Determination of mercury methylation potentials in the water column of lakes across Canada

Chris S. Eckley a,b,*, Holger Hintelmann a

a Trent University, Department of Chemistry, 1600 West Bank Drive, Peterborough, ON, Canada K9J 7B8
b University of Toronto at Mississauga, Department of Geography, 3359 Mississauga Rd. N., Mississauga, ON, Canada L5L 1C6

Received 16 October 2004; received in revised form 19 August 2005; accepted 12 September 2005
Available online 10 October 2005

Abstract

A stable isotope technique was used to trace the formation of methylmercury in lake water incubation assays at in situ conditions in five lakes across Canada. Methylation activity was only detected in the anoxic hypolimnia of lakes. The stable isotope was methylated at varying rates between lakes and depths within lakes ranging from 0.56%/day to 14.8%/day. A peak in methylation potential was typically observed just below the oxycline, which decreased with increasing depth. The depth and rates of methylation potential changed seasonally with no methylation activity occurring after fall turnover. A decrease in the sulfate concentration was concomitant with the zone of mercury methylation potential indicating the likely involvement of sulfate reducing bacteria in the methylation process. A simple correlation test between DOC concentrations and methylation rates indicated a positive relationship ($r^2 = 0.62$; $p = 0.006$; $n = 27$). The demethylation rate constant in the anoxic hypolimnia was less than 0.12 d$^{-1}$.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Mercury; Methylmercury; Methylation; Stable isotope; Water column

1. Introduction

Anthropogenic activity during the 20th century has tripled the amount of mercury in the environment compared to the global background level (Mason et al., 1994). Elevated levels are a concern to humans because mercury has highly toxic neurological effects even at low concentrations. The largest route of mercury exposure to humans is through the consumption of contaminated fish (US EPA, 1997). Most mercury emitted to the environment is in an organic form; however, essentially all the mercury accumulating in fish is methylmercury (MeHg) (Hildebrand et al., 1980). Therefore, effective management of this pollutant necessitates understanding all of the sources of MeHg to aquatic systems.

Numerous studies have linked MeHg production to anaerobic microbial activity in lake sediments (Korthals and Winfrey, 1987; Gilmour and Henry, 1991) with sulfate reducing bacteria (SRB) identified as the main bacteria involved in mercury methylation (Compeau and Bartha, 1985; King et al., 2000). Korthals and Winfrey (1987) observed that the zone of greatest methylation was just below the oxic/anoxic transition zone in sediments underlying oxygenated water where sulfate reduction is the greatest (King et al., 2000). A similar redox boundary exists in the hypolimnetic water
of dimictic lakes during summer stratification. However, it is commonly reported that methylation in the water column is minimal due to lower concentrations of bacteria and nutrients, and hence this potential zone of methylation has been studied less than lake sediments (Korthals and Winfrey, 1987; Ullrich et al., 2001). Even if methylation in the water column occurred at much lower rates than in sediments, the water column could be a significant source of MeHg when the volume of water containing an oxic/anoxic boundary is larger than the volume in surficial sediments. In addition, MeHg generated in the water column may be more available for entry into the aquatic food web because it does not need to diffuse out of the sediments.

Rates of mercury methylation in lakes remain difficult to predict largely because the links to pertinent biogeochemical cycles are numerous and complex. Watras et al. (1995a,b, 1998) found that within a 30 km region with identical mercury inputs, there was a 50-fold range of dissolved MeHg in the water column. This indicates that the processes occurring within the lakes (or within the watershed) are very important in determining MeHg concentrations and can be quite different between lakes in the same region.

Historically, methodological constraints made measuring methylation rates very difficult and subject to error (Hintelmann et al., 1995). Most early studies of mercury methylation were conducted using a radioisotopic method that required the addition of commercially available 203Hg. The radio-isotope was delivered along with a non-isotopic mercury carrier; which resulted in mercury levels orders of magnitude greater than ambient concentrations, especially in the water column where mercury concentrations tend to be much lower than in sediments. Methylation rates at such high mercury levels, however, may not reflect the rates that occur at environmentally relevant concentrations. The radiotracer technique was subsequently optimized using a high specific activity 203Hg isotope, allowing much lower spike concentrations (Gilmour and Riedel, 1995; Stordal and Gill, 1995). However, the availability of the custom made material necessary for such experiments is limited. Hintelmann et al. (1995) and Hintelmann and Evans (1997) developed a new methodology for simultaneously measuring rates of mercury methylation and demethylation using low level additions of mercury stable isotopes in short term (<1 day) incubations. While research has been conducted on the methylation processes over the last few decades, there are still many unanswered questions concerning rates of methylation in lakes and the factors influencing them. The isotope tracer technique provides a powerful new tool to address these questions.

The objective of this study is to determine where and under what conditions mercury is methylated in the water column of lakes. Various zones in the water column were sampled and the methylation potentials obtained were compared with other water parameters that have been associated with methylation, such as sulfate/sulfide concentrations, dissolved oxygen (DO), redox potential, and dissolved organic carbon (DOC).

2. Materials and methods

2.1. Site descriptions

Five lakes were sampled during the spring, summer, and fall of 2002. The lakes occurred in three regions across Canada (two boreal, and one temperate). Within the boreal region, two proximate pairs of lakes were chosen with the same pristine quality and sulfate concentrations, but differing degrees of anoxic hypolimnia (Table 1). All of the lakes studied were part of separate ongoing limnological investigations.

L658 and L442 are both within the Experimental Lakes Area (N 49°37' and N 49°47'; W 93°35' and W 93°50') about 6 km apart in northwestern Ontario, Canada. This region lies on the Precambrian Shield and with the exception of limnological research experiments is devoid of all anthropogenic activity. Both lakes are dimictic and are typically covered with ice 5 months a year. L658 was sampled as part of the large whole-ecosystem project METAALICUS (Mercury Experiment to Assess Atmospheric Loading in Canada and the United States), where three mercury stable isotopes were added to the ecosystem (202Hg to the lake, 200Hg to the upland forest, and 199Hg to the adjoining wetland) to elucidate the cycling and fate of mercury.

Plastic Lake (N 45°11' W 78°50') and Red Chalk Lake (N 45°11' W 79°45') are located about 10 km from each other near Thunder Bay, Ontario, Canada. Both lakes are temperate lakes and are located about 10 km from each other. Both lakes are adjacent to the Sand River, which is a major tributary of the nearby Lake Superior.

