FACTORS REGULATING METHANE PRODUCTION AND OXIDATION IN TWO SHALLOW ARCTIC ALASKAN LAKES

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ABSTRACT
Dendy Diane Lofton: Factors Regulating Methane Production and Oxidation in Two Shallow Arctic Alaskan Lakes
(Under the direction of Dr. Stephen C. Whalen)

Methane (CH₄) is second only to CO₂ as a greenhouse gas and is produced in the terminal step of organic matter decomposition in anaerobic environments, including lake sediments. Given the widespread distribution of lakes in Arctic Alaska, CH₄ emission from these lakes may significantly contribute to the atmospheric CH₄ budget. Aerobic methane oxidizing bacteria consume CH₄ diffusing from anaerobic zones of production, thereby modulating the flux of CH₄ to the atmosphere. Multiple research efforts indicate a significant source strength for arctic environments in the atmospheric CH₄ budget. Predicted climate induced alterations to the arctic landscape include increased organic matter loading from the terrestrial environment and increased temperature. These environmental changes can influence both rates of CH₄ production and oxidation, possibly altering rates of CH₄ exchange between shallow arctic lakes and the atmosphere.

I assessed rates and controls on CH₄ production and oxidation in two shallow arctic lakes to provide insight into the response of these two microbial groups to projected future climates. Rates of total methanogenesis and the fractional contribution of the acetoclastic pathway decreased with increasing depth below the sediment surface to 5 cm in both lakes. Substrate additions indicated substrate limitation to both methanogenic pathways (acetoclastic and hydrogenotrophic). Rates of total methanogenesis varied spatially in the horizontal dimension
in one lake only. However, there was no consistent relationship between rates of methanogenesis and depth of the overlying water as labile organic matter in the shallow sediments are likely resuspended and deposited unevenly by wind action. Under extant conditions, rates of methanogenesis responded positively to increases in temperature, while rates of CH$_4$ oxidation remained unchanged. The former were controlled by substrate x temperature interactions, while the latter were regulated strictly by substrate supply. Analysis of CH$_4$ oxidation kinetics for water samples points to a community of CH$_4$ oxidizing bacteria that is capable of oxidizing CH$_4$ at concentrations far in excess of observed levels. Increases in organic matter supply and temperature under future climates will likely increase rates of methanogenesis, but the impact may be fully mitigated by the excessive capacity of the CH$_4$-oxidizing community to process the added substrate.
To my parents, for always believing in me, even when I did not.
ACKNOWLEDGEMENTS

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<td>MG</td>
<td>methanogenesis</td>
</tr>
<tr>
<td>MOB</td>
<td>methane oxidizing bacteria</td>
</tr>
<tr>
<td>Mox</td>
<td>methane oxidation</td>
</tr>
<tr>
<td>OM</td>
<td>organic matter</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
</tr>
<tr>
<td>dw</td>
<td>dry weight</td>
</tr>
<tr>
<td>$J_s$</td>
<td>flux of CH$_4$ ([µmol CH$_4$ m$^{-2}$ s$^{-1}$])</td>
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<tr>
<td>$\phi$</td>
<td>sediment porosity (unitless)</td>
</tr>
<tr>
<td>$\Delta c/\Delta x$</td>
<td>change in CH$_4$ concentration with depth ([µmol CH$_4$ cm$^{-3}$ cm$^{-1}$])</td>
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<tr>
<td>$D_s$</td>
<td>effective diffusivity (cm$^2$ s$^{-1}$)</td>
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</tr>
<tr>
<td>$\theta$</td>
<td>tortuosity (unitless)</td>
</tr>
<tr>
<td>$Q_{10}$</td>
<td>change in rate associated with a 10°C temperature increase</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>maximum rate of CH$_4$ oxidation ([µmol CH$_4$ L$^{-1}$ d$^{-1}$])</td>
</tr>
<tr>
<td>$K_m$</td>
<td>half-saturation constant (µM CH$_4$)</td>
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CHAPTER 1: INTRODUCTION

1.1 Methane

Methane (CH₄) is a radiatively and chemically important trace atmospheric constituent that is over twenty times more influential in terms of radiative forcing than CO₂ on a per molecule basis (Whalen 2005). Production of methane results from multiple natural and anthropogenic sources with wetlands comprising roughly 20% of total atmospheric emissions (Whalen and Reeburgh 2000). Global atmosphere concentrations of CH₄ have been increasing in recent decades (Whalen 2005) with the highest observed concentrations appearing over the arctic and subarctic regions due to the high concentration of wetlands (Semiletov 1999). Tundra environments encompass approximately 7% of earth’s surface (Whalen and Reeburgh 1992) with up to 50% of that areal coverage comprised of shallow ponds in some regions. On a global basis, shallow arctic lakes may occupy approximately 2% of total land surface (Sheath 1986). Although arctic lakes have been studied less frequently than terrestrial tundra environments in terms of CH₄ efflux to the atmosphere, their contribution to the global CH₄ cycle is likely significant due to shallow depth and broad areal coverage (Bastviken et al. 2004; Semiletov 1999).

1.2 Methanogenesis

Methane is produced by methanogenesis (MG) as a terminal step of organic matter (OM) degradation in anaerobic sediments. A consortium of microorganisms is responsible for
the decomposition of OM through a series of reduction-oxidation reactions with each step providing substrate(s) for the next phase. Physiologically, microbes carrying out these processes utilize the energy obtained from redox reactions for cellular growth and maintenance (Zehnder and Stumm 1988). Energy yield for microbial oxidation of OM is greatest when O$_2$ is an oxidant, while the least amount of energy is available through fermentative CH$_4$ producing pathways. Consequently, methanogens are outcompeted for substrates by other microbial groups (Capone and Kiene 1988; Schink 1997). Direct competition with methanogens for substrates is most likely with groups capable of synthesizing acetate (acetogens) and/or reducing sulfate (SO$_4^{2-}$) and iron (Fe$^{3+}$) (Zinder 1993). Sulfate concentrations are typically low (< 200 µM) in freshwater sediments (Capone and Kiene 1988), so direct substrate competition with SO$_4^{2-}$ reducers should be minimal compared to acetogens and Fe$^{3+}$ reducers.

Methanogens are *Archaeobacteria* that exhibit a relatively large substrate range, although most species can only utilize one or two substrates (Zinder 1993). The three catabolic pathways are acetoclastic, hydrogenotrophic and methylotrophic, which enable production of CH$_4$ from acetate, formate, H$_2$/CO$_2$, methanol, carbon monoxide and methylated amines (Zinder 1993). Energy yields for each substrate vary with the highest availability from utilization of acetate followed by H$_2$/CO$_2$ (Oremland 1988). Consequently, these two pathways are dominant in freshwater sediments with most studies reporting a 2:1 ratio of acetoclastic to hydrogenotrophic pathways (Nusslein and Conrad 2000).

Many factors can influence both rates of MG and the ratio of the dominant processes. The most notable factors influencing methanogenic activity are availability of direct substrates, quantity and quality of methanogenic precursors as well as temperature (Conrad 2005; Schulz
Substrate availability depends largely on the presence and activity of the microbial consortium leading to the terminal stages of OM degradation. The variation in the relative proportion of the two dominant pathways has rarely been examined in lake sediments. However, Conrad (2005) found that sediments characterized by younger OM often show higher activity of the acetoclastic pathway whereas sediments characterized by older, more recalcitrant OM are typically dominated by the hydrogenotrophic pathway. In addition, the hydrogenotrophic pathway may be enhanced in higher temperatures due to the increased activity of the bacterial consortium responsible for $\text{H}_2$/CO$_2$ generation required by hydrogenotrophic methanogens (Schulz et al. 1997). Temperature will control rates of both processes, but may differentially influence the relative contribution of each pathway to total MG by increasing availability of direct substrates at different rates.

Terrestrial inputs of dissolved organic carbon (DOC) to a lake system may also influence rates of MG. Houser et al. (2003) found a positive correlation between epilimnetic DOC concentrations (allochthonous origin) and hypolimnetic CH$_4$ accumulation. In their study, hypolimnetic concentration of DOC decreased proportionally with increases in dissolved inorganic carbon (DIC) + CH$_4$ in 19 of 21 lakes, suggesting hypolimnetic metabolism was supported by incoming DOC (Houser et al. 2003). Autochthonous DOC resulting from decomposition of algal biomass also strongly influence rates of MG, particularly the acetoclastic pathway (Schulz and Conrad 1996; Schwarz et al. 2008). DOC within a lake system may be derived from allochthonous or autochthonous sources; however, a large portion of the terrestrially derived DOC in oligotrophic lakes (> 90%) is decomposed in the sediments (Hershey et al. 2006; Wetzel 2001). Arctic Alaskan lakes currently receive large inputs of allochthonous
OM, particularly during snowmelt and precipitation events (Michaelson et al. 1998). Several studies suggest that melting permafrost and elevated terrestrial plant productivity will increase OM loading to arctic lakes as a result of ongoing climate change (Neff and Hooper 2002; Rouse et al. 1997; Shaver et al. 1992). Consequently, increased inputs of terrestrially derived OM may lead to higher rates of CH$_4$ production in shallow Arctic Alaskan lakes.

1.3 Spatial Variation in Sediment Methane Production

Sediment methane flux varies spatially and temporally within a lake ecosystem. Most studies of MG have historically focused on the profundal regions of the lake. However, the proportion of OM (quantity and quality), disturbance frequency (e.g., sediment resuspension), sediment temperature and composition can vary significantly among habitats within a lake (Bastviken et al. 2008; Bussman 2005; Casper 1996) and all are known to influence methanogenic activity. Higher sediment temperatures along lake margins can also contribute to higher rates of MG in comparison to profundal zones (Murase et al. 2005). Therefore, any extant variation of these characteristics within a lake ecosystem can result in variations in CH$_4$ production rates among lake zones. Consequently, targeted research centered on one region of a lake may not capture the range of methanogenic activity in that water body.

Flux of CH$_4$ from the sediments (and subsequent efflux to the atmosphere) may vary seasonally as well. Seasonal differences in terrestrial OM loading (e.g., snowmelt) to lakes can influence rates of MG as well as intermittent pulses of OM from runoff following periodic precipitation events (Michaelson et al. 1998). In addition, variations in autochthonous production of OM during the growing season will directly influence rates of MG (Schulz and Conrad 1995). Therefore, understanding the spatiotemporal variation in methanogenic activity
within a lake ecosystem is imperative for adequate estimation of a lake’s potential CH₄ productivity although incorporation of all these factors into a single study is challenging and costly.

1.4 Effect of Temperature on Methanogenesis

Several studies have reported the positive influence of temperature on rates of MG (Zeikus and Winfrey 1976; Thebrath et al. 1993; Schulz and Conrad 1996; Schulz et al. 1997; Segers 1998; Duc et al. 2010). The individual pathways leading to MG may be differentially altered by fluctuations in temperature. Schulz et al. (1997) reported an increase in the hydrogenotrophic pathway relative to the acetoclastic pathway with increased sediment temperature. In general, however, it is not clear if increased temperature specifically impacts the activity of the methanogens and/or the bacterial consortium responsible for MG substrate generation (Schulz et al. 1997). Methanogenic response to temperature varies considerably across ecosystems with higher values occurring in regions with higher quantities and qualities of OM (Segers 1998; Duc et al. 2010). Thus, predicted increases in surface air temperatures and OM delivery to the lakes as a consequence of a changing climate may significantly stimulate rates of MG in shallow arctic lakes. Consequently, measurement of the effect of temperature on MG rates in shallow arctic lakes under the present climactic conditions may provide insight into the future response of MG in lake sediment.

1.5 Methane Oxidation

Methane diffusing upwards from the sediments is often oxidized by methanotrophic bacteria (MOB) both in oxic surficial sediment and overlying water. These bacteria belong to a
larger group, the methylotrophs, which utilize one-carbon compounds as their carbon and energy source for cellular growth and maintenance (Hanson and Hanson 1996). Oxidation of CH$_4$ (MOx) is most common in aerobic habitats with highest rates generally proceeding at the oxic-anoxic interface (Kankaala et al. 2006). Methane oxidizing bacteria also represent an important ecological linkage between benthic and pelagic food webs (Bastviken et al. 2003; Kankaala et al. 2006; Sundh et al. 2005). Consumption of methanotrophs by pelagic invertebrates (e.g., zooplankton) functions to transfer CH$_4$ derived carbon to higher trophic levels (Bastviken et al. 2003). Similarly, methanotrophy may also fuel benthic invertebrate metabolism. Hershey et al. (2006) found that off-shore macroinvertebrates, particularly, chironomids, were depleted in $\delta^{13}$C, indicating utilization of MOB as a food resource. Consequently, MOB play a major role in the food web of some arctic lake ecosystems. The importance of methane derived carbon in foodwebs is likely to be most important in smaller oligotrophic lakes with well oxygenated bottom waters where MOB are abundant at the sediment water interface (Hershey et al. 2006). Consequently, increased CH$_4$ consumption induced by greater rates of MG may significantly alter lake food web ecology. Furthermore, increased rates of MG without a proportional increase in MOx in lacustrine arctic environments may increase their relative contribution to the global atmospheric CH$_4$ budget.

1.6 Effect of Temperature on MOx Rates

As MOx regulates CH$_4$ efflux from the lake and land surface to the atmosphere, it plays a significant role in the global atmospheric CH$_4$ budget. Changes to the arctic environment, such as increasing water temperature and terrestrial OM inputs, may directly and indirectly influence MOx rates. Methane oxidizing bacteria appear to be mesophilic although community
adaptation to different temperatures may occur (Hanson and Hanson 1996). Considerable activity below 7°C suggesting the presence of psychrophilic methanotrophs (Sundh et al. 2005). Methanotrophic response to temperature can be explained by a $Q_{10}$ value which reflects the change in Mox rates associated with a 10°C increase in temperature (Duc et al. 2010). The literature values of $Q_{10}$ for CH$_4$ oxidation in northern peatlands range from 1.4 to 2.1 (Dunfield et al. 1993) although temperature is likely to be a more important driver of Mox rates at higher CH$_4$ concentrations as activity shifts from substrate limitation to enzyme-activity limitation, particularly if populations prove to be psychrophillic (Sundh et al. 2005; Whalen and Reeburgh 1996). The role of temperature as a regulator of Mox rates is poorly understood in freshwater lakes and determinations of the temperature-dependence of Mox rates in arctic lakes is non-existent. Therefore, evaluation of the influence of temperature on rates of Mox is essential to understanding how this regulatory process may be impacted by rising mean air temperatures in the arctic region as increasing lake water temperatures will likely follow (Overpeck et al. 1997; Christoffersen et al. 2008; Post et al. 2009; Flury et al. 2010).

1.7 Methane Oxidation Kinetics

Oxidation of CH$_4$ in lakes is controlled largely by substrate concentration, O$_2$ availability and temperature to a lesser degree in most cases (Liikanen et al. 2002). The maximum CH$_4$ uptake rate ($V_{max}$) is dependent upon substrate concentration and is indicative of the population size of MOB (Buchholz et al. 1995; Whalen and Reeburgh 1996). Similarly, the half-saturation constant for Mox ($K_m$) reflects community structure and physiology as it provides information regarding the presence of high or low affinity MOB (Bender and Conrad 1992; De Visscher et al. 2001). The apparent $K_m$ for low affinity MOB typically exceeds 1 µM (Roslev et al.
1997; Knief and Dunfield 2005) while the $K_m$ for atmospheric CH$_4$ oxidizers is much lower (10 to 280 nM CH$_4$) (Knief and Dunfield 2005). This lower $K_m$ is generally accompanied by low $V_{max}$ as well (Bender and Conrad (1992). Comparison of these constants will indicate differences in community structure and physiology which may lend insight into the ability for Mox communities in arctic lakes to accommodate higher CH$_4$ concentrations that may be associated with ongoing climate change.

1.8 Arctic Lakes and Future Climate Change

Methane is derived from a number of natural and anthropogenic sources (Whalen 2005) with large uncertainties regarding quantification of the magnitude of the global sources and sinks (Dlugokencky et al. 2009; Isaksen et al. 2011). The relative contribution of the Arctic to the global CH$_4$ budget is predicated upon wetland sources (Whalen and Reeburgh 1992). However, many arctic lakes are currently considered to be net sources of CH$_4$ to the atmosphere (Bartlett et al. 1992; Zimov et al. 1997; Bastviken et al. 2004; Semiltov 1999; Walter et al. 2007; Mazeas et al. 2009). It is hypothesized that OM loading to high latitude lakes will increase through interactions between changing terrestrial vegetation composition, increased terrestrial plant productivity and alterations to hydrological regimes (Wrona et al. 2006). Arctic lakes currently receive considerable inputs of dissolved organic carbon (DOC) from the surrounding landscape (Whalen and Cornwell 1985; Kling et al. 1991; Michaelson et al. 1998). Amplified carbon inputs to lakes due to warming-induced changes to the terrestrial landscape (Rouse et al. 1997) have the potential to increase lacustrine CH$_4$ emissions (Chapin et al. 2000; Zimov et al. 1997; Walter et al. 2006) if not accompanied by increased CH$_4$ consumption. Since MG is the terminal step involved in decomposition of OM in anaerobic sediments (Nusslein and Conrad 2000), it plays a
key role in the carbon cycling of lakes (den Heyer and Kalff 1998). Further, the extensive areal coverage of lakes across the arctic landscape dramatically increases their relative contribution to the global atmospheric budget in a disproportionate manner. Consequently, continued climate change may augment the magnitude of CH$_4$ emitting from arctic lakes if microbial consumption of CH$_4$ does not mediate increased CH$_4$ flux from the sediments. Therefore, increased OM loading from the terrestrial landscape has the potential to significantly impact CH$_4$ cycling in these shallow lakes with ramifications for positive feedback to lake CH$_4$ emissions in the arctic.

1.9 Research Objectives

The overall goal of this research was to evaluate the landscape and within-lake scale factors regulating microbial production and consumption of methane in two representative Arctic Alaskan lakes under current climatic conditions in an effort to better understand the response of these communities to predicted changes in environmental variables that control their activities. Specific research objectives are as follows:

1. Determine the relative importance of dominant methanogenic pathways in arctic lakes, the factors regulating vertical distribution of methanogenic activity and the community response to added substrates;

2. Quantify within lake spatial variability of CH$_4$ production in three major lake zones (maximum water depth, ½ maximum water depth and littoral zone);

3. Quantify the relationship between temperature and rates of MG in lake sediments and Mox in lake waters. I further explored substrate dependence of Mox rates and substrate-temperature interactions in rates of Mox. Analogous experiments were not
conducted for MG due to community involvement in production of methanogenic precursors and the presence of multiple pathways of microbial CH$_4$ production.

