were examined using simple effects and contrasts specific for RM-ANOVA

(Keppel 1982). Where data did not meet assumptions of normality and heteroscedacity Box-Cox transformations were employed.

5.4 Results

5.4.1 Short-term effects of elevated pH on bacterial communities isolated from the food web

In our short-term experiments, *in situ* pH had a dramatic effect on the response of bacterial communities to our experimental manipulations. In high pH L56, early-summer pH was similar to baseline Delta levels, but increased in a dramatic, sustained fashion as the summer progressed (to pH 10.5; Fig. 5.2). Concurrent with this seasonal pH increase, the effect of alkalization on BA decreased (Table 5.2, Fig. 5.3a). In June, alkalization had a strong and significant effect on BA, with abundances 58% lower, on average, in high relative to low pH treatments. In July, just after the pH increase (Fig. 5.2), the pH effect on BA continued to be significant, but the mean decline was less severe (22%, Table 5.2, Fig. 5.3a). In August, the effect of pH on BA was non-significant. For BP, the pH effect was significant throughout the summer. However, the magnitude of this effect was also lower when ambient pH was higher (a mean 100% decline in June, but 35 and 65% decline in July and August between low and high treatments; Table 5.2, Fig. 5.3a). The results for BR in L56 largely mirror the BP results; the effect of alkalization remained significant throughout the summer, but the magnitude of mean decline between low and high treatments was considerably lower in July and August (27 and 44%, compared to 90% in June, Fig. 5.3a). This trend of community adaptation was even more striking for comparisons between the low and mid pH treatments: amongst the latter summer comparisons only BP in July differed significantly between these 2 treatments (Table 5.2, Fig. 5.3a).

In contrast, pH in low pH L129 remained near Delta baseline levels throughout the summer (Fig. 5.2), and the effect of short-term pH manipulations on BA, BP and BR was much more pronounced across all months than for L56 communities. BA declined significantly with increased pH in all 3 study months (Table 5.2, Fig. 5.3b), although the magnitude of the mean decline did decrease as the summer progressed (57% in June, 41%

in July, and 26% in August; Fig. 5.3b). The effect of alkalization on BP in L129 was also significant throughout the summer, with mean declines between low and high pH treatments always ranging between 98 and 100% (Table 5.2, Fig. 5.3b). Declines in BR with increased pH were statistically consistent throughout the summer (lack of significant pH x month simple interaction; Table 5.2, Fig. 5.3b), with levels highest at low pH, and lowest at high pH (Table 5.2, Fig. 5.3b).

Similar trends were found for BGE. In high pH L56, BGE declined substantially in both the high and mid pH treatments, compared to the low pH treatment, early in the season, but levels between treatments converged as the season progressed (Fig. 5.3c). In contrast, for low pH L129, BGE was depressed throughout the summer in mid and high pH treatments, relative to the low pH treatment (Fig. 5.3d). Regardless of the lake from which experimental inocula were obtained, increases in pH above the *in situ* level resulted in clear, predictable decreases in BGE (Fig. 5.3e, r^2=0.94, p<0.0001). In contrast, where the *in situ* pH was higher than the treatment pH, as occurred in July and August experiments for L56, decreasing pH to a more neutral value did not further increase BGE. Because replicate measurements of BA were not matched to BP or BR replicates, we did not undertake statistical analyses of SBP and SBR. However, given that BP and BR declined in high ambient pH communities when BA was stable in response to elevated pH, it seems likely that SBP and SBR also declined. SBP and SBR are illustrated in Fig. 5.4.

5.4.2 Long-term effects of elevated pH on bacteria in situ

In contrast to results from the short term incubations, where *in-situ* exposure to high pH led to bacterial community adaptation, BA was significantly reduced in the presence of high pH throughout the duration of our mesocosm experiment (Table 5.3, Fig. 5.5a). The effect on community BP was less striking, with elevated pH significantly reducing BP only at the beginning of the experimental period (Table 5.3, Fig. 5.5b). There was no effect of elevated pH on community BR (Table 5.3, Fig. 5.5d). In contrast, SBP and SBR showed a significant response to alkalization throughout much of the experimental period. SBP increased significantly at high pH towards the end of the experimental period (Table

5.3, Fig. 5.5c), while SBR was significantly higher at high pH during the middle 2 sampling periods (Table 5.3, Fig. 5.5e). BGE was depressed at high pH early in the experimental period, but was unaffected by pH over the longer term (Table 5.3, Fig. 5.5f).

5.4.3 Long- term effects of elevated pH on higher trophic levels in situ

The conflicting response of BA to pH in our short- and long-term experiments likely occurred because of strong effects of pH on bacterial consumers in our mesocosms. Initial mesocosm concentrations of rotifers, copepods, and cladocerans were well within the range expected for L129 (Riedel 2002). HNAN abundances increased strikingly at high pH (Table 5.3, Fig. 5.6b), concurrent with the declines in BA that we observed (Table 5.3, Fig. 5.5a). Bacterial and HNAN abundances were strongly negatively correlated with one another in the high pH treatment, across all sampling times and high pH mesocosms (correlation not shown; $r^2 = 0.701$, $p<0.001$). Small (6-12 µm), filose amoebae were also observed in great numbers in our high pH mesocosms on d 9 and 19 (average density 1,700 L^{-1} and 10,000 L^{-1}, respectively), but were only sporadically observed at low densities in other samples (data not shown).

In contrast to the response of HNAN to alkalization, ciliates and rotifers decreased considerably at high pH (Table 5.3, Fig. 5.6c, d), and responded in tandem to this stress (correlation not shown, $r^2 = 0.918$, $p < 0.001$). This alternating pattern, with decreased BA at high pH, increased HNAN, and decreased ciliates and rotifers, resulted in a positive correlation between bacteria and ciliate abundances in the high pH mesocosms, when examined across all sampling times (correlation not shown, $r^2 = 0.378$, $p = 0.019$). A similar correlation between bacteria and rotifers was not significant ($r^2 = 0.231$, $p = 0.154$). Of the dominant rotifer taxa, *Asplancha* sp., *Conochilus* sp., and *Ascomorpha* sp. disappeared almost immediately after the manipulation in high pH mesocosms, while *Polyarthra* sp. and *Keratella* sp. showed a strong decline under high pH early in the experiment, but rebounded very slightly towards its end.

Neither copepods nor their nauplii were significantly affected by pH (Table 5.3, Fig. 5.6e, f). In contrast, cladoceran abundance showed a sharp, significant increase in high pH

mesocosms on the last sampling day (Table 5.3, Fig. 5.6g), which was driven by increased abundances of *Bosmina longirostris* and *Ceriodaphnia* sp. (data not shown). Chl *a*, which is relatively low in our study lakes, also responded equivocally to the stress of elevated pH, although there were significant differences between treatments at some points during our experiment (Table 5.3, Fig. 5.6a).

5.5 Discussion

5.5.1 pH as an ecological driver in productive aquatic systems

Although its consequences have not been extensively investigated, elevated pH is well documented in aquatic systems, particularly as a result of high productivity. In marine systems, pH values ranging from 9.75 to 10 can occur in coastal regions (Pedersen and Hansen 2003). In freshwaters pH as high as 10 is certainly not uncommon, particularly in shallow (Mitchell and Prepas 1990) and/or eutrophied systems (Jeppesen et al. 1990). In particular, dense macrophyte beds are known to result in localized pH peaks (Goulder 1969); although high, the macrophyte biomasses observed in productive Mackenzie Delta lakes can certainly be found elsewhere (Goulder 1969, Wetzel 1990, Rooney and Kalff 2000). The common practice of measuring physicochemical parameters in the open water zone of lakes may mask spikes in pH that occur on the microscale in lakes with well developed nearshore macrophyte beds.

5.5.2 The short-term effect of pH on bacteria in isolation from the food web

In the absence of indirect effects, bacterial communities experiencing high ambient pH adapted to this stress. The fact that communities from both high pH L56 (short-term experiments) and low pH L129 (long-term mesocosms) adapted to high pH suggests that the results we observed are specifically attributable to the effect of long-term alkalization, rather than being the result of a 'lake effect'. This adaptation effect is perhaps best demonstrated by the explicit response of BGE to deviation from the ambient pH level, rather than to absolute pH. The resilience to elevated pH that we observe suggests an *in situ* shift

in bacterial community composition in response to a selection pressure exerted by high pH, although we note that species-specific physiological adjustments may also have occurred. Change in community structure is a well known response to stress, and often affords continued functionality to the affected system (Yachi and Loreau 1999). Several studies have shown pHs ranging from mildly acidic to circumneutral to be an important predictor of bacterial composition (e.g., Lindström et al. 2005, Yannarell and Triplett 2005). However, information on the effect of high pH on bacterial community composition is scant, and we did not undertake taxonomic measurements in this study.

Despite this clear community adaptation response, it was not absolute across all the bacterial attributes we measured in our short-term incubations. While BA was unaffected by alkalization in communities experiencing high pH *in situ*, the response for BP and BR was somewhat more subtle: although the decrease was of a lower magnitude when *in situ* pH was high, both community-wide and per cell BP and BR fell under the stress of alkalization throughout the season in both lakes (Figs. 5.3, 5.4). Thus, even in adapted communities, high pH may continue to affect C cycling and energy flow by reducing rates of bacterially mediated C mineralization and C incorporation into biomass.

In unexposed communities, the response to elevated pH was severe, and again variable across the measured attributes. The strong decreases in BGE for communities taken from a circumneutral *in situ* pH occurred because BP was more strongly affected by high pH than BR. Decreased BP in response to elevated pH has been shown previously for productive lakes (Jeppesen et al. 1997). Respiration was measurable in high pH treatments when BP was indistinguishable from 0, indicating that maintenance respiration continued to occur even when biomass production had stopped. Thus, the range in BP across treatments was much wider than for BR (by approximately 44-fold), exhibiting a difference in range greater than has been found previously (10-fold; Roland and Cole 1999). The differential response of BP relative to BR indicates that in the face of rapid pH fluctuations, alkalization could critically affect functioning of the microbial loop, through a shift away from biomass creation, and towards CO_2 efflux.

5.5.3 Elevated pH elicits a trophic cascade that affects BA in situ

Over the long-term, there was a clear signal of top-down effects of pH on BA. Although BA in communities experiencing high *in situ* pH was unaffected by pH manipulation in the isolated short-term incubations, BA was consistently depressed in high pH mesocosms. This decrease was significantly correlated to the concurrent increase in HNAN and filose amoebae, whose abundances rose sharply at the onset of alkalization, and decrease in ciliates and rotifers abundances in response to alkalization. Although HNAN can be abundant in alkaline soda lakes (Finlay et al. 1987), research on the effects of elevated pH on this group of organisms has been sparse. The pulse of HNAN that appeared in our high pH mesocosms was comprised of smaller organisms than were observed at lower pH. This, coupled with the appearance of small filose amoebae, suggests a species shift occurred as other species reacted negatively to high pH (e.g., Vinebrooke et al. 2003).

In contrast, ciliate and rotifer communities appeared to not possess the functional redundancy necessary for resistance to our experimental stress. Previous studies have shown decreased growth of ciliates outside of species-specific pH optima (Weisse and Stadler 2006), and a strong influence of pH on the natural variability of ciliate community structure (Andrushchyshyn et al. 2003), while rotifer taxa are known to exist within strict, species specific pH ranges (Bērziņš and Pejlir 1987). In particular, *Asplancha* sp., *Polyarthra* sp., and *Keratella* sp. have been shown to not survive at pH 10.5, although other rotifer species not found in our experimental communities can tolerate the stress (Hansen et al. 1991, Hessen and Nilssen 1985).

The lack of effect of high pH on copepod species contrasts sharply with the cascade observed at lower trophic levels. Although several studies have shown a pronounced response by zooplankton to elevated pH, it is clear that not all species are equally sensitive to the stress. The dominant copepod in our enclosures, *Cyclops* sp. has been found both to decline at pH > 10 (Beklioğlu and Moss 1995), and be unaffected by the stress (Hansen et al. 1991). As was the case in our study, others have found copepod nauplii to persist in the face of elevated pH (Hansen et al. 1991). *Bosmina longirostris* and *Ceriodaphnia* sp.

abundances both increased sharply under high pH at the end our experiment. Although other studies have found these species to decline at high pH (Hessen and Nilssen 1985, Hansen et al. 1991, Beklioğlu and Moss 1995), cladoceran species can survive the stress of alkalization (*Daphnia longispina*, Hessen and Nilssen 1985; *Daphnia hyalina*, Beklioğlu and Moss 1995). It seems likely that these cladoceran species in our enclosures were released from the effects of competition or juvenile predation at elevated pH.

5.5.4 The long-term effect of pH on bacterial metabolism in the presence of other trophic levels

The decrease in SBP (albeit marginal), BP, and BGE seen at high pH early in our mesocosm experiment suggests adaptation occurs over several (10+) days. Once communities had adapted to high pH, levels of SBP and SBR were typically greatest in high pH treatments, resulting in similar measures of total-community BP and BR across pH, despite the marked drop in BA with alkalization. This result differed from the reductions in bacterial metabolism observed in pre-exposed communities during our short-term experiments. Several authors have shown that increased grazing by bacterial consumers, as likely occurred with the rise in HNAN at high pH, increases both SBP and SBR through mechanisms such as nutrient recycling by predators and the release of bacterial growth-promoting substances (Sherr et al. 1982, Pernthaler et al. 1997). An increase in DOM substrate quality could also lead to a rise in SBP, although would likely not affect SBR (Smith and Prairie 2004). In our high pH mesocosms, DOM molecules were smaller towards the end of the experiment (250: 365 nm absorbance ratio significantly higher at high pH; RM-ANOVA followed by simple contrasts, $F_{2,24(pH,\ time19)}=6.023$, $p=0.008$; $F_{2,24(pH,\ time29)}=3.551$, $p=0.045$), suggesting that DOM bioavailability may have increased.

5.5.5 The effect of elevated pH on aquatic bacterial community function

Given that large diurnal pH fluctuations (Maberly 1996) and spatially discrete pH peaks (Goulder 1969) are known byproducts of rapid aquatic photosynthesis, consideration

of short-term effect is particularly relevant for the study of high pH. We show short-term alkalization events to cause a dramatic decreases in BP, and substantial decreases in BA and BR, which may have profound effects on C cycling. Because such pulses of increased pH are photosynthetically-mediated, they may also be accompanied by the release of high quality DOM leachates from senesced vegetation (Findlay et al. 1992), and photosynthetic exudates (Wetzel and Manny 1972). Short-term decreases in BP and BR suggest that bacterial uptake of this DOM slowed substantially, indicating that the production of autotrophic DOM could become uncoupled from its processing by bacteria at high pH. At the same time, BP decreased dramatically relative to BR, suggesting alkalization provoked a fundamental switch in microbial loop functioning, with relatively less C available for flow to higher trophic levels (BP) and more mineralized to CO_2 (BR). Finally, alkalization likely changed the relative source of dissolved CO_2 available for photosynthesis, away from that remineralized by BR, and towards that derived from atmospheric flux. Such changes in bacterial function may be particularly important in macrophyte-dominated systems, where the bulk of primary production is unavailable to planktonic consumers, and bacteria may act as a C shunt to higher trophic levels.