Table 1

<table>
<thead>
<tr>
<th>Lake</th>
<th>Max. depth (m)</th>
<th>Area (ha)</th>
<th>Depth of anoxia (m)</th>
<th>Sulfate (mg/L)</th>
<th>Hypolimnetic MeHg (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L658</td>
<td>13</td>
<td>8</td>
<td>~1</td>
<td>1.9</td>
<td>1.48</td>
</tr>
<tr>
<td>L442</td>
<td>17</td>
<td>15</td>
<td>~6</td>
<td>2.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Plastic</td>
<td>16</td>
<td>32</td>
<td>~2</td>
<td>5.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Red Chalk</td>
<td>18</td>
<td>13</td>
<td>~8</td>
<td>5.2</td>
<td>0.42</td>
</tr>
<tr>
<td>Elk</td>
<td>13</td>
<td>246</td>
<td>~2</td>
<td>5.5</td>
<td>0.14</td>
</tr>
</tbody>
</table>

a Based on summer 2002 data.
b Based on summer 2002 epilimnetic average for L658, L442 and Elk Lake and fall turnover value for Plastic and Red Chalk Lakes.
c Based on summer hypolimnetic 2002 average.
apart near the southern margin of the Precambrian Shield in south-central Ontario. They are both oligotrophic lakes located in pristine watersheds. Red Chalk Lake contains two basins connected by a narrow channel. Throughout the paper, all data from Red Chalk Lake refers only to the East basin.

Elk Lake (N 48° 31' W 123° 23') is located on Vancouver Island, BC in the suburban Saanich Peninsula. It is a relatively eutrophic warm monomictic lake. Unlike the other lakes in this study, Elk Lake is in a temperate region where lakes do not freeze in the winter and summer thermal stratification is less pronounced.

2.2. Sampling schedule

All sampling took place between March and November of 2002. Elk Lake was sampled in early July; L442 were sampled in mid-July and again in mid-September; L658 was sampled in March under ice-cover, mid-July, and mid-September; Plastic Lake was sampled in early-August, mid-October, and late-November; and Red Chalk Lake was sampled in mid-October, and late-November.

2.3. Sample collection

Water samples were collected from the deepest section of each lake. The transition zone between the oxic and anoxic water was of particular interest for sampling due to the biogeochemically active nature of this region, though a few samples were also collected from the oxic epilimnion of L658 for comparison. A multiprobe (YSI, 650 MDS) with DO, temperature, conductivity, TDS, pH, turbidity, and Chlorophyll a (Chl a) was used when sampling L658 and L442 in July and September. An ORP (Consort C535) probe was used at L658 and L442 in September, and Plastic and Red Chalk Lake in October and November. A DO and temperature probe (YSI model 58) was used at Plastic Lake in August, October, and November and at Red Chalk Lake in October, and November.

All samples were collected from a boat, except in March at L658, where a hole was cut into the ice. Unfiltered water samples were collected using a peristaltic pump and a teflon line. Water samples were collected using the clean hands/dirty hands protocol into pre-cleaned, 160 ml opaque glass serum bottles. To preserve in situ redox conditions, the bottles were overfilled with twice their volume and capped by displacing air and water through a needle inserted into the rubber stopper. Samples were double bagged for transport in a cooler back to the laboratory. The cooler was monitored with a thermometer and approximate in situ temperatures were maintained during transportation (less than 2 h from collection).

At L658 in addition to samples collected by suspending a weighted line from the boat into the lake, a Close Interval Sampler (CIS) was used to sample the water at the sediment water interface. The CIS (described in Babiarz et al., 2003) is a structure that is anchored into the sediment and rises 80 cm above the sediment water interface with multiple sample lines fixed at measured depths. The CIS allows samples to be collected at 5, 10, 20, 40, and 80 cm from the sediment water interface. The lines extend to the lake surface where they are attached to a buoy and can be connected to a peristaltic pump as needed. Having access to this sampling tool provided insight into a community that would otherwise be very difficult to sample, and it allowed a very consistent way of sampling the same region over multiple months.

2.4. Mercury transformation assays

To determine mercury methylation and demethylation, incubations of lake water were performed following modified procedures originally developed for sediment assays (Hintelmann et al., 1995; Hintelmann and Evans, 1997). Mercury methylation activity was determined by adding inorganic 199Hg to each sample and monitoring the formation of Me199Hg. To determine demethylation, Me201Hg was added and was monitored for a decrease in concentration. In all samples, except L658, the 199Hg and Me201Hg spikes were added to the same bottle. At L658, methylation and demethylation assays were performed in separate bottles collected at the same time from the same depth. This was necessary because 3 stable isotopes had already been added to the L658 ecosystem as part of the METAALICUS project. If 199Hg and Me201Hg were added to the same bottle there would not have been two unadulterated isotopes left for an internal standard and ambient calculations. All isotopic spikes were administered to the samples through their re-sealable septum with an analytical syringe (Hamilton Gastight #84880, 25 μL).

All isotopes were obtained from Trace Sciences International, and stored in 0.2% HCl. The MeHg isotopes were synthesized from 201HgO using methylcobalamin following the technique in Hintelmann, 1999. The same concentration of isotope enriched mercury was added to all samples from a particular lake, but different concentrations were added among lakes as
shown in Table 2. All mercury additions increased the ambient mercury concentration by less than an order of magnitude and were only slightly higher than levels typically observed in pristine lakes (Bloom, 1989; Ulrich et al., 2001).

Samples were incubated using refrigerators set at in situ temperatures (± 2 °C) from the sample zones. Incubations were terminated by addition of 0.5 ml of concentrated HCl at 5-h intervals for the first 25 h to obtain methylation and demethylation rates. To account for abiotic methylation or demethylation, a time-zero sample was obtained by adding HCl before adding the isotopes. In a separate experiment, using the same technique, the incubations were found to maintain in situ conditions over the 25-h time period (Eckley et al., 2005).

2.5. Methylmercury determination

The formation and degradation of MeHg was determined by monitoring the concentrations of the respective isotopes Me199Hg and Me201Hg. Ultra-clean analysis techniques were used during every stage of the analysis based on Hintelmann and Ogrinc (2003). 50.0 ml from each incubation bottle was transferred to glass distillations vessels and 20 pg of Me202Hg was added as an internal standard to correct for procedural recoveries (except at L658 where Me199Hg was used in demethylation assays and 201Hg in methylation assays). 0.500 ml 9M H2SO4 was added to liberate the MeHg from sulfur and organic complexes in the sample matrix and 0.200 ml of 20% KCl was added for the distillation of methylmercury chloride into 5 ml of Milli-Q water. The samples were distilled at 115 °C with a mercury-free nitrogen gas flow of 60 ml/min until approximately 90% of the sample had been transferred to the receiving vessel (approximately 5 h). Following the distillation, MeHg was ethylated with 0.100 ml tetraethylborate at a pH of 4.9 (0.200 ml sodium acetate buffer) and allowed to react for 20 min before purging with 200 ml/min nitrogen gas onto Tenax™ traps for an additional 20 min. The mercury was thermo-desorbed from the traps at a temperature of 200 °C and carried on Hg-free argon gas through a gas chromatography column (GC) filled with 15% m/m OV-3 on Chromosorb W, AW, DMCS at 106 °C to separate the mercury species. The mercury was detected by connecting the GC column to an inducively coupled plasma mass spectrometer (ICP-MS) (Element 2, Thermo Finnigan or Platform, Micromass). Chromatographic data were collected from the ICP-MS and peak areas were used to calculate concentrations using a programmed spreadsheet that accounts for procedural blanks and the purities of the isotopes (as described in Hintelmann and Ogrinc, 2003). Me200Hg was used to represent the ambient MeHg concentrations, except at L658 where Me198Hg was used. It should be noted that although inorganic 198Hg was added to the L658 wetland as part of the METAALICUS project the preceding year no enriched Me198Hg was detected in the lake in 2002 (Hintelmann, 2003, unpublished).

