Methylmercury Cycling at the Aquatic-Terrestrial Interface in a High Arctic Freshwater Continuum

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Geography and Planning
University of Toronto

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Abstract

Methylmercury (MeHg) is a global toxin which bioaccumulates and biomagnifies through food webs. To discern how MeHg enters Arctic food webs, it is important to understand spatial and seasonal cycling of MeHg at the terrestrial-aquatic interface, processes which are sensitive to climate change. This research determined hotspots of MeHg production and degradation in a High Arctic freshwater continuum, and how these processes varied between ice-on and ice-off conditions. Different compartments of the Skeleton Continuum of Lake Hazen, Nunavut were sampled to capture variation in MeHg production and degradation. Examining interconnectedness of the landscape revealed that production hotspots occur in multiple compartments (lake/pond sediments, pore water), yet downstream degradation hotspots and storage (lake water, wetland soils) prevent MeHg from entering the downstream system. Additionally, high MeHg concentrations found in the springtime (water column, snow) solidify the theory that spring freshet is an important source of MeHg to freshwater systems.
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Chapter 1
Introduction

1 Introduction to Mercury (Hg)

1.1 Hg Background

1.1.1 Hg as a Contaminant

Mercury (Hg) is a globally distributed trace metal that is released into the environment through natural and anthropogenic processes, producing toxic effects in high concentrations (Morel et al., 1998; Mergler et al. 2007). Hg is found naturally as cinnabar and is released through igneous rock erosion, produced during the burning of biomass and emitted during volcanic eruptions (Pirrone et al., 2010). Emissions of Hg from natural sources are now surpassed by anthropogenic sources which include gold extraction, cement processing and the burning of fossil fuels (AMAP, 2011). Due to an expansion of anthropogenic Hg producing processes, Hg emissions have increased several-fold since the industrial revolution (NCP, 2012). Human perturbations to Hg’s biogeochemical cycle have resulted in three times the amount of Hg being found in the atmosphere and ocean surface waters (Laurier et al., 2004; Mason et al. 2012; Mason et al. 1994; Lamborg et al. 2002a). Anthropogenic increases in Hg emissions and altered patterns of Hg cycling due to anthropogenic climate change are concerning as Hg in its organic form methylmercury is toxic to humans and biota.

Hg (0) and Hg (II) are the most common of Hg's three possible oxidized states. The elemental form of mercury has an atmospheric residence time of 0.5-1 year, allowing for long-range transport via wind currents (Pacyna et al. 2006; Schroeder and Munthe 1998). Hg(II), in contrast, has a short atmospheric residence time, is commonly found bound to carbon, and is deposited in terrestrial and aquatic environments (Mason and Sheu, 2002). Deposited Hg(II) can undergo conversion into methylmercury (MeHg), an organic form of mercury which enters the food web and causes neurological deficiencies. MeHg is lipophilic and attaches to sulfur binding sites on amino acids, allowing it to incorporate into fats and proteins (Hintelmann, 2010). MeHg has a slow excretion rate allowing it to bioaccumulate in tissue, meaning that with prolonged exposure Hg concentrations increase over time (Morel et al., 1998). MeHg also biomagnifies, resulting in higher Hg concentrations as higher trophic levels consume Hg containing tissue (Morel et al.,
Mammal's tissues assimilate 95% of MeHg when consumed, compared to less than 15% expected for inorganic Hg (Mori et al., 2012). Differences in Hg uptake highlight the importance of understanding the controls on mercury speciation in the environment and what implications it might have on ecosystem and human health.

1.1.2 Hg in the Arctic

Hg(0) emissions from point sources at southern latitudes reach the Arctic due to their long residence time (Pacyna et al., 2006). Air masses carry Hg(0) to northern latitudes, and extreme seasonal changes in the spring oxidize elemental Hg into its shorter lived form Hg(II). During polar sunrise, Hg reacts with strong oxidizing agents such as ozone or other halogens (most commonly bromine), resulting in atmospheric mercury depletion events (AMDE’s) (Lindenberg, 2002) which deposit Hg (II) into terrestrial or aquatic ecosystems (Mason and Sheu, 2002).

Long range Hg transport has resulted in elevated Hg concentrations deposited onto Arctic landscapes, subsequently entering the diet of Northern Indigenous People through in situ conversion of Hg(II) to MeHg (UNEP, 2002). MeHg is primarily produced in aquatic systems, resulting in high levels of Hg being recorded in aquatic biota, most notably fish species (Clarkson and Magos 2006; Fitzgerald and Lamborg 2004). In the Artic more than 92% of the Hg burden in higher trophic levels organisms is of anthropogenic origin, demonstrating the impact human Hg emissions are having on Northern communities (Dietz et al., 2009).

Due to observations of high Hg concentrations in biota, the United Nations Environment Program agreed upon the Minamata Convention on Mercury in January 2013 with the goal of reducing Hg emissions globally, the fate of Hg in the Arctic is unclear because climate change will alter its biogeochemical cycling. Understanding how and where MeHg is being produced across both aquatic and terrestrial landscapes in the Arctic provides the underpinnings to understand how MeHg is being incorporated into biotic components of the ecosystem. The investigation of landscape features which potentially produce MeHg such as lake and pond sediments, water, wetland soil, and snowpack is important to understand inputs of MeHg to freshwater systems. It is critical that we study these systems as interacting components of the landscape and address seasonal change as a key component of MeHg cycling in the Arctic to get a better understanding of Hg cycling in the north, and how climate change may alter these processes.
1.2 Controls on MeHg Production and Degradation

1.2.1 MeHg Cycling

Hg that is deposited to the landscape and is not re-volatile has potential to be methylated under certain ranges of biotic and abiotic conditions, most commonly found at the terrestrial-aquatic boundary. Net Hg methylation is determined by the sum of net methylation and net demethylation in a system. In aqueous solution, the transfer of a methyl unit must be facilitated by a photochemical process or catalyzed by microorganisms (Morel et al., 2008). Methylation of Hg has been determined to be a primarily biotic process, however, small amounts of methylation have been attributed to abiotic pathways (Bigham et al., 2016; Ullrich et al. 2001). MeHg demethylation can occur through photochemical reactions in water or through microbial means in water, sediments and soil (Schaefer et al., 2004; Marvin-Dipasquale et al., 2000; Sellers et al., 1996; Oremland et al., 1991). In some cases, MeHg concentrations in a system may be close to zero, however, this could be a result of high rates of both methylation and demethylation, highlighting the importance of obtaining both measurements (Eckley and Hintelmann, 2006). Based on past literature it has been suggested that methylation controls MeHg concentrations in most ecosystems (Tjerngren et al., 2012; Drott et al. 2008a; Hammerschmidt and Fitzgerald 2006). Both Hg methylation and MeHg demethylation are determined by a combination of biotic mechanisms, abiotic mechanisms and the availability of Hg.

1.2.2 Biotic Mechanisms of MeHg Cycling

Biotic methylation of Hg and demethylation of MeHg are facilitated mostly by anaerobic microbes found commonly in freshwater environments (Driscoll et al., 2013; Ullrich et al., 2001). Anaerobic sulfate-reducing bacteria (SRB) in temperate systems are primarily responsible for these processes, while iron-reducing and methanogenic bacteria contribute a lesser amount to MeHg cycling (Hamelin et al., 2011; Yu et al., 2012; Kerin et al., 2006). These bacteria methylate Hg through the active transport of Hg into the cystol of bacteria, followed by an enzyme catalyzed transfer of a methyl group to the Hg, and finally, the export of MeHg from the cell (Schaefer et al., 2011). Demethylation is performed by the same groups of bacteria and occurs through one of two processes. First, through reduction via the mer operon resulting in elemental Hg and methane or through oxidation via methylotrophic metabolism creating carbon dioxide and a small amount of methane (Barkay et al., 2003; Oremland et al., 1991). Biotic Hg
methylation is the main producer of MeHg in aquatic systems, while biotic demethylation of MeHg plays a lesser role. These biotic methylation and demethylation processes are determined by the metabolic activity and composition of the microbial community present in a system.

Metabolic activity of anaerobic bacteria responsible for Hg cycling is determined by multiple physical factors within a system including, sulfate, dissolved organic carbon (DOC), pH, redox conditions and temperature (Ullrich et al., 2001). Sulfate and DOC are two of the most complicated relationships that regulate MeHg cycling. In systems where sulfate is limiting and SRB are the dominant methylators, the presence of sulfate stimulates Hg methylation (Kampalath et al., 2013) because it acts as an electron acceptor for SRB (Bigham et al., 2016). However, high levels of sulfate limit bacterial activity through the production of sulfide which creates complexes with Hg making it unavailable for conversion (Gilmour & Henry, 1991).

Carbon quality and quantity are also major drivers in the metabolic activity of methylating/demethylating bacteria. Multiple studies have found that changing the source of carbon can create large divergences in microbial functions, in one case doubling microbial activity (Bridou et al., 2011; Kwon et al., 2016). Carbon source also acts as a driver behind which microbial communities will form in a system because some SRB and FeRB cannot reduce more complex carbon sources (Handley et al., 2015; Akob et al., 2008). High amounts of carbon in a system have been found to decrease demethylation because of its ability to bind with MeHg, making it unavailable for breakdown (Marvin-Dispasquale et al., 2000).

Physical parameters such as redox potential, pH and temperature have a more straightforward relationship with MeHg cycling. The low redox potential needed for anaerobic bacteria to survive is primarily determined by low oxygen availability (Horn & Goldman, 1994). Stimulation of microbes in a system increases anoxia due to the consumption of oxygen (Eckley & Hintelmann, 2006). Methylating bacteria often function best in slightly acidic environments as acidification of systems increases the ability of Hg to pass bacteria’s cell membrane, promoting methylation (Winfrey et al., 1990; Xun et al., 1987). Lastly, higher temperature increases the metabolism of bacterial communities, increasing rates of Hg methylation (Hintelmann & Wilken, 1995; Watras et al., 1995). These physical characteristics work together to determine the capability of microbial communities to produce and breakdown MeHg in aquatic systems.
Pathways of methylation have not been fully understood, but recent literature has identified genes HgcA and HgcB as those responsible for Hg methylation (Parks et al., 2013; Gilmour et al., 2013). These genes are said to be responsible for initiating the intracellular enzymatic process which methylates Hg and have homologs that have been found in 52 bacterial and methanogenic archaea (Parks et al., 2013). Podar et al. (2015) found the HgcAB genes present in nearly all anaerobic environments that they evaluated from over 3500 microbial metagenome sequences, however, SRB and FeRB are by far the most abundant methylators found in natural systems. Identifying specific genes, and analyzing microbial assays allows for a better understanding of how and where Hg methylation can occur across the landscape, especially at Arctic latitudes where microbial assemblages are adapted to the cold.

1.2.3 Abiotic Mechanisms of MeHg Cycling

Photodemethylation of Hg has been identified as the most important pathway of MeHg export in certain lakes and is a main portion of lake Hg budgets (Lehnerr & Louis, 2009; Hammerschmidt et al., 2006; Sellers et al., 2001). Photodemethylation has been identified as the only important pathway of MeHg degradation in surface waters (Ullrich et al., 2001). Photodemethylation can occur directly through photolysis, and can produce both Hg(II) and Hg(0) (Inoko et al., 1981; Krabbenhoft, nd). Studies have attributed high rates of photodemethylation to DOC’s ability to absorb lights energy and then transfer it to break the Hg-C bonds of MeHg (Jeremaison, 2015; Qian et al., 2014; Zhang & Hsu-Kim, 2010). Previous research has demonstrated that both filtered and unfiltered water demethylate MeHg at similar rates, highlighting the importance of photolithically driven reactions in the decomposition of MeHg (Hammerschmidt & Fitzgerald, 2006; Sellers et al., 1996). Photodemethylation can be responsible for the loss of 31-83% of MeHg produced in sediments of wetland systems, 83% of inflow inputs in boreal lakes and ~30% of inflow inputs for humic lakes, highlighting the importance of this pathway in detoxifying aquatic systems (Hammerschmidt & Fitzgerald, 2006; Sellers et al., 1996).

MeHg produced through abiotic processes may occur through a series of chemical reactions, however, the donating methyl group is often produced through biological processes. The most common pathways for abiotic MeHg production occur in seawater because Cl⁻ is needed as a complexing agent (Celo et al., 2006). In freshwater systems, humic acids are another potential
source for abiotic Hg methylation. A study by Guevera et al. (2008) found that in sterilized lake water production of MeHg still occurred, producing 8.8-17.3% of the MeHg spike injected, demonstrating that there are abiotic pathways of Hg methylation in lake waters. These findings are supported by multiple other studies that have found small amounts of abiotic methylation to occur at environmentally relevant conditions (Sunderland et al., 2008; Cossa et al., 2009; Hintelmann et al., 1997).

1.2.4 Role of Hg Availability in MeHg Cycling

MeHg in aquatic and terrestrial ecosystems is partially determined by the amount of total Hg (THg) present in the system, but more so by the fraction of THg that is bioavailable (Bigham et al., 2016; Cossa et al., 2014). The relationship between MeHg and THg is often strong, but varies greatly within and between sites, highlighting that THg is not equivalent to the amount of Hg available for methylation by microbial communities (Heyes et al. 2006; Cossa et al. 2014). The bioavailability of Hg for methylation is determined by speciation of Hg, which is influenced by complexing agents and environmental conditions.

Complexing agents that regulate Hg bioavailability include sulfide and dissolved organic matter (DOM). As previously mentioned, SRB oxidize sulfate to produce sulfide which decreases Hg bioavailability by creating Hg-S complexes that are not able to pass through the cell membrane for methylation. Under certain conditions charged Hg-S complexes can be available for methylation but charged complexes or solid Hg-S particles are not (Kampalath et al. 2013). Age of Hg may play a role in its interaction with sulfide, where new Hg deposits in the form of dissolved Hg are more available for methylation than older nanoparticulate and crystalline Hg complexes (Zhang et al., 2014). In freshwater ecosystems, a high percentage of Hg can be attached to organic compounds when there are low sulfide concentrations (Hurley et al., 1991; Benoit et al. 2001a; Haitzer et al. 2002). DOM can act as a complexing agent in low sulfide conditions, but it can also act to slow and reduce the growth of Hg-S compounds in moderate to high sulfide conditions (Gerbig et al., 2011; Deonarine et al., 2009). Chiasson-gould et al. (2014) identified a mechanism behind DOM and Hg’s relationship, finding that at non-equilibrium there was a peak of bioavailable Hg at mid-ranges of DOM over time. This pattern was attributed to that tendency of new Hg to first rapidly bind to labile DOM molecules creating bioavailable complexes, where over time (24 h) Hg becomes less available due to ligand exchanges with less
labile molecules. The interaction between Hg, DOM, and S species plays a key role in determining the availability of Hg for methylation as particle size exerts control on reactivity (Dehner et al., 2011) and bioavailability (Liu et al., 2009) of nanoparticles.

In addition to complexing agents, environmental factors such as low pH, high temperature and ionic composition alter Hg bioavailability. Increased Hg methylation rates are observed at low pH which causes reduced binding of DOC and Hg (Miskimmin et al., 1992; Xun et al., 1987). Solid Hg complexes in solution are affected by the kinetic energy associated with higher temperatures, increasing complex solubility (Klapstien et al., 2016). Fe has potential to bond to sulfide, in turn decreasing the quantity of Hg-S complexes and increasing the amount of bioavailable Hg (Han et al., 2008). Additionally, the presence of cations in aqueous solution have been found to change the permeability of bacterial membranes, resulting in a decrease in Hg uptake (Daguene et al., 2012). In summary, these factors imply that it is the availability of Hg rather than the quantity that determines a system's potential for producing MeHg.

1.3 MeHg in Arctic Freshwater Ecosystems

1.3.1 MeHg in Lakes & Ponds

Bottom sediments have been identified as a significant source of Hg methylation within High Arctic Lakes (Gilmour and Riedel 1995; Lehnerr et al, 2012b). Sediment production of MeHg has been found to be highest at the water-sediment boundary decreasing with depth (Lehnerr et al., 2012b). However, other studies have identified the possibility of Hg methylation occurring at depths as far as 30cm due to deep anoxic conditions (Jiang, Liu, & Chen, 2011). Previous studies demonstrate that measurements of methylation within sediments in High Arctic freshwater systems are similar to rates found in temperate lakes and wetlands, which are known sources of MeHg (Lehnerr et al. 2012a). Additionally, in Arctic tundra with no wetland complexes sediments appear to be the major source of MeHg (Hammerschmidt et al. 2006). It has been reported that sediment demethylation of MeHg is greatest at the sediment-water boundary but compared to methylation is negligible in the overall ecosystem net methylation (Lehnerr et al., 2012a).

Anoxic hypolimnetic lake waters have also shown the ability to produce MeHg at temperate latitudes (Eckley and Hintelmann 2006; Eckley et al. 2005). In temperate regions summer lake
stratification can result in Hg methylation at all anoxic depths within the water column (Eckley and Hintelmann 2006). Methylation in the water column is often attributed to biotic processes similar to those found in sediments, but there is also possibility that a small portion is methylated via abiotic pathways (Celo et al., 2006). Though summertime bottom water anoxia in Arctic lakes is uncommon because lakes are well mixed, in small lakes an anoxic layer has been observed to form in the spring due to prolonged ice cover (St. Pierre, Unpublished). During the spring melt period in Arctic small lakes, anoxia occurs, inputs of nutrients and light are beginning, and atmosphere-water exchange is limited which provides a potential site for MeHg production (Stern et al., 2012).

Water column photodemethylation has been identified as a factor dominating net water column methylation. In High Arctic Lakes 66-88% of total MeHg inputs can be demethylated within the water column (Fitzgerald et al., 2006). MeHg photodemethylation occurs independently of site-specific characteristics and depends on light intensity, Hg availability and a source of DOM or nitrates, making it potentially universal to all lakes (Lehnher et al., 2008). The rate of photodemethylation is dependent on the attenuation of light making it exceeding Ly important in oligotrophic and shallow lakes, which are commonly found in the Arctic (Hammerschmidt et al., 2006; Sellers et al., 2001).

Another important landscape feature that determines MeHg concentrations in Arctic freshwater systems are wetland and permafrost thaw ponds, which have been observed to have MeHg concentrations higher than surrounding larger ponds or lakes (Benoit et al., 2001; Sellers et al., 1996; St Louis et al., 2005). Ponds may have a small surface area but are the most abundant aquatic ecosystem type at boreal and arctic latitudes, making their contribution to MeHg cycling significant (Pienitz 2008; Verpoorter et al. 2014). Ponds are ideal locations for the net production of MeHg as they are shallow, warmer and DOM rich when compared to surrounding water bodies (St Louis et al., 2005). These properties encourage the microbial production of MeHg in the sediment and allow for easy export of MeHg as they are hydrologically sensitive due to their small volume.

1.3.2 MeHg in Arctic Wetland Systems

Wetlands are characterized as being rich in organic material, warmer than surrounding environments, and tend to be periodically inundated, creating anoxic conditions that are ideal for
Hg methylation (Hammerschmidt & Fitzgerald, 2004; Gilmour et al., 1992). Wetlands have been identified as significant sources of Hg methylation at temperate regions, and a source of MeHg to downstream ecosystems (St. Louis et al., 1994, 1996; Branfireun and Roulet 2002). However, Arctic research on wetlands role in Hg cycling has been inconclusive (Oiffer & Sicilliano, 2009; Loseto et al., 2004). In recent years an increase in wetland area due to permafrost melt in the arctic has revealed an increase in MeHg concentrations associated with pore water found in soils (Gordon et al., 2016). Understanding current dynamics of wetland MeHg cycling is an important component of determining MeHg concentrations at the watershed level, as their role in ecosystem’s MeHg budget is often not proportionate to their size (Louis et al., 1996).

Arctic wetlands contrast those at mid-latitudes in climactic factors as well as exposure to Hg and MeHg. High latitude wetlands experience extreme changes in temperature and light availability, experiencing freezing for majority of the year and lengthy periods of light and thaw during the short growing season (Bliss, 1997). Arctic wetlands have a poor nutrient status when compared to temperate latitudes due to a short growing season and permafrost layer which underlies the wetlands. In addition, Arctic wetlands are far removed from point sources of Hg, meaning that MeHg concentrations are a function of in situ methylation or upstream production, rather than small amounts of deposition that can occur near point sources (AMAP, 2011). These distinct characteristics and removal from point sources result in THg concentrations that are much lower than temperate latitudes, and less consensus around whether these areas are conducive to Hg methylation.

