Mercury methylation in the hypolimnetic waters of lakes with and without connection to wetlands in northern Wisconsin

C.S. Eckley, C.J. Watras, H. Hintelmann, K. Morrison, A.D. Kent, and O. Regnell

Abstract: Rates of Hg methylation and demethylation were measured in anoxic hypolimnetic waters of two pristine Wisconsin lakes using stable isotopes of Hg as tracers. One of the lakes is a clear-water seepage lake situated in sandy terrain with minimal wetland influence. The other is a dark-water lake receiving channelized inputs from a relatively large terrestrial wetland. Methyl mercury (MeHg) accumulated in the anoxic hypolimnia of both lakes during summer stratification, reaching concentrations of 0.8 ng·L^{-1} in the clear-water lake and 5 ng·L^{-1} in the dark-water lake. The stable isotopic assays indicated that rate constants of $Hg^{(II)}$ methylation (K_m) ranged from 0.01 to 0.04·day^{-1} in the clear-water lake and from 0.01 to 0.09·day^{-1} in the dark-water lake, depending on the depth stratum. On average, K_m was threefold greater in the dark-water lake. Hypolimnetic demethylation rate constants (K_{dm}) averaged 0.03·day^{-1} in the clear-water lake and 0.05·day^{-1} in the dark-water lake. These methylation rates were sufficient to account for the observed accumulation of MeHg in hypolimnetic water during summer in both lakes. Despite substantial export of MeHg from the wetland to the dark-water lake, our study indicates that in-lake production and decomposition of MeHg dominated the MeHg cycle in both lakes.

Résumé : L'utilisation des isotopes stables de mercure comme traceurs nous a servi à mesurer les taux de méthylation et de déméthylation de Hg dans les eaux anoxiques de l'hyplimnion de deux lacs non perturbés du Wisconsin. Un des lacs est un lac à eau claire alimenté par le ruissellement et situé sur un terrain sablonneux avec peu d'influence des terres humides. L'autre est un lac à eau foncée qui reçoit des apports par canalisations provenant d'une grande région de terres humides terrestres. Le méthylmercure s'accumule dans l'hypolimnion anoxique des deux lacs durant l'été, pour atteindre des concentrations de 0,8 ng·L^{-1} dans le lac à eau claire et 5 ng·L^{-1} dans le lac à eau foncée. Les dosages d'isotopes stables indiquent que les constantes des taux de méthylation de $Hg^{(II)}$ (K_m) varient de 0,01 à 0,04·jour^{-1} dans le lac à eau claire et de 0,01 à 0,09·jour^{-1} dans le lac à eau foncée, en fonction de la profondeur. En moyenne, K_m est trois fois plus élevé dans le lac à eau foncée. Les constantes du taux de déméthylation (K_{dm}) dans l'hypolimnion sont en moyenne de 0,03 jour^{-1} dans le lac à eau claire et de 0,05 jour^{-1} dans le lac à eau foncée. Ces taux de méthylation suffisent à expliquer l'accumulation observée de MeHg dans les eaux de l'hypolimnion au cours de l'été dans les deux lacs. Malgré une importante exportation de MeHg des terres humides vers le lac à eau foncée, notre étude indique que la production et la décomposition de MeHg dans le lac lui-même dominent le cycle de MeHg dans les deux lacs.

[Traduit par la Rédaction]

Introduction

For decades, elevated levels of Hg in aquatic systems have been responsible for health advisories on fish consumption in many countries (US Environmental Protection Agency 1997). Pristine lakes receive Hg inputs through long-range atmospheric transport from industrial sources (Trip and Allan 2000). The majority of Hg entering lakes from the atmosphere is in the inorganic form; however, essentially all of the Hg accumulating in fish is methyl mercury (MeHg) (Bloom 1992). Understanding all of the sources of MeHg to aquatic systems is imperative for the management of Hg pollution.

There are three main sources of MeHg to remote lakes: direct precipitation, watershed runoff (especially from wet-

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lands), and the in-lake methylation of inorganic Hg (Rudd 1995). The relative importance of these sources reportedly varies with the rates of MeHg deposition from the atmosphere, lake type, and catchment hydrology (Rudd 1995). In-lake methylation is known to occur in both littoral sediments (e.g., Gilmour and Riedel 1995) and the anoxic water column (Watras et al. 1995*a*; Mauro et al. 2002). Historically, there has been an emphasis on methylation in the sediments (Korthals and Winfrey 1987; Ullrich et al. 2001) and inputs from the watershed (St. Louis et al. 1994; Lee et al. 1998) as important sources of MeHg to lakes. Several mechanisms have been proposed to explain the seasonal accumulation of MeHg in the anoxic hypolimnia of dimictic lakes including (*i*) diffusion from profundal sediments to the water column under anoxic conditions (Gagnon et al. 1997; Gill et al. 1999; Ullrich et al. 2001), (*ii*) methylation in the anoxic water column (Henry et al. 1995; Watras et al. 1995*a*; Mauro et al. 2002), and (*iii*) sedimentation of catchment derived particulate MeHg and methylation in the anoxic water column (Verta and Matilainen 1995).