Over the longer term bacterial communities adapted to elevated pH. In Delta lakes, this adaptation response may be accelerated somewhat by diurnal pH fluctuations that are muted in comparison to those often observed in productive temperate lakes. In adapted communities, the bacterial response will likely depend on an intricate balance between multiple indirect effects, the prevalence of which will certainly vary among aquatic systems. Such differences in indirect effects among systems could provide a fundamental mechanism to explain variations in the response of bacterial metabolism to elevated pH. In our study, cascading trophic interactions, which often differ in incidence and structure among aquatic environments (Pace et al. 1999), emerged as a key driver of bacterial response to alkalization. Increased bacterial predators decreased BA, but likely stimulated SBP and SBR such that BP and BR did not differ among treatments. Although we specifically excluded fish from our mesocosms, other studies have found elevated pH to decrease fish feeding rates and recruitment (Beklioğlu and Moss 1995, Jeppesen et al. 1998), causing effects that

cascade downwards but dampen significantly by the time they reach protozoans and bacterioplankton (Jeppesen et al. 1998). In such systems, where trophic cascades at the microbial level are weak or do not occur, results from our short term incubations of bacteria experiencing high pH *in situ* suggest BP and BR may decrease slightly, with BA remaining relatively stable. Conversely, in systems where cascades decrease bacterial predators, BA, and subsequently BP and BR, should increase.

On a more general level, we demonstrate that an ecological stressor occurring over both the short- and long-term can regulate bacterial metabolism and therefore C cycling. To date, the effect of stressors on bacterially-mediated ecosystem processes has been largely overlooked, perhaps because these communities are thought to be resistant as a result of their large functional redundancy (Yachi and Loreau 1999, Vinebrooke et al. 2003). Given the importance of bacteria for C cycling and energy flow in aquatic systems, we suggest that their regulation by other stressors that may similarly occur over the short-term (some examples include pollutant spikes during runoff events, increased UV exposure during near-surface thermoclines [Xenopoulos and Schindler 2001]) and long-term should be a priority for future research.

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5.7 Tables

Table 5.1: Calculated changes in alkalinity and ionic strength in response to short-term and long-term experiments, in comparison to measured ambient concentrations. Changes in dissolved inorganic carbon (DIC) and total cations were calculated assuming equilibrium concentrations of $CaCO_3$ at elevated pH, and resultant $CaCO_3$ precipitation[a]. In Mackenzie Delta lakes, ambient total alkalinity is composed almost entirely of DIC.

	L129		L56		
	Season-wide average		June	July	August
Ambient concentration[b]					
Cl^- (µM)	158.1		222.2	222.2	222.2
DIC (µM)	1996.5		2010.4	1727.7	934.9
Total anions (µM)	2396.4		2466.1	2183.4	1395.1
Na^+ (µM)	242.8		348.5	348.5	348.5
Total cations (µM)	1445.5		1705.2	1602.0	1314.3
Salinity (mg L^{-1})	199.4		208.1	186.7	127.1
Concentration at maximum NaOH addition	*Short-term experiment*	*Mesocosm experiment*	*Short-term experiment*		
DIC (µM)	1139.8	1139.8	1124.7	945.2	444.6
Total alkalinity	2106.2	2106.2	2082.5	1800.6	1014.5
Na^+ (µM)	1867.8	3692.8	2673.5	623.5	598.5
Total cations (µM)	1971.0	4038.8	3019.4	969.4	944.4
Salinity (mg L^{-1})	150.2	192.2	167.8	109.7	78.6
Concentration at maximum HCl addition					
Cl^- (µM)				608.1	583.1
Total anions (µM)	na	na	na	2569.4	1756.1
Salinity (mg L^{-1})				200.4	139.9

[a] Although $CaCO_3$ supersaturation can occur in natural waters, it is unlikely for our experiments given that $CaCO_3$ precipitates were readily visible both in our lakes as carbonate crusts on macrophyte leaves, and in our high pH experiments. The presence of these precipitates suggests that the seed crystals necessary to induce $CaCO_3$ precipitation, and thus inhibit supersaturation, were in abundance. However, we note that by assuming equilibrium $CaCO_3$ concentrations in our calculations, we present the maximum possible decrease in DIC and Ca^{2+} (total cations) brought about by our pH treatments.

[b] Obtained from previously collected concentrations of water chemistry parameters for Mackenzie Delta lakes (Anema et al. 1990a, b; Tank and Lesack, unpublished data), known to be stable inter-annually.

L129: concentrations are given as a seasonal average, from multiple measurements taken in this lake over 2 years.

L56: Because of seasonal changes in [DIC] and [Ca^{2+}] that occur throughout the summer as a result of $CaCO_3$ precipitation at high pH, concentrations for L56 are separated by month. Reported ambient values are as follows: [DIC] from weekly measurements taken by S. Tank during the summer of 2005, reported as monthly means. [Ca++] derived from [DIC], using known relationships between these species in Delta lakes. All other anions and cations are known to be stable throughout the ice-free season, and were obtained from late summer measurements taken by L. Lesack during 2 consecutive years.

Table 5.2: Short-term incubations: Results of 3-way ANOVA, and subsequent simple interactions and effects for the effect of pH, season, and lake on bacterial abundance (BA), production (BP) and respiration (BR). Where higher order interactions were significant for the 3-way ANOVA, an analysis of simple interactions at each level of lake was undertaken. When this 2-way simple interaction was not significant, simple contrasts for the effect of pH were conducted at the 2-way level, across seasons. Where the simple interaction was significant, an analysis of the simple effect of pH at each level of lake and month was undertaken. Significant simple effects were followed by simple contrasts. 'na' indicates inappropriate contrasts. Contrasts are shown from high to low treatment means; treatments that share a common underscore are not significantly different from one another.

		BA			BP			BR	
Source	*df*	F		*df*	F		*df*	F	
Month	2	533.71 ***		2	640.03 ***		2	60.53 ***	
pH	2	256.61 ***		2	1493.90 ***		2	33.70 ***	
Lake	1	174.89 ***		1	2461.85 ***		1	77.31 ***	
month x pH	4	27.41 ***		4	93.00 ***		4	3.50 *	
month x lake	2	115.39 ***		2	16.11 ***		2	12.09 ***	
pH x lake	2	15.85 ***		2	51.81 ***		2	4.00 *	
month x pH x lake	4	12.27 ***		4	199.21 ***		4	2.09	
Error	34			53			70		
Simple interaction		*L56*	*L129*		*L56*	*L129*		*L56*	*L129*
month x pH	4	33.86 **	5.541 **	2	222.84 ***	72.43 ***	2	5.47 **	2.01
Error	34			53			35		
Simple effects		*L56*	*L129*		*L56*	*L129*		*L56*†	*L129*†
Jun	2	118.82 ***	88.26 ***	2	392.67 ***	263.15 ***	2	9.47 ***	
Jul	2	23.04 ***	82.15 ***	2	1035.60 ***	511.56 ***	2	16.58 ***	22.66§ ***
Aug	2	1.48	33.47 ***	2	222.84 ***	72.43 ***	2	6.21 **	
Error	34			53			35		
Simple contrasts		*L56*	*L129*		*L56*	*L129*		*L56*	*L129*
Jun		L M H	L M H		L M H	L <u>M H</u>		L <u>M H</u>	
Jul		<u>M L</u> H	L M H		L M H	L M H		<u>M L</u> H	L M H §
Aug		na	L M H		<u>L M</u> H	L M H		<u>M L</u> H	

*p < 0.05, **p < 0.01, ***p < 0.001.

†2-way, rather than simple, interaction, as a result of non-significant higher order interaction in the 3-way ANOVA.

§Effects and contrasts for across all months

Table 5.3: Long-term mesocosm experiment: Degrees of freedom (df), F-statistics, and significance levels for RM-ANOVA, and subsequent simple effects for the effect of pH on measures of bacterial abundance (BA), production (BP) and respiration (BR), and abundances of other food web components. Where the pH x time RM-ANOVA interaction term was significant an analysis of the simple effect of pH within time was undertaken. Significant simple effects were followed by simple contrasts. 'na' indicates inappropriate contrasts. Contrasts are shown from high to low treatment means; treatments that share a common underscore are not significantly different from one another.

Source	*df*	BA	BP	SBP	BR	SBR	BGE	Chl *a*	HNAN	Ciliates	Rotifers	Nauplii	Copepods	Cladocera
pH	2	14.03**	1.58	0.60	2.95	9.54*	5.07	0.07	5.86*	25.29**	120.00***	0.26	0.29	2.19
$Error_{pH}$	6													
time	3	22.13***	9.98***	4.64*	4.09*	12.76***	11.12***	71.21***	15.75***	27.48***	30.30***	3.36	13.24***	43.37***
pHxtime	6	13.72***	3.84*	4.34**	2.02	4.13**	4.92**	9.02***	7.26***	17.79***	10.63***	1.42	0.64	4.90**
$Error_{time}$	18													
Simple effects														
time 0	2	0.81	0.79	1.30	na	4.50*	2.12	0.54	0.43	0.095	0.34	na	na	0.86
time 9	2	17.95***	9.33**	2.17	na	14.48***	16.69**	11.36***	13.08***	65.83***	40.06***	na	na	0.79
time 19	2	19.74***	1.42	4.28*	na	4.13*	0.60	2.36	8.92**	13.35***	18.47***	na	na	2.26
time 29	2	17.16***	0.27	4.00*	na	1.02	0.42	6.69**	5.47*	7.67**	37.07***	na	na	10.31***
Error	24													
Simple contrasts														
time 0		na	na	na	na	M H L	na	na	na	na	na	na	na	na
time 9		L M H	L M H	na	na	H M L	L M H	H L M	H M L	M L H	M L H	na	na	na
time 19		L M H	na	H M L	na	H L M	na	na	H M L	M L H	M L H	na	na	na
time 29		M L H	na	H M L	na	na	na	L M H	M L H	M L H	M L H	na	na	H M L

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

5.8 Figures

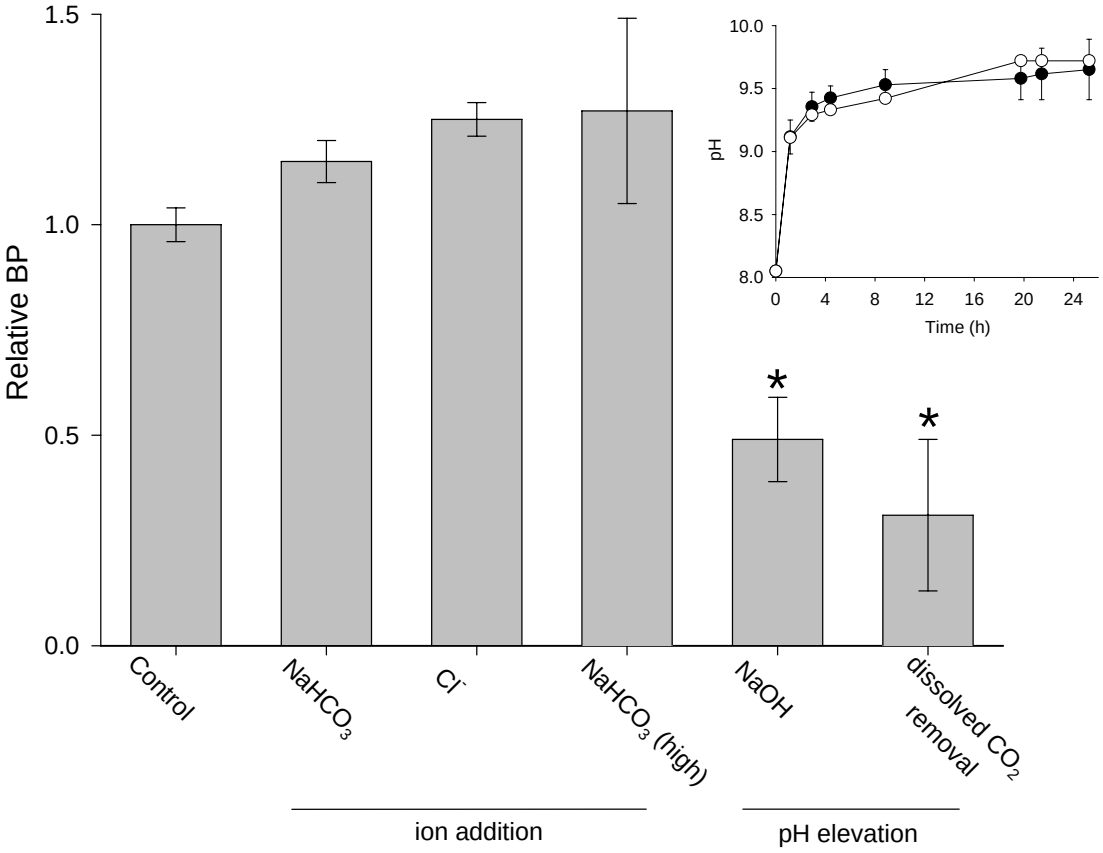

Figure 5.1: Effect of increased ionic concentrations, and different methods of pH elevation, on bacterial production (BP). Results are expressed as relative BP, normalized to the control treatment. Error bars represent standard error. Results were assessed using planned contrasts that compared each treatment with the control, and the two "pH elevation" treatments against one another. The two starred treatments have rates of BP that are significantly lower than the control, but not significantly different from one another. Experimental ion additions had no significant effect on BP.

Inset: measured pH over the course of the experiment for bacterial cultures exposed to elevated pH through NaOH addition (white circles) and dissolved CO_2 removal (black circles).

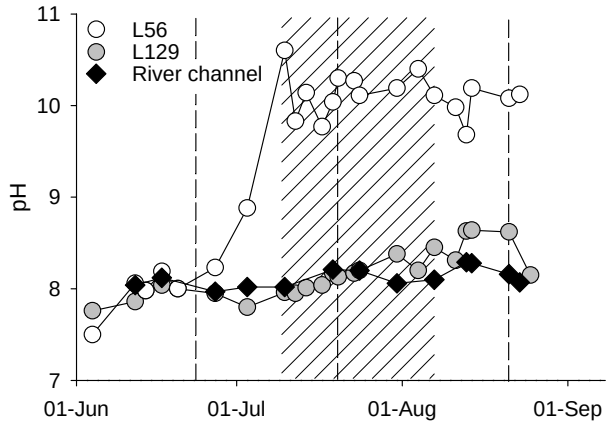

Figure 5.2: *In-situ* mid-day pH values throughout the study period for high pH L56 (white circles), low pH L129 (grey circles) and the river channel within the Mackenzie Delta that lies closest to these 2 lakes (black diamonds). Dashed lines indicate the dates of the June, July and August short-term experiments, the hatched area indicates the duration of the mesocosm experiment.

