Mercury Cycling in Hydroelectric Reservoirs of Northern Manitoba Decades After Impoundment

by

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Abstract

As the global climate changes and demand for renewable electricity increases, construction of hydroelectric dams is increasing globally though the impacts of regulating the worlds rivers are still understudied. Northern Manitoba, Canada, has extensive hydroelectric development since the 1950s; fish mercury (Hg) concentrations in on-system lakes were observed to have increased above human consumption guidelines upon impoundment and have taken decades to decrease towards natural concentrations. To better understand the long-term impacts of hydroelectric regulation on Hg in fish and other biota in Northern Manitoba, we determined methylmercury (MeHg) production potential in soil from the water fluctuation zone in on- and off-system lakes through a soil flooding incubation experiment in the laboratory. We further studied the historic flux of MeHg and Hg to the sediments in on- and off-system lakes and links to organic matter in these waterbodies. We found that MeHg production was highest in the water fluctuation zone of the on-system lakes, which may represent an increased source of MeHg to the food web in these environments even decades after impoundment. In addition, sedimentation rates were found to greatly affect Hg fluxes to the sediment in those waterbodies where increased water flows result in higher erosion and sedimentation. These findings provide new insight in our understanding of the long-term recovery of Hg cycling within on-system lakes decades after impoundment.

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List of acronyms

CRD	Churchill River Diversion
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
ELA	Experimental Lakes Area
ELARP	Experimental Lakes Area Reservoir Project
FLUDEX	Flooded Upland Dynamics Experiment
GS	Generating station
Hg	Mercury
HgS	Mercury sulfide
KHP	Potassium hydrogen phthalate
MeHg	Methylmercury
NSERC	Natural Sciences and Engineering Research Council of Canada
OC	Organic carbon
OM	Organic matter
RBR	Rat-Burntwood River
SRB	Sulfate reducing bacteria
THg	Total mercury
TSS	Total suspended sediments
UCTEL	Ultra-Clean Trace Elements Laboratory
Ww	Wet weight

Chapter 1: Introduction and literature review

1.1 Context

Anthropogenic activities have resulted in large global increases in mercury (Hg) in the environment and in biota. For instance, the present-day atmospheric Hg concentrations are estimated to be roughly 450 % above natural concentrations prior to industrialization (Outridge et al., 2018). Once in the aquatic environment, a fraction of inorganic Hg is methylated to become methylmercury (MeHg). MeHg biomagnifies in the aquatic food web, resulting in the accumulation of MeHg at high concentrations in high trophic-level animals such as fish and marine mammals. Human exposure to Hg is primarily through consumption of fish and other seafood (AMAP, 2012). After global efforts to reduce emissions, including new technologies (Li et al., 2018) and policies, anthropogenic emissions of Hg have decreased in recent decades, and are projected to decrease further with the recent signing and enforcement of the Minamata Convention on Mercury. However, ecosystem recovery from Hg contamination is anticipated to take a long time, as bioaccumulation in biota is increasingly driven by changes in biogeochemical and ecological processes (Wang et al., 2010; Wang et al., 2019). This includes both processes within waterbodies, but also larger scale changes such as river regulation and climate change (Wang et al., 2019).

In the 1970s, extensive hydroelectric development occurred in Manitoba, Canada. This included diverting ~75 % of the Churchill River water flow using a man-made channel and a series of dams and dikes to manipulate water height and flow through the Rat and Burntwood Rivers towards the Nelson River, maximizing the electrical output of a series of hydroelectric generating stations (Manitoba Hydro, 2015). These hydrologic changes resulted in increases to Hg in fish above human consumption guidelines in most cases and took decades to decrease

towards normal levels (Bodaly et al., 2007). However, there have been recent increases in fish Hg levels in several waterbodies in Northern Manitoba, including both "on-system" lakes (impacted by river regulation) and "off-system" lakes (natural lakes un-impacted by river regulation) that remain unexplained (Munson et al., in prep).

1.2 Objectives

This thesis research is part of Team 5 (Mercury) of the Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development Grant project "BaySys" that is co-funded by Manitoba Hydro. The objective of the BaySys-Mercury Team is to examine how Hg transport and transformation in the Hudson Bay ecosystem responds to hydroelectric regulation and a changing climate. The goal of my thesis research was to investigate impacts of river regulation on Hg cycling in reservoirs of Northern Manitoba during a period of changing emissions and climate.

The specific research objectives of this thesis were to:

1) Determine how water level fluctuations influence the mobilization of total Hg (THg) and the production and mobilization of MeHg into on-system and off-system lakes in Northern Manitoba. To accomplish this, soils from the water fluctuation zone of two on-system lakes and an off-system lake nearby were flooded in a laboratory incubation chamber and monitored for increases of MeHg in sediment as well as fluxes of THg and MeHg to the overlying water; and,

2) Measure historical changes in THg and MeHg in Northern Manitoba on-system lakes compared to off-system lakes to assess changes in regional Hg cycling throughout time. To accomplish this, sediment cores from two on-system lakes and two off-system lakes were collected, dated and analyzed for THg and MeHg. These results were examined together with

the characterization of organic matter (OM) in the watershed done by other team members of the BaySys project, as well as with physical observations from previous reports.

1.3 Literature Review

1.3.1 MeHg in the environment

Hg is a naturally occurring element in the Earth's crust where it is present largely as the mineral cinnabar (HgS) (Steffen and Morrison, 2016). Hg enters the environment, primarily as gaseous Hg(0), from natural and anthropogenic sources, and from re-emission processes. Dominant anthropogenic sources include: burning of coal, artisanal gold mining, metal production and cement production (AMAP, 2012). Natural sources of Hg include volcanic emissions and weathering of Hg-containing rocks. The re-emission processes release "legacy Hg" (which can be natural or anthropogenic in origin) that was previously emitted to the environment (Mason, 2009). Emitted Hg(0) gets oxidized in the atmosphere to Hg(II) and deposited through wet and dry deposition to the Earth's surface including freshwater environments (Mason, 2009). Once in freshwater environments Hg(II) can be methylated, primarily by anaerobic bacteria to produce MeHg (Compeau and Bartha 1985; Yu et al., 2012). This is known to happen both in anoxic bottom water and in sediment (Eckley et al., 2005). Sulfate reducing bacteria (SRB) were first identified as those responsible for Hg methylation in estuarine sediments (Compeau and Bartha, 1985). Since then, other biologically mediated pathways have been discovered. For example: in South River, Virginia, USA, sulfate reducing and iron reducing bacteria have been shown to be responsible for Hg methylation in the sediment, having higher activity in the summer (Yu et al., 2012). In other systems, methanogens are also reported to play a role in MeHg formation (Parks et al., 2013). MeHg from the

sediments can diffuse into the water column where it biomagnifies in the food web by several orders of magnitude. MeHg builds up faster than it can be removed in most biota due to its affinity for fatty tissue and sulfur-containing compounds (Roos et al., 2010). The sinks for MeHg in these environments are demethylation, eventual burial in the sediments or export via dissolved or particle phases downstream (Steffen and Morrison, 2016).

1.3.2 MeHg toxicity

At high enough concentrations, MeHg exhibits toxic effects in fish and other animals including humans (AMAP, 2005; AMAP, 2012; Nabi 2014). In freshwater fish, sublethal poisoning has been observed at levels ranging from 6-20 μg Hg/g ww (wet weight), depending on the species (AMAP, 2012). In a laboratory experiment, walleye fed with food containing 1 μg MeHg/g for 6 months displayed significantly impaired growth and gonadal development compared to those fed a control diet (Friedmann et al., 1996). MeHg is a known developmental neurotoxin to humans, making pregnant women and children especially vulnerable (Steffen and Morrison, 2016; Nabi, 2014).

Due to the toxic effects of MeHg, commercial limits of THg in fish (assumed to be entirely MeHg) have been established by governments including in Canada to protect human health (Steffen and Morrison, 2016). Commercial limits on fish Hg concentrations in Canada are: 0.5 μg Hg/g for most fish, while fish higher in the food web such as tuna and swordfish have limits of 1.0 μg Hg/g in fish and it is advised to eat them less frequently (Health Canada et al., 2007).

1.3.3 Factors known to impact Hg levels in biota

Hg goes through complex cycling in the environment and there are many factors affecting THg levels in biota within lakes and rivers. As MeHg is the only form of Hg that biomagnifies in aquatic food webs (Wang et al., 2019), the factors associated with increased Hg in biota will be considered as three categories: 1) those that change the balance of methylation/demethylation reactions, 2) those that impact the assimilation of MeHg into the food web; and 3) those that affect bioaccumulation and biomagnification of MeHg in the ecosystem.

1) SRB and other anaerobic bacteria have been shown to dominate MeHg production (Compeau and Bartha, 1985; Yu et al., 2012) and therefore their activity will largely determine Hg methylation (Regnell and Watras, 2019). MeHg has been shown to be negatively correlated with dissolved oxygen levels and positively correlated with sulfide in boreal lakes, conditions that favor activity of anaerobic bacteria (Small and Hintelmann, 2014). Examples of forcings that draw down oxygen and can result in increasing MeHg production are: stratification and flooding of OM (Kelly et al., 1997, Steffen and Morrison, 2016).

Temperature affects the metabolism rates of biological reactions, increasing both methylation and demethylation rates, though appearing to favor an increase in methylation/demethylation ratios (Yang et al., 2016). Yang et al. incubated Arctic soil at both -2 °C and 8 °C over 144 days and monitored for Hg methylation and found that the warming of permafrost by 10 °C produced up to a 10-fold increase in net methylation (Yang et al., 2016). As permafrost warms and thaws in the North, this is expected to be a large source of Hg which could potentially be methylated and enter the food web (AMAP, 2012).

Sulfate deposition has been shown to lead to increases in MeHg from increased activity of SRB (Wasik et al., 2012). For example, a full lake experiment which loaded a boreal peatland with sulfate over several years resulting in a 4-fold increase in sulfate, showed a corresponding 4-fold increase in MeHg (Wasik et al., 2012). As the MeHg concentrations increased, sulfate loadings on a portion of the lake were stopped, which led to a rapid decrease in MeHg in that part of the lake. Given the efforts and success limiting sulfate emissions in response to concerns over acid rain, decreased sulfate depositions should be considered as a confounding factor when looking at historical Hg concentrations in biota, especially in regions nearby industrial sources of sulfate (Wasik et al., 2012). MeHg production has also been correlated with neutral Hg-sulfide complexes in both laboratory with pure cultures of SRB (Benoit et al., 2001) and modelled concentrations in different environments (Benoit et al., 1999). It is generally assumed that neutral Hg-sulfide species are more bioavailable as they can cross cell membranes of bacteria due to their relatively non-polar and uncharged chemistry. Once inside the membrane, Hg can be methylated (Benoit et al., 1999).

Another factor that has been observed in many studies is that pH is inversely related to MeHg levels in fish, with Hg concentrations in fish higher from more acidic waters (Allen et al., 2005; Watras et al., 2006; Wiener et al., 1990). A study in which a lake was divided into two and one side was experimentally acidified with sulfuric acid shows significantly higher fish Hg concentration in the acidified side after just one year (Wiener et al., 1990). Watras et al. however interpreted this increase as sulfate stimulating the SRB activity and the increasing available Hg due to high deposition during the study (Watras et al., 2006). Because carbonate rather than sulfuric acid, would dominate acidification on a global scale due to climate change, a similar increase in fish Hg concentrations may not be a uniform response to pH changes (Watras

et al., 2006). However, because pH affects the speciation of Hg, and more specifically Hg-sulfide species that influence MeHg formation, the response to changing pH is complex (Liu et al., 2017).

2) For biomagnification to occur, the MeHg produced has to enter the base of the foodweb. MeHg has been shown to preferentially bind to dissolved organic carbon (DOC) over algae resulting in lower bioaccumulation in the base of food web when high DOC is present (Gorski et al., 2006). A laboratory study conducted by Gorski et al. shows that when dissolved organic matter (DOM) concentrations were varied, concentrations of MeHg in freshwater algae increased with decreasing levels of DOM, with increases occurring as concentrations of DOM decreased below 10 mg/L (Gorski et al., 2006). DOM has also been shown to play an important role in photodemethylation of Hg, with MeHg degradation occurring in solution with DOM, but not with deionized water (Tai et al., 2014). Furthermore, degradation, burial or export of MeHg are all mechanisms which would limit bioaccumulation.

3) Several "top down" ecological processes play an important role in MeHg concentrations in fish including metabolism of fish, change in fish habitat, and length and structure of the food web. Metabolism of fish can increase with increasing temperatures leading to faster growth and lower bioaccumulation for a given size, but changes in methylation can confound these trends (Steffen and Morrison, 2016). Fish biomagnify MeHg with each additional trophic level of a food web as they receive more MeHg from their diet. Therefore, a shorter food web results in less Hg in biota. For example, simple food webs in aquaculture-dominated reservoirs in China can help explain low Hg in biota despite high environmental Hg (Liu et al., 2012). Further, a

change of aquatic habitat can completely change the populations that occupy a given waterbody affecting entire food webs. For example, change in riverine habitat to more lacustrine from hydroelectric regulation has been shown to change the fish species that inhabit those waters (Manitoba Hydro, 2015), which in turn alters fish diets and the Hg present in those fish.

At higher trophic levels in the food web, bioavailability also plays an important role in biomagnification of MeHg. MeHg bioavailability in fish has been shown to vary greatly depending on the amount of amino acids present during digestion, which controls the amount of MeHg that becomes soluble during digestion and remains in the fish of different species (Leaner and Mason, 2002). Leaner et al. digested sediment and bloodworms in the presence of gastric and intestinal fluids from different fish species and showed that sediment MeHg was less bioavailable to fish because it was less digestible (Leaner and Mason, 2002).

1.3.4 MeHg in hydroelectric reservoirs

Though reservoir impoundment has been shown to increase concentrations of Hg in fish, earlier studies of flooded systems lacked methods capable of detecting trace levels of MeHg and THg reliably in waters (Kelly et al., 1997). In order to determine the effects of flooding of a reservoir on Hg methylation, several sites were selected to create experimental reservoirs where variables could be controlled in natural systems (Bodaly et al., 2004; Kelly et al., 1997). After monitoring a wetland for 2 years, as part of a project known as the Experimental Lakes Area Reservoir Project (ELARP), the wetland in the Experimental Lakes Area (ELA) of Canada was experimentally flooded and monitored for increases in THg, MeHg, CH_4, CO_2 and various other parameters. ELARP showed an increase in % MeHg of THg after flooding from 4 % to an average of 32 % post-flood over the two years of flooding (Kelly et al., 1997). These increases

coincided with increased production of by-products from OM decomposition. The results of ELARP suggested that the breakdown of OM resulted in the increase of MeHg production (Kelly et al., 1997). A follow up study termed the Flooded Upland Dynamics Experiment (FLUDEX) aimed to determine if reducing the amount of organic carbon (OC) would result in less MeHg production (Hall et al., 2005). FLUDEX flooded low, medium and high OC reservoirs (all of which had less OC than ELARP) while trying to keep other environmental variables similar. The high OC reservoir had higher MeHg production over the 3 years and larger amounts of MeHg were retained in the sediment; however, the medium OC reservoir had the highest MeHg concentrations in the water column. The medium carbon reservoir also had higher concentrations of DOC in the water column and exported more MeHg (Hall et al., 2005). The results suggest that different types of OM, and their rates of decomposition may play roles in Hg transformation, storage and transport (Hall et al., 2005). The results of the FLUDEX experiment also showed that all of the reservoirs were experiencing net demethylation by the third year. This suggests that the decades long increases in fish Hg concentrations observed by Bodaly et al. (2007) may have been caused by a short term burst in MeHg production (Hall et al., 2005).

After three years of flooding experiments at the FLUDEX reservoirs, the reservoirs were drained and soil core samples were collected from post-flooded and nearby reference soil. Overall the results showed MeHg production peaked within weeks to months, but the majority of MeHg stayed in the soil throughout the experiment (Rolfhus et al., 2015). Nine years later additional cores were collected from the previously flooded soil and from nearby reference soil. On average 86% of the measured MeHg concentrations in the soil during the last flooding season was retained after drawdown and nine years of drying (Rolfhus et al., 2015). From separate experiments in Newfoundland, Canada, this source of MeHg was attributed to the

breakdown of OM. Schartup et al. (2015) recently incubated flooded soil after removing the vegetation and litter layers rich in OM. The result of these core incubations was very low MeHg production from soil compared to the reservoir experiments (Schartup et al., 2015). This confirms that the production of MeHg is related to the breakdown of the OC (Schartup et al., 2015).

Though, the MeHg production was related to the amount of OC in ELA experiments (therefore the highest in ELARP), the concentrations in the food web were higher in the FLUDEX reservoirs (Bodaly et al., 2004). For example, Hall et al. studied zooplankton in the four experimental reservoirs and found that increases in zooplankton bioaccumulation factors of MeHg were most strongly related to the concentration of unfiltered MeHg and not the amount of OC stored in the reservoir (Hall et al., 2009). After flooding however, the reservoirs with higher OC had zooplankton with elevated bioaccumulation factors of MeHg compared to natural concentrations for longer periods of time. For example, the ELARP wetland reservoir still had elevated zooplankton Hg concentrations compared to natural lakes after 14 years, while the FLUDEX started declining rapidly at least a decade earlier (Hall et al., 2009). Though more MeHg was produced in higher OC reservoirs, concentrations in the food web were lower, likely due to a combination of other factors including both the DOC in higher OC reservoirs preferentially binding to DOC over algae and differences in retention and release of MeHg.

Historically, Hg increases in fish following reservoir flooding was often blamed on industrial sources (Bodaly et al., 1984). In recent years, several wide scale studies on the impacts of hydroelectric regulation on fish Hg concentrations have been conducted in different parts of the globe including: Canada, United States, Finland, and China (Bodaly et al., 2007; Porvari, 1998; Tremblay and Schetagne, 2000; Willacker et al., 2016; Xu et al., 2018).

Piscivorous fish in reservoirs within Quebec showed peak concentrations up to 7 times the background concentrations in nearby lakes that took 20-30 years to fall within background concentrations (Tremblay and Schetagne, 2000). Finnish reservoirs originally impounded between 1964-80 were monitored from 1979-94. Reservoirs showed high levels of fish Hg after impoundment and remained above background for 15-25 years (Porvari, 1998). At the time of the study, two reservoirs still showed pike above 1 μg/g even after 20 years or longer (Porvari, 1998).

In addition to the flooding experiments, large scale studies of fish Hg concentrations across many reservoirs provide insight into controls on Hg cycling within the reservoirs. A recent study by Willacker et al. conducted a large-scale study of reservoirs across Western United States and Canada, across many different environments and recognized several patterns (Willacker et al., 2016). A three-phase general pattern emerged with first rapid increase of fish Hg upon flooding peaking after 3.04 ± 0.71 years, followed by a decline until 12.48 ± 1.33 years post flooding, and finally a levelling off/slow decline towards background concentrations (Willacker et al., 2016). This is consistent with what is found in Manitoba, Quebec and Finland though the timing of peak concentrations varies (Bodaly et al., 2007; Porvari, 1998; Tremblay and Schetagne, 2000). In addition, Willacker et al. also found that high water levels in between-year flooding resulted in significant Hg increases in fish, while within-year flooding did not (Willacker et al., 2016). Furthermore, they found that the timing of minimum water storage was important. When low water levels occurred in the months of maximum plant growth, higher fish Hg were measured in most environments (Willacker et al., 2016).

In China, a unique pattern is observed compared to other regions (Xu et al., 2018). China has extensive hydroelectric development including the largest hydroelectric reservoirs in

the world. Throughout China, most notably in the Three Gorges Reservoir where water level fluctuates by 30 m, reservoirs do not show the same "reservoir effect" that was identified from hydro-regulated flooding in Canada, the United States and Finland (Bodaly et al., 2007; Porvari, 1998; Tremblay and Schetagne, 2000; Willacker et al., 2016; Xu et al., 2018). At Three Gorges Reservoir, there was no increase in fish Hg upon flooding, which Xu et al. (2018) attributed to high pH and low OC resulting in low Hg availability for methylation as well as short food webs. This happens despite relatively high THg compared to other regions and flooding more than 350 km^2 of land (Xu et al., 2018). Another example occurs in the reservoirs in the Guizhou Province, where fish Hg concentrations remain well below human consumption limits, due to low OC in the soil and fast fish growth rate because of intensive aquaculture activities in these reservoirs (Liu et al., 2012).

1.3.5 Hg and hydroelectric development in Northern Manitoba

Hydroelectric development in northern Manitoba started in the 1950s and intensified through the 1970s. Kelsey Generating Station (GS), the first hydroelectric GS on the Nelson River was constructed between 1957-61, flooding 102 km^2 of land (Manitoba Hydro, 2015). The subsequent development of the Lower Nelson River occurred in three phases. Phase I (1966-1976) diverted ~75% of the Churchill River through the Rat-Burntwood River system to the Nelson River where Kettle GS was also constructed. Phase II (1971-79) involved building Long Spruce GS and Phase III (1978-1992) involved building Limestone GS (Manitoba Hydro, 2015).

Kettle GS increased the water level on the Nelson River by ~ 31.5 m and flooded an area of 221 km^2, now known as Stephens Lake (Manitoba Hydro, 2015). The diversion of the Churchill River involved first a control structure (Missi Falls Control Structure) at Southern

Indian Lake to limit the flow into the Lower Churchill River and created a reservoir to hold more water (Manitoba Hydro, 2015). Water was then moved through a man-made channel to the Rat-Burntwood River system and was further regulated by another control structure (Notigi Control Structure) on the Rat River to control water for release downstream to the Kettle GS as the water is needed for electricity production (Manitoba Hydro, 2015). Hydroelectric development is ongoing in the region, with Keeyask GS currently under construction for operation beginning in 2020.

The extensive flooding and regulation of Northern Manitoba rivers has had significant effects on the physical environment and those that rely on it (Bodaly et al., 2007; Manitoba Hydro, 2015). The creation of the Missi Falls Control Structure led to the dewatering of ~ 204 km^2 downstream of Southern Indian Lake on the Lower Churchill River, resulting in an overall loss of fish habitat and freshwater source for the town of Churchill (Manitoba Hydro, 2015). Much of the area flooded saw a change in habitat from riverine to more lacustrine resulting in changes to fish communities. In addition, the quality of water was decreased and increased suspended sediment due to erosion were noted (Manitoba Hydro, 2015). Extensive loss of wetland habitat and altered shoreline habitat was observed by First Nations communities that rely on the habitat (Manitoba Hydro, 2015). Furthermore, manipulation of the dams maximizes water flow in winter when the demand is highest. This differs from natural flows where highest flows would occur during the spring freshet and lowest flows would occur in winter (Déry et al., 2011; Manitoba Hydro, 2015).