Methylation potentials were determined by linear regression of the methylation activity (% 199Hg methylated) versus time for each depth with the slope yielding the specific methylation rate constant, km (d−1). At all the depths where methylation was detected, the amount of Me199Hg generated increased linearly for the first 25 h. This concurs with the findings of Hintelmann (2000, unpublished) and Xun et al. (1987) that found the rate of methylation to be constant for the first 24 h. The concentration of the inorganic 199Hg spike is assumed to be constant over the incubation time period. With the exception of two depths at L658 (where ~ 15% of the spike was methylated), the remaining lakes had less than 5% of the inorganic spike converted to Me199Hg, leaving the inorganic concentration more than 95% unchanged throughout the experiment. To determine if two or more potentials were different, an analysis of covariance test for parallelism (using the F statistic) was performed using Statistica™. The residuals of the methylation data were normally distributed based on the Shapiro–Wilk’s test (W = 0.69; p < 0.00001) and the residuals tended towards homoscedasticity using the Levene test for homogeneity of
variances \((p > 0.06\) for all lakes, except for L442: \(p = 0.03\) and \(0.02\) for July and September, respectively).

Five to seven water samples were collected from each depth. However, to screen for methylation potential, initially only three samples were analyzed from each depth (first, last and one intermediate time point). Only if an increase in \(\text{Me}^{199}\text{Hg}\) over time was observed was the entire time series analyzed.

2.6. Detection limit

The detection limit for newly formed \(\text{Me}^{199}\text{Hg}\) (based on Hintelmann and Evans, 1997) is dependent on the concentration of the ambient \(\text{MeHg}\) and therefore varied between \(0.001–0.004\) ng/L for different lakes. The detection limit for the degradation of \(\text{Me}^{201}\text{Hg}\) was constrained by the spike addition technique (relative uncertainty of 11%), not by the precision of the isotope ratio measurement.

2.7. Other water parameters

DOC was analyzed from sub-samples of incubation bottles using an organic carbon analyzer (TOC 5000, Shimadzu). Cation and anion samples were analyzed from sub-samples of incubation bottles using ion chromatography (Dionex, model DX600). Sulfide at Red Chalk Lake and Plastic Lake in October were analyzed using the methylene blue method (Tang and Santschi, 2000).

3. Results

3.1. Calculation of methylation and demethylation potentials

Net mercury methylation rates can only be calculated directly using data from the methylation assay, where \(\text{MeHg}\) levels increase linearly over time and demethylation activity is insignificant. From the outset, it was unknown over which time period methylation will proceed at a constant rate in the water column. Consequently, samples were collected frequently during the first day of incubations and less frequently thereafter. After sample analyses, it became obvious that \(\text{MeHg}\) concentrations increased linearly during the first 25 h. Longer incubations not always followed this trend and frequently a decrease in production rates was observed, possibly for two reasons: 1) once sufficient \(\text{MeHg}\) was produced, the demethylation activity may be significant enough to slow down the net increase in \(\text{MeHg}\) and 2) it is conceivable that incubation condition change over longer time periods and original in situ conditions cannot be preserved for more than one day. Previous experiments confirmed that bacterial communities remain unchanged for at least 24 h, but information for extended periods are not available (Eckley et al., 2005). Consequently, net methylation rates were calculated using data from the initial 25 h of incubation, where a linear increase in \(\text{MeHg}\) concentration with time was observed.

Methylmercury demethylation potentials (based on a decrease in spiked \(\text{Me}^{201}\text{Hg}\) concentration) were below the detection limit (11%/day) for all of the lakes surveyed. This equates to a specific demethylation rate constant \((k_{\text{dm}})\) of \(<0.12\) d\(^{-1}\) based on the linear regression of \(\ln [\text{Me}^{201}\text{Hg}]\) versus time assuming a maximum 11% loss per day. Though not measured directly, demethylation rates are believed to be greater than zero for two reasons: 1) many of the methylation assays show a plateau in the rate of methylation after 25 h indicating that demethylation may be occurring and a steady state concentration is being established, and 2) using the specific methylation rates to estimate the ambient \(\text{MeHg}\) concentrations in lakes quickly over-predicts the ambient concentrations unless some demethylation is assumed (Eckley et al., 2005).

3.2. L658

At L658 in July, less than half a meter of anoxic water extended above the sediment-interface (Fig. 1). Seven depths were sampled for methylation activity in the bottom 1.5 m of the lake. The CIS sampler was used to sample the depths closest to the sediment (the depth of 80 cm from the sediment corresponds to a depth of \(\sim12.2\) m from the lake surface). In addition, three samples from the epilimnion were collected corresponding to a peak in Chl \(\alpha\) (5.8 \(\mu\)g/L compared to an average of 3.7 \(\pm\) 0.5 \(\mu\)g/L for the rest of the water column).

Methylation was only detected between 5 and 40 cm from the sediment–water interface, which were the only depths that were completely anoxic (Table 3, Fig. 1). The methylation potential was the lowest at 40 cm (4.1%/day) and increased moving toward the sediment interface. The maximum methylation potential occurred at 5 cm above the sediment (14.8%/day). The ambient \(\text{MeHg}\) profile showed low \(\text{MeHg}\) concentrations in the epilimnion (0.24 \(\pm\) 0.09 ng/L), which increased almost 4-fold to 1.11 ng/L at 40 cm from the sediment/water interface (Fig. 1). The sulfate concentration varied between 1.8 and 2.1 mg/L in the epilimnion and did not drop until 5 cm from the sediment where it was 0.3 mg/L. The DOC
profile was fairly uniform throughout the water column (12.8 ± 0.9 mg/L), with the only peak occurring at 5 cm (20.9 mg/L). The turbidity was low throughout the water column (0.8 ± 0.6 NTU), with a peak occurring at 40 cm (8.4 NTU) corresponding to where methylation activity was first detected.