Arctic wetland soils ability to methylate Hg has been suggested in lab-based experiments using low MeHg soil from 18 wetlands across Nunavut (0.065 ng g⁻¹). Incubation experiments which warmed the soils resulted in 100-fold MeHg concentration increases in the first 30 days (Loseto et al., 2004a). Additionally, in situ measurements revealed that concentrations at wetland outflows were consistently higher than wetland inflows (1.21 ng L⁻¹ vs. 0.02 ng L⁻¹), implying that there is MeHg production within the wetland. Though these concentrations were consistent spatially, an additional study by the same authors revealed that THg and MeHg concentrations varied seasonally (Loseto et al., 2004b). THg and MeHg were both highest at the onset of spring snowmelt and lowest during the summer, indicating that snowmelt is likely an important source of Hg to wetlands.
In contrast to wetland Hg methylation being observed in Nunavut, wetlands in Canada’s NWT demonstrate a decrease in MeHg concentration over time (Oiffer & Sicilliano, 2009). During the summer (late July to early August) MeHg concentrations dropped from 1.5 ng g\(^{-1}\) to 0.42 ng g\(^{-1}\). The decrease of MeHg over time is indicative of one of two processes occurring: demethylation or desorption and export of MeHg. The authors indicate that in this case desorption is unlikely as discharge is low and there is no spatial pattern in MeHg concentrations, leaving demethylation as a potential reasoning for the trends observed. It is important to note that though the wetland studies showed contrasting results, both studies revealed little to no activity present from SRB, indicating that other processes may play a role in Hg methylation and MeHg demethylation in Arctic wetlands (Loseto et al., 2004b; Oiffer & Sicilliano, 2009).

There has not been a consensus on arctic wetlands role in MeHg contributions to freshwater ecosystems, but more recent research has demonstrated that wetland ponds on the arctic landscape are hotspots of MeHg production. Small Arctic wetland ponds have been shown to have %MeHg concentrations over 4 times higher than large lakes and ponds (St. Louis et al., 2005). Work by Lehnerr et al. (2012) measured MeHg concentrations in wetland ponds on Ellesmere Island, NU, Canada. This study found 19% to 62% of Hg as MeHg, potentially due to high rates of anaerobic activity (interpreted from CH\(_4\) levels). Additionally, Arctic wetland ponds have been reported to have methylation rates similar to temperate wetlands (Lehnerr et al., 2012b), highlighting their importance to freshwater MeHg inputs.

1.3.3 MeHg in the Cryosphere

In High Arctic systems snow can be responsible for 60-80% of available water (Losetto et al., 2004), making snow reservoirs a key component to understanding MeHg cycling in Arctic freshwater systems. Investigating the mechanisms which result in Hg deposition and evasion, chemical and physical processes within snow, and how snow melt acts to deliver Hg/MeHg to downstream systems through melt helps to understand a major pathway of how Hg enters freshwater systems.

AMDE’s have been reported to contribute up to 100 ng L\(^{-1}\) of Hg to some Arctic snow packs (St. Louis et al., 2007). A portion of the deposited Hg is photoreduced and evades from the snow, with 25-75% remaining in the snows surface layer (Dommergue et al., 2003; Brooks et al., 2006; Kirk et al., 2006). In addition to AMDE’s, atmospheric scavenging and condensation deposit free
and particulate bound Hg to the snow (Tranter et al., 1986; Loseto et al., 2004; Tranter et al., 1986). Deposition of Hg to surface snow has been thoroughly investigated, but elevated levels of MeHg detected in surface snow in inland environments are less understood. These observations have been explained in near marine environments by dimethylmercury deposition and conversion into MeHg in the atmosphere or snowpack (St. Louis et al., 2008). In freshwater systems these observations are harder to explain, leading to hypothesis that chemical and physical transformations within the snow may be responsible for elevated snowpack MeHg (Lehnherr et al., 2014).

Snowpack in the High Arctic is normally shallow (<40cm) and its distribution is dependent on blowing snow events (Sturm and Liston, 2003; Derkson et al., 2009). Come spring, Arctic snow is characterized a hoar layer deposited the previous fall and a surface layer characterized by newly blown or precipitated snow (Domine et al., 2002). Within these layers chemical and physical transformations occur throughout the life of the seasonal snowpack, during melt-freeze events (Johannessen and Henriksen, 1978) and snow metamorphism (Colbeck, 1989; Kuhn, 2001). The changes in snow characteristics over time allow for the redistribution of the snows constituents, including Hg. Once deposited on the surface layer of the snow, Hg can accumulate and undergo reactions resulting in changes in speciation and phase association (Mann et al., 2014). In some cases, high concentrations of MeHg have been found in Arctic snowpack, representing between 5-100% of THg found in the snow (St. Louis et al., 2005; Lahoutifard et al., 2005).

Potential pathways that could explain these elevated levels of MeHg in snowpack could be the chemical or microbial methylation of Hg, something that has been suggested in literature but has yet to be fully explored (Constant et al. 2007; Dommergue et al., 2010). Bacterial activity in Arctic snow has been identified in multiple studies as a potential pathway for MeHg production (Felip et al. 1995; Priscu et al. 1998; Carpenter et al. 2000; Junge et al. 2004, Amato et al. 2007). In addition to pathways of transformation within the snow, it is also important to consider that this contribution of MeHg to the watershed could be occurring through snow melt processes.
1.4 Research Gaps

1.4.1 Addressing Spatial Variability in MeHg cycling

The importance of landscape heterogeneity in the transport and cycling of MeHg has been highlighted in both temperate and arctic studies. It is important to understand the production and degradation of MeHg across the terrestrial-aquatic interface in freshwater sub-catchments because *in situ* production of MeHg dominates the delivery of MeHg to downstream environments (Rudd, 1995; Sellers et al., 2001). In previous studies it has been shown that biogeochemical hotspots vary over space and time within a watershed, demonstrating the importance of examining watershed components as a system, rather than discrete areas (Mitchell et al., 2008a; Hedin et al., 1998; Hill et al., 2000).

In previous studies at temperate latitudes watershed spatial patterns of MeHg transformation and transport have been investigated. Variation in MeHg concentrations as water flows through wetlands has been observed where high concentrations are associated with MeHg production hotspots created by the delivery of limiting reactants (Mitchell et al., 2008a). Additionally, snow melt has been identified as an important mode of MeHg production and transport, in some cases delivering 22-23% of the annual MeHg flux in a two-week period (Mitchell et al., 2008b). Though these trends are quite clear at mid-latitudes, high arctic systems are vastly different in terms of climate, hydrology and water chemistry.

In High Arctic systems snow can account for 80% hydrologic inputs in the early spring and glacial and permafrost hydrological sources dominate in the summer, both pathways result in delivery of Hg into sub-catchments (Young, 2008). During snowmelt there is a pulse of ions and contaminants entering the system that were deposited and transformed within the snow pack over the winter (Douglas et al., 2017). In the summer, water from glacial melt rivers and active layer seeps (water delivered from seasonally thawing ice within the landscape) can deliver stored DOC and legacy contaminants to the sub-catchment (Lehnherr et al., 2018). Both water sources interact with the landscape as they move downstream undergoing transformations as they pass through distinct landscape compartments, eventually delivering Hg to downstream lakes (Semkin et al., 2005). In some systems larger watersheds result in higher concentrations of Hg reaching the downstream lake, highlighting the importance of understanding Hg cycling as it moves from source into lake systems (Hammerschmidt et al., 2006).
In High Arctic freshwater systems hotspots of MeHg production have been identified across the landscape, implicating small wetland ponds and snow melt as major sources of MeHg to downstream systems (Douglas et al., 2017; Lehnherr et al., 2012a; Macmillan et al., 2015). However, compartment interactions have not been investigated to determine how MeHg delivery varies over space and time. Understanding the interaction of each compartment of the landscape as water moves downstream will provide a better conceptual model of how MeHg is processed as it moves across the landscape by identifying what processes are controlling net MeHg production. Ultimately, this will help predict how processes that govern the amount of MeHg that enters downstream systems might be altered in the face of climate change.

1.4.2 Addressing Seasonal Variability and the Impact of Climate Change on MeHg cycling

Biogeochemical processing in High Arctic freshwater systems is thought to occur during a very short window of time during spring melt and through the short summer season, which spans a maximum of 12 weeks but often 6-10-weeks (Lehnherr et al., 2012b). Most research on Hg cycling in the Arctic focuses on the ice-free productive period as this period dominates Hg cycling at temperate latitudes (Wildman, 2016). However, recent research has shown that the spring melt period in snow dominated landscapes is a key part of understanding biogeochemical transformation, and delivery of contaminants and nutrients (Douglas et al., 2017). Additionally, understanding how Hg is cycled during the 9 months when the landscape is ice covered and freshwater processes are limited to snow and unfrozen bottom lake waters will provide key insights to how the changing climate might impact Hg cycling in the future.

The predicted increase in temperature in the High Arctic due to climate change has potential to alter the cycling of Hg in freshwater ecosystems at a multitude of levels. Increasing temperatures not only implies increased methylation potential in water due to available heat, but also have potential to change other driving factors that control Hg processing (Vincent et al., 2016; Lehnherr et al., 2012b). It is believed that the thawing of permafrost that has been seen in recent decades has potential to release Hg, accompanied by carbon and nutrients into ultra-oligotrophic freshwater systems. The addition of legacy Hg and potentially limiting nutrients could result in increased productivity and therefore increased Hg methylation (Quinton et al., 2011a). Increases in dissolved organic matter also have the potential to decrease attenuation of light in lakes and ponds, in turn decreasing the depth and rate of photo demethylation (Fitzgerald et al., 2006;
Melting of glaciers, ice and snow has the potential to increase the amount of bioavailable Hg in freshwater systems in the form of legacy Hg that was stored during formation and is released at quicker rates than it was originally accumulated (St. Louis et al. 2005; Selin et al. 2008; Sunderland and Mason 2007). In contrast, decreased ice cover due to warming has the potential to increase photo-demethylation rates as there will be a longer exposure (White et al., 2007; Rawlins et al., 2010; Prowse et al., 2011). Though these predictions provide a glimpse into what the fate of arctic freshwater MeHg may be, it is difficult to determine what effect this will have on overall methylation rates and food web dynamics without a comparison of seasonal MeHg dynamics and a better understanding of what drives MeHg production in the ice-covered months.

1.5 Objectives & Study Site

The first objective of this research is to identify hotspots of Hg methylation and MeHg demethylation as Hg moves through a High Arctic freshwater compartments at the terrestrial-aquatic interface. The second objective is to quantify seasonal changes in MeHg production across a High Arctic freshwater continuum to infer impacts of climate change on Hg cycling.

To address these objectives the Skeleton Continuum of Lake Hazen (81°49′ N, 71°20′ W) (Figure 1) was used as a study site to represent similar sub-catchments in the region. The Skeleton Lake continuum allows for the quantification of MeHg concentrations and methylation/demethylation rates at the terrestrial-aquatic interface as water flows from an active layer seep, into a small lake, 2 ponds, a grass dominated wetland and a tundra creek channel, into Lake Hazen itself.

1.6 Conclusion

Biogeochemical constraints on Hg methylation and MeHg demethylation control MeHg concentrations in freshwater ecosystems, determining the degree of contamination. Ultimately, Hg methylation is dominated by microbial activity in water, sediments and soils, which is controlled by Hg bioavailability (Ullrich et al., 2001). In contrast, MeHg demethylation is primarily a function of photodemethylation, which is largely driven by UV-rays (Lehnherr & Louis, 2009). Both abiotic methylation and biotic demethylation play smaller, but still significant roles in contributing to the overall MeHg concentration of an ecosystem. Understanding the
dynamics of MeHg concentrations allows for better explanation of observations made in compartments of freshwater ecosystems. It is clear from freshwater ecosystem studies that large amounts of variation in MeHg concentrations can be explained by the unique biogeochemical properties in a system which vary over space and time. Additionally, the seasonal changes experienced in the Arctic are extreme and even slight changes in seasonality have potential to greatly impact the cycling of MeHg. Understanding the mechanisms of MeHg cycling at the freshwater-terrestrial boundary is an integral part to identifying how MeHg enters the biotic system, and ultimately affects human health. Being able to piece together the factors which determine net production of MeHg will allow for the better prediction of what future ecosystem changes may mean for the Hg cycle at both temperate and Arctic latitudes.

1.7 Thesis Structure

This thesis contains five chapters. Chapter One reviews MeHg as a contaminant and its production/degradation across different compartments of the freshwater landscape. Chapter Two provides a description of the study site and methods used throughout this research. Chapter Three explores the spatial variation in MeHg cycling across the summer arctic landscape, capturing changes in MeHg concentrations and potential net methylation rates. Chapter Four addresses the seasonal variation in MeHg concentrations and MeHg production to make inferences about how climate change may alter the delivery of MeHg to downstream systems. Chapter Five summarizes the main findings of this research and explores limitations and future research directions.
Chapter 2
Methods

2 Methods

2.1 Site Description

This research was carried out in a small sub-catchment of the Lake Hazen region (81.78333°N, -70.98333°W), Quttinirpaaq National Park, northern Ellesmere Island, Nunavut (2.1). Lake Hazen is the largest freshwater lake by volume north of 80 degrees latitude (area: 542 km²; max. depth: 267 m), and the watershed (6901 km²) is located on the northwestern, leeward side of the Grant Land Mountain range (Figure 2.1b). The underlying geology of the area is characterized by Palaeozoic limestone, sandstone, slate, and chert pebble conglomerate, overlain by Quaternary glacio-fluvial sediments (Smith, 1999). The region is classified as a polar desert climate, with exceptionally warm summer temperatures (6°C daily average) and limited annual precipitation (~95 mm yr⁻¹). Due to the continentally and topography of the region, Lake Hazen and the surrounding landscape has been defined as a “thermal oasis” during the summer months (Smith, 1999), resulting in a diverse and productive landscape (Thompson, 1994).

The Lake Hazen region is comprised of both glacial and non-glacial sub-catchments. The rivers have a seasonal flow regime, where channel runoff typically begins in early- to mid-June, with flow cessation in late-August to early-September during freeze up. In the glaciated sub-catchments of the watershed, early season river discharge is dominated by seasonal snowmelt runoff until snowpack exhaustion, and then mid- to late-season river discharge is fed by a combination of glacial runoff and groundwater from active layer seeps (Emmerton et al., 2016). Similarly, the non-glacial sub-catchments are dominated by early season snowmelt runoff and mid- to late-season groundwater inputs from active layer seeps. Rivers in the non-glacial portions flow through small lakes, ponds, and wetlands, resulting in a landscape that fosters biogeochemical transformations as water moves from the terrestrial environmental, downstream into Lake Hazen (France, 1993).

The Skeleton Continuum (81.81666°N, -71.33333°W) was used as the study site for this research within the Lake Hazen watershed (Figure 2.2). This site was chosen for this research because it is an ideal site to assess how MeHg is transformed at the terrestrial-aquatic interface, and it is
representative of similar sub-catchments in the region. In this continuum, early season spring discharge is dominated by snowmelt runoff and flow is sustained throughout the summer by an active layer seep fed by melt from surrounding glaciers. Continuum runoff first flows through Skeleton Lake, a 4m deep (1.84 Ha) lake that becomes well mixed in the summer season allowing for distribution of nutrients throughout the water column. The outflow from Skeleton Lake flows into two ponds (Pond 4 & 11, unofficial names), which are <1 m in depth and freeze to the bottom during non-summer seasons. Runoff from the ponds flow into a wetland complex (Craig’s Wetland), which flows through a tundra creek channel (Skeleton Creek), and downstream into Lake Hazen (Figure 2.2). During the summer season the active layer seep provides seasonally variable water flow that creates connection between Skeleton Lake and the downstream ponds in mid-July through to late August. The continuum has some channelized flow at the inflow of Skeleton Lake and throughout Skeleton Creek, however the wetland is often characterized by pooling and eventually downstream export of surface water, making it difficult to quantify flow rates. However, there are no dominant downstream sources of water in the wetland and the amount of water entering from the Seep and flowing through Skeleton Lake and the Ponds is similar in quantity to that exiting through Skeleton Creek, suggesting that other sources of water within the continuum are negligible.

The wetland complex is characteristic of a shallow water wetland with central pond areas surrounded by a wet sedge meadow composed of water sedge (Carex aquatilis), cotton grass (Eriophorum spp.), two-flowered rush (Juncus biglumis), alpine foxtail (Alopecurus alpinus), and arctic willow (Salix arctica). Vegetation within the ponds includes Mare’s tail (Hippuris vulgaris), floating buttercup (Ranunculus hyperboreus), and semaphore grass (Pleuropogon sabinei), as well as aquatic mosses (e.g., Calliergon giganteum) (Lehnherr et al., 2012a). The spatial differences in continuum characteristics allows for sampling of multiple substrates that are connected through runoff and provide potential for advancement in knowledge of the fate of MeHg as it is transported downstream, particularly at the interface between terrestrial and aquatic ecosystems, such as riparian zones of streams and in saturated wetland soils.
Figure 2.1 Skeleton Lake Continuum, Lake Hazen, Nunavut, Canada. (a) Map of Canada with Lake Hazen Region denoted by red dot (b) Digital Elevation Map of Lake Hazen with Skeleton Lake Continuum denoted by red box (c) Skeleton Lake Continuum with water flow denoted by blue lines and elevation denoted by black contour lines.
Figure 2.2 Skeleton Continuum Sampling Schematic. Labels identify the sampling sites for water surveys in 2015/6 and points identify substrates sampled in 2016/17.
Figure 2.3 Skeleton Continuum in (a) spring and (b) summer. (a) The continuum is covered by ~30cm of snow and ponds are completely frozen, Skeleton Lake is covered by ~1.5m of ice. (b) the continuum is void of snow and ice by early June and is fed by active layer seeps, providing intermittent connectivity throughout. Both seasons experience 24 hours of sunlight.

2.2 Methods

To identify hotspots of Hg methylation and MeHg demethylation (Objective 1) and to quantify spatiotemporal changes in MeHg production (Objective 2), water and sediment samples were collected during three seasons (summer 2015, 2016 and spring 2017) from different terrestrial and aquatic ecosystems of the Skeleton Lake Continuum (Figure 2.3). Each sampling site was chosen to represent the flow of MeHg as it is transported along a continuum from the inflow of the continuum to the outflow into Lake Hazen (Figure 2.2). The following sections of this chapter outline the experimental design, field and laboratory methods used to investigate the specific objectives of this research. In the first section, the field and laboratory methods for the (de)methylation experiments are presented. This is followed by an account of the laboratory and data analysis methods used for this dataset. The second section presents the field, laboratory and data analysis methods for the spatial survey of MeHg production.

2.3 Methylation Experiments

To identify MeHg production hotspots, potential methylation and demethylation were quantified in different media (e.g., water and sediment) throughout the Skeleton Continuum using stable Hg isotope incubations (Hintelmann et al., 1995; Hintelmann and Evans, 1997, Eckley et al., 2006). Potential Hg methylation is determined by the addition of a known amount of $^{198}$Hg isotope tracer to the sample for a set period of time and monitoring the production of Me$^{198}$Hg. Potential MeHg demethylation is determined using the same procedure but is quantified by examining the transformation of Me$^{199}$Hg to $^{199}$Hg. Hg isotopes $^{198}$Hg (purity: 90.5%) and Me$^{199}$Hg (purity: 91.9%) were obtained from the Biogeochemical Analytical Service Laboratory (BASL, University of Alberta). Me$^{199}$Hg (purity: 91.9%) was synthesized in the laboratory from $^{199}$HgO using methylcobalamin (Hintelmann, 1999). The amount of isotope injected into each assay was determined based on previous field experiments of ambient Hg concentrations at the field site (Wong et al., Unpublished; Lehnherr et al., 2012a, b). Final isotope injection concentrations were selected so that the additions did not increase ambient concentrations by more an order of one,
but where production was large enough to detect chemical transformation over a 24-hour period (Eckely et al., 2006; Lehnerr et al., 2011). Method Detection Limits were calculated based on calculations established by Hintelmann and Evans (1997). All samples were collected using US EPA ‘Clean hands, dirty hands’ method 1669 (USEPA, 1996).

2.3.1.1 Water column methylation

Water column incubations were performed on water samples collected from Skeleton Lake (81.82965°N, -71.4820°W) during the spring (May 22 and June 4, 2017) before ice and snow melt and during the summer (July 15, 2016) while the landscape was ice-free and productive. 250 mL water samples were collected from the center of the lake at depths of 2 and 4 m during the spring and at depths of 0, 1.5 and 4 m during summer. For each incubation date the experiments were performed in duplicates.

A peristaltic pump was used to collect samples into pre-cleaned 250 ml amber boston round bottles with teflon lined caps and pre-drilled injection ports. To maintain in situ redox conditions, bottles were filled with their volume twice, then capped so there was no headspace and transported back to the field laboratory in a dark environment. In the field laboratory, 100 μl of a $^{198}$Hg and Me$^{199}$Hg solution in MilliQ water (referred to as Hg isotope solution from here on) was injected into incubation bottles using a 100 μl gas tight syringe for a final concentration of 2.160 ng L$^{-1}$ $^{198}$Hg and 0.109 ng L$^{-1}$ Me$^{199}$Hg. Sample bottles were kept in a dark cooler to maintain in situ temperature (± 2 °C). At time steps of 0, 6, 16 and 28 hours after injection, one bottle from each time step was acidified to 0.2% trace pure concentrated hydrochloric acid (HCl).

2.3.1.2 Sediment and wetland soil methylation

Sediment core incubations occurred on May 22, 2017 and July 16, 2016. Sediment cores were collected from Skeleton Lake during spring ($n = 3$) and summer ($n = 4$) sampling periods. Sediment cores were also collected from Pond 11 (81.83178°N, -71.46810°W) during the summer ($n = 4$), but not in the spring sediments due to inaccessibility to the pond bottom during ice-on. Cores were collected during each sampling period from random locations at the center of each site using 5 cm diameter Lexan core tubes with pre-drilled injections ports (with silicone septa) distributed vertically at 1 cm intervals along the core. During the spring sampling period
sediment cores were taken using a gravity-corer through an augured hole in the center of the lake ice and were sub-sampled using pre-drilled 5 cm core tubes (Figure 2.4).