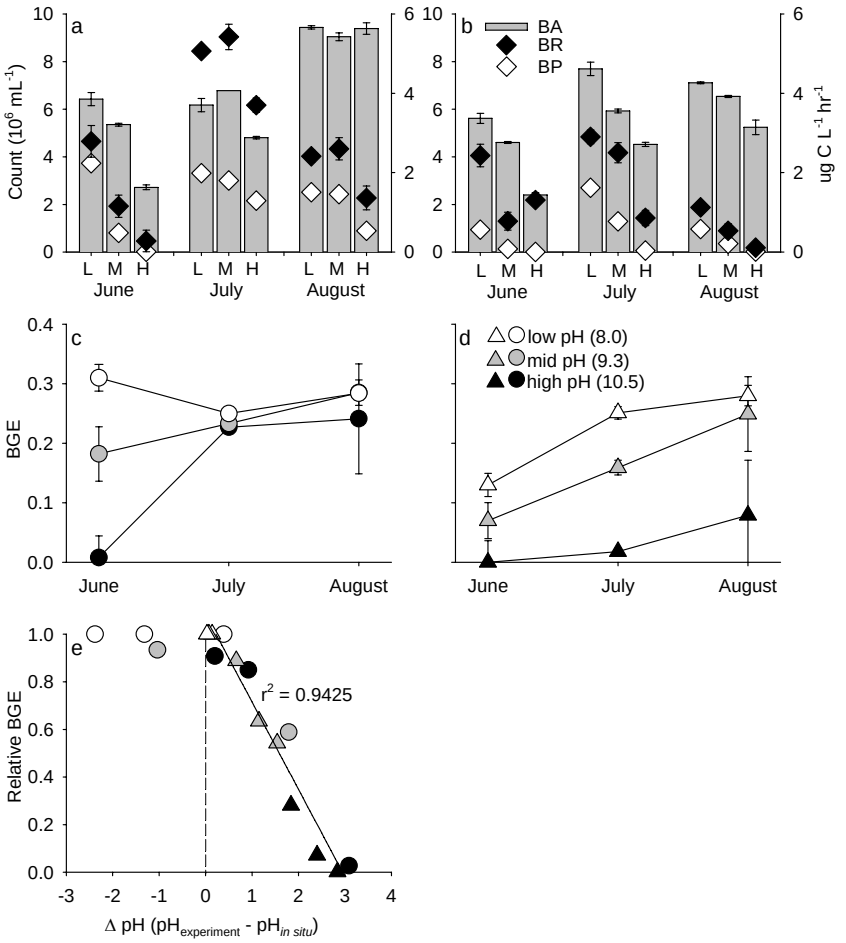

Figure 5.3: Short term incubations: Effect of manipulated pH on bacteria. Panels a (L56) and b (L129) show the effect of low, medium, and high pH levels on bacterial abundance (BA; grey bars; left axis), production (BP; white diamonds, right axis), and respiration (BR; black diamonds, right axis). Error bars indicate ± 1 standard error for 3 treatments, and are hidden by the symbol where not visible. Panels c (L56) and d (L129) show bacterial growth efficiency (BGE) in the 3 pH treatments in June, July and August, with standard errors calculated using formula for the standard error of a ratio. L56 is represented by triangles and L129 by circles, for low (white) medium (grey) and high (black) pH treatments. Panel e shows the difference between *in situ* and treatment pH, versus BGE relative to that of the low pH treatment for each incubation. Symbols are as in panels c and d.

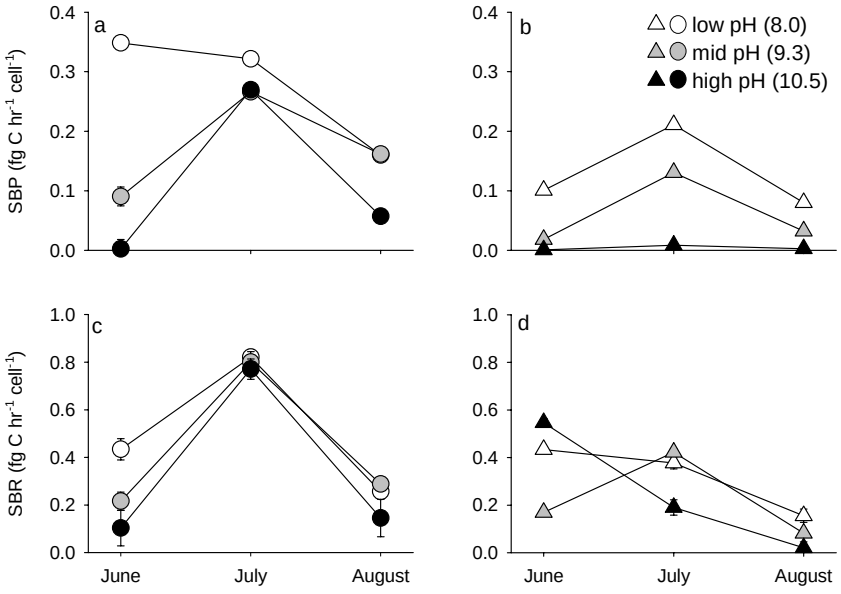

Figure 5.4: Short term incubations: Effect of manipulated pH on specific bacterial production (SBP) and bacterial respiration (SBR) in L56 (panels a and c) and L129 (panels b and d). Shown are data for the low (white), medium (grey), and high (black) pH treatments. Error bars are 1 standard error of the mean, calculated using formula for the standard error of a ratio.

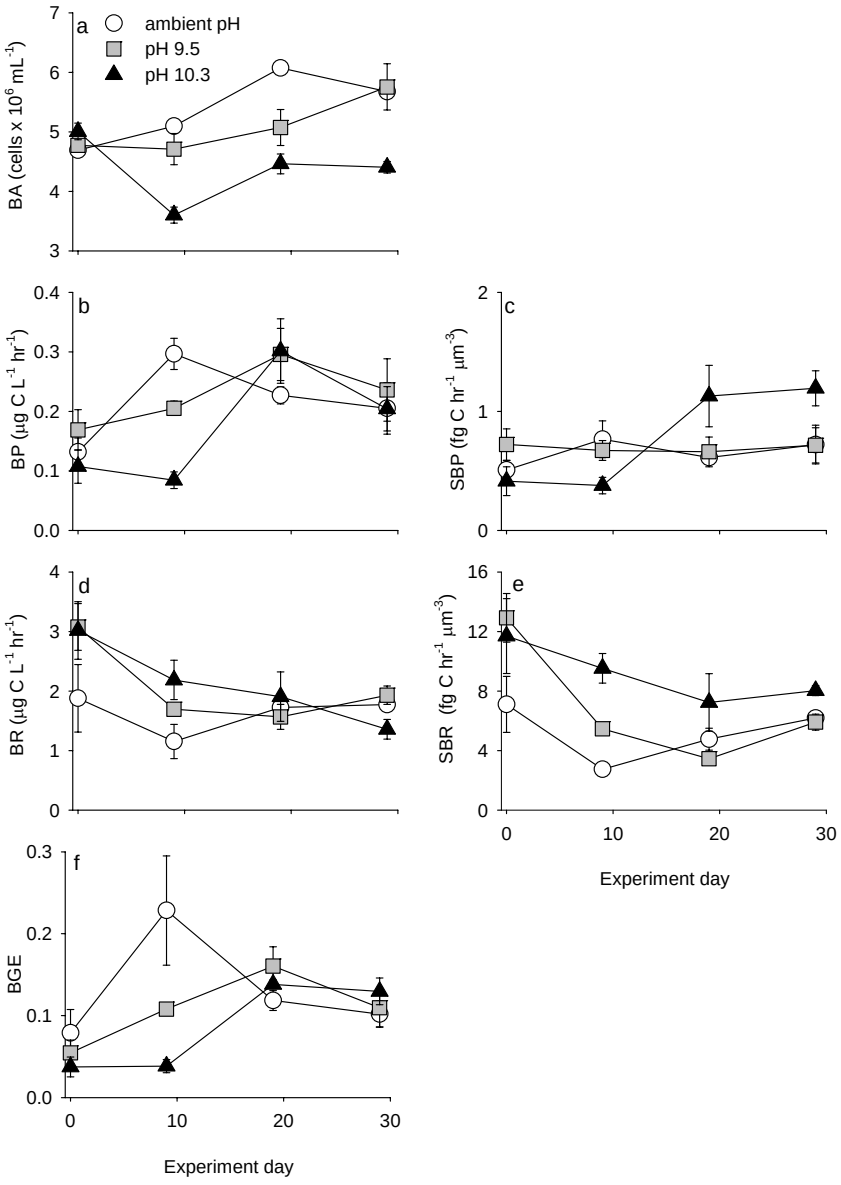

Figure 5.5: Long term mesocosm experiment: Effect of manipulated pH on bacterial abundance (BA), bacterial production (BP), specific bacterial production (SBP), bacterial respiration (BR), specific bacterial respiration (SBR), and bacterial growth efficiency (BGE) under ambient (white circles), pH 9.3 (grey squares) and pH 10.5 (black triangles) treatments. Error bars indicate ± 1 standard error for 3 replicates.

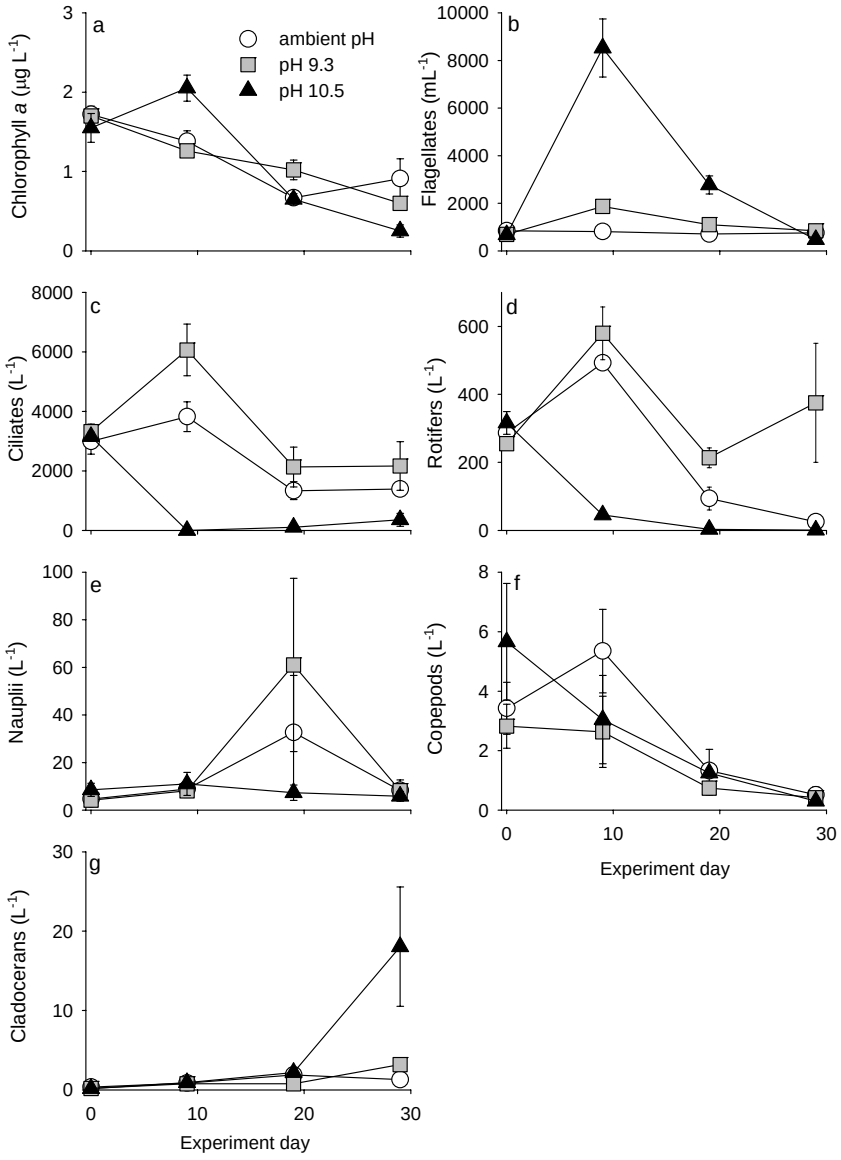

Figure 5.6: Long term mesocosm experiment: Effect of manipulated pH on various trophic levels. Shown are results for chlorophyll *a*, heterotrophic nanoflagellates (HNAN), ciliates, rotifers, copepod nauplii, copepod juveniles and adults, and cladocerans under ambient (white circles), pH 9.3 (grey squares) and pH 10.5 (black triangles) treatments. Error bars indicate ± 1 standard error for 3 replicates.

CHAPTER 6 THE CONTRIBUTION OF BACTERIA TO HIGHER TROPHIC LEVELS IN LAKES OF CONTRASTING DISSOLVED ORGANIC MATTER QUALITY

6.1 Abstract

Bacteria may alternately form an energy "link" between dissolved organic carbon (DOC) and higher trophic levels, or act as an energy "sink" for their DOC substrate. In part, the ability of bacterial biomass to subsidize aquatic foodwebs may depend on the lability of their DOM food source. We investigated the importance of bacterial biomass to zooplankton by undertaking a survey of zooplankton stable isotopes (^{13}C and ^{15}N) and taxonomically diagnostic fatty acids, and performing an experiment that assessed whether incubation with different DOM substrates affects the growth rates of higher trophic level organisms. We did this work in a series of waterbodies comprised of two endmember types: first, lakes that are strongly influenced by either terrestrially-derived riverwater, or terrestrial matter derived from permafrost slumping, and have relatively low bacterial productivity, and second, lakes that receive only moderate terrestrial inputs, but contain dense stands of submergent macrophytes and display relatively rapid bacterial growth *in situ*. Zooplankton from lakes where bacterial production is known to be rapid had a greater proportion of bacterial fatty acids in their tissue, and higher $\delta^{15}N$ values relative to zooplantkon, suggesting a greater contribution of bacteria to zooplankton biomass and longer food webs in bacterially-productive lakes. Zooplankton $\delta^{13}C$, however, was almost universally depleted compared to $\delta^{13}C$ for seston and heterotrophic bacteria, indicating that methanotrophic bacteria, which are known to be heavily $\delta^{13}C$-depleted, may additionally contribute to zooplankton biomass in our study lakes. Zooplankton $\delta^{13}C$ depletion was greatest in lakes with high levels of macrophyte production or permafrost slumping, indicating that the organic matter produced by both of these processes could augment methanotroph activity. In contrast, our relatively short-term experimental incubations appeared to occur over too short of a time span to assess whether bacterial food webs contribute to zooplankton biomass. This study demonstrates that the combined use of multiple tracers can be a powerful tool for elucidating energy flow in food webs.

6.2 Introduction

Bacteria and phytoplankton dominate the production of organic matter in pelagic (open water) environments, through their use of dissolved organic carbon (DOC) or dissolved inorganic carbon (DIC) as a substrate, respectively. Because DOC is typically assumed to be unavailable for direct consumption by higher trophic levels, DOC-fuelled bacterial growth has long been suggested to subsidize algal-based food webs in aquatic environments (Pomeroy 1974). However, the ability of bacteria to provide this subsidy may depend on the quality of the DOC substrate. DOC that is relatively recalcitrant as a bacterial food source tends to support relatively high rates of respiration (and thus conversion of DOC to CO_2), and low rates of production of new bacterial biomass (del Giorgio and Cole 1998). As such, recalcitrant DOC is poorly passed between trophic levels within the aquatic environment (Pace et al. 2007). In contrast, bacteria fed highly labile substrates can contribute significant biomass to higher trophic levels (e.g., glucose; Koshikawa et al. 1996, Havens et al. 2001).

In practice, the DOC available for consumption by bacteria is derived from a number of different sources, and can differ substantially in its lability. For example, while watershed-derived terrestrial organic matter is known to be a poor bacterial substrate, DOC derived as a byproduct of algal photosynthesis (Kritzberg et al. 2006), or macrophyte photosynthesis (Chapter 4) is readily incorporated into bacterial biomass. Although there is a considerable body of work that explores the importance of watershed-derived terrestrial organic matter to higher trophic levels in lakes (Pace et al. 2007), we know little about how other DOM substrates might act to structure aquatic foodwebs.

In this paper, we explore how the importance of bacteria to higher trophic levels varies among lakes that have DOC derived from different sources, and subsequent differences in bacterial productivity. We undertook this work in the Mackenzie Delta, where lakes tend to, alternately (1) be strongly influenced by inputs of terrestrially-derived riverwater, (2) contain dense macrophyte stands, which appear to produce highly labile DOC as a byproduct of their photosynthesis (Chapter 4), or (3) be strongly influenced by permafrost melting (thermokarst), which appears to contribute recalcitrant terrestrial-like,

thermokarst-derived DOC to the water column (Chapters 3 and 4). We hypothesized that in lakes where DOC is known to be most labile, and thus result in higher rates of bacterial production (i.e., macrophyte dominated lakes; Chapter 4), bacteria will be a greater contributor to higher trophic levels, such as zooplankton.

