Mercury methylation in high and low-sulphate impacted wetland ponds within the Prairie Pothole Region of North America

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Version Post-print/accepted manuscript

Citation (published version) Hoggarth, C.G.J., Hall, B.D., Mitchell, C.P.J., 2015. Mercury methylation in high and low-sulphate impacted wetland ponds within the prairie pothole region of North America. *Environmental Pollution* 205, 269–277. <https://doi.org/10.1016/j.envpol.2015.05.046>.

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Manuscript Number: ENVPOL-D-15-00088R1

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Article Type: Research Paper

Keywords: methylmercury, sediment, surface water, sulphate, wetland ponds, North American Great Plains

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Abstract: Using enriched stable ^{201}Hg injections into intact sediment cores, we provide the first reported Hg methylation potential rate constants (k_m) in prairie wetland ponds (0.016-0.17 d⁻¹). Our k_m values were similar to other freshwater wetlands and did not differ in ponds categorized with high compared to low surface water concentrations of sulphate. Sites with high sulphate had higher proportions of MeHg in sediment ($2.9 \pm 1.6\%$ vs. $1.0 \pm 0.3\%$) and higher surface water MeHg concentrations (1.96 ± 1.90 ng L⁻¹ vs. 0.56 ± 0.55 ng L⁻¹). Sediment-porewater partitioning coefficients were small, and likely due to high ionic activity. Our work suggests while k_m measurements are useful for understanding mercury cycling processes, they are less important than surface water MeHg concentrations for assessing MeHg risks to biota. Significant differences in MeHg concentrations between sites with high and low sulphate concentrations may also inform management decisions concerning wetland remediation and creation.

- Wetlands of the PPR provide many vital ecosystem services, but can have high MeHg concentrations
- Methylation potentials in prairie ponds are similar to other freshwater wetlands
- MeHg and %MeHg in surface water of high sulphate ponds was greater than low sulphate ponds
- Sediment-porewater partitioning coefficients were small compared to other systems
- Potential methylation rate constants did not correlate to surface water concentrations

Mercury methylation in high and low-sulphate impacted wetland ponds within the Prairie Pothole Region of North America

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Capsule: Prairie wetland ponds with higher sulphate concentrations have greater sediment and surface water methylmercury concentrations, but potential methylation rates do not differ.

ABSTRACT

Using enriched stable ²⁰¹Hg injections into intact sediment cores, we provide the first reported Hg methylation potential rate constants (k_m) in prairie wetland ponds (0.016-0.17 d⁻¹). Our k_m values were similar to other freshwater wetlands and did not differ in ponds categorized with high compared to low surface water concentrations of sulphate. Sites with high sulphate had higher proportions of MeHg in sediment ($2.9 \pm 1.6\%$ vs. $1.0 \pm 0.3\%$) and higher surface water MeHg concentrations (1.96 ± 1.90 ng L⁻¹ vs. 0.56 ± 0.55 ng L⁻¹). Sediment-porewater partitioning coefficients were small, and likely due to high ionic activity. Our work suggests while k_m measurements are useful for understanding mercury cycling processes, they are less important than surface water MeHg concentrations for assessing MeHg risks to biota. Significant differences in MeHg concentrations between sites with high and low sulphate concentrations may also inform management decisions concerning wetland remediation and creation.

INTRODUCTION

Methylmercury (MeHg) is a neurotoxin produced via microbial activity of a range of microbes including sulphate reducing bacteria (SRB), iron reducing bacteria, and methanogens

in wetlands and aquatic sediment. Methylmercury production rates depends on both availability of inorganic mercury (Hg) to, and metabolic activity of, methylating organisms (Hintelmann 2010). Thus, factors that influence Hg bioavailability (such as pH (Kelly et al. 2003) and sulphur speciation (Benoit et al. 1999)) and the activity of both methylating and demethylating bacteria (such as temperature (Loseto et al. 2004) and organic matter lability (Mitchell et al. 2008)) control net MeHg production rates. Sediment MeHg can be taken up by benthic invertebrates or diffused to surface water, and made available to pelagic organisms, ultimately bioaccumulating in fish. In small, depressional wetland ponds that do not support fish communities, such as those in the prairie region, benthic invertebrates may be important vectors of MeHg to terrestrial organisms such as insectivorous birds (Bates and Hall 2012). Overall, net MeHg production (rate of Hg methylation less the rate of MeHg demethylation) in sediments can ultimately result in biomagnification in aquatic food webs and thus harmful effects to humans and wildlife (Mergler et al. 2007; Scheuhammer et al. 2007).

Wetlands have been identified as important sites of net MeHg production and MeHg sources to downstream systems (St. Louis et al. 1994), but there is significant variability in Hg methylation among different wetland types. The Prairie Pothole Region (PPR) of the North American Great Plains extends over 850,000 km² and holds ~5-8 million small, depressional wetland ponds (Johnson et al. 2010). A wide range of hydrologic (permanent water to seasonal drying) and chemical variability (e.g. electrical conductivity, pH, dissolved organic carbon [DOC] concentrations) combined with shallow ponds and warm temperatures, may result in high MeHg production rates in these systems. Concentrations of MeHg are elevated in surface water and sediment of some prairie wetlands in Saskatchewan and North Dakota (Hall et al. 2009; Sando et al. 2007), suggesting substantial net production of MeHg.

Many wetlands and lakes within the PPR are saline, with high sulphate concentrations, although significant spatial and temporal chemical variability has been observed across the region (Last, 1999). The PPR is underlain by pyrite-rich sediment; the weathering and oxidation of pyrite, climatic factors, and groundwater dynamics are responsible for observed high sulphate concentrations (Goldhaber et al. 2014). Sulphur isotopes also show strong support for microbial sulphate reduction within this system (Goldhaber et al. 2014). The possible stimulative or inhibitive links between sulphate dynamics and MeHg production have not been explored in the PPR, however in other wetland systems impacted by sulphate such as the Florida Everglades,

MeHg production is greatest at low to moderate sulphate concentrations (2-20 mg L⁻¹) with moderate pore water sulphide concentrations (5-150 µg L⁻¹; Gilmour et al. 1998, Axelrad et al. 2007). Very low sulphate concentrations are unsupportive for the SRB responsible for most Hg methylation, whereas high sulphate concentrations may lead to high sulphide concentrations, which reduce the bioavailability of Hg for methylation (Benoit et al. 2003). Thus, the balance between bacterial stimulation and reduced Hg bioavailability is an important aspect of the sulphate-Hg methylation dynamic.