Once the cores were collected, rubber stoppers were placed on either end of the core tube to preserve the sediment-water interface and retain overlying water and lake redox conditions. Sediment cores were carefully transported back to camp vertically to maintain core structure. At camp the overlying water was drawn down to ~10 cm using a large syringe and c-flex tubing. 100 μl of the isotope solution was injected every 1 cm in the sediment core in a fan like pattern using a 100 μl gas-tight syringe. Injections began at 1 cm above the sediment-water interface and ended at 4 cm depth in the sediment core (Lehnherr et al., 2012b). Injections resulted in 77.88 ng $^{198}$Hg and 0.257 ng Me$^{199}$Hg at every 1 cm interval. One core from each set was reserved as a blank and was sectioned into 2 cm intervals, placed in whirlpack bags, and frozen immediately. The remaining cores were stored in a dark cooler at in situ temperature (± 2 °C) and incubated for 5 hours. After 5 hours, the remaining cores were sectioned into 2 cm intervals and frozen.

Wetland soil incubations occurred during the snow-free productive period on July 16, 2016. Soil cores were collected at two locations along the wetland water flow: (1) near the pond outflow (81.83176°N, 071.46491°W) and (2) 1 km from the outflow into Lake Hazen (81. 83412°N, 071. 37783°W) (Figure 2.5). Cores were collected and incubated using a similar methodology as the sediment samples, but samples were collected to the entire depth of the core tube (12 cm) and the isotope solution was injected at each 1 cm intervals throughout the entire soil profile. Soil cores were then placed back into the soil and left in the field to incubate and after 5 hours were sectioned into 2 cm intervals and frozen.
Figure 2.4 Spring time sediment sampling method: a) initial core taken from Skeleton Lake through ice and b) core sub-samples taken from larger core with sediment-water interface in tact to maintain in site redox conditions.
2.3.1.3 Snowpack and snowmelt methylation

Snowpack and snowmelt incubations were performed on May 21, 2017 at two sites along the Skeleton Continuum (Figure 2). Sites were located on top of Skeleton Lake (81.82964°N, -71.48136°W) and where Skeleton Creek flows (81.83106°N, -71.33599°W) in the summer. Snowpack experiments were conducted to elucidate any processes that might occur during the deposition or transport of mercury throughout the snow column during the winter season. Snowmelt experiments were intended to investigate chemical and physical processes that might occur during the melting process, because snowmelt has been identified as a significant source of Hg to downstream systems (Douglas et al., 2017; Loseto et al., 2004).

For the snowpack methylation experiments, six snow cores (n=3, in duplicate) were collected at each site using acid washed 7.3 cm ID polycarbonate core tubes with pre-drilled injection ports.
every 2 cm on both sides of the snow core (in alternating positions). Once transported back to the field laboratory, cores were injected with 100 μl of the isotope solution in a fan-like pattern at each injection port covered by snow, using a 100 μl gas-tight syringe. Injections resulted in 0.131522 ng and 0.000572 ng of $^{198}$Hg and Me$^{199}$Hg per cm, respectively. Cores were then taken outside and incubated in the surrounding snowpack. One of each duplicate core was deposited into pre-cleaned 500 ml glass jars at 0, 24, and 48 hours and acidified to 0.05% trace pure concentrated HCl.

For the snowmelt methylation experiments, six samples ($n$=3, in duplicate) were collected at each site using a pre-cleaned 5 cm ID stainless steel corer and spatula. Enough snowmelt was collected to fill pre-cleaned 500 ml glass jars and injected with ten 100 μl spikes of $^{198}$Hg + Me$^{199}$Hg solution throughout the jar. Injections resulted in 4.1035 ng L$^{-1}$ and 0.0229 ng L$^{-1}$ of $^{198}$Hg and Me$^{199}$Hg, respectively. Jars were then stored in the dark in a Weatherhaven™ tent above 0 °C to promote gradual melting. At 0, 24, and 48 hours after injection, one of each duplicate was acidified to 0.5% trace pure concentrated HCl. At 24 hours, samples were mostly melted, but with a small amount of floating snow in the jar, at 48 hours all snow had entirely melted.

### 2.3.1.4 Laboratory Analysis

Samples collected in 2016 were processed at BASL at the University of Alberta, while samples collected in 2017 were processed at University of Toronto Scarborough. Water, snow, and freeze-dried solid phase samples were analyzed for MeHg isotope concentrations to identify concentrations of injected stable Hg isotope solutions from the incubation experiments. All solid phase samples were freeze-dried and homogenized prior to analysis. Liquid and solid phase samples were distilled in Teflon vessels using a Tekran© 2750 distillation unit following US EPA Method 1630 (US EPA Method 1630, 1998). 0.3 g of solid samples (+38.7 g MilliQ) were inserted into Teflon distillation vials with 500 μl 9M sulphuric acid (H$_2$SO$_4$), 200 μl 20% potassium chloride (KCl), and 1ml copper sulfate (Cu(SO$_4$)). In 2016, an internal standard of 5 μl and 40 μl of Me$^{201}$Hg (purity 96.2%) was added to each liquid and solid sample, respectively. In 2017, an internal standard of 40 μl and 150 μl Me$^{201}$Hg (purity 84.7%) was added into each liquid and solid sample, respectively. This internal standard was added before distillation to correct for procedural recoveries during this process. Samples were distilled on a hot plate at 500
°C for approximately four hours until 80% of the sample volume was in the receiving vial. Samples were then ethylated with 125 μl of sodium tetraethyl borate (NaTEB) and 50 μl of Me\textsuperscript{201}Hg was added to each receiving vial to correct for procedural recovery during analysis. After 15 minutes of reaction time, samples were bubbled in a glass vessel using ultra-pure nitrogen (N\textsubscript{2}) flow (350 mL/min) for 15 minutes. Following this, volatile species were then purged and trapped onto Tenax using N\textsubscript{2} flow for seven minutes. Hg was then thermally desorbed onto argon (Ar) flow (30 mL/min) and trapped using a GC glass column packed with 15% OV-3 Chromosorb (60/80 Mesh) heated to ~100 ± 2°C and analyzed on an Agilent Technologies 7700 series ICP-MS to perform Isotope Dilution-Gas Chromatography-Inductively Coupled Mass Spectrometry (ID-GC-ICP-MS) as described by Hintelmann and Evans (1997).

Total Hg isotope concentrations were measured in soils and sediments to calculate potential methylation and demethylation rates. 0.3 g of homogenized, freeze-dried samples were added to glass vials and diluted 10x with water and spiked with 150 μl of \textsuperscript{201}Hg standard (purity 84.7%). Samples were oxidized through the addition of 0.5ml of BrCl and subsequent heating on a hot plate at 250°C for 3? hours. The samples were then reduced to Hg(0) with the addition of 0.2 mL Hydroxyl Ammonium Chloride (NH\textsubscript{2}OH•HCl) (react for 5 minutes), added to the bubblers, spiked with 20μl of \textsuperscript{201}Hg standard, and 0.5 mL Stannous Chloride (SnCl\textsubscript{2}) and purged onto a gold trap using N\textsubscript{2} flow (30 mL/min) for seven minutes. The Hg(0) from the gold trap was thermally desorbed and carried on Ar flow (30 mL/min) and analyzed using a Tekran© 2700 coupled with an Agilent Technologies 7700 series ICP-MS for ID-GC-ICP-MS analysis. Quality assurance/quality control (QA/QC) was performed by incorporating standard reference materials (SRM’s), blanks, matrix spikes and duplicates every ten samples. Marine sediment reference material (MESS-3) was used for solid samples; all results were within 5% of each other and within accepted range. See SI for additional QA/QC information.

2.3.1.5 Data Analysis

Potential methylation rates (\(k_m\), %d\textsuperscript{-1}) in soil and sediment samples were calculated as the proportion of added \textsuperscript{198}Hg that was converted into Me\textsuperscript{198}Hg, divided by the incubation time. Potential demethylation rates (\(k_d\), d\textsuperscript{-1}) were calculated using equation 1:

\[
k_d = -\frac{1}{t} \ln \left( \frac{MeHg_t}{MeHg_0} \right)
\]
Using first order kinetics, the potential demethylation rate ($k_d$) is calculated using the start (MeHg0) and end (MeHgt) concentration of MeHg and incubation time (t). The theoretical amount of Me$^{199}$Hg added is used to estimate MeHg0. Potential methylation rates in liquid samples were determined using data from the time series incubation experiments, where the methylation rate was equal to the slope of the linear regression of Me$^{198}$Hg concentrations throughout time. The Me$^{198}$Hg concentrations used in the linear regressions were corrected to account for the changing bioavailability of spike in the incubation bottles over time. The bioavailability of reactive Hg spike over time for snow incubations was determined by adapting experimental rates from the pristine snow site used in Willis et al., 2018.

Standard statistical analyses were performed to determine if sets of samples were significantly different from one another. Each dataset was assessed using two-tailed, non-paired t-tests (ttest2) ($p = 0.05$) using MATLAB® R2016a. Each dataset was assessed to ensure that it met the assumptions of each test (normality, normal distribution of residuals, independence of residuals where applicable).

2.3.2 Spatiotemporal survey

To quantify the temporal variation in concentrations of THg and MeHg along the Skeleton Continuum, a spatial survey was conducted in the spring (2017) and summer (2015 and 2016). The survey targeted areas identified as potential MeHg sources or sinks and included the Skeleton Lake water column and sediment, pond water and sediment (Ponds 11 and 4), snow, and wetland soils. In addition to these targeted areas, surface water was sampled along the continuum during the summer of 2015 and 2016 (Figure 2). Samples were collected throughout the transition period from a snow and ice-covered landscape to an ice-free productive summer landscape. The sample collection dates allow for changes in MeHg and other water chemistry parameters to be examined on a weekly basis, to identify subtle changes associated with the transition of the landscape.

2.3.2.1 Water column survey

A spring water column survey for THg and MeHg concentrations were taken at Skeleton Lake on May 22 and June 4, 2017. These concentrations were also used to obtain %MeHg values, which represents longer-term methylation potential of a system (Gilmour & Henry, 1991;
Lehnerr et al., 2012b). Ice thickness on Skeleton Lake was 1.5-1.75 m during this period and samples were taken 2 and 4 m below the ice surface. Ponds were not sampled during the spring period as they freeze to near bed depths. Summer water column surveys of Skeleton Lake, Pond 4 and 11 occurred on July 9, 17, and 21, 2016. Skeleton Lake water samples were collected at 0, 2 and 4 m depth while all pond samples were collected near surface (ca. 0 m).

Water samples were collected using a Teflon lined geo-pump into amber glass bottles (250 ml, 125 ml) in duplicates and with filtered/unfiltered marked bottles at each depth. Filtered duplicates were processed in the field laboratory using Nalgene MF75 series filter-units equipped with a 0.45μm cellulose nitrate membrane pre-cleaned with a 1% HCl solution (Lehnerr et al., 2012a). Filtrate was then transferred into clean amber glass bottles. All samples were acidified to 0.2% trace pure concentrated HCl. A water survey was also conducted at Skeleton Creek on June 9, 2017 at the onset of water flow. Samples were collected in 250 ml amber glass bottles in duplicate, by hand (US EPA method 1669) from the deepest part of the creek to avoid contamination from terrestrial input.

2.3.2.2 Water Continuum Survey

Surface water samples were collected for THg and MeHg concentrations from five locations along the Skeleton Continuum throughout the summer field seasons of 2015 and 2016. Sampling locations included: just after the active layer seep (81.83232°N, -71.52210°W), Skeleton Lake (81.82928°N, -71.4797°W), post-ponds (81.83192°N, -71.46518°W), post-wetland (81.83423°N, -71.35803°W) and Skeleton Creek (81.83057°N, -71.33055 °W) (Figure 2). In 2015, samples were collected on July 9, 13, 18, 23 and August 1st. In 2016, samples were collected on July 3, 9, 17, 25, 31, and August 6th. During both years there were periods where all sites could not be collected due to insufficient water flow.

Samples were collected by hand (US EPA method 1669) from the shoreline at the surface of the water using 250 ml amber boston round bottles. For each date and site, samples were collected for both filtered and unfiltered water analysis in duplicate. Filtered water samples were processed in the field laboratory using 0.45μm Nalgene filter packs and then transferred into clean amber bottles. All samples were acidified to 0.2% trace pure concentrated HCl for preservation. In 2016, water samples were collected from the shoreline and from the center of the lake to examine differences in sampling techniques.
In addition to Hg samples, water samples were collected and analyzed for water chemistry parameters at the BASL, University of Alberta and at the University of Waterloo (See Table S2 in SI).

2.3.2.3 Wetland Soil Survey

A summer wetland soil survey was conducted to examine THg and MeHg concentrations as water moves out of Pond 11, through 600 m of wetland and into Skeleton Creek (Figure 2.2). To address uncertainties identified in the literature (Losetto et al., 2004; Oiffer & Sicilliano, 2008), wetland soils were surveyed using four transects that were set up perpendicular to water flow. Each transect contained 5-7 sampling points and spanned from 20-100 m in length, with the center of the transect representing the most obvious point of water flow to best capture variation in MeHg across a saturation gradient. Samples were taken at each transect point using a 1-inch diameter stainless steel hand soil corer to maximum depth (~30 cm). Depending on the depth obtained, 2-4 cores were collected at each point to ensure sufficient soil for analysis. Soil from each point were extracted by gloved hands with the vegetative layer removed, placed into whirlpack bags and homogenized by hand before being frozen.

2.3.2.4 Snow Survey

A snow survey was conducted on May 20, 2017 to quantify concentrations and areal loading of THg, MeHg, and Particulate MeHg (pMeHg) in snowpack along the Skeleton Continuum. Three sites were sampled along the location of summertime water flow to address any spatial variation along the continuum (Figure 2.2). The sites were selected to represent: (1) Glacial input/active layer seep (81.83106°N, -71.33599°W), (2) Skeleton Lake (81.82964°N, -71.48137°W), and (3) Skeleton Creek (81.82645°N, -71.53738°W) At each site, four samples were collected; one for unfiltered Hg analysis, one for filtered Hg analysis, and two for particulate Hg analysis. Samples were collected from the cleaned edge of a snow pit using HNO3- washed stainless steel corer with 5 cm diameter and a stainless-steel spatula. Three cores were collected for each sample and placed into pre-cleaned 500 ml glass jars and stored in a cold dark freezer.

Samples were melted at room temperature in the dark for processing. Samples intended for filtered MeHg/THg analysis were processed using Nalgene MF75 series filter-unit equipped with a 0.45 μm cellulose nitrate membrane pre-cleaned with a 1% HCl solution. Once melted and
filtered, the snowmelt was transferred into 250 ml amber bottles, weighed, and acidified to 0.2% trace pure concentrated HCl. Samples intended for particulate MeHg analysis were weighed and then filtered through a Q-MA quartz filter (AQFA 47 mm, 2.5 μm nominal pore size) using an acid washed Teflon tower filter. Filters were pre-weighed before use and reweighed after the snowmelt sample was filtered to estimate particulate mass, and then stored in individual acid washed petri dishes and frozen until analysis.

To determine snow water equivalence (SWE), ten snow depth measurements were taken around each site and the mass of each core was recorded using a spring scale. SWE was calculated using equation 2 and areal loadings were calculated by multiplying SWE by snow MeHg concentrations.

\[
\text{SWE (kg/m}^2\text{)} = \frac{\text{snow core weight (kg)}}{\pi \text{[corer radius (m)]}^2}
\]  

[2]

2.3.2.5 Zooplankton Survey

Bulk zooplankton samples were collected to quantify MeHg at the first trophic level in the Skeleton Continuum. Samples were taken on May 30, 2017 during full ice cover and July 13, 17 and 21, 2016 during ice-off. During the ice-covered period, two holes were augured adjacent to each other in the center of Skeleton Lake. A 15 cm diameter zooplankton net with 80 μm netting was used to make ten ~2m vertical tows throughout the water column. The collected zooplankton samples were emptied into a whirlpack bag using a wash bottle filled with lake water. During the ice-free period samples were taken from the shoreline or from the center of the lake using horizontal 2 m tows.

2.3.2.6 Lab Analysis

Samples were analyzed for THg and MeHg at BASL, University of Alberta in 2016, and at Environment and Climate Change Canada’s Canadian Centre for Inland Waters (CCIW) and the University of Toronto Mississauga and Scarborough in 2017.

2.3.2.6.1 Liquid phase samples (water/snow)

Liquid phase samples were analyzed for THg through BrCl oxidation, SnCl₂ reduction, gold trap amalgamation and Cold Vapour Atomic Fluorescence Spectroscopy (CVAFS) detection following USEPA method 1631 (Bloom et al., 1983). 100 ml of each sample was poured into a
fluoropolymer bottle and 500 μl of BrCl was added. Samples were left to digest for 12 hours. 0.2ml of Hydroxylamine (NH$_2$OH) was then added to each sample and allowed to react for five minutes. A gold trap was added to each bubbler and samples were bubbled with an addition of 0.5 ml of SnCl$_2$ with N$_2$ flow (350 mL/min) for 20 minutes. Following this, traps were flushed with N$_2$ (30 mL/min) for two minutes to drive off condensed water. Hg on gold traps were then thermally desorbed onto the analytical trap and analyzed using a Tekran© 2600.

Liquid phase samples were analyzed for MeHg through distillation, aqueous ethylation and analysis using CVAFS following USEPA method 1630 (Bloom et al., 1989). 40 ml of each sample was deposited into an acid cleaned Teflon vial and each vial had 240 μl of 50:50 HCl:MilliQ + 9 ml concentrated KCl added. 20 μl of 1 ppm methylmercuric chloride (MeHgCl) was added to each sample as an internal standard to assess sample recovery. Distillation vials were then heated on a Tekran© 2750 Distillation Unit at 550°C for approximately two hours until 85% of the sample volume had been distilled into the receiving vessels. Prior to analysis, a calibration curve with a range of 0-20 pg MeHg was created using 1 ppm MeHgCl standard solution and was verified throughout the day using 1 ppm MeHgOH standard. 500 μl of acetate buffer, the sample, and 50 μl of sodium tetraethyl borate (NaTEB) were added to each analysis Teflon vial. 20 μl of 1 ppm MeHgCl standard solution was used to assess sample recovery (100.1%). Samples were then analyzed on a Tekran© 2700.

2.3.2.6.2 Solid phase samples (soil & zooplankton)

Prior to analysis all solid phase samples were freeze-dried for three days. Soil samples were homogenized using a stainless-steel grinder cleaned with MilliQ water before and between each sample to avoid any cross-contamination. Soil samples were analyzed for THg using a tri-cell Direct Mercury Analyzer 80 (DMA-80), through thermal decomposition, amalgamation, and release of Hg vapour which is measured by atomic absorption spectrometry. Instrument calibration was performed using High Purity Standards aqueous 1000 ug/mL Hg solution (2% HCl) to create ten calibration points, which ranged the expected raw Hg ranges of 0-5 ng. 50 mg of each soil sample was run in a pre-combusted nickel boat using the DMA-80 program method, which dried samples at 200 °C for one minute and decomposed the sample at 650 °C for two minutes (Milestone SRL, 2014). 50 mg of each soil sample was run after verifying that no mass dependency existed between accepted sample masses (p = 0.3-0.4) (see SI). Samples were run in
duplicates, with blanks, and MESS-3 standard reference material (0.45 mg) run every 12 samples. All instrument and boat blanks had an absorbance of <0.001 and the RSD for SRM’s was 0.71%.

Soil samples were analyzed for MeHg using gas chromatography–inductively coupled plasma mass spectrometry (GC-ICP-MS) (Hintelmann et al., 1995; Hintelmann et al., 1997). Before analysis, 0.3 g of each sample was distilled in a mixture of 500 μl 9M H₂SO₄, 200 μl 20% KCl and 1ml Cu(SO₄) using an acid cleaned Teflon distillation vials. A 400 μl spike of 1 ppm Me¹⁹⁹Hg (purity 91%) was added to each sample prior to distillation as a species-specific internal standard to account for procedural recoveries. Samples were distilled on a hot plate at 500 °C for approximately four hours until 80% of the sample volume was in the purging vial. Samples were then ethylated in glass bubblers with the addition of 125 μl of NaTEB and 50 μl of 1ppm Me¹⁹⁹Hg (purity 91%) as an internal standard. After 15 minutes of reaction time, samples were bubbled in a glass vessel using N₂ flow (350 mL/min) for 15 minutes. Following this, volatile species were then purged and trapped onto Tenax® using N₂ flow for seven minutes. Hg was thermally desorbed, transferred on Ar flow (30 mL/min) and trapped using a gas chromatography (GC) glass column packed with 15% OV-3 on Chromosorb® (60/80 Mesh) heated to ~100 ± 2°C and analyzed on an Agilent Technologies 7700 series ICP-MS. ²⁰²Hg was used as a surrogate for ambient MeHg; concentrations were calculated using measured isotope ratios with a precision of ± 1.4%.