We employed two contrasting approaches to test our hypotheses. First, we used stable isotopes (^{13}C and ^{15}N) and taxonomically diagnostic fatty acids (FA) as tracers to help elucidate the contribution of bacteria to zooplankton biomass. In lakes, the ratio of stable isotopes of C ($^{13}C/^{12}C$, expressed as parts per thousand relative to a standard; $\delta^{13}C$) is often used to differentiate between different basal resources, because phytoplankton $\delta^{13}C$, the $\delta^{13}C$ of watershed-derived particulate organic matter, and the $\delta^{13}C$ of attached algae and macrophytes often differ from one another. In Mackenzie Delta lakes, bacteria tend to be $\delta^{13}C$-enriched (display more positive values) relative to the bulk DOC pool, presumably because of preferential uptake of DOC derived from macrophytes and attached algae (Chapter 3). Because the ratio of light, to heavy, stable C isotopes alters (fractionates) very little as biomass is passed through trophic levels (Peterson and Fry 1987, Post 2002), the $\delta^{13}C$ of a consumer organism reflects the relative input of different basal pools. Thus, if bacteria and phytoplankton represent the major basal resources within a lake, and differ in their $\delta^{13}C$ signature, zooplankton $\delta^{13}C$ values should be intermediate between these two endmembers, and represent the proportional consumption of each. In contrast, $\delta^{15}N$ ($^{15}N/^{14}N$, relative to a standard) is often used to estimate the trophic position of an organism, because consumer $\delta^{15}N$ tends to be enriched by 3.4‰, on average, relative to its diet (Peterson and Fry, 1987, Post et al. 2002). Because bacterially-based food webs require additional trophic steps between the basal resource and higher trophic levels (Porter 1986), zooplankton receiving a greater proportion of their diet from the bacterial component of the overall foodweb may be $\delta^{15}N$ enriched (Berglund et al. 2007). Finally, the composition of FA in zooplankton tissue is also a useful indicator of zooplankton diet. Both algae and heterotrophic bacteria produce taxonomically-diagnostic FA (Napolitano 1999), while zooplankton lipids are largely a reflection of diet, because very little *de novo* synthesis occurs (Goulden and Place 1990). It is not possible to use FA to determine the absolute

proportion of bacterial, or algal, biomass ultimately consumed by higher trophic level organisms, because both algae and bacteria also produce many ubiquitous FA. However, changes in the relative importance of algal- and bacterial-specific FA within zooplankton tissue has been shown to be a good indicator of diet (Desvilettes et al. 1997, Napolitano 1999).

Second, we used an experimental approach to elucidate whether incubation with different DOC substrates results in variable growth rates of higher trophic level organisms. To do this, we measured how the abundance, or biomass, of individual trophic levels leading to and including zooplankton, as well as the activity of chitobiase, an enzyme released by crustacean zooplankton to facilitate moulting, varies in the presence of DOC from different sources. Chitobiase activity has been shown to increase proportionally to the overall growth rate of crustacean zooplankton *in situ* (Oosterhuis et al. 2000). Throughout the remainder of this work, we refer to the combined, inseparable pool of dissolved organic matter (DOM), which includes dissolved organic C, nutrients (i.e., dissolved organic nitrogen and phosphorus), and other organic constituents.

6.3 Methods

6.3.1 Study site

The Mackenzie Delta is situated in the western Canadian Arctic, at the entry point of the Mackenzie River into the Beafort Sea. Every spring, freshet water from southern tributaries flows north to meet ice still present in this northern delta. The resultant rapid rise in water levels causes most Delta lakes to flood. However, the period of connection between a lake and the system of deltaic river channels is regulated by lake elevation: Delta lakes occur along a flood frequency gradient that ranges from infrequently flooded (once every several years) perched lakes, to lower elevation lakes that are connected to the river throughout the ice-free season (Marsh and Hey 1989). DOM concentration and composition has been shown to change dramatically across this lake elevation gradient (Chapter 3, Spears and Lesack 2006, Gareis 2007). This variation in DOM appears, in part, to regulate the productivity of heterotrophic bacteria (HB) in these waterbodies (Chapter 4).

Low elevation, connected (CON) lakes receive inputs of terrestrially-derived riverwater DOM throughout the ice-free season, and are dominated by this DOM type (Chapter 3). Because suspended sediment loads are continually high in CON lakes, light penetration is low, and primary production is limited (Squires et al. 2002, Squires and Lesack 2002). In contrast, higher-elevation, perched Delta lakes are often extremely macrophyte-rich (MAC). The short burst of spring floodwater provides one of the few sources of terrestrially-derived DOM to these lakes. After floodwaters subside, the water column of these lakes quickly clears, and this clarity, coupled with flood-delivered nutrients and the shallow depths of Delta lakes create conditions ideal for submergent macrophyte growth, with summer macrophyte biomasses reaching 350 g m^{-2} (dry weight; Squires et al. 2002). In addition, a small subset of high-elevation lakes has been considerably deepened by the action of thermokarst (TK). TK lakes are characterized by visible slumps at the lake's edge, and mature, dead, trees rising vertically through the water column. These deep lakes also have dense multi-year mats of the macroalgae *Chara* sp. along the lake bottom.

6.3.2 Field surveys: fatty acids and stable isotopes

To assess trophic contributions to zooplankton in Mackenzie Delta lakes, we collected zooplankton samples for analyses of phospholipid FA in 2007, and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in 2005 and 2007. Samples of seston (2005 and 2007) and bivalves (2007) were also collected for baseline correction of stable isotope analyses (e.g., Post 2002). In 2007, a series of 9 lakes (Table 6.1) was surveyed in the early summer (30 June – 03 July). Zooplankton were collected with a standard net (80 μm mesh), using repeated tows through the water column. Bivalves were collected using a dip net to collect the top layer of lakebed sediments, which were manually sorted. Lakewater for seston samples were collected immediately below the lake surface. Collected zooplankton and bivalves were kept cool and in lake water, and collected lakewater kept cool, and returned to the laboratory for processing within 5 hours. Zooplankton were sorted using differentially-sized mesh sieves and hand-picking, to obtain samples for the dominant copepod (*Cyclops* sp.) common to all surveyed lakes. Cladocera were universally rare, and thus were not

collected for our analyses. Samples for *Cyclops* sp. were sorted to discard naupliar and early copepodid organisms. Once cleaned, zooplankton samples for FA analyses were immediately frozen on dry ice, while samples for stable isotope analyses were immediately frozen at -20°C, and stored frozen until later processing. Samples for seston were collected by filtering pre-screened (37 μm) lakewater onto pre-combusted (475°C for 4 h) GF/F filters (0.7 μm nominal pore size), and immediately frozen at -20°C. Bivalve samples were allowed to clean their guts in filtered lakewater for 24 h, and then stored frozen at -20°C until later processing. As soon as samples had been processed from all of the lakes, samples for fatty acid analyses were shipped on dry ice to Simon Fraser University, where they were immediately stored at -80°C until further processing.

In 2005, a series of 6 lakes spanning the lake elevation gradient (Table 6.1) were surveyed for stable isotopes of zooplankton and seston in June (14-16), July (21-26), and August (22-26), and HB in June and August. Zooplankton samples were cleaned to result in mixed samples of adult and copepodid copepods, *Daphnia* sp., and *Bosmina longirostris.* *Daphnia* sp. and *Bosmina longirostris* always constituted <10% of the samples. Cleaned samples were immediately dried at 60°C, and stored frozen until later processing. Samples for seston were collected as in 2007, except filters were immediately dried at 60°C and stored frozen until later processing. Samples for HB stable isotopes were collected as in Chapter 3: briefly, a bacterial inocula (GF/A filtered lakewater, 1.6 μm nominal pore size) was combined with 0.22 μm filtered lakewater at a dilution of 1:10, and distributed among a series of closed lengths of dialysis tubing (SpectraPor 2, 12,000-14,000 Da molecular cutoff, 45mm flat width; 6 replicate 1L incubations), which were anchored just below the lake surface. Samples were left to incubate *in situ* for 48 hours, at which time they were harvested, and samples collected by filtering 2L per sample through precombusted GF/F filters, which were frozen until further processing.

6.3.3 Experimental incubations

We further undertook an experimental incubation to assess whether planktonic food webs typical of Delta lakes experience variable energy flow to higher trophic levels

when incubated on different DOM substrates. 18 L incubations were initiated using a 50:50 dilution, by adding 9 L of 0.2 µm-filtered DOM to 9 L of lakewater. Aliquots of lakewater included a full complement of bacteria, protozoans, micro- and macro-zooplankton, and were collected from a high elevation, thermokarst lake (Table 6.1) using an integrated tube sampler. Each DOM treatment was undertaken in triplicate, with collected lakewater aliquots distributed randomly between the treatments. We used DOM obtained from the different lakewater types characteristic of Delta lakes (DOM from CON, TK, and MAC lakes abbreviated as CON_{DOM}, TK_{DOM}, MAC_{DOM}), and riverwater (RIV_{DOM}). Lake- and riverwater DOM was collected 4 d before the experiment commenced, immediately filtered (0.2 µm; Pall maxi culture capsule), and stored at 4°C until the experimental setup. The experiment was allowed to run for 7 d. Samples for bacterial, flagellate and ciliate abundances, and chitobiase activity were collected after 2 d, and at the end of the 7 d experiment. Samples for chlorophyll *a* (Chl *a*), rotifer, and larger zooplankton abundances were collected at the end of the 7 d experiment. Initial DOM samples were also collected for the analysis of DOC, and total dissolved nitrogen (TDN) and phosphorus (TDP), as outlined previously (e.g., Chapter 4). Experimental replicates were enclosed in 20 L cubitainers (Reliance Fold-a-Carrier, Reliance Products LP, Winnipeg, MB), and kept at *in-situ* temperatures using a recirculating water bath. To discourage the growth of phytoplankton, the incubations were kept shaded throughout the duration of the experiment, using a screening treatment that excluded 77% of total downwelling radiation (J. Gareis, Simon Fraser University, personal communication).

6.3.4 Laboratory analyses

Fatty acids

Samples for FA analyses were further sorted and cleaned at Simon Fraser University, to ensure uniform samples of late juvenile to adult zooplankton. Samples were thawed, and kept cool during processing by continued immersion in ice while under a dissecting microscope. Copepod samples were cleaned of any remaining naupliar and early copepodid (those with less than 5 segmented legs) organisms, and any non-cyclopoid

organisms. Samples were returned to a -80°C freezer directly after processing and freeze-dried once re-frozen.

All samples were sent to the laboratory of M.T. Arts (Canada Centre for Inland Waters, Burlington, ON) for analyses. Lipids were extracted from freeze-dried zooplankton samples as outlined in Schlechtriem et al. (2008). Extracted lipids were separated into polar and neutral (non-polar) fractions using a Sep-Pak silica cartridge (Waters Corp., Milford MA; Juaneda and Rocquelin 1985), and the neutral fraction retained. While the polar fraction of zooplankton fatty acids is largely comprised of membrane lipids which are relatively unaffected by diet, the neutral fraction is predominantly composed of storage lipids (such as triaclyglycerols) which reflect the dietary uptake of copepods (Caramujo et al. 2008). Fatty acids from the neutral fraction of the extracted lipid pool were derivatized to their methyl esters in a (2:1 vol:vol) solution of 1% sulfuric acid in methanol: toluene which served as the methylating (derivatizating) reagent (24 h at 50°C; Christie 1989). Lipids were analysed using gas chromatography, as outlined in Schlechtriem et al. (2008).

Stable isotopes

Samples collected in 2007 for stable isotope analyses were freeze dried before processing. A subset of the 2007 zooplankton samples were lipid extracted, following the modified Bligh and Dyer (1959) technique described in Tank et al. (2003). Lipid extraction accounts for the fact that lipids are ^{13}C depleted compared with whole organisms, and the lipid content of zooplankton is variable (Peterson and Fry 1987, Kling et al. 1992). We extracted a subset of samples to confirm a consistent relationship between zooplankton C:N and lipid extracted ^{13}C, as described in Smyntek et al. (2007). Samples for $\delta^{13}C$ and $\delta^{15}N$ of zooplankton were ground to a fine powder using a mortar and pestle, and powdered samples were acidified using 2N HCl to remove carbonates. Acidified, dried zooplankton samples then weighed into tin capsules (Elemental Microanalysis Limited). Filtered seston samples were fumed for 24 h with 2N HCl, and folded into tin capsules after the top layer of the filtered sample had been separated from the remaining filter. Filtered bacterioplankton samples were folded into tin capsules. All samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ by the G.G. Hatch Stable Isotope Laborotory (University of Ottawa, Canada) using a Vario III

elemental analyser (Elementar, Germnay) coupled to a DeltaPlus XP IRMS (ThermoFinnigan, Germany) via a ConFlo II interface.

Bacterial, protozoan, and zooplankton abundance

Samples for counts of bacteria, flagellates, and zooplankton were immediately preserved after collection from the experimental incubations. Samples for bacteria were preserved in glutaraldehyde (2.5% final concentration), and stored in the dark at 4°C until filtration and counting (within 6 months). To ensure even sample dispersal once filtered, samples were prepared following Bae Yoon and Rosson (1990). TWEEN-80 (10 ppm) was added to samples, which were incubated for 2 h at 4°C and then sonicated (30 s at 10 W). After sonication, DAPI was added to samples (final concentration 10 mg L^{-1}), which were filtered onto 0.22 µm black polycarbonate filters (GE Osmonics). Filters were rinsed with 2 mL of phosphate buffered saline both before and directly after filtering to clear excess humic material. Filtered samples were counted at 1000 x using a Zeiss Axioplan microscope fitted with a 50 W bulb (Osram HBO) and filters specific for the DAPI stain (Zeiss filter set 02).

To analyse protozoan (ciliate and heterotrophic nanoflagellate) abundance, triplicate 40 mL samples of unfiltered water from each experimental replicate was immediately fixed with a mixture of Lugol's solution, formalin, and sodium thiosulfate (Sherr and Sherr 1993). Samples were filtered onto 0.8 µm black polycarbonate filters (GE Osmonics) after staining with DAPI (1 mg mL^{-1}), within 1 week of collection. Samples were kept frozen (-20°C) until counting with epifluorescence microscopy, within 6 months of filtration. Ciliates were counted at 400x, by scanning multiple lengths of the filtered area. For flagellates, 15 fields of view or a minimum of 50 cells were counted at 1000x. All cells were checked for chlorophyll autofluroescence to ensure that phytoflagellates, which were rare, were not included in our counts. Samples collected for rotifers, copepods and cladocerans were preserved with sugared formalin. Samples were counted and sized at 50x, by fully enumerating triplicate subsamples taken from each preserved replicate, and sizing each enumerated organism using an ocular micrometer. Organism sizes were converted to biomass using the equations described in McCauley (1984) for copepods, and

Ruttner-Kolisko (1977) for rotifers, assuming a rotifer dry weight: biovolume ratio of 0.1 (McCauley 1984). Because of their infrequent occurrence (see Results), cladoceran biomass estimates were not undertaken.

Chitobiase assay

The activity of chitobiase within our experimental incubations was measured following Oosterhuis et al. (2000). Water samples from each experimental replicate were filtered (0.2 μm) prior to analysis to exclude particulate chitobiase from the assay. From each experimental replicate, duplicate 4.7 mL aliquots of filtered water were incubated with 100 μL of 150 μM Methylumbelliferyl N Acetyl β-d glucosaminide (MUFNAG, Sigma #M2133; dissolved in methylcellosolve, Sigma #360503) and 200 μL of a 0.5 M Tris/HCl buffer prepared to pH 7.5. The concentration of methylumbelliferon (MUF), which chitobiase acts to cleave from MUFNAG, was assessed as by measuring its fluorescence on a Turner 112 fluorometer (Sequoia-Turner Corp., Mountain View, CA), using broad-band filters (excitation = 310-390 nm; emission = 380-480 nm). Samples were incubated in a water bath at 25°C for 2 h, and fluorescence measured every 30 min. The change in fluorescence over time was converted to MUF liberated (nM h^{-1}) using a standard calibration curve created by measuring the fluorescence of MUF (Sigma #M1381) in filtered lakewater (4.7 mL) and Tris/HCl buffer (200 μL), at concentrations ranging from 0 to 38 nM.