Table 3
Summary of L658 data

<table>
<thead>
<tr>
<th>Month</th>
<th>Depth a</th>
<th>Methylation (%/day)b</th>
<th>p</th>
<th>n</th>
<th>Ambient MeHg (ng/L)</th>
<th>Sulfate (mg/L)</th>
<th>Temp (°C)</th>
<th>DOC (mg/L)</th>
<th>pH</th>
<th>Redox (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 4.0 m</td>
<td>ND</td>
<td>–</td>
<td>3</td>
<td>0.29</td>
<td>NA</td>
<td>11.22</td>
<td>12.4</td>
<td>5.86</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 4.25 m</td>
<td>ND</td>
<td>–</td>
<td>3</td>
<td>0.30</td>
<td>2.1</td>
<td>10.41</td>
<td>12.6</td>
<td>5.80</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 11.85 m</td>
<td>ND</td>
<td>–</td>
<td>3</td>
<td>0.50</td>
<td>2.1</td>
<td>4.49</td>
<td>NA</td>
<td>5.62</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 12.1 m</td>
<td>ND</td>
<td>–</td>
<td>3</td>
<td>0.58</td>
<td>2.2</td>
<td>4.57</td>
<td>12.6</td>
<td>5.83</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 80 cm</td>
<td>ND</td>
<td>–</td>
<td>3</td>
<td>0.75</td>
<td>1.7</td>
<td>4.53</td>
<td>11.3</td>
<td>5.60</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 40 cm</td>
<td>4.1</td>
<td>0.02</td>
<td>5</td>
<td>1.11</td>
<td>2.0</td>
<td>4.52</td>
<td>13.6</td>
<td>5.60</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 20 cm</td>
<td>8.3</td>
<td>0.001</td>
<td>6</td>
<td>1.34</td>
<td>1.7</td>
<td>NA</td>
<td>14.3</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 10 cm</td>
<td>7.5</td>
<td>0.035</td>
<td>6</td>
<td>1.58</td>
<td>1.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>July 5 cm</td>
<td>14.8</td>
<td>0.005</td>
<td>5</td>
<td>1.39</td>
<td>0.3</td>
<td>4.46</td>
<td>21.1</td>
<td>5.48</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Sept. 3.25 m</td>
<td>0.11</td>
<td>0.67</td>
<td>5</td>
<td>0.26</td>
<td>1.8</td>
<td>19.12</td>
<td>9.3</td>
<td>7.06</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>Sept. 4.0 m</td>
<td>0.24</td>
<td>0.34</td>
<td>5</td>
<td>0.14</td>
<td>1.7</td>
<td>15.24</td>
<td>9.4</td>
<td>6.24</td>
<td>307</td>
<td></td>
</tr>
<tr>
<td>Sept. 11.5 m</td>
<td>0.82</td>
<td>0.007</td>
<td>5</td>
<td>1.11</td>
<td>1.0</td>
<td>4.69</td>
<td>9.0</td>
<td>6.12</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Sept. 80 cm</td>
<td>13.8</td>
<td>0.011</td>
<td>5</td>
<td>1.58</td>
<td>1.6</td>
<td>4.64</td>
<td>10.5</td>
<td>6.35</td>
<td>– 3</td>
<td></td>
</tr>
<tr>
<td>Sept. 40 cm</td>
<td>7.4</td>
<td>0.013</td>
<td>5</td>
<td>1.57</td>
<td>0.5</td>
<td>NA</td>
<td>11.8</td>
<td>NA</td>
<td>– 34</td>
<td></td>
</tr>
<tr>
<td>Sept. 20 cm</td>
<td>7.4</td>
<td>0.001</td>
<td>5</td>
<td>1.62</td>
<td>0.8</td>
<td>NA</td>
<td>12.7</td>
<td>NA</td>
<td>– 51</td>
<td></td>
</tr>
<tr>
<td>Sept. 5 cm</td>
<td>6.4</td>
<td>0.028</td>
<td>5</td>
<td>1.82</td>
<td>0.2</td>
<td>NA</td>
<td>13.1</td>
<td>NA</td>
<td>– 52</td>
<td></td>
</tr>
</tbody>
</table>

ND: not detected.
NA: not analyzed.
a Depths expressed in cm were measured moving up the water column from the sediment–water interface. Depths expressed in m were measured moving down the water column from the surface of the lake.
b Expressed as the percent of the added 199 Hg isotope methylated per day.
In September at L658, the region of anoxia had increased up the water column by approximately a meter (Fig. 1). Five samples were collected from within the anoxic region, as well as two samples from the oxic epilimnion. Methylation was not observed in the epilimnion (less than 1% of Hg methylated and regression $p > 0.3$), but was detected at all of the anoxic depths (Table 3). Methylmercury formation was lowest (0.82%/day) at the transition zone between oxic/anoxic conditions (11.5 m), followed by a large peak (13.8%/day) just below the oxycline at 80 cm from the sediment/water interface. This depth is also where the redox potential became negative. Below the peak at 80 cm, methylation activity decreased by half (to 7.4%/day) and remained at a relatively constant rate down to 5 cm from the sediment ($F_{2,9} = 0.24$, $p = 0.74$). The ambient MeHg profile shows a low MeHg concentration in the epilimnion similar to levels observed in July (Fig 1). In
contrast, the hypolimnion showed a marked increase in MeHg. For example, between July and September ambient MeHg at 11.5 m doubled as the depth switched from being oxic to anoxic. The sulfate concentration was 1.5 ± 0.3 mg/L, until it dropped to 0.5 mg/L at 40 cm and remained low down to the sediment. A large peak in turbidity was observed where methylation was first detected (depth: 11.5 m; turbidity: 8.6 NTU) compared with the low average in the rest of the water column (0.54 ± 0.23 NTU). The September DOC profile did not change much from the July profile with little variation with depth (10.8 ± 1.7 mg/L).

L658 was sampled under ice cover in March at three depths (5.5, 9.0, and 13.0 m). Methylation activity was not observed at any of the depths sampled.

3.3. L442

In July at L442, four depths were sampled from the meter and a half deep anoxic hypolimnion (Fig. 2a). Methylation activity was detected at all the depths sampled, with a potential peak just below the oxycline (15.75 m: 2.6%/day) and decreasing methylation potential with increasing depth (1.6%/day to 0.99%/day) (Table 4). However, none of the rates were statistically different from each other ($F_{3, 15} = 2.26, p = 0.12$). The ambient MeHg concentration was 0.63 ± 0.03 ng/L, and the DOC was 11.5 ± 1.3 mg/L. Sulfate levels were low at all depths sampled showing a decrease from 0.8 mg/L at 15.75 m to 0.4 mg/L at 16.5 m.

In September at L442, four depths were sampled from the anoxic hypolimnion, which had increased to above 4 m from the sediment (Fig 2a). Methylation was detected at all the depths sampled with a peak at 12.5 m ($F_{3, 25} = 8.59, p = 0.001$) just below the oxycline where the redox potential became negative (Table 4). The methylation potentials at the deeper depths were not statistically different and averaged $2.3 ± 0.2%/day$ ($F_{2, 12} = 0.13, p = 0.88$). Sulfate was low at all the depths sampled, decreasing from 0.9 mg/L at 12.0 m to 0.3 mg/L at 15.0 m. DOC did not vary with depth, averaging $7.4 ± 0.6$ mg/L. The turbidity was low in the epilimnion (0.8 NTU average), before sharply increasing at 12.0 m (4.0 NTU) and remained high throughout the rest of anoxic hypolimnion (4.3 NTU average).