No investigations of *in situ* Hg methylation in prairie sediment, likely the principal source of MeHg to these ecosystems, have been done. Our primary objective was to measure Hg methylation potential rate constants wetland sediment from the PPR; measurements that have previously been made in sediment from aquatic systems including freshwater (Lehnherr et al. 2012b) and saltwater wetlands (Mitchell and Gilmour 2008), streams, lakes (Gentès et al. 2013), and marine sediment (Hammerschmidt and Fitzgerald 2004). A secondary objective was to investigate the role of surface water sulphate concentrations on Hg methylation potentials and MeHg and THg concentrations in prairie wetland ponds. This study is the first to report Hg methylation potential rate constants in prairie region wetland sediment. Furthering our understanding of MeHg production and Hg accumulation in wildlife is paramount to adequately evaluating ecotoxicological risk in this valuable ecosystem, which is estimated to contain habitat for over 80% of migrating North American waterfowl (Batt et al. 1989).

METHODS

Study Site

Sediment and water were collected from nine prairie wetland ponds within Saskatchewan's St. Denis National Wildlife Area (SDNWA; Fig. S1; Table S1). The 361 hectare SDNWA is located ~40 km east of Saskatoon and is comprised of areas of cultivated annual crops, perennial grass, and birch and willow that surround many wetland ponds (Pennock et al. 2010). This region has a humid continental climate with daily mean temperatures from 1971-2000 of 2.2°C; ranging from -17.0°C in January to +18.2°C in July, with daily mean temperatures >0°C from April through October. Over the same period, mean annual precipitation was 350 mm with ~25% as snowfall .

Within the SDNWA, four wetland ponds (Ponds 2, 3, 130, and 139) were located in a lowland area that forms a ring at the base of an upland area 5 to 10 m above. Five wetland ponds located in upland areas (Ponds 100, 103, 110, 113, and 118) were also sampled. Our grouping of wetland ponds was originally based on topographic position, but this delineation also falls along distinct differences in surface water sulphate concentration. Upland wetland ponds were characterized by surface water sulphate concentrations lower than 20 mg L^{-1} (henceforth “Low SO_4 ”), whereas lowland wetland ponds had sulphate concentrations between 150 and 3250 mg L^{-1} (henceforth “High SO_4 ”). These sulphate groupings provide a clear comparison with previous work where our “Low SO_4 ” sites coincide with sulphate concentrations expected to stimulate MeHg production and our “High SO_4 ” sites coincide with sulphate concentrations expected to reduce Hg bioavailability for methylation (Benoit et al. 2003; Axelrad et al. 2007). The High SO_4 ponds tended to have significant riparian vegetation and duckweed (Lemnoideae) communities. All wetlands studied were classified as shallow, Type III marshes which are seasonally flooded (Stewart and Kantrud 1971; additional details in supplemental section). Depending on annual and summer precipitation, timing of spring snow melt, and temperature patterns, it is typical that these wetland ponds dry completely by the end of the growing season; most likely due to infiltration to shallow groundwater and evapotranspiration (Hayashi et al. 1998; Nachson et al. 2014). However, during our study period none of the ponds completely dried up (Table S1).

Sample Collection

Sediment Sampling and Isotope Incubations

Wetland sediment cores were collected in July and August 2011 and analyzed for total Hg (THg) and MeHg concentrations and organic matter content (%OM; Heiri et al. 2001 and supplemental information). Core tubes were 30 cm long, 5 cm diameter acrylic cylinders with a beveled bottom edge and 1/16” silicone-sealed septa spaced 1 cm apart in a column down the side of the cylinder. Three cores at each wetland pond were taken 1-3 m apart from sediment submerged in 5-25 cm of water. All cores had an organic sediment layer of at least 9 cm. Cores with thick roots or more than 3 cm of sediment compression were discarded. Patches of dense vegetation and disturbed areas were avoided. In July 2011 only, we measured potential methylation rate constants in six wetland cores using standard enriched stable Hg isotope

techniques (Mitchell and Gilmour 2008; Lehnher et al. 2012b). 100 µl of an ~4 µg mL⁻¹ solution of 96.17% enriched ²⁰¹Hg was injected, using a gas-tight borosilicate glass syringe and stainless steel needle, through each of the septa within the top 10 cm of each core. The injected solution was prepared using a ²⁰¹HgCl stock solution equilibrated for 1-2 hours with native pore water. The intended amount of Hg injected into cores was less than double the THg in sediment. Direct analyses of THg isotope in sediment indicated that Hg contents were increased by 8-66% of the ambient THg. After injection of the enriched Hg isotope solution, cores were incubated at ambient field temperature (mean 19.6°C; range = 17-22 °C) for 4 hours, then sectioned into 2 cm sections, placed in 120 ml polypropylene containers, frozen on dry ice, and subsequently freeze dried and homogenized.

Water Sampling

Porewater was sampled for THg, MeHg, DOC and sulphate concentrations, pH, and electrical conductivity. The day before the incubations a 10-cm diameter acrylic core tube was used to create 10-20 cm deep holes adjacent to the core sampling areas. Sampling holes were left overnight to allow water to accumulate and sediment particles to settle. Porewater samples were then taken from approximately 1-2 cm below the accumulated water surface using a peristaltic pump outfitted with acid-washed Teflon sample tubing and dispensed into pre-cleaned 125 mL glass bottles with fluoropolymer resin lined lids. Using ultraclean handling techniques, a perfluoroalkoxy filter cartridge containing an ashed 47 mm, 45µm Whatman quartz fibre filter, was used to filter porewater collected for THg and MeHg concentrations. Samples for porewater THg and MeHg concentrations were preserved with trace metal grade HCl (0.2% and 0.4% by volume, respectively) and refrigerated until analysis. Methylmercury results from one of the wetland ponds in the July sampling event (Pond 139) are not reported due to filter damage. Porewater electrical conductivity and pH were measured with a YSI 556 multiprobe sonde.

Surface water was sampled for THg, MeHg, DOC and sulphate concentrations, pH, and electrical conductivity exactly as described for pore waters except that samples were collected by wading into the centre of the wetland and sampling water from ~5-10 cm beneath the surface.