6.3.5 Calculations and statistical analyses

Heterotrophic bacterial fatty acids (BFA) were calculated as the proportion of odd and branched-chain fatty acids relative to the total apolar pool extracted from zooplankton samples (Napolitano 1999, Dalsgaard et al. 2003). Phytoplankton (algal) fatty acids (AFA) were calculated as the proportion of algal-diagnostic fatty acids (18:3ω3, 20:5ω3, and 22:6ω3) relative to the total apolar pool (e.g., Napolitano 1999). Changes in the relative proportion of taxonomically-diagnostic fatty acids were assessed using both 1-way ANOVA, with lake type (CON, TK, MAC) as the factor, and a simple regressions comparing the prevalence of taxonomically-diagnostic fatty acids to sill elevation. When the ANOVA result

was significant, it was followed by a Tukey's HSD post-hoc test. For all regression analyses, adjusted r^2 values are reported.

Zooplankton $\delta^{15}N$ values were normalized to seston $\delta^{15}N$ in 2005, and seston and bivalve $\delta^{15}N$ in 2007, by subtraction. This normalization is necessary because the $\delta^{15}N$ signature at the base of aquatic food webs is known to vary between lakes (Post 2002). While seston $\delta^{15}N$ provides a 'snapshot' measurement of the isotopic baseline within a lake, filter feeders, such as bivalves, provide a baseline $\delta^{15}N$ that is integrated over a longer time period (Post 2002). In 2005, the variation in seston-corrected $\delta^{15}N$ zooplankton signatures across the lake elevation gradient was assessed using regression. In 2007, regression was again employed, in addition to a 1-way ANOVA analysis that compared differences in seston- and bivalve-corrected *Cyclops* sp. $\delta^{15}N$ between the 3 lake types (CON, MAC, TK).

Zooplankton $\delta^{13}C$ signatures were corrected for lipid content using the equation in Smyntek et al. (2007), after confirming that the $\delta^{13}C$ we measured for lipid-extracted zooplankton conformed to this calculation. Initially, we considered HB and phytoplankton as foodweb baselines for direct or indirect zooplankton consumption. Phytoplankton $\delta^{13}C$ was calculated in two ways: first, by using seston $\delta^{13}C$ as a proxy for the phytoplankton signature, and second, by calculating phytoplankton $\delta^{13}C$ following Bade et al. (2006), as outlined in Chapter 3, and constraining these results based on a maximum photosynthetic fractionation of 20‰ that is typically assumed between CO_2 and phytoplankton (Goericke et al. 1994, $^{13}CO_2$ derived from $DI^{13}C$ values given in Table 3.1, using carbonate equilibrium constants and equilibrium fractionation factors; Mook et al. 1974, Stumm and Morgan 1996). HB are typically $\delta^{13}C$ enriched in Delta lakes relative to seston and terrestrial DOM (Chapter 3), presumably as a result of preferential incorporation of ^{13}C-enriched macrophyte-derived DOM. We hypothesized that the HB-mediated contribution of macrophytic C to zooplankton biomass would be highest in high elevation, macrophyte-rich lakes, resulting in elevated zooplankton $\delta^{13}C$. Thus, we compared seston-normalized zooplankton $\delta^{13}C$ to sill elevation in both 2005 and 2007, using regression. For the 2007 samples, we additionally used 1-way ANOVA to investigate the difference in seston-normalized zooplankton $\delta^{13}C$ between the 3 lake types (CON, MAC, TK).

However, zooplankton $\delta^{13}C$ was almost always depleted when compared to both seston/phytoplankton, and bacteria (see Results). Therefore, we also assessed the contribution of methanotrophic bacteria (methane oxidizing; MOB), which are heavily ^{13}C depleted (e.g., Jones et al. 1999, Bastviken et al. 2003) to zooplankton C content. We used 2 contrasting methods. First, we compared seston-corrected zooplankton $\delta^{13}C$ to partial pressures of CH_4 (pCH_4), which were measured concurrently with zooplankton stable isotope collection in 2005 (pCH_4 methods outlined in Chapter 2). We hypothesized that increased CH_4 substrate would lead to increased MOB production (Kankaala et al. 2006), and thus greater zooplankton ^{13}C depletion if MOB consumption is proportional to MOB activity. Second, we undertook a single-isotope ($\delta^{13}C$ alone) mixing model that assessed the relative contribution of phytoplankton, HB, and MOB to zooplankton in June and Aug. of 2005, when isotopic measurements for each of these endmembers were available. We used measured $\delta^{13}C$ values for seston and heterotrophic bacteria, modeled $\delta^{13}C$ for phytoplankton (as described above), and literature $\delta^{13}C$ values for methanotrophic bacteria, which ranged from -50 to -86‰ (Bastviken et al. 2003, Taipale et al. 2007, Taipale et al. 2008). We used the IsoSource mixing model outlined in Phillips and Gregg (2003), and described in detail in Chapter 3. For each sampling period, in each lake, we considered 4 different models, by using measured seston $\delta^{13}C$ and modeled phytoplankton $\delta^{13}C$ as the endmember phytoplankton signature, and the two extreme literature values of MOB $\delta^{13}C$ as the MOB endmember.

The results of our incubation experiments were assessed using 1-way ANOVA for response variables measured only at the experiment's end, and repeated measures ANOVA (RM-ANOVA) for response variables measured repeatedly over time. All variables were assessed for normality before statistical analyses, which were conducted using SYSTAT 12 (Systat Software Inc., 2007). For the 1-way ANOVAs, a significant DOM effect was followed by post-hoc comparisons using a Tukey's HSD test. For RM-ANOVA, a significant DOM effect was followed by post-hoc pairwise comparisons, following Keppel (1982). Pairwise comparisons were assessed for significance using Dunn-Šidák corrected p-values (Sokal and Rohlf 1995) for n=6 total contrasts.

6.4 Results

6.4.1 Fatty acids

The proportion of BFAs in *Cyclops* sp. tissue, relative to the total apolar pool, differed significantly between lake types (Fig. 6.1a, 1-way ANOVA $F_{2,6}$=5.406, p=0.046). BFAs were highest in *Cyclops* sp. from MAC lakes and lowest in *Cyclops* sp. from TK lakes (Tukey's HSD contrasts; MAC>TK, p=0.040), while proportions did not differ between MAC and CON lakes (p=0.479), or between CON and TK lakes (p=0.187). Although the linear relationship between lake sill elevation and BFA was not significant (p=0.331), a marginal quadratic relationship did exist (p=0.057, Fig. 6.1a). In contrast, the proportion of AFA increased significantly with increasing sill elevation (p=0.028, Fig. 6.1b), but showed a marginal response to differences among lake types (1-way ANOVA $F_{2,6}$=4.308, p=0.069). Concentrations of BFA and AFA in *Cyclops* sp. tissue were also highly negatively correlated (Fig. 6.1c; p=0.041).

6.4.2 Stable isotopes: $\delta^{15}N$

In contrast to our expectation that zooplankton $\delta^{15}N$ would be highest in lakes with high rates of bacterial production, seston-normalized zooplankton $\delta^{15}N$ decreased unimodally with increasing sill elevation in the June 2005 samples (Fig. 6.2a), and was unrelated to sill elevation in July and August of 2005 (Fig. 6.2 b,c). In 2007, there was a non-significant tendency for normalized *Cyclops* sp. $\delta^{15}N$ to decrease with increasing sill, for seston-corrected samples (p=0.069; Fig. 6.3a), while bivalve-corrected $\delta^{15}N$ was uncorrelated to sill (p=0.123; Fig 6.3b). Comparisons between lake types using 1-way ANOVA were marginally non-significant for seston-corrected ($F_{2,6}$=4.388; p=0.067) and non-significant for bivalve-corrected ($F_{2,5}$=2.494; p=0.177) samples in 2007 (Fig. 6.3). Although the value of $\delta^{15}N$ is generally expected to increase by an average of 3.4‰ (range of 2-5‰) per trophic level (Post 2002), values of zooplankton $\delta^{15}N$, corrected to either seston, or bivalves, were often quite low. In both 2005 and 2007, a majority of samples fell below the average trophic fractionation of 3.4‰, and several fell below the lower expected

limit of fractionation of 2‰. Despite this low apparent trophic fractionation, however, zooplankton $\delta^{15}N$, corrected to seston, was positively correlated to the BFA/AFA ratio in zooplankton (Fig. 6.3c). The seasonal patterns for seston and uncorrected zooplankton $\delta^{15}N$ in 2005 revealed seston $\delta^{15}N$ to be relatively stable over time, within lakes, and zooplankton values to be much more variable (Fig. 6.2d). The range of variation of within-lake $\delta^{15}N$ signatures spanned from 0.32 to 2.16 for seston, and 1.47 to 3.39 for zooplankton. Paired t-test comparisons showed within-lake seasonal variation of seston $\delta^{15}N$ to be significantly less than that for zooplankton (t_4=2.754, p=0.026).

6.4.3 Stable Isotopes: $\delta^{13}C$

Contrary to our expectation that zooplankton $\delta^{13}C$ would lie between values measured for phytoplankton and bacteria, signatures (as mixed zooplankton in 2005, and *Cyclops* sp. in 2007) were almost universally ^{13}C depleted relative to measured $\delta^{13}C$ for seston, modeled signatures for phytoplankton, and, when applicable, measured signatures for bacteria (Fig. 6.4a-d). There was no relationship between sill elevation and seston-corrected zooplankton $\delta^{13}C$ in either 2005 (p=0.084, June; p=0.170, July; p=0.826, Aug.), or 2007 (p=0.763, data not shown). ANOVA analysis comparing seston-corrected zooplankton $\delta^{13}C$ across lake types in 2007 was also not significant ($F_{2,6}$=1.611, p=0.275). Zooplankton signatures that are $\delta^{13}C$ depleted have been attributed to the consumption of MOB (e.g., Jones et al. 1999, Bastviken et al. 2003, Kankaala et al. 2006), which exhibit $\delta^{13}C$ values that are considerably lower than those for phytoplankton and HB. Although we did not measure the abundance or isotopic signature of MOB in Delta lakes, we did measure pCH_4 concurrently with zooplankton $\delta^{13}C$ in 2005. The degree of zooplankton $\delta^{13}C$ depletion relative to seston $\delta^{13}C$ increased with increasing pCH_4 in June of 2005 (Fig 6.4e, p=0.035). Correlations for July and August of 2005 were not significant (Fig. 6.4f,g).

Mixing model results indicated that on average, the relative contribution of phytoplankton C to zooplankton biomass was greater than for HB (Fig. 6.5). However, the mixing models were extremely poorly constrained for contributions from these 2 endmembers, and the model output often spanned the full range of possible contributions.

The median potential contribution of both of these 2 endmembers to zooplankton biomass was not correlated to sill elevation (data not shown, $p>0.20$ in all cases). The relative contribution of MOB to zooplankton biomass was also not correlated to sill elevation ($p>0.40$ in all cases), or within-lake pCH_4 ($p>0.10$). However, results for MOB were considerably more constrained than for phytoplankton or HB (Fig 6.5). Except for in one low elevation lake, in which phytoplankton and HB could account for all zooplankton biomass, MOB always accounted for a small, but significant proportion of overall zooplankton biomass, ranging between 4 and 51% when MOB $\delta^{13}C$ was assumed to be -50‰, and 2 and 22% when MOB $\delta^{13}C$ was assumed to be -86‰ (Fig. 6.5).

6.4.4 Experimental incubations

The mesozooplankton community in the experimental incubations was dominated by copepods. The calanoid *Diaptomus* sp. occurred in greatest abundance, while *Cyclops* sp. was also common (Fig. 6.6). *Heterocope* sp. was observed sporadically in our samples (data not shown). Although cladocerans were also observed, they were rare, and did not occur in every sample. *Daphnia longiremis*, *D. middendorffiana*, *Ceriodaphnia* sp., *Bosmina longirostris*, and *Simocephalus serrulatus* were each observed in no more than 2 of the 12 experimental replicates, at densities ≤ 1.2 organisms L^{-1} (data not shown). The microzooplankton community was dominated by the rotifers *Keratella cochlearis* group, and *Kellikottia* sp. Although *Polyarthra* sp. was observed in the initial samples at a density of 7.0±1.2 (standard error) organisms L^{-1}, this genus was absent from all incubated samples. Concentrations of DOC, TDN, and TDP were similar across incubations (Table 6.2).

Lower trophic level organisms showed little response to being incubated with naturally-occurring DOM from different sources (CON_{DOM}, TK_{DOM}, MAC_{DOM}, and RIV_{DOM}). While bacterial abundances were greater on MAC_{DOM} than CON_{DOM}, they did not differ between any other substrate type (Table 6.3, Fig. 6.6). Similarly, there were no significant differences between treatments for flagellate or ciliate abundances (Table 6.3, Fig. 6.6).

Rotifers and nauplii showed a stronger response to the DOM incubation treatment (Table 6.4, Fig. 6.6). Rotifer biomass was highest when incubated with TK_{DOM} and RIV_{DOM},

and lowest when incubated with MAC_{DOM} and CON_{DOM}. Nauplii biomass was higher on TK_{DOM} than on MAC_{DOM} or CON_{DOM}, and higher on RIV_{DOM} than on MAC_{DOM} (Table 6.4, Fig. 6.6). In contrast, *Diaptomus* sp. and *Cyclops* sp. biomass, and chitobiase activity, did not differ between treatments. There was a marginal effect of DOM substrate on Chl *a* concentrations ($p=0.097$; Table 6.4), and a marginal tendency for concentrations to be higher in the RIV_{DOM} treatment than in the MAC_{DOM} treatment (Tukey's post-hoc test, $p=0.079$; Fig. 6.6). Overall, there was a tendency for the biomass of higher trophic level organisms to be correlated to Chl *a* concentrations (rotifer $r^2=0.321$, $p=0.032$; nauplii $r^2=0.289$, $p=0.039$; *Diaptomus* sp. $r^2=0.320$, $p=0.032$), while there were no obvious relationships between zooplankton and bacteria. None of these correlations were significant when corrected for multiple comparisons, however.

6.5 Discussion

6.5.1 Evidence from multiple tracers for the importance of differing basal resources to zooplankton

Analysis of BFAs suggests that zooplankton derive a greater proportion of their biomass from HB-based food webs in lakes where HB production and HB growth efficiency tend to be greatest. BFAs in zooplankton tissue were highest in MAC lakes, where both DOM substrate quality for bacteria, and bacterial production and growth efficiency, are known to be elevated (Chapter 4). The importance of bacteria as a biomass "link" to higher trophic levels, as opposed to a metabolic "sink" for C, has long been a focus of study in aquatic ecology (e.g., Ducklow et al. 1986, Sherr et al. 1987). Previous studies have shown bacteria fed highly labile DOM substrates to be contributors to zooplankton biomass, albeit with a very low transfer efficiency (Ducklow et al. 1986, Koshikawa et al. 1996, Havens et al. 2000), while zooplankton from experimental systems manipulated to have extremely high bacterial, relative to algal, production have been shown to derive a substantial proportion of their biomass from HB-based food webs (Blomqvist et al. 2001, Berglund et al. 2007). To our knowledge, however, this is the first time that evidence has been presented for variable transfer of bacterial C to higher trophic levels in concert with variations in DOM quality, and

as a result HB production, in natural systems. Mackenzie Delta lakes are somewhat unique when compared to the deep, temperate lakes that are commonly the basis for limnological study. As in other productive ecosystems, high levels of primary productivity in these lakes appear to generate labile bacterial substrates as a byproduct. However, autotrophic production in Delta waterbodies is overwhelmingly macrophtyic, rather than algal, in origin. While high levels of planktonic algal productivity will likely swamp the importance of bacterially-based food chains, phytoplankton-poor Delta lakes may be particularly well suited to detecting a signal of bacterial biomass in higher trophic levels.