3.4. Plastic Lake

At Plastic Lake in August, only about 0.25 m of anoxia existed above the sediment. Three depths were sampled to encompass the region just above and below the oxycline (Table 5, Fig. 2b). Methylation activity was observed at 15.5 and 15.75 m with an average rate of $0.88 ± 0.10%/day$ ($F_{3, 8} = 0.25, p = 0.63$). Above the oxycline (14.5 m) the ambient MeHg concentration was 0.24 ng/L and just below the oxycline it more than doubled to 0.58 ng/L. The sulfate concentration did not change much above or below the oxycline and averaged $3.4 ± 0.1$ mg/L. The DOC concentration increased markedly from 4.1 mg/L above the oxycline to 12.7 mg/L just above the sediment.

In October at Plastic Lake, the oxycline had moved up the water column almost 2 m since August (Fig. 2b). Methylation activity was detected at the two anoxic depths sampled, with a peak of 4.3%/day at 15.25 m ($F_{1, 6} = 5.64, p = 0.054$) which corresponded to where the redox potential became negative. The ambient MeHg concentrations in the anoxic hypolimnion (14.0 m: 0.80 ng/L) were 3-times higher than in the epilimnion (7.0 m: 0.26 ng/L). Sulfide was first

Table 4

<table>
<thead>
<tr>
<th>Month</th>
<th>Depth (m)</th>
<th>Methylation (%/day)$^a$</th>
<th>$p$</th>
<th>$n$</th>
<th>Ambient MeHg (ng/L)</th>
<th>Sulfate (mg/L)</th>
<th>Temp ($^\circ$C)</th>
<th>DOC (mg/L)</th>
<th>pH</th>
<th>Redox (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>15.75</td>
<td>2.6</td>
<td>0.022</td>
<td>6</td>
<td>0.62</td>
<td>0.8</td>
<td>4.36</td>
<td>11.4</td>
<td>5.89</td>
<td>NA</td>
</tr>
<tr>
<td>July</td>
<td>16.0</td>
<td>1.6</td>
<td>0.012</td>
<td>6</td>
<td>0.62</td>
<td>0.9</td>
<td>4.35</td>
<td>10.9</td>
<td>5.98</td>
<td>NA</td>
</tr>
<tr>
<td>July</td>
<td>16.25</td>
<td>1.3</td>
<td>0.003</td>
<td>6</td>
<td>0.62</td>
<td>0.6</td>
<td>4.34</td>
<td>10.5</td>
<td>6.08</td>
<td>NA</td>
</tr>
<tr>
<td>July</td>
<td>16.5</td>
<td>0.99</td>
<td>0.103</td>
<td>5</td>
<td>0.67</td>
<td>0.4</td>
<td>4.34</td>
<td>13.4</td>
<td>6.15</td>
<td>NA</td>
</tr>
<tr>
<td>Sept.</td>
<td>12.0</td>
<td>2.5</td>
<td>0.025</td>
<td>6</td>
<td>0.39</td>
<td>0.9</td>
<td>4.65</td>
<td>6.5</td>
<td>6.20</td>
<td>50</td>
</tr>
<tr>
<td>Sept.</td>
<td>12.5</td>
<td>5.5</td>
<td>0.003</td>
<td>5</td>
<td>0.58</td>
<td>0.7</td>
<td>4.62</td>
<td>7.5</td>
<td>6.29</td>
<td>−58</td>
</tr>
<tr>
<td>Sept.</td>
<td>13.1</td>
<td>2.1</td>
<td>0.020</td>
<td>6</td>
<td>0.61</td>
<td>0.7</td>
<td>4.36</td>
<td>7.1</td>
<td>6.39</td>
<td>−30</td>
</tr>
<tr>
<td>Sept.</td>
<td>14.0</td>
<td>2.3</td>
<td>0.007</td>
<td>6</td>
<td>0.63</td>
<td>0.4</td>
<td>4.55</td>
<td>7.9</td>
<td>6.42</td>
<td>−58</td>
</tr>
<tr>
<td>Sept.</td>
<td>15.0</td>
<td>−2.4$^b$</td>
<td>–</td>
<td>1</td>
<td>0.67</td>
<td>0.3</td>
<td>4.52</td>
<td>7.9</td>
<td>6.44</td>
<td>−71</td>
</tr>
</tbody>
</table>

NA: not analyzed.

$^a$ Expressed as the percent of the added $^{199}$ Hg isotope methylated per day.

$^b$ Based on only two samples—time zero, and after 25 h. No other samples were collected at this depth.
detected at 14.0 m (6.6 μM) and increased with depth to 33.0 μM at 15.25 m.

The sampling at Plastic Lake in November occurred after fall turnover. The lake was well mixed, oxygenated, and had a positive redox potential at all depths (Fig. 2b). The same depths sampled in October that showed methylation activity were re-sampled, but neither showed any methylation potential (less than 0.4% ¹⁹⁹Hg methylated; regression p value >0.18). The average DOC concentration was 2.2 ± 0.1 mg/L, which was a decrease from the summer.

### 3.5. Red Chalk Lake

Red Chalk Lake in October contained a large anoxic hypolimnion 8 m deep (Fig. 2c). Five depths from the anoxic region were sampled with methylation detected at all of them (Table 6). A peak in methylation activity occurred just below the oxycline at a depth of 10.0 m (1.1%/day; F₁,₅ = 7.92, p = 0.037). At deeper depths (12.0 and 15.0 m in particular) the methylation potentials were much lower resulting in regression p values greater than 0.05 (0.16 and 0.12, respectively) reflecting the increased variability of measuring methylation at very low levels. The ambient MeHg concentration in Red Chalk Lake was low compared to the other study lakes, with 0.12 ng/L measured in the epilimnion and 0.40 ± 0.03 ng/L in the hypolimnion. The DOC concentration increased with depth, from 3.9 mg/L at 10.0 m to 6.1 mg/L at 16.0 m. The redox potential became negative at a depth of 11.0 m. Sulfide was detected beginning at 10.0 m and increased with depth to 28.3 μM at 16.0 m.