When extensive areas were impounded and flooded for hydroelectric regulation in Northern Manitoba during the Churchill River Diversion (CRD) in 1976, fish Hg concentrations from several lakes were measured before and after flooding and were found to increase in all

flooded waterbodies with no other point sources or explanations, while fish from nearby lakes unaffected by flooding did not display the same increases over that time period (Bodaly et al., 1984). These increases in fish Hg concentrations resulted in closure of several fisheries in Northern Manitoba (Manitoba Hydro, 2015). Decades after impoundment and continued collection of fish Hg data by commercial and survey data, Bodaly et al. (2007) showed that peak Hg in fish across all waterbodies flooded increased with area flooding up until ~100 % flooded area where it leveled off. Downstream effects were apparent, though variable (Bodaly et al., 2007). After 20-30 years, fish concentrations were similar to natural lakes nearby suggesting that the reservoirs and flooded lakes may be similar to the nearby lakes in their Hg cycling (Bodaly et al., 2007). Munson et al. (in prep) show significant increases in these same waterbodies from 2004-2010 but also in 4 of 5 natural lakes nearby suggesting that these key lakes may be responding to a factor other than regulation, as has been seen in other non-regulated waterbodies (Wang et al., 2019), such as emissions that influence both reservoirs and lakes (Munson et al., in prep).

1.3.6 Knowledge gap

There have been extensive studies of Hg in the environment which have advanced our understanding of its fate and effects considerably; however due to its presence in trace levels and complex cycling significant knowledge gaps remain. Though the post-impoundment increases in fish Hg are well documented (Bodaly et al., 2007; Bodaly et al., 1984), the impacts of river regulation decades after impoundment are not well understood. Flooded soil was likely a methylation hotspot during flooding for the CRD and other development in Manitoba. However,

further study of the MeHg production potential in the water fluctuation zone would be useful for understanding the impact of river regulation decades after impoundment.

As mentioned in section 1.3.4, several ELA experiments suggest that OM can regulate MeHg production and uptake in the food web. Assessing current and historical links between OM and Hg in the sediment history in areas that have been previously flooded for hydroelectric development would provide insight into Hg cycling in these environments. A better understanding of how OM controls MeHg production is needed to help project how complex environmental systems will respond to regional and global changes in climate and land use.

1.4 Study area

The study area (Figure 1.1) comprises an area of Northern Manitoba in the Hudson Bay Watershed along the Churchill and Nelson Rivers. The total area drained by the Nelson River includes more than 1,000,000 km^2 with the water coming from central Canada and United States, from east of the Rocky Mountains to northwestern Ontario and discharges an average of 2480 m^3/s into Hudson Bay (Rosenberg et al., 2005). Due to hydroelectric regulation, the highest flows occur in the winter (Manitoba Hydro, 2015). Major riparian plants along the Nelson River include: black spruce, tamarack, willow, alder, swamp birch, paper birch, trembling aspen and white spruce (Rosenberg et al., 2005). The region falls within the sporadic discontinuous permafrost range encompasses areas with permafrost ranging from 10-50 % of aerial coverage (Natural Resources Canada, 1993).

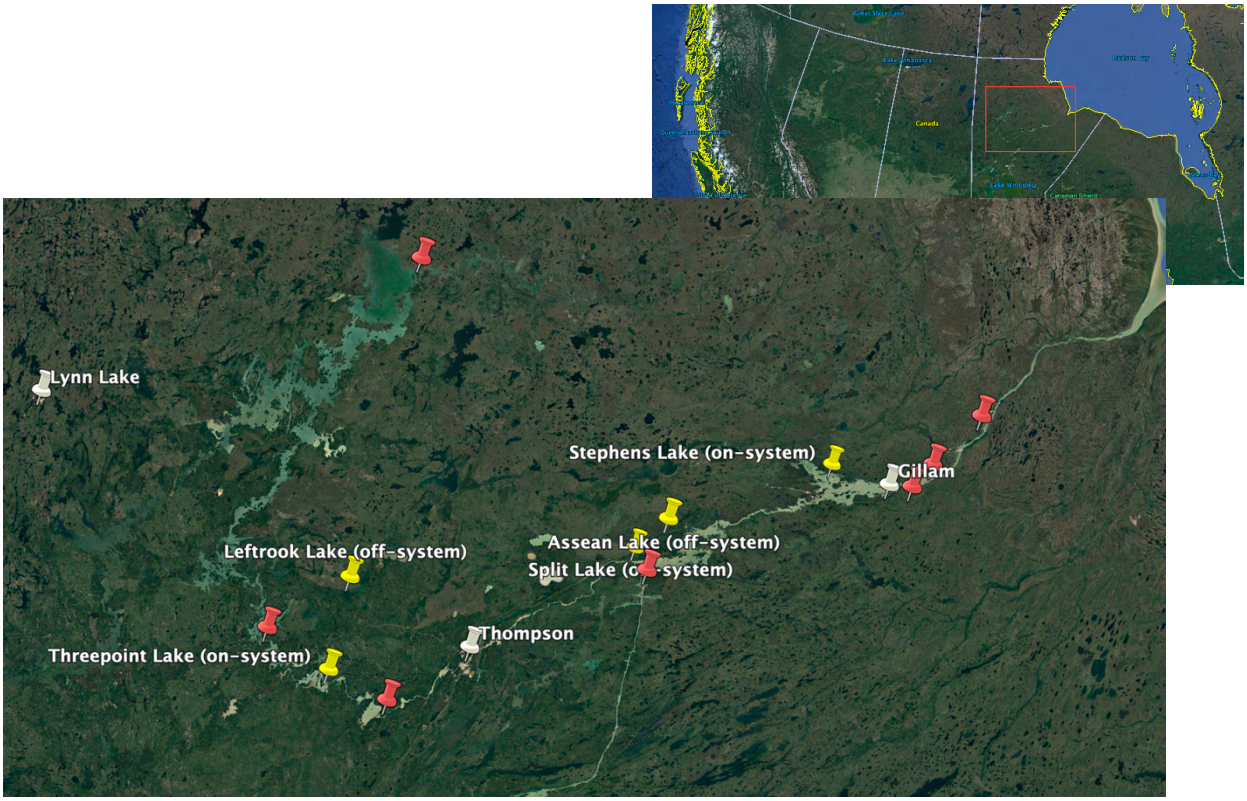

Figure 1.1: The study area on the Western Hudson Bay Watershed. The waterbodies studied are represented by yellow pins, nearby generating stations (GS) and control structures are represented by red pins and nearby towns are represented by white pins. The satellite image was sourced from Google Earth on October 3rd 2019.

The communities of Thompson, Lynn Lake and Gillam were chosen to represent regional climate data as they are the closest Environment and Climate Change Canada monitoring sites to the study area. The most recent climate data set available for the area is from 1981-2010. Thompson had average minimum precipitation in February of 16.5 mm and maximum in July with 80.9 mm. The coldest average temperatures occur in January with ranges of -29.3 to -18.3 °C and warmest in July with ranges of 9.1 to 23.1 °C (Environment and Climate Change Canada,

2011). Gillam received minimum average precipitation in February with 19.0 mm and the maximum occurring in July with 78.6 mm. Average temperatures in Gillam ranged from 9.7 to 21.8 °C in July, while minimum temperatures were reached in January ranging from -19.7 to -29.0 °C (Environment and Climate Change Canada, 2011). In Lynn Lake precipitation reached minimum average values in February at 16.3 mm and maximum values in July at 85.4 mm. Temperatures reached average lows ranging from -29.3 to -19.3 °C in January and highs in July of 10.3 to 22.1 °C (Environment and Climate Change Canada, 2011).

Canada's Changing Climate Report shows the Canadian prairies have seen air temperature increases between 1.1 ºC in the fall to 3.1 ºC in the winter from 1948 to 2016 (Environment and Climate Change Canada, 2018). Temperatures are expected to continue to increase rapidly compared to global temperatures, with the magnitude of the increase depending on global emissions of greenhouse gas, principally CO_2 (Environment and Climate Change Canada, 2018). Average precipitation in the prairies has increased by 7.0 % overall between 1948 and 2012, with decreases in the winter and increases in all other seasons, the largest occurring in the spring (Environment and Climate Change Canada, 2018). Precipitation in the region is expected to continue to increase, with the increases also depending on emissions and with large uncertainties. The model projections for the scenario RCP 2.6 is an increase of 5.0 % by 2050 over 1986 to 2005 levels with 25% of the model projections falling below -0.7 % (a small decrease) and 25 % of the model projections falling above a 10.8 % increase for the region (Environment and Climate Change Canada, 2018).

1.5 Thesis structure

This thesis is formatted in a "sandwich style" structure with four chapters starting with an introduction chapter, followed by two manuscript style chapters that each have their own corresponding abstract, introduction, methods, discussion, conclusion and reference sections. Finally, a conclusion chapter presents with major conclusions and ideas for future research. Chapter 1 (this chapter) serves as an introduction chapter, and provides a background and set up for the thesis. Chapter 2 will focus on a soil flooding experiment to understand how the frequent flooding of the soil through river regulation impacts Hg cycling decades after impoundment. Chapter 3 will focus on analytical results of water and sediment samples collected in Northern Manitoba and what changes in Hg cycling in recent history are observed in this data. Chapter 4 is a conclusion chapter, summarizing major conclusions and future research.

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Chapter 2: Sulfide and organic matter in nearshore soils influence methylmercury production in Northern Manitoba in on-system and off-system lakes

Abstract

Mercury (Hg) increases in fish have been commonly observed upon the creation of reservoirs in North America and Europe, but the impacts of river regulation on Hg cycling decades after impoundment remain largely unknown. The aim of this study was to better understand how Hg methylation and release from soil are altered by repeated flooding for hydroelectric regulation in Northern Manitoba, Canada, where extensive hydroelectric development has occurred for decades. Nearshore soils that have been periodically submerged in water and offshore soils that are 10-20 m farther inland were collected from two on-system lakes (lakes that were affected by hydroelectric development) and one off-system lake (a lake unaffected by hydroelectric development) in the region and studied for their methylmercury production potential in the laboratory. The soil was flooded with natural water in the laboratory and incubated for 196 hours to measure methylmercury (MeHg) and associated variables in soil, porewater and overlying water. Concentrations of MeHg in the flooded soil increased between 3- and 38-fold throughout the incubation with the highest concentrations found in the flooded nearshore soil of Stephens Lake, an on-system lake that serves as a hydroelectric reservoir. The elevated MeHg production in nearshore soils relative to offshore soils contrasts an earlier study in eastern Canada, and appears to depend on the chemistry of regional soils. The MeHg concentrations in porewater after the incubation correlated with the modelled concentrations of

neutral Hg-sulfide complexes. The flux of MeHg from the flooded soil was shown to consistently increase throughout the experiment. These results provide new insight into the role of Hg speciation in soil in controlling methylation and food web entry following repeated inundation.

2.1 Introduction

Mercury (Hg) can enter the environment by natural sources such as volcanoes or anthropogenic sources such as the combustion of coal and artisanal gold mining (AMAP, 2012). Once in the environment, Hg can undergo biotic methylation, with the resulting methylmercury (MeHg) being readily bioaccumulated and biomagnified in the aquatic food web (AMAP, 2012). All human populations surveyed have been shown to have MeHg exposure, but some populations have been shown to have higher exposure due to frequent consumption of fish and seafood (Basu et al., 2018). MeHg is a potent neurotoxin in humans and poses a particular risk to developing brains (AMAP, 2012).

Previous studies show that MeHg production in sediment is associated with the breakdown of organic matter (OM) (AMAP, 2012; Kelly et al., 1997). Reservoir construction, which can flood large areas of terrestrial soils rich in OM, is known to result in high Hg concentrations in fish from the reservoir and therefore present an increased risk of Hg toxicity to fish consumers (Bodaly et al., 2007; Willacker et al., 2016). The impact from the initial flooding of these environments is observed in fish Hg concentrations sampled after impoundment (Bodaly et al., 2007; Willacker et al., 2016). Though it is known that Hg in fish rises rapidly after impoundment, many of the processes that are responsible for this remain unknown and require further study. The effects of impoundment on fish Hg concentrations can impact local

communities dependent on the fish as their food or economic source. For example, in Northern Manitoba, Canada, the fisheries from Notigi, Rat, Mynarski, Wapisu and Issett lakes were closed in the late 1970s due to high Hg concentrations (Manitoba Hydro, 2015). With the current global increase in the development of hydroelectricity as a form of renewable energy, it is important to understand how to manage the ecosystem changes caused by our current and future hydroelectric development (Zarfl et al., 2015).

In the 1970s, the Churchill River Diversion (CRD) project diverted ~75% of the flow from the Churchill River in Northern Manitoba through the Rat and Burntwood River systems to increase hydroelectricity output from the generating stations on the lower Nelson River (Bodaly et al., 2007). After the CRD-driven flooding, Bodaly et al. showed that the fish Hg concentrations from all 10 affected lakes that they sampled increased to near or over export limits, while no increase was observed from nearby off-system lakes (Bodaly et al., 1984). In 2007, upon re-examination of the fish Hg data from the CRD-driven flooding, Bodaly et al. showed that peak Hg concentrations in the CRD area were related to the area of flooded soil. In addition, downstream effects were observed.

Similar increases in fish Hg levels from reservoir creation have been observed in other regions of Canada, the US and Finland (Bodaly et al., 2007; Porvari, 1998; Willacker et al., 2016). These studies show large increases in fish Hg levels after impoundment and slow decadal timelines to decrease toward background ranges (Bodaly et al., 2007). Willacker et al. conducted a broad study of Western US and Canada which showed that fish from reservoirs have higher Hg concentrations than those from natural lakes in many ecoregions regardless of the amount of time that has passed since reservoir creation (Willacker et al., 2016). They also saw significant rises based on interannual water level fluctuations and timing of minimum water storage, suggesting

that changes in the operation of these reservoirs could make a significant difference in fish Hg (Willacker et al., 2016).

Some of the most important studies that assess flooding and the resulting effects on the Hg cycle occurred in the Experimental Lakes Area in Northwest Ontario, where experimental reservoirs were created to study the impact of hydroelectric development (Hall et al., 2005; Hall et al., 2009; Rolfhus et al., 2015; St Louis et al., 2004). These experiments flooded a wetland and three small lakes that differed in the amount of OM present in soils. They found that the soil OM content could affect how long elevated Hg was observed in biota in those reservoirs but could not predict the timing or height of the peak concentrations (Hall et al., 2009). The type of soil OM, rather than the amount, may therefore affect the timing of peak Hg in the food web. A laboratory study in Finland flooded both peat and humus in aerated and non-aerated conditions. Throughout a 117-day study, the non-aerated incubations produced more MeHg while the OM source affected the timing of MeHg increase and amount produced (Porvari and Verta, 1995). Further than just the type of OM flooded, Eckley et al. (2017) studied a reservoir and compared MeHg in the occasionally flooded soil and the always flooded sediment and measured higher MeHg in the occasionally flooded soil. Liu et al. (2017) studied Hg cycling in the water fluctuation zone, incubating sediment from the Three Gorges Reservoir in China and found that sulfur speciation due to repeated drying and wetting affects methylation.

Recently Schartup et al. (2015) studied the potential impact of reservoir creation on the east coast of Canada. They incubated soil in lab experiments with both occasionally flooded nearshore soils and offshore soils and found a higher flux to the water column from the offshore soil compared to the nearshore soil. The duration of the experiment, 120 hours, did not capture the maximum flux of Hg to the overlying waters, suggesting that the measured flux

underrepresented the total potential for flooded soil as a source of MeHg. However, their experimental design eliminated photodemethylation, which could occur in shallow nearshore waters, and mixed overlying waters throughout the incubation, which could decrease the concentration gradient across the sediment-water interface relative to in situ conditions. Both of these experimental conditions could lead to measured fluxes that overrepresented flooded soil as a source of MeHg.

Three water bodies in Northern Manitoba, Stephens Lake, Assean Lake and Split Lake, were selected to study the impacts of soil inundation on MeHg fluxes. These three water bodies were chosen because they have long term observation data sets including fish Hg concentrations and accompanying aquatic chemistry (Bodaly et al., 2007; Manitoba Hydro, 2015). In addition, these waterbodies allow a contrast between a natural waterbody and regulated waterbodies that are subject to similar environmental conditions, such as temperature, precipitation and soil composition. Our study was designed to examine the net effect of flooding these areas and using realistic summertime conditions such as incubation temperature and light exposure. In addition, our study extended the incubation time used in a similar study (Schartup et al., 2015) from 120 hours to 196 hours in hopes of catching the peak flux of MeHg from the flooded soil.

2.2 Methods

2.2.1 Area of study

The study area includes Stephens Lake and Split Lake, both "on-system" lakes affected by hydroelectric development and Assean Lake, an "off-system" lake unaffected by hydroelectric development, all in the Lower Nelson River Basin. Stephens Lake is a reservoir created by the construction of the Kettle Generating Station on the lower Nelson River. The

construction began in 1966, with impoundment completed in 1970, flooding 221 km^2 and creating Stephens Lake. Split Lake is ~10 km downstream of Kelsey Generating Station on the Nelson River which was in operation by 1961 as a run-of-the-river dam (Manitoba Hydro, 2015). Both Split Lake and Stephens Lake began to receive water from the CRD in 1977 (Bodaly et al., 2007; Manitoba Hydro, 2015). Assean Lake is a natural lake that is in the same geographic region, about 2 km north of Split Lake, and is not affected by these reservoirs nor by the CRD (Manitoba Hydro, 2015).

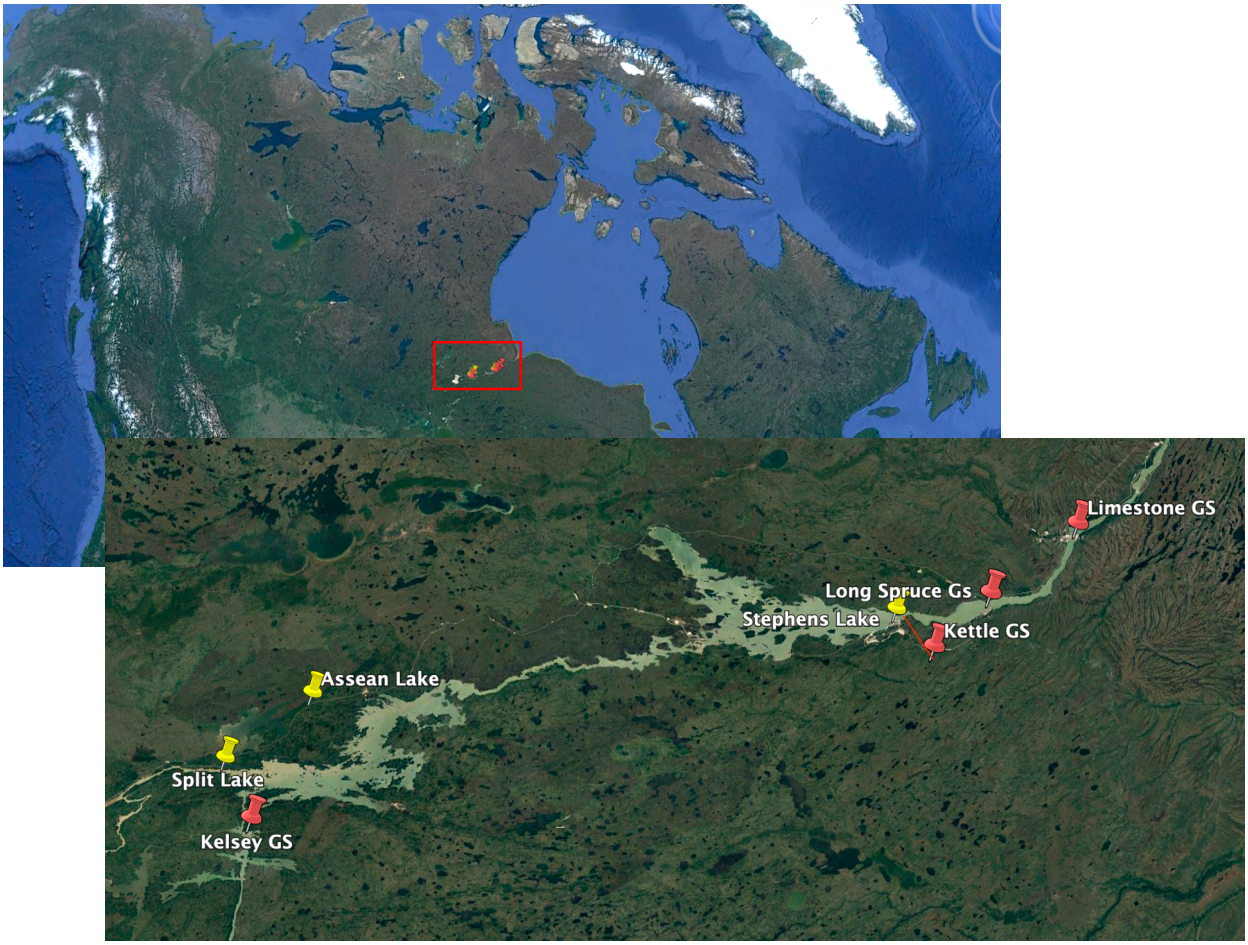

Figure 2.1: Map of the study area in the Lower Nelson River Basin in Northern Manitoba with sampling stations represented in yellow pegs and nearby generating stations in red pegs. The satellite image was sourced from Google Earth in 2019.

Table 2.1: Sampling site coordinates and description for soil samples collected in Northern Manitoba.

Site	Latitude	Longitude	Material collected
Stephens Lake	56°22'38.12"N	94°38'42.23"W	Nearshore and offshore soil (~10-20 m apart)
Split Lake	56° 8'32.18"N	96°36'27.11"W	Nearshore and offshore soil (~10-20 m apart)
Assean Lake	56°14'52.99"N	96°21'13.73"W	Nearshore and offshore soil (~10-20 m apart)

2.2.2 Sampling

In July 2017, the top 10 cm of occasionally flooded soil was collected from near the shore (within 5 m of the water line) of Stephens Lake, Assean Lake and Split Lake and offshore soil was collected 10-20 m inland. Samples were collected using a steel spade and stored in Ziploc bags in a cooler in the field. Natural water samples were also collected from the surface of Nelson-Burntwood River system, including soil sampling sites, to be used as the "flood water". The unfiltered water was collected into acid-cleaned 2.2 L wide mouth Teflon (Nalgene) bottles using the clean hands/dirty hands technique (Davidson, 1999). Both the soil and flood water samples were stored at 4 °C prior to the incubation experiment.