Although Hultberg et al. (1994) found that inputs of MeHg from the watershed could account for all of the MeHg accumulated by fish in a Swedish Lake, and although wetlands have been implicated as important sources of MeHg to receiving waters (St. Louis et al. 1994; Hurley et al. 1995; Lee et al. 1998), the relative importance of internal and external inputs of MeHg remains unclear — perhaps varying widely with hydrologic conditions (Sellers et al. 2001). Here, we investigate the internal production of MeHg in two Wisconsin lakes from very different hydrologic settings. One is a clear-water, precipitation-dominated seepage lake in a relatively small, sandy terrestrial catchment. Previous studies indicate that direct atmospheric deposition is the major source of $Hg^{(II)}$ and that internal production is the major source of MeHg for this lake, although the relative importance of littoral versus profundal methylation remains in question (Watras et al. 1995*a*, 1995*b*; Krabbenhoft et al. 1998). The other is a dark-water drainage lake that receives most of its water via channelized runoff from a relatively large wetland.

This study uses a new analytical tool (stable isotopes of Hg) to build on the findings of previous research on water column methylation in Wisconsin lakes (Watras et al. 1995*a*; Mauro et al. 2002). The present study uses short incubation times under ambient conditions with low-level spikes of stable $Hg^{(II)}$ and a more rigorous time series (six time points in 25 h) to better quantify rates during the critical early stages of the incubations. We also monitored changes in water chemistry and microbial community structure during the incubations to assess the preservation of in situ lake conditions. A first attempt was made to quantify demethylation rate constants (K_{dm}) in these lakes, since K_{dm} is expected to play a critical role in determining net MeHg production and accumulation (Hintelmann et al. 2000; Sellers et al. 2001).

Materials and methods

Site descriptions

Pallette Lake (45°59′N, 89°42′W) and Devils Lake (45°31′N, 88°52′W) are both mesotrophic lakes located in northern Wisconsin, USA. Pallette Lake is a clear seepage lake with a surface area of 70 ha and a maximum depth of 18.3 m. Anoxia develops at approximately 14 m by mid-August in this lake and the stratification of microbial communities above and below the oxic–anoxic boundary is striking (Watras and Bloom 1994). Devils Lake is a dystrophic drainage lake with a surface area of 12.5 ha and a maximum depth of 7 m. Anoxia develops at around 3.5 m by mid-August. The inlet stream to Devils Lake emanates from a 24-ha wetland roughly 0.75 km from the lake. Stream water flowing from the wetland has high concentrations of dissolved organic C (DOC) (30–85 mg $C \cdot L^{-1}$), total Hg (HgT) (7–16 $ng \cdot L^{-1}$), and total MeHg (MeHgT) (0.2–0.8 $ng \cdot L^{-1}$) and low pH (3.5–4.5) (C.J. Watras et al., unpublished data). While both lakes are in remote locations in undeveloped watersheds, Devils Lake has a notably higher (roughly four times) MeHg concentration than Pallette Lake (0.2–0.4 versus 0.03–0.09 $ng \cdot L^{-1}$, surface).