In November, Red Chalk Lake was sampled during fall turnover while the lake had a uniform temperature, DO and redox profile (Fig 2c). The same depths that were anoxic and showed methylation activity in October were re-sampled in November and did not display any

<table>
<thead>
<tr>
<th>Month</th>
<th>Depth (m)</th>
<th>Methylation (%/day)</th>
<th>p</th>
<th>n</th>
<th>Ambient MeHg (ng/L)</th>
<th>Sulfate (mg/L)</th>
<th>Sulfide (μM)</th>
<th>Temp (°C)</th>
<th>DOC (mg/L)</th>
<th>Redox (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct.</td>
<td>4.0</td>
<td>NA</td>
<td></td>
<td></td>
<td>0.12</td>
<td>NA</td>
<td>&lt;1.5</td>
<td>17.6</td>
<td>NA</td>
<td>207</td>
</tr>
<tr>
<td>Oct.</td>
<td>7.0</td>
<td>NA</td>
<td></td>
<td></td>
<td>0.21</td>
<td>NA</td>
<td>&lt;1.5</td>
<td>12.0</td>
<td>NA</td>
<td>246</td>
</tr>
<tr>
<td>Oct.</td>
<td>10.0</td>
<td>1.1</td>
<td></td>
<td></td>
<td>0.013</td>
<td>4.7</td>
<td>10.2</td>
<td>6.4</td>
<td>3.9</td>
<td>34</td>
</tr>
<tr>
<td>Oct.</td>
<td>11.0</td>
<td>0.59</td>
<td></td>
<td></td>
<td>0.050</td>
<td>3.7</td>
<td>17.1</td>
<td>5.8</td>
<td>4.1</td>
<td>38</td>
</tr>
<tr>
<td>Oct.</td>
<td>12.0</td>
<td>0.26</td>
<td></td>
<td></td>
<td>0.160</td>
<td>2.7</td>
<td>22.1</td>
<td>5.6</td>
<td>4.8</td>
<td>49</td>
</tr>
<tr>
<td>Oct.</td>
<td>15.0</td>
<td>0.27</td>
<td></td>
<td></td>
<td>0.120</td>
<td>NA</td>
<td>26.1</td>
<td>5.2</td>
<td>5.8</td>
<td>103</td>
</tr>
<tr>
<td>Oct.</td>
<td>16.0</td>
<td>0.56</td>
<td></td>
<td></td>
<td>0.014</td>
<td>NA</td>
<td>28.3</td>
<td>5.2</td>
<td>6.1</td>
<td>113</td>
</tr>
<tr>
<td>Nov.</td>
<td>10.0</td>
<td>ND</td>
<td></td>
<td></td>
<td>0.30</td>
<td>5.3</td>
<td>NA</td>
<td>3.8</td>
<td>3.4</td>
<td>211</td>
</tr>
<tr>
<td>Nov.</td>
<td>12.0</td>
<td>ND</td>
<td></td>
<td></td>
<td>0.69</td>
<td>5.2</td>
<td>NA</td>
<td>3.9</td>
<td>3.4</td>
<td>222</td>
</tr>
<tr>
<td>Nov.</td>
<td>15.0</td>
<td>ND</td>
<td></td>
<td></td>
<td>0.75</td>
<td>5.0</td>
<td>NA</td>
<td>3.9</td>
<td>3.4</td>
<td>227</td>
</tr>
<tr>
<td>Nov.</td>
<td>16.0</td>
<td>ND</td>
<td></td>
<td></td>
<td>0.97</td>
<td>5.1</td>
<td>NA</td>
<td>4.0</td>
<td>3.4</td>
<td>207</td>
</tr>
</tbody>
</table>
methylation potential (less than 0.3% 199Hg methylated; regression p values > 0.30). The ambient MeHg concentration was uniform and low (0.17 ± 0.005 ng/L) and the DOC concentration had decreased to 3.4 ± 0.03 mg/L.

3.6. Elk Lake

The oxygen profile of Elk Lake decreased slowly with depth, with only about half a meter of completely anoxic water occurring in the hypolimnion (Fig 2d). Six depths just above and below the zone of oxygen depletion were sampled for mercury methylation, but none was detected (less than 0.2% of 199Hg methylated; regression p values > 0.2). The ambient MeHg concentration in Elk Lake was lowest of all the lakes studied (0.13 ± 0.02 ng/L) and did not show any increase in the anoxic hypolimnion. The sulfate concentration was 5.3 ± 0.2 mg/L, which also did not decrease in the anoxic region. The DOC concentration varied between 14.1 and 32.8 mg/L, but did not display a visual trend with depth.

4. Discussion

4.1. Tracer isotope incubations and mercury methylation

Incubation experiments performed by Mauro et al. (2002) on macrophytes, found the % mercury isotope methylated was affected by the amount of inorganic isotope added. In their experiment, spikes ranged from 1 to 23 ng and the resulting fraction of mercury methylated ranged from 38% to 16%, respectively. They concluded that the amount of mercury methylated is controlled by its bioavailability, which appears to be mediated by the formation of sulfur complexes (Benoit et al., 1999). If the inorganic Hg additions are unrealistically high, only a relatively small fraction of the spike may be present as the bioavailable neutral complex resulting in a smaller percentage being methylated. Another explanation of this phenomenon could result from enzyme saturation at higher concentrations of inorganic mercury as Choi et al. (1994) showed that mercury methylation is an enzymatic process. Previous studies on water column mercury methylation used the radioactive-isotope tracer technique, requiring large additions of inorganic 203Hg, which resulted in inorganic mercury concentrations up to 66 μg/L (Korthals and Winfrey, 1987). The methylation potentials detected in these studies are reported as the % of the spike methylated per day, and were all very small (< 1%/day) (Korthals and Winfrey, 1987; Xun et al., 1987; Matilainen, 1995; Verta and Matilainen, 1995) (Table 7), giving the appearance that the water column is not a major zone of MeHg production. Relative to these early studies, the methylation potentials in this paper are much larger (ranging from 0.56%/day to 14.8%/day) (Tables 3–6). However, comparing the absolute amount (as opposed to the percent) of Me203Hg produced in the early studies shows that an order of magnitude more MeHg was produced in the earlier studies than in our study. Therefore, the low rates (expressed as percents) previously reported are likely a function of the large tracer spike overwhelming the ligand or enzymatic capacity of the system.

4.2. Anoxia and mercury methylation

Mercury methylation was only detected under anoxicic conditions (defined for this study as less than 0.5 mg/L DO detected using a probe). When Red Chalk and Plastic Lake were sampled after fall turnover creating a well-oxygenated water column, the same depths that displayed methylation activity a month earlier, showed no methylation. This agrees with the generally accepted notion that methylation is mediated by anaerobic bacteria (Compeau and Bartha, 1985; Gilmour et al., 1992). Methylation was detected in all lakes with anoxic waters, with the exception of Elk Lake. The lack of methylation activity observed in the anoxic region of Elk Lake could have many explanations. In many regards, Elk Lake is unique from the other study lakes in that it is in a temperate region, monomictic, and has slow decrease in temperature and DO with depth. While these factors may create conditions where the redox potential is not low enough to favor methylation activity, the lack of methylation may simply be a result of timing—Elk Lake was visited earlier in the summer than the other lakes and the sampling
may have preceded the emergence of SRB into the water column (as explained below) as there was only half a meter of anoxic water.