2.2.3 Incubation

Upon arrival to the laboratory, the soil samples were homogenized by gloved hands after surface vegetation was removed. The soil was packed gently into duplicate acid-cleaned polycarbonate incubation tubes (4.4 cm I.D. by 30 cm height) to roughly 10 cm. The incubation tubes were fixed vertically in a dedicated environmental chamber maintained at 20 ± 1 ºC on a 24-hour diurnal cycle with 12 hours light and 12 hours darkness. The tubes were flooded with 10 cm of

the flood water, filtered to 0.2 μm (AcroPak 500 Supor membrane capsule filter, Pall Corporation) in the laboratory to remove larger particles and to minimize potential microbial methylation in the overlying water. During the 8-day incubation, the overlying water was sampled roughly every 24 hours for total Hg (THg) and MeHg by carefully mixing the water column and removing half of the water with acid cleaned syringes. An equal amount of flood water was then added to each experiment tube to keep the volume constant. The sample water was filtered to 0.45 μm (Supor 200, 47 mm Pall Corporation) using re-usable filter cups (47mm, Nalgene) and analyzed as described below in the class-100 cleanroom of the Ultra-Clean Trace Elements Laboratory (UCTEL). Duplicate controls for the experimental setup used ultrapure water (< 18 MΩ·cm, Milli-Q) in incubation tubes in the absence of soil. A third control tube contained only the flood water to account for changes in the absence of soil.

At the end of the incubation, the water was sampled again for THg, MeHg as well as dissolved organic carbon (DOC). The remaining water was carefully removed from the incubation tubes using syringes and the tubes containing flooded soil were transferred to a N_2 filled glove box (LABmaster by MBRAUN, < 1 ppm O_2). In the glove box, the flooded soil was subsampled in four sections by depth. Porewater was extracted from each of these sections using a centrifuge (Heraeus Megafuge 40R, Thermo Scientific) at 3000 rpm and 22 °C for 30 min and was filtered to 0.02 μm using a syringe filter (Anatop 25 inorganic membrane filter, 25 mm, GE Healthcare). The filtrate was analyzed for pH (Orion Model 420A+, Thermo Scientific), sulfide, THg and MeHg. The flooded soil was stored at –20 °C until it was processed for analysis.

2.2.4 Analysis

Detection limits were calculated for the methods presented below according to the following formulas (Skoog et al., 1998).

$$S_m = \bar{S}_{bl} + ks_{bl} \qquad \text{Equation 2.1}$$

$$detection\ limit = \frac{s_m - \bar{s}_{bl}}{m} \qquad \text{Equation 2.2}$$

Where S_m is the minimum detected signal, calculated as the sum of the average blank signal $\bar{S}_{bl}$ and a multiple of k standard deviations of the blanks (s_{bl}). A value of $k = 3$ was used throughout. The value of S_m then gets plugged into equation of the line used for quantification as the y value, rearranged in the form of Equation 2.2 (Skoog et al., 1998).

THg in water and pore water was analyzed on a Tekran 2600 Mercury Analyzer following EPA method 1631 (USEPA, 2002). Briefly, this method consists of a reduction of all Hg to Hg(0), followed by the phase separation of the Hg(0) gas from solution, concentration on gold traps and detection by fluorescence. Quantification was done by standard curves verified by a certified reference material, BCR579 (Hg = 9.5 ± 2.5 pmol kg^{-1}, Institute for Reference Materials and Measurements, European Commission – Joint Research Centre). The detection limit was calculated according to Equations 2.1 and 2.2 to be 0.15 ng/L.

MeHg in water and porewater was determined on a Brooks Rand MERX instrument following EPA method 1630 (USEPA, 1998). This method involves ethylation of Hg species, a separation step by gas chromatography and quantification by fluorescence detection (USEPA, 1998). Quantification was done by standard curves and with a 2 pg internal standard of n-propyl mercuric chloride (prepared from a 10.1 mg/L stock solution in HPLC-grade isopropanol, Anachemia Science, ERA/Waters) to correct for changes in ethylation efficiency. The detection limit was calculated according to Equations 2.1 and 2.2 to be 0.16 pg.

The flooded soil was freeze dried (Laconco Freezone 2.5), ground with a mortar and pestle and sifted with a 300 μm sieve to remove large particles. The remaining soil was then analyzed for THg and MeHg. Soil THg was analyzed using a standard method on a Hydra II Direct Mercury Analyzer (Uthe et al., 1970). This method pyrolyzes the sample and measures the released Hg using atomic absorption. The THg was quantified using an external standard curve prepared with the certified standard material MESS-3 (Marine Sediment Reference Materials for Trace Metals and other Constituents from the National Research Council of Canada; THg = 0.091 ± 0.009 mg kg^{-1}). The detection limit was calculated according to Equations 2.1 and 2.2 to be 0.2 ng.

Soil MeHg was first distilled (Bowles and Apte, 2000; Horvat et al., 1993) and then diluted in ultrapure water to be analyzed using the same method as water samples described above. In brief, ~ 0.05 g of the soil sample was distilled in 12.5 mL ultrapure water containing 1.2 % m/v KCl (ACS grade, EMD Millipore), 2 % m/v $CuSO_4$ (ACS grade, ≥ 98.0 %, Sigma Aldrich) and 1 % v/v H_2SO_4 (ACS grade, 95.0-98.0 %, Sigma Aldrich) in acid cleaned Teflon distillation tubes into receiving vials assisted by N_2 (grade 4.8 high purity, Praxair, Winnipeg) into 5 mL of ultrapure water to ~ 80 % completion. The MeHg recoveries of this method were verified using the certified reference material ERM CC580 (estuarine sediment, MeHg = 0.075 ± 0.004 mg kg^{-1}, Institute for Reference Materials and Measurements, European Commission – Joint Research Centre). The detection limit was calculated according to Equations 2.1 and 2.2 to be 0.9 pg.

DOC in the overlying water was analyzed at the end of the incubation following filtration to 0.45 μm (pre-combusted GF/F, Whatman) using a Thermalox TOC/TN Analyzer (Ducklow et al., 2007). Samples were quantified using a calibration curve made from a potassium hydrogen

phthalate (KHP) standard (1000 ± 10 mg/L, Sigma-Aldrich). The detection limit (calculated according to Equations 2.1 and 2.2) is 0.2 mg/L.

Sulfide in porewater samples was complexed to 1 mL of 4 % w/v zinc acetate (Trace metal basis, 99.99 %, Sigma-Aldrich) for increased stability and measured by the methylene blue method (Cline, 1969). Briefly, this method involves a chemical reaction between sulfide and N, N-dimethyl-p-phenylenediamine followed by an oxidation with Fe(III) to produce methylene blue which can be detected at 667 nm using a UV-Vis Spectrometer. This reaction is quantified through the use of a standard curve prepared with sodium sulfide in deoxygenated water and validated by iodometry. The method detection limit using a 1 cm cuvette (calculated according to Equations 2.1 and 2.2) is 0.08 μM.

Sulfide speciation was estimated by a chemical equilibrium model MINEQL+ (Environmental Research Software). THg, MeHg, pH and sulfide were used to model the speciation of sulfide in each porewater profile. Recently updated constants were used (Liu et al., submitted).

The flux of THg and MeHg were calculated according to the following equations:

$$Flux_{MeHg} = ([MeHg]_2 - [MeHg]_1)/(t_2 - t_1) \quad \text{Equation 2.3}$$

$$Flux_{THg} = ([THg]_2 - [THg]_1)/(t_2 - t_1) \quad \text{Equation 2.4}$$

$$[MeHg_1] = \frac{[MeHg_{flood\,water}]}{2} + \frac{[MeHg_{-1}]}{2} \quad \text{Equation 2.5}$$

$$[THg_1] = \frac{[THg_{flood\,water}]}{2} + \frac{[THg_{-1}]}{2} \quad \text{Equation 2.6}$$

where $[MeHg]_t$ and $[THg]_t$ are the concentrations of MeHg and THg in the overlying water at an incubation time t. The time of sampling is t_2, while t_1 is the time of flood water was added after the previous sample and t_{-1} is the time of the previous sampling.

Statistical analysis was carried out using Prism software (version 8.2.1, Graphpad). A significance threshold of $p < 0.05$ was used throughout. As all data was random and interval/ratio data, if the variables tested passed the Kolmogorov-Smirnov test at $\alpha = 0.05$, the data was assumed to be normally distributed and parametric tests were used. If the Kolmogorov-Smirnov test did not pass, the non-parametric tests were used. Correlations were tested for association using Pearson correlation for normally distributed variables and Spearman correlation for variables that were not normally distributed. If a strong significant association was found for data that was normally distributed, linear regression was calculated. A Mann-Whitney test was used to test if MeHg concentrations differed significantly in nearshore and offshore flooded soil from each site.

2.3 Results

2.3.1 MeHg concentrations

MeHg was produced in the flooded soil of all experiments after flooding as seen in Figure 2.2. An average of all depths from duplicate tubes showed MeHg increased 3-38 times over the 196-hour incubation. After flooding and incubating, Stephens Lake nearshore soil increased to an average of 13 % MeHg of THg, the highest observed.

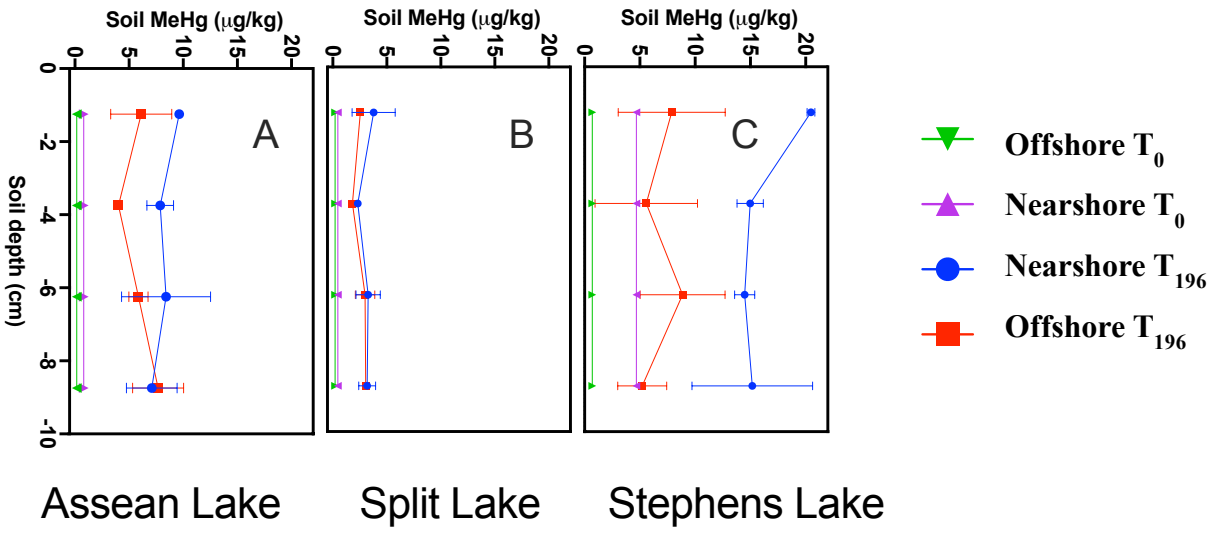

Figure 2.2: Vertical distribution profiles of methylmercury (MeHg) in the flooded soil from Assean, Split and Stephens Lakes throughout the 196-hour flooding incubation. Initial concentrations (T_0) are uniform throughout the 10 cm depth of homogenized soil from both offshore (green triangles) and nearshore (purple triangles) samples. At the end of the incubation (T_{196}), offshore (red squares) MeHg were lower than nearshore (blue circles) samples. The error bars represent one standard deviation between duplicate experiments.

After the 196-hour incubation, the DOC in the overlying water was negatively correlated to the proportion of larger particles (> 300 μm) in the soil, as seen in Figure 2.3. This suggests that the larger particles are mostly inorganic. In addition, the larger particles have less surface area relative to sediment volume available for decomposition of OM to take place and release into the overlying water. Figure 2.4 shows a subsequent negative correlation between the MeHg in the soil at the end of the flooding incubation and the percentage of soil greater than 300 μm (representing the OM content and particle size). This results in more MeHg in the higher OM content soils following the incubation.

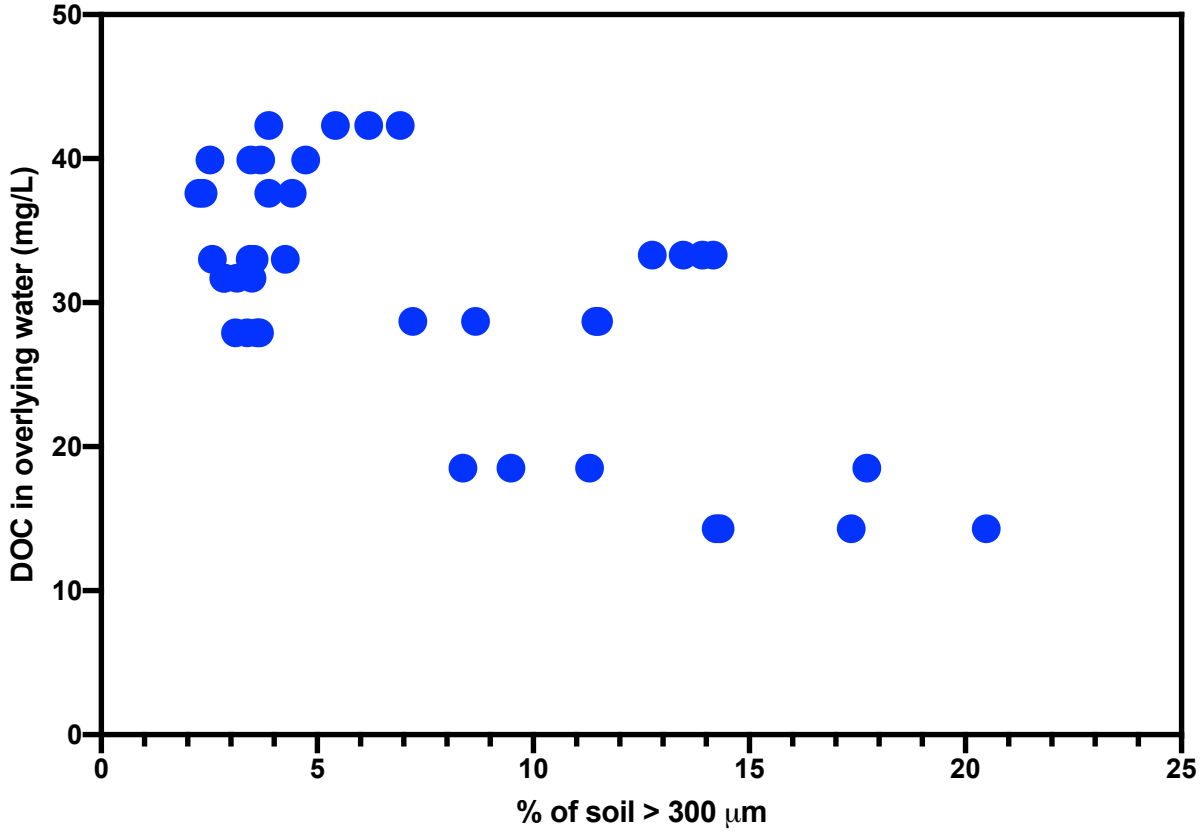

Figure 2.3: Dissolved organic carbon (DOC) in overlying water of all soil incubation experiments plotted against the percentage of soil particles larger than 300 μm after 196-hour flooding incubation. Spearman correlation shows $\rho = -0.39$ and $p = 0.01$.

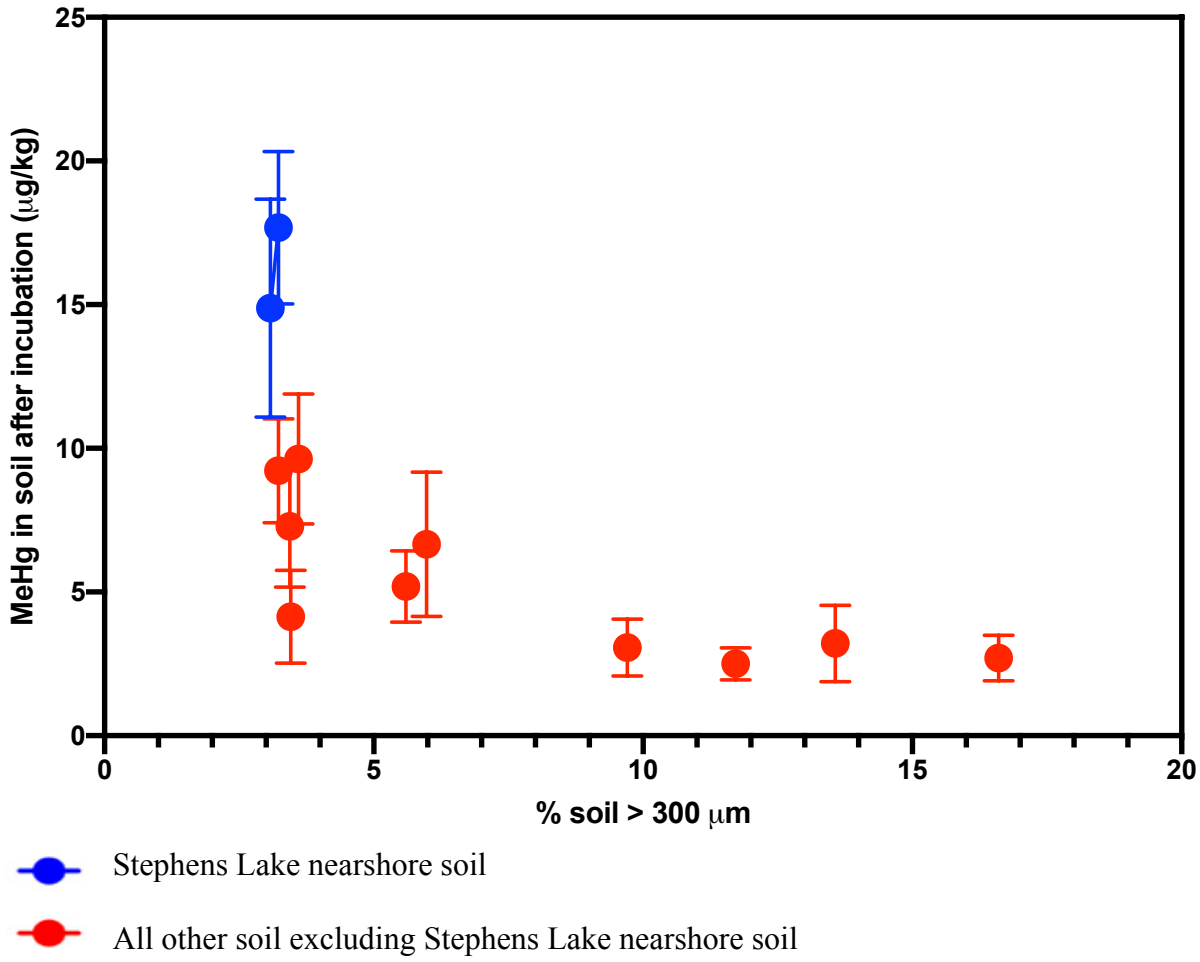

Figure 2.4: Spearman correlation of the relationship between methylmercury (MeHg) in the flooded soil after the 196-hour incubation period and the percentage of soil particles greater than 300 μm in size ($\rho = -0.79$, p value = 0.009) in Stephens Lake offshore and Assean and Split Lake soil flooding experiments (red circles). Flooded Stephens Lake nearshore soils (blue circles) are excluded from the correlation. The MeHg in the flooded soil is represented by an average concentration taken every 2.5 cm of the 10 cm core. The error bars represent one standard deviation between duplicate samples.

Porewater MeHg concentrations and modelled $Hg(HS)_2^0$ species in porewater were correlated across all experiments (Figure 2.5) at the end of the incubation.

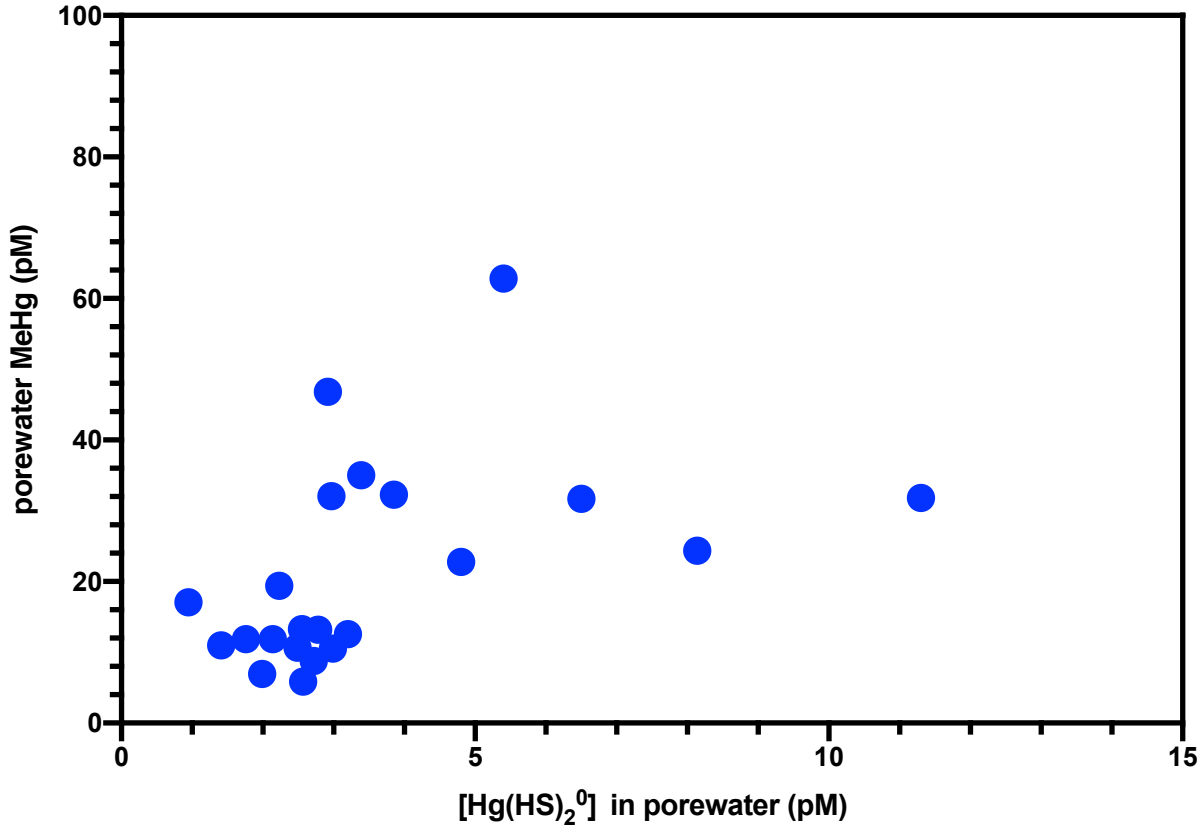

Figure 2.5: Porewater methylmercury (MeHg) concentrations versus modelled porewater sulfide speciation in flooded soil porewater after the 196-hour incubation from all soil flooding experiments. Spearman correlation showed a positive correlation ($\rho = 0.62$, p value = 0.002) between the porewater MeHg in flooded soil and the modelled $Hg(HS)_2^0$ concentration in the porewater.