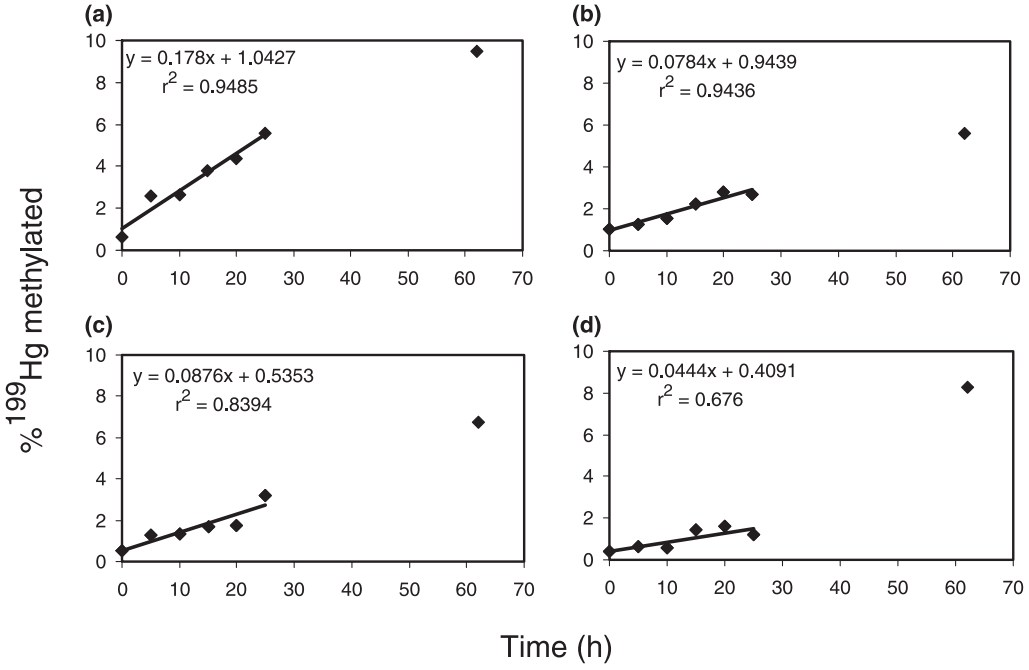
Field sampling

In August 2002, Devils Lake and Pallette Lake were sampled using a vertical profiling system (Seabird 19 plus conductivity–temperature–depth (CTD) instrument) that measures several parameters such as dissolved oxygen (DO), beam attenuation, chlorophyll fluorescence, and pH throughout the water column at a frequency of 4 Hz. The fine-scale bio-optical profiles were used to target discrete depths for sampling Hg species and other analytes. Clean techniques were followed throughout all phases of sample collection, transport, storage, and analysis (US Environmental Protection Agency 1996). Water samples were collected from discrete depths using an all-plastic submersible pump attached to an acrylic outrigger that was fixed to the Seabird CTD. The inlet of the pump was aligned with the CTD sensors and the outlet was attached to 1.3-cm internal diameter C-Flex™ tubing that ran to the surface. An in-line Nitex screen (64 μm) was used to remove zooplankton.

Samples for ambient Hg analyses were collected in 2-L acid-cleaned glass bottles that were rinsed with lake water three times to condition the bottle walls. Water for methylation and demethylation assays was collected in acid-cleaned 160-mL darkened glass serum bottles that avoided exposure to sunlight. To preserve in situ redox conditions, the incubation bottles were overfilled thrice their volume and capped by displacing air and water through a needle injected into the rubber stopper. Samples were double ziplock bagged for transport in a cooler back to the laboratory. The cooler was monitored with a thermometer and approximate in situ conditions were maintained during transportation (less than 2 h from collection).

At the Trout Lake Station Laboratory, 460 pg of inorganic ^{199}Hg (92% pure (Trace Sciences International), stock stored in 0.2% HCl and diluted with Milli-Q water to yield the working standard less than 2 weeks before sampling) and 350 pg of $Me^{201}Hg$ (98% pure (Trace Sciences International), synthesized from ^{201}HgO using methylcobalamin, stored in 50/50 (*v*/*v*) isopropanol–water and 0.2% HCl, and diluted with Milli-Q water to yield the working standard less than 2 weeks before sampling) were added to each sample through the ziplock bags via an analytical syringe (Hamilton

Fig. 1. Methylation time series and regression analysis used to estimate K_m at four hypolimnetic depths in Pallette Lake: (*a*) 14.0 m, (*b*) 15.0 m, (*c*) 16.0 m, and (*d*) 17.5 m.



Gastight No. 84880, 25 μL) that punctured the resealable rubber stopper. In Devils Lake, the inorganic ^{199}Hg spike increased the HgT concentration between 30% and 50% and the Me^{201}Hg spike increased the MeHgT concentration between 40% and 125%. In Pallette Lake, the inorganic ^{199}Hg spike increased the HgT concentration between 200% and 300% and the MeHgT was increased between 300% and 750%. The increase in isotopic Hg in Pallette Lake may appear large, but the isotopic concentrations of 2.9 ng inorganic ^{199}Hg·L^{-1} and 2.2 ng Me^{201}Hg·L^{-1} were less than the ambient levels in Devils Lake. Samples were incubated using two refrigerators set to the in situ lake temperatures. 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. An additional sample at each depth was incubated for 48 h at Devils Lake and 62 h at Pallette Lake. The time zero sample was obtained by adding HCl before the isotopes.

MeHg determination

Analysis of ambient Hg species was performed at the Trout Lake Station Laboratory and followed Morrison and Watras (1999). The methylation and demethylation samples were analyzed at Trent University, Ontario, following Hintelmann and Ogrinc (2003). The formation and degradation of MeHg were determined by monitoring the concentrations of the respective isotopes Me^{199}Hg and Me^{201}Hg. MeHg was distilled from the incubation samples, which was followed by ethylation, purging with nitrogen gas, trapping on Tenax®, thermal desorption, separation by gas chromatography, and detection with an inductively coupled plasma mass spectrometer. Me^{202}Hg (99% pure (Trace Sciences International)) was added as an internal standard to correct for procedural recoveries. Chromatographic data were collected from the inductively coupled plasma mass spectrometer 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).