In addition to their variability across lake types, our zooplankton BFA data also suggest that HB provide a measureable component of the organic matter transferred to zooplankton across all Delta lakes. Fatty acids diagnostic of direct, or indirect, consumption of HB were found in all zooplankton samples, ranging from ~2-5% of the total pool. While bacteria are a known component of the gut flora of zooplankton, BFAs from this source alone typically occur at concentrations lower than the levels we observed (Desvilettes et al. 1997). The values we obtained for zooplankton BFAs are well within the range of other literature values (e.g., Ederington et al. 1995, Kainz and Mazumder 2005, Perga et al. 2006), suggesting that Delta zooplankton do not receive a particularly remarkable proportion of organic matter from HB sources. However, we note that many investigations of zooplankton BFA content have been conducted on pure samples of cladocerans, or mixed samples dominated by these filter feeders (e.g., Kainz and Mazumder 2005, Perga et al. 2006), which typically feed at a lower trophic level than copepods (Matthews and Mazumder 2003, Karlsson et al. 2004). Thus, the zooplankton BFA signal that we observe may be somewhat muted compared to other literature values.

In lakes where the BFA/AFA ratio in zooplankton tissue was greatest, there was a tendency for food webs to be longer, as assessed by $\delta^{15}N$. A clear increase in zooplankton $\delta^{15}N$ has also been observed in experimental systems where bacterially-based food webs are manipulated to dominate over their phytoplankton-based counterparts (Berglund et al. 2007), as would be expected given the greater number of trophic linkages required between bacteria and zooplankton. The bacterial $\delta^{15}N$ signature in Delta lakes is, on average,

enriched relative to seston $\delta^{15}N$ (by 2.3‰±0.5, 95% confidence interval; data not shown), which might also contribute to higher zooplankton $\delta^{15}N$ where the bacterial contribution to higher trophic levels is greatest. Despite these trends, however, zooplankton $\delta^{15}N$ values normalized to seston were typically low, and often less than the amount expected as a result of a single trophic transfer (2-5‰, Post 2002): a notable result given that copepods are typically omnivorous or carnivorous (Hopp et al. 1997, Brandl 2005). A low C:N ratio for zooplankton food is one potential explanation for the small trophic fractionation that we observe: laboratory studies have shown fractionation to decrease with decreasing food source C:N (Adams and Sterner 2000). The C:N of seston collected during our surveys ranged from 4 to 9.5, which would be expected to lead to fractionation between 0.8 and 2.2‰ based on previously calculated relationships (Adams and Sterner 2000); clearly lower than those reported for broad-based lake surveys. Additionally, the C:N of microzooplankton and bacteria should lie close to the Redfield ratio of ~7, which might also result in a relatively low $\delta^{15}N$ fractionation in trophic transfers to copepods. Alternatively, however, the low $\delta^{15}N$ observed in Delta zooplankton relative to seston could indicate switching between multiple basal resources, one of which possesses a basal isotopic signature that is relatively $\delta^{15}N$ depleted. Large seasonal variations in zooplankton $\delta^{15}N$, relative to the sestonic baseline (Fig. 6.2), suggest that switching between different trophic pathways, with different basal $\delta^{15}N$ signatures, may indeed be occurring for zooplankton in these lakes (e.g., Matthews and Mazumder 2007).

Although HB tend to be $\delta^{15}N$ enriched relative to seston in Delta lakes, MOB do have the potential to serve as a $\delta^{15}N$-depleted basal resource in these waterbodies. MOB preferentially use nitrate as an N source (aquatic $\delta^{15}NO_3$ values typically range from -5 to +10‰; Fogg et al. 1998), which undergoes an estimated fractionation of ~-8.6‰ during incorporation into MOB tissue (Taipale et al. 2008). Thus, MOB $\delta^{15}N$ would be expected to be at, or below the ~1.5-6‰ $\delta^{15}N$ values observed for seston and HB in these lakes. Our $\delta^{13}C$ results, in particular, point to methanotrophs as a consistent source of organic matter to zooplankton in Delta lakes. Zooplankton across a wide range of lakes have often been observed to be $\delta^{13}C$ depleted relative to seston (del Giorgio and France 1996), which has

been attributed to the ingestion of MOB (Jones et al. 1999, Kankaala et al. 2006). Because of their extreme $\delta^{13}C$ depletion (typical range of -50 to -86‰; see Methods), even a small proportion of direct, or indirect, MOB consumption by zooplantkton will significantly affect $\delta^{13}C$ signatures. Based on the observed relationship between zooplankton $\delta^{13}C$ and pCH_4, it appears that MOB might be most important to zooplankton in the spring to early summer, when pCH_4 levels can be extremely high as a result of recent under-ice accumulation (Pipke 1996), and MOB production might therefore be substantial (Kankaala et al. 2006).

Mixing models showed a small, but consistently significant contribution of MOB organic matter to zooplankton biomass across all lake types, in both June and August. The results of our mixing models would have differed, however, if the $\delta^{13}C$ of the other two basal resources (HB, phytoplankton) were not universally $\delta^{13}C$-enriched when compared to zooplankton. In particular, phytoplankton $\delta^{13}C$ is notoriously difficult to calculate. Seston is never entirely composed of algae, so sestonic $\delta^{13}C$ can only provide an estimate of phytoplankton $\delta^{13}C$. In particular, seston often contains terrestrial detritus, which typically has a $\delta^{13}C$ of -28‰, and could therefore result in an enriched phytoplankton $\delta^{13}C$ estimate, if based solely on seston $\delta^{13}C$. Phytoplankton $\delta^{13}C$ is often calculated from measured $^{13}CO_2$, using an estimated fractionation factor (ε_p) for the incorporation of this C into phytoplankton biomass during photosynthesis. However, although the theoretical maximum for ε_p is well understood (~-29‰; Goericke et al. 1994; Hecky and Hesslein 1995), this is likely closer to -20‰ in aquatic systems (Goericke et al. 1994, Chanton and Lewis 1999), and modeled values of ε_p based on realistic *in situ* conditions from various lake regions are generally much lower than this (e.g., ~-6‰, Cole et al. 2002; 0 to -20‰, Bade et al. 2006; -8 to -15‰, McCallister et al. 2008). Recent work comparing phytoplankton $\delta^{13}C$ estimates obtained by calculating ε_p to methods such as measuring the $\delta^{13}C$ of phytoplankton that had been physically separated from bulk seston, or calculating phytoplankton $\delta^{13}C$ based on Chl *a* and total organic C within the sestonic pool, suggests that estimating phytoplankton $\delta^{13}C$ using $^{13}CO_2$ and an assumed ε_p could lead to unrealistic results (Bade et al. 2006, Marty and Planas 2008). In particular, ε_p is known to decrease with decreasing CO_2 concentrations (Hollander and McKenzie 1991), and extremely low

concentrations of CO_2 are commonly observed in macrophyte-rich Delta lakes (Hesslein et al. 1991, Chapter 2). Calculating the magnitude of this decrease *in situ*, for multiple species assemblages, is difficult (e.g., Popp et al. 1998). Direct use of HCO_3^-, rather than $CO_{2(aq)}$, for photosynthesis, can also increase phytoplankton $\delta^{13}C$ and thus decrease ε_p, because of differences in isotopic signature between these C species (Mook et al. 1974). In this study, we estimated phytoplankton $\delta^{13}C$ using measured planktonic Chl *a* concentrations, and previously published estimates of C:Chl and total organic C in Delta lakes (Anema et al. 1990, Ramlal et al. 1991, Bade et al. 2006), rather than by calculating fractionation using an assumed ε_p. The method we used has been shown to give results for phytoplankton $\delta^{13}C$ that are statistically indistinguishable from signatures obtained by measuring phytoplankton physically separated from bulk seston (Marty and Planas 2008). Except for when pCO_2 was substantially below atmospheric equilibrium in these lakes (<100 μatm), the ε_p values that result from these phytoplankton $\delta^{13}C$ calculations varied between -14 and -20‰; well within the range cited above for lakes. In lakes experiencing extremely low pCO_2 (sill elevations of 3.4 and 4.6 m in August) calculated phytoplankton $\delta^{13}C$ is nearly identical to measured bulk $DI^{13}C$ (Table 3.1), indicating non-discriminatory uptake of DIC, which would largely be composed of HCO_3^- under these conditions. Thus, we feel confident that our phytoplankton $\delta^{13}C$ estimates are reasonable, and provide a useful supplement to estimates of phytoplankton $\delta^{13}C$ based solely on seston. However, if a maximum fractionation of -20‰ were assumed for all lakes, zooplankton from low elevation, connected lakes would typically be $\delta^{13}C$ enriched relative to phytoplankton (i.e., display $\delta^{13}C$ signatures between those of phytoplankton and bacteria), while zooplankton from macrophyte-rich and thermokarst lakes would typically continue to be $\delta^{13}C$ depleted when compared to phytoplankton (data not shown).

In addition, we found differences in algal contribution to zooplankton biomass among lake types, based on the proportion of AFAs in zooplankton tissue. Zooplankton AFA was proportionately highest in the highest elevation Delta lakes (in our survey, TK lakes; Fig 6.1), while proportions of AFA and BFA in *Cyclops* sp. tissue were strongly negatively correlated (Fig. 6.1). Algal biomass, as assessed by Chl *a*, does not tend to vary among Delta

lake types (Chapter 4; Table 4.2, Fig. 4.3). However, this result suggests that HB may substitute for, rather than augment, algae as a basal resource in some Delta lakes. In lakes where DOM is relatively nutrient rich and dissolved inorganic P levels are low (as appears to occur in macrophyte rich Delta lakes, Chapter 4), bacteria may be both abundant, as well as a nutrient-dense basal resource. While phytoplankton C:nutrient ratios inherently track the availability of inorganic nutrients, bacterioplankton tend to be relatively nutrient rich, with stoichiometric ratios that are much more tightly constrained (Healey and Hendzel 1979, Sterner and Elser 2002).

6.5.2 Experimental evidence for the importance of differing basal resources to zooplankton

In contrast to the results for our tracer investigations, experimental incubations showed no evidence that variation in DOM quality, leading to concurrent variation in bacterial abundance, resulted in differential transfer of bacterial organic matter to higher trophic levels. There are several possible reasons for the overall lack of effect seen at the highest trophic level for our experimental incubations. First, the duration of our experiment (7 d) may not have been substantial enough to observe a significant effect. We chose the experimental length based on previously published studies that had that had found significant differences in energy transfer to higher trophic levels, based on variation of several different experimental parameters (e.g., Urabe et al. 2003, Karlsson et al. 2007). However, our target zooplankton taxa were dominated by omnivorous/carnivorous species, rather than filter feeders, while energy transfer to zooplankton through bacterially-based food webs requires an elevated number of trophic links. Either of these factors could have inhibited our ability to see a significant effect over the course of this relatively short-term experiment. Second, the DOM substrates that we used varied relatively modestly in comparison to one another. Although substrates taken from different Delta lake and river sources do support concomitant variations in HB productivity (Chapter 4), and did result in variable bacterial abundance in this study (Fig. 6.6), the use of extremely labile DOM substrates may have produced a more pronounced effect. Because of the high

concentrations of soluble reactive phosphorus in macrophyte leachates from Delta lakes (Chapter 4), and thus potential confounding effects of rapid algal growth in leachate-amended treatments, we chose to not use this substrate for our experiments.

Even in the relatively inorganic-nutrient poor natural DOM substrates used in our experiment, we observed a non-significant tendency for Chl *a* to vary between treatments. This occurred despite the fact that all algae were filtered out of the DOM substrates, and the unfiltered (lakewater) component used for replicate experimental incubations was obtained within several hours, from one lake, with samples randomized over treatments. Significant differences in mid-trophic level organisms (rotifers, nauplii) largely mirrored the Chl *a* trend, suggesting that algal, rather than bacterial, production was most important for structuring higher trophic levels during our experiment. Given the shorter trophic distance between algae, compared to bacteria, and zooplankton, it seems that this unexpected variation in algal productivity, while less pronounced than differences between treatments for bacteria, could have overwhelmed the bacterial signal to higher trophic levels.

Although chitobiase is a much more sensitive measure of zooplankton productivity than measurements of overall change in zooplankton biomass, we did not see a significant difference between treatments in total enzyme activity. In part, this may have occurred because the production of chitobiase is concurrently balanced by its breakdown in the water column, largely as a result of bacterial activity (Oosterhuis et al. 2000). Thus, if bacterial decomposition of chitobiase varies between treatments, the measurement of static enzyme activity does not give an accurate assessment of variability in enzyme production between treatments. Although these measurements are possible (Oosterhuis et al. 2000), we were unable to accurately assess enzymatic breakdown in our experimental incubations. Based on measured bacterial abundance, and previous measurements of bacterial production on these DOM substrates (Chapter 4), it seems likely that bacterial decomposition of the chitobiase enzyme was greatest in MAC_{DOM} incubations, where total chitobiase activity was also greatest (albeit not significantly) by the end of the experiment. Overall, our experimental results suggest that the experimental duration may have been too short to detect changes in the transfer of bacterial biomass to higher trophic levels, and that

small changes in algal biomass may swamp the bacterial signature. In concert with the results from our surveys, this indicates that variation in bacterial transfer to higher trophic levels might be most easily detected under *in situ*, rather than experimental, conditions, where multiple factors acting over long time scales are inherently incorporated into measured trends.

6.5.3 Study implications

Overall, this work supports a modest, but significant, difference in the importance of HB to higher trophic levels between the contrasting lake types found within the Mackenzie Delta ecosystem. If the trophic transfer of bacterial organic matter to higher trophic levels is adding to the energy flow from algal-based food webs, then bacteria may truly be acting as a trophic "link" in bacterially-productive Delta lakes; increasing the amount of biomass available to pelagic consumers as a result of bacterial growth on DOM, which is otherwise unavailable to higher trophic levels (Pomeroy 1974). However, the strong negative relationship between AFA and BFA in zooplankton suggests that bacterially-based food webs may substitute for, rather than augment, food webs with phytoplankton as the basal resource. Bacterially-based food webs typically require an additional 1-2 trophic linkages to transfer biomass to zooplankton (Porter 1996). Thus, even when bacterial communities use their DOM substrate with high growth efficiencies (and thus, a majority of DOM is transformed into living biomass, rather than lost as CO_2 during respiration), bacterial foodwebs may act as an energy sink. The transfer efficiency of biomass between trophic levels is typically around 10% (Wetzel 2001). Because of the extra trophic levels in bacterially-based food webs, aquatic systems experimentally modified to have high bacterial, relative to algal, production rates, often show sharp declines in zooplankton biomass (Blomqvist et al. 2001, Berglund et al. 2007). Thus, in Delta lakes where bacteria may be able to outcompete algae as a result of the availability of highly labile bacterial substrate, or the poor availability of inorganic C for phytoplankton photosynthesis as a result of rapid macrophytic growth, decreased energy transfer to higher trophic levels may in fact result.