1.10 Dissertation Structure

My dissertation is structured with five major chapters such that the internal data chapters (Chapters 2 - 4) address each of the research objectives described above and are designed to serve as standalone manuscripts for publication. Therefore, the first chapter serves as an introduction, while the fifth chapter summarizes the conclusions of all major research objectives. As a consequence of this structure, some repetitive overlap with background information and discussion of findings may prevail throughout the document.
REFERENCES


CHAPTER 2: VERTICAL DISTRIBUTION OF METHANOGENIC PATHWAYS IN THE SEDIMENTS OF TWO SHALLOW ARCTIC LAKES

2.1 Introduction

Methane (CH$_4$) is produced in the terminal step of organic matter (OM) degradation in anaerobic sediments. A consortium of microorganisms is responsible for decomposition of OM through a series of reduction-oxidation reactions with each step providing substrate(s) for the next phase. Physiologically, microbes carrying out these processes utilize the energy obtained from redox reactions for cellular growth and maintenance (Zehnder and Stumm 1988). Energy yield for microbial oxidation of OM is greatest when O$_2$ is an oxidant, while the least amount of energy is available through fermentative CH$_4$ producing pathways. Consequently, methanogens are outcompeted for substrates by other microbial groups (Capone and Kiene 1988; Schink 1997). Direct competition with methanogens for substrates is most likely with groups capable of synthesizing acetate (acetogens) or reducing sulfate (SO$_4^{2-}$) or iron (Fe$^{3+}$) (Zinder 1993). Sulfate concentrations are typically low (< 200 µM) in freshwater sediments (Capone and Kiene 1988), so direct substrate competition with SO$_4^{2-}$ reducers should be minimal compared to competition with acetogens and Fe$^{3+}$ reducers.

Methanogens belong to the anaerobic group *Archaea* (Schimel and Gulledge 1998) which exhibits a relatively large substrate range, although most species can only utilize one or two substrates (Zinder 1993). The three catabolic pathways are acetoclastic, hydrogenotrophic
and methylotrophic, which enable production of CH₄ from acetate, formate, H₂/CO₂, methanol, carbon monoxide and methylated amines (Zinder 1993). Energy yields for each substrate vary with the highest availability from utilization of acetate followed by H₂/CO₂ (Oremland 1988). Consequently, these two pathways are dominant in freshwater sediments with most studies reporting a 2:1 ratio of acetoclastic to hydrogenotrophic pathways (Nusslein and Conrad 2000).

Many factors can influence both rates of methanogenesis (MG) and the ratio of these processes. The most notable factors influencing methanogenic activity are availability of direct substrates, quantity and quality of methanogenic precursors as well as temperature (Schulz and Conrad 1996; Conrad 2005). Substrate availability depends largely on the presence and activity of the microbial consortium leading to the terminal stages of OM degradation. The variation in the relative proportion of the primary methanogenic pathways has rarely been examined in lake sediments. However, Conrad (2005) found that sediments characterized by younger OM often show higher activity of the acetoclastic pathway whereas sediments characterized by older, more recalcitrant OM are typically dominated by the hydrogenotrophic pathway. Temperature will control rates of both pathways, but may differentially influence the relative contribution of each pathway to total MG. Activity of the bacterial consortium responsible for H₂/CO₂ generation increases with sediment temperature (Schulz et al. 1997), and may play a larger role in controlling MG activity in the future due to climate induced warming (McGuire et al. 2009; Meuller et al. 2009). Further, increased air temperature in the Arctic is expected to release large amounts of stored organic carbon through permafrost degradation (Weller et al. 1995; Pastor et al. 2003; Finlay et al. 2006; Guo et al. 2007). Consequently, both major methanogenic pathways in lake sediments will likely respond positively to increased inputs of
terrestrial OM as well as increased temperature as a function of climate change (Michaelson et al. 1996; Roehm et al. 2009; Mazeas et al. 2009). Characterization of these pathways and the factors controlling their distribution has not been examined in Arctic Alaskan lakes. Therefore, experiments were conducted to determine: (a) the relative proportion of these pathways in shallow arctic lakes and its distribution with depth below the sediment surface, and (b) the response of the methanogenic community to substrate additions.

2.2 Materials and Methods

2.2.1 Field Sites

My study site is located in the Arctic Foothills province of Alaska which is a region of the Arctic that is characterized by a tundra landscape underlain with continuous permafrost (Whalen et al. 2006). Mean annual temperatures range from −7 to −11°C with annual precipitation ranging from 140 to 267 mm (Bowden et al. 2008). The trophic state of most of the lakes in this region ranges from ultraoligotrophic to oligotrophic with heavy reliance on spring snowmelt for terrestrial subsidies of dissolved organic matter (Kling 1995; Whalen and Cornwell 1985). A full description of the region including vegetation characteristics and glacial geology can be found in Ping et al. (1998) and Hamilton (2002).

The two lakes chosen for this study, E4 and GTH 114, possess similar geomorphological characteristics, with GTH 114 having a larger total volume and catchment area (Table 2.1). The selected study lakes are regionally representative with respect to surface and catchment area, and lack fish, permanent inlets and rooted macrophytes. Additionally, these lakes exhibit
polymictic mixing patterns, thermally stratifying on an intermittent basis during the summer growing season (Figures 2.1 and 2.2).

2.2.2 Field Sampling

Sediments for CH$_4$ production studies were collected from GTH 114 on 4 July 08 and from E4 on 18 July 08. Sediment cores were extracted from the lakes using a KB gravity corer (Wildlife Supply Company) deployed from an inflatable raft. Polycarbonate sleeves (4.8 cm inside diameter x 50 cm length) were inserted into the KB corer which was then lowered carefully into the sediments. Once removed from the sediments, sleeves were capped on each end with rubber stoppers. Additional sediments were similarly collected for dissolved organic carbon (DOC) and %OM analysis. Intact sediment cores were transported undisturbed via foot, vehicle, or helicopter to TFS for processing.

Samples for DOC analysis were also taken from temporary water tracks entering E4 (n = 1) on 3 July 2009 and GTH 114 (n = 2) on 17 June 2009. Additional water samples were collected in 2010 for fluorescence index (FI) determination, which is an index of whether the precursor material was autochthonous or allochthonous (McKnight et al. 2001; Cory et al. 2007; Cory et al. 2010). Single samples were collected from the epilimnion and hypolimnion of E4 on 4 July 2010 and similarly from GTH 114 on 25 June 2010 using a Van Dorn water sampler (Wildlife Supply Company) deployed from an inflatable raft.

2.2.3 Laboratory Studies

Triplicated sediment slurries were prepared from discrete core sections using a total of 6 cores per slurry. Cores were sectioned in 1 cm increments to a depth of 5 cm and sediments from each depth interval were homogenized into three polycarbonate 1-L beakers. Anoxic
Deionized water (Dh₂O) was prepared in separate polycarbonate beakers by purging with ultra-pure helium (He) and an air stone affixed to the end of tygon tubing. The uppermost sediment interval (0 – 1 cm) contained a visibly higher proportion of water relative to deeper sediment intervals. Therefore, 50 ml of anoxic Dh₂O were added to the surface layer (0 – 1 cm interval) while 100 ml of anoxic Dh₂O were added to subsequent depth intervals in an effort to approximate similar water: sediment ratios among samples. To account for any variation in the water: sediment ratios among depths intervals, rates of MG were based on sediment dry mass. Following addition of Dh₂O, all homogenized sediment slurries were continually purged with He. A polycarbonate syringe with a bored tip was used to measure 20 ml of anoxic sediment slurry that was then placed into 160 ml serum bottles. Serum bottles were sealed with butyl rubber stoppers and capped with aluminum crimp seals. Each serum bottle was evacuated and purged with He a minimum of 5 times to ensure anoxia, filled to 1 atm with He then placed in a 10°C water bath to acclimate for approximately 12 h prior to treatment additions (i.e., methanogenic substrates or a pathway inhibitor). Substrate additions included acetate and H₂ while methyl fluoride (CH₃F) was used as a specific inhibitor of the acetoclastic pathway. One of the following amendments were added to triplicate sediment slurries to obtain a final concentration for each treatment: acetate (1 Mm), H₂ (4% v/v), CH₃F (1% v/v). The selected concentrations for each amendment have been previously found to be effective for this type of study (Nusslein and Conrad 2000; Nozhevnikova et al. 2007) and thus, were similarly implemented in my study. Headspace samples were collected in 3 ml plastic syringes that had been previously tested to confirm no loss of CH₄ over 4 h.
Analysis of CH₄ was conducted by a gas chromatograph equipped with a flame ionization detector (FID-GC; Shimadu GC-8A) and was completed within 2 h of headspace sampling. Operating conditions for the FID-GC were as follows: Column = ½” diameter x 1 m length mol sieve 5A (60/80); column temperature = 90°C; injector and detector temperatures = 140°C; carrier gas = ultra-high purity N₂ at 33 ml min⁻¹ flow rate. Vial headspaces were sampled 4 times over 10 d, resulting in the linear production of CH₄. Rates were calculated using linear regression of CH₄ production versus time and were normalized to 1 g dry sediment matter. Methods were adapted from Nusslein and Conrad (2000).

Triplicate cores for porewater analysis of DOC were carefully sliced in 1 cm increments, which were then added to 15 ml glass centrifuge tubes and sealed with rubber septa without a headspace. Tubes were centrifuged at < 2000 rpm for 30 min. The supernatant for each core section was filtered (ashed Whatman GF/F filter) and acidified (0.1 ml of 0.1N HCl). Water track samples were similarly filtered and acidified for DOC analysis. All DOC samples were stored at 4°C, and transported to UNC-CH for analysis. DOC was measured on a Shimadzu TOC-VCPH Combustion-Infrared instrument (Shimadzu Corp., Kyoto, Japan) using Standard Method 5310B (Standard Methods for the Examination of Water and Wastewater 1998). Solid phase sediments (in 1 cm increments) were weighed, dried for 2-3 d at 45°C and reweighed. Dry sediments were ashed at 550°C for 4 h.

The non-acidified water samples for FI determination were filtered (ashed Whatman GF/F) and analyzed twice on a Fluoromax-4 fluorometer (analytical error ± 0.005) equipped with a xenon lamp. The FI is determined by the ratio of emission intensity at 470/520 nm produced to excitation at 370 nm (Cory et al. 2010).
2.2.4 Calculations and Statistics

Headspace CH$_4$ in serum vials as a mixing ratio was converted to a moles per liter basis. Methane concentration in the aqueous phase at each sampling point was calculated from temperature corrected solubility coefficients (Yamamoto et al. 1976) andHenry’s law (Stumm and Morgan 1996). The total amount of CH$_4$ produced in each bottle at each time point was computed as the sum of the aqueous phase and headspace masses.

The rates of MG from the unamended slurries (control) provided an estimate of total MG. Since CH$_3$F inhibits the acetoclastic pathways, any CH$_4$ produced in the presence of CH$_3$F was assumed to be produced via the hydrogenotrophic pathway. Thus, the relative proportion of MG attributed to the acetoclastic pathway was calculated as the difference between the mean rate of MG in the control treatments and the mean rate of MG in the CH$_3$F treatments for each lake at each depth.

The effect of sediment depth increment on unamended rates (controls) of MG was evaluated using separate One-way ANOVAs. Post-hoc comparison of means was conducted using Tukey’s Honestly Significant Difference (HSD) for within-lake comparisons and Student’s t-test for between-lake comparisons. The effect of substrate addition (acetate or hydrogen) on MG rates was also evaluated using One-way ANOVA followed by Dunnett’s test for direct comparison with the control. The effect of sediment depth increment on amended rates of MG was assessed using One-way ANOVA following by Tukey’s HSD for comparison of multiple means (Sokal and Rohlf 1995; Zar 1996; McDonald 2009).

Initially, the dependence of DOC or % OM on sediment depth was evaluated with simple linear regression. In the case of each environmental variable, either significant linear
relationships in all profiles among sediment zones were not found (see Results), or some regression assumptions were violated preventing use of linear regression. Therefore, One-way ANOVAs were used to compare within-lake and between-lake differences in DOC and % OM with respect to sediment depth increment. Post-hoc comparisons of means were conducted with Tukey’s HSD for within lake evaluations or Student’s t-test for between lake evaluations. The % OM was arcsine transformed for all statistical analyses (Sokal and Rohlf 1995; Zar 1996; McDonald 2009).

Due to the non-normal distribution of environmental variables, the relationship between rates of MG and DOC, or MG rates and % OM were evaluated using Spearman’s rank correlation ($r_s$) (Sokal and Rohlf 1995; Zar 1996; McDonald 2009).

Organic content (%) was calculated from the difference in mass between oven dried sediments (40-60°C) and sediments combusted at 550°C for 4 h (den Heyer and Kalff 1998; Wetzel and Likens 2000).

2.3 Results

Within lake comparison of unamended sediment slurries in E4 and GTH 114 exhibited a general trend of decreasing rates of total MG with increasing depth below the sediment surface (Figure 2.3). A comprehensive list of statistical comparisons between unamended rates of MG and vertical sediment depth can be found in Table 2.2. Mean rates of total MG in E4 varied from 118 to 1696 nmol CH$_4$ g$_{dw}^{-1}$ d$^{-1}$ (dw = dry weight). Rates in the 4-5 cm interval were significantly lower than all other intervals while the rates in the 1-2 cm interval were significantly greater than rates in deeper sediment intervals. Mean rates of MG in GTH 114
ranged from 118 to 3291 nmol CH₄ g⁻¹ dw⁻¹ with the rate in the 0-1 cm depth interval significantly higher than rates in deeper sediments. Between lake comparisons showed some significant differences in rates of MG at comparable depth intervals. Rates of MG in the 1-2 cm and 2-3 cm depth intervals were higher in E4 than in GTH 114. No other pairs of sediment depth intervals differed significantly with respect to rates of MG.

Substrate additions generally enhanced MG rates relative to controls at all depths in both lakes, but not all rate increases were significant. A complete list of statistical tests evaluating the effect of substrate additions on rates of MG can be found in Table 2.3. Briefly, in E4, H₂ significantly stimulated MG relative to the control in the 0-1 cm interval, but showed no significant difference relative to the control in the 1-2 cm interval (Figure 2.4). Hydrogen and acetate both significantly increased rates of MG compared to controls at depths below 2 cm. Rates of MG increased significantly in the 0-1 cm interval in response to the addition of H₂ when compared to acetate. No other significant differences between acetate or H₂ treatments were found at subsequent depth intervals in E4. In GTH 114, H₂ significantly stimulated rates of MG at all depths relative to unamended controls whereas acetate significantly increased CH₄ production only at depths below the 0-1 cm depth interval (Figure 2.5). No significant differences were found at any depth interval in GTH 114 when comparing MG rates for samples supplemented with acetate versus hydrogen. In comparison to unamended treatments, substrate additions generally resulted in a greater percent increase in MG rates in the 0-1, 1-2 and 2-3 cm depth intervals in GTH 114 than E4 (Figure 2.6). Furthermore, the stimulatory effects of H₂ was comparable between lakes in the 3-4 and 4-5cm intervals while acetate was more influential in E4 than in GTH 114 at these depths.
The acetoclastic and hydrogenotrophic pathways were inversely distributed with depth in E4, with the importance of the acetoclastic pathway generally decreasing with increasing depth below the sediment surface (Figure 2.7). A similar but less clear trend was observed in GTH 114 (Figure 2.8). The ratio of the acetoclastic:hydrogenotrophic pathway transitioned in E4 from 17:1 in the 0-1 cm depth interval to 2:1 in the 4-5 cm interval. In contrast, the 0-1 cm depth interval in GTH 114 displayed a 6:1 ratio for the acetoclastic:hydrogenotrophic pathway, but showed a slightly greater than even contribution by the hydrogenotrophs in the 2-3 and 4-5 cm sediment depth intervals. Interestingly, only the 1-2 cm interval showed a 2:1 ratio of acetoclastic:hydrogenotrophic MG in GTH 114.

In E4, the mean DOC concentration at the 4-5 cm depth interval was significantly greater than the mean for the 0-1 cm depth interval, while no significant differences were found among sediment depth intervals in GTH 114 (Table 2.4; Figure 2.9). There was a significant linear relationship between DOC and sediment depth increment in E4, but not in GTH 114 (Table 2.5). Therefore, the difference for overall means in DOC between lakes was assessed using One-way ANOVA (Table 2.4). The mean DOC of 24.5 mg L⁻¹ in GTH 114 was significantly greater than the mean of 14.9 mg L⁻¹ for E4. Samples from temporary water tracks in 2009 had DOC concentrations that were more than two-fold higher near GTH 114 (18 mg L⁻¹; n = 2) than near E4 (8 mg L⁻¹; n = 1). Values of FI for samples taken (n=1 per stratum per lake) in 2010 from the epi- and hypolimnetic waters of E4 were 1.37 and 1.39 and with respective values of 1.36 and 1.40 in GTH 114. The measured FI values are indicative of a high terrestrial influence for both lakes (Cory et al. 2010).
Neither lake exhibited a significant change in % OM with increasing sediment depth (Table 2.4; Figure 2.10). In E4, the surficial sediment layer (0-1 cm) was comprised of approximately 42 ± 1.08 % OM (\(\bar{x} \pm SE\)) with the 4-5 cm interval comprising 43 ± 8.9 % OM (\(\bar{x} \pm SE\)). No clear pattern was observed between unamended rates of MG and % OM in each sediment depth interval in E4, but the highest unamended rate of MG in GTH 114 occurred in the surficial sediment interval (0-1 cm) which also coincided with the depth interval containing the greatest % OM (Figure 2.11). Despite the lack of a significant difference in % OM among depth intervals, GTH 114 had a higher organic content in the 1-2 and the 3-4 cm depth intervals than E4 (Table 3.4). There was a significant linear relationship between % OM and sediment depth increment in GTH 114, but not in E4 (Table 2.5). Therefore, the overall mean % OM content between lakes was compared by One-way ANOVA (Table 2.4). The mean % OM content of 45% in the solid phase (0-5 cm) of E4 sediment was significantly higher than the value of 32% in GTH 114 (paired t-test), although considerably more variability was evident in E4 sediments relative to GTH 114 sediments.

In E4, no clear pattern was observed between rates of MG attributed to the acetoclastic or hydrogenotrophic pathways versus % OM at the corresponding sediment depth intervals (Figure 2.12). Rates of MG via the acetoclastic pathway in GTH 114 occurred in the depth interval (0-1 cm) that contained the highest observed % OM (Figure 2.13). However, no other clear pattern between MG rates and % OM in the discrete depth intervals was observed.

Spearman’s rank correlation revealed a significant correlation between sediment depth increment and rates of MG in E4 and GTH 114 (Table 2.6). There was a moderately significant correlation between rates of MG and sediment DOC in GTH 114, but no significant relationship
in E4. Sediment DOC, however, was not significantly correlated to pooled rates of MG for E4 and GTH 114. No significant relationship was found between pooled % OM and rates of MG, but rates of MG were strongly correlated to % OM in GTH 114.

2.4 Discussion

2.4.1 Total Methanogenesis

Rates of methanogenesis in freshwater lake sediments are somewhat scarce and vary globally (Tranvik et al. 2009). Rates of total MG observed in lakes E4 and GTH 114 generally fall within the range of other reported studies worldwide. Duc et al. (2010) measured MG rates in lakes of the temperate and boreal areas of Sweden and found a wide range (2 – 3990 nmol CH$_4$ g$_{dw}$ d$^{-1}$). In contrast, rates in soil slurries from unflooded rice fields in Italy depended on temperature regimes (4 – 30°C) and agricultural impacts, with values varying from 264 – 552 nmol CH$_4$ g$_{dw}$ d$^{-1}$ (Dannenberg et al. 1997). Rates in slurries from Lake Biwa in Japan were considerably lower than rates found in my lakes at < 2 nmol CH$_4$ g$_{dw}$ d$^{-1}$ (Dan et al. 2004). Conrad et al. (2010) reported rates between 1092 – 1519 nmol CH$_4$ g$_{dw}$ d$^{-1}$ in sediments from two clear-water Amazonian lakes.