Sample Analysis

Sediment MeHg and THg Concentrations and Methylation Potentials

Freeze dried sediments collected for both enriched Hg isotope and ambient THg and MeHg concentrations were analyzed at the University of Alberta's Biogeochemical Analytical Service Laboratory. Budgetary constraints limited analysis to duplicate 0-2 cm sections from three wetland ponds selected from each of the High SO₄ and Low SO₄ groups of wetlands. Cores with incubation lengths closest to the four-hr target incubation times were chosen for analysis. For THg analyses, freeze dried sediment was digested with trace metal grade HNO₃, H₂SO₄, and BrCl and analyzed using a Tekran 2600 upstream of a PerkinElmer Elan DRC-e inductively coupled plasma mass spectrometer (ICP-MS). Sediment MeHg samples were distilled with small additions of Me¹⁹⁹Hg as an internal standard (Hintelmann et al. 2000), ethylated with sodium tetraethylborate, and analyzed on a Tekran 2700 MeHg analyzer connected to an ICP-MS. Ambient THg and MeHg concentrations were calculated using the dominant ²⁰²Hg isotope after correction for ²⁰²Hg in the injected enriched isotope, as per Hintelmann and Evans (1997).

Methylation potentials were calculated as the amount of spike ²⁰¹Hg methylated to Me²⁰¹Hg over the incubation (Hintelmann and Evans 1997):

$$^{201}\text{Hg methylation potential } (k_m) = -\ln(1 - (\text{Me}^{201}\text{Hg} / ^{201}\text{Hg})) / (\text{incubation time} * (1 \text{ day} / 24\text{h}))$$

where: Me²⁰¹Hg is the excess Me²⁰¹Hg (concentration of spiked enriched stable isotope above the ambient concentration) in ng g⁻¹, ²⁰¹Hg is excess ²⁰¹Hg in ng g⁻¹ from added spike, and incubation time is incubation length in hours. Despite our equilibration with native pore water, the newly added ²⁰¹Hg is likely more bioavailable than ambient inorganic Hg, thus the resulting values represent *potential* methylation rate constants (Mitchell and Gilmour 2008).

Surface and Porewater MeHg, THg, DOC, and Sulphate Concentrations

Surface water and porewater THg concentrations were analyzed at the University of Regina by CVAFS using a Tekran 2600 following EPA Method 1631. Methylmercury concentrations were quantified using a Tekran 2700 at the University of Western Ontario, following distillation on a Tekran 2750 at the University of Regina. Sulphate and DOC were analyzed by standard methods (Stainton et al. 1977).

Quality Assurance, Quality Control

Approximately 10% of samples were analyzed in duplicate. Standard reference materials included MESS-3 (National Research Council) for THg and IAEA-405 (International Atomic

Energy Agency) for MeHg and recoveries were generally greater than $95\pm 2\%$ and $99.7\pm 0.1\%$ for THg and MeHg respectively (Table S2). Average spike recoveries were between $85\pm 4\%$ and $107\pm 10\%$ (Table S2). Minimum detection limits for water and sediment were less than 0.3 ng L^{-1} for THg and 0.02 ng L^{-1} for MeHg. Samples below the MeHg detection limit were assigned a value of half the detection limit (0.01 ng L^{-1}).

Partitioning coefficients

The apparent porewater / sediment partitioning coefficients ($\log K_D$) were calculated as the base-10 logarithm of the concentration in sediment (ng/kg) divided by the concentrations in porewater (ng/L) (Lyon et al. 1997).

Statistical Analysis

Data with non-normal distributions were identified using Kolmogorov-Smirnov tests and natural log transformed when necessary. For negative data, values were uniformly translated to positive values. Differences in potential methylation constants in ponds grouped into High SO_4 and Low SO_4 ponds were determined by t-tests. For sediment geochemistry and mercury data, two-way ANOVAs were used to test differences due to sulphate classification and time of sampling (July vs. August). For all statistical tests, $\alpha = 0.05$ and error bars represent standard error. Associations between potential methylation rate constants and other parameters (listed in Table S2) were quantified through linear regression. All interaction effects were not significant. SigmaPlot 13® was used for statistical tests.

RESULTS

Geochemical Differences Between Low SO_4 and High SO_4 Wetland Ponds

Our groups of wetland ponds had significant geochemical differences among sediment, and surface and pore waters. Sediment %OM ranged from 5.5 to 29.6 % (Table S3), tended to be higher (although not significantly; $p = 0.079$) in High SO_4 ponds compared to Low SO_4 ponds (Fig. S2), but did not differ between July and August ($p=0.218$). Both surface water and porewater had significantly greater sulphate concentrations (Fig. 1A/B) and related electrical conductivities (Fig. 1C/D) in our High SO_4 ponds. Our observed sulphate concentrations ranged from 10.16 to over 3200 mg L^{-1} and 5.6 to 2600 mg L^{-1} in surface water and porewater, respectively (Tables S3 and S4). pH and DOC concentrations were significantly higher in High

SO₄ ponds in both surface water and porewater (Fig. 1E-1H). Concentrations of DOC ranged from 9.62 to 40 mg L⁻¹ and 12.73 to 88.92 mg L⁻¹ for surface and porewater, respectively. Values for pH in all ponds were between 6.41 and 8.33 in surface water and 6.72 and 7.34 in porewater. There were no significant differences in any parameters between July and August (See Fig. 1 for *p* values).