2.3.2 Hg fluxes

MeHg fluxes from the flooded soil start off negative and increase throughout the course of the experiment (Figure 2.6). The overall THg fluxes (Figure 2.7) have a bimodal shape, first decreasing, then increasing and finally decreasing again. All changes to the overall trend were significant (p value < 0.05).

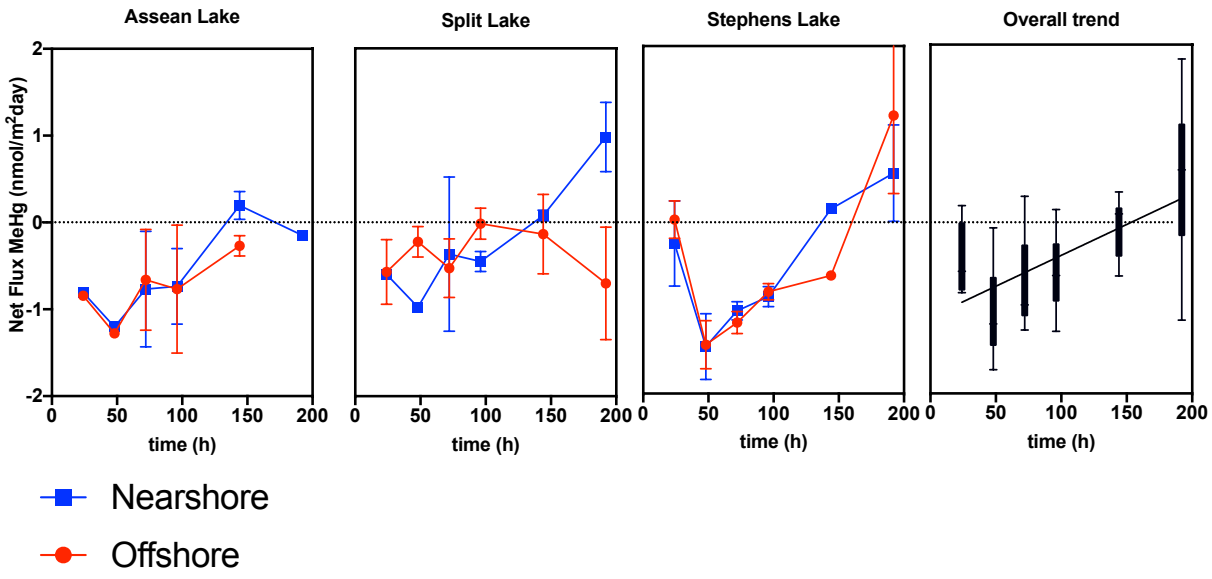

Figure 2.6: The net methylmercury (MeHg) fluxes to the overlying water in both nearshore (blue squares) and offshore (red circles) cores throughout duplicate experiments from Assean, Split and Stephens Lake and the overall trend (right panel). The error bars in the left three panels represent one standard deviation between duplicate experiments. The overall trend, displayed as a box and whisker plot, shows a linear regression p value of < 0.0001 and $R^2 = 0.32$. The control water tube (incubation water with no soil) was used for correction.

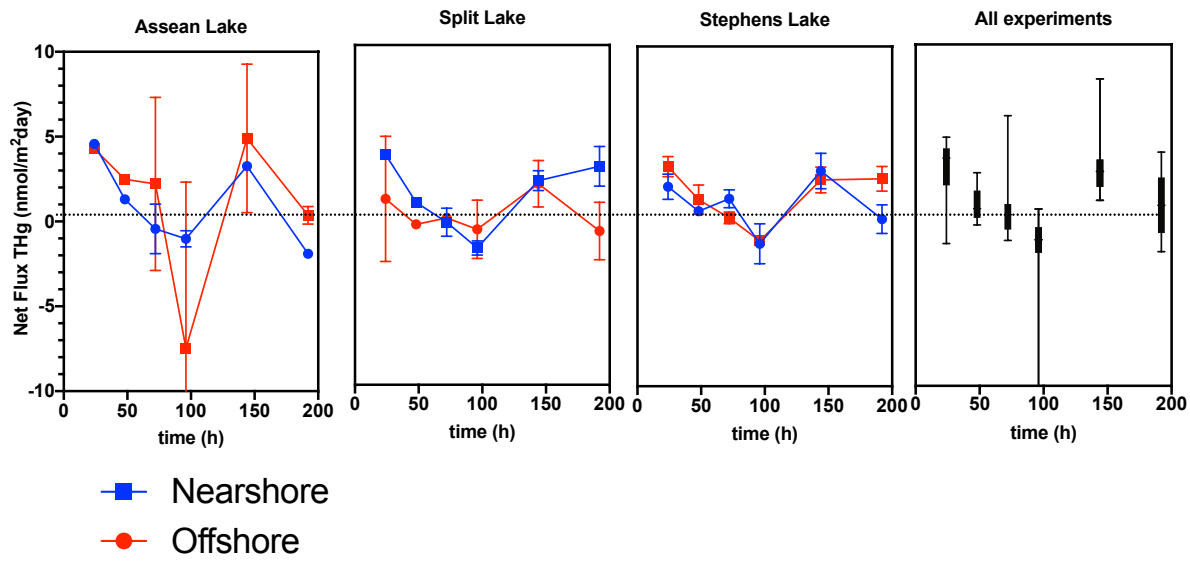

Figure 2.7: The net total mercury (THg) net fluxes to the overlying water in both nearshore (blue squares) and offshore (red circles) flooded soil throughout duplicate experiments from Assean, Split and Stephens Lakes and the overall trend (right panel). The error bars in the left three panels represent one standard deviation between duplicate experiments. The overall trend is represented by a represented in a box and whisker plot and shows a significant increase after 96 hours and a significant decrease after 144 hours (Mann Whitney test). The control water tube (incubation water with no soil) was used for correction.

2.4. Discussion

2.4.1 Nearshore vs offshore soil

All nearshore soil contained higher MeHg than the offshore soil after the incubation, but the difference was only significant in Stephens Lake (Mann-Whitney test: p value = 0.03). The nearshore soil of Stephens Lake has experienced the greatest impact from frequent inundation due to hydroelectric development and showed the highest MeHg concentration in the flooded soil after the incubation. The initial concentration in the Stephens Lake nearshore soil was 4.7

μg/kg, while all others were 0.8 μg/kg or lower. The elevated MeHg in impounded soil however is consistent with the findings of Willacker et al. (2016), which showed that reservoirs in Western United States and Canada had fish Hg concentrations 44% above those of lakes. The higher initial MeHg concentrations in the soil from Stephens Lake and the high levels after flooding are consistent with previous studies that found that soils in water fluctuation zones are primed for methylation due to their unique chemical properties prior to flooding (Eckley et al., 2017; Liu et al., 2017).

As seen in Figure 2.6, the overall net flux of dissolved MeHg from the flooded soil to the water column starts off negative and trends towards positive values by the end of the experiment. The net flux was calculated by the change in dissolved MeHg concentration in the water column over time (Equation 2.3). Possible reasons for the initially strong negative net flux despite the marked increase in MeHg of the flooded soil are: loss of MeHg to photodemethylation and particle scavenging. Previous studies show that MeHg in the presence of DOC is subject to photodemethylation (Tai et al., 2014), which can take place in the shallow water column when exposed to 12 hours of daylight in the incubation chamber. Light exposure was included in this experiment in order to replicate the shallow (< 0.5 m) coastal waters that are likely to cover nearshore soils during changes in water levels. Other experiments have used dark incubations and isotopically enriched Hg species additions to simultaneously quantify the rates of methylation and demethylation (Schartup et al., 2015). We did not use isotopically enriched Hg species as they are known to act differently than the Hg species bound to natural organic matter and are not needed to determine the net changes in MeHg fluxes or concentrations (Liang et al., 2004). In addition, the light/dark cycle and the incubation temperature were used to model realistic local summertime conditions.

After an initial negative flux in all incubations, the incubations of nearshore soils showed increasing MeHg flux to the water column throughout the experiment, while increases in the MeHg concentrations of offshore soils were only significant in Stephens Lake soil. This result is contrary to that of a flooding experiment from eastern Canadian coastal soils conducted by Schartup et al., which showed greater fluxes from offshore soil than the occasionally flooded nearshore soil (Schartup et al., 2015). The study in eastern Canada showed a small pulse of MeHg, peaking after three hours in nearshore soil and an increasing flux of MeHg to the overlying water in offshore soils beyond their 120-hour incubation time. These differences may be due to local soil composition. Soil composition has been shown to influence long-term increases in MeHg in experimentally flooded humus relative to peat. Flooded humus took longer than peat to rise in MeHg (lower in the first 46 days), but increased throughout the remaining time of the experiment and was higher after 117 days (Porvari and Verta, 1995). These results further showed that humus acted as a MeHg carrier, releasing MeHg as the OM was degraded, while methylation occurred more rapidly in peat and was released faster (Porvari and Verta, 1995). These experiments suggest that the source of OM is very important in both the rates of MeHg production and release.

Split Lake nearshore soil had the highest flux of MeHg at the end of the experiment and the highest pore water MeHg concentrations, despite relatively low MeHg concentrations in the flooded soil. Contributing factors to the elevated Split Lake MeHg may include lower demethylation in the pore water and water column of Split Lake as well as differing adsorption of Hg and MeHg to particulate matter present in the soil and water column, as has been seen in lacustrine sediment from a Canadian Shield lake (Feyte et al., 2010). The increased movement

of MeHg from Split Lake flooded soil to the water column may result in lower MeHg storage, which influence higher peak concentrations in the food web and greater export downstream.

In our experimental setup we only measured filtered water and therefore cannot estimate how much particulate THg or MeHg was present throughout the experiment. In order to ensure accurate sampling of overlying water in the flooded soil tubes to quantify MeHg and THg fluxes, the water was carefully mixed before sampling. The mixing of overlying water precluded the differentiation between changes in particulate matter from the water column and the re-suspended flooded soil during sampling. Full quantification of dissolved and particulate MeHg and THg has been used in previous studies that have utilized mesh to separate the flooded soil from overlying water (Porvari and Verta, 1995). However, unfiltered MeHg has been shown to be a good predictor of biomagnification (Hall et al., 2005; Wang et al., 2018).

2.4.2 Role of OM

Figure 2.7 shows a time dependent THg flux to the water column. Overall the fluxes of THg from the flooded soil to the overlying water column are mostly positive. This results in a large increase in the THg in the overlying water, but because the pool of THg is much larger in the flooded soil, the concentration in the soil does not change by a measurable amount. The flux of THg from the flooded soil shows a multimodal shape, with high fluxes at the beginning, which decrease over time, presumably representing the breakdown of OM releasing its associated Hg. The inflection point may represent a change in metabolism of anaerobic bacterial methylation in the flooded soil, as was observed by Schartup et al. (2015) due to a change in electron acceptors after a similar amount of time. A subsequent increase in MeHg within the

water column begins at this time. This is consistent with activity of sulfate reducing bacteria acting as a key player in Hg methylation, active under anoxic conditions.

Results show a general trend of the flooded soil with higher OM having a greater MeHg concentration after the incubation (Figure 2.4), consistent with results from experimental reservoirs which showed the influence of high OM degradation on MeHg production (Hall et al., 2005). However, the Stephens Lake nearshore soil, which is most influenced by hydroelectric development, did not fit into this model as the concentration of MeHg could not be adequately explained by the OM content of the incubated soil. Due to differences in Stephens Lake nearshore soil, values from these soils were excluded in the calculation of the Spearman correlation. The difference in chemistry of the nearshore soil of Stephens Lake reservoir relative to natural water bodies is consistent with differences in the MeHg cycling and entry into the aquatic food web.

2.4.3 Role of sulfide

Previous work shows that the wetting and drying cycles that occur due to river regulation can increase the neutral sulfur species, which stimulate sulfate reducing bacteria producing sulfide and MeHg (Liu et al., 2017). Porewater had measurable sulfide consistent with previous observations of methylation by sulfate reducing bacteria (Compeau and Bartha, 1985). The presence of sulfide in the soil indicates rapid establishment of anoxic conditions in the soil following inundation. The modelled sulfide data in Figure 2.5 shows a positive correlation between the concentration of $Hg(HS)_2^0$, a neutral Hg-sulfide complex that is presumed to readily cross biological membranes (Benoit et al., 1999), in the porewater and the porewater MeHg

concentration. This suggests that the Hg-sulfide speciation affects the bioavailability of the inorganic Hg to methylators and thus plays a significant role in these environments.

The pore waters were filtered to 0.02 μm prior to analysis of sulfide speciation and therefore the Spearman correlation excludes the dynamics of particles, which may contribute to its variability. The variation may be due to differing adsorption constants of Hg and MeHg in the presence of the organic species present in the water column, the water bound particulate and the flooded soil (Feyte et al., 2010). These smaller particles will be taken up and released by the methylators and likely make up a large component of the MeHg (Hill et al., 2009). The chemistry of the neutral mercury sulfide species allows them to cross cell membranes of Hg methylators passively (Hsu-Kim et al., 2013).

2.5. Conclusions

We flooded soil in an incubation experiment to monitor increases in MeHg in flooded soil, pore water and the overlying water column. MeHg increased in the flooded soil by 3-38 times over the 196-hour incubation time course. Concentrations of MeHg in nearshore soil from Stephens Lake, a hydroelectrically influenced reservoir, were higher than the offshore soil both before and after the incubation reaching levels of 13 % MeHg of THg. In comparison with natural waterbodies, the elevated MeHg concentrations after the incubation of nearshore soil from a hydroelectric reservoir are consistent with the bioavailability of Hg sulfide complexes controlling Hg methylation. Furthermore, these changes in bioavailability appear related to the priming of the sulfur cycling by repeated wetting and drying cycles in nearshore soils. These differences persist despite the influence of photodemethylation and higher temperatures that control net summertime MeHg production in natural waterbodies.

Overall, MeHg fluxes to the water column started off negative and increased over the course of the incubation in soil from all locations, which held for soils at each individual nearshore site and offshore Stephens Lake. These results contrast incubations of east coast soils that showed only a short pulse, peaking at three hours, of MeHg to the overlying water from nearshore soils. As this was not seen in either the on-system or off-system lakes, we conclude that these differences must be due to the regional composition of soils. Though the nearshore soil of Stephens Lake reached higher concentrations of MeHg, the flux of MeHg between the nearshore and offshore soil were not significantly different. Both were continuing to increase however and the nearshore soil that was higher in MeHg may result in higher fluxes of MeHg or high fluxes over a longer duration if flooding time was increased. We therefore conclude that due to the chemistry of on-system waterbodies, the increased MeHg production upon flooding in the nearshore soils within the water fluctuation zone of older reservoirs may represent an important source of MeHg that can be transferred to the water column and subsequently the food web, which may contribute to variability in the long-term recovery of regional fish Hg following reservoir impoundment.

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Chapter 3: Sedimentation rate drives the flux of mercury to the sediments of on-system and off-system lakes in Northern Manitoba

Abstract

Hydroelectric development and climate change are altering processes by which mercury (Hg) moves through the environment. To better understand the effects of these changes on the waterbodies within the Hudson Bay Watershed, sediment cores were collected from on-system lakes (lakes that were affected by hydroelectric development) and off-system lakes (lakes that were not affected by hydroelectric development) in Northern Manitoba and were subsequently dated by ^{210}Pb and analyzed for total mercury (THg) and methylmercury (MeHg) concentrations and fluxes. Profiles of water quality parameters including dissolved oxygen, temperature and pH were measured along with concentrations of THg and MeHg from the water column both under ice at the time of sediment collection (March/April) and during an open water season (August). Results of this study show that sedimentation is driving the flux of THg in both on-system and off-system lakes. Fluxes of THg to sediments in sampled waterbodies are still increasing despite global reductions in anthropogenic Hg emissions. Greater knowledge of Hg cycling in these environments is key to helping ensure a safe food source for Northern populations that depend on the bounty of fish that these environments provide.

3.1 Introduction

Mercury (Hg) is predominantly released into the environment from anthropogenic sources as gaseous elemental Hg(0), which gets oxidized in the atmosphere to form Hg(II). Hg(II) enters the aquatic environment by both wet and dry deposition and can be biologically methylated to form methylmercury (MeHg), a developmental neurotoxin that biomagnifies in the aquatic food web (AMAP, 2012). MeHg presents a risk for Northern Populations that depend on fish and marine mammals as a traditional food source (AMAP, 2012). Recognition of the global sources and transport of Hg led to ratification of the Minamata Convention that entered into effect in 2017 aiming to reduce anthropogenic Hg emissions (Wang et al., 2019).

Northern Manitoba has few large sources of Hg, though at one time a smelter near Flin Flon, Manitoba, Canada, was the largest source of Hg in North America (Wiklund et al., 2017). Although atmospheric Hg(0) concentrations and North American Hg(II) deposition are declining (Steffen et al., 2015; Prestbo and Gay, 2009), Hg concentrations in many biota continue to increase (Wang et al., 2019). The sub-Arctic, northern environment in Manitoba is changing rapidly both from climate change (AMAP, 2012) and hydroelectric development (Bodaly et al., 2007). Climate change is affecting Hg cycling by raising temperatures, altering precipitation, increasing coastal erosion, thawing permafrost, and changing biological communities (AMAP, 2012). Hydroelectric regulation in Manitoba has been extensive with 15 hydroelectric generating stations currently in operation (Manitoba Hydro, 2018), many of which rely on enhanced flow from the Churchill River Diversion (CRD), which has diverted ~75 % of the flow of the Churchill River through the Rat-Burntwood River (RBR) system towards the Nelson River, to enhance electricity output (Manitoba Hydro, 2015). These changes affect water residence times, flow dynamics, shoreline erosion, sedimentation, oxygen and also required

flooding large areas of soil to form reservoirs (Manitoba Hydro, 2015), all of which affect Hg sources and cycling.

Various federal and provincial agencies, including the Department of Fisheries and Oceans (DFO) and the Manitoba Department of Natural Resources, have led efforts, some since 1970, to measure Hg in fish following impoundment in Northern Manitoba. Since 2008, these efforts have been continued with the Coordinated Aquatic Monitoring Program (CAMP) between the province of Manitoba and Manitoba Hydro. Results show that upon CRD-driven flooding, fish Hg concentrations show a similarly shaped Hg profile for all waterbodies in the flooded region in earlier decades. A rapid significant increase in fish Hg occurred within the first decade of flooding followed by subsequent declines towards background levels (Bodaly et al., 2007). However, in recent years fish Hg concentrations have increased within many waterbodies, both in on-system and off-system lakes (Munson et al., in prep), as well as in other regions including the Great Lakes (Blukacz-Richards et al., 2017; Zhou et al., 2017). These increases contradict expectations that decreases in Hg emissions would result in lower fish Hg concentrations (Wang et al., 2019). The complex controls on Hg cycling likely play a role in this response.

Although CAMP sampling parameters include water quality data, such as THg concentrations, in an effort to understand the impact of hydroelectricity production on water quality, Hg cycling in various waterbodies remains unclear. CAMP THg concentrations are analyzed to comply with drinking water quality standards (1000 ng/L) (Health and Welfare Canada,1993) and therefore measured values are almost always below the detection limit. These measurements fail to quantify water Hg concentrations that are capable of influencing fish Hg concentrations, as THg itself does not biomagnify in the food web but when transformed into

MeHg will biomagnify by several orders of magnitude. Given the lack of available water THg trends, this study aims to provide historical trends in Northern Manitoba THg and MeHg cycling from sediment records from two natural lakes and two regulated lakes in different parts of the Hudson Bay Watershed. These cores were used in conjunction with water column measurements to improve on the understanding of the effects of hydroelectric development on northern aquatic systems that are undergoing climate change against the backdrop of decreasing emissions.

3.2 Methods

3.2.1 Area of study

Two on-system lakes (Threepoint Lake and Stephens Lake; lakes impacted by hydroelectric development) and two off-system lakes (Leftrook Lake and Assean Lake; lakes unimpacted by hydroelectric development) in different regions of the Hudson Bay Watershed (Figure 3.1) were chosen to compare regulated and natural waterbodies within the study region. To avoid confusion between on-system and off-system lakes, the waterbodies will be referred to as: Threepoint, Stephens, Assean and Leftrook from this point on. Threepoint is on the RBR system along the CRD region surrounded largely by coniferous forest cover. The surface area of Threepoint increased by 47.7% in 1977 following the CRD (Bodaly et al., 2007). Since then, river flows have further increased as part of the Augmented Flow Program, which began in 1986 (Manitoba Hydro, 2015). Stephens is an on-system lake situated on the Lower Nelson River upstream of Kettle GS, Longspruce GS and Limestone GS surrounded largely by cultivated land. Stephens surface area increased by 236.6 % in 1970 during the CRD-driven flooding (Bodaly et al., 2007).