2.4.2 Unamended Treatments

Rates of total MG in unamended slurries declined with increasing depth below the sediment surface in both lakes. I expected overall rates of MG in GTH 114 to be higher than in E4 as a consequence of higher DOC concentrations in GTH 114 compared to E4. With the exception of the uppermost sediment layer, unamended rates of MG in E4 were generally higher at each depth interval than in GTH 114 although only the 1-2 and 2-3 cm depth intervals
showed rates of MG that were significantly different between lakes. High variability and few replications limit statistical power making trends difficult to identify.

Although the difference was not significant, the higher rates of MG observed in the uppermost sediment layer of GTH 114 compared to E4 could be attributed to several factors. As oxygen inhibits growth of methanogens (Zinder 1993), intermittent oxic conditions in the uppermost surficial sediments in E4 likely suppress growth of methanogenic communities in contrast to the favorable environment provided by consistently anoxic surficial sediment layer in GTH 114 (Figures 2.1 and 2.2). Yuan et al. (2009) found methanogens in rice field soils repeatedly exposed to oxygen for 72 h did not resume production of CH$_4$ until 23 days after cessation of oxygen exposure. In contrast, the deepest zone in GTH 114 is approximately 2 m deeper than that of E4 and covers approximately 3 times less surface area (668 vs. 2309 m$^2$ respectively). It is likely that this small surface area of sediments at maximum water depth (Zmax) in GTH 114 is somewhat buffered from wind driven sediment resuspension events thereby promoting consistently anoxic conditions in the surficial sediment layer. Furthermore, the surficial sediments of Zmax in GTH 114 are much more flocculent than those found in E4. Highly flocculent material, derived from autochthonous and allochthonous sources, overlying sandy sediments can increase sediment oxygen demand, thereby depleting oxygen concentrations at the sediment surface (Sweerts et al. 1986).

The contrasting rates between lakes in unamended slurries below the 0-1 cm depth interval could be caused by differences in methanogenic community composition. In eutrophic Lake Dagow (Germany), Chan et al. (2005) found denser populations of the acetoclasts in the upper sediment layers (0-3 cm) while the hydrogenotrophs were more prevalent in deeper
sediments (i.e., down to 20 cm). Concomitant reductions in total numbers of *Bacteria* with increasing sediment depth were also observed. Consequently, the authors suggested that the decline in total *Bacteria* likely drive the lower overall rates of MG in those sediments as *Bacteria* can regulate the availability of MG substrates. Given that a host of symbiotic bacterial processes regulate the availability of methanogenic substrates (Glissman et al. 2004) a similar mechanism may explain the depth distribution of MG pathways in E4 and GTH 114.

Pelagic primary production in shallow lakes characteristic of the Alaskan Arctic is limited significantly by nutrient availability (Levine and Whalen 2001). Benthic primary production can be an important component of whole-lake primary productivity in shallow lakes due to high light availability to the epipelic algae in close proximity to nutrient rich sediments (Vadeboncoeur et al. 2001; Whalen et al. 2008; Rautio et al. 2011). To supplement these autochthonous limitations, whole-lake metabolism currently depends heavily on terrestrial inputs of nutrients and OM occurring in snowmelt and periodic flushing from storm events (Whalen and Cornwell 1985; Kling 1995; Michaelson et al. 1998; Cory et al. 2007). Increases in the magnitude and frequency of nutrient and OM delivery to aquatic systems from the terrestrial landscape may significantly alter lacustrine ecosystem processes in response to climate change (Rouse et al. 1997). Whalen and Levine (2001) showed that nutrient additions increased phytoplankton uptake rates in bioassays from 45 lakes in the same region as my study lakes. In a separate study, direct deposition of algal biomass to sediment cores from Lake Kinneret (Israel) significantly increased CH$_4$ production (Schwarz et al. 2008). In my study, acetate amendments significantly stimulated MG relative to the controls in nearly all depth intervals in both lakes. The magnitude of the projected increases in nutrient and OM delivery to
lentic systems will depend on vegetation composition, precipitation, hydrological flow paths and localized permafrost thaw depths (Prowse et al. 1996; Rouse et al. 1997; Michaelson et al. 1998; Post et al. 2009; Kittel et al. 2011). Collectively, my results and these previous reports suggest that increased nutrient delivery from the terrestrial landscape in response to climate change may indirectly fuel MG through stimulation of autochthonous production in shallow arctic lakes.

Terrestrial OM inputs to shallow arctic lakes under current climatic conditions may also influence MG activity through photodegradation of recalcitrant DOM into labile substrates (Moran and Zepp 1997; Judd et al. 2007; Zhang et al. 2009; Rautio et al. 2011). In the present study, FI values of DOC samples collected from the epilimnion and hypolimnion of E4 and GTH 114 indicated a high degree of terrestrial source material (Cory et al. 2010) and were consistent with measurements from Toolik Lake, a large, deep lake in the region of my study (Cory et al. 2007). Terrestrially derived OM typically shows FI indices of < 1.4 while FI values > 1.4 are indicative of autochthonous OM (Cory et al. 2010). In GTH 114, the FI values did not differ significantly between the epilimnion and hypolimnion. However, in E4, epilimnetic FI values were significantly lower than FI values for the hypolimnion. Bacterial processing of terrestrial OM can increase the FI value relative to the source material (Cory et al. 2007). Similarly, photobleaching can lower the FI value (Cory et al. 2007) which is likely why the epilimnetic samples in E4 and GTH 114 exhibited lower FI values than the respective hypolimnnetic samples, a result that is consistent with observations in nearby surface waters (Dr. Rose M. Cory, personal communication). Acetate is a byproduct of photodegradation of DOM (Moran and Zepp 1997; Bertillson and Tranvik) which as previously discussed, is a primary substrate for MG.
Consequently, photodegradation of DOM may represent an important linkage between MG substrate supply and terrestrial OM inputs to shallow arctic lakes.

Increased frequency and magnitude of terrestrial DOC inputs to arctic lakes may lead to alterations in lake-ecosystem function. Under current conditions, E4 and GTH 114 periodically exhibit intermittent and weak thermal stratification throughout the open water season (Figures 2.1 and 2.2). Allochthonous OM differs substantially from autochthonous DOM in its capacity to absorb light (Rautio et al 2011). Terrestrially derived OM absorbs more light than autochthonous OM (Reche et al. 2001; Pace and Cole 2002; Sobek et al. 2007). Thus, high inputs of allochthonous OM can affect the thermocline structure and depth of the mixed layer in response to light absorption and heat retention (Houser et al. 2003; Sobek et al. 2007; Fortino 2010). In a 2008 survey of 15 lakes near Toolik Field Station, Fortino (2010) found a significant correlation between light attenuation and DOC, and between light attenuation and thermocline depth. The results of that study indicated that shallower thermoclines develop in response to greater light attenuation as a function of higher DOC concentrations. Increased light attenuation by terrestrial DOC also results in reduced light availability to the benthos which can be particularly important in shallow lakes where benthic primary production is a major contributor to whole-lake primary production (see above). Additionally, deeper hypolimnetic waters and extended stratification periods may lead to hypoxic or anoxic bottoms water (Rouse et al. 1997). Accordingly, production of CH$_4$ under these conditions can result in a hypolimnetic buildup of CH$_4$ that would be directly released to the atmosphere at turnover (Kankaala et al 2006).
2.4.3 Substrate Additions

The stimulatory effect of acetate or hydrogen relative to the unamended treatments generally increased with increasing sediment depth (Figure 2.4). However, in the deeper sediment intervals (i.e., 3-4 and 4-5 cm), H\textsubscript{2} exerted a much stronger influence on MG rates than acetate supplementation compared to the controls in GTH 114 than in E4. Two possible mechanisms could be driving this observation. Methanogens are capable of dormancy for extended periods (Rothfuss et al. 1997; Watanabe et al. 2007), but could be readily stimulated by substrate additions. Alternatively, compositional differences in methanogenic communities could be driving the observed patterns if hydrogenotrophic methanogens are more densely populated at these depth intervals than acetoclastic methanogens.

2.4.4 Pathway Delineation

Although the acetoclastic pathway appeared to dominate the upper sediment layers in each lake, the down core shift in pathway importance in each lake was not parallel. The data suggest that the acetoclastic pathway was the dominant mechanism for CH\textsubscript{4} production in all depth intervals measured in E4, but two sediment depth intervals (i.e., 2-3 and 4-5 cm) in GTH 114 indicated a slightly higher importance of the hydrogenotrophnic pathway than the acetoclastic pathway. Depth integrated rates of MG in each lake shows that the approximate ratio of the acetoclastic: hydrogenotrophic pathway was 8:1 in E4, whereas the ratio in GTH 114 was 3:1 indicating that the acetoclastic pathway is a more important mechanism in E4 than in GTH 114 in the upper 5 cm of sediment. Some studies have found that complete dominance of CH\textsubscript{4} production from acetate is indicative of acetate production via homoacetogenesis (Schulz and Conrad 1996; Conrad 1999; Nusslein and Conrad 2000). Homoacetogenetic bacteria
synthesize acetate directly from fermentation products, effectively bypassing the degradation step by syntrophic bacteria that typically produces alcohols and fatty acids which are then degraded into methanogenic substrates (i.e., acetate, H\textsubscript{2}/CO\textsubscript{2}) (Conrad 1999). Because the acetoclastic pathway shows nearly complete dominance in the upper sediment layers of E4, high rates of homoacetogenesis could be causing the observed ratios in the MG pathway with increasing sediment depth in E4, but the factors controlling the overall higher contribution of the hydrogenotrophic pathway in GTH 114 and at comparable depths is not clear.

The predominance of the hydrogenotrophic pathway over acetoclastic fermentation is typically only found in marine systems (Crill and Martens 1986) or in peat bogs (Horn et al. 2003; Prater et al. 2007; Rooney-Varga et al. 2007). Occurrence of a higher contribution from the hydrogenotrophic pathway than the acetoclastic pathway in lake sediments is uncommon and not very well understood. Potential reasons for the importance of the hydrogenotrophic pathway to exceed acetate fermentation in freshwater lake sediments could be attributed to excessively high concentrations of acetate, which inhibit acetoclastic MG (Nozhevnikova et al. 1997), or additional sources of H\textsubscript{2} (Conrad 1999). Substantial differences in acidity and vegetational composition can exist on surfaces of different glacial age on the North Slope of Alaska (Hobbie et al. 2002), which could contribute to the variation in landscape-scale control on MG pathways between these two catchments. Both of these lakes are on surfaces that were glaciated during the middle Pleistocene (i.e., approximately 125,000 to 780,000 years ago), but they differ considerably with age since deglaciation. GTH 114 exists in a region that is much older than E4 in terms of glacial histories (Hamilton 2002). Therefore, it is possible that OM age,
vegetation composition, and consequently the quality of DOC entering the lakes influences the relative proportion of MG pathways in these shallow lakes.

The functional linkage between arctic lakes and their surrounding landscapes will likely be altered in the future as a consequence of global and regional climate change. Some of the most notable predictions contend that terrestrial OM delivery to the lakes will be amplified due to widespread thawing of permafrost (Weller et al. 1995; Mazeas et al. 2009; Karlsson et al. 2010). Mobilization of the previously sequestered OM into arctic lake sediments will likely enhance production of CH$_4$ in these environments (Zimov et al. 1997; Walter et al. 2007; Mazeas et al. 2009; Karlson et al. 2010). The degree of influence on individual MG pathways is not yet clear. Given the widespread distribution of these lakes across the arctic landscape, increased CH$_4$ production without complementary offsets by CH$_4$ oxidation will considerably impact the atmospheric CH$_4$ budget, serving as positive feedback to climate warming. This study suggests that methanogenic communities in these two shallow arctic lakes are currently substrate limited. Consequently, increased OM loading to arctic lakes will in all probability positively impact rates of MG in these lake sediments. While the relative ratio of the acetoclastic: hydrogenotrophic pathways did change with increasing sediment depth, individual response of each pathway to increased terrestrial OM inputs is not clear. The variation in the relative proportion of these pathways between E4 and GTH 114 suggests that landscape scale factors (i.e., glacial histories and DOC loading) may play a role in governing the functional distribution of methanogenic pathways.
2.5 Conclusion

Rates of MG in unamended treatments differed drastically between these two geomorphologically similar lakes. Within each lake, rates of MG decreased with increasing sediment depth, and responded positively to substrate additions of acetate or hydrogen. Mean DOC concentrations were significantly higher in the upper 5 cm of GTH 114 than in lake E4. In contrast, %OM was significantly greater in E4 than in GTH 114 in the upper 5 cm sediment layer. The acetoclastic pathway dominates the surficial sediments in these two shallow arctic lakes, but transitions to increased importance of the hydrogenotrophic pathway in the deeper sediments, which is consistent with previous reports (Falz et al. 1999; Chan et al. 2005). However, considerable within and between lake variations in the relative proportion of these pathways with vertical sediment depth were also present. Proliferation of oxic or anoxic conditions in the surficial sediment layer as dictated by mixing regimes represents internal controls on MG rates within a lake. Factors explaining the between lake variation may be DOC quality and quantity as well as characteristics inherent to each catchment (e.g., hydrology and plant cover type). To what degree the relative proportion of the MG pathways will be altered may ultimately depend on the quantity and quality of the OM reaching lake sediments. The results from this study suggest that currently rates of MG are likely controlled by internal mixing regimes, substrate availability as well as landscape scale factors (e.g., allochthonous OM inputs). Consequently, methanogenic bacteria will likely respond positively to increases in OM delivery to lake sediments associated with future climate change. Given the variability in rates of MG within and between these two lakes, future studies should focus on multiple lakes within
catchments, as well as between catchments, in order to gain a comprehensive assessment of CH$_4$ cycling in arctic lakes.
REFERENCES


### Table 2.1. Morphological characteristics of study lakes.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Catchment area (m²)</th>
<th>Lake surface area (m²)</th>
<th>Catchment: lake area ratio</th>
<th>Lake volume (m³)</th>
<th>Maximum depth (m)</th>
<th>Mean depth (m)</th>
</tr>
</thead>
<tbody>
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<td>E4</td>
<td>476,856</td>
<td>38,955</td>
<td>12</td>
<td>55,234</td>
<td>4.4</td>
<td>2.0</td>
</tr>
<tr>
<td>GTH 114</td>
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<td>39,583</td>
<td>15</td>
<td>87,111</td>
<td>6.7</td>
<td>2.2</td>
</tr>
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</table>
Table 2.2. Results of individual One-way ANOVA’s evaluating the effect of sediment depth increment or substrate additions on unamended (control) rates of methanogenesis (MG) in E4 and GTH 114.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Lake</th>
<th>Post-hoc analysis</th>
<th>$F$-ratio</th>
<th>$p$-value</th>
<th>Means comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG rates (controls)</td>
<td>Sediment depth</td>
<td>E4</td>
<td>Tukey’s HSD</td>
<td>$F_{4,10} = 7.89$</td>
<td>0.004</td>
<td>1-2</td>
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<td>MG rates (controls)</td>
<td>Sediment depth</td>
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<td>Tukey’s HSD</td>
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<tr>
<td>MG rates (controls)</td>
<td>Lake</td>
<td>E4 &amp; GTH 114</td>
<td>Student’s t</td>
<td>$F_{1,4} = 1.94$</td>
<td>0.23</td>
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<td>$F_{1,4} = 8.81$</td>
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* In descending order of least square mean value.

** Levels connected by the same letter are not significant ($\alpha = 0.05$).
Table 2.3. Results of individual One-way ANOVA’s evaluating the effect of acetate (Ac) or hydrogen (H₂) substrate additions on rates of methanogenesis (MG).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Lake</th>
<th>Post-hoc analysis</th>
<th>F ratio</th>
<th>p-value</th>
<th>Sediment depth interval (cm)*</th>
<th>Significant depth differences**</th>
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<td><strong>MG rates</strong></td>
<td>Treatment (Control, Ac, H₂ only)</td>
<td>E4</td>
<td>Dunnett’s test</td>
<td>F₂,₆ = 15.43</td>
<td>0.004</td>
<td>0-1</td>
<td>Ac &gt; control; H₂ &gt; control</td>
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<td>F₂,₆ = 3.55</td>
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<td></td>
<td></td>
<td>F₂,₆ = 21.42</td>
<td>0.002</td>
<td>2-3</td>
<td>Ac &gt; control; H₂ &gt; control</td>
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<td></td>
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<td>F₂,₆ = 23.27</td>
<td>0.002</td>
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<td></td>
<td></td>
<td>F₂,₆ = 149.2</td>
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<td>4-5</td>
<td>Ac &gt; control; H₂ &gt; control</td>
</tr>
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<td><strong>MG rates</strong></td>
<td>Treatment (Control, Ac, H₂ only)</td>
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<td>Dunnett’s test</td>
<td>F₂,₆ = 6.96</td>
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<td>F₂,₆ = 88.94</td>
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<td>F₂,₆ = 23.23</td>
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<td>4-5</td>
<td>Ac &gt; control; H₂ &gt; control</td>
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<tr>
<td><strong>MG Rates</strong></td>
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<td>Student’s t</td>
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<td><strong>MG Rates</strong></td>
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<td><strong>MG Rates</strong></td>
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* In descending order of least square mean value.

** Levels connected by the same letter are not significant (α = 0.05)
Table 2.4. Results of individual One-way ANOVA’s evaluating the effect of sediment depth increment or lake on dissolved organic concentrations (DOC) or percent organic content (OM) of sediment in E4 and GTH 114.