Furthermore, zooplankton samples were analyzed for MeHg using the same process as liquid phase samples but were freeze-dried and extracted using HNO₃ digestion rather than distillation (Hintelmann et al., 2005). For this analysis, lobster hepatopancreas (TORT-5) was analyzed as a standard reference material.

2.3.2.6.3 Data Analysis

Standard statistical analyses were performed to determine if sets of samples were significantly different from one another. Each dataset was assessed using two-tailed, non-paired t-tests (ttest2) and two-way ANOVA (anova2) tests (p = 0.05) using MATLAB® R2016a. Each dataset was assessed to ensure that it met the assumptions of each test (normality, normal distribution of residuals, independence of residuals where applicable).
To determine how much Hg and MeHg are contributed to the Skeleton Continuum during snowmelt, the drainage basin for the sub-catchment was delineated using ArcMap 10.5™ hydrology tools. First, a digital elevation model (DEM) was obtained for Skeleton Lake and the surrounding area. The digital elevation model was processed using the ‘Fill’ tool to smooth over any imperfections in the DEM raster. The ‘Flow direction’ tool was used to determine which way water would flow from each cell, this new raster was then processed using the ‘Basin’ tool to delineate each basin based on water flow (Figure 2.6). The area of the basin was calculated by converting the basin into a polygon and using the geometry calculator (Ha).

Figure 2.6 Deliniation of Skeleton Continuum spring drainage catchment within the Lake Hazen watershed, Site names refer to sampling sites along the continuum.
Chapter 3
Spatiotemporal variation of summer Hg concentrations and cycling in the Skeleton Continuum

3 Spatiotemporal variation of summer Hg concentrations and cycling in the Skeleton Continuum

3.1 Spatial distribution and delivery of Hg within the Continuum

3.1.1.1 Skeleton Lake and Pond Water Hg

To address the spatial evolution of THg and MeHg as it is transported downstream, surface water was sampled along the Skeleton Continuum during the summer ice free period on July 9, 18, 23 and August 1 in 2015, and July 3, 9, 17, 25, 31 and August 6 in 2016. From upstream to downstream, samples were collected (when there was sufficient water flow), at (1) an active layer seep, (2) Skeleton Lake, (3) downstream of Pond 4, where Skeleton Creek enters the wetland complex, henceforth referred to as “post-ponds” (4) the outflow to Craig’s Wetland (unofficial name), and (5) the mouth of Skeleton Creek where it flows into Lake Hazen (Figure 2.2).

Overall, Skeleton Continuum surface water THg and MeHg concentrations fit well within other surface water measurements reported at temperate and Arctic latitudes. For example, Skeleton Lake water column and downstream pond waters had mean (± standard deviation) THg concentration of 0.76 (±0.14) ng L$^{-1}$ (Figure 3.1), similar to, but on the lower end of concentrations reported for other water bodies in the Canadian Arctic (0.5-3.2 ng L$^{-1}$, Chételat et al., 2015). These THg concentrations are relatively low compared to temperate latitudes, which is not surprising considering that Arctic regions have fewer point sources of Hg, leading to lower THg concentrations in Arctic freshwaters (Pacyna et al., 2012; AMAP, 2011).

MeHg concentrations in Skeleton Lake and the downstream ponds (Ponds 4 and 11) averaged 0.064 (±0.001) ng L$^{-1}$. These MeHg concentrations are similar to those measured in Canadian Arctic lakes (0.02-0.1 ng L$^{-1}$) but were interestingly on the low end of MeHg concentrations measured in other ponds in this region (Lehnherr et al., 2012a and b; St. Louis et al., 2005). However, the lower MeHg concentrations observed at these sites relative other ponds reflect the flow-through nature of these systems, where MeHg produced by in situ methylation can be
exported downstream, rather than being left to accumulate as is the case in closed-system ponds. Lower MeHg concentrations, relative to other ponds, may also be the consequence of shallow mean depth and lower DOC concentrations, both of which favour the photodemethylation of MeHg within the water column (Lehnerr et al., 2012a; Lehnerr and St. Louis, 2009; Krabbenhoft et al., 2002). In previous studies, photodemethylation has been identified as a major sink of water column MeHg (Girard et al., 2016; Lehnerr et al., 2012a; Sellers et al, 2001). The water bodies sampled in this study had relatively low DOC concentrations (5-6 ng L\(^{-1}\)) compared to other ponds/small lakes in the Lake Hazen watershed (7.9-40.6 ng L\(^{-1}\), Lehnerr et al., 2012a), facilitating higher photodemethylation rates. Both THg and MeHg concentrations in Skeleton Lake and the downstream ponds are consistent with measurements going back a decade or more at these same site (Lehnerr et al. 2012a), suggesting that while climate change has had dramatic impacts on the Lake Hazen watershed (Lehnerr et al. 2018), it has not yet impacted open-water THg and MeHg concentrations of small upland lakes and ponds.
Figure 3.1 Depth integrated averages of (a) THg (ng L$^{-1}$), (b) MeHg (ng L$^{-1}$) and (c) % MeHg in Skeleton Lake, Pond 11 and Pond 4 water column, Summer 2016

MeHg concentrations in the Skeleton Continuum as a whole showed similar patterns to those reported for temperate water MeHg concentrations by Selvendiran et al. (2008), which ranged from 0.02-0.04 ng L$^{-1}$ in upland waters, and from 0.1-0.27 ng L$^{-1}$ in wetland waters. Multiple other studies have shown similar wetland water MeHg concentrations ranging from 0.1-0.6 ng L$^{-1}$, highlighting the important role that wetlands and wetland ponds play in the production of
MeHg (Branfireun et al., 1996; Babiarz et al., 1998; Lee et al., 1998, 2000; Waldron et al., 2000).

Spatial patterns of THg and MeHg concentrations in Skeleton Continuum waters were generally the same between 2015 and 2016 (p>0.2) (Figure 3.2), implying that the observed trends are relatively consistent from year to year, and representative of the system. Within seasons THg and MeHg generally follow the same trends, with some variation observed at the Post-pond and Post-wetland sites (see Appendix D-3). Concentrations of THg in the Skeleton Continuum ranged from 0.55-1.12ng L$^{-1}$ and typically increased moving downstream from the Seep (site 1) to the Skeleton Creek mouth (site 5) (Figure 3.2). THg nearly doubles over the course of the catchment, suggesting that the organic matter rich soils in the wetland and wetland stream are acting as an in-catchment source of THg. This is further supported by high THg levels measured in pore water in wetland soils near Skeleton Lake (1.45 ng L$^{-1}$), suggesting that soil Hg leached into solution acts as a source to surface water flow. THg in surface waters was mostly in the dissolved phase (76-96%), and thus more bioavailable for microbial methylation than in other systems where most of the THg is bound to particulate matter (e.g., glacier-fed rivers in the Lake Hazen watershed, St. Pierre et al., 2019).

MeHg concentrations in the surface water were low in the active layer seep (0.01-0.02 ng L$^{-1}$) and increased substantially moving downstream through Skeleton Lake and the downstream ponds, peaking at the post-pond site (site 3; 0.25-0.35 ng L$^{-1}$) (Figure 3.2). MeHg concentrations then decreased between the post-pond site and the wetland outflow, back to the levels present in seep water entering the continuum. The observed increase in MeHg concentrations and %MeHg (the fraction of THg occurring in the MeHg form) in surface waters in the upstream portion of the continuum (i.e., between the active layer seep waters and lake and pond waters) demonstrates the occurrence of in-situ Hg microbial methylation and net production of MeHg, likely due to favourable biogeochemical conditions for methylation within Skeleton Lake, the downstream ponds, and potentially in saturated wetland soil pore water. %MeHg is often used as a proxy for methylation activity (Mitchell et al., 2008) and %MeHg values in Skeleton Lake and the downstream ponds (Figure 3.1) were typically higher than in the seep and in Skeleton Creek waters flowing into Lake Hazen (Figure S8). However, compared to other wetland ponds on Ellesmere Island and across the Canadian Arctic, the %MeHg in the water column of Skeleton Lake is relatively low (Lehn herr et al., 2012a, MacMillan et al., 2015; St. Louis et al., 2005).
Additionally, THg and MeHg concentrations and composition in Skelton Lake, Pond 4 and Pond 11 were not significantly different (p>0.1) and were consistent throughout the summer season (p>0.5). This implies that though the lake and the ponds do produce MeHg, buildup of MeHg in the water column is limited partially by photodemethylation and by downstream export.

The spatial survey results demonstrate that the wetland complex acts as a MeHg sink and plays an important role in regulating the amount of MeHg delivered downstream potentially through particle settling, sorption onto soil and/or demethylation within the soils. These trends highlight the importance of understanding MeHg production, degradation, storage and transport in the Continuum rather than in individual compartments as the ponds and wetland clearly tell contrasting stories that would not be captured by studying these compartments separately.

Though THg and MeHg concentrations followed the same general trends in 2015 and 2016, there were some notable differences between years, particularly with how Craig’s Wetland functioned as a MeHg source/sink. The summer season was wetter in 2015 than in 2016, with higher flow velocity and discharge through the continuum, resulting in a higher water table in Craig’s Wetland. Increased soil saturation in the wetland is known to enhance anaerobic microbial activity (Emmerton et al., 2012), and thus Hg methylation. This likely explains the higher MeHg concentrations measured at the wetland outflow (site 4) in 2015 (Figure 3.2). Additionally, higher water flow through the wetland decreased the interaction time between MeHg and soils, reducing the potential for MeHg to sorb to soil organic matter and/or to be demethylated. Year-to-year difference in hydrology and flow conditions also had an important impact on the partitioning of THg and MeHg between dissolved and particulate phases (Figure 3.2). For example, in 2015, MeHg became increasingly bound to particulate matter in going from the seep (site 1) to the wetland outflow (site 4). Contrastingly, in 2016, MeHg in the active layer seep and at the post-pond site was at times primarily particulate-bound, but by the time surface waters exited the wetland, virtually all of the MeHg was in the dissolved phase. Therefore, the wetland acted as a sink for particulate matter, effectively filtering out particulate-bound MeHg and preventing it from being transported further downstream and entering the Lake Hazen ecosystem and food.
Figure 3.2 (a, c) THg (ng L\(^{-1}\)) and (c,d) MeHg concentrations (ng L\(^{-1}\)) in Skeleton Continuum surface water in (a-b) 2015 (a-b) and 2016 (c-d), measured in surface waters at the (1) active layer seep, (2) Skeleton Lake, (3) Post-ponds, (4) Wetland Outflow and (5) Skeleton Creek.

In contrast to a number of previous studies in temperate and boreal wetlands (Branfireun et al., 1996; Babiarz et al., 1998; Lee et al., 1998, 2000; Waldron et al., 2000), the wetland complex in the Skeleton Lake Continuum does not appear to export large quantities of MeHg despite high MeHg concentrations occurring in surface waters at some sites along the Skeleton Continuum. This indicates that some Arctic wetlands may play a key role in capturing and breaking down MeHg, rather than production and export as seen in wetlands at lower latitudes.

Despite the potential for downstream transport of MeHg from Skeleton Lake and the ponds, MeHg concentrations in Skeleton Creek waters flowing into Lake Hazen (0.028 ±0.01ng L\(^{-1}\))
were not much more elevated relative to the active layer seep waters (0.013 ±0.02 ng L⁻¹). The MeHg inputs from Skeleton Creek to Lake Hazen are therefore minimal compared to those measured in glacial rivers in the Lake Hazen watershed which have both higher mean MeHg concentrations (0.049 ±0.016 ng L⁻¹) and dramatically higher discharge (St. Pierre et al., In Press). This is contrary to most predictions that wetland outflow would be more elevated in MeHg relative to glacier-fed rivers and highlights the ability of the Skeleton Continuum wetland complex to mitigate the delivery of MeHg to downstream lakes and food webs. However, the differences observed between the summers of 2015 and 2016 also demonstrate the vulnerability of this MeHg sink to warmer and wetter conditions, which are predicted to become more prevalent as a result of climate change (ACIA, 2005).

To better understand the controls on MeHg concentrations in the Skeleton Continuum, a principal components analysis including major water chemistry parameters was carried out. The PCA separated sites along two components which represented a nutrient gradient (principal component 1) and parameters that are associated with bioavailable Hg (THg, THgdis, DOC) (principal component 2) (Figure 3.3). Each sampling site formed distinct clusters within the PCA, indicating that they were unique in their water chemistry. The seep had low concentrations for all parameters when compared to the other sites, even if throughout the summer season seep water became more enriched in nutrients. The sites with the highest MeHg concentrations and %MeHg both clustered around the (0,0) point, indicating that MeHg production may be driven by microbial activity fueled by nitrogen, phosphorus and DOM in the system, rather than being limited by the bioavailability of Hg (Figure 3.4). However, it is important to note that the concentrations of THg, nutrients and ions experienced in sites 2-5 were well within the range of biogeochemical parameters that would be conducive to the production of MeHg (Benoit et al, 2001). Though total phosphorus (TP) did not load as a distinguishing water chemistry variable in the PCA, it had a significant positive relationship with MeHg concentrations and follows identical spatial patterns suggesting that phosphorus limitation has implications for MeHg production and potentially controls microbial activity at this site (Sosa, 2017).
Figure 3.3 Principal Components Analysis of water chemistry parameters in Skeleton Continuum surface water identified by site. Samples collected throughout the Summer sampling campaigns from June-August 2015-16. Vectors represent Percent of Total Mercury Dissolved (THg_{dis}), Total Mercury (THg), Sulfate (SO4), Total Dissolved Solids (TDS), Chloride (Cl), Total Phosphorus (TP), Total Dissolved Organic Nitrogen (TDN) and Total Nitrogen (TN).
3.1.2 Sediments

Sediment cores were collected from random locations in the littoral zones of Skeleton Lake ($n=3$) and Pond 11 ($n=3$) in July of 2016. In the Skeleton Lake sediments, the depth-integrated (0-4 cm) average ($\pm$ standard deviation) concentrations were 22.26 ($\pm$17.2) ng g$^{-1}$ and 0.57 ($\pm$0.29) ng g$^{-1}$ for THg and MeHg respectively, yielding a %MeHg value of 3.6 ($\pm$0.3) %. In Pond 11 sediments, average values were 11.26 ($\pm$3.4) ng g$^{-1}$, 1.58 ($\pm$1.35) ng g$^{-1}$, and 13.2 ($\pm$9.1) % for THg, MeHg and %MeHg, respectively (Figure 3.5).
THg concentrations in Skeleton Lake and Pond 11 sediments are relatively low when compared to THg concentrations reported in some Arctic sediments (4-113 ng g\(^{-1}\); Lehnherr et al., 2012b) and average THg concentrations in temperate/boreal sediments (76-330 ng g\(^{-1}\), CMSAP, 2016; Hammerschmidt et al., 2006; Fitzgerald et al., 2005; Gilmour and Riedel, 1995). The Skeleton Continuum has a small catchment size, resulting in relatively smaller THg catchment inputs to
the lake and sediments (Chetelat et al., 2008). Despite relatively low sediment THg concentrations, MeHg concentrations were the higher end of ranges reported for lacustrine sediments in Arctic (0.1-4.6 ng g$^{-1}$ Lehnherr et al., 2012b; 0.13 ng g$^{-1}$, Chetelat et al., 2008) and boreal/temperate (0.14-1 ng g$^{-1}$, Bertillon et al., 2017; 0.2-2.9 Bravo et al., 2017, 0.01-5 ng g$^{-1}$, Gilmour et al., 1998) regions. High MeHg concentrations in Skeleton Lake and pond sediments are likely a result of in situ Hg methylation, which is a factor of Hg availability and other in-pond factors, supported by measured potential methylation rates (see below paragraphs) and mass balance observations made in other ponds on the landscape (Lehnherr et al., 2012b).

%MeHg values measured in Skeleton Lake sediments are on the low end of the range compared for other small freshwater ecosystems on Ellesmere Island (0.4-18.5%, Lehnherr et al., 2012), while Pond 11 sediments are on the higher end of this range, indicating that these net MeHg production is taking place within these sediments, and that they are potentially a source of MeHg to overlying waters as seen in other lakes (Lehnherr et al., 2012b; Drott et al., 2008; Hammerschmidt et al., 2006; Gilmour et al., 1998). In comparison to other studies at temperate and boreal latitudes, Skeleton Lake and Pond 11 %MeHg values are relatively high (0.5-3% Hintelmann et al.; 2010, Watras et al., 2006; Ulrich et al., 2001). Higher MeHg concentrations and %MeHg in Pond 11 (p<0.05) indicate that the biogeochemical conditions within the sediments were more conducive to Hg methylation. Pond 11 is shallow, has a higher water temperature, DOC and Chl-a concentrations (and thus productivity), which likely explains higher MeHg concentrations (Lehnherr et al., 2012b).

%MeHg in Pond 11 varied significantly throughout the different depth horizons (p = 0.05), and higher values were observed in the 0-2 cm horizon (Figure 3.6). This suggests enhanced methylation near the sediment-water interface, likely coinciding with the oxic-anoxic boundary and the presence of fresh organic matter, stimulating microbial metabolism (Lehnherr et al. 2012b). Contrasting this, MeHg concentrations did not vary significantly by depth in Skeleton Lake, which could be explained by the fact that the sediments near the sediment-water interface are very loosely compacted with high water content, allowing porewater exchange of MeHg. Overall, these observations support the idea that MeHg is produced within lake and pond sediments and is the main source of MeHg to the water column above.
Incubation experiments were performed on the same sediment cores from Skeleton Lake and Pond 11 to obtain potential methylation ($k_m$, % d$^{-1}$) rates for sediments. Mean methylation rates were 4.01 (±3.35) % d$^{-1}$ and 6.63 (±1.73) % d$^{-1}$ in Skeleton Lake and Pond 11, respectively. $k_m$ did not vary significantly by site (p=0.12) or depth (0-2 cm and 2-4 cm) (p=0.85) (Figure 3.7). $k_m$ values reported in this study are similar to previous measurements made in sediments of ponds within the Lake Hazen watershed (2-15 % d$^{-1}$) (Lehnherr et al., 2012b). These methylation rates are similar, but on the higher end of those found in arctic sediments (2-8 % d$^{-1}$, Hammerschmidt et al., 2006) and boreal/temperate sediments (0-12 % d$^{-1}$, Gilmour et al., 1998)). It is important to note, that though rates from other studies are expressed in the same units they must be interpreted with caution as methodological differences in isotope injection, spike equilibration and incubation duration may have an impact on rates and could account for some of the variability detected across studies.
Figure 3.7 Average methylation potentials ($k_m$, % d$^{-1}$) by depth (cm) measured in sediments of in Skeleton Lake and Pond 11, Summer 2016

High methylation potentials in these sediments may be related to the quantity and quality of DOC available, which act as an electron donor to anaerobic bacteria found in the sediments, stimulating microbial metabolism and hence methylation. In previous studies in the Lake Hazen area, sediment OM did not correlate with $k_m$, but it was suggested that DOC quality may be a better predictor of methylation potential (Lehnherr et al., 2012b). The DOC in Skeleton Lake water was particularly labile with relatively high SUV-A (4.6) (Aukes et al., 2016, personal comm.), an indicator of carbon aromaticity which has been connected to higher rates of MeHg production (Graham et al., 2012). Additionally, the DOM within Skeleton Lake comes mostly from autochthonous plankton sources, which have been associated with elevated %MeHg in lake sediments (Bravo et al., 2017). Though DOM quality was not assessed in Pond 11, this could potentially account for the slight difference in $k_m$ between the two sites.

There was no clear relationship between sediment methylation and water $SO_4^{2-}$ (the electron acceptor for Hg methylating sulfate reducing bacteria), despite many studies suggesting that sulfate plays an important role in controlling Hg methylation. The lack of relationship between $SO_4^{2-}$ and Hg methylation observed here is likely due to an absence of large differences in sulfate concentrations across sites and the fact that $SO_4^{2-}$ concentrations were in the optimal range for methylation, as previously suggested (Lehnherr et al. (2012). A recent study of
Skeleton Lake sediments identified that 14 of the Operational Taxonomic Units (OTU’s) from the microbial communities mapped to mercury methylation in a functional database (Ruuskanen et al., 2018). These OTU’s were genetically similar to *Methanosphaerula palustris*, a methanogenic species that possesses the *hgcAB* gene known to be responsible for Hg methylation (Gilmour et al., 2013). Neither Sulfate-reducing bacteria or Iron reducing δ-proteobacteria, which are both dominant methylators (CMSAR, 2016), were not dominant in Skeleton Lake and Pond 11 sediments implying that other potential microorganisms, such as methanogenic archaea, could be responsible for Hg methylation.

### 3.2 Craig’s Wetland

#### 3.2.1 Wetland Soil Survey

Wetland soil samples were collected in July 2016 at 4 transects (*n*=5-7 per transect) running perpendicular to the direction of water flow to include both saturated and unsaturated soils within each transect. The four transects were located along the Skeleton continuum, at (1) the post-pond site just downstream of the outflow of Pond 11 (i.e., wetland inflow), (2) 350m downstream of the wetland inflow, (3) in the middle of a large sedge meadow known as Craig’s wetland (wetland mid-point, 2 km downstream) and (4) across the mouth of Skeleton Creek where it flows into Lake Hazen’s inflow (2.8 km downstream) (Figure 2.2). Loss on ignition (LOI, %) measured in these soils showed that percent organic matter ranged from 5.3-64.5%, with an average of 22.7% (Table 3.1).