Differences in Hg and MeHg Chemistry in Low SO₄ and High SO₄ sites

In situ sediment k_m values in July ranged from 0.019 to 0.166 d⁻¹ (Fig. 2A). Mean k_m values in High SO₄ ponds (0.051 ± 0.017 d⁻¹) did not differ significantly from those in Low SO₄ ponds (0.102 ± 0.034 d⁻¹; $p = 0.250$; Fig. 2B). Concentrations of MeHg in sediment (0.18 - 2.12 ng g⁻¹; Fig. 3A), surface water (below detection limit - 5.72 ng L⁻¹; Fig. 3B) and porewater (0.19 to 55.61 ng L⁻¹; Fig. 3C) tended to be higher in High SO₄ sites; this difference was only significant in surface water ($p = 0.014$; Table S3-S5). Total Hg concentrations in surface (0.61 – 6.82 ng L⁻¹; Fig. 3E) and pore (3.40 to 50.6 ng L⁻¹; Fig. 3F) waters also tended to be higher (again, not significantly) in High SO₄ sites compared to Low SO₄ sites. In sediment (Fig. 3D), THg concentrations did not differ either by sulphate grouping or month ($p = 0.575$ and 0.936). With the exception of sediment where there was no observed difference by month, MeHg and THg concentrations were higher in July compared to August in both surface and pore waters (see Fig. 3 for *p* values). The proportion of THg that was MeHg (%MeHg) was higher in surface (1.02 – 90.65%; Fig. 3H) and pore (4.60 – 109.85%; Fig. 3I) waters compared to sediment (0.63 – 4.83%; Fig. 3G). Sediment and surface water %MeHg were significantly greater in High SO₄ sites compared to Low SO₄ sites ($p = 0.027$ and 0.001 , respectively). Month to month differences were observed in %MeHg in surface water ($p = 0.036$) and porewater ($p = 0.018$) but not sediment ($p = 0.740$). Log sediment/porewater K_D values for MeHg and THg ranged from 0.88 to 3.23 L kg⁻¹ and 2.73 to 4.06 L kg⁻¹, respectively (Table S6). Partitioning coefficients for both MeHg and THg were higher in August ($p = 0.016$ for MeHg and $p = 0.005$ for THg) suggesting that there was less MeHg and THg in porewater in August compared to July. Partitioning coefficients for both MeHg and THg were similar between High and Low SO₄ ponds ($p = 0.376$ for MeHg and $p = 0.069$ for THg).

Relationships among geochemical factors and Hg methylation potentials

Linear regression was used to identify potential relationships among methylation potentials and factors that may impact the production of MeHg. Porewater THg, sulphate, and DOC concentrations, and sediment %OM, THg concentrations and %MeHg, were tested as possible predictors of methylation potentials. There were no significant relationships observed between any of these variable and potential methylation rate constants (see Table S7 for r^2 and p values). Linear regressions also showed that neither pore water THg and MeHg concentrations nor porewater %MeHg predicted surface water MeHg concentrations (Table S7). However, there were significant relationships between surface water MeHg concentrations and %MeHg in sediment in July and August (Fig. 4A) and sediment THg and MeHg concentrations and %OM in July (Fig. 4 B-D).

DISCUSSION

A novel measurement of methylation potentials in prairie wetland sediment

Wetlands are the functional ecological unit of the PPR of the North American Great Plains and provide vital roles in groundwater aquifer recharge, flood control and water quality, carbon and nutrient sequestration, and wildlife biodiversity (Millet et al. 2009; Poiani et al. 1991). Wetland ponds in this region have a wide range of MeHg concentrations in unfiltered surface water, with a range of reported values from 0.02 to over 9 ng L⁻¹ (Bates and Hall 2012; Hall et al. 2009; Sando et al. 2007). Elevated water MeHg concentrations in these wetlands suggest a strong potential for elevated k_m values. Our study is the first to measure *in situ* Hg k_m values via enriched stable isotope methods in the PPR. Despite elevated MeHg concentrations measured in prairie ponds, we found that the range of k_m values from wetland sediment in the region was generally similar to k_m values from other freshwater and saltwater wetlands (Table 1). Mean k_m values from SDNWA wetlands (0.02-0.17 d⁻¹) were most similar to values from surface sediments of freshwater Arctic wetlands (0.04–0.16 d⁻¹; Lehnher et al. 2012b) and were greater than those in Florida Everglades (0–0.12 d⁻¹; Gilmour et al. 1998) and in Swedish boreal (0.01-0.06 d⁻¹; Tjerngren et al. 2012a) wetlands.

Concentrations of MeHg and THg, and %MeHg in SDNWA wetland sediments were also generally similar to, or lower than, those in other freshwater ecosystems (Table 1). The most apt comparison is for sediment data collected from North Dakota's Lostwood National Wildlife Refuge (LNWR), located in the PPR of the northern United States. There, both MeHg (<0.4 -

4.16 ng g⁻¹) and THg (6.77 - 99.0 ng g⁻¹) concentrations were similar to those in our sites (Sando et al. 2007). Sediment %MeHg values at SDNWA were similar to those from ponds at LNWR (0.3 – 8.4%; Sando et al. 2007) and other freshwater wetlands (Table 1). Freshwater wetlands generally have higher %MeHg than estuarine or marine sediments because of higher rates of net methylation (Hall et al. 2008; Mitchell and Gilmour 2008).

Surface water MeHg and THg concentrations compared similarly to those from studies of other wetlands in the PPR. Porewater MeHg and THg concentrations were within the range of concentrations observed in studies in other freshwater wetlands (for example, Louisiana: Hall et al. 2008), although three of our sites sampled in July had relatively higher MeHg and THg concentrations (Fig. 3), which was reflected in partitioning coefficients from SDNWA being lower than those in other freshwater systems. Thus relatively more MeHg and THg is found in the more mobile porewater phase, suggesting greater ability to transfer MeHg produced in sediment into overlying waters.

Differences in MeHg in High SO₄ ponds compared to Low SO₄ ponds

The most striking difference between our two pond groups was the wide range of sulphate observed in our ponds. Higher sulphate concentrations in both surface and pore waters in High SO₄ wetlands suggest that these systems may experience less sulphate limitation, in turn more continuously stimulating sulphate reducing bacteria, which are a significant methylating organism in wetlands (Ullrich et al. 2001) and resulting in increased k_m values. However, evidence suggests that systems with high sulphate concentrations and high SRB activity result in a corresponding increase in sulphide concentrations, which can have an inhibitory impact on methylation (Benoit et al. 2001). Unfortunately, we did not sample our sites for sulphide concentrations; however, qualitative observations of high sulphide, such as a distinct odor of H₂S gas and bands of dark sediment in the cores, suggest that sulphide concentrations in our High SO₄ wetlands were higher than in Low SO₄ wetlands. As a result, we expected lower potential methylation rate constants in these high sulphate wetlands because sulphate concentrations were well into the range in wetland systems wherein sulphide accumulation is expected to inhibit Hg bioavailability (>~10-100 mg L⁻¹; Gilmour et al., 1998; Benoit et al. 2003). It was thus surprising that we did not observe significant differences in k_m values between High SO₄ and

Low SO₄ wetlands. Our small amount of data does suggest that rate constants were lower in High SO₄ wetlands (Fig. 2).