<table>
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<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Lake</th>
<th>Post-hoc analysis</th>
<th>F-statistic</th>
<th>p-value</th>
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<tbody>
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<td>Depth</td>
<td>E4</td>
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<td>1-2: A B</td>
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<td>2-3: A B</td>
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<td>4-5: A B</td>
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<td>0-1: B</td>
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<td></td>
<td></td>
<td>3-4: No difference</td>
</tr>
<tr>
<td>DOC</td>
<td>Lake by depth</td>
<td>E4 and GTH 114</td>
<td>Student’s t</td>
<td>$F_{1,4} = 7.96$</td>
<td>0.047</td>
<td>0-1: GTH 114 &gt; E4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F_{1,3} = 3.35$</td>
<td>0.16</td>
<td>1-2: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F_{1,3} = 0.99$</td>
<td>0.39</td>
<td>2-3: No difference</td>
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<td></td>
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<td></td>
<td>$F_{1,3} = 2.29$</td>
<td>0.22</td>
<td>3-4: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F_{1,4} = 0.14$</td>
<td>0.73</td>
<td>4-5: No difference</td>
</tr>
<tr>
<td>DOC</td>
<td>Lake</td>
<td>E4 and GTH 114</td>
<td>Student’s t</td>
<td>$F_{1,25} = 7.67$</td>
<td>0.01</td>
<td>GTH 114 &gt; E4</td>
</tr>
<tr>
<td>arcsine OM</td>
<td>Depth</td>
<td>E4</td>
<td>Tukey’s HSD</td>
<td>$F_{4,9} = 0.41$</td>
<td>0.80</td>
<td>2-3: No difference</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>4-5: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-2: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>0-1: No difference</td>
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<td>3-4: No difference</td>
</tr>
<tr>
<td>arcsine OM</td>
<td>Depth</td>
<td>GTH 114</td>
<td>Tukey’s HSD</td>
<td>$F_{4,9} = 1.57$</td>
<td>0.26</td>
<td>0-1: No difference</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>2-3: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4-5: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-4: No difference</td>
</tr>
<tr>
<td>arcsine OM</td>
<td>Lake by depth</td>
<td>E4 and GTH 114</td>
<td>Student’s t</td>
<td>$F_{1,4} = 6.34$</td>
<td>0.07</td>
<td>0-1: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F_{1,3} = 48.33$</td>
<td>0.006</td>
<td>1-2: E4 &gt; GTH 114</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F_{1,3} = 9.32$</td>
<td>0.06</td>
<td>2-3: No difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F_{1,4} = 13.08$</td>
<td>0.02</td>
<td>3-4: E4 &gt; GTH 114</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$F_{1,4} = 1.88$</td>
<td>0.4</td>
<td>4-5: No difference</td>
</tr>
<tr>
<td>arcsine OM</td>
<td>Lake</td>
<td>E4 and GTH 114</td>
<td>Student’s t</td>
<td>$F_{1,26} = 27.00$</td>
<td>&lt;0.0001</td>
<td>E4 &gt; GTH 114</td>
</tr>
</tbody>
</table>

* In descending order of least square mean value.
** Levels connected by the same letter are not significant (α = 0.05)
Table 2.5. Results of simple linear regressions of porewater dissolved organic carbon (DOC) or sediment percent organic matter content (% OM) on sediment depth.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Model</th>
<th>Equation</th>
<th>n</th>
<th>$R^2$</th>
<th>p  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4</td>
<td>arcsine %OM = depth</td>
<td>arcsine %OM = 0.45 + 0.0011*depth</td>
<td>14</td>
<td>0.0003</td>
<td>0.95</td>
</tr>
<tr>
<td>GTH 114</td>
<td>arcsine %OM = depth</td>
<td>arcsine %OM = 0.36 – 0.013*depth</td>
<td>14</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>E4</td>
<td>DOC = depth</td>
<td>DOC = 4.34 + 3.62*depth</td>
<td>13</td>
<td>0.44</td>
<td>0.01</td>
</tr>
<tr>
<td>GTH 114</td>
<td>DOC = depth</td>
<td>DOC = 29.91 – 1.74*depth</td>
<td>14</td>
<td>0.07</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Table 2.6. Spearman’s rank correlation coefficients (\(r_s\)) describing the association between rates of methanogenesis and environmental variables in E4 and GTH 114.

<table>
<thead>
<tr>
<th>Lake</th>
<th>E4</th>
<th>GTH 114</th>
<th>Both lakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment depth</td>
<td>-0.83***</td>
<td>-0.61*</td>
<td>-0.71***</td>
</tr>
<tr>
<td>DOC</td>
<td>-0.37</td>
<td>0.62*</td>
<td>0.09</td>
</tr>
<tr>
<td>arcsine OM</td>
<td>0.08</td>
<td>0.68**</td>
<td>-0.24</td>
</tr>
</tbody>
</table>

Values of \(r_s\) significant at \(\alpha = 0.05\) are denoted by “*”, \(\alpha = 0.01\) are denoted by “**”, and \(\alpha = 0.0001\) are denoted by “***”.
Figure 2.1. Dissolved oxygen and temperature profiles in the water column of E4 taken during the summer of 2008. Dotted lines represent water column temperature profiles and solid lines represent dissolved oxygen profiles.
Figure 2.2. Dissolved oxygen and temperature profiles in the water column of GTH 114 taken during the summer of 2008. Dotted lines represent water column temperature profiles and solid lines represent dissolved oxygen profiles.
Figure 2.3. Depth distribution of unamended rates of methanogenesis in E4 and GTH 114. Homogenized slurries from each depth interval were incubated in triplicate at 10°C in 2008. Error bars are ±1 SEM (n = 3).
Figure 2.4. Depth distribution of rates of methanogenesis in E4 in response to amendments with methanogenenic substrates for samples incubated at 10°C in 2008. Error bars are ± 1 SEM (n = 3).

Figure 2.5. Depth distribution of rates of methanogenesis (MG) in GTH 114 in response to amendments with methanogenenic substrates for samples incubated at 10°C in 2008. Error bars are ± 1 SEM (n = 3).
Figure 2.6. Percent increase in rates of methanogenesis (MG) in response to methanogenic substrate additions relative to unamended treatments in E4 and GTH 114 in 2008.
Figure 2.7. Depth distribution of the relative proportion of acetoclastic and hydrogenotrophic pathways in E4 in 2008.

Figure 2.8. Depth distribution of the relative proportion of acetoclastic and hydrogenotrophic pathways in GTH 114 in 2008.
Figure 2.9. Dissolved organic carbon (DOC) concentrations plotted at the midpoint of each sampling interval for sediment samples in E4 and GTH 114 porewater taken from the deepest point in each lake in July 2008. Error bars are ± 1 SEM (n = 3).
Figure 2.10. Organic matter profiles plotted at the midpoint of each sampling interval for sediment samples taken from the deepest point of lakes E4 and GTH 114 in 2008. In most cases, n = 3. Error bars are ± 1 SEM.
Figure 2.11. Scatterplot of unamended rates of methanogenesis (MG) versus organic matter (OM) content at a corresponding depth interval in E4 and GTH 114 in 2008.
Figure 2.12. Scatterplot of the rates of methanogenesis (MG) from the acetoclastic or hydrogenotrophic pathways versus organic matter (OM) at the corresponding depth interval in E4 from 2008.

Figure 2.13. Scatterplot of the rates of methanogenesis (MG) from the acetoclastic or hydrogenotrophic pathways versus organic matter (OM) at the corresponding depth interval in GTH 114 from 2008.
CHAPTER 3: SPATIAL VARIATION OF METHANOGENESIS AMONG LAKE ZONES

3.1 Introduction

Arctic Alaskan lakes currently receive large inputs of allochthonous organic matter (OM), particularly during snowmelt and precipitation events (Michaelson et al. 1998). Several studies suggest that thawing permafrost and elevated terrestrial plant productivity will increase OM loading to arctic lakes as a result of ongoing climate change (Neff and Hooper 2002; Rouse et al. 1997; Shaver et al. 1992). Consequently, increased inputs of terrestrially derived OM may lead to higher rates of methanogenesis (MG) in arctic lakes, and OM inputs may influence this activity in shallow sediments to a larger degree than in profundal sediments due to proximity to the terrestrial-aquatic interface. Measurements of MG have been conducted traditionally in the deepest region of lakes (e.g., Kuivila et al. 1989; Schulz and Conrad 1996; Nozhevnikova et al. 1997; Nusslein and Conrad 2000; Huttenen et al. 2006; Conrad et al. 2007; Schwarz et al. 2008). However, research targeted on one sediment zone of a lake may not capture the range of methanogenic activity in that ecosystem. Bastviken et al. (2008) found that shallow epilimnetic sediments are important regions of methane (CH$_4$) production and there is considerable evidence that littoral zones may produce more CH$_4$ than profundal areas (Thebath et al. 1993; Casper 1996; Rolletschek 1997; den Heyer and Kalff 1998). The proportion of OM (quantity and quality), disturbance frequency (e.g., sediment resuspension), sediment temperature and sediment composition can vary significantly among zones within a lake (Casper 1996; Bussman 2005; Bastviken et al. 2008) and all are known to influence
methanogenic activity. In particular, microbial metabolic activity in the littoral region is highly influenced by inputs of terrestrial OM (Michmerhuizen et al. 1996; Juutinen et al. 2003). Therefore, terrestrial inputs of OM may fuel MG activity in nearshore sediment to a larger degree than in deeper sediment zones in shallow arctic lakes.

The ratio of the littoral zone to the entire lake area is often higher in smaller lakes (Michmerhuizen et al. 1996) like those that dominate the arctic (Wetzel 2001; Juutinen et al. 2003). Consequently, the relative importance of CH₄ efflux from the littoral zone (or shallow sediments) may be more important than CH₄ release from deeper zones in terms of the total areal CH₄ emissions from the sediment surface. By focusing primarily on the deepest point within a lake, previous studies may have underestimated the total CH₄ production from a given lake, particularly in arctic lakes that generally display an extensive shallow littoral region. Therefore, this research objective focused on determination of MG rates in sediments of three lake zones in two shallow arctic lakes; profundal (maximum water depth), epilimnetic (one half the maximum water depth) and littoral (along lake margins). I hypothesized that the rates of MG and CH₄ release to overlying water in the shallow sediments (epilimnetic and littoral zones) would be greater than rates of MG and CH₄ efflux in the profundal region in these arctic lakes due to proximity to important terrestrial OM inputs.

3.2 Materials and Methods

3.2.1 Field Sites

This study was conducted on the North Slope of Alaska within a few kilometers of Toolik Field Station (TFS), which is located at 68°N, 149°W. This region of the Arctic is characterized by
a tundra landscape underlain with continuous permafrost (Whalen et al. 2006). Mean annual temperatures range from –7 to –11°C with annual precipitation ranging from 140 to 267 mm (Bowden et al. 2008). The trophic state of most lakes in this region ranges from ultraoligotrophic to oligotrophic and rely heavily on spring snowmelt for terrestrial subsidies of dissolved organic matter (Whalen and Cornwell 1985; Kling 1995). A full description of the region including vegetation characteristics and glacial geology can be found in (Ping et al. 1998) and Hamilton (2002). The two lakes chosen for this study, E4 and GTH 114, possess similar geomorphological characteristics, with GTH 114 having larger total volume and catchment area (Table 2.1; Chapter 2). Typical in size and catchment area of lakes in the Arctic Foothills region of northern Alaska, these lakes are also fishless, lack permanent inlets and rooted macrophytes are absent.

3.2.2 Field Sampling

I sampled littoral, epilimnetic and profundal sediments of E4 on 29 June 2010 and GTH 114 on 30 June 2010. The term profundal typically refers to deeper lakes than those included in my study, while the littoral zone can be further divided into distinct sub-regions (Wetzel 1999). For simplicity and consistency with related studies (Casper 1996; Rolletschek 1997; Den Heyer and Kalff 1998; Bastviken et al. 2008), these broad definitions were extended to my lakes. In my study, the profundal zone refers to the sediments at the maximum water depth (Table 3.1), the epilimnetic zone refers to sediments at ½ of the maximum water depth and the littoral zone is defined by sediments with approximately 0.25 m of overlying water. Epilimnetic and profundal sediments for CH₄ production studies were collected using a KB gravity corer (Wildlife Supply Company) deployed from an inflatable raft while littoral sediments were collected from the
shore exercising care to minimize sediment disturbance. Polycarbonate sleeves (4.8 cm inside diameter x 50 cm length) were inserted into the KB corer, which was then lowered carefully into the epilimnetic and profundal sediments. Cores sleeves were inserted by hand into littoral sediments. All sleeves were capped on each end with rubber stoppers once sediment was collected. Additional sediments from the profundal and epilimnetic zones were similarly collected on 26 July 2008 (E4) and 30 July 2008 (GTH 114) for porewater dissolved organic carbon (DOC) determination, while sediments from all three sediment zones were obtained for determination of % OM content and porewater CH$_4$ concentration between 18 – 20 July 2009. Intact sediment cores were transported undisturbed via foot, vehicle, or helicopter to TFS for processing. Duplicate cores for sediment temperature profiles were collected from E4 on 24 July 2009 and GTH 114 on 25 July 2009. Cores were taken one at a time from each zone and immediately returned to shore for temperature measurements. After the overlying water was removed from the core, a thermometer was incrementally advanced vertically at 1 cm intervals midcore to a final depth of 20 cm. Additionally sediment cores were collected from each zone (n=2) and lake on 22 July 2009 (E4) and 24 July 2009 (GTH 114) for porewater CH$_4$ analysis. Water column samples for DOC analysis were collected with a Van Dorn water sampler at 1 m below the water surface (epilimnion) and approximately 1 m above the sediment (hypolimnion) in each lake on multiple sampling dates in 2009.

**3.2.3 Laboratory Studies**

Replicated (5) sediment slurries were prepared using a total of 10 cores per zone. The upper 2 cm of two sediment cores were homogenized into a polycarbonate 1-L beaker. Anoxic deionized water (dH$_2$O) was prepared in separate polycarbonate beakers by purging with high
purity N\textsubscript{2} and an air stone affixed to the end of tygon tubing. Following addition of dH\textsubscript{2}O, all homogenized sediment slurries were continually purged with N\textsubscript{2} during the following experimental setup. A polycarbonate syringe with a bored tip was used to measure 20 ml of anoxic sediment slurry that was then transferred to 160 ml serum bottles. Serum bottles were sealed with butyl rubber stoppers and capped with aluminum crimp seals. Each serum bottle was evacuated and purged with N\textsubscript{2} a minimum of 10 times to ensure anoxia, filled to 1 atm with N\textsubscript{2} then placed in a 10°C water bath to acclimate for approximately 12 h before headspace sampling was initiated.

Headspace samples were collected from serum bottles in 3 ml plastic syringes that had been previously tested to confirm no loss of CH\textsubscript{4} over a 4 h test period. Analysis of CH\textsubscript{4} was conducted by a gas chromatograph equipped with a flame ionization detector (FID-GC; Shimadzu GC-8A) and was completed within 2 h of headspace sampling. Operating conditions for the FID-GC were as follows: Column = ¼” diameter x 1 m length mol sieve 5A (60/80); column temperature = 90°C; injector and detector temperatures = 140°C; carrier gas = ultra-high purity N\textsubscript{2} at 33 ml min\textsuperscript{-1} flow rate. Vial headspaces were sampled 4 times over 10 d, resulting in the linear production of CH\textsubscript{4}. Rates of CH\textsubscript{4} production were calculated using linear regression of CH\textsubscript{4} accumulation versus time and were normalized to 1 g dry sediment matter.

Triplicate cores for porewater analysis of DOC were sliced in 1 cm increments, which were then added to 15 ml glass centrifuge tubes and sealed with rubber septa without a headspace. Tubes were centrifuged at < 2000 rpm for 30 min. The supernatant for each core section was filtered (ashed Whatman GF/F filter) and acidified (0.1 ml of 0.1N HCl). Dissolved organic carbon samples were stored at 4°C, transported to UNC-CH for analysis, and
subsequently analyzed on a Shimadzu TOC-V$_{CPH}$ Combustion-Infrared instrument using Standard Method 5310B (Standard Methods for the Examination of Water and Wastewater 1998). Solid phase sediments (in 1 cm increments) were weighed, dried for 2-3 d at 45°C and reweighed. Dry sediments were ashed at 550°C for 4 h. In addition, 20 mL of lake water was filtered through pre-combusted Whatman GFF filters into amber scintillation vials with Teflon caps. Samples were acidified with 0.1 mL 1 N HCl, stored at 4°C, and transported back to UNC-CH for analysis.

Sediment CH$_4$ profiles (n=2) at each lake zone were determined using a modified polycarbonate sediment core squeezer adapted from Jahnke (1988). The original design described in Jahnke (1988) would not accommodate the ultra-soft sediments in these lakes so a more stationary design for core porewater sampling was implemented. The squeezer apparatus contained pre-drilled sampling holes at 1 cm intervals in the first 10 cm, then at 2 cm intervals from 10-20 cm. Black electrical tape was placed over the holes for the duration of sampling. Intact sediment cores were vertically extruded into the core squeezer such that the position of the sediment-water interface corresponded with the first vertical sampling port. Beginning at the top of the core, a syringe with a 20 gauge needle was inserted horizontally through the taped holes to the center of the core within each sediment interval and 1 ml of porewater and associated sediment was extracted. The sample was then placed into an N$_2$- filled 30 ml serum vial containing 200 µL 1 N HCl. Vials were inverted and CH$_4$ was allowed to equilibrate between the aqueous and gas phases for 24 h prior to headspace analysis for CH$_4$ by FID-GC.
3.2.4 Calculations and Statistics

Headspace mixing ratios for CH$_4$ in serum vials were converted to a moles per liter basis. Methane concentrations in the aqueous phase at each sampling point were calculated from temperature corrected solubility coefficients (Yamamoto et al. 1976) and Henry’s law (Stumm and Morgan 1996). The total amount of CH$_4$ produced in each bottle at each time point was computed as the sum of the aqueous phase and headspace masses.

Rates of CH$_4$ production were log transformed to correct departures from normality and homoscedasticity. Two-way ANOVA was used to test the effect of lake, sediment zone and lake x sediment zone interactions on log-transformed rates of MG. Comparisons of means were conducted using either Tukey’s Least Significant Difference test or Student’s t-test, where appropriate (Sokal and Rohlf 1995; Zar 1996; McDonald 2009). The effect of zone on log-transformed rates MG within each lake was analyzed individually using One-Way ANOVA. Pair-wise differences among log-transformed mean rates of MG in each sediment zone were determined using Tukey’s HSD test (Sokal and Rohlf 1995; Zar 1996; McDonald 2009).

Within-lake and among-lake variations in environmental variables (i.e., DOC, % OM content, sediment temperature, and porewater CH$_4$ were evaluated using the entire downcore sediment profile for each variable. Initially, the linear dependence of each environmental variable with sediment depth was evaluated with simple linear regression. In the case of each environmental variable, either significantly linear relationships in all profiles among sediment zones were not found, or some regression assumptions were violated preventing use of these procedures. Therefore, One-way ANOVA’s were used to compare within-lake and between-lake differences in DOC, %OM, sediment temperature and porewater CH$_4$ profiles. Post-hoc
comparisons of means were conducted with Tukey’s HSD or Student’s t test where appropriate. Values for % OM content were arc-sin transformed for all statistical analyses (Sokal and Rohlf 1995; Zar 1996; McDonald 2009).

To determine the correlation between environmental variables (DOC, % OM and temperature) and log-transformed rates of MG, the mean values for the upper 2 cm of sediment were used as this coincided directly with the sediment layer homogenized in MG rate determinations. Log-transformation of environmental variables (i.e., mean DOC, % OM, and temperature in the upper 2 cm sediment interval) did not correct violations of normality, so the degree of association between each environmental variable and log-transformed rates of MG was assessed using Spearman’s rank correlation ($r_s$). The effect of sediment zone on porewater CH4 profiles was assessed using One-way ANOVA per lake followed by multiple comparison of means using Tukey’s HSD test (Sokal and Rohlf 1995; Zar 1996; McDonald 2009). All analyses were performed using JMP 9.0 or SAS 9.2 (SAS Institute, Inc.) statistical software.

Organic content (%) was calculated from the difference in mass between oven dried (40-60°C) and combusted (550°C for 4 h) sediments (den Heyer and Kalff 1998; Wetzel and Likens 2000).