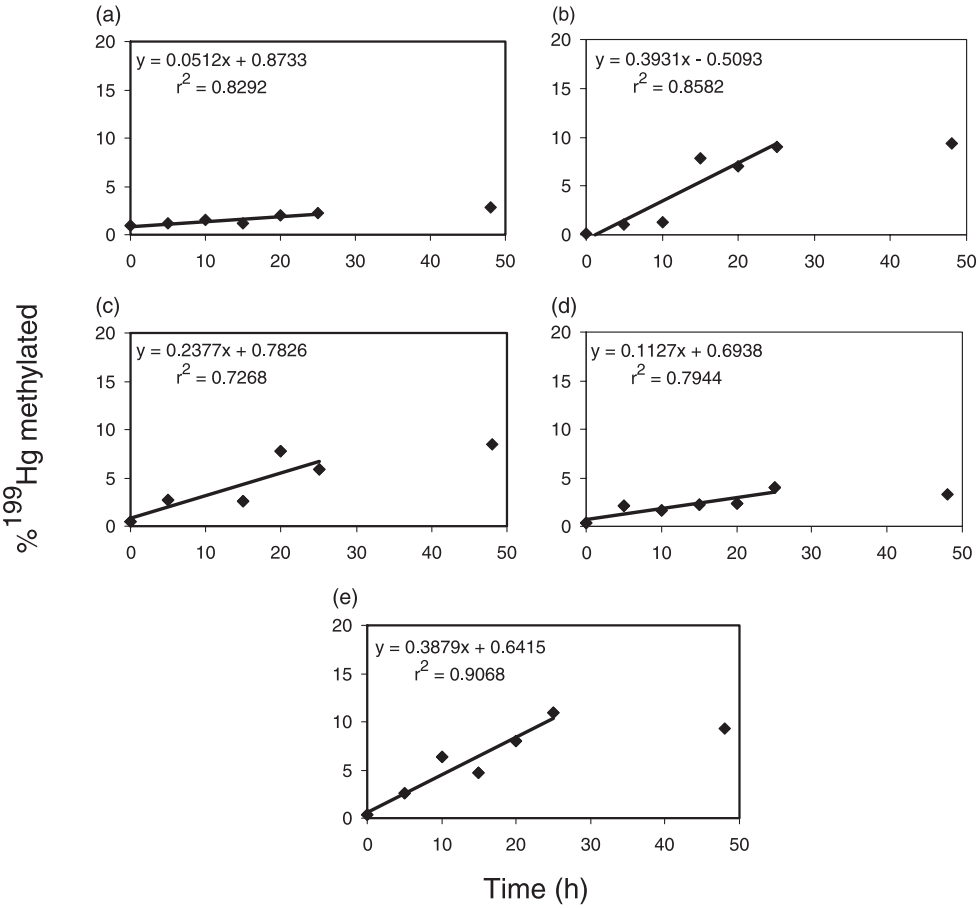
Methylation and demethylation rate constants

Following Hintelmann et al. (2000), we used eq. 1 below to calculate the methylation rate constant:

$$(1) \qquad [\mathrm{Me}^{199}\mathrm{Hg}_t] = [\mathrm{Me}^{199}\mathrm{Hg}_{t-1}] + K_m[^{199}\mathrm{Hg}_t^{(II)}]$$

where t represents incubation time (h). The methylation rate at each depth was obtained from the slope of the regression line [Me^{199}Hg] versus time fitted to the points for the first 25 h of the incubation (Figs. 1 and 2). The methylation rate constant (K_m) was obtained assuming that [^{199}Hg] remained constant during this time. Considering the negligible consumption of [^{199}Hg] by methylation (the maximum was <10%), this approximation seemed justified, as indicated by the linearity of [Me^{199}Hg] versus time. The methylation potential is expressed as the percent Me^{199}Hg formed from the inorganic ^{199}Hg spike (%·day^{-1}) or as the methylation rate constant K_m (day^{-1}). To determine differences between K_m values at different depths, an analysis of covariance test for parallelism (using the F test) was performed using Statistica™. The methylation data from Pallette Lake met the parametric assumptions of the analysis of covariance test — the residuals showed homoscedasticity (Levene test, $p > 0.05$) and a normal distribution (Shapiro–Wilk's test, $p > 0.05$), and there was a linear trend between the response variable and covariate (Fig. 1). At Devils Lake, there was a linear trend between the response variable and the covariate (Fig. 2), but the residuals from two of the five sample depths were not homoscedastic (Levene test, $p < 0.05$; however, by graphical assessment, no trend was visually discernable), nor