Both on-system lakes are monitored for water quality and fish THg as part of CAMP as are the two off-system lakes that were selected as reference for the on-system lakes. Leftrook provides a reference natural off-system lake for Threepoint, while Assean serves as a CAMP reference off-system lake for nearby Stephens. The proximity of each reference off-system lake to its regional on-system lake allowed us to compare water bodies with similar temperature variations, precipitation, bedrock and permafrost extent (Manitoba Hydro, 2015).

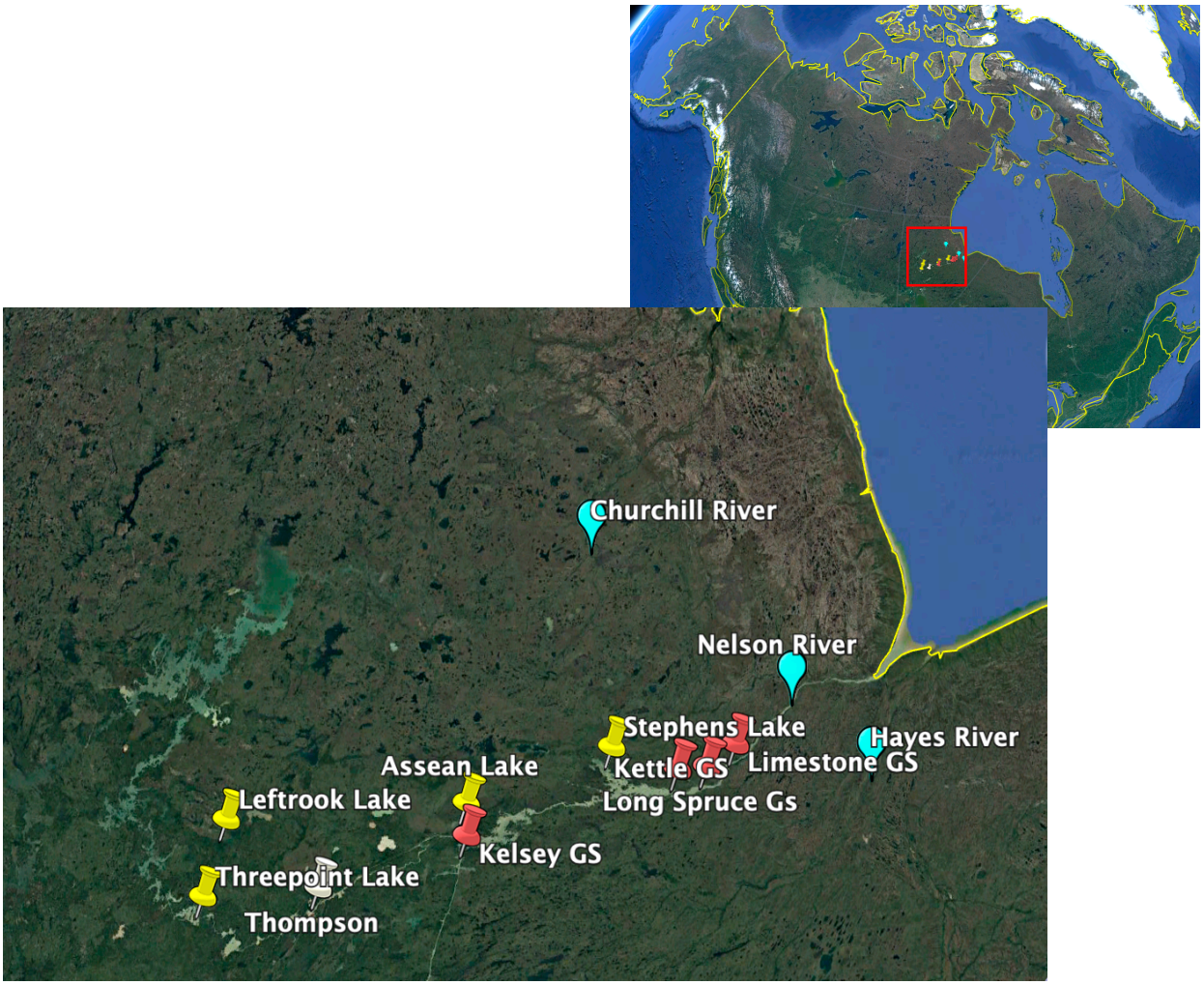

Figure 3.1: Map of the study area, showing waterbodies sampled in yellow, hydroelectric generating stations (GS) in red and nearby notable rivers in blue. The satellite image was sourced from Google Earth in 2019.

3.2.2 Site selection

For sediment sampling, a deep site and a shallow site were selected for each waterbody, although the only waterbody where two sediment cores could be successfully dated was Assean (Table 3.1). The deep sites were targeted to provide historical Hg fluxes both before and after water diversion, while shallow sites were targeted to determine Hg in soil prior to river regulation in on-system lakes and to compare soil Hg to analogous cores collected from the off-system reference lakes. Sites were chosen using sediment maps (North/South Colsultants Inc., 2016) to maximize the likelihood of successful coring. The shallow cores had to be collected in less than 2 m water depth as this was the limitation of the wetland push corer used for collection of samples.

3.2.3 Sediment core collection and preservation

Sediment cores were sampled in March/April, 2017, for ease of sampling from a stable ice cover. Most waterbodies were accessed using a de Havilland Twin Otter on wheel-skis. Assean sites were accessed on foot from a nearby road (route MB-280). Snow was removed from the ice surface and a 10" hole was drilled through the ice using a gas-powered auger. For the deep sites, a KB-style gravity corer with a plastic 10 cm diameter core barrel fastened to a pulley with a rope was used to acquire the cores. The gravity corer was manually pulled up at a steady rate and a plug was inserted into the bottom of the core tube when it neared the water-air interface. Overlying water was carefully removed with a syringe and the cores were kept upright until they could be sectioned later the same day. For shallow sites, a wetland style push corer was used. The push corer involves manually pushing the plastic bevelled core tube through the water column and into the sediment at a steady rate. Cores were sectioned into labeled plastic

bags (Whirl-Pak) in 1 cm sections using an extruder and both plastic and metal spatulas that were rinsed between sections. Any large rocks were manually picked out during this process. The core sections were kept refrigerated until transported to the University of Manitoba in Winnipeg.

At the University of Manitoba, all sections were transferred to pre-weighed 50 mL centrifuge tubes (Falcon polypropylene, Corning) and stored at -20 °C until they could be freeze dried. Samples were weighed before and after freeze drying on a four-point balance to determine water content. Samples were freeze dried (FreeZone 2.5, Labconco) according to manufacturer instructions and were then ground using a mortar and pestle. Ground samples were passed through a metal 300 μm sieve to remove larger particles that may have interfered with analysis. The mass of both sections of sediment was recorded.

Table 3.1: Sediment coring site descriptions for the cores that could be dated. Shallow cores collected from Leftrook and Threepoint could not be dated. The sediment corer bounced off the bottom at multiple locations at Stephens deep sites possibly due to current or bottom material.

Site	Latitude (N)	Longitude (W)	Surface area[30] (km^2)	Water depth at coring site (m)	Core length (cm)	Collection date
Threepoint	55°40'33.60"	98°53'6.00"	62.2	6.7	8	March 2nd, 2017
Leftrook	56° 3'21.60"	98°42'54.00"	46.3	10.0	53	March 3rd, 2017
Stephens	56°29'16.80"	95°14'13.20"	307	1.8	20	April 4th, 2017
Assean deep site	56°11'56.40"	96°32'42.00"	76.3	8.3	54	March 4th, 2017
Assean shallow site	56°14'34.43"	96°22'9.98"	76.3	1.0	20	March 5th, 2017

3.2.4 Water sample collection and preservation

Winter water samples were collected from a site roughly 10 m upstream of the coring site to allow for tandem collection of sediments and water without risk of contaminating water samples. Surface snow was removed using a plastic shovel and a 10" hole was augured. After checking the depth with a depth finder, a Water Quality Instrument (Pro Plus, YSI) including a temperature sensor (± 0.2 °C), a galvanic dissolved oxygen (DO) probe (± 0.2 mg/L or 2 %, whichever is greater) and a pH sensor (± 0.2 units) was lowered throughout the water column to collect water quality data. The water was left to settle for ~2 minutes before sampling. Water samples were collected using a 2 L GO-FLO bottle (General Oceanics) with Teflon parts. Sampling for THg and MeHg was performed using the clean hands/dirty hands technique to prevent contamination (Davidson, 1999). The water was added to acid-cleaned and spot tested amber glass bottles (250 mL, IChem) after rinsing three times with the matrix. Water was filtered in the field or in a hotel bathroom using disposable 0.45 μm filters (GH Polypro, hydrophilic polypropylene, Pall Life Sciences) and a hand-operated vacuum pump (Nalgene, Thermo Scientific) using the clean hands/dirty hands technique (Davidson, 1999). Samples were preserved to 0.5% HCl (concentrated, JT Baker). Water samples were kept cool and dark until analyses. Open and poured blanks were taken at all sampling sites and during filtration using ultrapure water (< 18 MΩ·cm, MilliQ) transported from the University of Manitoba.

Summer water samples were collected from the pontoon of a float plane from a central and coastal site of both on-system and off-system lakes. Water was drawn from the water column using silicon tubing (size 24, MasterFlex) using a peristaltic pump (Environmental Sampler, MasterFlex) through 0.45 μm filters (GH Polypro, hydrophilic polypropylene, Pall Life

Sciences) in a PFA single-stage filter assembly (Savillex). Blank collection and sample preservation were performed as described above for winter sample collection.

3.2.5 THg and MeHg analysis

THg and MeHg analyses were performed at the University of Manitoba in the Ultra Clean Trace Element Laboratory. Analyses of THg in water samples was performed on a Tekran 2600 using Cold Vapour Atomic Fluorescence Spectroscopy (CVAFS) according to EPA method 1631 (USEPA, 2002) following reduction to Hg(0) and phase separation from solution. Quantification was verified by a certified reference material, BCR579 (Hg = 9.5 ± 2.5 pmol kg^{-1}, Institute for Reference Materials and Measurements, European Commission – Joint Research Centre). The detection limit was calculated to be 0.15 ng/L. THg in sediments was analyzed using Cold Vapour Atomic Absorption (CVAA) according to EPA method 7473 on a Hydra II Mercury Analyzer (USEPA, 2007). The THg was quantified using an external standard curve prepared with the certified standard material MESS-3 (Marine Sediment Reference Materials for Trace Metals and other Constituents from the National Research Council of Canada; THg = 0.091 ± 0.009 mg kg^{-1}). The detection limit was calculated to be 0.2 ng.

MeHg in water was analyzed on a Brooks Rand MERX using CVAFS according to EPA method 1630 (USEPA, 2002) following ethylation of Hg species and separation by gas chromatography. Internal n-propyl mercuric chloride standards (2pg; prepared from a 10.1 mg/L stock solution in HPLC-grade isopropanol, Anachemia Science, ERA/Waters) were added to correct for changes in ethylation efficiency. The detection limit was determined to be 0.16 pg. MeHg in sediments was first distilled (Bowles and Apte, 2000; Horvat et al., 1993) and then diluted and run as a water sample. The MeHg recoveries were verified using the certified

reference material ERM CC580 (estuarine sediment, MeHg = 0.075 ± 0.004 mg kg^{-1}, Institute for Reference Materials and Measurements, European Commission – Joint Research Centre). The detection limit was calculated to be 0.9 pg. All samples were analyzed against standard curves prepared daily. Full methods for THg and MeHg analyses and the calculation of detection limits are further described in section 2.2.3.

3.2.6 Dating sediment

Well established ^{210}Pb alpha dating methods were used for dating the sediment at the University of Manitoba (Vesterbacka and Ikäheimonen, 2005). The method consists of a digestion step, a plating step and a counting step. For the digestion, 0.5 to 1.0 g of freeze-dried sediment were weighed into a glass beaker on an analytical balance. An aliquot of ^{209}Po in 1.5 N HCl (ACS-Pur, Fisher Scientific) solution was added as an internal standard to each beaker. Next, 30 mL of 6N HCl (ACS-Pur, Fisher Scientific) was added to each beaker and covered with a watch glass. The samples were digested on a hot plate set at 180 °C for ~4h. Before plating, the samples were allowed to cool and were decanted into 55 mL of 1 % ascorbic acid (USP/FCC, Fisher Scientific) solution. A silver counting disc was added to each sample and left overnight on the hotplate set at 80 °C. The following day, the plating discs were carefully rinsed with DI water. Each counting disc was put on an alpha detector (576 dual alpha spectrometers, Ortec) for 3-5 days to reach threshold counts for the internal standard. The activity of ^{210}Po, the daughter isotope of ^{210}Pb was measured and ^{210}Pb was calculated assuming secular equilibrium. For QA/QC, periodically reference soil (IARMA-001) samples were run for quantitation. In addition, duplicate samples were run roughly every 10^{th} sample.

To verify the ^{210}Pb profiles, ^{137}Cs was measured in all sediment cores. Freeze-dried sediment was weighed out to fill petri dishes (Falcon, 50 x 9 mm, Corning) and allowed to equilibrate for a period of at least 21 days. The ^{137}Cs was counted on one of two gamma detectors (BE3830 or GC3020, Canberra) at the University of Manitoba at 661 keV for 24 to 48 hours. Absolute counts and the mass of the sediments were used to calculate the activity of each sample.

Unsupported ^{210}Pb concentrations were calculated by subtracting the background concentration within each sediment core from total ^{210}Pb. Constant rate of supply (CRS) methods were used with the unsupported ^{210}Pb activity to calculate the date of the sediment (Last and Smol, 2001). Agreement between gamma ^{137}Cs and ^{210}Pb analysis was compared but was not always a good fit, as discussed below (section 3.3.2).

The following CRS equations were used to date the sediment (Equations 3.1-4) and calculate the sedimentation rate (Equation 3.5) within waterbodies (Last and Smol, 2001):

$$\hat{A}_n = \hat{A}_{n-1} + \frac{C_{n-1} - C_n}{\ln (C_{n-1}/C_n)}(m_n - m_{n-1}) \qquad \text{Equation 3.1}$$

Where $\hat{A}_n$ is the ^{210}Pb inventory above and including depth n measured in Bq/m^2, m_n is the cumulative mass above and including the section being measured (n) measured in kg/m^2 and C_n is the concentration in section n measured in Bq/m^2.

$$A(0) = \sum \hat{A} \qquad \text{Equation 3.2}$$

$$A = A(0) - \hat{A}_n \qquad \text{Equation 3.3}$$

A_n is the unsupported ^{210}Pb inventory beneath the section being dated and A(0) is the total inventory of unsupported ^{210}Pb.

$$t = \frac{1}{\lambda} \ln \left(1 + \frac{\hat{A}}{A}\right) \qquad \text{Equation 3.4}$$

$$r = \frac{\lambda A}{C}$$ Equation 3.5

t is the time in years since deposition, r is the sedimentation rate and λ is the radioactive decay constant for ^{210}Pb (0.03114 y^{-1}).

3.2.7 Hg Flux calculations

The following equations were used to calculate the fluxes of THg (Equation 3.6) and MeHg (Equation 3.7) in each waterbody:

$$Flux_{THg} = ([THg] * m_{sed})/(area * yr)$$ Equation 3.6

$$Flux_{MeHg} = ([MeHg] * m_{sed})/(area * yr)$$ Equation 3.7

Where [THg] and [MeHg] are measured concentrations of THg and MeHg respectively in each sediment section; m_{sed} is the mass of deposited sediment in each measured section; area is cross section of the core tube and yr is the number of years since the previous core slice was deposited.

3.2.8 Statistical analysis

Statistical analysis was carried out using Prism software (version 8.2.1, Graphpad). A significance threshold of $p < 0.05$ was used throughout. As all data was random and interval/ratio data, if the variables tested passed the Kolmogorov-Smirnov test at $\alpha = 0.05$, the data was assumed to be normally distributed and parametric tests were used. If the Kolmogorov-Smirnov test did not pass, non-parametric tests were used.

3.3 Results

3.3.1 Water [THg], [MeHg] and other properties of the waterbodies

The water quality parameters indicate seasonal differences between water bodies. During August sampling, Threepoint, Stephens, and Assean displayed well mixed water columns, which were fully oxygenated (Figure 3.2a). Stephens and Assean displayed similar temperature profiles, ~17.5 °C, while Threepoint and the surface water of Leftrook were slightly warmer. Leftrook was the only site that appeared stratified during August sampling, which approached anoxic conditions near the bottom of the lake (Figure 3.2a).

During March/April sampling, Threepoint and Leftrook both exhibited stratification with potentially suboxic water in bottom depths of both lakes (Figure 3.2b). Assean displayed a weaker oxycline, while Stephens appeared fully oxygenated throughout the water column (Figure 3.2b). Threepoint and Stephens, both of which are influenced by river regulation, had colder, uniform water columns compared to the unregulated control lakes (Figure 3.2b), where warmer water ~4 °C was maintained beneath the thermocline.

Little vertical structure was observed in water column THg and MeHg concentrations during either sampling season (Figure 3.3a, 3.3b), despite the August stratification observed in Leftrook (Figure 3.2a) and the March/April stratification observed in Leftrook, Threepoint and Assean (Figure 3.2b).

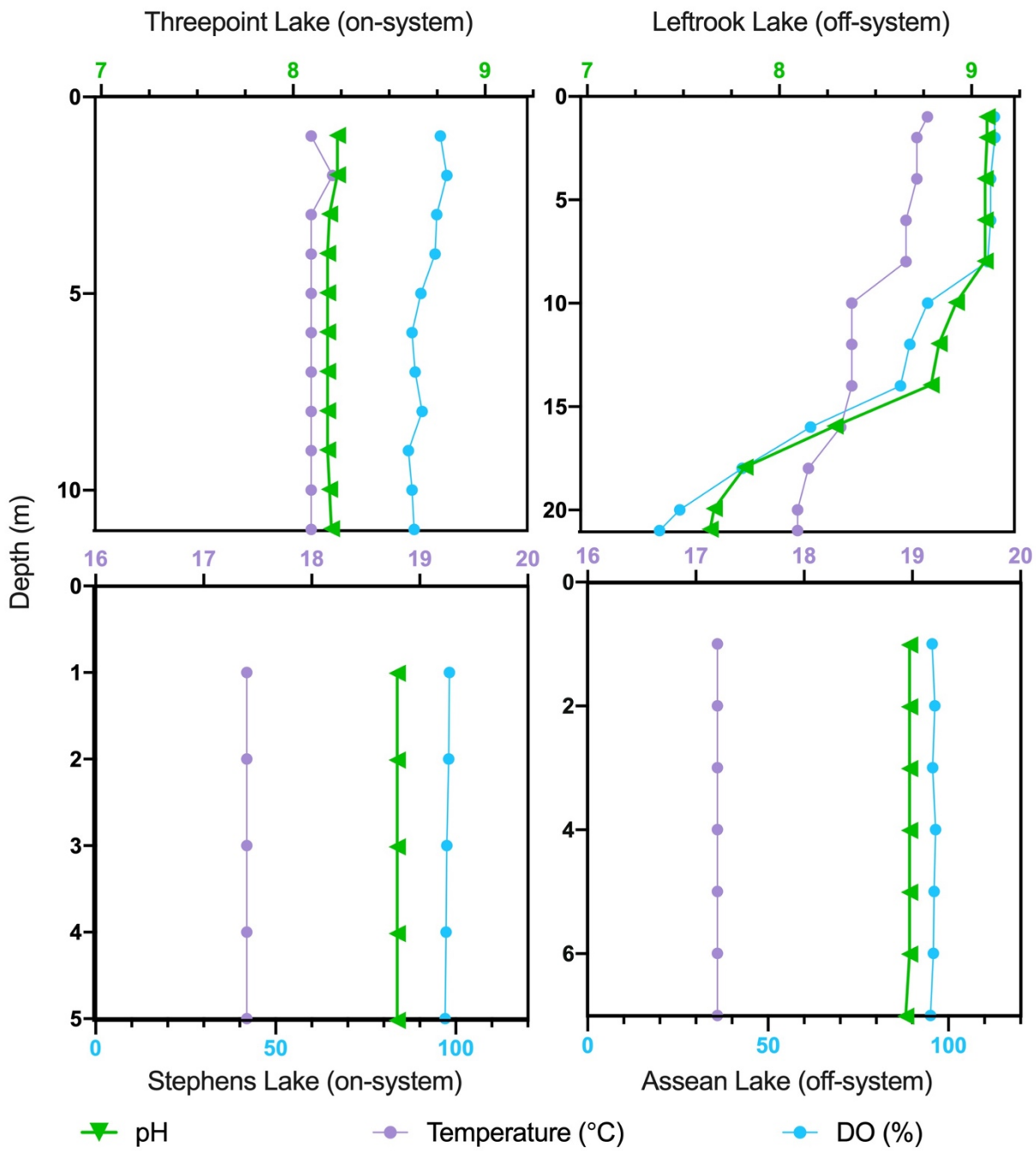

Figure 3.2a: Water quality parameters in on-system (left) and off system (right) lakes collected from a float plane using a Water Quality Instrument (Pro Plus, YSI) in August 2016.