**Table 3.1** THg (ng g⁻¹), MeHg (ng g⁻¹), %MeHg and LOI (%) in wetland soils measured at the (1) outflow of Pond 11, (2) 350 m’s downstream of Pond 11, (3) wetland mid-point and (4) wetland outflow.

<table>
<thead>
<tr>
<th></th>
<th>THg (ng g⁻¹)</th>
<th>MeHg (ng g⁻¹)</th>
<th>%MeHg</th>
<th>LOI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43.21</td>
<td>1.23</td>
<td>2.58</td>
<td>35.11</td>
</tr>
<tr>
<td>2</td>
<td>40.33</td>
<td>1.26</td>
<td>3.54</td>
<td>26.57</td>
</tr>
<tr>
<td>3</td>
<td>32.37</td>
<td>0.98</td>
<td>2.89</td>
<td>19.26</td>
</tr>
<tr>
<td>4</td>
<td>40.29</td>
<td>0.58</td>
<td>1.44</td>
<td>11.26</td>
</tr>
</tbody>
</table>

Soil THg concentrations measured in this study (20.52-50.10 ng g⁻¹ dw) were similar to that found in other High Arctic wetland soils (̅= 46 ng g⁻¹ (10-250), Loseto et al., 2004), but lower than majority of concentrations observed at temperate latitudes (65.8-186.6 ng g⁻¹, Liu et al.,
2003; 193-287 ng g⁻¹, Warner et al., 2003; 16-347 ng g⁻¹, Selvindiran et al., 2008; 113-287 ng g⁻¹, Tjerngren et al., 2012). In contrast, MeHg concentrations (0.16-2.69 ng g⁻¹ dw) were on the high end of MeHg ranges previously reported for in arctic soils (0.065-0.32 ng g⁻¹, Losetto et al., 2004; 0.43-1.66 ng g⁻¹, Oiffer and Sicilliano, 2009), and on par with wetland soil concentrations measured in at boreal and temperate latitudes (0.16-1.19 ng g⁻¹, Liu et al., 2003; 0.25-0.64 ng g⁻¹, Warner et al., 2003; 0.1-13.4 ng g⁻¹, Skyllberg et al., 2003; 0.1-7.8 ng g⁻¹, Selvindiran et al., 2008; 3.5-21 ng g⁻¹, Tjerngren et al., 2012). Relatively high soil MeHg concentrations resulted in %MeHg values (0.75-9.41%) that were on the higher end for Arctic soils, and similar to those at temperate latitudes, indicating that wetland soils at this site represent a significant pool of MeHg and could act as a source to the downstream system if mobilized under the right conditions.

To examine spatial variation of Hg in wetland soils, the means of each transect were compared for THg concentrations, MeHg concentrations, %MeHg and LOI (Table 3.1), which revealed that there was no significant difference between transects moving downstream (p>0.1), which is in stark contrast to the spatial variability in surface water MeHg through the continuum. Though differences were not significant, it is important to note that MeHg concentrations were highest at the wetland inflow and just downstream of the inflow. %LOI was highest at the wetland inflow and decreased in downstream soil samples. Surprisingly, saturated and unsaturated soils did not vary significantly in their THg concentrations, MeHg concentrations, %MeHg and LOI (p >0.2) (Table S4). Though there was no significant difference, MeHg and %MeHg were higher in the saturated soils.

The lack of variability in THg concentrations and MeHg concentrations in soils and declines in surface water MeHg concentrations as it flows downstream is in contrast to other studies that have reported spatial variation of MeHg in wetlands (Loseto et al., 2004; St. Louis et al., 1996). However, these studies looked solely at wetland surface waters rather than soils. A lack of spatial variability in Hg was also observed in wetland soils in Nunavut (Oiffer and Sicilliano, 2009). This could suggest that the THg being delivered to the system includes both upstream inputs and snowmelt sources, giving soils along the transect gradient equal opportunity to capture Hg. It should be noted that these data represent depth integrated samples, where the organic layer and mineral layer of the soil profile were combined for analysis, therefore potentially explaining the absence of observed spatial differences in Hg or LOI. In previous studies it has been identified that MeHg concentrations are highest just under the water table, decreasing rapidly with depth,
and that the organic layer of the soil carries most of the Hg (Branfireun et al., 2002). However, Hg concentrations were measured at discrete depths in a subset of cores (see below) and additional samples have been collected for analysis in 2018 to examine Hg variability in different horizons of the Skeleton Continuum wetland soils.

Hg concentrations were also measured at discrete depths (0-12cm; 2cm intervals) in a smaller number of cores from two sites (n=3 per site). THg concentrations varied significantly when analyzed by depth and site. Mean THg concentrations average values were significantly different between transects (p<0.001), with concentrations of 39.11 ng g⁻¹ and 51.33 ng g⁻¹ for the wetland inflow and mid-point, respectively. MeHg concentrations did not vary significantly by site (x̄ = 1.73 ng g⁻¹, range = 0.11-6.71 ng g⁻¹), but each site had distinct MeHg concentrations when examined by depth. MeHg concentrations at the wetland inflow were significantly higher at 0-4 cm (4.16 ng g⁻¹) (p<0.001) compared to the lower depths (0.45-1.51 ng g⁻¹) (Figure 3.8 a-c). MeHg concentrations at wetland transect 3 were not significantly different by depth.
Figure 3.8 (a) THg concentrations (ng g⁻¹), (b) MeHg concentrations (ng g⁻¹) and (c) %MeHg in Skeleton Continuum wetland soils by transect and depth (cm), Summer 2016.

Higher MeHg concentrations and LOI at the wetland inflow, and a more variable distribution of MeHg within the soil when compared to the wetland mid-point implies that different processes are determining the distribution of Hg in the soil at each site. The mismatch between THg and MeHg trends at the wetland inflow suggests that MeHg is being produced at the surface of the
wetland. The ponds were not hydrologically connected to the wetland until July 25th, and the largest peak in post-pond MeHg concentration is detected on July 17th. This suggests that there may have been flooding and pooling near the end of the pond, turning the wetland inflow soils into a newly submerged extension of the pond which could have stimulated MeHg production in the soil pore water. It appears a similar phenomenon occurred on July 31, where a peak in delivery of total suspended solids (TSS), particulate carbon (PC) and TP stimulated microbial activity resulting in %MeHg approaching 50% in surface waters at this site. Contrasting this, the downstream wetland mid-point site had lower and more evenly distributed MeHg concentrations, reflecting the lower soil saturation and suggesting that MeHg production there may be limited in summers that are not particularly wet.

Both MeHg concentrations and %MeHg exhibited a significant positive relationship with LOI. 88% of the variability within MeHg concentrations in wetland soils can be explained by LOI (p<0.001), while 63% of the variability of %MeHg can be explained by LOI (p<0.001) (Figure 3.9). LOI is not significantly related to THg in soils, and interestingly THg concentrations were not correlated with MeHg concentrations (p>0.05). This suggests that THg was not a limiting factor for MeHg production within the soils, and organic matter (OM) played a key role in the production and retention of MeHg within these soils. The contrasting relationship of THg and MeHg concentrations with LOI suggests that soil OM content can be used as a predictor of MeHg production, a trend that was also observed in temperate wetlands (Drott et al. 2007, 2008a; Windham-Myers et al. 2009; Lambertsson and Nilsson 2006).

![Figure 3.9 Wetland soil (a) MeHg concentrations (ng g⁻¹) and (b) %MeHg in relation to LOI (%), Summer 2016](image)

\[ y = 0.0398x + 0.1065, \quad R^2 = 0.776 \]
\[ y = 0.0965x + 0.447, \quad R^2 = 0.6507 \]
3.2.2 Wetland Soil Methylation Assays

Methylation ($k_m$) and demethylation ($k_d$) potential were measured in wetland soil cores ($n=2/site$) from wetland transect 1 (wetland inflow) and at wetland transect 3 (wetland mid-point). $k_m$ ranged from 0.16-4.98 % d$^{-1}$, with a mean value of 1.45 % d$^{-1}$. $k_d$ ranged from 0-10.84 d$^{-1}$, with a mean of 3.45 d$^{-1}$. When comparing $k_m$ values there was no significant variation in potential rates between depth or site (Figure 3.10a) ($p=0.43, 0.94$). When comparing $k_d$ values there was no significant differences between sites ($p=0.78$), but suggestions of differences with depth ($p=0.056$) (Figure 3.10b) with $k_d$ higher at 10-12 cm ($k_d=8.16$ d$^{-1}$) than at 0-8cm ($k_d=1.22-4.24$ d$^{-1}$).

**Figure 3.10** (a) Hg methylation potential ($k_m$) and (b) MeHg demethylation potential ($k_d$) by transect and depth (cm) in Skeleton Continuum wetland soils. (n/a= data not available).

%MeHg is often used as proxy for methylation rates, but a comparison of $k_m$ and %MeHg in soils reveals that this relationship is complex and varies significantly with depth. In the top 6cm of the soil cores the relationship between km and %MeHg is not significant ($r^2=0.01$, $p=0.98$). In the bottom 6 cm of the soil cores, $k_m$ and %MeHg are strongly positively correlated ($r^2=0.80$, $p=0.001$) (Figure 3.11). $k_m$ values are significantly higher (1.8 % d$^{-1}$) at the lower depths (6-12 cm) compared to the top 6 cm (0.98 % d$^{-1}$) ($p=0.046$). However, the %MeHg in soils follows the opposite trend, where the top 6 cm are significantly higher (6.24%) than the bottom 6cm (2.18%) ($p=0.003$).
Figure 3.11 Variation in methylation potential ($k_m$, % d$^{-1}$) with %MeHg in wetland (a) near surface soils (0-6 cm) and (b) subsurface soils (6-12 cm). Compiled soil cores from the wetland inflow and wetland mid-point ($n=12$).

The variation in relationship between %MeHg and $k_m$ implies that there were two different drivers behind the MeHg concentrations in the soil. The top 6cm of wetland soil had interacted with surface waters, allowing for the input of MeHg from upstream sources (ponds, upstream wetland porewaters), which provided an additional source of MeHg in addition to in situ methylation. Lower $k_d$ values measured in the upper (0-6 cm) soils, relative to deeper soils, may provide an additional explanation for elevated %MeHg values in the soil horizons. The strong relationship between $k_m$ and %MeHg in 6-12 cm soils implies that methylation was the primary mechanism responsible for high MeHg concentrations in these horizons. This is consistent with the values of $k_m$ measured at different depths, as $k_m$ peaked between 4-8 cm in the wetland inflow and 8-12 cm at the wetland midpoint. The differences in depth of maximum MeHg production most likely represents a difference in biogeochemical hotspots based on the water table/saturation of the soil (Mitchell et al., 2008; Branfireun et al., 2002).

$k_d$ generally followed an increasing trend as you move deeper in the soil from 4-12 cm. This implies that the microbial communities found deeper in the soils were more favourable for demethylation. Demethylation was also present in the first two centimeters of the soil, but not detected in the 2-4 cm interval in all cores. Methylation to demethylation ratios were calculated.
to determine whether the wetland soils were acting as a site of net methylation or demethylation. This ratio was calculated using rate constants multiplied by ambient THg and MeHg concentrations for methylation and demethylation, respectively and assumes that the ambient fraction of bioavailable Hg and MeHg is the same. Average soil Hg methylation to MeHg demethylation had a ratio of 0.21, which suggests that wetlands were acting as a net sink for MeHg. However, it is clear that MeHg can be produced in the soils, which is consistent with observations from a previous arctic study (Loseto et al., 2004).

Though the wetland soils were likely a net sink of MeHg during the sampling period, as supported by the surface water data presented in the previous section, the wetland inflow represented a significant biogeochemical hotspot for Hg methylation in Skeleton Continuum. This is demonstrated by the fact aqueous MeHg concentrations on July 13 increased from 0.07 ng L$^{-1}$ in Pond 11 to 0.30 ng L$^{-1}$ at the post-pond site in a distance of less than 15 m. This increase indicates that there was a methylation hotspot occurring where the pond meets the wetland. Because the $k_m$ at the wetland inflow was not significantly different from the mid-point site despite higher MeHg concentrations being present there in both the soils and surface water, it suggests that MeHg is being produced elsewhere at this interface, for example in the more saturated soils that were too moist to core for incubation experiments. As suggested in previous literature, this may be due to the meeting of two different systems, where the pond is providing fresh Hg and other limiting reactants to the biogeochemically active anaerobic soils at the wetland inflow, creating a pulse of methylation, most likely in wetland pore waters rather than the wetland soils themselves (McClain et al., 2003; Mitchell et al., 2008).

The significant increase in the dissolved fraction of both THg and MeHg in the surface water when moving from the post-pond site to the post-wetland site implies a larger fraction of the Hg entering the wetland complex was particulate bound compared to the rest of the continuum, and that the wetland plays a significant part in filtering it out. As discussed in the previous section, the wetland plays a significant role in retaining particulate bound MeHg that enters the complex in surface waters, and this is most likely due to the high affinity of organic matter for MeHg (Heyes et al., 2000). It appears that the storage of MeHg in the soils may be largely driven by wetland hydrology, where in high flow years particulate bound Hg does not have the chance to be fully filtered out and instead are transported downstream into Skeleton Creek (as observed in 2015). This observation is congruent with what was reported by Branfireun et al. (2002) in a
temperate wetland. This hypothesis can be further supported by looking at the changes in total suspended solids (TSS) in surface water from the post-ponds to the post-wetland site. In 2016, there was a decrease in TSS from 13.6 to 0.64 mg L\(^{-1}\) which suggests that in the lower flow years the water likely had a longer period to interact with the wetland soils, allowing for particulate matter to settle and/or MeHg to bind to soils and potentially undergo demethylation within the soil profile.

It is important to note that though this balance of M:D is a significant contribution to the wetlands ability to act as a MeHg sink, it only captures a snapshot in time and may not be representative of MeHg cycling within the wetlands at other times throughout the year. Other studies have identified clear seasonal changes in the production of MeHg in soils, and the mid-summer snapshot presented in this study (July 16) is most likely representative of a system that has been depleted of most of the labile Hg, DOC and nutrients delivered during the spring melt, and therefore methylation may be limited (Oiffer and Sicilliano, 2009, Gilmour et al., 1998). Upstream waters supply some Hg to the wetland, but the post-pond water has 2.3 times the THg compared to the Pond 11 water, which suggests that the Hg in the wetland is either sourced from retention of Hg from snowmelt, atmospheric deposition, or Hg from previous years, rather than recent stream flow. Though hydrological linkage between Pond 11 and the wetland during the 2016 sampling campaign did not occur until July 15-17, there is no proof that it stimulated any additional MeHg production, as Hg concentrations at the post-pond site remained relatively similar from July 16-August 1\(^{st}\). The picture painted by the mid-summer soil incubations could be starkly contrasted by what occurs during the spring snowmelt period, where Hg would be more bioavailable, and periods of high flow might provide a net source of MeHg to Skeleton Creek.

The Skeleton Continuum wetland soils may not have been net producers of MeHg at the time or location where they were sampled, but MeHg and THg concentrations within these soils are not negligible in the overall Hg budget of the system and demonstrate that they play a role in storing THg and MeHg. The storage of THg in Arctic wetlands is important in the context of climate change based on the predictions that 1) permafrost will continue to thaw due to warming temperatures and earlier onset of snowmelt (Schuster et al., 2018) and that 2) precipitation will increase and become more variable (Wrona et al., 2016). With permafrost temperatures increasing (Walker et al., 2006), there will be release of legacy Hg and carbon entering the active
portion of the soil, contributing to the Hg pool available for methylation (Schuster et al., 2018; Klaminder et al., 2008; Schuur et al., 2009). Additionally, soils may be microbially active for a longer period of time due to earlier spring melt (Stern et al., 2012). However, based on the observations from this study an extended growing season may also result in a longer period that facilitates demethylation in the soil. However, if there is an increase in water flow through the wetland, or more intermittent, high flow events throughout the summer season, we may see that the ability of the wetland to filter out particulate bound THg and MeHg will be diminished, with methylation likely simultaneously stimulated. This is demonstrated by the higher MeHg in water and saturated soils at the post pond site, relative to the rest of the wetland, and the higher concentrations of MeHg in Skeleton Creek in 2015 during the higher flow year. Additionally, more intermittent but intense precipitation events could lead to prolonged periods of pore water interacting with soils, with Hg leaching into solution, followed by flushing from a precipitation event, as observed in other boreal ecosystems (Oswald et al., 2014).

In summary, MeHg concentrations in Skeleton Continuum soils are relatively high when compared to other Arctic and temperate studies, which highlights their important role in the Hg budget of this continuum. MeHg found in the soils had a strong relationship to soil organic matter, likely due to the ability of soils to bind MeHg, but also because soil OM likely stimulates MeHg production, which was shown to occur in these wetland sites. Differences of Hg distribution within the soil profile imply that there are distinct processes occurring at the wetland inflow and mid-point. The methylation incubations show that, in agreement with previous literature, Arctic soils have the ability to methylate Hg, but the less saturated soils sampled were sites of net demethylation in mid-summer. (M:D=0.21). Production and degradation of MeHg appears to be a function of soil saturation, which is a function of water table depth, exemplified by the fact that both processes have gradual peaks but occur at different depths in each transect.

Based on the surface water Hg survey a hotspot of Hg methylation was identified near the wetland inflow where soils were saturated and where surface water MeHg concentrations reached up to 0.35 ng L$^{-1}$. This suggests that the soils sampled to estimate methylation and demethylation within the wetland may represent less saturated soils that are less conducive to methylation, contrasting the surface water samples which were taken from areas with more saturated soils that were likely hotspots of Hg methylation. Despite the detection of MeHg production hotspots within the wetland, it was a net sink of MeHg during the summer sampling
period through its ability to filter, sorb and demethylate MeHg within the soils. In the future, altered hydrology due to climate change influenced precipitation regimes could result in increasing MeHg transport to downstream lakes and food webs.
Chapter 4
Contrasting seasonal variation in MeHg cycling in a High Arctic freshwater continuum

4 Contra{sting seasonal variation in MeHg cycling in a High Arctic freshwater continuum

4.1 Skeleton Lake

4.1.1 Water column mercury

Under ice THg and MeHg has not been frequently measured previously because of logistical reasons and because temperate and boreal lakes, where Hg cycling has been more thoroughly studied are not likely conducive to Hg methylation in the winter due to well oxygenated waters and cold temperatures limit anaerobic microbial metabolism (Lehnher et al., 2014). Therefore, peak Hg methylation in temperate and boreal lakes has typically been detected during summer months. Consequently, northern studies have also focused on the summer season, despite drastic differences in climate and water chemistry. Recently, relatively high MeHg concentrations and %MeHg have been observed under ice in shallow arctic lakes/ponds that form anoxic bottom waters in the spring (Pilote et al., 2017). High under-ice MeHg (0.47±0.23 ng L⁻¹) concentrations were also measured in the Skeleton Lake spring water column, which represents an increase of almost 10-fold relative to MeHg concentrations measured in the summer. Given the modest THg concentrations under the ice (1.06 ±0.24 ng L⁻¹), which were less than 2-fold greater than in the open water summer season, average spring %MeHg in the Skeleton Lake water column was very high (40.35±2.56 %), and much greater than in summer (13.5%). Spring MeHg concentrations in the Skeleton Lake water column varied significantly with depth (p=0.002), where concentrations in unfiltered waters at 2m (0.43 ± 0.01 ng L⁻¹) were lower than those at 4m (0.68± 0.22 ng L⁻¹).
Figure 4.1 Comparison of unfiltered Hg concentrations (ng L$^{-1}$) in the Skeleton Lake water column during the ice-covered spring season and the open water summer season. (a) THg, (b) MeHg and (c) %MeHg. There is no zero depth in the spring ice-covered season, because the upper 1.5 m of the water column is lake ice at this time.

Comparing the Skeleton Lake spring and summer water column, there were significant differences between THg concentrations (p<0.001), MeHg concentrations (p<0.001), %MeHg dissolved (p=0.007) and %MeHg (p<0.001) (Figure 4.1). In all cases, spring values were significantly higher than summer. For comparison, the THg reservoir in the portion of the water
column that does not freeze (1.5-4m) in Skeleton Lake was 15.87 mg in the spring versus 10.17 mg in the summer, a 1.5 times increase. MeHg storage in Skeleton Lake waters in the (7.49 mg) is almost 7.5 times higher than in the summer.