Fig. 2. Methylation time series and regression analysis used to estimate K_m at five hypolimnetic depths in Devils Lake: (*a*) 3.0 m, (*b*) 4.5 m, (*c*) 4.9 m, (*d*) 5.5 m, and (*e*) 6.0 m.



were the residuals normally distributed (Shaprio–Wilk's test, $p < 0.0001$). Lindman (1974) noted that the F test is remarkably robust to deviations from normality, indicating that it is still appropriate to use this test without transforming the Devils Lake data. For both lakes, the range and average of the Durbin–Watson test statistic for the individual regression time series suggested that significant autocorrelation was not likely to exist because the values were close to 2 (overall: $d = 2.0 \pm 0.4$; Devils Lake: $d = 2.2 \pm 0.4$; Pallette Lake $d = 1.8 \pm 0.3$). The detection limit for the formation of $Me^{199}Hg$ (Hintelmann and Evans 1997) is affected by the ambient MeHg concentration in the lakes and was 0.011 ng·L^{-1} at Devils Lake and 0.002 ng·L^{-1} at Pallette Lake (corresponding to 0.40% and 0.07% of the added $^{199}Hg^{(II)}$ methylated, respectively).

The demethylation rate constant K_{dm} was obtained from the linear regression of ln[$Me^{201}Hg$] versus time (Hintelmann et al. 2000). Based on the variability of the time = 0 samples, the precision associated with the $Me^{201}Hg$ isotope additions was determined to be 11% (mostly attributed to variability associated with the analytical syringe). None of the demethylation assays had demethylation occurring above the 11% variability. A K_{dm} value of 0.12·day^{-1} (based on a maximum 11% loss of $Me^{201}Hg$ over time) gives an upper limit to demethylation activity. Since we were unable to quantify K_{dm} at specific depths with reasonable certainty, we pooled the available data and regressed ln[$Me^{201}Hg$] versus time for all depths to obtain a global estimate of K_{dm} in the hypolimnion of each lake. The 90% confidence intervals bounding the slopes of the two regressions add additional constraints to the probable range of K_{dm} for a given depth and lake.

Ancillary water chemistry

Samples for water chemistry analysis were collected in 4-L plastic carboys, except for DOC and sulfide. The DOC samples were filtered through precombusted 0.4-µm glass fiber filters (using a precleaned all-glass syringe) into precombusted glass vials with Teflon-lined screw caps. Sulfide samples were collected into 60-mL serum bottles using the overflow technique to exclude oxygen. The serum bottles were sealed with Teflon septa. Dissolved sulfide (detection limit 0.1 µmol·L^{-1}) was determined using an Orion model 94-16BN silver/sulfide electrode with an Orion double-junction reference as described by van Gemerden (1987). Analysis for other constituents followed Watras et al. (1995*b*).

Microbial community analysis

To assess potential biological changes during the in vitro incubations, we estimated bacterial community composition at the beginning and end of each experiment by automated ribosomal intergenic spacer analysis (ARISA) following a modification of the method of Fisher and Triplett (1999). Similarity of the bacterial community profiles before and after incubation of the samples was analyzed using the

Table 1. Mercury, MeHg, and ancillary constituents in the water column of Pallette Lake and Devils Lake.

Lake	Depth (m)	HgT ($ng \cdot L^{-1}$)	MeHgT ($ng \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	SPM ($mg \cdot L^{-1}$)	pH	ANC ($\mu equiv. \cdot L^{-1}$)	Specific conductivity ($\mu S \cdot cm^{-1}$)	Total P ($\mu g \cdot L^{-1}$)	NO_3-N ($mg \cdot L^{-1}$)	SO_4 ($mg \cdot L^{-1}$)
Pallette	3.0	0.38	bd	5.05	2.17	6.9	108	18.8	7	<0.006	1.82
Pallette	6.5	0.49	bd	5.36	4.89	9.4	109	28.0	13	<0.006	1.90
Pallette	8.5	0.42	bd	5.31	6.43	9.3	110	32.6	19	<0.006	1.94
Pallette	9.8	0.38	bd	4.71	4.07	6.4	111	20.0	21	0.082	1.99
Pallette	14.0	0.97	0.22	4.60	5.24	6.9	142	23.6	49	<0.006	2.12
Pallette	15.0	1.40	0.62	4.85	6.21	6.2	185	29.4	81	<0.006	1.80
Pallette	16.0	1.42	0.72	4.91	6.43	6.3	193	28.3	101	<0.006	1.58
Pallette	17.5	1.43	0.76	4.91	6.67	6.3	207	32.4	109	0.021	1.52
Devils	1.5	2.25	0.34	10.75	1.17	5.6	84.6	19.1	13	<0.006	2.80
Devils	3.0	5.02	1.72	11.04	5.34	5.5	28.3	16.9	22	<0.006	2.98
Devils	4.5	7.40	3.36	12.15	4.94	5.7	114	25.5	32	<0.006	2.29
Devils	4.9	7.08	3.55	12.28	4.01	5.8	34.4	27.8	37	<0.006	2.10
Devils	5.5	8.32	4.85	12.95	3.60	6.0	63.7	27.2	75	<0.006	1.51
Devils	6.0	9.49	5.27	13.56	4.95	6.1	69.1	32.2	116	<0.006	0.86