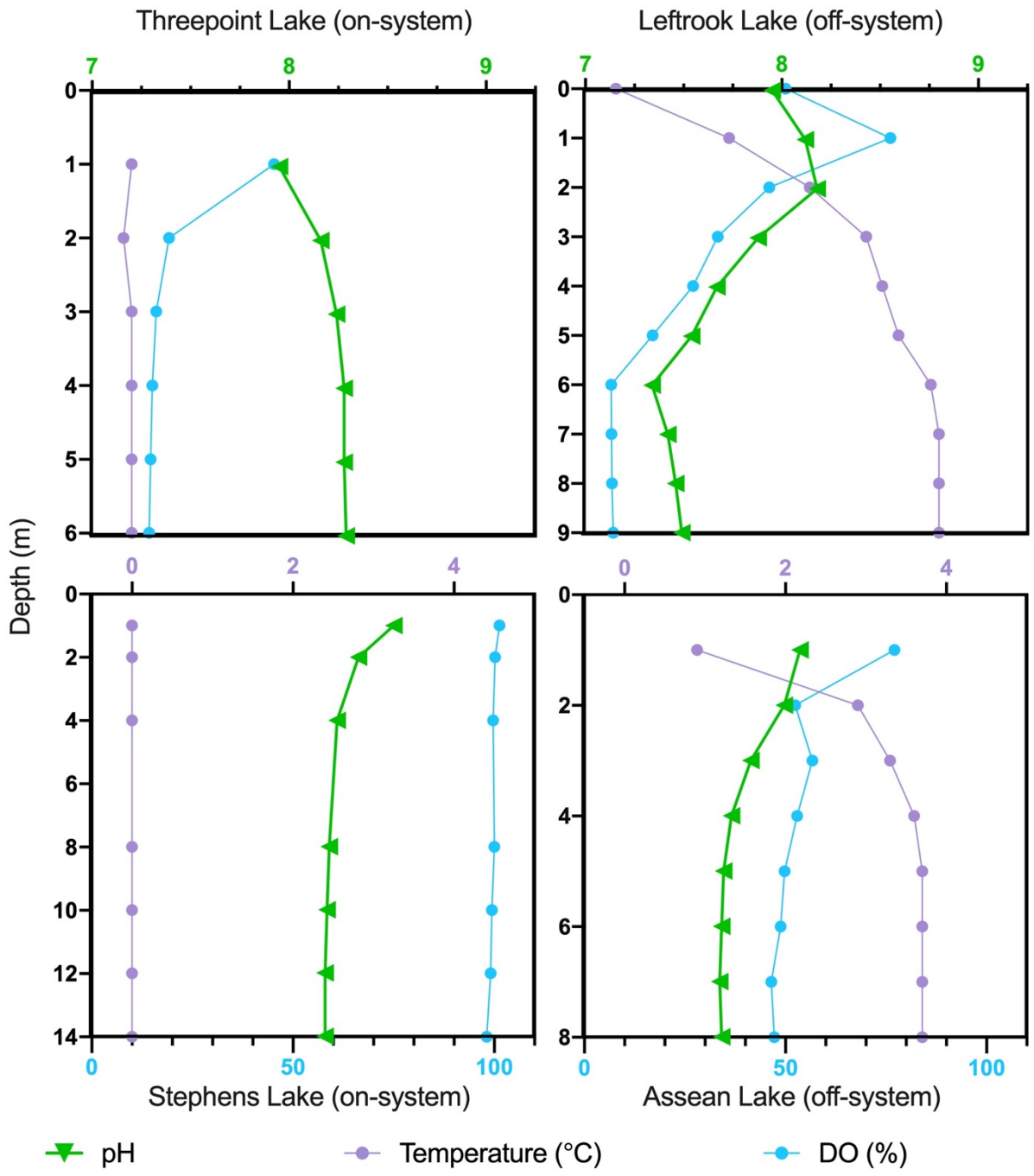

Figure 3.2b: Water quality parameters in on-system (left) and off-system (right) lakes collected from the ice surface using a Water Quality Instrument (Pro Plus, YSI) in March/April 2017.

Despite the vertical uniformity of the water column THg and MeHg (Figure 3.3a and b), we observed differences between waterbody average concentrations. The on-system lakes had higher THg in both August and March/April than the off-system lakes (Table 3.2). The on-system lakes also displayed more variable concentrations of THg between seasons compared to natural off-system lakes. In contrast, to THg concentrations, MeHg concentrations are uniform, both between waterbodies and sampling seasons. Leftrook however had the highest percentage MeHg of THg in both August and March (Table 3.2). This may be related to the fact that it was stratified with a drawdown of oxygen near the bottom of the lake during both sampling seasons (Figure 3.2a and b), providing conditions where mercury methylation is known to take place (Compeau and Bartha, 1985; Fleming et al., 2006).

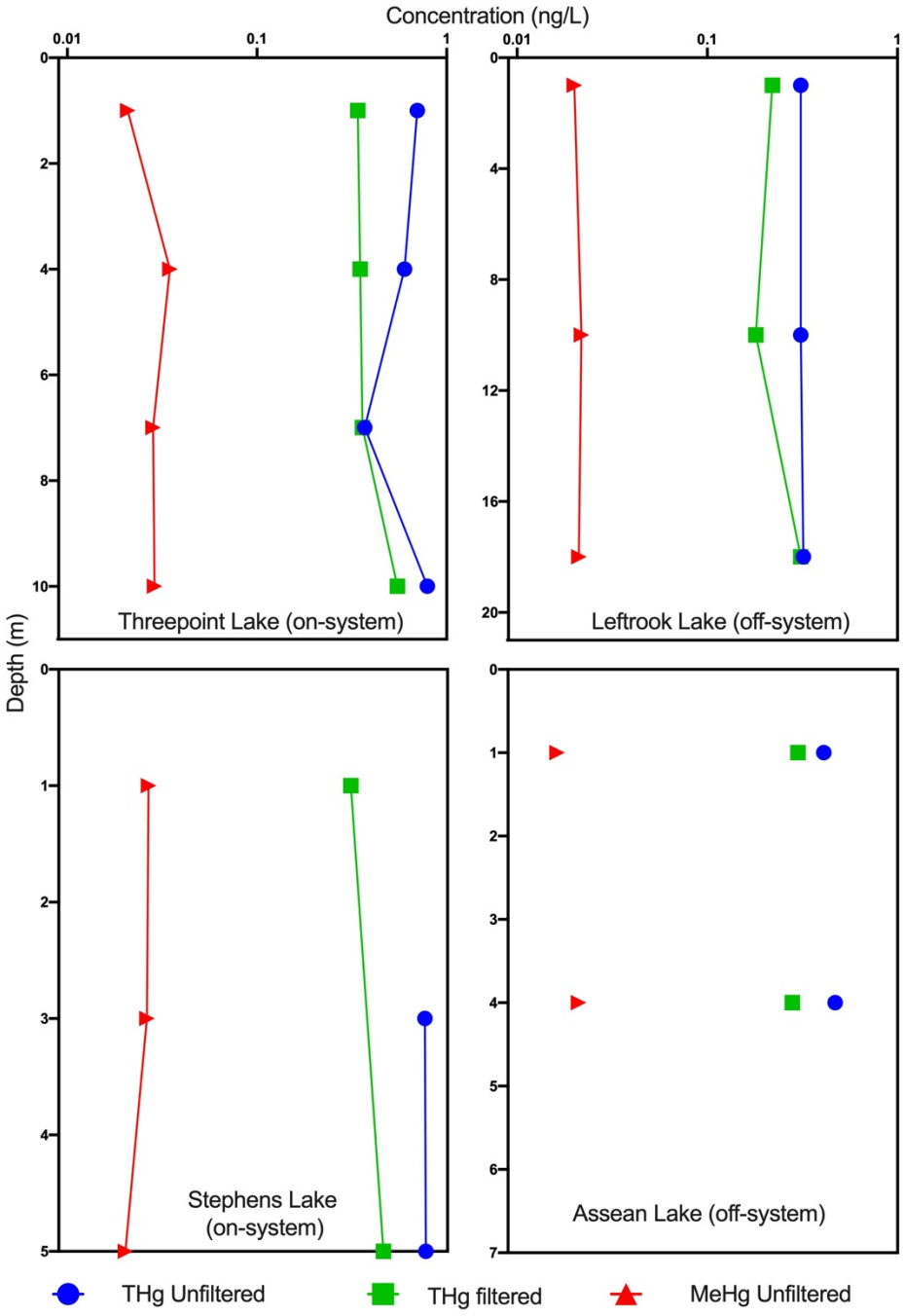

Figure 3.3a: Concentrations of total mercury (THg) unfiltered (blue), THg filtered (green) and methylmercury (MeHg) unfiltered (red) in Threepoint Lake (top left), Leftrook Lake (top right), Stephens Lake (bottom left) and Assean Lake (bottom right) collected in August 2016 from a float plane.

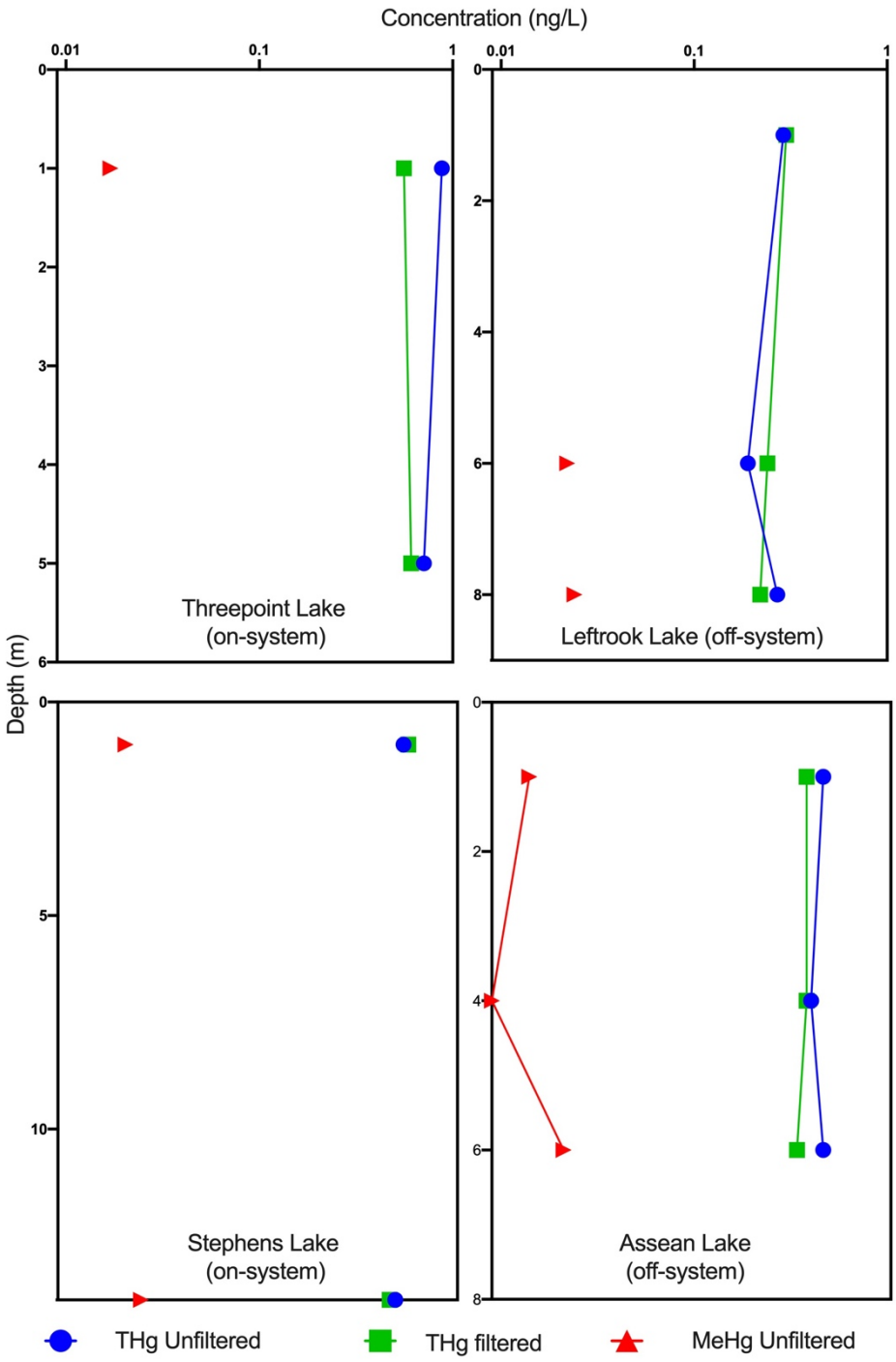

Figure 3.3b: Concentrations of total mercury (THg) unfiltered (blue), THg filtered (green) and methylmercury (MeHg) unfiltered (red) in Threepoint Lake (top left), Leftrook Lake (top right),

Stephens Lake (bottom left) and Assean Lake (bottom right) collected in March/April 2017 from the ice surface.

Table 3.2: Average total mercury (THg) and methylmercury (MeHg) concentrations in Northern Manitoba waterbodies sampled during different seasons.

	Site	Unfiltered THg (ng/L)	Filtered THg (ng/L)	Unfiltered MeHg (ng/L)	% MeHg of THg (unfiltered)
August 2016	Threepoint	0.62	0.40	0.028	5.0
	Leftrook	0.31	0.24	0.021	6.7
	Stephens	0.77	0.39	0.024	3.0
	Assean	0.44	0.29	0.019	4.2
March 2017	Threepoint	0.81	0.56	0.020	2.3
	Leftrook	0.31	0.25	0.023	10.
	Stephens	0.51	0.51	0.022	4.4
	Assean	0.42	0.35	0.015	3.5

3.3.2 Sediment dating results

Using the CRS model, we quantified cores sections ranging between 2008-2016 in surface sediments and beyond 1900 for bottom sections (Figure 3.4), with the exception of Stephens, which lacked sufficient data for meaningful modeling (Figure 3.4c). Sedimentation rates for all the waterbodies ranged between 0.01 and 0.45 $kg/m^2/yr$ (Figure 3.5).

Peaks in ^{137}Cs were quantified in upper sections of all cores (Figure 3.4c). However, agreement between the ^{137}Cs peak and the CRS model dating were generally limited due to low ^{137}Cs abundance or poorly defined peaks (Figure 3.4). We would expect to see a peak around 1963 from the maximum levels due to testing of nuclear weapons and another possible peak in

1986 from the Chernobyl accident (Last and Smol, 2001). Stephens did not show any measureable ^{137}Cs activity throughout the sediment profile. Threepoint showed a very low surface peak, not representative of where we would expect the peak to occur. All other sites showed elevated ^{137}Cs activity in the region we would expect, but the peaks were not well resolved. The broadening of peaks can be explained by chemical diffusion of the more mobile ^{137}Cs, broadening the peak over time to above and below the section of the core where it was deposited (Last and Smol, 2001). This diffusion can be enhanced by increased porosity and low clay content (Crusius and Anderson, 1995). A missing ^{137}Cs peak in the on-system lakes may be due to re-suspension of the newly deposited sediment containing the ^{137}Cs from the recent nuclear testing at the time of the CRD flooding or by low absorption/adsorption to the more inorganic sediment to levels below detection.

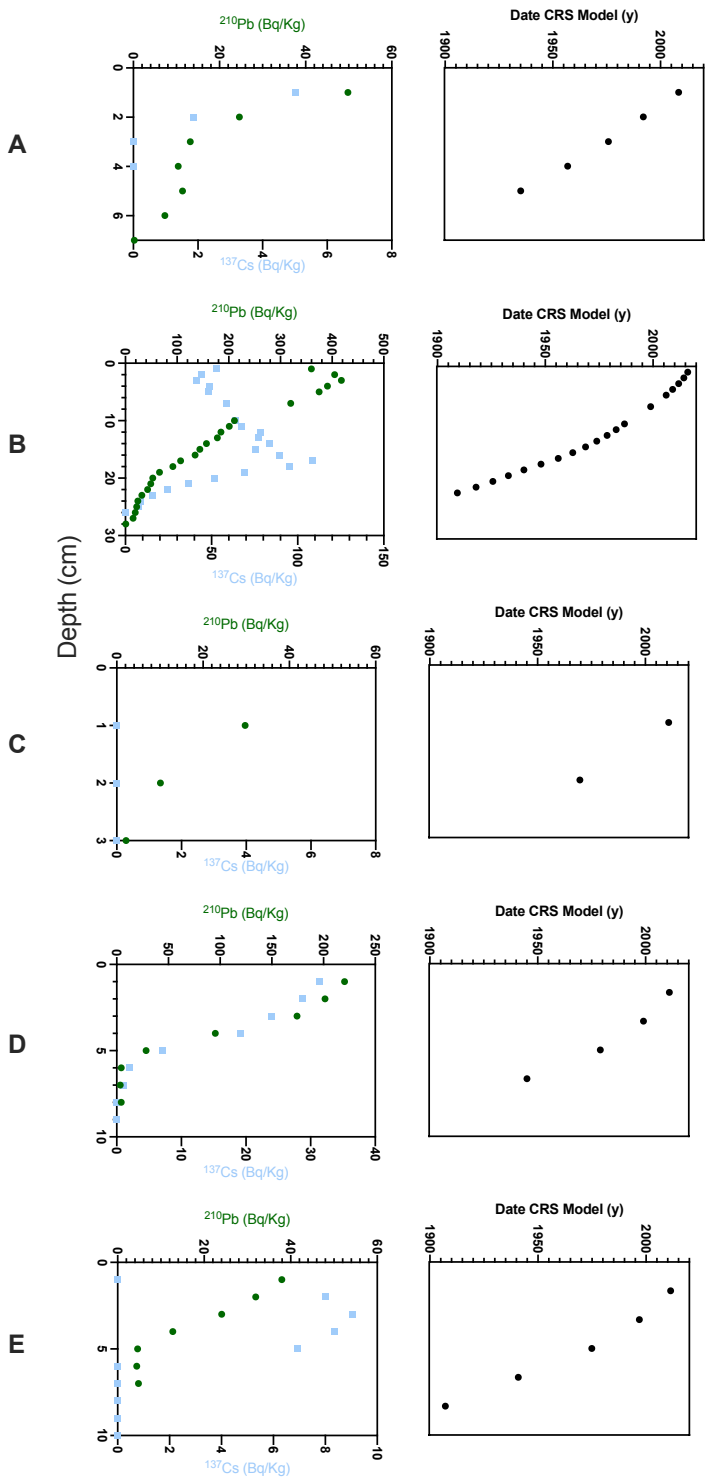
A
B
C
D
E
Depth (cm)
210Pb (Bq/Kg)
137Cs (Bq/Kg)
Date CRS Model (y)

Figure 3.4: Left: Unsupported ^{210}Pb (green circles) and ^{137}Cs (teal squares) activity in the sediment profiles of A) Threepoint B) Leftrook C) Stephens D) Assean deep site C) Assean shallow site. Right: Date in the Constant Rate of Supply (CRS) model using unsupported ^{210}Pb concentrations.

3.3.3 Sediment concentrations and fluxes

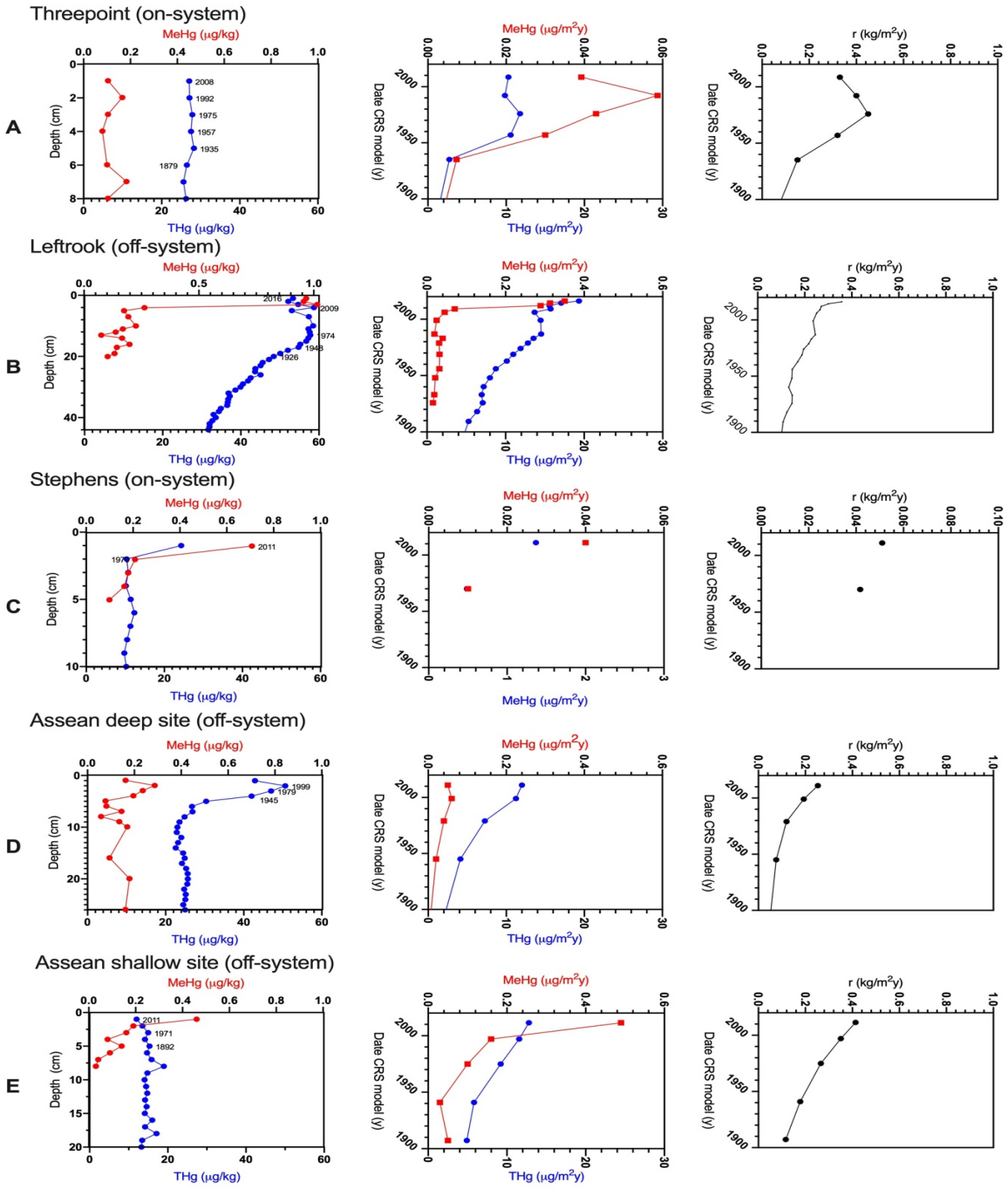

Figure 3.5: Left graphs: Concentrations of total mercury (THg) (blue) and methylmercury (MeHg) (red) throughout sediment core profiles. Center graphs: fluxes of MeHg (red) and THg (blue) to the sediment throughout the history of the core. Right graphs: sedimentation rate throughout sediment profiles.

Sediment profiles of THg were either uniform throughout the cores, as seen in Threepoint and the Assean shallow site, or had elevated THg concentrations in the upper core sections (Leftrook, Assean deep site). In most cores THg concentrations were stable in deeper core sections, with concentrations between ~ 10 and 25 μg/kg. In contrast, the THg concentrations in Leftrook sediment continued to decrease with core depth and did not plateau (Figure 3.4b). Concentrations of THg in the sediment of all on-system and off-system lakes ranged between 9.4 μg/kg in Stephens to 59 μg/kg in Leftrook and were within the range of other northern lakes such as several lake sediment cores in Greenland which ranged from 6-183 μg/kg in the bottom of the cores to 25-440 μg/kg in the top of the cores (Bindler et al., 2001).