Methane flux from the sediment surface into the overlying water column was estimated using Fick’s first law of diffusion (Sweerts 1991):

$$J_s = -\varphi D_s (\Delta c/\Delta x)$$

where: $J_s$ is the flux of CH$_4$ ($\mu$mol CH$_4$ m$^{-2}$ s$^{-1}$); $\varphi$ is the sediment porosity (unitless); $\Delta c/\Delta x$ is the change in CH$_4$ concentration with depth ($\mu$mol CH$_4$ cm$^{-3}$ cm$^{-1}$); and $D_s$ is the effective diffusivity.
cm² s⁻¹). $D_s$ was estimated using the following equation (Sweerts 1980; Boudreau 1996; Huttenen et al. 2006):

$$D_s = \frac{D_0}{\theta^2}$$

where $D_0$ is the diffusivity of CH₄ at 10°C (1.25 x 10⁻⁵ cm² s⁻¹; Jahne et al. 1987), $\theta$ is the tortuosity (unitless) which can be estimated by the easily measured sediment porosity ($\varphi$) through the following equation (Boudreau 1996):

$$\theta = 1 - \ln(\varphi^2)$$

Porosity was calculated using the following equation (Percival and Lindsay 1997):

$$\varphi = \frac{100}{\rho_w} \times (M_{ws} - M_{ds})/V_{ws}$$

where $\rho_w$ is the density of water (1 g cm⁻³), $M_{ws}$ is the empirically determined wet sediment mass (g), $M_{ds}$ is the empirically determined dry sediment mass (g) and $V_{ws}$ is the volume of wet sediment (cm³).

Methane flux from each zone into the overlying water column was estimated by multiplying the sediment surface area of each zone within each lake by the CH₄ diffusive flux. The cumulative sediment surface area of each zone was estimated from bathymetric maps that were constructed by concomitant measurements of lake perimeter (Tremble Geo Explorer GPS) and sonar transects (Garmin GPSMAP 180 sonar). One meter contour lines along the lake bottom were constructed from triangulated sonar measurements in ARC-GIS (ESRI, 2006) by the GIS specialists at the Toolik Field Station. A whole lake estimate of CH₄ efflux from the sediment in each lake was obtained by multiplying the area-based (m²) estimate of CH₄ release from each zone times the total area of that zone and summing the values. The littoral, epilimnetic and profundal zones in E4 were considered to be the area of sediment surface in
the 0-1, 1-3 and 3-4.5 m depth intervals. In GTH 114, the 0-1, 1-4 and 4-6.5 m depth intervals were assigned to the respective zones.

3.3 Results

Non-transformed rates of MG were highly variable in the littoral zone of E4 (Figure 3.1) and the profundal zone of GTH 114 (Figure 3.2). Two-way ANOVA showed significant relationships between log-transformed rates of MG and lake, log-transformed rates of MG and zone as well as significant interactions between lake and zone (Table 3.2). The significant interaction between lake and zone indicated that the effect of sediment zone on log-transformed rates of MG was dependent upon lake. Therefore, One-way ANOVAs were conducted to evaluate within lake differences in log-transformed rates of MG among zones and the between lake differences in sediment zone with respect to log-transformed rates of MG. Tukey’s HSD showed that log-transformed rates of MG in the littoral zone and in the profundal zone were significantly greater than the log-transformed rates of MG measured in slurries from epilimnetic sediments in E4 (Table 3.3). No significant differences were found among zones in the GTH 114 (Table 3.4). Between lakes, no significant difference was found between log-transformed rates of MG in the profundal zone (Table 3.5) or epilimnetic sediments (Table 3.6). In the littoral zone, however, Student’s t test showed that E4 had significantly higher log-transformed rates of MG than in the littoral zone of GTH 114 (Table 3.7).

The mean pelagic DOC concentration in GTH 114 was significantly greater than the respective value in E4 ($F_{1,18} = 133.62; p < 0.0001$; Figure 3.3). No significant difference was found between mean downcore porewater DOC (± 10 cm sediment depth) concentrations in
the profundal (32 mg L$^{-1}$) and epilimnetic (21 mg L$^{-1}$) sediments of E4 ($F_{1.51} = 1.80$, $p = 0.19$; Figure 3.4) or GTH 114 (26 and 35 mg L$^{-1}$, respectively) ($F_{1.54} = 2.12$, $p = 0.15$; Figure 3.5). The mean porewater DOC concentration was significantly higher in the E4 epilimnetic sediments compared to GTH 114 epilimnetic sediments ($F_{1.52} = 6.33$, $p = 0.015$). The mean porewater DOC concentration in the profundal sediments did not differ significantly between lakes (One-way ANOVA, $F_{1,53} = 0.38$, $p = 0.54$).

Significant differences in mean % OM were found among zones in E4 and GTH 114 (Figure 3.6). In E4, the mean OM content of 47% in the littoral zone was significantly greater than the values of 36% and 29% for profundal and epilimnetic sediments ($F_{2,57} = 32.66$, $p < 0.0001$). The mean % OM in profundal sediments of E4 was also significantly greater than that of epilimnetic sediments. In GTH 114, the mean % OM in profundal (25%) and epilimnetic sediments (25%) were not statistically different, but both were significantly higher than the mean % OM in the littoral zone (12%) ($F_{2,57} = 22.68$, $p < 0.0001$). Between lakes, One-way ANOVA using Tukey’s HSD showed that the mean % OM in E4 was significantly greater in the epilimnetic sediments ($F_{1,38} = 11.27$, $p = 0.0018$), the littoral sediments ($F_{1,38} = 165.63$, $p < 0.0001$) and the profundal sediments ($F_{1,38} = 18.74$, $p = 0.0001$) compared to the respective zones in GTH 114.

The mean sediment temperature in E4 epilimnetic sediments (13.7 °C) was significantly greater than mean sediment temperature in the littoral (12.8°C) and profundal (10.9°C) sediments (Table 3.8; Figure 3.7). The mean sediment temperature in the littoral zone of E4 was also significantly greater than mean sediment temperature in the profundal zone. Significant differences in sediment temperature among zones were also found in GTH 114 (Table 3.9;
Figure 3.8). Here, the lowest mean sediment temperature was found in profundal sediments (7.0°C), and this value was significantly lower than the mean sediment temperature in the littoral (13.2°C) and epilimnetic (11.2°C) zones. The mean sediment temperature in the littoral zone was significantly greater than that of the other two zones. Between lakes, mean sediment temperature in the epilimnetic sediments of E4 was significantly greater than the corresponding mean sediment temperature in GTH 114 ($F_{1,78} = 209.53; p < 0.0001$). The mean sediment temperature in the profundal zone of E4 was also significantly greater than the corresponding mean sediment temperature in GTH 114 ($F_{1,38} = 1080.92; p < 0.0001$). No significant difference was found the mean sediment temperatures in littoral zones of the two lakes ($F_{1,51} = 1.55; p = 0.23$).

Spearman’s rank correlations ($r_s$) were used to evaluate the association between log-transformed rates of MG and the mean %OM or mean sediment temperature of the 0 to 2 cm sediment interval, which corresponded with the depth interval used in the sediment slurries to measure MG. A strong significant correlation between log-transformed rates of MG and %OM was found in E4 (Table 3.10). The strength of that association is evident by the proportional changes between rates of MG and %OM content for each sediment zone (Figure 3.9). All pairs of values for mean %OM in the 0 to 2 cm sediment interval among sediment zones were significantly different in E4 ($F_{2,9} = 123.13; p < 0.0001$) and GTH 114 ($F_{2,9} = 80.13; p < 0.0001$). However, no clear pattern between log-transformed rates of MG and %OM was found among sediment zones in GTH 114 (Figure 3.10), which is consistent with the lack of significant correlation between those variables (Table 3.10). When both lakes were combined, a
significant association between log-transformed rates of MG and %OM was detected (Table 3.10).

No correlation was found between log-transformed rates of MG and sediment temperature in E4 (Table 3.10). Mean sediment temperature in the profundal sediments was only slightly lower than the mean sediment temperature in the epilimnetic or littoral zones in the upper 0-2 cm sediment depth interval ($F_{2,9} = 112.82; p < 0.0001$). No clear pattern emerged between sediment temperature and log-transformed rates of MG (Figure 3.11). A significant negative correlation was found between log-transformed rates of MG and temperature in GTH 114 (Table 3.10). In GTH 114, the mean sediment temperature in the 0 to 2 cm interval of the profundal sediments was significantly lower than the mean sediment temperature in the other two zones ($F_{2,9} = 314.80; p < 0.0001$). Although significant differences in log-transformed rates of MG among zones were not detected in GTH 114, the highest mean rate of MG observed in that lake corresponds to the sediment zone with the lowest mean sediment temperature (Figure 3.12). No correlation was observed between log-transformed rates of MG and sediment temperature when both lakes were combined.

Sediment CH$_4$ profiles generally showed increasing CH$_4$ concentrations with increasing depth below the sediment surface in all zones in each lake (Figure 3.13). Mean sediment porewater CH$_4$ concentrations were significantly different among sediment zones in E4 (Table 3.11) and in GTH 114 (Table 3.12). The mean (± SEM) sediment CH$_4$ concentration in E4 littoral sediment (621 ± 78 µM CH$_4$) was significantly greater than the mean concentration in the profundal sediment (196 ± 53 µM CH$_4$). The mean CH$_4$ concentration in E4 epilimnetic sediment (432 ± 55 µM CH$_4$) was also significantly greater than the value for profundal sediment. In GTH
114, the mean sediment CH$_4$ concentration in the littoral zone (573 ± 128 µM CH$_4$) did not differ significantly from those in any other zone. However, the mean sediment CH$_4$ concentration in profundal sediment (933 ± 91 µM CH$_4$) was significantly greater than the mean sediment CH$_4$ concentration in epilimnetic sediment (588 ± 94 µM CH$_4$).

Area based (m$^2$) fluxes of CH$_4$ into the overlying water column from each zone were inversely proportional with overlying water depth in E4 but were directly proportional to overlying water depth in GTH 114 (Table 3.13). However, when areal fluxes were weighted to include total surface area for each zone, shallow zones assumed greater importance with respect to total sediment CH$_4$ efflux in each lake. Consequently, the importance of the littoral zone in E4 and the epilimnetic zone in GTH 114 increased dramatically due to their disproportionately high areal coverage relative to the profundal zones.

3.4 DISCUSSION

3.4.1 Rates of Methanogenesis Among Zones

Rates of MG among lake zones in E4 and GTH 114 varied from 28 – 565 nmol CH$_4$ g$_{dw}$ d$^{-1}$ and were generally in the range of similarly expressed rates reported in studies from around the world. Duc et al. (2010) found rates ranging from 2-3990 nmol CH$_4$ g$_{dw}$ d$^{-1}$ in eight temperate and boreal lakes in Sweden. In their study, boreal lake sediments exhibited lower rates of MG than the temperate lake sediments incubated at a temperature similar to that of my study, 10°C. Dannenberg et al. (1997) reported an average rate of 552 nmol CH$_4$ g$_{dw}$ d$^{-1}$ in Italian rice fields while much higher rates of MG (1092-1519 nmol CH$_4$ g$_{dw}$ d$^{-1}$) were reported in sediments from clear water Amazonian lakes (Conrad et al. 2010).
The hypothesis for this component of my study, rates of MG would differ among lake zones, was only partially supported. The only significant differences in mass-based rates of MG among lake zones were found in E4. Interestingly, the mass-based MG rates in the epilimnetic sediment of E4 were significantly lower than the rates of MG measured in the profundal and littoral regions. Although the differences were not significant, mass-based rates of MG in the profundal zone of GTH 114 were generally higher than rates of MG in the epilimnetic or littoral sediments. Logistical difficulty with sediment collection and processing prevented experimentation with higher sample numbers, thereby limiting statistical power. The only statistically significant difference between lakes occurred in the littoral zone. The mean rate of MG in the E4 littoral zone was higher than the mean rate of MG in the littoral zone of GTH 114 and the difference between means was likely a function of OM availability, as % OM in the upper 2 cm of sediment was four times greater in E4 than in GTH 114 (discussed further below). Within the studies that have directly compared rates of MG among lake zones (Thebrath et al. 1993; Casper 1996; Rolletschek 1997; den Heyer and Kalff 1998; Murase et al. 2005; Bastviken et al. 2008), most have found that MG activity is higher in littoral zones than profundal zones in oligotrophic lakes while the opposite is true in eutrophic lakes (Thebrath et al. 1993; Casper 1996). In a study of 9 lakes in Quebec, OM mineralization rates in littoral regions were approximately 3 times greater than in profundal regions (den Heyer and Kalff 1998), demonstrating the importance of littoral regions as intense sites of OM mineralization, and by extension MG.
3.4.2 Environmental Controls on Rates of MG

Rates of MG are mostly controlled by OM availability and temperature (Conrad 2005; Schulz and Conrad 1996). In E4, the Spearman’s rank correlation coefficients suggest that rates of MG in E4 are more strongly controlled by %OM availability than by in situ temperature. Conversely, in situ sediment temperature was the only environmental variable significantly correlated to MG rates in GTH 114. The mean in situ sediment temperature in the upper 2 cm of the profundal zone of GTH 114 was only 7.5°C in late July (Figure 3.12). Despite no effect of sediment zone on log-transformed rates of MG in GTH 114, the highest MG rates in laboratory experiments corresponded to the sediment zone with the lowest in situ temperature (profundal) while the lowest rates in that lake corresponded to the highest in situ sediment temperature (littoral). Such a negative correlation is not expected, nor easily interpreted. Thus, this particular metric is limited in its ability to predict rates of MG among zones in these two lakes. Future studies examining the effect of in situ temperature on MG rates should incorporate more intense monitoring of sediment temperature to capture seasonal or diurnal fluctuations that may influence bacterial metabolism.

Dissolved organic carbon was expected to exert a stronger influence on rates of MG in my study than the data revealed. Sediment profiles of DOC collected in 2008 were highly variable within each zone illustrating the heterogeneity of DOC cycling within lake sediments. The only significant difference in mean porewater DOC was found between epilimnetic lake pairs where the mean DOC was higher in E4 than in GTH 114. Surprisingly, no significant correlation was found between sediment DOC and rates of MG among zones in either lake. Consequently, solid phase organic content appears to be a better predictor of the observed
spatial variation in MG rates than in E4 than pelagic or porewater DOC. The lack of significant variation in rates of MG among sediment zones in GTH 114 limits evaluation of cause and effect relationships between rates of MG and environmental variables.

3.4.3 Estimation of Methane Flux from Zones

Sediment CH$_4$ profiles showed considerable variability among zones and lakes (Figure 3.7). The diffusive flux of CH$_4$ calculated from the sediment cores provided an areal flux in each zone. On a per unit area basis ($m^2$), the flux of CH$_4$ into the overlying water column of E4 was greatest from the littoral zone, while the largest contributor to area-based flux in GTH 114 was from the profundal zone. However, the overall contribution of each sediment zone to total lake sediment CH$_4$ emission changed considerably when the normalized data were extrapolated to include the entire sediment surface area of each zone (Table 3.4). In E4, the littoral zone contributed roughly 77% of the total CH$_4$ sediment flux while the epilimnetic sediments of GTH 114 contributed approximately 73% of the total sediment CH$_4$ flux. The flux of CH$_4$ from the littoral zone in E4 was larger than the combined emissions from profundal and epilimnetic sediments. Given that the littoral zone is approximately 42% of the total lake sediment surface area in E4, this result points to the importance of the littoral zone to whole lake sediment CH$_4$ flux. Conversely, the greatest contribution to whole-lake sediment CH$_4$ flux in GTH 114 was from the epilimnetic sediments. In that lake, the epilimnetic sediment zone comprises approximately 71% of the total lake sediment surface area. Combined, the data from both lakes emphasize the relative importance of shallow sediments with regard to total lake sediment CH$_4$ flux. Therefore, the areal extent of each dominant sediment zone within a lake ecosystem should be taken into consideration when evaluating of whole-lake sediment CH$_4$ flux.
Landscape-scale factors may greatly impact the disproportionate role shallow sediments play in whole-lake sediment CH$_4$ efflux. Wind-events create wave currents and shear stress on the lake bottom that can redistribute shallow sediments (Hamilton and Mitchell 1997) thereby releasing sediment CH$_4$ (Hofman et al. 2003; Bastviken et al. 2008) in association with both complete and incomplete water column mixing events (Hilton 1986). The degree of sediment redistribution from wind or storm events will vary among lake systems as it is a function of wind speed, storm event duration, fetch and position in the landscape (Hilton 1985; Hilton 1986; Hamilton and Mitchell 1997). Wind-driven sediment resuspension was found to be a particularly important process in these two lakes during the open water season (Fortino et al. 2009) pointing to the potential influence on CH$_4$ efflux from the shallow sediments. However, sediment resusupension can displace MOB thereby reducing their capacity to consume diffusive CH$_4$ (Bussman 2005). Alternatively, turbulence-induced rapid sediment CH$_4$ flux may bypass MOB altogether (Bastviken et al. 2008). Given the polymictic nature of E4 and GTH 114, sediment resuspension and redistribution may influence CH$_4$ cycling in the shallow sediments.

3.5 Conclusion

Methanogenic rates on a dry mass basis among zones within a lake measured in the sediment slurries were not as variable as I had expected. In E4, the littoral zone was an intense region of MG activity, likely due to a large percentage of OM in sediment in that zone. In GTH 114, %OM content was lowest in the littoral zone which coincided with the lowest rates of MG in that lake. Although dry mass-based MG rates in GTH 114 did not reveal significant differences between zones, mean porewater CH$_4$ was significantly greater in the profundal zone.
of GTH 114 than in the epilimnetic zone. The sediment CH$_4$ profiles allowed calculation of area-based CH$_4$ fluxes from the sediment into the overlying water column, which pointed to a higher rate of CH$_4$ flux in the profundal zone of GTH 114 than the other zones. The area-based flux calculations in E4 were slightly more consistent with the results from the dry mass based MG rates determined in the slurries. However, when area-based CH$_4$ flux was weighted to account for the fractional coverage of each zone, the littoral region proved to be the most important sediment zone with regard to sediment-water CH$_4$ exchange in E4, while the epilimnetic sediments were the most important sediment zone with regard to sediment CH$_4$ flux into the overlying water in GTH 114. Sediment in situ temperature did not appear to exert a strong influence on mass-based rates of MG in either lake. However, variations between timing of sediment collection for MG experiments and sediment temperature measurements may limit my ability to draw direct conclusions on the relationship between in situ temperature and rates of MG. Solid phase OM appeared to strongly correlate with mass-based rates of MG in E4 with no direct correlation of porewater DOC to MG in either lake. The lack of a significant difference in mass-based rates of MG among sediment zones in GTH 114 prevents determination of the influence of the measured environmental variables. Sediment CH$_4$ profiles indicated a potential for substantial CH$_4$ efflux into the overlying-water column with large contributions from shallow sediment zones. Based on the large areal composition of shallow sediments relative to whole-lake area, these zones may play a disproportionate role in whole lake sediment CH$_4$ efflux. However, the magnitude of this contribution will greatly depend on the Mox capabilities in the overlying oxic environment. Although significant spatial variation was found in E4 only, my research shows that evaluation of MG activity within a lake ecosystem should incorporate the
relative contribution of individual zones and multiple methodologies to assess whole-lake estimates of methanogenesis. Furthermore, future studies may benefit from more discrete monitoring of *in situ* sediment temperature, OM, or DOC across the summer growing season to assess any temporal influences these variables may have on MG activity.
REFERENCES


Table 3.1. Sampling depth of sediment zones in E4 and GTH 114.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Sampling Zone</th>
<th>Sampling Depth (m)</th>
<th>Maximum depth (m)</th>
<th>Mean depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4</td>
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<td>4.4</td>
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</tr>
<tr>
<td></td>
<td>Epilimnetic</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Littoral</td>
<td>&lt; 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Profundal</td>
<td>6.0</td>
<td>6.7</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Epilimnetic</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>Littoral</td>
<td>&lt;0.5</td>
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Table 3.2. Results of a Two-Way ANOVAs analyzing the lake, zone and the interaction of lake versus zone on log transformed rates of methanogenesis.