Note: The water column was not sampled at a sufficiently fine spatial scale to reveal maxima of Hg or MeHg in the hypolimnion, as previously regions of the anoxic–sulfidic water column. The DOC in Pallette Lake is not chromophoric dissolved organic matter (CDOM) of terrestrial origin; Lake DOC is predominantly CDOM originating from a wetland in the terrestrial watershed. SPM, suspended particulate matter; ANC, acid-neutralizing

Sorenson's index of similarity (Magurran 1988). Calculation of Sorenson's indices was carried out using the PRIMER 5 software package (PRIMER-E, Plymouth, UK).

Results and discussion

Vertical profiles of Hg species and other analytes

In Pallette Lake, HgT and MeHgT concentrations both increased in the hypolimnion (Table 1; Fig. 3). The percentage of MeHgT to HgT increased from less than 12.1 ± 1.4% (MeHgT was below the detection limit of 0.05 $ng \cdot L^{-1}$) in the epilimnion to 49.4 ± 4.6% in the anoxic hypolimnion. Dissolved MeHg ($MeHg_d$) in the epilimnion (above 8.5 m) was below the detection limit of 0.05 $ng \cdot L^{-1}$ and particle-associated MeHg ($MeHg_p$) ranged from 0.01 to 0.04 $ng \cdot L^{-1}$. At 17.5 m, almost all of the MeHg was dissolved. In the hypolimnion, the amount of HgT_p that was $MeHg_p$ was 25 ± 15%, while the percentage of HgT_d that was $MeHg_d$ was 75 ± 14%, potentially reflecting lower particle reactivity and (or) higher solubility of MeHg than of $Hg^{(II)}$ during sulfidic conditions (Gagnon et al. 1997) or reflecting depletion of dissolved $Hg^{(II)}$ by microbial cells and subsequent release of Hg as MeHg (Benoit et al. 2001).

In Devils Lake, the MeHg concentrations were roughly four times higher than in Pallette Lake. As in Pallette Lake, the concentration of HgT and MeHgT both increased with depth (Table 1; Fig. 3). In the epilimnion, the percentage of MeHgT to HgT was 15%, which increased to 52 ± 6% in the anoxic hypolimnion. The amount of Hgd that was MeHg was 13% in the epilimnion and increased to 65 ± 8% in the anoxic hypolimnion. The percentage of $MeHg_p$ to HgT_p was 17 ± 10% with no visible trend with depth. Almost all of the MeHg in the lake was in the dissolved form, with $MeHg_p$ <10% throughout the water column, except at 1.5 m where $MeHg_p$ was slightly higher.

The pH of hypolimnetic water in Devils Lake and Pallette Lake was similar, ranging from 6.1 to 6.3, which is characteristic of sulfate-reducing environments (Morel 1983). Specific conductivity and concentrations of major nutrients, anions, and cations were also similar in both lakes, with the exception of potassium, iron, and aluminum, which were higher in Devils Lake. Devils Lake also had slightly higher sulfate concentrations in the epilimnion and slightly lower sulfate concentrations in the hypolimnion compared with Pallette Lake. Consistent with the larger decline in sulfate, there was also a larger buildup of sulfide in the anoxic waters of Devils Lake than in Pallette Lake. The DOC concentration at Devils Lake was roughly twofold higher than at Pallette Lake, but this difference underestimates the relative importance of dissolved allochthonous humic matter in Devils Lake. As evidenced by the high water clarity and the spectral distribution of downwelling light (Watras and Baker 1988), most of the DOC in Pallette Lake comprises autochthonous plankton exudates of low color. In Devils Lake, the deep brown stain and low transparency indicate high concentrations of humic matter of wetland origin.

Possible explanations for the elevated levels of MeHg in the two hypolimnia include diffusion of MeHg from the profundal sediments, downward transport of MeHg by settling particles, and methylation occurring within the anoxic water column. A possible explanation for the higher MeHg concentrations in Devils Lake than in Pallette Lake is inputs of MeHg from the wetland affecting Devils Lake. Another possibility is that humic matter, $Hg^{(II)}$, and sulfate received from the wetland stimulated the production of MeHg and increased the stability and retention of MeHg in the water column of Devils Lake. For both lakes, we used the results from the Hg methylation and demethylation assays to assess if MeHg production in the water column could explain the observed buildup of MeHg during summer stratification (see below).