Sediment profiles of MeHg were often elevated in the first 0-5 cm of the sediment cores, with the strongest concentration gradients observed in Leftrook, Stephens, and Assean shallow cores (Figure 3.5b, 3.5c, 3.5e). Concentrations of MeHg ranged from 0.015-1.0 μg/kg or 0.15-3.8 % of THg measured. The MeHg results are within range of an extensively sampled lake in Sweden which ranged in concentration from 0.07-2.45 μg/kg MeHg (Rydberg et al., 2012).

Overall, THg fluxes to the sediments studied increase over time (Figure 3.5). The THg flux in Threepoint also increases over time, but reaches a maximum flux of THg in 1976. Figure 3.6a shows that the sedimentation rate explains 67 % of the variation in the THg flux to the sediments (p value < 0.0001). This is across all on-system and off-system lakes studied and throughout the whole sediment profile, suggesting that the sediment flux is dominating the THg flux in these environments. When split into on-system and off-system lakes, both showed linear regressions with $R^2 = 0.90$ and 0.95 respectively (Figure 3.6b) and the slopes are significantly different ($P < 0.0001$). When divided into each individual sediment core (Figure 3.6c), significant correlations were reported for each individual site ($P < 0.05$).

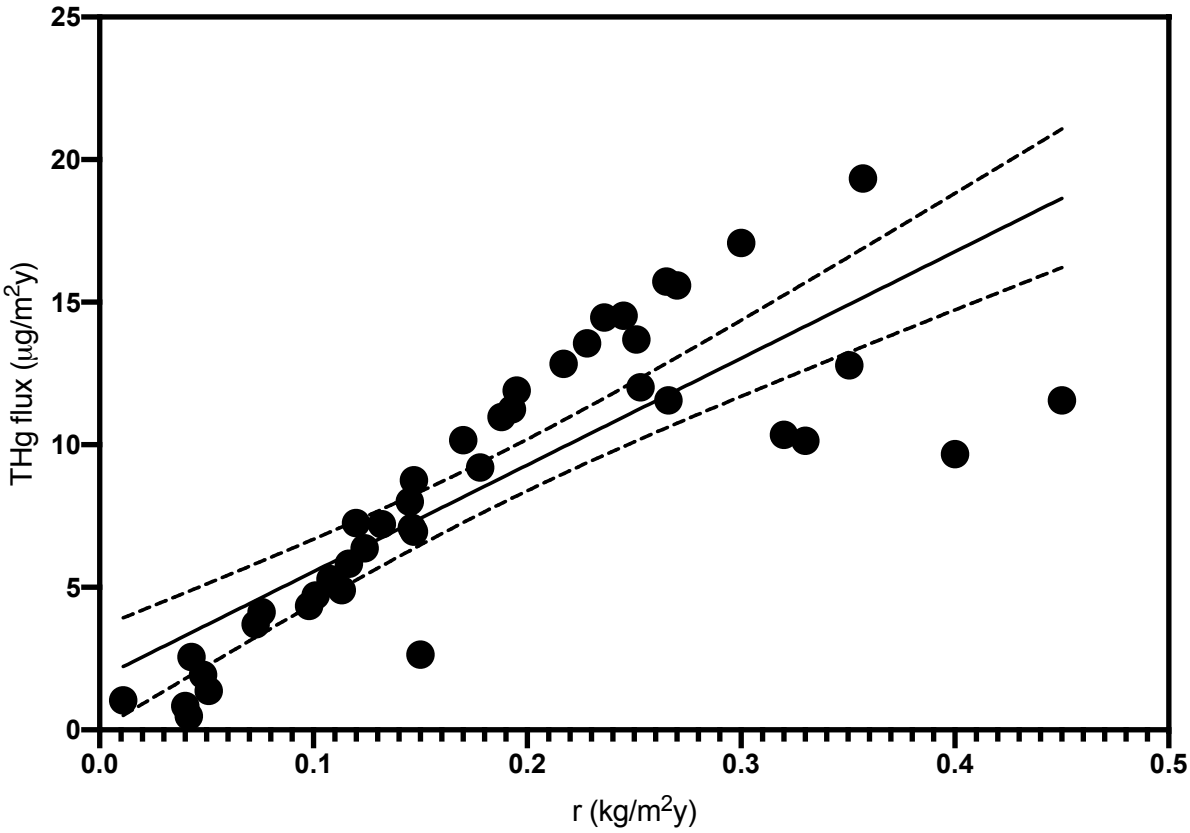

Figure 3.6a: Linear regression of sedimentation rate (r) calculated using the Constant Rate of Supply method for sediment cores of on-system and off-system lakes collected in Northern Manitoba in March/April of 2017 and total mercury (THg) flux in waterbodies studied throughout the sediment profiles ($R^2 = 0.67$, p value < 0.0001).

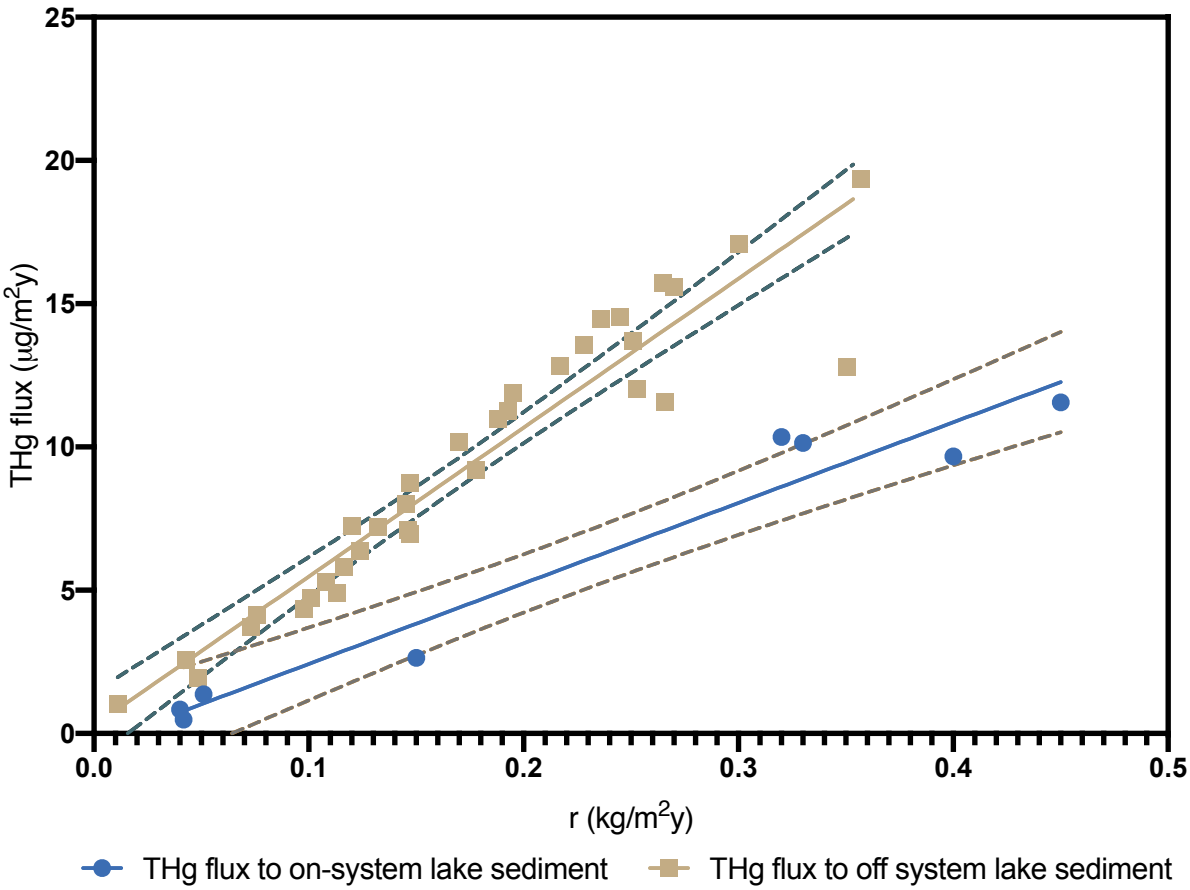

Figure 3.6b: Linear regression of sedimentation rate (r) calculated using the Constant Rate of Supply method for sediment cores of on-system and off-system lakes collected in Northern Manitoba in March/April of 2017 and total mercury (THg) flux to the sediments when grouped into off-system ($R^2 = 0.90$) and on-system ($R^2 = 0.95$) lakes. Slopes are significantly different from each other and from zero (p value < 0.0001).

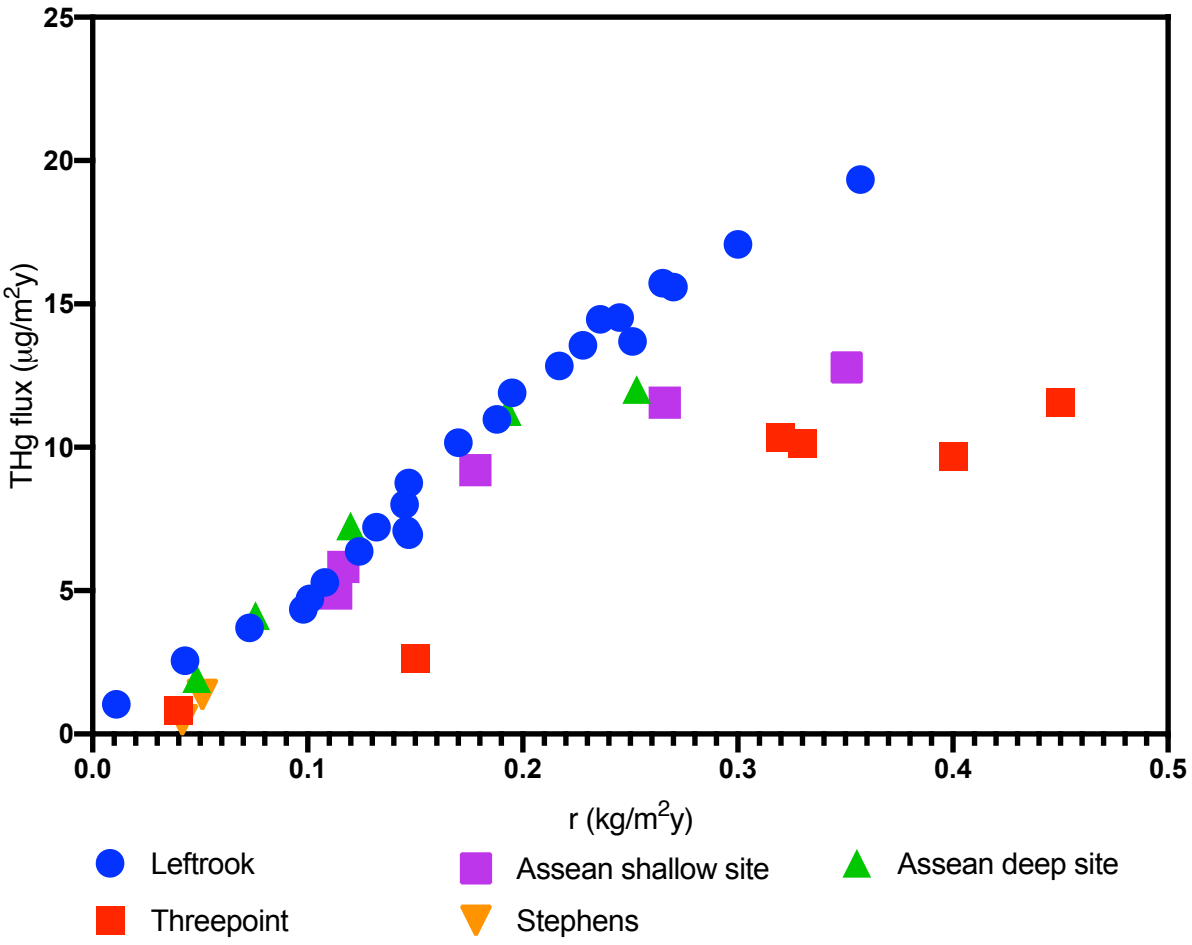

Figure 3.6c: Sedimentation rate (r) calculated using the Constant Rate of Supply method for sediment cores of waterbodies collected in Northern Manitoba in March/April of 2017 correlated to total mercury (THg) flux in each of the sediment profiles. All correlations are significant (p value < 0.05).

A Spearman correlation (ρ = 0.65, p value < 0.0001) is observed for the MeHg flux to the sediment versus the sedimentation rate across all waterbodies studied as seen in Figure 3.7a. When categories are split between on-system and off system lakes (Figure 3.7b), both categories show a stronger correlation (ρ = 0.72, p value < 0.0001 and ρ = 0.79, p value = 0.03 respectively). The flux of MeHg was also correlated to the flux of THg within sediment profiles (Figure 3.8a: ρ = 0.63, p value = 0.0001).

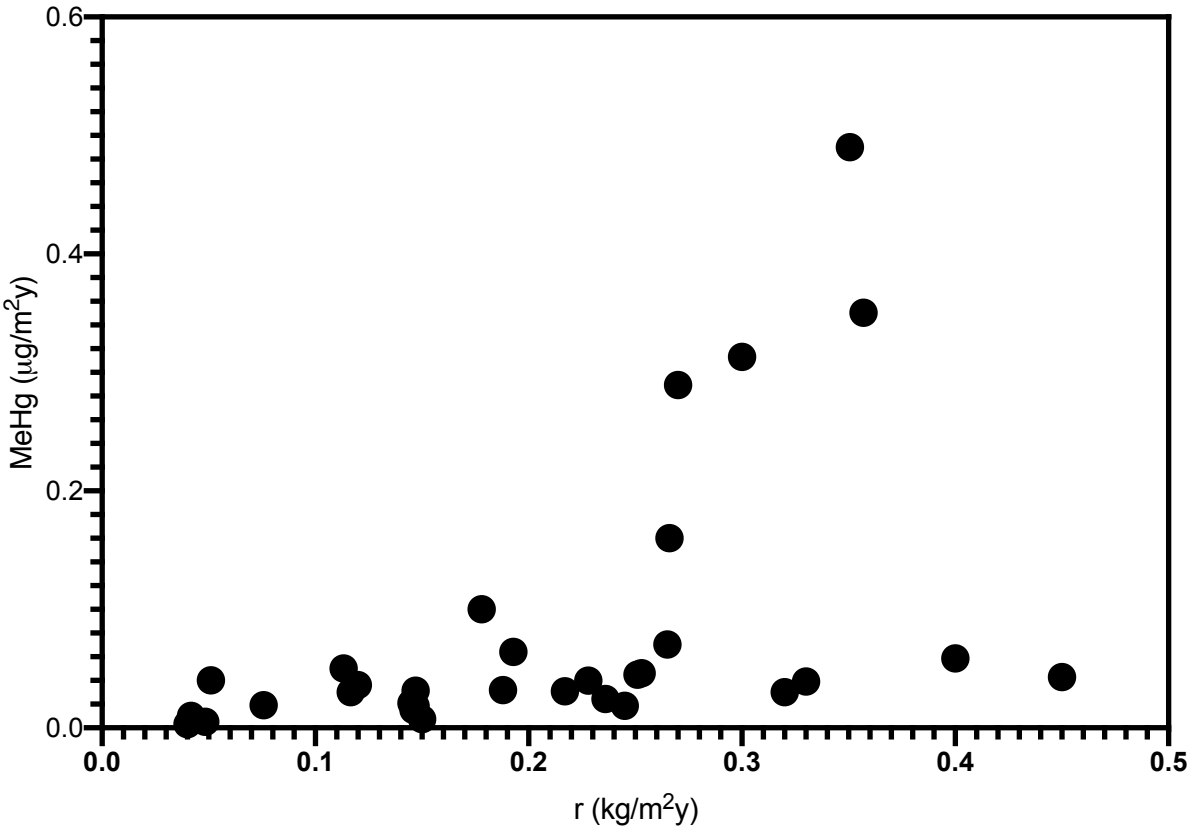

Figure 3.7a: Sedimentation rate (r) calculated using the Constant Rate of Supply method for sediment cores collected from Northern Manitoba waterbodies in March/April of 2017 correlated to MeHg flux throughout the sediment profile in all on-system and off-system lakes ($\rho = 0.65$, p value < 0.0001).

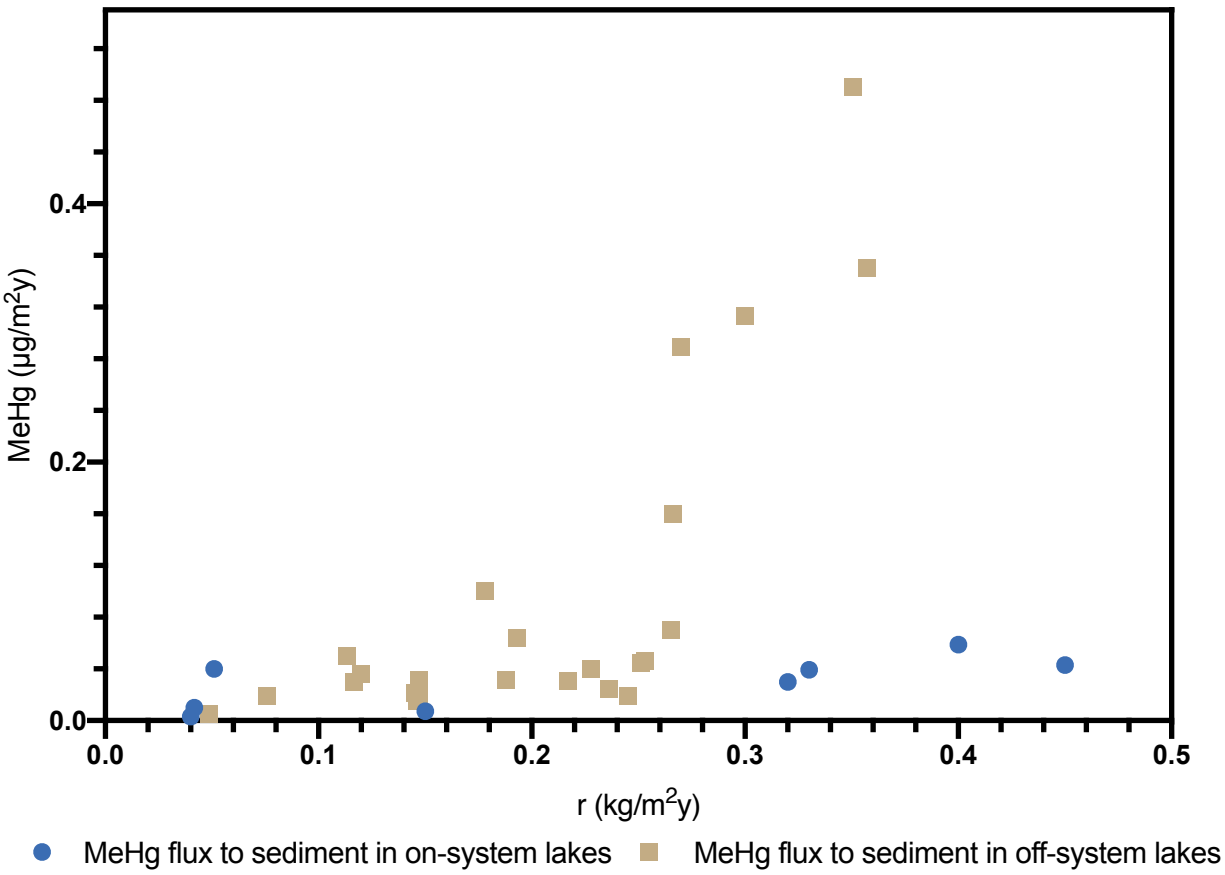

Figure 3.7b: Sedimentation rate (r) calculated using the Constant Rate of Supply method for sediment cores split into the categories of on-system and off-system lakes collected in Northern Manitoba in March/April of 2017 correlated to methylmercury (MeHg) flux to sediment. On-system (ρ = 0.79, p value = 0.03, N=8) and off-system (ρ = 0.72, p value < 0.0001, N=24) lakes both showed strong correlations.

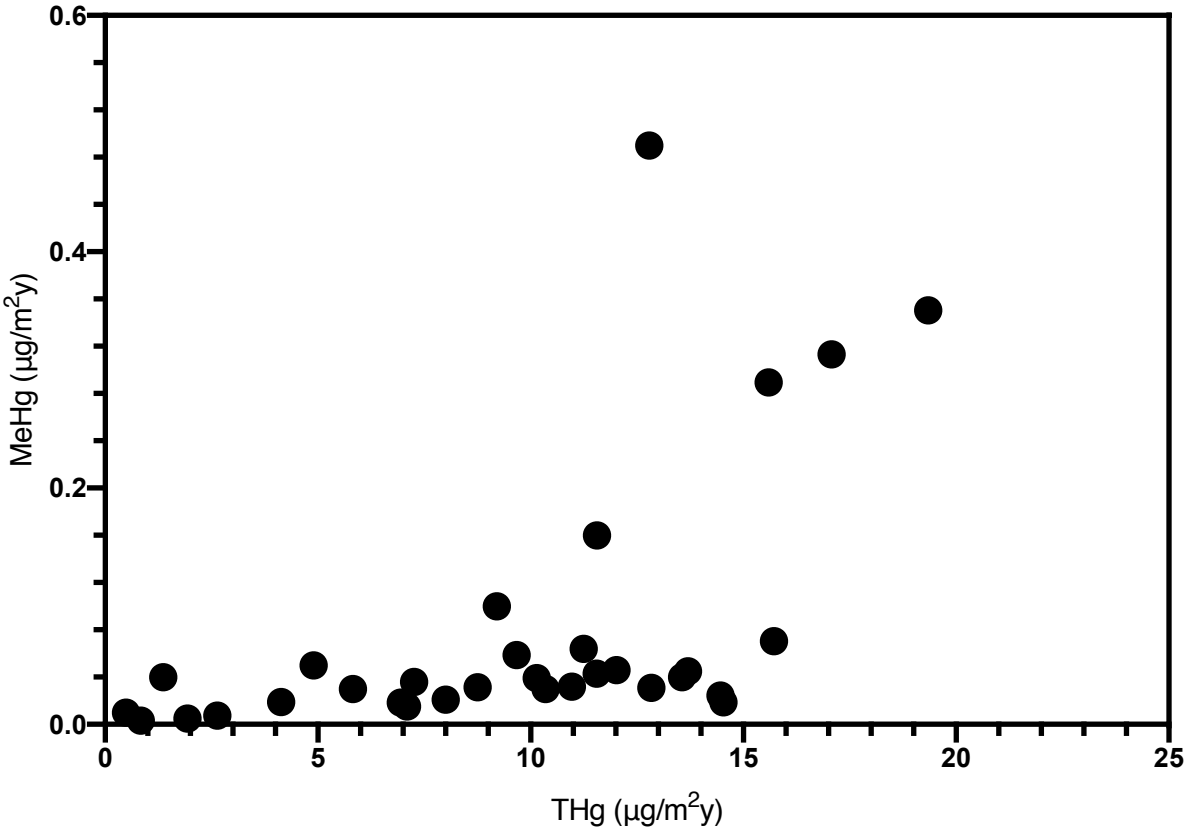

Figure 3.8a: Total mercury (THg) fluxes to the sediment are correlated to methylmercury (MeHg) fluxes to the sediment (ρ = 0.63, p value = 0.0001) in combined sediment profiles of on-system and off-system lakes collected in Northern Manitoba in March/April of 2017.