Our work also suggests that MOB could be an important component of the overall foodweb in Delta lakes. Given the extremely high levels of CH_4 that can occur in these waterbodies (Pipke et al. 1996, Chapter 2), MOB activity could be considerable in comparison to many other systems. CH_4 concentrations in Delta lakes appear to increase with increasing lake elevation, and are considerably elevated in TK lakes (Chapter 2). This has two potential implications: first, that the high CH_4 observed in macrophyte-rich lakes, which likely occurs as a result of the anaerobic breakdown of this autotrophic biomass (Chapter 2), allows for increased productivity of MOB. Thus, in addition to augmenting HB productivity, the high rates of macrophyte growth observed in Delta lakes could also be augmenting MOB growth in the oxic water column (as well as the production of methanogens in anaerobic sediments). Second, thermokarst, which is known to result in extremely high levels of CH_4 (Walter et al. 2006, Chapter 2), could cause a shift in the importance of different classes of bacteria in the lakes in which it occurs. The growth of MOB may be particularly strong in TK lakes given the longer passage time of CH_4 through the water column in these relatively deep lakes (Bastviken et al. 2008). Understanding how differences in the relative importance of HB, compared to MOB, might affect microbial, and higher, food webs, could therefore add significantly to our understanding of the future functioning of northern, thermokarst-affected lakes.

6.6 References

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6.7 Tables

Table 6.1: Overview of lakes surveyed for stable isotopes in 2005 (6 lake survey; 6LS), and stable isotopes and fatty acids in 2007 (9LS). Lakes from which DOM samples were collected for the incubation experiment are also indicated. Lake types are specified as connected (CON), macrophyte-rich (MAC) and thermokarst (TK), with lake numbers given in brackets. Macrophyte biomasses from thermokarst lakes are largely composed of *Chara* sp., a macroalgae that forms dense stands of multi-year growth.

Lake Type	Sample collection	Sill Elevation (masl)	Macrophyte biomass (g m^{-2})[a]	Thermokarst extent (%)[b]
CON (L129)	6LS, 9LS, DOM	2.363	15.9	0
CON (L80)	6LS, 9LS	2.631	7.3	0
CON (L79a)	9LS	2.631	ND	0
MAC (L87)	6LS	3.389	177.2	0
MAC (L280)	6LS, 9LS	3.838	89.0	0
MAC (L278)	9LS	4.077	359.0	0
MAC (L56)	6LS, 9LS, DOM	4.623	87.9	0
TK (L520)	6LS, 9LS, DOM	4.913	2446 (multi-year)	100
TK (L181)	9LS	5.169	453.4 (multi-year)	100
TK (L143)	9LS	5.169	280.2 (multi-year)	75

[a] Data from Squires et al. 2002

[b] As determined in Chapter 2.

Table 6.2: Measured dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) concentrations for DOM from riverwater (RIV), and macrophyte-rich (MAC), thermokarst (TK), and connected (CON) lakes used as substrates in the incubation experiments.

DOM Source	DOC ($\mu g\ L^{-1}$)	TDN ($\mu g\ L^{-1}$)	TDP ($\mu g\ L^{-1}$)
RIV	6773	249	4
MAC	9919	461	8
TK	9324	419	4
CON	5866	217	4

Table 6.3: Results of repeated measures ANOVA for experimental incubations. Significant results are highlighted in bold. Significant main effects for the DOM treatment were followed by contrasts, which are shown from lowest to highest treatment mean. DOM treatments for connected (CON), macrophyte-rich (MAC), and thermokarst (TK) lakes, and riverwater (RIV) are shown; treatments sharing a common underscore are not significantly different from one another. NSD indicates inappropriate contrasts because of non-significant main effects.

		Bacteria		Flagellates		Ciliates		Chitobiase	
Source	df	F	p	F	p	F	p	F	p
Main effects									
DOM	3	4.261	**0.045**	0.883	0.490	1.941	0.202	0.230	0.873
Error	8								
Time	1	2.864	0.129	0.407	0.541	49.087	**0.000**	0.087	0.776
Time x DOM	3	0.211	0.886	3.352	0.076	2.491	0.134	1.636	0.257
Error	8								
Contrasts		CON RIV TK MAC		NSD		NSD		NSD	

Table 6.4: Results of one way ANOVA for experimental incubations. Significant results are highlighted in bold. Significant ANOVA results were followed by contrasts, which are shown from lowest to highest treatment mean; treatments sharing a common underscore are not significantly different from one another. DOM treatments for connected (CON), macrophyte-rich (MAC), and thermokarst (TK) lakes, and riverwater (RIV) are shown. NSD indicates inappropriate contrasts because of non-significant ANOVA results.

Source	*df*	F	p	Contrasts
Chl *a*	3	2.968	0.097	NSD
Error	8			
Rotifer DW	3	15.021	**0.001**	MAC CON RIV TK
Error	8			
Nauplii DW	3	12.818	**0.002**	MAC CON TK RIV
Error	8			
Cyclops sp. DW	3	1.190	0.373	NSD
Error	8			
Diaptomus sp. DW	3	2.169	0.170	NSD
Error	8			

6.8 Figures

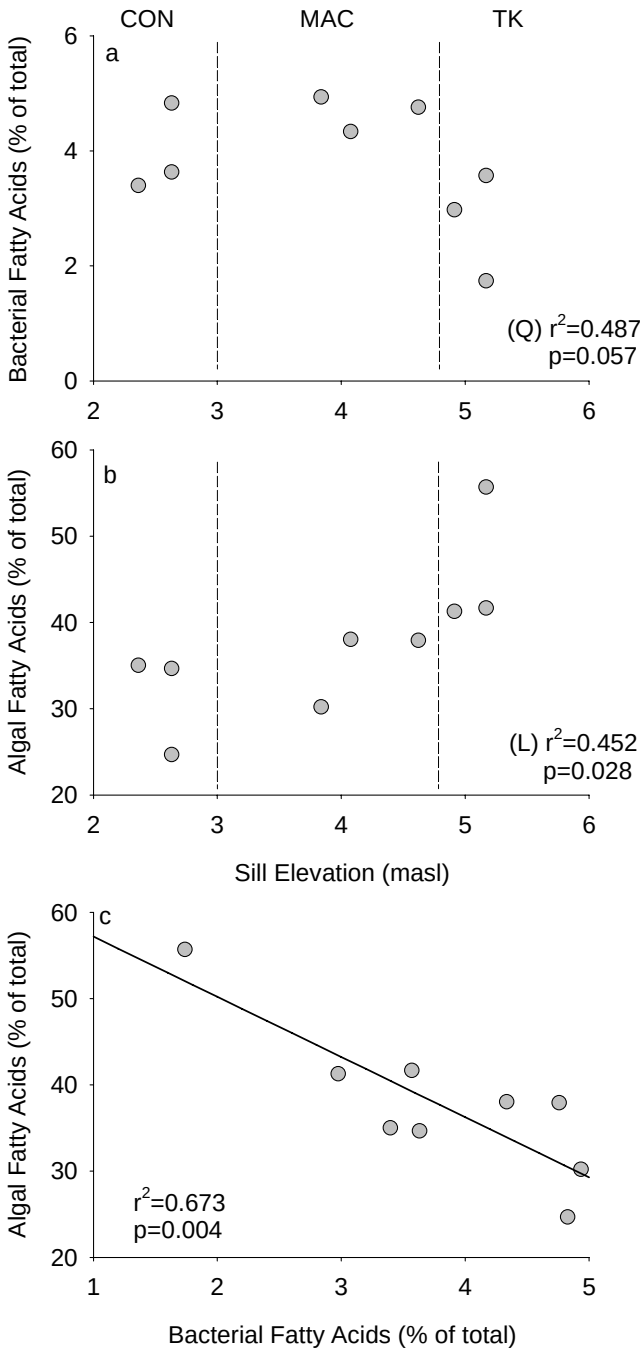

Figure 6.1: Bacterial- and algal- specific fatty acids in zooplankton, expressed as a percentage of the overall apolar pool. Panels (a) and (b) show the relationship between bacterial- and algal-specific fatty acids, respectively, and sill elevation, with dashed lines separating connected (CON), macrophyte-rich (MAC) and thermokarst (TK) lakes. r^2 and p values given are for the best fit quadratic (panel a) and linear (panel b) relationships for the data. Panel (c) shows the relationship between bacterial- and algal-specific fatty acids.

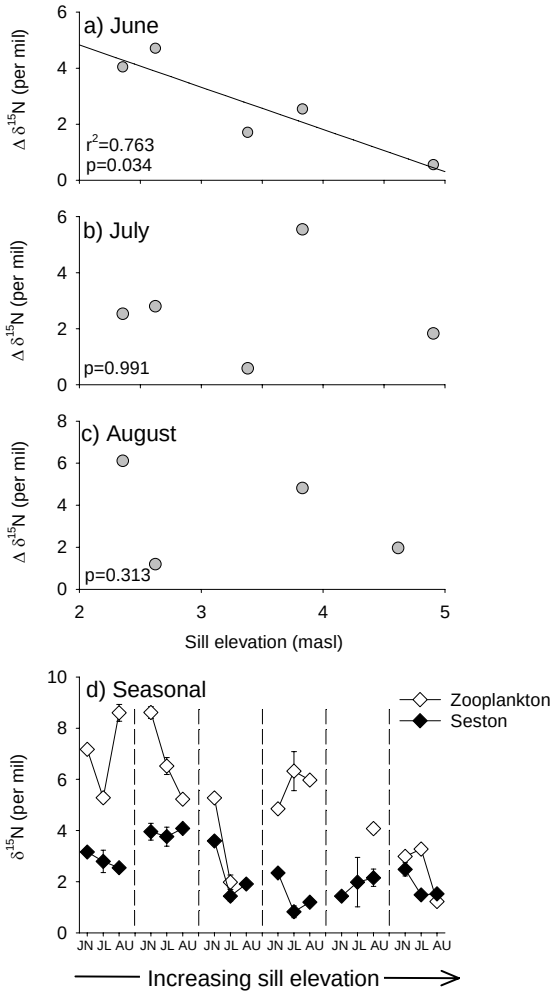

Figure 6.2: Survey results for 2005 zooplankton $\delta^{15}N$ values. Shown are zooplankton values normalized to seston for the June (panel a), July (panel b), and August (panel c) surveys, and non-normalized zooplankton and seston values displayed seasonally (panel d). r^2 and p values are displayed for the best linear fit through the data in panels a through c, with r^2 values and the line of best fit given for significant relationships only.

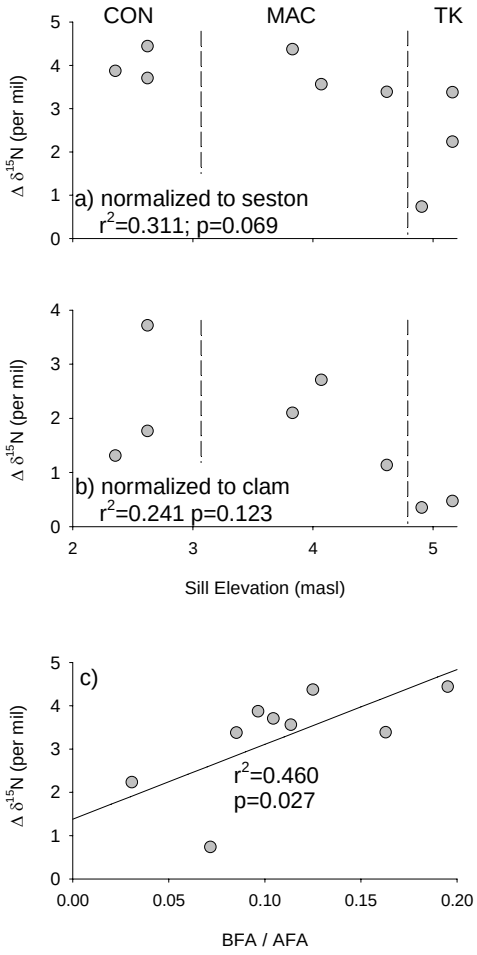

Figure 6.3: Survey results for *Cyclops* sp. $\delta^{15}N$ in 2007. Results are normalized to seston in panels a and c, and normalized to the bivalve signature in panel b. r^2 and p values are shown for the best linear fit through the data. Panels (a) and (b) show the relationship with sill elevation, while panel (c) shows the relationship with the ratio of bacterial fatty acids to algal fatty acids (BFA/AFA) in zooplankton. In panels (a) and (b), dashed lines separate connected (CON), macrophyte-rich (MAC), and thermokarst (TK) lakes.

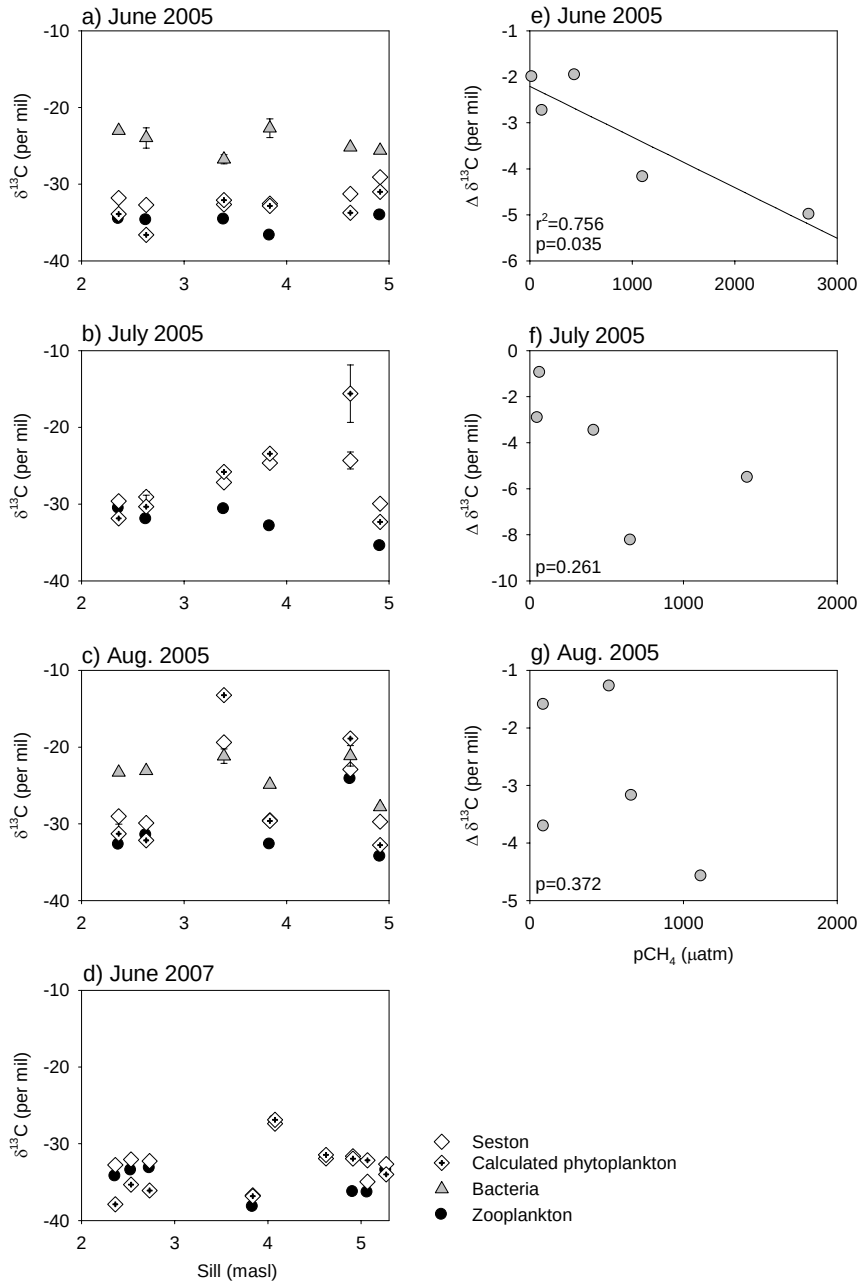

Figure 6.4: Survey results for $\delta^{13}C$ during the 2005 and 2007 surveys. Panels (a) through (d) show results for zooplankton (black circles), bacteria (grey triangles; panels a and c only), seston (white diamonds) and calculated phytoplankton (dotted white diamonds) signatures. Data in panels a-d are grouped by sill elevation; each lake surveyed has a unique sill. Panels e – g show the relationship between zooplankton $\delta^{13}C$ corrected to seston and the partial pressure of CH_4 (pCH_4) measured concurrently in the surveyed lakes in June (e), July (f), and August (g) of 2005. p-values are shown for the best linear fit through the data. Where the regression is significant, r^2 values are also provided.