We also suspect that high electrical conductivities (driven by high sulphate concentrations) were a major driver of low K_D 's in our systems. Partitioning coefficients for both THg and MeHg in our systems were among the lowest presented in the literature (see summary in Liu et al. 2011) which indicates a preferential movement of Hg into porewater, compared to other freshwater environments. While we lack quantitative sulphide data, the very high sulphate concentrations observed in High SO₄ wetlands, coupled with the reducing conditions of these wetlands and distinct H₂S odours, lead us to speculate that significant porewater sulphide accumulation may be preferentially pulling Hg into the porewater phase. Sulphides are very strong ligands for Hg species, with either dissolved sulphides themselves, polysulphides, or thiol functional groups on dissolved organic matter likely responsible for the significant partitioning of Hg into porewater observed in our study (Skylberg 2008). As sulphide concentrations increase, Hg solubility increases according to the formation of the species HgS_2H^- $>\text{Hg}(\text{SH})_2^0>\text{HgS}_2^{2-}$ (Skylberg, 2008) and thus the abundant sulphate (and presumably sulphide) in some of our wetland systems likely led to greater Hg solubility than is normally observed.

High SO₄ wetlands did have significantly higher MeHg concentrations and %MeHg in both sediment and surface water (Fig. 3). Two possibilities may explain this difference. First, Low SO₄ wetlands were located at higher elevations of the study site and had lower DOC concentrations and less overhanging riparian vegetation surrounding them. As a result, these sites potentially experienced greater rates of photodemethylation, which in turn would result in lower concentrations of MeHg in the water column of Low SO₄ wetlands (Lehnher et al. 2012a). Since our sites were shallow, usually less than 1.5 m deep, there is the potential for light penetration to the sediment, which might also result in more photodemethylation at the sediment surface. Second, and perhaps most important, the flux of MeHg from sediment porewater to surface water may have been higher in High SO₄ wetlands compared to Low SO₄ wetland ponds. This could explain the higher surface water MeHg concentrations in those systems. In reality, it is likely that a combination of these possibilities explains increased MeHg concentrations (and corresponding %MeHg) in water in High SO₄ wetlands compared to Low SO₄ wetlands.

Why do k_m values not relate to MeHg concentrations in surface water?

In our study, methylation potentials were not correlated with porewater THg, sulphate, and DOC concentrations or sediment %OM, THg concentrations or %MeHg. This was unexpected: all of these parameters have been shown to impact methylation in other sediment and wetland systems, though not all in every system. Most surprising was the absence of a relationship between measured k_m values and sediment MeHg concentrations, which may be due to significant variation in demethylation rates which were not measured in our study. Loss of MeHg via demethylation occurs within wetland sediment (Lehnherr et al. 2012a) and since net Hg methylation depends on the gross rates of Hg methylation and MeHg demethylation, both rates of methylation and demethylation may influence the proportion of MeHg in sediment (Tjerngren et al. 2012a). If rates of demethylation are relatively similar among sites, %MeHg may be related to Hg potential methylation rates (Lehnherr et al. 2012b), however there was no significant relationship between potential rate constants and %MeHg in sediment ($r^2 = 0.164$, $p = 0.426$). The lack of a significant relationship between %MeHg and k_m values suggests that methylation was not the main driver of sediment MeHg concentrations in the summer and that demethylation plays a greater role in controlling MeHg accumulation (Mitchell and Gilmour 2008). We suggest the measurement of potential demethylation rates (especially in systems like prairie wetlands which have significant amounts of incident radiation) accompany future studies of potential methylation rate constants.

It is also surprising that we did not see a positive relationship with sediment %OM. In systems with higher organic matter content, we would expect two processes to impact potential methylation. First, sediments rich in OM tend to have higher oxygen penetration compared to sediment with less OM (Cai and Sayles 1996), which may inhibit the activity of anaerobic Hg methylating microbes. This has been observed in other systems; methylation potentials in organic sediment of Chesapeake Bay were highest in surface sediments as oxygen penetration was limited to several millimetres, while in sandy sediments of the mid-Atlantic continental shelf oxygen penetration was several centimetres and methylation potentials were higher below the oxic surface sediment (Hollweg et al. 2009). Secondly, systems with higher %OM tend to have higher rates of microbial activity, and hence greater rates of both methylation and demethylation, depending on redox conditions (Marvin-Dipasquale et al. 2014). However, there is also the possibility of an inhibitory effect of increased OM on methylation rate constants in that organic

matter complexation with Hg may decrease the bioavailability of inorganic Hg (Hsu-Kim et al. 2013).

Studies examining MeHg concentrations in PPR biota show that MeHg is bioaccumulating in waterfowl (Hall et al. 2009; Hall et al. 2014; Ofukany et al. 2012), fish (Hall et al. 2014), tadpoles (unpublished data – BDH), and aquatic invertebrates (Bates and Hall 2012) that use the ecosystem services available in wetlands. For example, both Hall et al. (2013) and Ofukany et al. (2012) show that MeHg in double-crested cormorants nesting on more northern, less productive lakes were lower than those nesting on prairie lakes. Predatory insects, and waterfowl preying on those organisms, have higher concentrations than those at lower trophic levels (Hall et al. 2009; Bates and Hall 2012). Regardless of the organisms studied, the fact remains that the vast majority of MeHg exposure to biota is as a result of *in situ* MeHg production and therefore in order to adequately understand the potential risk to organisms, we must understand net MeHg production. However, our work suggests that, although the measurement of potential methylation rate constants are important to the understanding of mercury cycling in wetlands, from a management perspective, measuring MeHg concentrations, as well as the %MeHg, in surface water may be sufficient to predict and assess MeHg risks to biota.