The relatively small change in THg concentrations between seasons, combined with a much greater increase in MeHg, and thus %MeHg, indicates that net production of MeHg is taking place during the winter ice-covered period. High MeHg could be due to methylation within the hypoxic water column, as has been observed in summer anoxic hypolimnetic temperate lake waters (Eckley et al., 2006), reduced demethylation rates during the ice-covered season, and/or significant sediment methylation and diffusion into overlying waters (Fitzgerald et al., 2006). However, methylation incubations carried it in the spring season show that there is no detectable methylation occurring within the water column (MDL= 0.022-0.046 d\(^{-1}\) and 0.064-0.132 d\(^{-1}\) for \(k_m\) and \(k_d\), respectively), and that some demethylation is occurring at 4m depth on one date (0.043 % d\(^{-1}\)). This suggests that water column methylation is not the driving factor for the high MeHg concentrations observed in the spring. These findings counter our original hypothesis that low dissolved oxygen (DO) concentrations (Table 4.1) and increased anaerobic microbial activity provide suitable conditions for water column methylation in Skeleton Lake during the ice-covered season. Water column methylation has been observed in other temperate lakes (Eckley et al., 2005), but could potentially be limited in Skeleton Lake during the winter due to cold temperatures (Lehnherr et al., 2014). It is also possible that though the bottom water conditions could be conducive to Hg methylation, the Hg that is available in the water column has become recalcitrant through extensive processing during the summer/fall or has bound to DOC in the water column (Table 4.1).

**Table 4.1** Skeleton Lake water chemistry parameters, Spring 2017 and Summer 2016. (n/a= data not available).
A second possible reason for the absence of Hg methylation in the water column, is that methylation was restricted to such a small depth interval of the water column that it was not detected by the incubation experiments, which were only performed at two depths (1.5 and 4 m). Between the May 20 and June 6 when incubations were performed, a true oxycline formed, and DO decreased from 3.29 to 0.98 mg L\(^{-1}\) in bottom waters. Water column methylation may have not been detected because there was no true oxycline on the first sampling day (May 22) and later on because Hg methylation occurs within 80cm of the oxycline (Eckley et al., 2006), which occurred at 3m, contrasting our samples which were collected at 4m. However, if this is the case water methylation would likely only be occurring for a short period between May 28-June 4 when the DO drops and anaerobic respiration increases (increase in pCH\(_4\)). Even if we apply water column methylation rates (1.7 % d\(^{-1}\), Eckley et. al, 2006) found in anoxic waters with similar physical parameters (THg concentrations, temperature, DOC, pH), the 6 day span where water may be close to anoxic (decrease from 1.71 to 0.92 mg L\(^{-1}\)) would only produce 0.13 ng L\(^{-1}\) of MeHg within the water column, compared to the 0.5 ng L\(^{-1}\) observed in the summer water column (Eckley et al., 2006). This suggests that even if a short period of water column methylation was missed by the incubation experiments, it could only account for a maximum of 1/5\(^{th}\) of the concentration detected in the water. These findings suggest diffusion from sediments, combined with the absence of photodemethylation, are the mechanisms responsible for high water MeHg concentrations under the ice.

In the summer, photodemethylation appeared to be a key factor in modulating the buildup of MeHg in Skeleton Lake whereas in the spring little photodemethylation would be permitted to occur as a result of ~1.5m of ice and additional snow cover limiting penetration of solar radiation, particularly in the UV range which is responsible for MeHg photodemethylation (Lehnherr and St. Louis 2009). This suggests that majority of MeHg that would be produced over the ice-covered period would remain within the system and be allowed to accumulate in the sediments and water column over the 8 ice-covered months.
In the spring, Skeleton Lake contained a large pool of MeHg, but there is a large reduction in water column MeHg concentrations moving from the ice-covered to the ice-free period. Assuming water MeHg concentrations are relatively similar between years, which our data supports, we see a reduction in MeHg of 0.49 ng L\(^{-1}\) over 27 days (June 6 – July 3, note that July 3 sample is from 2016 and June 6 sample is from 2017). MeHg photodemethylation was estimated in the Skeleton Lake water column to represent the 11 days between ice-off (June 22) and the first summer sample date (June 3) based on spring time MeHg concentrations (0.295 ng L\(^{-1}\)) and DOC concentrations (6 mg L\(^{-1}\)) taking into account dilution from ice-melt, light attenuation (0.51 m\(^{-1}\)) and cumulative daily PAR (57.3 mol/m\(^2\)), following the method of Lehnherr and St. Louis, 2009. Dilution of MeHg by ice-melt and photodemethylation in the water column over this time frame can explain, on its own, a reduction in MeHg concentrations from 0.5 ng L\(^{-1}\) to 0.21 ng L\(^{-1}\). This suggests that though large amounts of MeHg can accumulate in during the ice-covered period, a portion of MeHg produced in the spring may undergo photodemethylation before it can be transported downstream during the summer growing season. However, these calculations suggest that there would still be a portion of the MeHg pool that is not removed by photodemethylation (summer concentrations are 0.05 ng L\(^{-1}\) compared to estimated 0.21 ng L\(^{-1}\) left after photodemethylation), implying that there would still be approximately 0.16 ng L\(^{-1}\) available for uptake by zooplankton or for export downstream. These calculations also assume that there is little downstream transport during the snowmelt spring freshet, although partial ice cover at this time allows for snow meltwaters to enter the lake and flush lake waters downstream.

4.1.2 Seasonal Comparison of Hg in Skeleton Lake Sediments

In spring 2017 during the ice-covered period, sediment cores were taken from Skeleton Lake (n=3, do you have the depths they were collected from? This might be important because these cores were taken from deeper in the lake). Mean THg, MeHg and %MeHg in these sediments were 26.74 (±7.70) ng g\(^{-1}\), 0.07 (±0.05) ng g\(^{-1}\) and 0.26 (±0.17) %, respectively. THg concentration did not vary significantly between spring and summer (p=0.57), but both MeHg concentrations and %MeHg were significantly lower in spring (p<0.01, 0.05, respectively). In contrast to summer, both MeHg and %MeHg were significantly different in the different depth horizons sampled, with higher values in the 0-2 cm interval (p=0.04, 0.01) (Figure 4.2).
Figure 4.2 Seasonal comparison of (a) MeHg concentrations (ng g⁻¹) and (b) %MeHg in Skeleton Lake sediments by depth (cm), Spring 2017 and Summer 2016.

Potential methylation and demethylation rates in Skeleton Lake sediments averaged of 0.86 (±0.18) % d⁻¹ and 5.32 (±1.84) d⁻¹ for $k_m$ and $k_d$, respectively. Spring $k_m$ and $k_d$ did not vary significantly by depth (0-2cm and 2-4 cm) ($p=0.85, 0.52$), similar to the lack of a trend with depth observed in measurements carried out during the summer open water season $k_m$ was significantly lower in spring than in summer (0.86 % d⁻¹ vs. 4.01 % d⁻¹; $p = 0.04$) (Figure 4.3). Given that THg concentrations did not differ significantly between seasons, and that the measured $k_m$ was approximately 4x higher in summer, it is reasonable to expect that actual sediment methylation rates would also be ~4x greater during the open water season than under the ice. Lower MeHg production rates in the spring were most likely due to decreases in microbial metabolism due to the colder temperatures and the absence of fresh autochthonous organic matter inputs. Furthermore, though THg concentrations were similar in spring and summer, a lack of fresh Hg entering the lake in might have meant that a greater fraction of the THg was in a form that was not bioavailable for microbial methylation. These conditions indicate that at some period during the winter the sediments are producing MeHg at a much slower rate, and potentially acting as a site of net demethylation (M:D=0.61). This compares to a ratio of 1.11 measured in Skeleton Lake summer sediments in previous years when sediments were identified as methylation hotspots (Lehnherr et al., 2012a), highlighting the different processes occurring between seasons.

Previous work in Arctic ponds revealed that ponds with higher anaerobic microbial activity, inferred from $\text{NH}_4^+:\text{NO}_3^-$ ratios and dissolved CH$_4$ concentrations, had higher %MeHg in water column and $k_m$ in sediments (Lehnherr et al., 2012b). In Skeleton Lake, higher $\text{NH}_4^+:\text{NO}_3^-$ and CH$_4$ concentrations were measured in the spring which suggests that there was anaerobic activity...
occurring either in the water column or in the bottom sediments, which is not surprising given the really low DO concentrations measured under the ice.

![Figure 4.3 Comparison of spring (2017) and summer (2016) sediment Hg methylation potential ($k_m$, % d$^{-1}$) and MeHg demethylation potential ($k_d$, d$^{-1}$) in Skeleton Lake.](image)

The decrease of MeHg concentrations from 0.57 (±0.29) ng g$^{-1}$ in summer, to 0.08 ng g$^{-1}$ in the spring sediments could be explained by some demethylation in the sediments, however, the increase of MeHg in the lakes bottom waters suggest that there may also be significant diffusion of sediment MeHg to overlying waters (Morel et al., 1998). In the spring time the bottom waters of Skeleton Lake become more oxygen depleted over time due to the respiration of OM and lack of oxygen input from the atmosphere; in turn this alters the redox conditions overlying the sediments. This change creates similar physical conditions in the sediments and water, allowing the MeHg formed sediments and stored in sediment pore water to easily move into the water column (Sellers et al., 2001). This can be further supported by comparing $\%$MeHg$_{dis}$ between season, where the proportion increases from 50-70% in the summer to 92% in the spring. A strong association between water MeHg and $\%$MeHg$_{dis}$ has been found in sediments (Lehnherr et al., 2012b) and could be result in MeHg being diffused from the sediments.

Low DO detected in the spring water column is likely a result of extensive microbial OM remineralization during the ice-covered period, supported by enhanced DOC concentrations in spring time waters (Table 4.1). In shallow lakes the decomposition of OM occurs primarily in the
sediments and would occur closer to the beginning of the ice-covered season (fall) rather than when the sediments were sampled in the spring. OM decomposition has potential to stimulate Hg methylating microbial communities, suggesting though there is no water column methylation detected and little methylation occurring in the sediment spring, early ice cover may be a time of peak under-ice MeHg production in sediments.

Despite a lack of water column and sediment methylation detected in incubations, estimates of change in MeHg mass stored in Skeleton Lake water column between the end of the summer and the spring time reveals that there is a 7.5x increase in water column MeHg. Cryoconcentration of MeHg, which is the exclusion of MeHg from ice during the freezing process could provide an explanation for a portion of the MeHg detected in the spring time water column (Semkin et al., 2005). Based on changes in conservative tracers such as Cl and TDS, there is 60% exclusion occurring which suggests that there is still a 5 times, or 0.47 ng/L, increase in MeHg concentrations between the summer and the spring that has not been explained. This change can be applied to the period where Skeleton Lake is ice covered between September and June to obtain an estimated Hg methylation rate of 2.74 ng m\(^{-2}\) d\(^{-1}\). This estimate is fits well within rates reported in other ponds near Skeleton Lake (1.75-18.5 ng m\(^{-2}\) d\(^{-1}\), Lehnheer et al., 2012b), Alaskan tundra lakes (1.5-4.5 ng m\(^{-2}\) d\(^{-1}\); Hammerschmidt et al, 2006), and boreal lakes (5 ng m\(^{-2}\) d\(^{-1}\); Sellers et al., 2001), suggesting that the MeHg detected in the spring water column could be produced by slow rates of Hg methylation in the sediments and a lack of photodemethylation in the water column.

4.1.3 Seasonal comparison of Zooplankton Hg

Skeleton Lake Zooplankton had THg and MeHg concentrations of 145 and 98 ng g\(^{-1}\) dw, respectively for July 13\(^{th}\) and 93.5 and 70.5 ng g\(^{-1}\) dw, respectively for July 17 (Table 3). The THg concentrations in Skeleton Lake zooplankton are relatively high compared to other Arctic studies (65 ng g\(^{-1}\), Chetelat et al., 2017) and northern temperate lakes (<50 ng g\(^{-1}\), Clayden et al., 2013; Chetelat et al., 2012), but lower than those found at newly flooded wetlands (100-500 ng g\(^{-1}\), Hall et al., 2009; 87-500 ng g\(^{-1}\), Patterson et al., 1998). The decrease in zooplankton Hg between dates is likely due to biodilution as a result of the turnover of the zooplankton community. Though biomass was only analyzed for one day, observations from the field season noted a large and visible increase in zooplankton over this time. Earlier blooming zooplankton...
(July 13) had lower biomass allowing for the MeHg to be more concentrated, where later (July 17) the zooplankton were in higher abundance, partitioning Hg concentrations among more cellular mass, therefor decreasing zooplankton concentrations. Additionally, a shift in taxonomic composition could alter how MeHg is distributed within the same biomass (Chetelat and Amyot, 2009).

**Table 4.2 Skeleton Lake water column zooplankton, Summer 2016.** Measurements are displayed for zooplankton biomass (mg dw m\(^{-3}\)), THg concentrations (ng g\(^{-1}\) dw), MeHg concentrations (ng g\(^{-1}\) dw), %MeHg, and water volume normalized zooplankton THg (ng L\(^{-1}\)) and MeHg (ng L\(^{-1}\)), Bioaccumulation Factor and the mass ratio of water MeHg concentrations to DOC.

<table>
<thead>
<tr>
<th></th>
<th>Biomass (mg m(^{-3}))</th>
<th>THg (ng g(^{-1}))</th>
<th>MeHg (ng g(^{-1}))</th>
<th>%MeHg</th>
<th>THg (ng L(^{-1}))</th>
<th>MeHg (ng L(^{-1}))</th>
<th>Log(BAF)</th>
<th>MeHg:DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>July 13</strong></td>
<td>174.0</td>
<td>145</td>
<td>98</td>
<td>67.6</td>
<td>0.025</td>
<td>0.017</td>
<td>6.2</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>July 17</strong></td>
<td>174.0</td>
<td>93.5</td>
<td>70.5</td>
<td>75.4</td>
<td>0.016</td>
<td>0.012</td>
<td>6.0</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Log Bioaccumulation Factor (BAFs), calculated as the ratio of the MeHg concentration in zooplankton (in ng g\(^{-1}\) dry weight) to the MeHg concentration in water (ng mL\(^{-1}\)) averaged 6.12 (±0.12), which fits within ranges reported on the Lake Hazen landscape (Lenherr et al., 2012a). The BAF values reported here are on the higher end of ranges reported for other Arctic marine studies (4.4-4.8, St. Louis et al., 2007) and temperate studies (5.2-5.9; Patterson et al., 1998), suggesting that this ecosystem is particularly sensitive to MeHg bioaccumulation. The percentage of THg as MeHg in zooplankton was 68% and 75.5% for the 13\(^{th}\) and the 17\(^{th}\), respectively, which is much higher than typical for zooplankton (30-50%, Hintelmann et al., 2010), but is similar to previous measurements in other shallow water bodies in the Lake Hazen watershed (Lehnerr et al., 2012a). This demonstrates the efficient uptake and trophic transfer of MeHg in the Skeleton Lake food web. Efficient bioaccumulation of Hg is likely due in part to low DOC present in Arctic water bodies (including Skeleton Lake). In previous literature low water column DOC concentrations (< 9 mg L\(^{-1}\)) resulted in fewer Hg-DOC complexes, leaving more Hg in a bioavailable form and enhancing Hg uptake in low trophic level species (Chetelat et al. 2017; French et al., 2017).
Efficient bioaccumulation and biomagnification of MeHg in Skeleton Lake, combined with relatively high zooplankton biomass, resulted in a reservoir of THg and MeHg in zooplankton of 0.25 (±0.06) mg and 0.15 (±0.04) mg, respectively. This equates to 6.4% of the MeHg found in the Skeleton Lake water column, which is similar to findings in the adjacent ponds on the landscape (3-12%; Lehnherr et al., 2012a) and to those in flooded peatland reservoirs (7-8%; Patterson et al., 1998), which are known sites of MeHg production, but much higher than the ~1% of the Hg pool stored in zooplankton that is typical of most lakes (Watras et al., 1998; Monson and Brezonik, 1998). This indicates that MeHg produced during the summer is easily incorporated into the food chain and has potential to act as a source of MeHg biota on the landscape.

In the spring (May 28), Skeleton Lake zooplankton biomass was 92.89 mg m$^{-3}$, ~2x lower than what was measured the previous summer. Concentrations of THg and MeHg in the zooplankton were 52.5 (±9.2) ng g$^{-1}$ and 14.5 (±6.4) ng g$^{-1}$, ~1/2 and ~1/5 of those measured in the summer. This resulted in much lower concentrations of zooplankton-bound THg in the Skeleton Lake spring time water column (0.009 ±0.019 ng L$^{-1}$ and 0.005 ±0.001 ng L$^{-1}$ for summer and spring, respectively). Additionally, MeHg was significantly higher in the summer than the spring (0.011 ±0.002 ng L$^{-1}$ and 0.001 ±0.001 ng L$^{-1}$, respectively; p=0.01). Spring zooplankton had a BAF of 4.6 and %MeHg of 27.5, both much lower than those experienced in the summer (Table 4.3).

Lower BAF and %MeHg in the spring, despite the higher MeHg concentrations and %MeHg in the water column suggests that the zooplankton present are not efficiently taking up water column MeHg. This results in MeHg in springtime zooplankton only representing 0.88% of the MeHg in the water column, similar to other studies of lentic freshwater systems (Watras et al., 1998; Monson and Brezonik, 1998). Zooplankton samples measured in the spring could be representative of the adult, overwintering community which would not experience consume as much during their second year and therefore would have less MeHg (Maclaren et al., 1964).

Table 4.3 Seasonal comparison of Skeleton Lake water column zooplankton, Spring 2017 and Summer 2016. Measurements are displayed for zooplankton biomass (mg m$^{-3}$), THg concentrations (ng g$^{-1}$ dw), MeHg concentrations (ng g$^{-1}$), %MeHg, zooplankton concentrations
within the water column for THg (ng L\(^{-1}\)), MeHg (ng L\(^{-1}\)), natural logarithm of Bioaccumulation Factor and the ratio of water MeHg concentrations (ng L\(^{-1}\)) to DOC (ng L\(^{-1}\)).

<table>
<thead>
<tr>
<th></th>
<th>Biomass (mg m(^{-3}))</th>
<th>THg (ng g(^{-1}))</th>
<th>MeHg (ng g(^{-1}))</th>
<th>%MeHg</th>
<th>THg (ng L(^{-1}))</th>
<th>MeHg (ng L(^{-1}))</th>
<th>Log(BAF)</th>
<th>MeHg:DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring</strong></td>
<td>92.9</td>
<td>52.5</td>
<td>14.5</td>
<td>27.6</td>
<td>0.005</td>
<td>0.001</td>
<td>4.6</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Summer</strong></td>
<td>174</td>
<td>110.7</td>
<td>79.7</td>
<td>72.0</td>
<td>0.019</td>
<td>0.011</td>
<td>6.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Summertime MeHg concentrations in zooplankton are controlled by MeHg concentrations and DOC within the water column (Chetelat et al., 2017; French et al., 2014; Lehn herr et al., 2012b). In a study by Chetelat et al. (2017) higher water column MeHg:DOC resulted in elevated MeHg in zooplankton, because of increased MeHg bioavailability at lower DOC concentrations, however, in this study, springtime zooplankton followed opposite trends. Additionally, the results of French et al. (2014) suggest that at DOC concentrations below \(\sim 8.5\) mg L\(^{-1}\), increases in DOC result in higher zooplankton MeHg concentrations. However, no such increase was observed in spring zooplankton relative to summer zooplankton, despite spring DOC (8.2 mg L\(^{-1}\)) being higher than summer DOC (6 mg L\(^{-1}\)) and still below the threshold at which DOC starts to inhibit Hg bioaccumulation. These seasonal differences in zooplankton MeHg uptake could be explained by decreased feeding in overwintering zooplankton communities resulting in lower MeHg intake, but it is clear that springtime zooplankton MeHg concentrations are controlled by different factors than in the summer. This suggests that more research needs to be done to determine driving factors of Hg bioaccumulation during ice-covered periods.

### 4.2 Snow

#### 4.2.1 Snow Survey

Snow samples were collected at three sites along the continuum in May 2017, representing (1) the area before Skeleton Lake where the active layer seep flows in the summer, (2) on top of Skeleton Lake, and (3) after Skeleton Lake where Skeleton Creek flows in the summer. THg and MeHg loadings were lowest at Skeleton Lake when compared to the other over land sites (Figure...
~80% of THg and MeHg in snowpack was particulate bound, excluding MeHg at the Skeleton Lake site which was 60% particulate bound (Table 4.4). The lower concentration of particulate bound THg and MeHg at the Skeleton Lake site is most likely due to its position at the bottom of a valley, which would be provided with less windblown snow when compared to the other sites which were located in open areas.

Figure 4.4 (a) THg loadings (mg ha\(^{-1}\)), (b) MeHg loadings (mg ha\(^{-1}\)), and (c) %MeHg in Skeleton Continuum snowpack samples, Spring 2017. Samples were collected (1) on an elevated area near where the active layer seep flows in the summer, (2) on top of Skeleton Lake and (3) After Skeleton Lake where Skeleton Creek flows during the summer.
Table 4.4 Unfiltered (U) and filtered (F) THg concentrations (ng L$^{-1}$), MeHg concentrations (ng L$^{-1}$) and %MeHg in Skeleton Continuum snowpack, Spring 2017.