<table>
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<tr>
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<th>P</th>
</tr>
</thead>
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<td>1.69</td>
<td>10.66</td>
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<td>0.006</td>
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<tr>
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</tr>
<tr>
<td>Total</td>
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Table 3.3. Results of One-Way ANOVA analyzing the effect of zone on log-transformed rates of methanogenesis in E4.

<table>
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<td>Total</td>
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Table 3.4. Results of One-Way ANOVA analyzing the effect of zone on log-transformed rates of methanogenesis in GTH 114.

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### Table 3.5. Results of One-Way ANOVA analyzing between lake differences in log-transformed rates of methanogenesis in the profundal zone.

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<td>0.42</td>
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<td>Total</td>
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### Table 3.6. Results of One-Way ANOVA analyzing between lake differences in log-transformed rates of methanogenesis in the epilimnetic zone.

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<td>Total</td>
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<td>1.38</td>
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### Table 3.7. Results of One-Way ANOVA analyzing between lake differences in log-transformed rates of methanogenesis in the littoral zone.

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<tr>
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<td>3.96</td>
<td>36.12</td>
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<td>Total</td>
<td>9</td>
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Table 3.8. Results of One-Way ANOVA analyzing the main effect of sediment zone on sediment temperature in E4.

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<td>95.59</td>
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<td>Error</td>
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<td>80.67</td>
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<tr>
<td>Total</td>
<td>100</td>
<td>24.01</td>
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Table 3.9. Results of One-Way ANOVA analyzing the main effect of sediment zone on sediment temperature in GTH 114.

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Table 3.10. Spearman’s rank correlation coefficients ($r_s$) for the association between log-transformed rates of methanogenesis and environmental variables in E4 and GTH 114.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E4  ((n = 15))</td>
<td>GTH 114 ((n = 15))</td>
<td>Both lakes ((n = 30))</td>
</tr>
<tr>
<td>DOC$^{a,b}$</td>
<td>-0.15</td>
<td>0.07</td>
<td>-0.02</td>
</tr>
<tr>
<td>% OM$^b$</td>
<td>0.81**</td>
<td>0.19</td>
<td>0.50*</td>
</tr>
<tr>
<td>Temperature$^b$</td>
<td>0.15</td>
<td>-0.66</td>
<td>-0.14</td>
</tr>
</tbody>
</table>

Values of $r_s$ significant at $\alpha = 0.05$ are denoted by “*” and $\alpha = 0.01$ are denoted by “**”.  
$^a$Includes profiles from profundal and epilimnetic sediments only taken in 2008.  
$^b$Represent values measured in surficial 2 cm of sediment.
Table 3.11. Results of One-Way ANOVA analyzing the effect of zone on sediment porewater CH$_4$ profiles in E4.

<table>
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<tr>
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Table 3.12. Results of One-Way ANOVA analyzing the effect of zone on sediment porewater CH$_4$ profiles in GTH 114.

<table>
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<td>75</td>
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<td>263285</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>77</td>
<td>22059269</td>
<td></td>
<td></td>
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</tr>
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</table>
Table 3.13. Potential methane flux from surficial sediments into the overlying water column in the lake zones in E4 and GTH 114.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Zone</th>
<th>Areal Flux (µmol CH(_4) m(^2) d(^{-1}))</th>
<th>Surface area (m(^2))</th>
<th>% of total lake surface area</th>
<th>Weighted CH(_4) flux (mmol CH(_4) d(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>E4</td>
<td>Profundal</td>
<td>5</td>
<td>4835</td>
<td>12</td>
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<td>Epilimnetic</td>
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<td>17743</td>
<td>46</td>
<td>4515</td>
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<td></td>
<td>Littoral</td>
<td>947</td>
<td>16377</td>
<td>42</td>
<td>15511</td>
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<tr>
<td>GTH 114</td>
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<td>5338</td>
<td>14</td>
<td>6271</td>
</tr>
<tr>
<td></td>
<td>Epilimnetic</td>
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</tr>
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<td>6116</td>
<td>15</td>
<td>1813</td>
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</tbody>
</table>
Figure 3.1. Distribution of rates of methanogenesis (MG) among sediment zones in E4 in 2010. Mean rates of MG in each zone are indicated by a single dot and median values are indicated by horizontal line within each box. Upper and lower whiskers show the maximum and minimum values within the range of rates while the box edges represent the 10% and 90% quantiles.
Figure 3.2. Distribution of rates of methanogenesis (MG) among sediment zones in GTH 114 in 2010. Mean rates of MG in each zone are indicated by a single dot and median values are indicated by horizontal line within each box. Upper and lower whiskers show the maximum and minimum values within the range of rates while the box edges represent the 10% and 90% quantiles.
Figure 3.3. Water column dissolved organic carbon (DOC) concentrations in the epilimnion (Epi) and hypolimnion (Hypo) of E4 and GTH 114 across multiple days in 2009.
Figure 3.4. Dissolved organic carbon (DOC) concentrations in E4 porewater taken from profundal and epilimnetic sediments in E4 in July 2008. Data are plotted at the midpoint for each sampling interval. Error bars are ± 1 SEM (n = 3).

Figure 3.5. Dissolved organic carbon (DOC) concentrations in GTH 114 porewater taken from profundal and epilimnetic sediments in July 2008. Data are plotted at the midpoint for each sampling interval. Error bars are ± 1 SEM (n = 3).
Figure 3.6. Percent organic matter content (% OM) plotted at the midpoint of each sampling interval for sediment samples collected from defined lake zones in E4 and GTH 114 in July 2009. Error bars are ± 1 SEM (n=2).
Figure 3.7. Sediment temperature profiles taken in July 2009 from profundal, epilimnetic and littoral sediments in E4. Error bars are ± 1 SEM (n = 2).

Figure 3.8. Sediment temperature profiles taken in July 2009 from profundal, epilimnetic and littoral sediments in GTH 114. Error bars are ± 1 SEM (n = 2).
Figure 3.9. The relationship between mean rates of methanogenesis (MG) and mean percent organic content (% OM) among sediment zones in E4. Data are for the 0 to 2 cm depth interval in each zone. Error bars are ± 1 SD.

Figure 3.10. The relationship between mean rates of methanogenesis (MG) and mean percent organic content (% OM) among sediment zones in GTH 114. Data are for the 0 to 2 cm depth interval in each zone. Error bars are ± 1 SD.
Figure 3.11. The relationship between mean rates of methanogenesis (MG) and mean sediment temperature among sediment zones in E4. Data represent are for the 0 to 2 cm depth interval in each zone. Error bars are ± 1 SD.

Figure 3.12. The relationship between mean rates of methanogenesis (MG) and mean sediment temperature among sediment zones in GTH 114. Data are for the 0 to 2 cm depth interval in each zone. Error bars are ± 1 SD.
Figure 3.13. Sediment CH$_4$ profiles taken in July 2009 from profundal, epilimnetic and littoral sediments in E4 and GTH 114 (n = 2). Data are plotted at the midpoint of each sampling interval.
CHAPTER 4: THE INFLUENCE OF TEMPERATURE ON METHANE PRODUCTION AND OXIDATION AND EVALUATION OF KINETIC CONSTANTS FOR METHANE OXIDATION

4.1 Introduction

Methane (CH₄) is a radiatively and chemically important trace atmospheric constituent that is over twenty times more influential in terms of radiative forcing than CO₂ on a per molecule basis (Dalton 2005; Whalen 2005). Methane production results from multiple natural and anthropogenic activities while reduction of CH₄ from terrestrial and aquatic environments occurs via microbial CH₄ oxidation (Whalen 2005). However, considerable uncertainties exist regarding the magnitude of the global sources and sinks of CH₄ (Dlugokencky et al. 2009; Isaksen et al. 2011). Atmospheric concentrations of CH₄ had remained somewhat stable (~1.7 ppm) in the decade preceding 2008, whereupon an increase to 1.8 ppm has been observed (Dlugokencky et al. 2009). This current level is roughly 2.5 times greater than the pre-industrial concentration of about 0.7 ppm (Dlugokencky et al. 2009; Isaksen et al. 2011). One of the suspected drivers for the 2008 increase in the atmospheric CH₄ concentration is the abnormally high temperatures in the Arctic in 2007 (Dlugokencky et al. 2009). The highest observed atmospheric CH₄ concentrations have appeared over the Arctic and subarctic regions due to the high areal extent of wetlands (Semiletov 1999). Tundra environments encompass approximately 7% of earth’s surface (Whalen and Reeburgh 1992) with up to 50% of that areal coverage comprised of shallow ponds in some regions. On a global basis, shallow arctic lakes
may occupy approximately 2% of total land surface (Sheath 1986). Therefore, their contribution to the atmospheric CH$_4$ budget is likely significant due to their shallow depth and broad areal coverage (Semiletov 1999; Bastviken et al. 2004). Decomposition of organic matter (OM) by a consortium of syntrophic bacteria produces the primary substrates for methanogenesis (MG), acetate and H$_2$. The terminal step to OM degradation is MG, where acetate or H$_2$/CO$_2$ is converted to CH$_4$ (Glissman et al. 2004). The regulatory process modulating the amount of CH$_4$ reaching the atmosphere is CH$_4$ oxidation (Mox) which occurs in the presence of O$_2$ in soils, sediment and water overlying zones of CH$_4$ production (King 1992; Whalen and Reeburgh 1996). Methane oxidizing bacteria (MOB) incorporate some of the CH$_4$ into cellular biomass and oxidize the remainder to CO$_2$ (Hanson and Hanson 1996; Kankalla et al. 2006). Bacterial oxidation of CH$_4$ is a highly efficient biogeochemical process (Frenzel et al. 1990; Casper 2000) as up to 90% of the CH$_4$ diffusing from the anaerobic sediments may be oxidized in the overlying oxic zones within a lake (Wetzel 2001; Bastiken et al. 2003; Kankaala et al. 2007). Consequently, Mox plays a significant role in the global atmospheric CH$_4$ budget.

Both MG and Mox rates exhibit limitations by temperature, substrate availability, and O$_2$, although MG may be more sensitive to temperature fluctuations than Mox (Segers 1998; Duc et al. 2010). Temperature influences all biogeochemical processes to some degree (Duc et al. 2010), but there is evidence that Mox is controlled more strongly by substrate availability (i.e, CH$_4$ concentration) than by temperature (Liikanen et al. 2002). Kelly and Chynoweth (1981) found that MG rapidly responded to increased temperature until substrates were depleted. Temperature is likely to be a more important driver of Mox rates at higher CH$_4$ concentrations.
as activity shifts from substrate limitation to enzyme-activity limitation, particularly if populations prove to be psychrophillic (Whalen and Reeburgh 1996; Sundh et al. 2005).

Methanotrophic bacteria differ primarily in their affinities for CH$_4$ at varying concentrations (King 1992; Segers 1998). The apparent half-saturation constant, $K_m$, is a measure of CH$_4$ affinity which points to the types of MOB present (Bender and Conrad 1992; De Visscher et al. 1999), while from a kinetic perspective the maximum CH$_4$ oxidation rate, $V_{\text{max}}$, is roughly indicative of the MOB population size (Whalen and Reeburgh 1996). The apparent $K_m$ for low affinity MOB usually exceeds 1 µM (Roslev et al. 1997; Knief and Dunfield 2005) while the $K_m$ for atmospheric CH$_4$ oxidizers typically exposed to low CH$_4$ mixing ratios is much lower (10 to 280 nM CH$_4$) and is accompanied by a low $V_{\text{max}}$ as well (Bender and Conrad 1992; Knief and Dunfield 2005). Since MOB use CH$_4$ as their sole source of carbon and energy (Buchholz et al. 1994; Dalton 2005), increased concentrations of CH$_4$ may lead to population growth. Consequently, in situ CH$_4$ concentrations are likely determinants, from a physiological perspective, of the dominant MOB group residing within or between lake ecosystems (Rahalkar and Schink 2007). It follows that evaluation of CH$_4$ oxidation kinetics is vital to holistically approaching the net effect that may occur with increased CH$_4$ production associated with regional and global climate change.

To my knowledge, no single study has evaluated the effects of temperature on both MG and Mox in shallow lakes that are a prominent feature of arctic landscapes. The coupled response of these two processes with regard to increased temperature associated with ongoing climate change is poorly understood. Therefore, one objective of this component of my study was to determine the effect of temperature on CH$_4$ production and oxidation. A second goal
was to quantify Mox kinetic constants in order to evaluate the influence of substrate limitation of the extant MOB populations in hypolimnetic waters in two representative shallow Arctic Alaskan lakes. This information regarding important environmental influences on CH$_4$ oxidation and production will aid in the development of process-based models aimed at evaluating the impact of projected future climates on microbial communities that directly affect atmospheric CH$_4$ concentrations in a region that figures prominently in the atmospheric CH$_4$ budget.

4.2 Materials and Methods

4.2.1 Field Sites

My study site is located in the Arctic Foothills province of Alaska which is a region of the Arctic that is characterized by a tundra landscape underlain with continuous permafrost (Whalen et al. 2006). Mean annual temperatures range from –7 to –11°C with annual precipitation ranging from 140 to 267 mm (Bowden et al. 2008). The trophic state of most of the lakes in this region ranges from ultraoligotrophic to oligotrophic with heavy reliance on spring snowmelt for terrestrial subsidies of dissolved organic matter (Kling 1995; Whalen and Cornwell 1985). A full description of the region including vegetation characteristics and glacial geology can be found in Ping et al. (1998) and Hamilton (2002).

The two lakes chosen for this study, E4 and GTH 114, possess similar geomorphological characteristics, with GTH 114 having a larger total volume and catchment area (Table 2.1). The selected study lakes are regionally representative with respect to surface and catchment area, and lack fish, permanent inlets and rooted macrophytes. Additionally, these lakes exhibit
polymictic mixing patterns, thermally stratifying on an intermittent basis during the summer growing season (Figures 2.1 and 2.2).

4.2.2 Field Sampling

Sediments for CH$_4$ production studies were collected using a KB gravity corer (Wildlife Supply Company) deployed from an inflatable raft. For MG experiments, sediments were collected on 27 July 2010 and 31 July 2010 from E4 and GTH 114, respectively. Polycarbonate sleeves (4.8 cm inside diameter x 50 cm length) were inserted into the KB corer which was then lowered carefully into the sediments. Once removed from the sediments, sleeves were capped on each end with rubber stoppers. Ten sediment cores were collected from the maximum water depth in each lake for CH$_4$ production studies. Intact sediment cores were transported undisturbed via foot, vehicle, or helicopter to TFS for processing.

Hypolimnetic water samples for substrate-saturated CH$_4$ oxidation studies were collected by deploying a Van Dorn water sampler (Wildlife Supply Company) from an inflatable raft. Samples were collected in E4 from 3.3 m water depth on 23 July 2010. In GTH 114, water was collected from 5.5 m on 9 July 2010. Hypolimnetic water samples for Mox studies at near in situ concentration were collected on 1 August 2009 and 31 July 2009 in E4 and GTH 114, respectively.

Lake water samples for water column CH$_4$ concentration profiles were collected on 24 June 2008 (E4) and 2 July 2008 (GTH114) as similarly described above except water was collected at 1 m intervals. A 5 mL plastic syringe with a 20 gauge needle was inserted into the Van Dorn outlet tube to extract a sample. The syringe was flushed three times with water from the sample depth and the fourth fill (3 ml) was expelled into He- filled 30 ml serum vials.
containing 100 µL of 1N HCl to prevent biological activity. The vials were then inverted to prevent gas loss through the rubber stopper and returned to the lab for CH₄ analysis.

4.2.3 Laboratory Studies

Five replicated sediment slurries were prepared using a total of 10 cores. The upper 2 cm of two sediment cores were homogenized in a polycarbonate 1-L beaker to form each slurry. Oxygen-free deionized water (dH₂O) was prepared in separate polycarbonate beakers by purging with high purity N₂ and an air stone affixed to the end of tygon tubing. Following addition of O₂-free dH₂O, all homogenized sediment slurries were continually purged with N₂. A polycarbonate syringe with a bored tip was used to measure 15 ml of anoxic sediment slurry that was then ejected into a 60 ml serum bottle and sealed with a rubber stopper and crimp. Each serum bottle was evacuated and purged with N₂ at least 10 times to ensure anoxia, filled to 1 atm with N₂ then one vial from each slurry was randomly placed in a water bath (0, 4, 8, 12, and 16°C) for a total of 5 vials at each temperature. Vials were allowed to acclimate for approximately 12 h before headspace sampling was initiated.

Headspace samples were collected from serum bottles in 3 ml plastic syringes that had been previously tested to confirm no loss of CH₄ over a 4 h test period. Analysis of CH₄ was conducted by a gas chromatograph equipped with a flame ionization detector (FID-GC; Shimadu GC-8A) and was completed within 2 h of headspace sampling. Operating conditions for the FID-GC were as follows: Column = ⅛” diameter x 1 m length mol sieve 5A (60/80); column temperature = 90°C; injector and detector temperatures = 140°C; carrier gas = ultra-high purity N₂ at 33 ml min⁻¹ flow rate. Vial headspaces were sampled 4 times over 10 d, resulting in the linear production of CH₄. Headspace mixing ratios for CH₄ in serum vials were converted to a
moles per liter basis. Methane concentrations in the aqueous phase at each sampling point were calculated from temperature corrected solubility coefficients (Yamamoto et al. 1976) and Henry’s law (Stumm and Morgan 1996). The total amount of CH$_4$ produced in each bottle at each time point was computed as the sum of the aqueous phase and headspace masses. Rates were calculated using linear regression of CH$_4$ accumulation versus time.

Methane oxidation experiments were conducted in 2010 using microbially produced $^{14}$CH$_4$ (Daniels and Zeikus 1983) with a specific activity of 517 MBq mmol$^{-1}$. Water samples for the $^{14}$CH$_4$ oxidation experiments were collected at approximately 1 m above the sediment surface at the maximum water depth in each lake. An aliquot (50 or 100 µl) of stock $^{14}$CH$_4$ was diluted with ultra-high purity N$_2$ in a calibrated vial of 24.3 ml volume to yield a working standard.