Conclusion

Mercury methylation potentials in July in prairie pothole wetland pond sediments were similar to those in other freshwater systems. Within prairie ponds, %MeHg and MeHg concentrations tended to be higher in High SO₄ wetland sediment compared to Low SO₄ wetlands. Our work suggests that measuring MeHg concentrations, rather than methylation potentials, in surface water may be a better method in predicting and assessing MeHg risks to biota. We also suggest the measurement of potential demethylation rates (especially in prairie wetlands which have significant amounts of incident radiation) accompany measurement of potential methylation measurements. Future research in prairie wetlands on the removal of MeHg through demethylation in sediments, and photodemethylation in the water column, will increase understanding of net methylation and factors leading to differences in MeHg concentrations in surface water, and hence biota, among wetlands. Differences in surface water MeHg concentrations in High SO₄ wetlands compared to Low SO₄ wetlands may be a consideration that stakeholders in wetland protection, reclamation, or creation consider. If it is

possible to influence sulphate concentrations of wetlands during reclamation or creation and subsequent management, it might be prudent to create low sulphate ponds preferentially.

Acknowledgements

Laboratory assistance provided by L. Bates, M. Collins, V. Bazira, S. Boczulak, and R. Rees Tiller at the Universities of Regina, Alberta, and Western Ontario. Dr. B. Branfireun supported analysis of MeHg in water through the Canadian Research Chair program. Dr. H. Baulch supported sulphate analysis. A. Bugajski, K. Gurniak, N. Hoggarth, K. Pangracs, and D. Wright were field assistants. The National Science and Engineering Research Council and the University of Regina provided funding.

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Table 1. Methylation potential ($k_m \text{ d}^{-1}$), methylmercury (MeHg) and total mercury (THg) concentrations and percent of total mercury as methylmercury (%MeHg) for freshwater wetland, saltwater wetland, and marine surface sediment.

Site	Type	$k_m (\text{d}^{-1})$	MeHg (ng g^{-1})	THg (ng g^{-1})	%MeHg	Reference
SDNWA ¹ SK	Freshwater	0.02-0.17	0.18-2.1	18.3-44.0	0.6-4.8	This study
LNWR ² ND	Freshwater	no data	<0.4 – 4.16	6.77 – 99.0	0.3-8.4	Sando et al. 2007
Arctic	Freshwater	0.04-0.16	0.6 – 18.5	4 - 69	1.2-12	Lehnherr et al. 2012b
Florida Everglades	Freshwater	0-0.12	~0.1-4.5	~60-410	0.1-1.7	Gilmour et al. 1998
Yolo Bypass CA	Freshwater	0.0003-1.3	0.53-6.17	107-433	0.31-3.88	Pers. comm. M. Marvin-Dipasquale
Boreal Sweden	Freshwater	0.011-0.057	3.5-21	113-287	2.3-17	Tjerngren et al. 2012b
Peatlands MN	Freshwater	~0-0.05	no data	no data	no data	Branfireun and Krabbenhoft, as cited in Mitchell and Gilmour 2008
Connecticut	Saltwater	~0.001-0.2	no data	no data	no data	Langer et al. 2001
San Pablo Bay	Saltwater	0.014	~290-360	~0.4-5.4	~1.8	Marvin-Dipasquale et al. 2003
Chesapeake Bay	Saltwater	0.002-0.07	~110-150	~1-5.5	0.2-4.6	Mitchell and Gilmour 2008
San Francisco	Saltwater	0.002-0.122	0.6-3.7	256-559	0.1-1.4	Windham-Myers et al. 2009
Long Island Sound	Marine	0.014-0.082	0.2-3.2	39-345	0.4-1.1	Hammerschmidt and Fitzgerald 2004
New England shelf	Marine	0.02-0.21	0.1-3.3	64-339	0.4-1	Hammerschmidt and Fitzgerald 2006
Chesapeake Bay	Marine	0.007-0.045	~0.2-11	~20-1700	0.3-1.6	Hollweg et al. 2009

¹SDNWA = St. Denis National Wildlife Area, Saskatchewan

²LNWR = Lostwood National Wildlife Refuge, North Dakota

Figure Captions:

Fig. 1: Average geochemical parameters (sulphate concentrations, electrical conductivity, dissolved organic carbon [DOC] concentrations, and pH) in surface water (A, C, E, and G) and pore water (B, D, F, H) in Low SO₄ (white) and High SO₄ (black) wetland ponds. P-values presented refer to differences between Low SO₄ and High SO₄ wetlands (Type) and July and August 2011 (Month). Error bars indicate one standard error.

Fig. 2: Sediment mercury methylation potential rate constants (d⁻¹) for A: six wetlands sampled in 2011 and B: averages in Low SO₄ (white) and High SO₄ (black) wetland ponds. Two cores per wetland were analyzed. Error bars indicate one standard error.

Fig. 3: Average Methylmercury (MeHg) and total mercury (THg) concentrations and the proportion of total mercury that is MeHg (%MeHg) in sediment (A, D, and G), surface water (B, E, and H) and porewater (C, F, and I) in Low SO₄ (white) and High SO₄ (black) wetland ponds. P-values presented refer to differences between Low SO₄ and High SO₄ wetlands (Type) and July and August 2011 (Month). Error bars indicate one standard error.

Fig. 4: Surface water methylmercury (MeHg) concentrations (ng L⁻¹) vs. A: sediment % of total Hg that is MeHg (%MeHg), B: sediment % organic matter (%OM), C: sediment MeHg concentrations (ng g⁻¹) and D: total Hg (THg) concentrations (ng g⁻¹). See Table S7 for r² and *p* values. Regression lines are only included for significant relationships.

Fig. 1.

Fig. 2.

Figure 3

Fig. 4.

Supporting Information

Mercury methylation in high and low-sulphate impacted wetland ponds within the Prairie Pothole Region of North America

Cameron G.J. Hoggarth, Britt D. Hall, and Carl P.J. Mitchell

Additional Site Description

Stewart and Kantrud's (1971) method of wetlands classification is based on vegetation characteristics but also reflects differences in water permanence. All of our wetlands were classified as Class III shallow marsh sites. Class III wetland sites are characterized seasonal ponds and lakes with a central pond that may dry up over the course of the ice-free season (typically April to October; van der Kamp et al. 2003). Representative vegetation communities are dominated by coarse grasses, sedges, and rushes that typically occur in the deepest zone. Willow rinds surrounded most of our wetlands. Typical mean water levels in our ponds ranged from 26 to 146 cm (Table S1).