<table>
<thead>
<tr>
<th>Site</th>
<th>THg U (ng L$^{-1}$)</th>
<th>THg F (ng L$^{-1}$)</th>
<th>%THg Dissolved</th>
<th>MeHg U (ng L$^{-1}$)</th>
<th>MeHg F (ng L$^{-1}$)</th>
<th>%MeHg Dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Sk. Lake</td>
<td>8.52</td>
<td>0.79</td>
<td>9.25</td>
<td>0.65</td>
<td>0.13</td>
<td>20.50</td>
</tr>
<tr>
<td>Skeleton Lake</td>
<td>2.47</td>
<td>0.39</td>
<td>15.81</td>
<td>0.19</td>
<td>0.07</td>
<td>38.79</td>
</tr>
<tr>
<td>After Sk. Lake</td>
<td>6.30</td>
<td>0.74</td>
<td>11.74</td>
<td>0.67</td>
<td>0.20</td>
<td>29.35</td>
</tr>
</tbody>
</table>

THg in the Skeleton Continuum snow pack (2.47-8.52 ng L$^{-1}$) was consistent with snow that has been measured at other sites in the Lake Hazen watershed in 2015, which ranged from 3.03-6.54 ng L$^{-1}$ (St. Pierre et al., Unpublished), and at other inland sites in the Canadian High Arctic (0.38-5.34 ng L$^{-1}$, St. Louis et al., 2005; 0.9-8.9 ng L$^{-1}$, Loseto et al., 2004b), and in Alaska (1-11 ng L$^{-1}$, Douglas et al., 2017). Additionally, it is clear that snowpack in the Skeleton Continuum are not influenced by atmospheric mercury depletion events because measured THg snowpack concentrations are much lower than those observed at AMDE impacted coastal sites, which can exceed 50-100 ng L$^{-1}$ (St. Louis et al., 2007, Dommergue et al., 2010, Poulain et al., 2007).

Though concentrations are similar to those reported in other Arctic snow packs (0.9-8.9 ng L$^{-1}$, Loseto et al, 2004b; 0.5-1.4 ng L$^{-1}$, Douglas and Sturm, 2004; 1-11 ng L$^{-1}$, Douglas et al., 2017), the THg loadings are on the lower end due to the rather shallow nature of the snow pack compared to other Arctic sites (6.04 (±2.17) mg Ha$^{-1}$, this study; 19.00 (±2.17) mg Ha$^{-1}$, Douglas et al., 2017; 11.75 (±13.25) mg Ha$^{-1}$, St. Louis et al., 2005).

To determine the driving factors of THg concentrations in the snow pack, snow chemistry variables were run through a stepwise regression model (Figure S1-2). The regression model indicated that THg concentrations had a significant negative relationship with Total Dissolved Nitrogen (TDN) and a significant positive relationship with Total Phosphorus (TP) ($r^2$=0.93, p=1.8x10$^{-13}$). The positive relationship with TP, high percentage of particulate bound THg, and lack of relationship with TDP suggests that dry deposition of particulate-bound Hg may be the driving factor behind deposition of THg to the snowpack. Previous literature has described that high TP can be accounted for majorly by dry deposition, supporting this point (Pearson et al.,
2015). In addition, it has been reported that nitrogen species found in the snow have been positively associated with wet deposition, which would help explain the negative relationship between THg and TDN (Pearson et al., 2015; Clow et al., 2002; Williams and Melack, 1991a). Furthermore, it has been observed in previous years that the concentrations of MeHg measured in rainfall are lower than snow MeHg concentrations, suggesting that there may be additional sources of MeHg to the snow pack (Wong et al., unpublished).

Despite THg concentrations being on the lower end of observed ranges in Arctic studies, MeHg concentrations were at the higher end of ranges presented by other studies. At the Skeleton Continuum, snow MeHg concentrations averaged 0.5 ng L\(^{-1}\), with a loading of 0.52 mg Ha\(^{-1}\), where other measurements on Ellesmere Island ranged from 0.022-0.18 ng L\(^{-1}\) (St. Louis et al., 2005). Even when comparing to the High Arctic (<0.015-0.2 ng L\(^{-1}\), 0.016-0.085 mg Ha\(^{-1}\), St. Louis et al., 2005; 0.02-0.22 ng L\(^{-1}\), Dommergue et al., 2010) and sub-Arctic studies (0.02-0.1 ng L\(^{-1}\), Constant et al., 2007) these values are relatively high. The MeHg concentrations observed within the continuum are comparable to snow pack near the Athabasca Oil Sands (0.02-0.28 mg L\(^{-1}\), 1.46-20.2 mg Ha\(^{-1}\)) which has been identified as having elevated levels of MeHg (Willis et al., 2018).

To examine parameters associated with snow MeHg concentrations a stepwise regression model was run using snow chemistry parameters and THg concentrations (Figure S3). The model revealed that MeHg concentrations has a significant positive relationship with THg and SO\(_4^{2-}\) concentrations (\(r^2=0.39, p=0.0008\)). The association of MeHg concentrations with THg concentrations imply that MeHg is following a similar depositional pattern as THg, or that MeHg is being produced \textit{in situ} and was limited by the amount of bioavailable Hg (St. Louis et al., 2007). The significant relationship between MeHg and THg concentrations contrast previous High Arctic studies that found no relationship between the two parameters (Ferrari et al., 2002; Lahoutifard et al., 2005, Constant et al., 2007). Studies which did not find a relationship between the two Hg parameters concluded that the MeHg present in snow packs was most likely from deposition from marine aerosols, due to the significant positive relationship of MeHg concentrations with Cl\(^{-}\) and SO\(_4^{2-}\) (Constant et al., 2007; St, Louis et al., 2007). We measured mass SO\(_4^{2-}\):Cl\(^{-}\) ratios ranging from 4.25 to 33.56, which is well over the ratio found in marine waters (0.140) (Riley and Skirrow, 1975). The excess of SO\(_4^{2-}\) and lack of relationship between MeHg concentrations and Cl\(^{-}\) indicate that the source of MeHg within the Skeleton Continuum is
not from marine deposition of aerosols. However, the relationship between SO$_4^{2-}$ and MeHg concentrations may be indicative of methylation as a sulfur-bacteria link has been suggested as a potential pathway for snow methylation in Svalbard, Norway (Larose et al., 2010). The relationship between MeHg and SO$_4^{2-}$ could also be indicative of Hg deposition during ‘Arctic Haze’ conditions or production of MeHg on atmospheric aerosols. It has been observed that Arctic Haze is associated with the deposition of both Hg and anthropogenic SO$_4^{2-}$ onto the landscape (Barrie, 1986; Brock et al., 1990; Shaw, 1995; Douglas and Sturm, 2004. Additionally, elevated SO$_4^{2-}$:Na ratios have been a signature of natural and anthropogenic combustion aerosols, providing an alternative source for MeHg (MacInnis et al., 2018), however, MeHg was not quantified in these studies.

In previous years wet deposition of MeHg through rainfall was measured in the summer, wet deposition ranged from 0.029 to 0.051 ng MeHg m$^{-2}$ d$^{-1}$ and concentrations ranged from <0.015-0.248 ng L$^{-1}$ (Lehn herr et al., 2012a). Both concentrations and loadings from summer rainfall in previous years were lower than those measured within the snow pack in this study, suggesting that rainfall is most likely not the sole contributing factor to snow MeHg concentrations. It is important to note that rainfall measured in previous years was from the summer, and that there is potential that MeHg deposition during other periods are important sources of MeHg. For example, in the spring when snow fall occurs, and large amounts of particulate matter are deposited to the landscape (Douglas and Sturm, 2004).

4.2.2 Snow Methylation

The Skeleton Continuum snowpack had relatively higher MeHg concentrations in the Skeleton Continuum snowpack (St. Louis et al., 2005, Constant et al., 2007, Dommergue et al., 2010), which resulted in above average %MeHg (7.6-10.6%). This fits within observed %MeHg values on Ellesmere Island (1.1-48%, St. Louis et al., 2005; high values due to DMHg at coastal sites), in the general Arctic (0.1-18.2%, St. Louis et al., 2007; Dommergue et al., 2010) and is higher than what has been observed at boreal sites (0.7-6.3% Graydon et al.) and distal sites of oil sands impacted snow (~2%, Kirk et al., 2014; 2.1-3.8%, Willis et al., 2017). The relatively high concentrations of MeHg in the Skeleton Continuum snowpack contrast some previous research that has identified snow as being a relatively insignificant pool of MeHg (St. Louis et al, 2007), and may provide more information on the recent observations that snowmelt provides a pulse of
Potential mechanisms that have been postulated to account for elevated %MeHg in snow include dry deposition of MeHg produced by the photodegradation of atmospheric gaseous dimethylmercury (DMHg) (Baya et al. 2014; St. Pierre 2015), wet MeHg deposition through scavenging during precipitation (Grannas et al., 2007), and Hg methylation within the snowpack (St Louis et al., 2007; Constant et al., 2007; Dommergue et al., 2010). DMHg originates from marine sources (Kirk et al., 2012), however, based on the SO$_4^{2-}$/Cl$^-$ ratios measured in snow (see above) and the distance from marine water (~80km), it is unlikely that this is the source of MeHg to the snowpack in the Skeleton Continuum. This leaves wet deposition and in situ methylation as proposed explanations for observed high MeHg concentrations and %MeHg in the snowpack.

To further investigate the possibility of Hg methylation occurring in the snow or in snow melt, stable Hg isotope incubations were performed in snow cores and in melting snow to estimate potential methylation ($k_m$) and demethylation rates ($k_d$). In the snow pack incubations $k_m$ values were low, measuring 0.0143 d$^{-1}$ at Skeleton Creek and below the detection limit at Skeleton Lake. (MDL= 0.022-0.046 d$^{-1}$), while $k_d$ values were 0.252 and 1.14 d$^{-1}$ for Skeleton Lake and Skeleton Creek area snow, respectively (Table 4.4). Estimated methylation/demethylation ratios were 0.03 and 0.12 for Skeleton Lake snow and Skeleton Creek snow, respectively. Snow stable Hg isotope experiments have been used in one additional paper to examine potential methylation and demethylation rates in snow pack near the Alberta Oilsands. Willis et al. (2017) found that there was methylation occurring in the snow pack, with methylation/demethylation ratios which ranged from 0.3-1 which suggested a balance of methylation/demethylation processes in the snow pack. This contrasts the present study where both sites are dominated by net demethylation, suggesting that in situ methylation is a very limited source of MeHg within the snow pack in the Skeleton Continuum.

Snow samples were collected in jars, injected with isotope solution and incubated in below 0°C temperatures to examine the possibility that MeHg is produced during the melting process, as suggested by observations that MeHg concentrations have been found to be higher in snow melt than in snow pack (St. Louis et al., 2005; Douglas et al., 2017). In snow melt, $k_m$ was below the detection limit at both sites. $k_d$ was below the detection limit in Skeleton Lake snowmelt, but 0.538 d$^{-1}$ in Skeleton Creek snow melt (Table 4.4). Willis et al. (2017) found low rates of methylation and no demethylation in snowmelt in snow melt, however our measurements were
performed during the process of snow melt, which would have different redox and physical conditions.

Table 4.5 Methylation ($k_m$, %d$^{-1}$) and demethylation ($k_d$, d$^{-1}$) constants in Skeleton Continuum snowpack and snowmelt, 2017

<table>
<thead>
<tr>
<th>Site</th>
<th>$k_m$ (%d$^{-1}$)</th>
<th>$k_d$ (d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeleton Lake Snowpack</td>
<td>&lt;MDL</td>
<td>0.0019</td>
</tr>
<tr>
<td>Skeleton Creek Snowpack</td>
<td>0.0143</td>
<td>1.144</td>
</tr>
<tr>
<td>Skeleton Lake Snowmelt</td>
<td>&lt;MDL</td>
<td>&lt;MDL</td>
</tr>
<tr>
<td>Skeleton Creek Snowmelt</td>
<td>0.538</td>
<td>&lt;MDL</td>
</tr>
</tbody>
</table>

These results imply that there is limited Hg methylation at one site and some MeHg demethylation in the snow pack and snow melt in the Skeleton Continuum. This lends support to the suggestion that high Hg delivery during snowmelt is due to the preferential elution of Hg in the melting process (Larose et al., 2010). Demethylation of MeHg was present in both snow pack sites, and only at the Skeleton Creek snow melt site. The MeHg spike in Skeleton Creek snow pack could have been more available for demethylation because it had significantly lower particulate bound MeHg and lower TSS when compared to other sites (Willis et al., 2017). Additionally, the fact that demethylation was detectable more consistently and with a higher rate constant in the snow pack experiments implies that photodemethylation in the snow pack may be a driving factor behind MeHg loss, which is congruent with observations of photochemical reactions occurring the quasi-liquid layer of the snowpack and the rapid loss of dissolved Hg in the interstitial space of snow pack (Grannas et al., 2007). The snow collected at Skeleton Creek was less dense when compared to Skeleton Lake, which would create a different light environment within the snow pack, also contributing to higher demethylation rates within the snow.

Skeleton Creek and Skeleton Lake most likely have different microbial communities existing in their snowpack, which in previous literature has been used to explain differences in snow MeHg concentrations (Constant et al., 2007). Skeleton Continuum snow contained the highest number of unique phyla compared to water measured at all sites along the continuum (during summer and spring), suggesting that there is large variation in the microbial communities detected in the
snow (Cavaco et al., 2018, unpublished). Unique microbial communities in Skeleton Creek snow pack could account for this snow being the only sample where Hg methylation was detected.

4.2.3 Snow Melt Implications

To estimate snows total Hg and MeHg contribution to the Skeleton Continuum, the watershed was delineated using the ArcMap hydrology package. The estimated area that contributes melt to the continuum is ~493 Ha (Figure 2.4). Based on this estimate, and assuming uniform distribution of snow (based on snow surveys), 2958 mg of THg and 197 mg of MeHg could be contributed to the catchment via snowmelt. The Skeleton Continuum snow pack THg and MeHg storage capacity is much larger than the Hg stored in Skeleton Lake (10.17 & 7.49 mg, respectively), highlighting the important role that snow plays in the delivery of Hg and MeHg to downstream systems during melt.

In contrast to the observation that the initial snowmelt period is associated with a pulse of major ions and Hg, which accounts for the higher THg concentration in snowmelt compared with their originating snowpack (Douglas et al. 2017), we measured lower THg concentrations in snowmelt (4.12 ng L\(^{-1}\), at Skeleton Creek on June 4) than in Skeleton Continuum snow pack (6.30 ng L\(^{-1}\), on May 22). MeHg concentrations followed a similar trend, with the snow pack having a concentration of 0.67 ng L\(^{-1}\) compared with 0.14 ng L\(^{-1}\) in snow melt. Interestingly, despite the lower Hg concentrations in snowmelt (vs. snow), we did observe a pulse of major ions and snow constituents such as SO\(_4^{2-}\) and DOC.

In congruence with observations in previous studies, this study does show a flushing of major ions, suggesting that at the onset of melt Hg is preferentially lost to the landscape (Douglas et al., 2017). This observation, in tandem with the fact that the THg snow melt measurements taken from the continuum had a higher dissolved fraction than in the snow pack suggests that the snow melt sample may represent melt that has already interacted with the landscape and the particulate bound THg and MeHg has already been lost to the landscape. This can be further supported by the fact that the snow melt samples used for the isotope incubation experiments (representing a closed system that doesn’t interact with the landscape) has similar MeHg concentrations as those measured in the snow pack samples. Loss of MeHg and THg to the landscape during snowmelt could prevent MeHg from entering the downstream system.
In summary, MeHg concentrations in Skeleton Continuum snow pack were elevated, and %MeHg was relatively high. The storage capacity of the Skeleton Continuum snow pack far exceeds that of Skeleton Lake, indicating that snow pack contributes a significant amount to the Hg pool in this ecosystem during spring melt. Based on snow chemistry data it appears that snow THg concentrations are driven by dry deposition (TP, TDN), and snow MeHg concentrations are significantly related to THg and SO$_4^{2-}$. Due to the fact that majority of the SO$_4^{2-}$ is from non-marine sources (Non-sea salt sulfide= 90%) and the site is ~80km away from marine sources, DMHg bound to marine aerosols does not appear to be the major source of MeHg to the regions snow pack. Methylation in the snow pack and snow melt were detectable in some cases but the snow appears to be a site of net demethylation, supported by low M/D ratios. The majority of the THg and MeHg measured in the snow pack was particulate bound, and the microbial communities present in the snow indicate significant contributions from the terrestrial and aeolian environment, suggesting that particulate deposition from blowing dust or as a result of scavenging from snowfall may also be a contributing source for snow MeHg.

The snow melt samples appear to have lower concentrations of THg and MeHg when compared to the snow pack. With further investigation, the systems snow melt in a closed system has statistically similar concentrations, and that the lower concentrations detected, and high fraction of dissolved Hg species indicates that the Hg in the snow melt was lost to soils in transport. This is also supported by the fact that the preferential elution of major ions as reported in other studies is observed in the continuum snow pack where major ions (SO$_4^{2-}$) are lost in early melt and snow melt is less depleted in $^{18}$O than the snow pack.
5 Conclusion

5.1 Overview

This research provides insights into how MeHg cycles across the High Arctic landscape as it passes through freshwater compartments at the terrestrial-aquatic interface. An understanding of Hg methylation and MeHg demethylation hotspots across the landscape provides significant insight into the delivery of MeHg to downstream aquatic systems, how they interact and how it may be altered in the face of climate change. Additionally, this research explores how sites of MeHg production and degradation, including hotspots, vary seasonally, elucidating how Hg is distributed and delivered in the ice and snow dominated spring period, and in the productive, ice-free summer growing season. These research questions address literature gaps that have been identified by the most recent Canadian Mercury Assessment Report (2016) and Arctic Monitoring and Assessment Program (2011), which suggest that understanding spatial variation of Hg pools through small catchments is critical for understanding contributions to downstream ecosystems and that capturing seasonal variability will help elucidate the fate of MeHg throughout the year, and potentially how changes in the duration of ice and snow cover may impact net MeHg production on an annual scale.

5.2 Conclusions and Future Directions

This study focused on examining the spatial variability of MeHg from its headwater source within the Skeleton Continuum to its delivery downstream into Lake Hazen to better understand the production and degradation of MeHg as it moves across the landscape. Seasonal differences in MeHg cycling between the pre-snowmelt season and the peak summer growing season were also examined to better understand how MeHg biogeochemistry may be impacted by climate change in the future.

Generally, trends in surface water Hg and MeHg concentrations were consistent across summers, where MeHg concentrations increased moving from the active layer seep to the ponds, with a large peak in MeHg occurring at the post-pond site at the upstream end of the wetland and decreasing back to low concentrations by the time the water flowing through this continuum reaches the Skeleton Creek mouth. This study demonstrates that though Hg methylation occurred within the sediments of shallow lakes and ponds and was likely a source of MeHg to these
freshwater systems, the downstream delivery of MeHg is modulated by MeHg sequestration and demethylation in the wetland, and to a lesser extent by water column photodemethylation in the lake and ponds themselves. Additionally, this research provides a better understanding of the role of Arctic wetlands in the regulation of MeHg production and transport. Surface MeHg concentrations and %MeHg suggests that the pooling of upstream waters in wetland soils created a hotspot of MeHg production at the terrestrial-aquatic interface, but that this MeHg is sequestered and/or demethylated by downstream wetland soils. The wetland complex appears particularly efficient at sequestering MeHg through both settling of particulate-bound MeHg and sorption of dissolved MeHg to soil organic matter. Although the general spatial patterns in MeHg concentration throughout the continuum were consistent across years, the ability of the wetland to act as a MeHg sink regulating downstream transfer of MeHg in Skeleton Creek was dependent on hydrological regime, with wetter conditions leading to higher MeHg concentrations at the wetland outflow.

Climate change predictions for the Arctic suggest that in the future the Skeleton Continuum will experience longer ice-free periods, shorter snow cover duration, increased and variable precipitation and increased microbial activity due to higher temperatures and a deeper soil active layer. A longer ice-free season could lead to increased productivity and carbon inputs in to the water bodies, stimulating methylation processes in lake, pond and wetland sediments, for example. However, it would also provide longer periods for photodemethylation to occur in the water column, enforcing the current regime where Hg transformation and transport in this system is modulated by photochemical reactions in the surface water, limiting export of MeHg produced in lacustrine sediments. Increased and more variable precipitation throughout the growing season may have the most impact in changing the delivery of MeHg downstream. Comparing surface water data between 2015 and 2016 suggests that in a higher flow year the ability of the wetland to sequester and demethylate Hg is dampened. This could imply that if there are intermittent, high flow precipitation events throughout the summer, MeHg delivery downstream would increase. An intermittent regime would allow the water at the post-pond site to pool creating conditions conducive to MeHg production, and a rainfall event, or period of warm temperature increasing permafrost thaw, could cause enough surface flow to efficiently transport this MeHg downstream, limiting sequestration and demethylation of MeHg in the wetland soils during temporary water storage in the wetland.
This study is, to the best of our knowledge, the first Arctic study to quantify Hg methylation and MeHg demethylation in snow and in an ice-covered water column. This information is a significant contribution to understanding the sources of high MeHg concentrations observed across the springtime landscape. Spring storage of MeHg in Skeleton Lake and in snow was much higher than that experienced on the landscape during the summer, demonstrating the importance of examining the role of ice-covered periods in MeHg transport in freshwater arctic systems. MeHg concentrations in the Skeleton Lake water column under the ice were approximately eight times higher than in the summer, suggesting the occurrence of *in situ* production of MeHg during the ice-covered season, at areal rates that are comparable to what has been measured during the summer in both Arctic and Boreal freshwater ecosystems. However, Hg methylation was, surprisingly, not detected within spring time hypoxic lake waters and sediment methylation rates were lower at this time compared to the summer open water season. The diffusion of MeHg from the sediments and the lack of water column photodemethylation during winter and spring because of ice cover provided ideal conditions for MeHg accumulation in the water column. Despite high levels of MeHg in the spring time water column, the MeHg concentrations decrease eight-fold in less than a month suggesting that a large portion of the MeHg is demethylated and/or delivered downstream. Zooplankton in Skeleton Lake had relatively high concentrations, relative to some other Arctic lakes, suggesting that zooplankton are a source of MeHg to higher trophic organisms in the aquatic food web. However, spring time zooplankton had lower MeHg concentrations to summer time zooplankton, despite the large difference in water column MeHg concentration between those two seasons, potentially due to slow growth and feeding rates during the ice-covered season. Bioaccumulation factors for summer zooplankton were at the high end of the normal range for lakes, potentially as a result of rapid uptake of MeHg from the large water column MeHg pool during or right after the spring melt season.