Dissolved oxygen concentrations change seasonally in boreal lakes with anoxia developing in many lakes in early summer and growing until fall turnover. The zone of methylation potential was observed to change seasonally as well, following the rise of anoxic conditions up the water column. For example, at L658 in July, no methylation potential was detected at 11.5 or 12.2 m (80 cm from the sediment/water interface), where the water was oxygenated. In September, 11.5 and 12.2 m were anoxic and displayed methylation potential (0.82%/day and 13.8%/day, respectively). L442 and Plastic Lake were also sampled twice during the summer and their regions showed the same trend of methylation activity following the rising oxycline up the water column.

Within anoxic hypolimnia, mercury is methylated at different rates as a function of depth. In all the lakes with methylation potentials, a peak in activity was observed at one depth—typically just below the oxycline, with decreasing potentials with increasing depth. At L658 in July, over a span of less than half a meter, three distinct methylation potentials were detected (varying over 10%/day), indicating narrowly defined microbial zones, or a concentration gradient of methylating bacteria. The hypolimnia of L442, Plastic and Red Chalk Lakes also showed statistically significant variations in methylation potentials even with their hypolimnia sampled at intervals of 0.5 to 1 m (compared to the 5 to 40 cm sample intervals at L658). Narrowly defined regions of methylation potentials may be missed when sampling at these larger intervals, but the distinctions in methylation activity between specific depths may become less pronounced as the oxycline moves further from the sediment interface. Bacteria populations are more prolific in the nutrient and carbon rich sediments than the water column. High rates of methylation have been observed in the top layers of the sediments underlying oxygenated water (Korthals and Winfrey, 1987). Sediments underlying anoxic water, however, display low rates of sulfate reduction and methylation potential (Watras et al., 1995a,b). In this study, the highest methylation potential was detected 5 cm from the sediment interface at L658 in July, less than a meter below the oxycline. In September, the oxycline had moved up the water column approximately 1 m as did the peak in methylation activity (13.8%/day, which was statistically equivalent to the peak detected in July of 14.8%/day; $F_{1,6} = 0.09, p = 0.77$). Of the lakes studied, L658 had the smallest anoxic hypolimnion (in depth and volume) and it had much higher methylation rates than the other lakes. Comparing L658, L442, and Red Chalk Lake, a trend of decreasing methylation potential with further distance from the sediment–water interface emerges. A possible explanation for these results is that bacteria communities originating in the sediment are proliferating upwards into the water column following favorable conditions (such as fresh sources of sulfate) as oxygen becomes depleted. The higher methylation potentials in lakes with small anoxic hypolimnia could result from bacteria populations being denser near the sediment, where bacteria are in much higher abundance. As the anoxic boundary layer moves further from the sediment, the bacterial communities may become more diffuse.

The best comparison to support the above hypothesis lies between L658/L442 and Plastic/Red Chalk Lake. The two pairs of lakes are over 1000 km apart, and have more than a two-fold difference in sulfate concentrations, but within the pairs (less than 10 km apart) the sulfate concentrations are essentially identical owing to similar geologic and atmospheric settings. As already established, the anoxic hypolimnion of L658 was small (~1 m) and contained large methylation potentials (~14%/day) less than a meter from sediment, while the hypolimnion of nearby L442 was much larger (~6 m by late summer) but had 3–4 times smaller methylation potentials (~3–5%/day) sampled many meters from the sediment interface. Similarly, Plastic Lake had a small anoxic hypolimnion (~2 m) compared to the large anoxic region of Red Chalk Lake (~8 m). The same trend as in L658/L442 emerged with methylation potentials in Plastic Lake (~4%/day) being 4-times higher than the methylation potentials (~1%/day) in Red Chalk Lake which were much further from the sediment interface.

### 4.3. Sulfate/sulfide and mercury methylation

While anoxic conditions are necessary for the activity of SRB, it is likely the changing sulfate concentrations that drive the SRB upward in the water column. SRB use sulfate as their terminal electron acceptor, creating sulfide in the process. The presence of SRB can be detected by observing the seasonal decrease of sulfide under anoxic conditions and the concomitant increase of sulfide. Data from our lakes show good agreement between the movement of methylation activity up the water column and a decrease in the sulfate concentration. For example, at L658 in July the sulfate concentration was constant at 1.9 ± 0.3 mg/L in the water column until it sharply decreased to 0.3 mg/L where the peak in methylation was occurring at 5 cm
from the sediment interface. By September, the zone of depleted sulfate had increased almost half a meter up the water column falling just below the peak in methylation activity. Similar results were found in L442 where the epilimnetic concentration of sulfate (2.0 mg/L) was decreased to less than 1 mg/L where methylation was occurring.

The relationship between mercury methylation and sulfate/sulfide chemistry is complex. While sulfate controls microbial activity, sulfide controls mercury speciation. At low sulfide concentrations, neutral mercury-sulfide complexes that can diffuse through cell membranes are thought to dominate; while at high sulfide concentrations the bioavailability can be reduced due to the formation of charged mercury-sulfide species (Benoit et al., 1999).

In Plastic Lake, peak methylation was detected at a sulfide concentration of 33.0 μM and in Red Chalk Lake peak methylation was detected at a sulfide concentration of 10.2 μM. In pore-water experiments, Benoit et al. (2003) report that sulfide concentrations above 10 μM are inhibitory to methylation activity due to the formation of charged mercury-sulfide complexes. While the findings from our study indicate that methylation can continue at much higher concentrations in the water column, as the sulfide concentration further increased with depth in Red Chalk Lake, there was a decrease in the methylation potential (likely due to decreased SRB activity from the depletion of sulfate and/or the formation of charged sulfide–mercury species).

Of the lakes studied, the highest hypolimnetic sulfate concentration was at Elk Lake (5.3 ± 0.2 mg/L), where no methylation was detected. The sulfate concentration at Elk Lake did not show any decrease over the 3.5 m sampled, likely indicating the absence of SRB activity, which may also explain the lack of methylation activity.

Ingvorsen et al. (1981) report that the rate of sulfate reduction is typically 10^3 times higher in lake sediments than in the water column. These high rates can rapidly deplete the available pool of sulfate in the sediment; however, this is not likely to occur in the water column because fresh pools of sulfate are constantly made available as the zone of anoxia increases up the water column during summer stratification. Therefore, while the rate of sulfate reduction in the water column is lower, it is likely sustainable over the entire summer.

Optimum conditions to sustain sulfate reduction are around −100 mV (pH 5) (Wetzel, 1983). In lakes, various redox reactions occur at the same depth which cannot be distinguished using a redox probe. Therefore, all redox potentials reported in this paper are a result of multiple reactions happening simultan-

ously (Stumm and Morgan, 1996) and our measurement represent general trends rather than specific processes. In lakes where the redox potential was measured, the boundary between oxidizing and reducing conditions was pronounced, showing a dramatic drop in the redox potential just after the onset of anoxia. Methylation activity was observed at low but positive redox potentials, but the higher methylation rates tended to occur just after the redox potential became negative. As the redox potential became increasingly negative, methylation rates decreased perhaps representing a shift away from SRB communities towards methanogens.