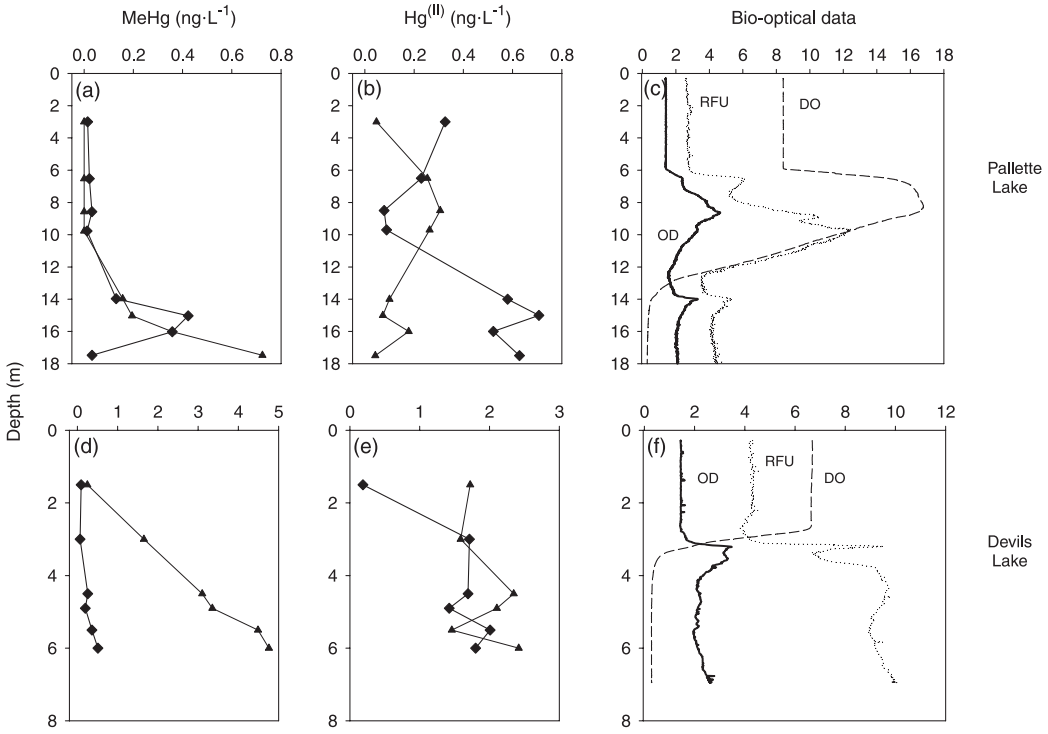
In vitro incubation conditions

ARISA profiles generated from the microbial communities present in the incubation bottles at the initial time point and after 25 h indicated that there was no significant change

H_2S ($mg \cdot L^{-1}$)	Cl ($mg \cdot L^{-1}$)	Ca ($mg \cdot L^{-1}$)	Mg ($mg \cdot L^{-1}$)	Total K ($mg \cdot L^{-1}$)	Na ($mg \cdot L^{-1}$)	Fe ($\mu g \cdot L^{-1}$)	Mn ($\mu g \cdot L^{-1}$)	Total Al ($\mu g \cdot L^{-1}$)
na	0.22	2.0	0.57	0.34	0.47	11	1.6	<17
na	0.22	2.0	0.57	0.33	0.46	18	3.2	<17
na	0.34	2.1	0.61	0.38	0.40	21	4.5	<17
na	0.24	2.1	0.59	0.39	0.44	35	7.6	<17
0.03	0.22	2.0	0.60	0.43	0.48	260	48	<17
0.20	0.22	2.4	0.60	0.44	0.47	660	63	19
0.45	0.24	2.5	0.60	0.46	0.47	800	70	<17
0.50	0.33	2.5	0.60	0.45	0.45	870	73	<17
0.01	0.18	1.5	0.48	0.62	0.25	360	47	110
0.01	0.22	1.9	0.49	0.80	0.27	970	71	150
0.19	0.23	2.1	0.51	0.85	0.30	1800	71	180
0.31	0.23	2.1	0.51	0.86	0.66	1900	71	190
0.72	0.24	2.2	0.53	0.86	0.28	2300	75	210
1.03	0.24	2.4	0.55	0.88	0.32	2800	78	240