High MeHg concentrations were measured within the springtime snow pack, however, these concentrations could not be explained by *in situ* production. MeHg production was detected at one site, but the snowpacks and snow meltwaters sampled appeared to be sites of net demethylation resulting from low or non-detectable methylation and the occurrence of (photo)demethylation. Snow chemistry and microbial data also suggest that the primary source of MeHg in snow is not methylation or marine aerosols, rather MeHg in snow most likely
originates from dry deposition and aeolian sources, as suggested by the fact that the majority of MeHg in the snowpack is bound to particulate matter. The fate of snow pack MeHg during melt is still unclear and MeHg concentrations measured in initial snowmelt in Skeleton Creek are lower than in snow. This either reflects that most of the snowmelt MeHg export occurs later in the melt season, or that during melt, MeHg partitions out of solution and onto soils across the landscape.

High MeHg concentrations that are found in the Skeleton Lake water column and the Skeleton Continuum snowpack at the onset of melt demonstrate that there is potential for the downstream transport of large amounts of MeHg when compared to the summer. Spring time may therefore play an important role in the delivery of MeHg downstream as snowmelt increases hydrological flow occurs during this period. Additionally, spring time MeHg concentrations in Skeleton Lake are approximately eight times higher when compared to the summer, contrasting temperate lakes which experience a late summer maximum in MeHg. This highlights that Arctic lakes have unique seasonal trends in MeHg cycling demonstrating the need for more research on MeHg cycling in high latitudes to better understand how MeHg is transformed across the landscape.

Future directions for this research should focus on obtaining a better understanding of the hydrology in the Skeleton Continuum, which would allow for the quantification of THg and MeHg fluxes through the continuum and provide more information as to what is driving the spatial variability and transport of MeHg downstream. More extensive methylation incubations, particularly in the saturated, would add to the understanding of what is creating the hotspot of MeHg in surface waters at the wetland inflow. Additionally, exploring how MeHg sequestration and degradation develop over the ice-free season in wetland soils, right after snowmelt, would provide a better understanding as to how methylation in wetlands soils and the delivery of MeHg downstream varies within the transition between spring and summer seasons under changing hydrological conditions. This information would provide more support to the hypotheses put forward in this thesis and would help fill in temporal gaps in seasonal MeHg variability.

Additionally, to better understand the fate of MeHg present at high concentrations in Skeleton Lake present under the ice in spring, it would be ideal to extend the period of measurements in the lake and in Skeleton Creek throughout the June snow and ice melt season. Such measurements would allow us to more conclusively state whether this large MeHg pool is primarily degraded in the lake water column following ice out, or flushed downstream during the
snowmelt freshet, both of which have completely different implications for the Arctic char food web in downstream Lake Hazen.
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## Appendix A: Acronyms

<table>
<thead>
<tr>
<th>Term</th>
<th>Acronym</th>
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<tr>
<td>Alkalinity</td>
<td>Alk</td>
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<tr>
<td>Ammonia+Ammonium</td>
<td>NH3+NH4</td>
</tr>
<tr>
<td>Chloride</td>
<td>Cl</td>
</tr>
<tr>
<td>Dissolved Methylmercury</td>
<td>MeHg&lt;sub&gt;dis&lt;/sub&gt;</td>
</tr>
<tr>
<td>Dissolved Total Mercury</td>
<td>THg&lt;sub&gt;dis&lt;/sub&gt;</td>
</tr>
<tr>
<td>Dissolved Organic Carbon</td>
<td>DOC</td>
</tr>
<tr>
<td>Dissolved Organic Matter</td>
<td>DOM</td>
</tr>
<tr>
<td>DOM Specific UV Absorbance</td>
<td>SUVA</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>DO</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe</td>
</tr>
<tr>
<td>Methylmercury</td>
<td>MeHg</td>
</tr>
<tr>
<td>Nitrate+Nitrite</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt;+NO&lt;sub&gt;3&lt;/sub&gt;</td>
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<tr>
<td>Sodium</td>
<td>Na</td>
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<tr>
<td>Sulphate</td>
<td>SO4</td>
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<td>Total Nitrogen</td>
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<td>Total Dissolved Organic Nitrogen</td>
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<td>Nitrogen</td>
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Appendix B: Methods and Study Site

Appendix B-1: Study Site

Appendix B-2: Water Chemistry Analysis Summary

All water chemistry parameters were analyzed at the Biogeochemical Analytical Service Laboratory at the University of Alberta. Tables S1 and S2 summarize the methodology, instrument and detection limit for each analysis type.

Table S1 Water Chemistry Parameters and Analysis Techniques used by the Biogeochemical Analytical Service Lab (University of Alberta) Table includes a lab specific
ID, method name, method literature reference, description of the method and analytical instruments used for each type of analysis.
<table>
<thead>
<tr>
<th>Method ID</th>
<th>Method Name</th>
<th>Reference</th>
<th>Method</th>
<th>Instrument</th>
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<tr>
<td>TM-IOG-001</td>
<td>Determination of Anion in Waters by Ion Chromatography</td>
<td>US EPA 300.1 (Modified)</td>
<td>Determination of Inorganic Anions in Drinking Water by Ion Chromatography</td>
<td>Dionex DX-600 Ion Chromatography</td>
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<tr>
<td>------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>---------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>TM-IOG-017</td>
<td>Turbidity using Hach Laboratory Turbidimeter 2100N</td>
<td>EPA 180.1 (Modified)</td>
<td>Determination of Turbidity by Nephelometry</td>
<td>Hach 2100N Turbidimeter</td>
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<tr>
<td>TM-IOG-019</td>
<td>Determination of Total Dissolved Solids in Water Sample</td>
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<td>Residue, Filterable (Gravimetric, Dried at 180°C).</td>
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<td></td>
<td></td>
<td>USGS 09-A6.6</td>
<td>Chapter A6. Section 6.6 Alkalinity and Acid</td>
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Table S2 Water Chemistry Parameters Detection Limit Determined by the Biogeochemical Analytical Service Lab (University of Alberta). Table identifies water chemistry parameters, acronyms for each parameter, the method ID (matching Table S1), detection limit units, and detection limit value for each method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbrev</th>
<th>Method ID</th>
<th>Unit</th>
<th>Method Detection Limit</th>
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<td>Total Phosphorus</td>
<td>TP</td>
<td>TM-IOG-007</td>
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<td>Total Dissolved Phosphorus</td>
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<td>Nitrite + Nitrate</td>
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<td>ppb</td>
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<td>NO2</td>
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<td>ppb</td>
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<td>ppb</td>
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Appendix B-3: Wetland Soil Mass Dependency and Summary

To ensure that there was no mass dependency for wetland soil samples run on the Tricell Direct Mercury Analyzer (DMA-80) two samples were run in triplicate at 50 mg and 100 mg. The
variation within each mass was not significantly different from the variation between masses (p>0.3) (Table S3). Subsequent samples were run using 50 mg for each sample.

**Table S3 Mass dependency test for wetland soils on DMA.** THg concentrations (ng g⁻¹) of samples run at 50ng and 100ng, testing for mass dependency on DMA-80.

<table>
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<th>Sample</th>
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</tr>
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<td>WTR1-E</td>
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</table>

Table S4 Wetland transect THg (ng g⁻¹), MeHg (ng g⁻¹), LOI (%) and Saturation raw data. Individual sample data is displayed by site (ID) and transect to demonstrate spatial variation in THg, MeHg, % MeHg, LOI and soil saturation throughout the wetland. Saturation was determined by qualitative field observations.

<table>
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<th>ID</th>
<th>Transect</th>
<th>THg (ng g⁻¹)</th>
<th>MeHg (ng g⁻¹)</th>
<th>%MeHg</th>
<th>LOI (%)</th>
<th>Saturated (S) /Unsaturated (U)</th>
</tr>
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<td>0.387</td>
<td>0.819</td>
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<td>S</td>
</tr>
<tr>
<td>WTR1C</td>
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<td>S</td>
</tr>
<tr>
<td>WTR1D</td>
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<td>Value 2</td>
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</tbody>
</table>
Appendix C Statistical Analysis

Appendix C-1: Snow Chemistry Analysis

To examine the relationship between snow chemistry parameters and Hg concentrations in the Skeleton Continuum snowpack, 2017 snow data (n=3) was combined with snow data collected for larger snow sampling campaigns in 2014 (n=9) and 2015 (n=18).

THg concentrations in the Lake Hazen Region snowpack in 2014 ranged from 8.14-40.27 ng L\(^{-1}\), with a mean concentration of 21.41. MeHg concentrations in 2014 ranged from 0.31-0.94 ng L\(^{-1}\), with a mean concentration of 0.61. %MeHg in 2014 ranged from 2.08-4.43%, with a mean of 3.05%. THg concentrations in the Lake Hazen Region snowpack in 2015 ranged from 3.02-6.54 ng L\(^{-1}\), with a mean concentration of 4.73 ng L\(^{-1}\). MeHg concentrations in 2015 ranged from 0.14-0.68 ng L\(^{-1}\), with a mean concentration of 0.36 ng L\(^{-1}\). %MeHg in 2015 ranged from 4.17-12.85% with a mean of 7.56%. Comparing each year reveals that MeHg concentrations were not significantly different between all 3 years (p=0.01). For THg concentrations and %MeHg, 2014 values are significantly higher than 2015 (p<0.001).

Though snow Hg values differ significantly between 2014 and 2015, a principal components analysis of both years show that the snow chemistry is driven by the same. This allows for the three data sets (2014, 2015 and 2017) to be combined to assess which snow chemistry factors are driving Hg concentrations. To assess the driving factors behind THg concentrations in snowpack a stepwise regression model was run using ln(THg), with all chemistry parameters that did not covary (variance inflation factor (VIF)<5).

For the ln(THg) model, two of the three data-points from 2017 fit within the 95% confidence interval of the data, and the last point fit within the natural variation of the data observed in 2014 and 2015, justifying the applicability of this model to 2017 Hg concentration in snowpack (Figure S2). For the MeHg model, all three data points from 2017 fit within the natural variation of the model’s data (Figure S3).
Figure S 1 Dataset distribution of THg (a) and transformed lnTHg (b) in snow. Panel (a) shows that the distribution of THg concentrations in snow is right skewed, panel (b) shows the natural logarithm transformed data which is normally distributed and fitting for further statistical analysis.

Figure S 2 (a) Multiple linear regression model fit for THg (ng L^{-1}) in snow (TDN and TP) and assessment of model (b) residuals and (c) leverage
Figure S 3 (a) Multiple linear regression model fit for MeHg (ng L⁻¹) in snow (THg and SO₄) and assessment of model (b) residuals and (c) leverage

Appendix D: Results

Appendix D-1: Skeleton Lake MET station summary from 2016 and 2017 sampling season

During the Summer 2016 and Spring 2017 field seasons a meteorological station was set up beside Skeleton Lake to monitor air temperature, barometric pressure, wind speed and photosynthetically active radiation (PAR) at the field site to explain any observed changes in lake input or in lake chemistry. In 2016, observations were recorded every 20 minutes from July 7 to August 4 (Figure S4). In 2017, observations were recorded every 20 minutes from May 24 to July 23 (Figure S5). Temperature and PAR demonstrated matching diurnal patterns in both years, due to the descension of the sun behind the adjacent mountains in the evening (Mt. McGill). In Summer 2016, the temperature ranged from
Figure S 4 (a) Temperature (°C), (b) Barometric Pressure (hPa), (c) Wind Speed (m/s) and (d) Photosynthetically Active Radiation (PAR) (μmol/m²/s) measured from a MET station beside Skeleton Lake, July 7-August 8, 2016
Figure S 5 (a) Temperature (C), (b) Barometric Pressure (hPa), (c) Wind Speed (m/s) and (d) Photosynthetically Active Radiation (PAR) (μmol/m²/s) measured from a MET station beside Skeleton Lake from May 23-July 7, 2017
# Appendix D-2: Skeleton Continuum Surface Water Chemistry

Table S5 Average water chemistry parameters in Skeleton Continuum surface waters, 2015

<table>
<thead>
<tr>
<th></th>
<th>TN (ug L⁻¹)</th>
<th>TDN (ug L⁻¹)</th>
<th>TP (ug L⁻¹)</th>
<th>TDP (ug L⁻¹)</th>
<th>Cl (mg L⁻¹)</th>
<th>SO₄ (mg L⁻¹)</th>
<th>TDS (mg L⁻¹)</th>
<th>DOC (mg L⁻¹)</th>
<th>Alkalinity (mg L⁻¹ as CaCO₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seep</td>
<td>n/a</td>
<td>92.80 (± 15.77)</td>
<td>2.94 (±1.56)</td>
<td>3.38 (±2.22)</td>
<td>0.41 (±0.14)</td>
<td>96.37 (±67.55)</td>
<td>208.40 (±123.55)</td>
<td>0.6 (±0.11)</td>
<td>47.29 (±3.16)</td>
</tr>
<tr>
<td>Skeleton Lake</td>
<td>383.88 (±7.05)</td>
<td>378.0 (±4.64)</td>
<td>5.60 (±0.74)</td>
<td>5.80 (±0.37)</td>
<td>2.30 (±0.11)</td>
<td>287.99 (±61.81)</td>
<td>478.20 (±42.73)</td>
<td>5.7 (±0.13)</td>
<td>86.44 (±1.48)</td>
</tr>
<tr>
<td>Post-ponds</td>
<td>298.38 (±24.36)</td>
<td>248.33 (±15.18)</td>
<td>3.67 (±0.76)</td>
<td>3.33 (±1.53)</td>
<td>1.13 (±0.07)</td>
<td>194.26 (±9.43)</td>
<td>408.33 (±60.39)</td>
<td>3.6 (±0.15)</td>
<td>89.68 (±2.44)</td>
</tr>
<tr>
<td>Post-wetland</td>
<td>368.70 (±19.80)</td>
<td>350.33 (±16.04)</td>
<td>5.25 (±0.35)</td>
<td>6.00 (±1.41)</td>
<td>0.58 (±0.39)</td>
<td>274.99 (±11.19)</td>
<td>606.5 (±14.85)</td>
<td>6.1 (±0.28)</td>
<td>140.78 (±0.18)</td>
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<tr>
<td>Skeleton Creek</td>
<td>350.03 (±23.12)</td>
<td>344.67 (±21.96)</td>
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<td>2.67 (±0.58)</td>
<td>0.49 (±0.42)</td>
<td>285.89 (±23.87)</td>
<td>632.67 (±46.72)</td>
<td>5.7 (±0.35)</td>
<td>150.09 (±3.43)</td>
</tr>
</tbody>
</table>

Table S6 Average water chemistry parameters in Skeleton Continuum surface water 2016

<table>
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<tr>
<th></th>
<th>TN (ug L⁻¹)</th>
<th>TDN (ug L⁻¹)</th>
<th>TP (ug L⁻¹)</th>
<th>Cl (mg L⁻¹)</th>
<th>SO₄ (mg L⁻¹)</th>
<th>TDS (mg L⁻¹)</th>
<th>TSS (NFR)</th>
<th>DOC (mg L⁻¹)</th>
<th>PC (mg L⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Seep</td>
<td>73.00 (±21.88)</td>
<td>84.80 (±21.37)</td>
<td>2.63 (±0.96)</td>
<td>0.36 (±0.14)</td>
<td>170.80 (±85.81)</td>
<td>288.0 (±123.25)</td>
<td>17.36 (±15.24)</td>
<td>0.68 (±0.19)</td>
<td>439.58 (±419.73)</td>
</tr>
<tr>
<td>Skeleton Lake</td>
<td>373.67 (±39.62)</td>
<td>375.17 (±17.31)</td>
<td>3.83 (±1.47)</td>
<td>1.67 (±0.51)</td>
<td>247.88 (±17.11)</td>
<td>491.33 (±21.15)</td>
<td>5.01 (±7.80)</td>
<td>6.21 (±0.30)</td>
<td>231.47 (±83.89)</td>
</tr>
<tr>
<td>Post-ponds</td>
<td>292.75 (±42.70)</td>
<td>286.50 (±20.53)</td>
<td>8.25 (±5.38)</td>
<td>1.69 (±0.30)</td>
<td>245.07 (±5.16)</td>
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<td>4.80 (±0.80)</td>
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<tr>
<td>Post-wetland</td>
<td>315.50 (±2.12)</td>
<td>312.0 (±4.24)</td>
<td>2.50 (±0.71)</td>
<td>1.25 (±0.57)</td>
<td>266.48 (±1.50)</td>
<td>558.5 (±6.37)</td>
<td>0.64 (±0.23)</td>
<td>4.85 (±1.06)</td>
<td>56.75 (±48.58)</td>
</tr>
<tr>
<td>Skeleton Creek</td>
<td>321.00 (±28.87)</td>
<td>313.0 (±19.80)</td>
<td>2.50 (±0.71)</td>
<td>0.93 (±0.86)</td>
<td>279.31 (±0)</td>
<td>610.50 (±33.23)</td>
<td>0.70 (±0)</td>
<td>5.70 (±0.14)</td>
<td>39.25 (±18.60)</td>
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</table>
Appendix D-3: Skeleton Continuum surface water trends within season (July-August 2015/2016)

In 2015, connectivity between the ponds and wetland was not established until between July 13-18. There were small amounts of variation in surface water THg concentrations within the season at each site. Most of the variation that was observed was accounted for by changes in the particulate bound Hg, which can be seen when comparing Figure S6 a and b. THg concentrations were higher at the Seep and Post-ponds on August 1st compared to all other dates which displayed similar trends. MeHg concentrations were more variable within the season, increasing after the wetlands on July 18 (0.26 ng L⁻¹) and i after the ponds on August 1 (0.49 ng L⁻¹) (Figure S6 c-d). The increase after the ponds on July 18 is produced by an increase in particulate bound MeHg and decrease in MeHg concentrations to 0.03 ng L⁻¹ by July 23 (Figure S6 d). The increase in MeHg concentrations after the ponds on August 1 was an increase in both dissolved and particulate bound MeHg (Figure S6 c-d).

In 2016, Skeleton Lake was free of ice by July2 and connectivity between the ponds and wetlands was established by July 17. Sufficient flow from the post-wetland site was measured between July 25-31. Surface water THg concentrations showed minimal variation and was primarily driven by changes in particulate bound THg, similar to 2015 (Figure S7 a-b). on July 31, THg concentrations deviated from its seasonal patter with a lower concentration at the post-ponds site and a higher value at the wetland outflow and Skeleton Creek. At the post-pond site THg concentrations on all other dates ranged from 1.17-1.32 ng L⁻¹, while July 31 had a concentration of 0.67 ng L⁻¹. THg concentrations on July 31 increased from the post-ponds to 0.94 ng L⁻¹ at the wetland outflow and 1.17 ng L⁻¹ at Skeleton Creek, with these changes being accounted for by an increase in dissolved MeHg. MeHg concentrations were more consistent throughout 2016, the most variation was seen at the post ponds site where concentrations ranged from 0.20 ng L⁻¹ on July 25 to 0.42 ng L⁻¹ on July 17, all other sites had standard deviations under 0.02 ng L⁻¹ for their seasonal averages (Figure S7 c-d).

In 2015 and 2016, %MeHg always peaked at the post ponds site with a range of 17.20-51.29 ng L⁻¹. In both years the high average %MeHg is largely driven by an increase in %MeHg that occurred on August 1 and July 31 for 2015 and 2016, respectively (Figure S8 a-b). In 2015, on August 1 high %MeHg is driven by an increase in both THg and MeHg concentrations. In
contrast, 2016’s peak on July 31 is driven by relatively low THg concentrations and high MeHg concentrations.

**Figure S 6** (a,b) THg (ng L\(^{-1}\)) and (c,d) MeHg (ng L\(^{-1}\)) concentrations in (a,c) unfiltered and (b,d) filtered surface water in the Skeleton Continuum by date, 2015.
Figure S 7 (a,b) THg (ng L$^{-1}$) and (c,d) MeHg (ng L$^{-1}$) concentrations in (a,c) unfiltered and (b,d) filtered surface water in the Skeleton Continuum by date, 2016.

Figure S 8 % MeHg Unfiltered in Skeleton Continuum surface water by date in (a) 2015 and (b) 2016