To assess the effect of temperature on rates of CH$_4$ oxidation under substrate-saturated conditions, hypolimnetic water from each lake was initially placed in a 1 L beaker on a magnetic stirrer for approximately 1 h to equilibrate with atmospheric gases. Then, seventeen 40 ml amber EPA vials (Fisherbrand Enviroware) were filled with the atmosphere-equilibrated hypolimnetic water from each lake and sealed with a teflon lined cap without a headspace. Triplicate vials from each lake were placed in each of 5 water baths (0, 4, 8, 12, or 16°C) for 1 h to acclimate prior to the addition of $^{14}$CH$_4$. I conducted preliminary experiments earlier in the season to determine the appropriate concentration of $^{14}$CH$_4$ required to achieve a substrate-saturated response of CH$_4$ oxidation. Accordingly, 0.5 ml of a $^{14}$CH$_4$ working standard (9.83 MBq) was added to each vial of E4 water while 0.5 ml of a second $^{14}$CH$_4$ working standard (4.92 MBq) was added to each vial containing GTH 114 water. Samples were also amended with 0.5
mL of N₂-diluted \(^{12}\)CH\(_4\) to result in a target concentration of 33 \(\mu\)M CH\(_4\) in the aqueous phase. With each addition of radiocarbon labeled and unlabeled CH\(_4\), a 22 gauge needle was inserted into the sealing septum to allow expulsion of a similar volume of water to maintain 1 atm pressure in the vials. Two samples were immediately injected with 4N NaOH to serve as killed controls. All samples were vigorously shaken to equilibrate CH\(_4\) between the gaseous and aqueous phases to initiate the experiment. Samples from E4 were incubated at specified temperatures for 48 h while samples from GTH 114 were incubated for 24 h. Samples from both lakes were periodically shaken by hand 2-3 times daily during the course of the incubations to eliminate phase transfer limitation. The experiments were terminated by the addition of 0.5 ml of 4N NaOH to the vials which were then shaken vigorously. Five milliliters of sample was removed and discarded to reduce sample volume for the following step. Unsealed vials were then placed on a rotary shaker (100 rpm) under the hood for 24 h to remove unreacted \(^{14}\)CH\(_4\) from solution. Eight milliliters from each vial was then added to 20 ml glass scintillation vials, followed by the addition of 10 ml of liquid scintillation cocktail (Aquasol-2). Radioactivity was assessed on a Packard Tri-Carb 2100 TR Scintillation Counter. Rates of Mox were calculated from the fractional utilization of \(^{14}\)CH\(_4\) and the aqueous phase CH\(_4\) concentration.

Rates of Mox at 5°C and 15°C were estimated from the linear regression of Mox rate on temperature, and were used to calculate \(Q_{10}\) values using the following formula (Duc et al. 2010):

\[
Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2-T_1}}
\]
where $R_1$ and $R_2$ equals the mean rate of Mox 5°C ($T_1$) and 15°C ($T_2$), respectively. High variability among replicates necessitating data transformation in the MG versus temperature experiments precluded calculation of a $Q_{10}$ value in this manner.

To assess the influence of temperature on Mox rates at near in situ CH$_4$ concentrations, bulk hypolimnetic water from each lake was first equilibrated with the atmosphere as described above. Following FID-GC confirmation that the equilibrated bulk water was depleted of any measurable dissolved CH$_4$, 20 ml of equilibrated lake water was added to 160 ml serum vials in triplicate. Methane was added to the headspace of each vial to target near in situ concentration of 0.5 µM CH$_4$ in the aqueous phase. Then vials were randomly placed in water baths (0, 4, 8, 12, and 16°C) for a total of 3 vials per lake at each temperature. Vial headspaces were sampled 4 times over approximately 9 h. The rate of CH$_4$ loss from the headspace was calculated from the linear regression of the CH$_4$ mixing ratios versus time.

The substrate dependence of CH$_4$ oxidation was determined through a series of incubations at room temperature (20°C). Hypolimnetic water from each lake was equilibrated with the atmosphere as described above and placed into 40 ml amber EPA vials without a headspace. A 0.5 ml aliquot of a $^{14}$CH$_4$ working standard (4.92 or 9.83 MBq) was added to each of 17 vials. In addition, a 0.5 ml volume of N$_2$-diluted $^{12}$CH$_4$ prepared at different mixing ratios was added to each vial to give aqueous phase CH$_4$ concentrations varying from 0.15 to 33.5 µM. Sealing septa were pierced with a 22 gauge needle to expel water with gas addition as described above. Vials were then vigorously shaken by hand to equilibrate gases between the aqueous and gas phases and incubated on a rotary shaker (100 rpm) for 12 (GTH 114) or 24 h (E4). The experiments were terminated by the addition of 0.5 ml 4N NaOH and the samples
were further processed as described above for other $^{14}$C addition experiments. Rates of Mox were calculated from the fractional utilization of $^{14}$CH$_4$ and the aqueous phase CH$_4$ concentration. Data for Mox rates as a function of aqueous phase CH$_4$ concentrations were directly fitted to the Michaelis-Menten relationship through nonlinear regression using the Gauss-Newton method (Systat 7.0) (Liikanen et al. 2002; Lehninger 1982):

$$V = \frac{V_{\text{max}} \cdot S}{K_m + S}$$

where $V$ is the rate of CH$_4$ oxidation (µmol CH$_4$ L$^{-1}$ d$^{-1}$); $V_{\text{max}}$ is the maximum rate of CH$_4$ oxidation (µmol CH$_4$ L$^{-1}$ d$^{-1}$); $K_m$ is the half-saturation constant (µM CH$_4$).

4.2.4. Statistics

Nonlinear regression (i.e, exponential model) of MG rate versus temperature in E4 did not correct severe violations of homoscedasticity. Therefore, rates of MG were log-transformed in both lakes (Sokal and Rohlf 1995; Zar 1996) and analyzed with simple linear regression. Simple linear regression was used to analyze the effect of temperature on Mox rates in E4 and GTH 114. Analysis of covariance (ANCOVA) was used to evaluate the effects of lake and temperature on log-transformed rates of MG or rates of Mox. Comparisons of log-transformed mean rates of MG or rates of Mox between lakes were conducted using Tukey’s Honestly Significant Difference (HSD). The presence of significant outliers was evaluated statistically using Grubb’s test (Grubb’s 1969). No significant outliers were detected and thus, were preserved in the analyses. Log-transformed rates of MG were compared to log-transformed rates of Mox using ANCOVA with temperature as a covariate for E4 and GTH 114 separately. All
analyses were performed at the $\alpha = 0.05$ significance level using SAS, JMP 9.0 (SAS Institute, Inc.) or Systat 7.0 (SPSS, Inc.) statistical software.

4.3 Results

4.3.1 Rates of CH$_4$ Production as a Function Temperature

Non-transformed rates of MG across the 0-16°C temperature range varied from 0.15 to 20.9 µmol CH$_4$ L$^{-1}$ d$^{-1}$ and 0.7 to 67.9 µmol CH$_4$ L$^{-1}$ d$^{-1}$ in E4 and GTH 114, respectively (Table 4.1). Within-group variation in MG rates was higher at higher temperatures as compared to lower incubation temperatures. Log-transformed MG rates in E4 positively responded to increasing incubation temperature (Figure 4.1) while in GTH 114, the linear response of log-transformed MG rates to temperature was much stronger (Figure 4.2). Analysis of covariance (ANCOVA) resulted in an overall significant effect of lake and temperature on log-transformed MG rates (Table 4.2). After adjusting for the effect of the covariate temperature, Tukey’s HSD showed that the least square mean log-transformed MG rates was significantly higher in GTH 114 than in E4 ($p < 0.0001$). The overall geometric mean rate of MG in E4 across the entire temperature range with 95% confidence limits was 0.979 (0.51 to 1.91) µmol CH$_4$ L$^{-1}$ d$^{-1}$. In GTH 114, the overall geometric mean rate of MG across the entire range of incubation temperatures was 9.75 (4.9 to 19.03) µmol CH$_4$ L$^{-1}$ d$^{-1}$.

Typical water column CH$_4$ concentrations in both lakes (Figure 4.3) were low, showing maximum concentrations of 1.52 and 0.78 µM CH$_4$ in E4 and GTH 114. In contrast, surficial sediments (Figure 3.13; Chapter 3) showed CH$_4$ concentrations of around 5.76 and 149.27 µM.
in the respective lakes, representing levels that exceeded water column concentrations by factors of 3 and 190, respectively.

4.3.2 Rates of CH$_4$ Oxidation as a Function of Temperature

Across all temperature conditions, rates of Mox under substrate-saturated conditions varied from 0.053 to 0.231 µmol CH$_4$ L$^{-1}$ d$^{-1}$ in E4 while substrate-saturated Mox rates in GTH 114 ranged from 1.17 to 8.16 µmol CH$_4$ L$^{-1}$ d$^{-1}$ (Table 4.3). Rates of Mox exhibited a strong linear response to incubation temperature in E4 (Figure 4.4) and GTH 114 (Figure 4.5). Overall, the ANCOVA model showed a strong significant relationship between lake and temperature on rates of Mox (Table 4.4). There was a significant interaction between lake and temperature indicating that the rate of change in CH$_4$ oxidation in response per unit increase in temperature was significantly different between lakes. After controlling for the effect of temperature, Tukey’s HSD showed that the least squares mean rate of Mox was significantly greater in GTH 114 than the least squares mean rate of Mox in E4 ($p < 0.0001$). The mean rate of Mox in E4 ($\pm$ 95% CI) across the entire temperature range was 0.14 ($\pm$ 0.08) µmol CH$_4$ L$^{-1}$ d$^{-1}$. In GTH 114, the least squares mean rate of Mox ($\pm$ 95% CI) across the entire range of incubation temperatures was 4.60 ($\pm$ 0.08) µmol CH$_4$ L$^{-1}$ d$^{-1}$. In contrast to the Mox rates measured under substrate-saturating conditions, experiments conducted in 2009 at near in situ CH$_4$ concentrations showed no significant response to temperature in either lake (Figure 4.6).

4.3.3 Methane Production versus Methane Oxidation

For direct comparison to log-transformed rates of MG, rates of Mox were also log-transformed. Mean log-transformed rates of Mox were positively related to mean log-transformed rates of MG in E4 and GTH 114 (Figure 4.7). ANCOVA was used to evaluate the
differences between slopes of the linear regression of log-transformed rates of MG on temperature and the linear regression of log-transformed rates of Mox on temperature in E4 (Table 4.5) and GTH 114 (Table 4.6). After controlling for the effect of temperature, Tukey’s HSD showed that the least squares mean log-transformed rates of MG were significantly greater than the least squares mean log-transformed rates of Mox in E4 (p = 0.0006) and in GTH 114 (p = 0.0002).

4.3.4 Kinetics of CH₄ Oxidation

Although the aqueous phase CH₄ concentration at which Mox were assessed were similar between experiments, the range of Mox rates determined in kinetics experiments was considerably lower in E4 than in GTH 114 (Figures 4.8 and 4.9). Methane oxidation rates in E4 ranged from 0.01 to 0.30 µmol CH₄ L⁻¹ d⁻¹ while the Mox rates in GTH 114 ranged from 0.10 to 6.25 µmol CH₄ L⁻¹ d⁻¹. The estimate for Vₘₐₓ was approximately 25 times greater in GTH 114 than in E4, while the values for Kₘ were roughly two-fold higher in GTH 114 than in E4. The calculated Vₘₐₓ with 95% confidence in E4 was 0.32 ± 0.06 µmol CH₄ L⁻¹ d⁻¹ compared with a value of 8.39 ± 0.67 µmol CH₄ L⁻¹ d⁻¹ in GTH 114. With 95% confidence, the half-saturation constants for CH₄ oxidation (Kₘ) were 4.45 ± 2.36 µM in E4 and 10.61 ± 2.03 µM in GTH 114. The lack of overlap in the 95% confidence intervals for both Vₘₐₓ and Kₘ values between E4 and GTH 114 suggest that these differences are statistically significant.
4.4 Discussion

4.4.1 Influence of Temperature on Methanogenesis

Consistent with other freshwater studies (Schulz et al. 1997; Nozhevnikova et al. 2007; Duc et al. 2010), temperature appears to play a major role in the determination of MG rates in these two shallow arctic lakes as evidenced by the significant increase in log-transformed MG rates with increasing temperature in both lakes. Non-transformed rates of MG exhibited a sharper response to higher temperatures than lower incubation temperatures in both lakes. High variability among replicates was observed in both lakes, particularly at higher temperatures. After adjusting for the influence of temperature, least square mean log-transformed rates of MG was significantly greater in GTH 114 than in E4. The variation in methanogenic response to temperature is likely related to differences in substrate availability among studies (Segers 1998; Duc et al. 2010). In my previous experiments, addition of methanogenic substrates to sediments from E4 and GTH 114 significantly enhanced rates of MG, suggesting that substrate supply was suboptimal in the sediments of these two lakes (Figures 2.4 and 2.5; Chapter 2). Methanogenic experiments conducted in the present component of my study did not receive additional methanogenic substrates and are therefore assumed to be representative of MG in these lakes under suboptimal conditions of substrate supply. While methanogenic substrate availability may be suboptimal currently, the data suggest that sufficient substrate were present to allow some degree of temperature dependence on MG rates, but MG rates are not strictly governed by enzyme activity.

It is widely recognized that MG occurs across a broad temperature range, but only a few studies have determined optimum temperatures for MG bacteria in freshwater sediments.
(Zeikus and Winfrey 1976; Schulz and Conrad 1996; Schulz et al. 1997). Zeikus and Winfrey (1976) found that MG activity was optimal in the 35-42°C temperature range in sediments from Lake Mendota. In Lake Constance, 30°C was found to be the optimal temperature for MG in the littoral zone (Thebrath et al. 1993) while 40°C was determined to be the optimum temperature for MG activity in the profundal zone, despite the fact that *in situ* temperatures are typically below 25°C in the littoral zone and remain near 4°C in the profundal zone in that lake (Schulz and Conrad 1996). Though it is not clear whether the driving force for these temperature optima relate to the methanogens and/or the symbiotic bacterial groups providing methanogenic substrates (Schulz et al. 1997). The optimum temperatures for MG activity were not determined in my study. However, given the significant response of log-transformed rates of MG to temperature in my study, it is probable that the temperature optimum for MG and/or the microbial community providing methanogenic substrates in E4 and GTH 114 is higher than the maximum temperature incubation in my study. Although it is unlikely that these arctic lake sediments will approach the optimum temperatures for methanogens, my data suggest that the MG response to temperatures will exhibit a positive response to any realistic increase in temperature without additional changes in substrate quantity or quality.

### 4.4.2 Influence of Temperature on Methane Oxidation

The influence of temperature on Mox rates appears to be more pronounced at higher CH$_4$ concentration than at low CH$_4$ concentration (De Visscher et al. 2001; Duc et al. 2010). The $Q_{10}$ estimates for Mox under non-substrate limiting conditions in landfill soil covers ranged from 2.8 to 8.4 (De Visscher et al. 2001; Einola et al. 2007), while $Q_{10}$ values for Mox rates are relatively low (1.1 - 1.9) in forest soils exposed to atmospheric CH$_4$ concentrations (King and
Adamsen 1992; Roslev et al. 1997). My calculated $Q_{10}$ values of 2.3 (E4) and 2.4 (GTH 114) under substrate saturated conditions ($\sim$ 33 µM CH$_4$) were slightly higher than the reported $Q_{10}$ values of 1.4 to 2.1 for northern peatlands (Dunfield et al. 1993), but lie between the range of $Q_{10}$ values reported for non-substrate limited and substrate limited Mox environments. Methane availability at 15°C in the latter study corresponded to approximately 1.72 µM CH$_4$. As previous studies have reported that Mox is saturated at CH$_4$ concentrations of 5-10 µM (Rudd and Hamilton 1975; Harrits and Hanson 1980; Liikanen et al. 2002), it is likely that MOB in the Dunfield et al. (1993) study were in an environment of suboptimal CH$_4$ concentration. Furthermore, Duc et al. (2010) found no dependence of temperature at a headspace concentration approximating 0.57 µM aqueous phase CH$_4$, which are also indicative of suboptimal substrate conditions. My data indicate that the influence of temperature on rates of Mox at substrate saturation (33 µM) will be modest compared with other studies and that rates of CH$_4$ oxidation at 0.5 µM CH$_4$ were uninfluenced by temperature. However, it is unclear at what CH$_4$ concentration a temperature x concentration effect will govern rates of CH$_4$ oxidation here.

Low concentrations of dissolved CH$_4$ in the water column are typical of these shallow arctic lakes (Figure 4.3). Further, the kinetic curves (discussed in more detail below) for E4 (Figure 4.8) and GTH 114 (Figure 4.9) show that Mox rates will be linear with substrate concentration well in excess of current in situ water column CH$_4$ concentrations (Figure 4.3). Consequently, the in situ concentration of dissolved CH$_4$ in the water column of these two lakes will need to increase dramatically to induce a strong temperature effect on Mox rates. Therefore, the effect of temperature on water column Mox is closely tied to CH$_4$ availability and
is minimal under extant temperature regimes. As noted earlier, it is unlikely that CH$_4$
concentrations will approach the substrate-saturated conditions necessary for Mox to be
governed strictly by temperature.

The optimal temperature range for CH$_4$ oxidizing bacteria varies considerably across
ecosystems. In the absence of O$_2$ or substrate limitation, the optimal temperature range for
Mox in freshwater lakes ranges from 25-35°C (Rudd and Hamilton 1975; Harrits and Hanson
1980; Liikanen et al. 2002). In a boreal peat bog, Whalen and Reeburgh (1996) determined that
the optimal temperature for Mox was 23°C. As Mox is a key factor in the regulation of CH$_4$
emissions from the lake surface, the response of MOB to changing temperature regimes could
have important implications for the global CH$_4$ cycle as temperatures increase towards the
optimum at high latitudes. However, in the case of these lakes, temperature will provide a
strong environmental control on Mox only if CH$_4$ increases significantly above current levels.

4.4.3 Kinetics of Methane Oxidation

Only one study evaluating Mox kinetics in the water column has been reported (Liikanen
et al 2002) whereas a few studies give values for kinetics parameters in lake sediments
(Lidstrom and Somers 1984; Kuivila et al. 1988; Remsen et al. 1989; Buchholz et al. 1995; Duc et
al. 2010). Both E4 and GTH 114 exhibited values of $V_{max}$ that were considerably lower than the
reported estimates in hypolimnetic waters in a eutrophic lake in Finland (Liikanen et al. 2002).
In that study, the $V_{max}$ estimates were 36 µmol CH$_4$ L$^{-1}$ d$^{-1}$ in the shallow water (4 m) and 140
µmol CH$_4$ L$^{-1}$ d$^{-1}$ in the deep water (9 m). In contrast, the apparent $K_m$ value in the hypolimnetic
water of E4 approximated the $K_m$ value in the shallow hypolimnetic water of the Finnish lake
(5.5 µM), but the $K_m$ value of 44 µM in the deeper zone of that study (Liikanen et al. 2002) was
over 4 times greater than the estimated $K_m$ values in E4 or GTH 114. Values of kinetic constants for Mox were generally in the range of another study that examined Mox kinetics in Lake Superior sediments. In that study, the apparent $K_m$ value was 4.6 µM while $V_{\text{max}}$ was determined to be 0.7 µmol CH$_4$ L$^{-1}$ d$^{-1}$ (Remsen et al. 1989). Kuivila et al. (1988) found comparable $K_m$ values in the floculent layer above the sediment surface (5.1 µM) and in the upper 1 cm sediment interval (10 µM) in Lake Washington, but $V_{\text{max}}$ values in their study (29 µmol L$^{-1}$ d$^{-1}$ and 26 µmol L$^{-1}$ d$^{-1}$, for the respective depth intervals) were considerably higher than my estimates. Kinetics parameters in terrestrial ecosystems also show high variability. Higher affinity MOB are typically found in forested soils with low CH$_4$ mixing ratios and show comparatively low values of $K_m$ (10-280 nM) whereas environments with higher CH$_4$ concentrations typically have much higher $K_m$ values (> 1 µM) (Knief and Dunfield 2005). Higher estimates of $V_{\text{max}}$ are indicative of higher MOB population density (Buchholz et al. 1995) and sediments support denser bacterial populations than the water column (den Heyer and Kalff 1998). Consequently, estimates of $V_{\text{max}}$ in the sediments are likely higher than those in my study, which was conducted with hypolimnetic water.