4.4. Organic carbon and mercury methylation

Besides a terminal electron acceptor, SRB also require a carbon source, however, the relationship between DOC and mercury methylation is complex and not well understood. On one hand, DOC has been shown to increase mercury methylation by stimulating microbial activity, while on the other hand it can complex inorganic mercury making it less biologically available for methylation (Ullrich et al., 2001). Due to the stability of humic–mercury complexes, Hurley et al. (1991) found that a high percentage of the mercury in lakes can be attached to organic compounds. However, Benoit et al. (2001), reports that in the presence sulfide above 0.01 μM (easily reached in the anoxic hypolimnia of our lakes), mercury–sulfide complexes will be favored and DOC binding will be less important.

The range in DOC between lakes with methylation activity was 3.9 to 21.1 mg/L. Correlation analysis between methylation rates in all the lakes and DOC, demonstrated a positive and significant relationship (r^2 = 0.62, p = 0.0006, n = 27). Because methylation activity was only detected in anoxic waters where sulfate reduction was occurring, complexation with DOC was likely minimal, allowing the DOC to simply act as a stimulant to microbial activity. While higher levels of DOC may directly stimulate SRB activity, it will also stimulate microbial species that consume oxygen and help create the anoxic conditions necessary for SRB activity. While abiotic methylation by organic material (particularly humic and fulvic substances) has been reported, this source of MeHg is thought to be of minor importance (Ullrich et al., 2001).

4.5. Water column methylation and MeHg in lakes

Many studies have observed higher levels of MeHg in the hypolimnion during the summer (Watras et al., 1995a,b; Verta and Matilainen, 1995; Mauro et al.,
While the increase is well documented, the sources responsible for the enrichment are not well understood. Possible sources include diffusion of MeHg out of the sediments (Wright and Hamilton, 1982), transport from the catchment on settling particles (Verta and Matilainen, 1995), or the production of MeHg directly in hypolimnion of the water column (Watras et al., 1995a,b).

Elevated hypolimnetic MeHg levels were observed in all of the lakes with methylation activity. In L658, the average MeHg concentration in the hypolimnion increased by 50% between July and September, while the epilimnion concentrations remained relatively constant (Fig. 1). The increase in MeHg concentration in the hypolimnion was mainly a factor of the MeHg concentration increasing following the oxycline. At 80 cm from the sediment/water interface, which was oxic in July, the MeHg concentration doubled after it became anoxic in September and methylation activity was detected. At 5 cm from the sediment–water interface where methylation was occurring in July and September, the MeHg concentration only increased by 30%. Therefore, the largest increase in MeHg concentrations followed the oxycline as it moved upward in the water column, indicating that water column methylation may be the source of enriched hypolimnetic MeHg. An alternative explanation for this MeHg profile is that mercury methylated in the epilimnetic sediments diffuses into the water column, attaches to particulates and settles to deeper sections of the lake. As the MeHg settling with the particles moves into the anoxic hypolimnion, the partitioning may change and the MeHg may be released into the water column resulting in a similar MeHg profile as observed in L658. As the origin of hypolimnetic MeHg was not directly measured, both scenarios offer plausible explanations for the observed phenomenon and may likely co-occur. Further investigation is needed to clarify the origin of hypolimnetic MeHg enrichment.

The amount of MeHg produced in the water column of lakes will depend on the methylation rate, the volume of the anoxic hypolimnion, and the length of time a lake experiences anoxic conditions. In L658 the rates of methylation were high, but only a very small volume of the lake was anoxic (~1 m in the deepest basin). In L442 and Red Chalk Lake, methylation rates were lower, but the regions of anoxia were larger. Precambrian Shield lakes tend to have conical basins, so even late in the summer, anoxic waters were probably less than 15% of the volume of Red Chalk Lake and less than 1% of the volume of L658, with the other lakes falling somewhere in between. During the summer, boreal lakes that develop anoxic hypolimnia will likely have methylation potential occurring for 3–4 months during stratification. Some boreal lakes never develop an anoxic hypolimnion during the summer and therefore are expected to not exhibit water column methylation activity.

4.6. Abiotic methylation

Under natural conditions, biotic methylation dominates MeHg production compared to abiotic methylation, which is generally considered insignificant (Benoit et al., 2003; Ullrich et al., 2001; Korthals and Winfrey, 1987). Time zero methylation assays from all lakes showed the formation of small amounts of Me$^{199}$Hg (0.04 ± 0.06 ng/L). While humic material has been reported to abiotically methylate mercury in lakes (Weber, 1993), a significant correlation between DOC and time zero Me$^{199}$Hg was not found ($r^2=0.51$, $p=0.16$, $n=9$). A probable explanation for the small amount of Me$^{199}$Hg in the time zero samples may come from the observation of Ramamoorthy et al. (1982), who found that dead bacteria cells could methylate mercury. Correlation analysis showed that time-zero Me$^{199}$Hg was highest where the biotic methylation potentials were high ($r^2=0.68$, $p=0.016$, $n=12$), indicating that the abiotic Me$^{199}$Hg may result from dead communities of bacteria that can continue to methylate by releasing enzymes into the water. The potential for extracellular methylation by the release of exoenzymes has also been observed by Parkman et al., 1994. If this is the case, then the formation of abiotic Me$^{199}$Hg could result from our experimental design (inactivating the bacteria with HCl) and may not reflect natural conditions. In all the lakes where abiotic methylation was detected, however, the levels were very minimal compared to the biotic methylation occurring during the time series. The calculation of methylation rates was not affected by the experimental formation of abiotic methylmercury because the linear regression intercepts were not forced through zero.

4.7. Demethylation

Our finding of low demethylation potentials in the water column is consistent with that of Compeau and Bartha (1984) which found demethylation to be 2% day in anoxic waters (as determined by a 7 day incubation) as well as multiple other studies (Xun et al., 1987; Matilainen, 1995; Winfrey and Rudd, 1990), which report that demethylation is predominately mediated by aerobic bacteria. Photochemical demethylation has been reported by Sellers et al. (1996).
However, with the exception of a few samples from the L658 epilimnion, all of the depths sampled in this study were more than 10 m below the surface, where light penetration would be minimal. For this reason, opaque bottles were incubated in the dark and the possibility of photochemical demethylation was excluded by experimental design. This is not believed to affect our results because studies on photodemethylation at the Experimental Lakes Area have shown that it is a surface water process and does not to occur below a depth of 5 m (Sellers et al., 1996).

Acknowledgements

This research was supported by an NSERC strategic research grant to Dr. Holger Hintelmann. Thanks to: Dr. Asit Mazumder and Dr. Martin Kainz for their support at Elk Lake, BC; Dr. Peter Dillon and Dolly Kothawala for their support at Plastic and Red Chalk Lake; Heather Broadbent for the sulfate analysis; Jajung Yang for the DOC analysis; Dr. Nives Ogrinc and Brian Dimock for their laboratory assistance; and Jeff Small and Beth Howe in assistance in field sampling.

References