Sediment Water and Organic Matter Content

Sediment sections for water and organic matter content (%OM) were processed from a single sediment core. The entire sediment section was split into three subsamples and each subsample was measured for wet weight, dry weight after drying the samples to constant weight in an oven at 100°C, and loss on ignition after heating the samples to 450°C in a muffle furnace for 8 hours. Water content was calculated as the average %water content of the three subsamples of sediment:

$$\% \text{water content} = ((\text{WW} - \text{DW}_{100}) / \text{WW}) * 100$$

where WW is the wet weight (g) of the sediment and DW₁₀₀ is the dry weight (g) of the sediment. Organic content was calculated as the average %organic content of each of the three sediment subsamples (Chasar et al. 2009):

$$\% \text{organic content} = ((\text{DW}_{100} - \text{DW}_{450}) / \text{DW}_{100}) * 100$$

where DW₁₀₀ is the dry weight (g) of the sediment after heating at 100°C, and DW₄₅₀ is the dry weight (g) after heating at 450°C.

663 Table S1. Latitude and longitude and water level data for wetlands sampled for sediment in
 664 2011. ND= no data.

Pond	Latitude	Longitude	Wetland type	Water level (cm)		
				May	July	October
100	52°12.605'	106°04.852'	Low SO ₄	89	88	26
103	52°12.567'	106°05.010'	Low SO ₄	98	105	41
110	52°12.486'	106°05.278'	Low SO ₄	94	89	30
113	52°12.634'	106°05.112'	Low SO ₄	ND	98	ND
118	52°12.587'	106°05.044'	Low SO ₄	92	96	35
2	52°12.883'	106°05.271'	High SO ₄	146.5	144.5	ND
3	52°12.869'	106°05.401'	High SO ₄	73.5	68.0	24.0
130	52°12.724'	106°04.688'	High SO ₄	111.5	129.0	105.0
139	52°12.859'	106°05.036'	High SO ₄	ND	ND	56.0

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Table S2. Quality assurance and quality control for total mercury (THg) and methylmercury (MeHg) analysis of water and sediment samples with recovery of standard reference materials (SRM) or spiked samples $\pm$ one standard deviation. Approximately 10% of samples for all parameters were analyzed in duplicate. Water Minimum detection limits for water and sediment were less than 0.3 ng L^{-1} for THg and 0.02 ng L^{-1} for MeHg. NA= none available.

Matrix and Analyte	SRM	%Recovery	Spike %Recovery
Sediment THg	MESS-3	$95 \pm 2\%$	$85 \pm 4\%$
Sediment MeHg	IAEA-405	$99.7 \pm 0.1\%$	$105 \pm 15\%$
Surface and porewater THg	MESS-3	$95 \pm 2\%$	$92 \pm 13\%$
Surface and porewater MeHg	NA	NA	$107 \pm 10\%$

Table S3. Sediment % organic matter, methylmercury (MeHg) and total Hg (THg) concentrations, and the proportion of THg that is MeHg (%MeHg) in sediment for wetlands sampled in 2011. ND indicates sites with no data. For OM, n=1. For MeHg and THg, n=2.

Pond	Organic Matter (%)		MeHg (ng g ⁻¹)		THg (ng g ⁻¹)		%MeHg	
	July	Aug	July	Aug	July	Aug	July	Aug
Low SO ₄ wetland ponds								
100	7.4	12.1	ND	ND	ND	ND	ND	ND
103	12.6	11.2	0.334	0.254	25.69	25.15	1.30	1.01
110	16.7	9.5	ND	ND	ND	ND	ND	ND
113	12.0	9.2	0.223	0.311	18.26	39.12	1.22	0.76
118	16.3	10.4	0.244	0.182	25.80	29.02	0.95	0.63
Low SO ₄	13.00	10.48	0.267	0.115	23.25	33.82	1.16	0.81
mean	±1.69	±0.54	±0.034	±0.516	±2.49	±5.16	±0.11	±0.11
High SO ₄ wetland ponds								
2	12.5	9.0	0.424	0.188	27.31	25.22	1.55	0.75
3	17.3	18.6	ND	ND	ND	ND	ND	ND
130	29.6	25.5	2.124	1.289	44.02	32.39	4.83	3.98
139	17.5	5.5	0.796	0.703	30.13	18.28	2.64	3.85
High SO ₄	19.23	14.67	0.249	0.727	31.10	25.30	3.01	2.86
mean	±3.66	±4.54	±0.037	±0.318	±4.16	±4.07	±0.96	±1.06

Table S4. Surface water electrical conductivity and pH, and dissolved organic carbon (DOC), sulphate, methylmercury (MeHg) and total mercury (THg) concentrations for wetlands sampled in 2011. For all parameters n = 1.

Pond	Surface water conductivity (mS cm ⁻¹)		Surface water pH		Surface water DOC (mg L ⁻¹)		Surface water sulphate (mg L ⁻¹)		Surface water MeHg (ng L ⁻¹)		Surface water THg (ng L ⁻¹)		Surface water %MeHg	
	July	Aug	July	Aug	July	Aug	July	Aug	July	Aug	July	Aug	July	Aug
Low SO ₄ s														
100	0.44	0.60	7.27	7.35	15.35	18.57	11.13	12.35	0.772	0.092	3.66	1.30	21.10	7.07
103	0.23	0.34	6.98	6.95	12.77	12.73	11.50	10.57	0.448	ND	3.58	0.99	12.52	1.02
110	0.30	0.51	6.58	6.53	16.01	21.73	12.11	16.10	1.678	0.282	3.37	2.24	49.79	12.60
113	0.27	0.57	7.26	6.41	9.62	12.24	12.60	10.25	0.043	0.111	2.52	0.61	1.72	18.23
118	0.29	0.47	6.86	6.82	10.96	12.59	19.70	13.82	1.155	0.417	3.25	1.29	35.60	32.47
Low SO ₄														
mean	0.31 ±0.03	0.50 ±0.05	6.99 ±0.13	6.81 ±0.17	12.94 ±1.23	15.57 ±1.94	13.41± 1.59	12.62± 2.42	0.819 ±0.282	0.226 ±0.069	3.27 ±0.20	1.28 ±0.27	24.15 ±8.47	14.28 ±5.37
High SO ₄														
2	3.89	4.40	8.16	8.33	28.64	26.42	3119.13	3246.31	0.886	0.116	2.27	1.46	39.11	7.95
3	2.58	3.15	7.24	7.73	29.81	28.74	1498.58	1970.47	2.544	0.930	3.53	1.64	72.07	56.90
130	0.75	1.01	7.41	7.67	20.75	37.32	164.57	197.62	5.715	0.766	6.82	2.08	83.87	36.93
139	1.30	1.81	7.1	7.79	33.09	39.99	714.39	770.24	3.667	1.030	4.05	1.8885	90.65	54.91
High SO ₄														
mean	2.13 ±0.07	2.59 ±0.75	7.48 ±0.24	7.88 ±0.15	28.07 ±2.62	33.12 ±3.28	1374.17±642.83	1546.16 ±676.46	3.203 ±1.01	0.711 ±0.205	4.16± 0.96	1.76± 0.13	71.43 ±36.39	39.17 ±11.34

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689 Table S5. Porewater electrical conductivity and pH, and dissolved organic carbon (DOC), sulphate, methylmercury (MeHg) and total
 690 mercury (THg) concentrations for wetlands sampled in 2011. For all parameters n = 1.