Although $V_{\text{max}}$ varies as a function of MOB population density, it may be influenced by the different types of MOB (Pester et al. 2004; Rahalkar and Schink 2007). Different MOB types occupy different niches and may exhibit shifts in population dominance in response to changes in environmental conditions (Borjesson et al. 2004). Further, differing populations of MOB have been found to occupy separate niches within a lake ecosystem (Rahalkar and Schink 2007). Different communities of MOB will exhibit different affinities for CH$_4$ (Hanson and Hanson 1996; King 1992; Segers 1998). Given the significantly greater values of $V_{\text{max}}$ and $K_m$ in GTH 114
relative to E4, it is plausible that different populations of MOB exist in the water column between the two lakes, although $V_{\text{max}}$ is also influenced by population size as noted earlier. Consequently, the estimated $V_{\text{max}}$ and $K_m$ values could be lower in E4 relative to GTH 114 in response to lower sediment efflux of CH$_4$ and/or differing populations of MOB.

Comparison of the dissolved CH$_4$ profile in the sediments (Figure 3.13; Chapter 3) and in the water column of E4 and GTH 114 (Figure 4.3) indicates considerable oxidation of the CH$_4$ diffusing up from the sediments. Based on the highest measured dissolved CH$_4$ concentration in the water column of E4 and GTH 114 from the 2008 profiles, the Michaelis-Menten equation provided estimates of Mox rates equal to 0.08 µmol CH$_4$ L$^{-1}$ d$^{-1}$ in E4 and 0.57 µmol CH$_4$ L$^{-1}$ d$^{-1}$ in GTH 114 at the corresponding water depths. Compared to the calculated $V_{\text{max}}$ for each lake, these estimates suggest that MOB are operating at 25 and 7% of their capacity at in situ water column CH$_4$ concentrations in E4 and GTH 114, respectively, assuming no diffusion limitation of substrate. These calculations also ignore the fact that the kinetic experiments were conducted at a temperature greater than in situ values, and therefore provide an upper estimate of $V_{\text{max}}$.

Profiles of porewater CH$_4$ in the 0-1 cm depth interval suggest high rates of CH$_4$ oxidation in surficial sediments. Indeed, Liikenanen et al. (2002) found higher rates of Mox in the sediments than in the water column of Lake Kevaton (Finland). While studies cited earlier show high $V_{\text{max}}$ for sediments, low water column CH$_4$ concentrations (Figure 4.3) and an unrealized capacity to oxidize diffusive CH$_4$ in the water of both lakes suggest that these lakes efficiently minimize CH$_4$ emissions to the atmosphere during the thaw season. Overall, my research indicates that MOB in the water column of E4 and GTH 114 are substrate limited currently and will likely respond
positively to increased CH₄ availability but will be little influenced by temperature increases unless dramatic increases in MG are observed.

4.4.4. Methane Production versus Methane Oxidation

Methane production and oxidation are integral components of within-lake carbon cycling. Methane oxidizing bacteria are an important food resource for secondary consumers and therefore, represent a substantial source of carbon and energy transfer for lake food webs (Hanson and Hanson 1996; Bastviken et al. 2003; Hershey et al. 2006). In the same study region as my lakes, Hershey et al. (2006) found that benthic macroinvertebrates were depleted in δ¹³C indicating utilization of MOB as a food resource. Similarly, numerous studies have indicated that zooplankton exhibit stable isotope ratios also consistent with consumption of MOB (del Giorgio and France 1996; Jones et al. 1999; Bastviken et al. 2003). The linkage between methane production and subsequent carbon transfer to zooplankton through consumption of MOB may be stronger in lakes with higher allochthonous inputs (Jones et al. 1999), which may be an important process in these shallow arctic lakes that rely heavily upon terrestrial inputs. As discussed earlier, increased CH₄ concentrations may result in MOB population growth which may positively impact their overall capacity to mitigate increased CH₄ in association with future climate change. Conversely, intense grazing on MOB by bacterivorous zooplankton has been found to significantly reduce MOB cell numbers (Kankaala et al. 2006). Consequently, trophic suppression of methanotrophic activity could lead to increased CH₄ flux to the atmosphere. Given the simple structure of arctic lake food webs (Sierszen et al. 2003) increased CH₄ availability as a function of higher allochthonous OM inputs could alter food web dynamics and subsequent ecosystem function in the face of a changing arctic climate. However, the direction
or magnitude of these particular changes as a consequence of future climate change is beyond the scope of my study.

My data indicate that under extant substrate conditions, MG will exert a stronger response to temperature increases than water column Mox in both E4 and GTH 114. The Mox kinetics constants estimated for water column MOB point to a high capacity for MOB to assimilate CH$_4$ at concentrations greater than present *in situ* conditions regardless of any temperature increase. Further, the Mox kinetics experiments were conducted in hypolimnetic water, so estimates of Mox rates may differ considerably in the oxic surficial sediment layer (upper 1 cm) in E4 and GTH 114. Bacterial density is often 2-3 orders of magnitude higher in the sediments than in the water column (den Heyer and Kalff 1998) and $V_{max}$ is at least partly dependent upon MOB population density (Buchholz et al. 1995; Hanson and Hanson 1996). Published values of $V_{max}$ for sediments greatly exceed my water column $V_{max}$ values as noted earlier. Measurements of sediment Mox potential were not conducted in this study due to logistical constraints. Therefore, future studies should examine the kinetics potential of sediment MOB in these oligotrophic shallow lakes in order to fully understand the capacity for Mox to offset concomitant increases in MG associated with future climate change.

### 4.5 Conclusion

Log-transformed rates of MG in the sediments of E4 and GTH 114 at presumed suboptimal substrate levels increased positively with incubation temperature to 16°C. Thus, the measured rate increase represented a temperature x substrate supply response and the response to temperature under substrate-saturated conditions is likely greater than in my
experiments. Rates of Mox under strictly substrate-saturated conditions displayed a strong linear response to temperature. Consequently, the coupled response of sediment MG and water column Mox to increased temperatures in arctic lakes as a function of ongoing climate change is likely disproportionate. Under current climatic conditions, MOB appear to be more strongly controlled by substrate availability than by temperature in these two shallow arctic lakes. My research shows that without a population increase, MOB will efficiently adjust to increases of > 1 order of magnitude in CH\textsubscript{4} availability without reaching their maximum uptake potential. My data further indicate that CH\textsubscript{4} must greatly exceed its current concentrations in order to induce strict dependence of water column Mox on temperature, although it is unclear at what point temperature x substrate interactions commence.

Kinetic constants for Mox differed considerably between lakes. The maximum CH\textsubscript{4} uptake rate (V\textsubscript{max}) was much greater in GTH 114 than in E4, which is likely driven by differences in MOB population density due to characteristically greater CH\textsubscript{4} availability due to diffusion from underlying sediments (Figure 3.7; Chapter 3), although differences could be influenced by population structure. In addition, the half-saturation constant (K\textsubscript{m}) was considerably higher in GTH 114 than in E4 further suggesting the possibility of physiologically different MOB groups existing between lakes. Future research should incorporate comparisons between sediment and water column Mox to compare CH\textsubscript{4} oxidation potential between environments. These factors would likely provide valuable information regarding the extent to which Mox rates in these shallow arctic lakes will offset any increases in CH\textsubscript{4} production and elevated sediment and water temperature associated with projected future climates.
REFERENCES


Table 4.1. Mean CH\(_4\) production ± 1 SEM (µmol CH\(_4\) L\(^{-1}\) d\(^{-1}\)) in sediment slurries from E4 and GTH 114 at various water temperatures (°C).

<table>
<thead>
<tr>
<th>Lake</th>
<th>0°C</th>
<th>4°C</th>
<th>8°C</th>
<th>12°C</th>
<th>16°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4</td>
<td>0.15 ± 0.05</td>
<td>1.38 ± 0.80</td>
<td>3.13 ± 0.88</td>
<td>8.93 ± 5.07</td>
<td>20.90 ± 5.71</td>
</tr>
<tr>
<td>GTH 114</td>
<td>0.73 ± 0.18</td>
<td>9.04 ± 4.94</td>
<td>16.10 ± 3.21</td>
<td>38.18 ± 5.99</td>
<td>67.95 ± 8.69</td>
</tr>
</tbody>
</table>
Table 4.2. Results of the Analysis of Covariance (ANCOVA) evaluating the effects of lake and temperature (°C) on log-transformed rates of methanogenesis (nmol CH4 L\(^{-1}\) d\(^{-1}\)).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type I SS</th>
<th>MS</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake</td>
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<td>12.47</td>
<td>12.47</td>
<td>23.94</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>22.02</td>
<td>22.02</td>
<td>42.26</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lake * Temperature</td>
<td>1</td>
<td>0.08</td>
<td>0.08</td>
<td>0.15</td>
<td>0.6975</td>
</tr>
<tr>
<td>Model</td>
<td>3</td>
<td>34.57</td>
<td>11.52</td>
<td>22.12</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residual Error</td>
<td>46</td>
<td>23.97</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>49</td>
<td>58.54</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.3. Mean CH₄ consumption ± 1 SEM (µmol CH₄ L⁻¹ d⁻¹) in sediment slurries from E4 and GTH 114 at various water temperatures (°C).

<table>
<thead>
<tr>
<th>Lake</th>
<th>0°C</th>
<th>4°C</th>
<th>8°C</th>
<th>12°C</th>
<th>16°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4</td>
<td>0.053 ± 0.008</td>
<td>0.073 ± 0.006</td>
<td>0.107 ± 0.007</td>
<td>0.215 ± 0.04</td>
<td>0.231 ± 0.034</td>
</tr>
<tr>
<td>GTH 114</td>
<td>1.17 ± 0.04</td>
<td>2.59 ± 0.19</td>
<td>4.57 ± 0.05</td>
<td>6.52 ± 0.10</td>
<td>8.16 ± 0.11</td>
</tr>
</tbody>
</table>
Table 4.4. Results of the Analysis of Covariance (ANCOVA) evaluating the effects of lake and temperature on rates of methane oxidation (µmol CH$_4$ L$^{-1}$ d$^{-1}$).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type I SS</th>
<th>MS</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake</td>
<td>1</td>
<td>149.66</td>
<td>149.66</td>
<td>6009.38</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Temperature</td>
<td>1</td>
<td>50.84</td>
<td>2.20</td>
<td>2041.26</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lake * Temperature</td>
<td>1</td>
<td>45.49</td>
<td>45.49</td>
<td>1826.47</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Regression</td>
<td>3</td>
<td>245.98</td>
<td>81.99</td>
<td>3292.37</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>26</td>
<td>0.65</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>29</td>
<td>246.63</td>
<td></td>
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</tr>
</tbody>
</table>
Table 4.5. Results of the Analysis of Covariance (ANCOVA) comparing log-transformed methane production to log-transformed methane oxidation after controlling for the influence of the covariate temperature in E4 from 2010. The reaction term categorizes methane production or oxidation.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type I SS</th>
<th>MS</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction</td>
<td>1</td>
<td>8.22</td>
<td>8.22</td>
<td>14.16</td>
<td>0.0006</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>9.29</td>
<td>9.29</td>
<td>16.01</td>
<td>0.0003</td>
</tr>
<tr>
<td>Reaction * Temperature</td>
<td>1</td>
<td>1.34</td>
<td>1.34</td>
<td>2.31</td>
<td>0.14</td>
</tr>
<tr>
<td>Regression</td>
<td>3</td>
<td>18.85</td>
<td>6.28</td>
<td>10.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>20.90</td>
<td>0.58</td>
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<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>39</td>
<td>39.75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6. Results of the Analysis of Covariance (ANCOVA) comparing log-transformed methane production to log-transformed methane oxidation after controlling for the influence of the covariate temperature in GTH 114 from 2010. The reaction term categorizes methane production or oxidation.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type I SS</th>
<th>MS</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction</td>
<td>1</td>
<td>1.62</td>
<td>1.62</td>
<td>17.78</td>
<td>0.0002</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>12.13</td>
<td>12.13</td>
<td>132.84</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Reaction * Temperature</td>
<td>1</td>
<td>1.56</td>
<td>1.56</td>
<td>17.07</td>
<td>0.0002</td>
</tr>
<tr>
<td>Regression</td>
<td>3</td>
<td>15.31</td>
<td>5.10</td>
<td>55.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>3.29</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>39</td>
<td>18.60</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Figure 4.1. Simple linear regression of log-transformed rates of methanogenesis (MG) on incubation temperature in E4 in 2010.

Figure 4.2. Simple linear regression of log-transformed rates of methanogenesis (MG) on incubation temperature in GTH 114 in 2010.
Figure 4.3. Water column methane profiles taken from zone of maximum water depth in 2008.
Figure 4.4. Simple linear regression of methane oxidation rate (Mox) on temperature in E4 in 2010.

Figure 4.5. Simple linear regression of methane oxidation rate (Mox) on temperature in GTH 114 in 2010.
Figure 4.6. Mean rates of CH4 loss from the headspace in serum vials containing hypolimnetic water from E4 and GTH 114 and incubated at varying temperatures under substrate-limiting conditions in 2009. Error bars are ±1 SEM (n = 3 in most cases).
Figure 4.7. Mean log-transformed rates of methanogenesis (MG) vs. log-transformed rates of methane oxidation (Mox) in 2010.

GTH 114
\[ y = 1.89 + 0.42x \]
\[ R^2 = 0.99 \]
\[ p < 0.0001 \]

E4
\[ y = 0.93 + 0.37x \]
\[ R^2 = 0.88 \]
\[ p = 0.02 \]
Figure 4.8. The effect of methane concentration (µM) on water column methane oxidation rates (V) in E4 in 2010.

Figure 4.9. The effect of methane concentration (µM) on water column methane oxidation rates (V) in GTH 114 in 2010.
CHAPTER 5: CONCLUSION

The Arctic landscape is currently undergoing multi-scale ecosystem transformations as a result of ongoing climate change (Overpeck et al. 1997; Rysgaard et al. 2003; Post et al. 2009). Despite widespread areal coverage of shallow lakes in the Arctic, research efforts have primarily focused on CH$_4$ dynamics and emission estimates from terrestrial environments. Projected alterations to the arctic environment under future climates may influence methanogenesis and methane oxidation, leading to possible changes in rates of CH$_4$ exchange between shallow arctic lakes and the atmosphere. Therefore, I conducted this research on two shallow arctic lakes to understand the factors controlling MG and Mox under present conditions in an effort to evaluate their response to predicted increases in temperature and OM availability that may result from ongoing climate change.

Rates of MG increased significantly in response to substrate (acetate or H$_2$) additions in most 1 cm intervals to 5 cm sediment depth of these two shallow lakes, indicating that both methanogenic pathways experience suboptimal substrate levels. Thus, any climate-induced influences on the syntrophic bacteria or acetogens providing methanogenic substrates will positively influence MG rates. The relative contribution of the acetoclastic pathway to total methanogenesis in both lakes decreased with increasing depth below the sediment surface to 5 cm, reflecting a down-core decrease in OM quality. The relative importance of the acetoclastic and hydrogenotrophic pathways differed between lakes, suggesting control by within-lake
processes related to internal mixing regimes as well as landscape scale factors such as quality and quantity of OM delivery from the terrestrial landscape.

Analysis of the spatial variability of CH$_4$ production in the horizontal dimension in these two shallow arctic lakes showed significant differences among lake zones in E4, but no zone-wise differences in rates of MG in GTH 114. Thus, studies using rate measurements at a central, deep station to characterize lakes with respect to MG may give misleading results and broader spatial coverage is warranted for a firmly based analysis. Similarly, porewater CH$_4$ profiles showed spatial variability within each lake and whole lake estimates of sediment CH$_4$ efflux will benefit from properly weighting fluxes from defined lake zones. Most sediment CH$_4$ exchange occurred in either the littoral or epilimnetic regions of these lakes, regions close to shore that can intercept diffuse terrestrial inputs.

Temperature may directly influence MG pathways by enhancing enzyme activity or indirectly via stimulation to symbiotic bacteria that provide MG substrates. Therefore, in situ sediment temperature was included into statistical models to assess the level of influence of in situ sediment temperature on rates of MG in sediment slurries incubation without additional substrates. My data showed that %OM was a better predictor of MG rates than in situ temperature. Consequently, under present conditions, MG appears to be more tightly regulated by availability of OM than by in situ temperature in these two shallow arctic lakes.

Although in situ sediment temperature did not appear to be a good predictor of MG rates under present conditions, separate laboratory incubations with unamended sediment slurries from E4 and GTH 114 exhibited a strong linear response to increasing temperature to 16°C. While my substrate addition experiments indicated that substrate supply to MG is
suboptimal, the supply of substrates must be sufficient to elicit a positive influence to
temperature on MG rates in unamended sediments slurries. Therefore, MG activity in these
two shallow arctic lakes will likely respond positively to any realistic increases in sediment
temperature without any concomitant increases in substrate supply.

Methane oxidation rates at CH$_4$ concentrations typical in these lakes showed no
response to temperature increases, indicating that MOB in the water column of E4 and GTH
114 are currently substrate limited. In contrast, addition of high concentrations of CH$_4$ induced
dependence of Mox on temperature. Comparison of the Mox kinetic constants and *in situ* CH$_4$
concentrations in the water column of E4 and GTH 114 revealed that MOB are operating at 25
and 7%, respectively, of their maximum CH$_4$ uptake capacity. Thus, my research shows that
while MG will be influenced positively by increasing temperatures associated with ongoing
climate change, Mox will be minimally impacted by changing temperature unless a drastic
increase in CH$_4$ concentration is observed.

While rates of MG will likely be stimulated with increased temperature and substrate
availability in association with future climate change, my data suggests that the overall impact
of changes in temperature and OM supply may be minimal with regard to CH$_4$ efflux to the
atmosphere due to the underutilized capacity for MOB to process CH$_4$.

Several future research directions have been identified through my study. Future
experiments should more firmly establish the interactive effects of temperature and substrate
availability on MG and Mox. Further, the coupled interaction between substrate availability and
sediment temperature as it influences the spatial variability of MG should also be considered.
Finally, future research should incorporate comparisons between sediment and water column Mox to evaluate overall CH₄ uptake potential.
REFERENCES