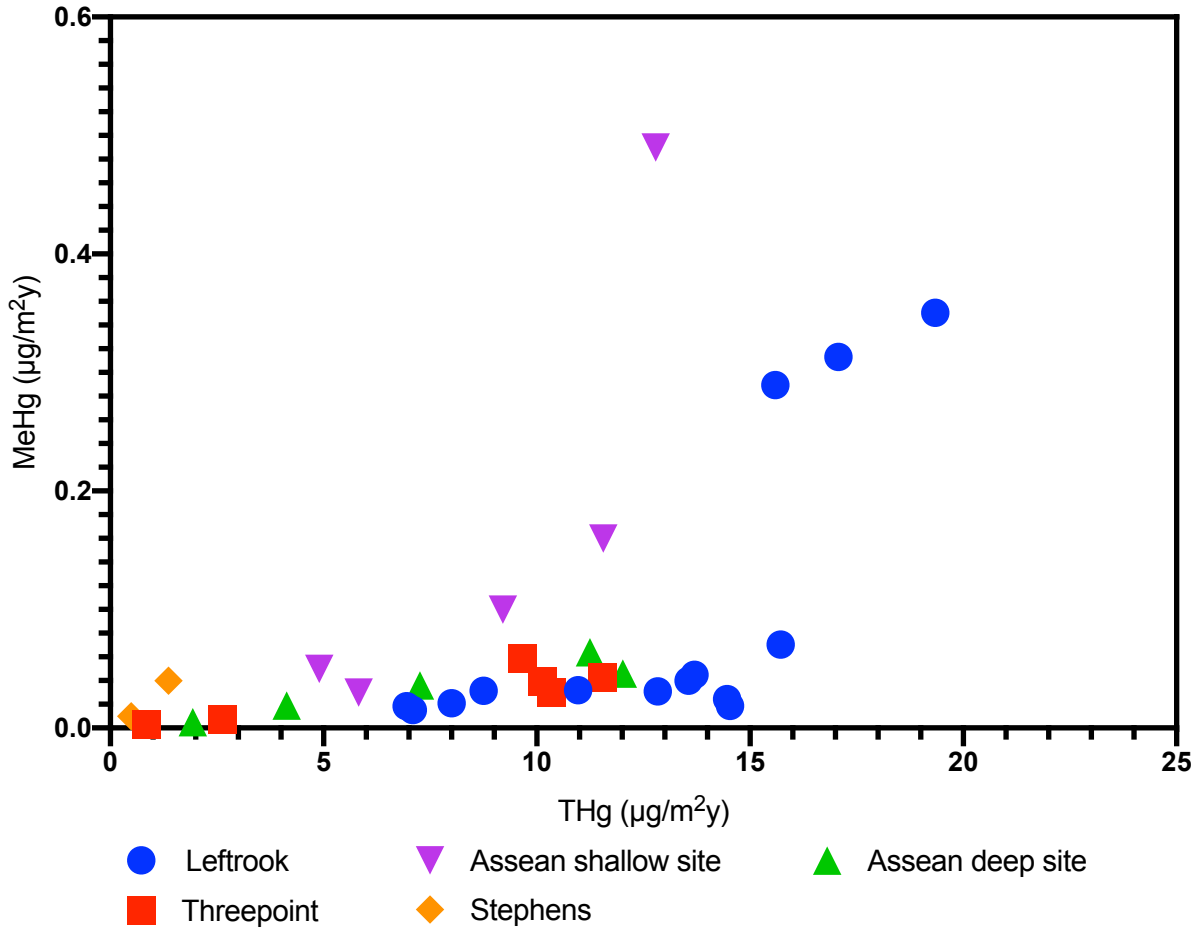

Figure 3.8b: Scatter plot showing total mercury (THg) and methylmercury (MeHg) fluxes to the sediment from individual sediment cores collected in Northern Manitoba from on-system and off-system lakes in March/April of 2017.

3.4 Discussion

3.4.1 Influence of sedimentation rate on THg and MeHg fluxes

In the current study the mean flux of THg to the sediments in Northern Manitoba waterbodies was greater from 1990-present than from the period 1960-1989 (Figure 3.5) despite decreases in Hg emissions and wet deposition in North America and Europe since the 1990s (Wang et al., 2019). This was also observed in lakes across the Arctic and Sub Arctic, north of

53 °N where 53 of 57 cores were shown to have increases in the flux of THg beyond 1990 compared to the period from 1960-1990 (AMAP, 2012). The recent increases may be partly due to increasing contributions of Hg stored in the catchments as suggested in a previous whole lake experiment (Wiklund et al., 2017). A Hg addition study at the Experimental Lakes Area, Ontario where different isotopes of inorganic Hg were spiked directly on the surface of a small lake, on nearby vegetation and over the catchment, labelled MeHg was found within 3 days in anoxic bottom water, while less than 1% was exported from the catchment in the first year (Harris et al., 2007). After six years of applying the spiked Hg, upon sampling the following year, the Hg in the catchment was beginning to move to areas where it could be exported to the lake. The majority had migrated into the mineral layer of the soil similar to the ambient Hg, where it would be expected to behave as ambient Hg (Oswald et al., 2014).

Large changes in sedimentation rate are observed throughout the sediment profile (Figure 3.5). Overall, it is apparent when represented visually that fluxes seemed to vary more with sedimentation rate than concentrations (Figure 3.5). For this reason, the hypothesis that sedimentation rate is driving THg and MeHg fluxes to the sediment was tested. Figure 3.6a shows that 67 % of the variation in the THg flux to the sediment can be explained by changes in sedimentation rate calculated by CRS methods across all on-system and off system lakes studied and throughout the whole sediment profile, suggesting that the sediment flux is dominating the THg flux in these environments. This holds true when separated into on-system and off-system lakes, where different slopes are observed for the two categories with off-system lakes having the greater slope (Figure 3.6b). The CRS model assumes a constant flux of ^{210}Pb from the atmosphere to the sediments. Sediment mixing and re-suspension of sediment will impact the distribution in the core and the resulting calculated dates of deposition. Given the ^{210}Pb

distribution increasing until the top sections of the sediment cores studied here (Figure 3.4), this is not suspected to play a major role in this case, while other cores that we did not report from Split Lake showed evidence of greater mixing.

Both climate change and hydroelectric development can play a role in affecting sedimentation in this area. Climate change is causing large changes to Sub-Arctic regions including rapid thawing and slumping of permafrost affecting the sedimentation rate in this area and mobilizing stored Hg (Nihoul and Kostianoy, 2009). Hydroelectric development is also playing a large role in sedimentation by: increasing the flow of water destabilizing river banks, flooding soil and changes in flow dynamics and residence times (Stainton, 2019). In the area of study, the already fragile banks due to thawing permafrost are made increasingly less stable by greater water flows compared to natural levels due to CRD (Stainton, 2019).

In contrast to THg, MeHg flux to the sediment is only moderately correlated ($\rho = 0.65$, p value < 0.0001) to the sedimentation rate (Figure 3.7a). One difference that contributes to more variability in the MeHg flux compared to THg flux is that MeHg is more dynamic than THg in the sediment, with methylation/demethylation reactions occurring long after deposition. For example Moneiro et al. (2016) observed large seasonal changes to the sediment profiles in the Tagus Estuary in Portugal due to a shift in the balance of methylation/demethylation reactions.

3.4.2 Influence of hydroelectric regulation on THg and MeHg fluxes

The flux of THg in Threepoint peaks at 1976, which is consistent with the timing of CRD flooding, while MeHg fluxes continue to increase until 1992 (Figure 3.5). Stephens surface sediment show flux increases since 1970 for both THg and MeHg. It is difficult to draw conclusions from Stephens sediment however, as there are only two sediment sections that could

be dated. Fluxes of THg and MeHg also increase in off-system lakes (Leftrook and Assean), unaffected by the CRD, likely due to the impacts of the confounding variables of higher emissions and deposition. Leftrook THg fluxes increase throughout the sediment core, but plateau periodically from 1986 to 2006 followed by an increase to the top section of the core. Both sites at Assean show an increase in the flux of both THg and MeHg since 1900. Off-system lakes do not show a local maximum in the mid 1970s, as Threepoint does. This local maximum at 1976 in Threepoint may be a subtle signal from the CRD-driven flooding.

Overall MeHg fluxes increase with THg (Figure 3.8a and b). Although core collection was attempted at both deep and shallow sites in each waterbody for spatial comparisons, cores could only be collected and dated at two sites in Assean. Both the deep and shallow sites at Assean showed similar THg flux profiles and correlated strongly with sedimentation rate with $\rho = 0.95$ and $\rho = 0.92$ respectively (Figure 3.6c). The shallow site at Assean had a higher percentage of THg as MeHg than the deep site (especially in the active layer) and had a greater flux of MeHg. The shallow site proximity to shoreline material may influence the higher MeHg concentrations and fluxes. A comparison of lake sediment cores to shoreline soils from both Stephens and Assean that were incubated for 196-hr with river water (Chapter 2), shows that sediment core MeHg concentrations were much lower than nearshore soils (Table 3.4). The Assean shallow site may therefore indicate erosion of nearshore soil.

In addition to shoreline proximity, a whole lake study in Sub-Arctic Sweden shows that distributions of MeHg can be quite variable even within a single lake (Rydberg et al., 2012). In the current study, the highest concentrations of MeHg are found within the top 2 cm of cores from all waterbodies measured, which is likely due to shifts in the balance between methylation/demethylation reactions over time and the diffusion of MeHg to overlying water.

Table 3.3: MeHg from Incubation results (Chapter 2) compared with the maximum concentration in the sediment profiles.

	Maximum [MeHg] in sediment core (µg/kg)	[MeHg] in nearby soil nearshore (µg/kg)	Max [MeHg] after 196-hr incubation nearshore (µg/kg)	[MeHg] in offshore soil (µg/kg)	Max [MeHg] after 196-hr incubation offshore (µg/kg)
Stephens	0.71	4.7	16	0.69	6.9
Assean	0.64	0.81	8.3	0.16	5.9

Overall the flux of MeHg to the sediments in on-system lakes (Stephens and Threepoint) is lower than in off-system lakes (Assean and Leftrook). In addition, when comparing the MeHg flux to the sediment in on-system lakes to that in off-system lakes, the flux in the off-system lakes increases faster than in the on-system lakes with increases in sedimentation rate (Figure 3.7b). In Threepoint it was surprising to see the sediment concentration so low compared to the off-system lakes given the similar MeHg water concentrations (Figure 3.3a and b), the fish concentrations have been high (Munson et al. in prep) and the oxygen near the sediment at the time of sampling was low (Figure 3.2 a and b). Collaborators have shown that there have been recent increases to the total suspended solids (TSS) in the RBR system where Threepoint is located (Stainton, 2019). The TSS however is low in organic carbon (OC) (Stainton, 2019), the breakdown of which would spur methylation of Hg where OC concentrations are high (Jackson, 2005). The TSS increase in the RBR system happens despite decreases in flow, which Stainton attributes to erosion from the instability of banks increased by regulation or other processes independent of flow or water level in on-system lakes (Stainton, 2019). Changes in sedimentation rate were observed in Threepoint that support this (Figure 3.5). Furthermore, Bravo et al. surveyed 10 boreal lakes in Sweden and showed that bacterial methylation in boreal

lake sediment was greater where OM is phytoplankton derived compared to terrigenous sources (Bravo et al., 2017). This phenomenon helps to explain the differences we observed, where in areas experiencing greater sources of sediment from erosion, lower MeHg was observed in the sediment. At the Stephens core site, the sedimentation rate is very low (Figure 3.5), resulting in a low flux of THg and MeHg. TSS has increased however in the Lower Nelson River, where Stephens is located, from 2005-2017 relative to the period 1987-1993, which Sainton (2019) attributes to the increases in the RBR system. However, sedimentation may be higher at other sites in the on-system lakes or much of the TSS may be exported downstream.

3.4.3 Additional sources of Hg to Northern Manitoba lake sediment

Previous work in Manitoba lake sediments has focused on the impact on regional deposition from the Flin Flon smelter and associated mining activities. At one time, Flin Flon was the site of the largest Hg emissions in North America making up as much as 6% of the total emissions around 1980 (Wiklund et al., 2017). Wiklund et al. (2017) reported sediment cores measured nearby the Flin Flon smelter stack (5-75 km) that showed peak fluxes of THg to the sediments from 30 to 2200 $\mu g/m^2y$, with closer lakes registering the higher values and decreasing with distance. Closer lakes peaked in the early 1980s and lakes on the fringe peaked in the early 2000s (Wiklund et al., 2017), possibly due to re-emission of nearby Hg. The sediment cores collected in the current study from off-system lakes were Leftrook (246 km from Flin Flon) and Assean (371 km from Flin Flon). Any contribution from the Flin Flon point source above background anthropogenic sources is thus very low in our sampled off-system lakes compared to lakes nearby Flin Flon. Our data agrees with previous cores measured that at the sampled distances from the smelter site, any contribution would be far less than 30 $\mu g/m^2y$ above

background (Wiklund et al., 2017), with the maximum total flux of THg being 24 μg/m^{2}y. There are no other large noteworthy point sources nearby, although the impact of deposition is complicated by internal processes that could mask differences in deposition between lakes.

3.5 Conclusions

THg and MeHg concentrations in the water and sediment in all on-system lakes (Stephens and Threepoint) and off-system lakes (Assean and Leftrook) surveyed of Northern Manitoba are within the range observed in similar environments. Sedimentation rate explained a large part of the variation in THg flux to the sediments and show significantly different slopes in on-system lakes relative to off-system lakes in waterbodies measured in Northern Manitoba from 1900 to present. Sedimentation rate was also correlated to MeHg fluxes to the sediments. THg and MeHg fluxes to the sediments were correlated.

Differences in TSS, which collaborators have shown to be released from weakened banks in response to the CRD, can help explain the different sources of OM, THg and MeHg. These different sources, along with low OC, help to explain low MeHg flux in the on-system lakes along the RBR system compared to nearby off-system lakes. Threepoint shows a local maximum of THg flux and a corresponding increase in sedimentation at the time of CRD flooding associated with reservoir creation. This may be a subtle signal of the CRD that is absent in off-system lakes in the corresponding time periods. In addition to differentiating between on-system and off-system lake sediment THg and MeHg, the cores analyzed in this study provide sediment fluxes in boreal regions of Northern Manitoba and can indicate the complex controls on THg and MeHg cycling away from point sources.

3.6 References

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Chapter 4: Conclusions and future research

4.1 Major contributions

The goal of my thesis research was to investigate impacts of river regulation on mercury (Hg) cycling in reservoirs of Northern Manitoba, Canada during a period of changing emissions and climate. This thesis has provided distinct contributions to the field through a realistic soil flooding experiment demonstrating the net methylmercury (MeHg) production potential in occasionally flooded nearshore soil of hydroelectric reservoirs, by filling data gaps in sediment and water total Hg (THg) and MeHg concentrations and by drawing links between organic matter (OM), THg and MeHg in the study area within the Hudson Bay Watershed.

The first distinct contribution of knowledge of this thesis is measuring the MeHg production potential within the water fluctuation zone in on-system lakes in Northern Manitoba by incubating both nearshore and offshore soils. The highest concentrations of MeHg both before and after flooding were in the soil from the water fluctuation zone in Stephens Lake, an on-system hydroelectric reservoir, which indicates long-term MeHg production potential in flooded soil. The Hg methylation throughout all experiments was related to the speciation of Hg-sulfide complexes in porewater. Nearshore and offshore soil in Stephens Lake did not show significantly different fluxes during the experiment, fluxes from both soil types were still increasing at the end of the incubation period and the MeHg in the soil was significantly higher in soil from the water fluctuation zone. We therefore concluded that soil in the water fluctuation zones may represent an increased source of MeHg or a source for a longer duration to the water and, in turn, the food web. In contrast to earlier studies focused on soil MeHg production and fluxes in recently impounded reservoirs, this study examined hydroelectric reservoirs that were impounded several decades ago and shows the impact river regulation is continuing to have in

this region. This also contrasts results of recent incubations of nearshore occasionally flooded soil and offshore soil from eastern Canada showing only a small pulse of MeHg, peaking after just a few hours from nearshore soil and greater flux in offshore soil (Schartup et al., 2015). Our incubations used more realistic summertime conditions and looked at net methylation to represent the natural conditions in the environment of Northern Manitoba.

This thesis also addressed additional data gaps in the long-term historical record of hydroelectric impacts on Hg cycling from the sediment profiles of THg and MeHg in both on-system regulated and off-system reference lakes. These profiles extend the sediment record in the local Northern Manitoba boreal forest ecosystem, where previous data for THg was largely limited to studies of the extent of point source contamination from the Flin Flon, Manitoba smelter and associated activities, and MeHg data in sediment is absent (Wiklund et al., 2017). Furthermore, THg and MeHg in on-system and off-system lakes provide insight into regional sedimentation rates in areas where results were not previously reported. These data provide increased coverage to improve models for the distribution of Hg in particulate matter and its cycling in the environment.

In addition to filling key gaps in sediment data, this thesis concluded from the sediment core analysis that the sedimentation rate explains the majority of the variation in THg in the sediment accumulation throughout the study area, while the physical processes affecting sedimentation varied. The sediment record from one regulated waterbody, Threepoint Lake, which was flooded by the Churchill River Diversion (CRD), showed a subtle increase in sedimentation rate and THg flux that can be attributed to the CRD-driven flooding.

Climate change trends and model output suggest that average precipitation and temperature as well as severe weather events in Northern Manitoba will continue to increase

(Environment and Climate Change Canada, 2018). Our results from Chapter 2 showed high MeHg production in soil flooding incubations at 20 ºC and suggest that climate-driven changes may increase the MeHg produced in nearshore soils. Furthermore, Chapter 3 data suggest the shoreline influences on sedimentation of THg. As a result, if increased precipitation and temperatures cause more erosion, this source of sediment to the water column may subsequently increase THg sedimentation. With the many other impacts of climate change such as thawing of permafrost and changing of biological communities, we cannot draw overall conclusions of the impact of climate change on the entire system but can draw conclusions based on our results on these areas.

4.2 Future research

This thesis was conducted as part of the larger BaySys-Mercury Team project, which has conducted additional research to connect the Northern Manitoba watershed to downstream impacts on Hudson Bay. The suggested research proposed below will focus on improving the strength of conclusions drawn in Chapters 2 and 3, and offer suggestions on how to use the Keeyask Generating Station project as a case study for improving understanding on initial impacts of impounding a reservoir in the boreal forest.

Results from our incubation study suggest that MeHg is higher in the soil within the water fluctuation zone both before and after flooding compared to offshore soil or nearshore soil in other lakes. Though we measured only a dissolved flux, it would be beneficial to have a total flux by measuring unfiltered concentrations or taking particle dynamics into account based on chemical equilibrium modeling between the dissolved and solid phases present. MeHg binding to particles was otherwise not accounted for. While unfiltered MeHg has been observed to be

correlated with entry into the food web by zooplankton uptake within boreal lakes as well as food webs in the Arctic Ocean (Hall et al., 2009; Wang et al., 2018), MeHg binding to particles would help determine the burial flux of MeHg along the extent of the regulated Nelson River system. This would provide more insight into the relative role of particle scavenging relative to uptake in determining MeHg in lacustrine food webs.

The sediment cores collected from Northern Manitoba that were discussed in Chapter 3 are catalogued in the Soil Science building at the University of Manitoba and can be used for future study. To further support the conclusions in this thesis, organic carbon characterization could be determined from sediment core sections. Minimally, organic carbon content could be analyzed, by techniques such as by loss on ignition. More complex characterization could be achieved through stable isotope analysis of a subset of the sediment core sections to provide "organic matter fingerprinting" and used to allocate organic matter sources in the sediment records relative to results of Stainton (2019). This will help to determine how sources have changed throughout regulation.

This thesis relied on sediment records and present-day incubations to identify potential long-term impacts of hydroelectricity in Northern Manitoba decades after impoundment, however, there are still large gaps remaining in our understanding of how current Hg cycling is linked to initial impoundment of reservoirs in this area. The ongoing construction of the Keeyask Generating Station, which is expected to become operational in fall 2020, provides an opportunity to collect missing baseline data and monitor Hg cycling in Gull Lake and the downstream impacts on Stephens Lake. Specific recommendations are listed below:

- A similar soil incubation experiment to the one described in Chapter 2 should be conducted with Keeyask area soil in summer and winter conditions to measure the potential production of MeHg in the soils and fluxes to Gull Lake water column.
- A transect of the surface soil/sediment for MeHg from the unflooded soil, through the flooded soil and into the basin of Gull Lake should be collected and analyzed at multiple time points throughout the flooding to track differences in MeHg within the waterbody.
- Seasonal concentrations of particulate and dissolved THg and MeHg should be monitored in Gull Lake, upstream and downstream sites, including Stephens Lake beginning in fall 2019 to provide a baseline and ongoing concentrations that can be related to particulate burial and transport in the Nelson River system.
- Zooplankton collected as part of CAMP or Keeyask project monitoring should be analyzed for MeHg content to monitor the entry into the lower food web and be compared to corresponding water column and sediment concentrations.
- Sediment traps should be deployed to determine the role of sinking particulate matter in balancing increased shoreline sources of OM and Hg.

4.3 References

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