Pond	Porewater conductivity (mS cm ⁻¹)		Porewater pH		Porewater DOC (mg L ⁻¹)		Porewater sulphate (mg L ⁻¹)		Porewater MeHg (ng L ⁻¹)		Porewater THg (ng L ⁻¹)		Porewater % MeHg	
	July	Aug	July	Aug	July	Aug	July	Aug	July	Aug	July	Aug	July	Aug
Low SO ₄														
100	1.04	0.72	6.97	6.89	22.87	32.08	9.93	14.11	1.980	0.388	10.25	4.66	19.28	8.34
103	1.47	0.95	6.77	6.84	37.40	12.73	19.26	18.98	5.927	1.006	19.52	6.00	30.36	16.76
110	0.99	0.65	6.72	6.99	31.13	40.59	15.34	33.42	3.061	1.809	14.15	8.38	21.63	21.60
113	0.97	1.00	6.83	6.76	19.62	16.48	5.62	20.04	0.457	0.192	4.12	3.40	11.09	5.64
118	2.07	0.76	6.83	6.85	20.70	20.75	15.44	10.83	2.055	1.083	10.94	4.69	18.79	23.12
Low SO ₄														
mean	1.31	0.82	6.82	6.87		24.53	13.12	19.47	2.695	0.896	11.80	5.42	20.23	15.09
	±0.21	±0.07	±0.04	±0.04	26.34±3.42	±5.16	±2.39	±3.86	±0.909	±0.286	±2.52	±0.85	±3.09	±3.50
High SO ₄														
2	4.17	3.33	7.05	7.01	70.55	56.72	148.50	2600.76	55.605	0.309	50.62	6.13	109.85	5.04
3	2.89	4.55	7.24	7.17	42.45	88.92	1573.30	801.57	0.458	0.220	5.45	4.79	8.39	4.60
130	1.92	1.06	6.73	7.34	44.07	37.32	123.35	142.14	30.161	0.767	28.94	3.90	104.22	19.67
139	2.08	1.87	6.67	7.11	ND	49.26	300.59	655.98	49.268	2.088	27.96	7.56	176.21	27.60
High SO ₄														
mean	2.77±	2.70±	6.92	7.16		58.05	536.43	1050	33.873	0.846	28.24	5.60	99.37	14.23
	0.52	0.77	±0.14	±0.07	52.36±9.11	±11.04	±347.83	±535.88	±12.381	±0.431	±9.22	±0.80	±34.54	±5.67

Table S6. Log sediment/porewater total mercury (THg K_D) and methylmercury (MeHg K_D) partitioning coefficients ($L\ kg^{-1}$) for wetlands sampled in 2011. ND indicates sites with no data. For all parameters $n = 1$.

Pond	THg K_D ($L\ kg^{-1}$)		MeHg K_D ($L\ kg^{-1}$)	
	July	August	July	August
Low SO_4				
100	ND	ND	ND	ND
103	3.12	3.62	1.751	2.403
110	ND	ND	ND	ND
113	3.65	4.06	2.688	3.210
118	3.37	3.79	2.075	2.225
Low SO_4	3.38	3.83	2.117	2.613
mean	± 0.15	± 0.13	± 0.275	± 0.303
High SO_4				
2	2.73	3.61	0.882	2.784
3	ND	ND	ND	ND
130	3.18	3.92	1.848	3.225
139	3.03	3.38	ND	2.539
High SO_4	2.982	3.64	1.365	2.846
mean	± 0.13	± 0.16	± 0.483	± 0.204

Table S7. Selected regression parameters related to methylmercury (MeHg) production and geochemical parameters. Bold type indicates significance at $\alpha = 0.05$. ND= no data. k_m = methylation potentials, THg = total Hg, DOC = dissolved organic carbon, %OM = % organic matter, %MeHg = proportion of THg that is MeHg.

Independent variable	Dependent variable	r^2 July	p - value July	r^2 August	p -value August
k_m	Porewater THg	0.292	0.268	ND	ND
k_m	Porewater sulphate	0.460	0.139	ND	ND
k_m	Porewater DOC	0.289	0.350	ND	ND
k_m	Sediment THg	0.329	0.234	ND	ND
k_m	Sediment %OM	0.114	0.512	ND	ND
Porewater MeHg	Surface MeHg	0.075	0.512	0.154	0.336
Porewater THg	Surface MeHg	0.070	0.493	0.022	0.726
Porewater %MeHg	Surface MeHg	0.253	0.204	0.192	0.271
Sediment %OM	Surface MeHg	0.809	<0.001	0.119	0.403
Sediment MeHg	Surface MeHg	0.879	0.006	0.497	0.184
Sediment THg	Surface MeHg	0.864	0.007	0.332	0.309
Sediment %MeHg	Surface MeHg	0.910	0.003	0.822	0.034
Sediment %MeHg	k_m	0.164	0.426	ND	ND

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709 Fig. S1. Location of sampling sites in St. Denis National Wildlife area (SDNWA),
710 Saskatchewan, Canada.

Fig. S2. Organic matter content (%) and porosity in sediment from Low SO₄ and High SO₄ wetland ponds. P-values presented refer to differences between Low SO₄ and High SO₄ wetland ponds (Type) and July and August 2011 (Month). Error bars represent one standard error.

Fig. S3. Surface water sulphate concentrations (mg L⁻¹) vs. potential methylation rate constants.

Figure
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1. Saskatoon, SK