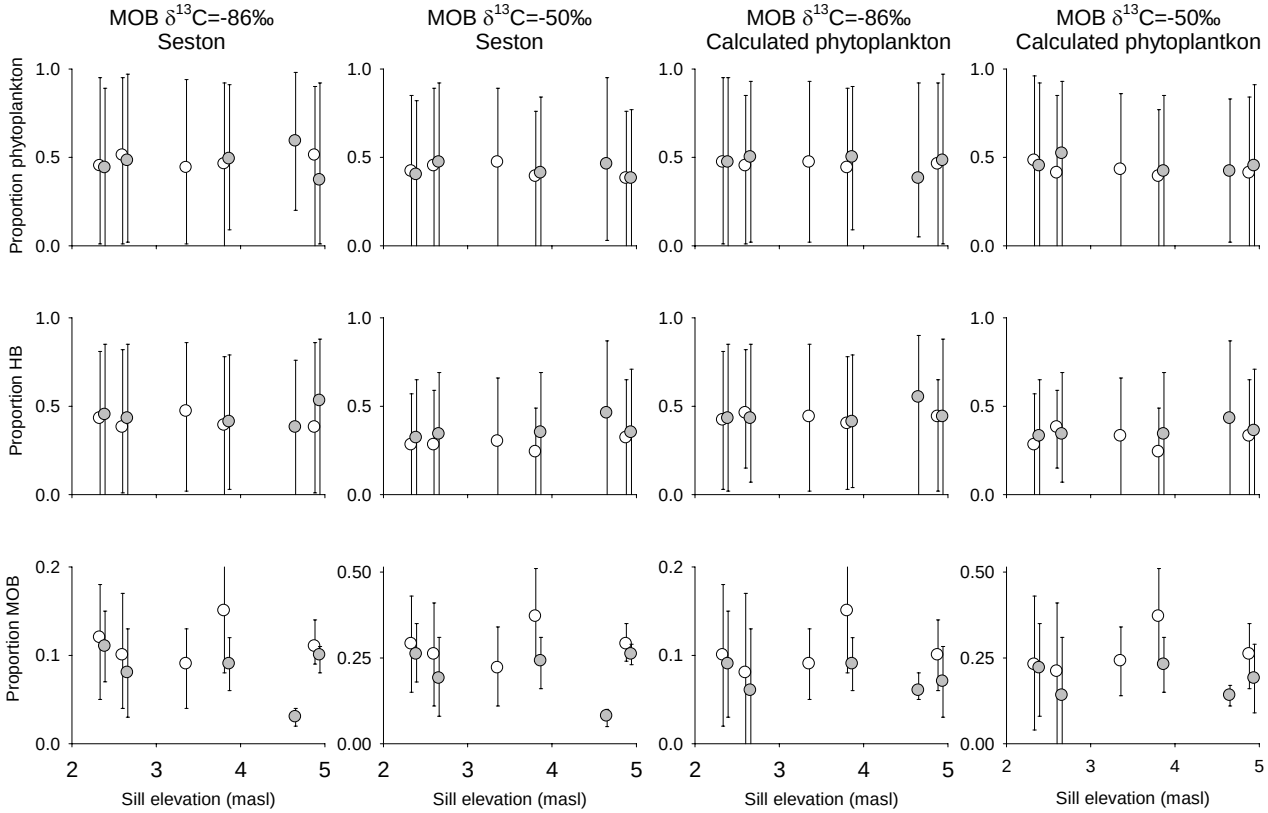

Figure 6.5: Results of the $\delta^{13}C$ mixing model. Each panel shows the median (circles) and total range (error bars) of possible values output from the mixing models for runs conducted using June (white) and August (grey) survey data. Shown are the proportion of phytoplankton (top row), heterotrophic bacteria (HB; middle row) and methanotrophic bacteria (MOB; bottom row) contributing to zooplankton biomass, for models run assuming two different ^{13}C values for MOB (-50 and -86‰) and ^{13}C values for phytoplankton that were either calculated, or based on ^{13}C measured for seston. Different model runs are shown as indicated in different columns.

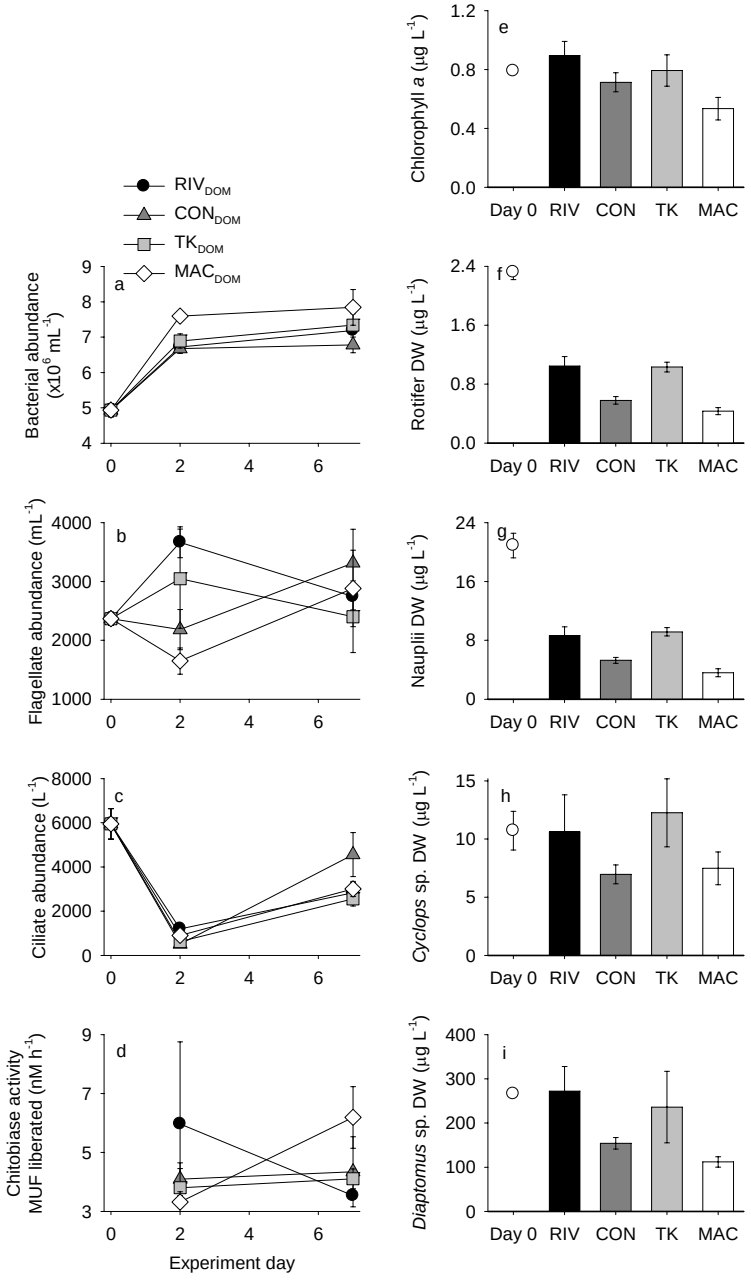

Figure 6.6: Abundances and total biomass estimates for bacteria, protozoans, and zooplankton, and total chitobiase activity measured during the incubation experiment,. In panels e-i, measurements taken at Day 0, and at the termination of the experiment, are shown. Error bars show ±1 standard error of the mean, for DOM from riverwater (RIV), and connected (CON), thermokarst (TK), and macrophyte-rich (MAC) lakewater.

CHAPTER 7 GENERAL CONCLUSIONS

7.1 Thesis summary

This study examined the importance of two relatively understudied sources of dissolved organic carbon (DOC) to aquatic environments. Overwhelmingly, the results indicate that the source of DOC to the within lake pool is an important regulator of energy flow and food web structure in Mackenzie Delta lakes. In Chapter 2, high DOC concentrations *per se* were not sufficient to push a lake towards net heterotrophy (*sensu* Sobek et al. 2005, Hanson et al. 2003). Instead, consideration of the source of the carbon (C) was clearly required in order to understand the effect of DOC on CO_2 balance. This is perhaps best demonstrated by the dichotomy between CO_2 flux from macrophyte-rich, and thermokarst affected lakes. While the DOC pool in the former appears to be derived largely from subsidies of long-sequestered C, the pool in the latter is produced through either evapoconcentration, which tends to results in a recalcitrant DOC pool (e.g., Arts et al. 2000), or as a byproduct of photosynthesis, and thus by definition requires CO_2 consumption for its creation.

The results of Chapter 3 indicate that the standing DOC pool within a lake is not necessarily a proportional representation of DOC delivered to the waterbody, or produced within the lake, because different DOC sources are differentially incorporated into bacterial biomass, as has been shown previously for algal versus terrestrial substrates (Kritzberg et al. 2006). Likewise, the results from Chapter 4 indicate clear differences in lability between DOC substrates, with macrophyte-derived DOC supporting rapid rates of C cycling overall, and bacterial production specifically. In Chapter 6, lakes known to contain pools of more labile DOC substrates appear to also show a greater contribution of bacteria to higher trophic levels.

The results of this thesis also demonstrate, however, that bottom up (substrate quality) and top down (food web interactions and ecological stress) processes must both be considered to gain a complete understanding of bacterially-mediated C cycling in aquatic environments. In particular, the work in Chapter 5 reveals that elevated pH can profoundly affect the ability of bacteria to process the DOC that is often formed as a byproduct of rapid photosynthesis, particularly over the short term. Given that elevated pH commonly

accompanies high levels of primary productivity, this effect may be common to many lakes and lake regions globally. While the importance of substrate quality for shaping bacterial metabolism is often considered (e.g., del Giorgio and Cole 1998), the role of ecological stress has been poorly examined.

Finally, this work suggests that future changes predicted for the Mackenzie Delta ecosystem in particular, and aquatic ecosystems more generally, could have a clear and lasting effect on energy flow and food web structure in lakes. In the Delta, falling peak water levels (Lesack and Marsh 2007) should increase water clarity in mid-elevation lakes, potentially acting to augment macrophyte densities in these waterbodies, while inputs of riverwater DOM decline. Similarly, the incidence of thermokarst is projected to increase. This raises the possibility of increasingly divergent sources of DOC to lakes across the Mackenzie Delta landscape, with consequent effects on energy flow and C cycling in these systems.

In the north more generally, the increased concentrations of terrestrially-derived DOC that are expected in northern lakes as the treeline advances (Pientz and Vincent 2000) could be magnified by additional inputs of aged allochthonous DOC in areas where thermokarst slumping is pronounced. This shift could have far-reaching implications in terms of light penetration (Kirk 1994), thermal structure (Kalff 2002), and the balance between algal and bacterial productivity in arctic lakes (e.g., Blomqvist et al. 2001). Globally, shallow lakes are expected to continue to shift from algal dominated, to macrophyte dominated systems as the incidence of eutrophication wanes (Scheffer et al. 1993). The increased incidence of subsidies of labile, macrophyte-derived DOM to the pelagic zone of these shallow lakes should increase the importance of bacteria in these ecosystems, and thus bacterially-based trophic pathways (e.g., Chapter 6, Cotner and Biddanda 2002). Our results suggest that this could compound the decreases in energy available to higher trophic levels expected as a result of the decreased productivity that accompanies a relaxation of eutrophication, as an increase in the proportion of biomass flowing through bacterially-based food webs causes greater energy loss as a result of an increased number of trophic linkages.

7.2 Future research directions

The outcomes of my thesis research suggest several avenues for future research, some of which I present here, grouped by chapter. In Chapter 2, detailed measurements of pCO_2 across multiple lakes indicate that as a whole, Delta lakes show net CO_2 fluxes that are near neutral when integrated over a yearly cycle. However, as is discussed in Section 2.5, this does not necessarily mean that these lakes are neutral from an overall greenhouse gas perspective. In particular, previous measurements of under-ice pCH_4 (Pipke 1996), and crude measurements of dissolved pCH_4 during the open water season (Fig. 2.9) show that Delta lakes are likely strong, consistent emitters of CH_4, a potent greenhouse gas, throughout the year. Notably, pCH_4 increases with sill elevation. Previous studies have already demonstrated that CH_4 emission from thermokarst-affected lakes is remarkably high (Walter et al. 2006, Walter et al. 2007). However, the fact that pCH_4 levels are also elevated in high elevation, macrophyte-rich lakes suggests the interesting potential for rapid macrophyte photosynthesis, which depletes CO_2, to instigate a net production of greenhouse gasses, as the large biomasses of macrophytic organic matter become available for anaerobic decomposition, and thus the production of CH_4 degradation products after senescence.

In Chapter 3, I show that DOC accumulates in the within-lake pool of thermokarst lakes, and that this DOC appears to be terrestrially derived. Because of low inputs of riverwater, floodwater, and overland flow to these high elevation lakes, I conclude that the most likely source of this DOC is the significant thermokarst slumping that occurs around the margins of these lakes. The analysis of DOC $\Delta^{14}C$ from thermokarst lakes would provide a much clearer understanding of the origin of this C. Along the same vein, it seems likely that different components of the C pool within thermokarst (and other Delta) lakes are derived from different sources. For example, the CH_4 and CO_2 produced during methanogenesis could occur as a result of processes that are occurring well below the lakebed, in aged, anoxic soils that have recently thawed, but are not in direct contact with the water column. In contrast, DOC within these lakes is more likely to be derived from

pools of carbon that are more directly exposed to lakewater. Gaining a better understanding of the origin of the different C pools that exist within Delta lakes would substantially increase our understanding of carbon cycling in this system.

The results from Chapter 4 suggest that rates of bacterial production and respiration on DOC from thermokarst lakes may fluctuate significantly over time, but do not suggest a clear mechanism for this variation. Detailed *in situ* measurements of bacterial metabolism in these lakes, with concurrent measurements that would allow a better understanding of the potential drivers of temporal changes in these processes, would greatly add to our understanding of bacterially-mediated C cycling in waterbodies affected by thermokarst. From a Delta-centric perspective, such work would allow a better understanding of the fate of DOC, and the importance of bacterioplankton, in this system. This increased knowledge will be particularly important for understanding the future functioning of Mackenzie Delta lakes if the incidence of permafrost melting increases in this system, as expected. From a broader circumpolar perspective, however, understanding the mechanisms that regulate bacterial metabolism in Mackenzie Delta thermokarst lakes will give much more certainty about the relevance of extrapolating from these waterbodies to draw conclusions about the future functioning of thermokarst-affected arctic lakes generally.

In Chapter 5, I show that bacterially-mediated C cycling can be profoundly altered by the stress of elevated pH. The effect of ecological stressors on bacterial functioning has generally been poorly investigated, likely because longer term studies have shown no effect (Kalff 2002). However, this work indicates that over the short term, the stress of elevated pH may profoundly affect bacterial metabolism. Understanding whether this effect carries over to other stressors that are known to commonly occur over the short term would greatly increase our understanding of bacterial processing of organic C in aquatic environments.

Finally, the results from Chapter 6 suggest that methanotrophs may provide considerable organic matter to zooplankton in Delta lakes, particularly when and where pCH_4 is high. However, direct measurements to assess of the importance of methanotrophs

in this system were not undertaken. Gaining a better understanding of the relative importance of methanotrophic versus heterotrophic bacteria in this system, and the specific importance of methanotrophs to energy flow and C cycling in Delta lakes, will add to our understanding of the functioning both of these waterbodies, and of other northern lakes that may generate significant CH_4 as a result of thermokarst processes (Walter et al. 2006).

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