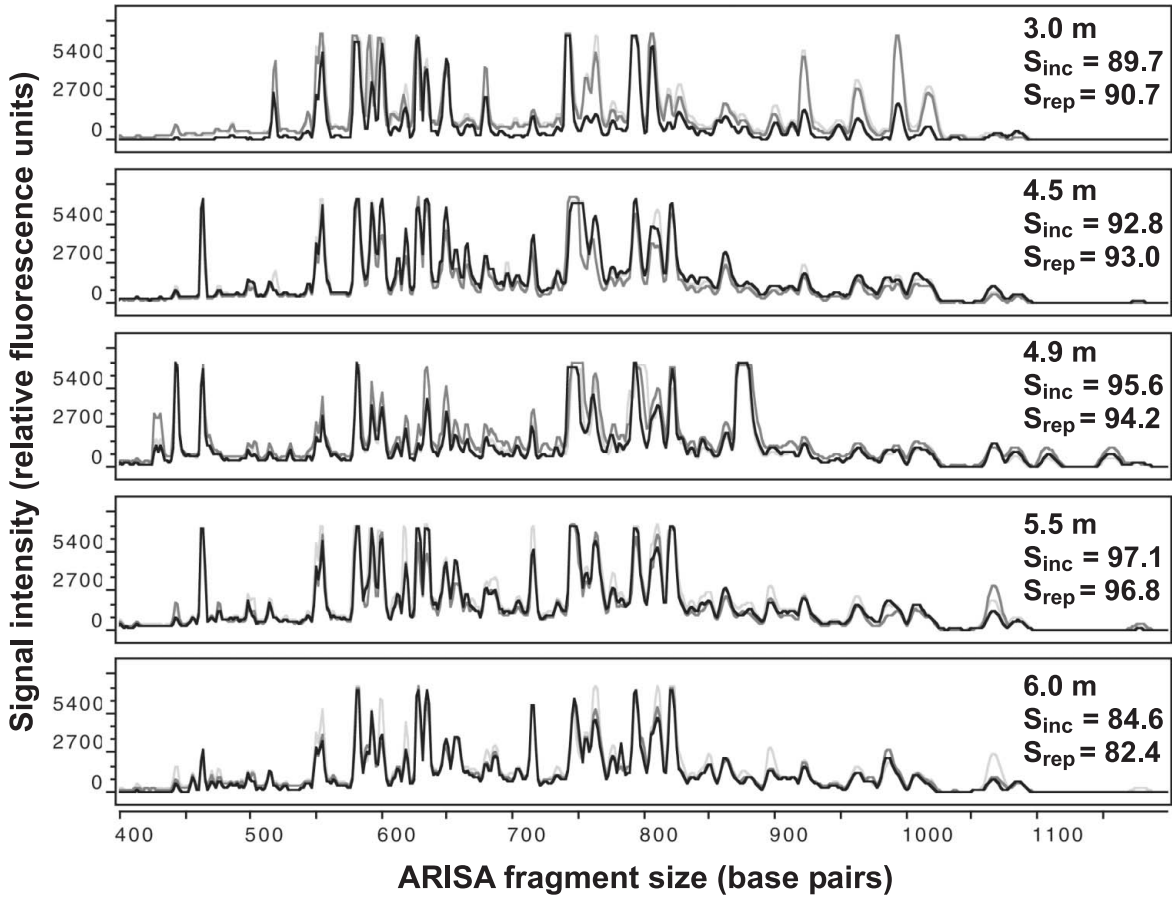
observed in other Wisconsin lakes. The objective here was simply to measure concentrations and rates in various rather, it is colorless dissolved organic matter (DOM), likely derived from plankton exudates. In contrast, Devils capacity; na, not available; bd, below detection limit.

Fig. 3. Depth distribution of waterborne Hg species (triangles, dissolved; diamonds, particulate) and selected lake parameters. (*a* and *d*) MeHg; (*b* and *e*) $Hg^{(II)}$; (*c* and *f*) DO ($mg \cdot L^{-1}$), chlorophyll fluorescence (RFU), and beam attenuation (OD) (m^{-1}). Note that concentrations of Hg species that were below detection in the upper water of Pallette Lake are plotted here at the detection limit.



in the composition of the microbial community at any of the depths sampled (Fig. 4). The magnitude of the ARISA peaks does not exactly reflect the abundance of the bacterial population in the original sample due to preferential amplification of some templates during polymerase chain reaction amplification. For this reason, we chose to evaluate only the presence or absence of peaks in comparing ARISA profiles. However, similar results are obtained when peak area is considered using the Bray–Curtis similarity index. Major anions (sulfate, fluoride, nitrate, and phosphate), cations (lithium, sodium, ammonium, potassium, calcium, and magnesium), and DOC were monitored at each incubation time interval and were shown to remain constant through the duration of the experiment (Fig. 5 shows data for sulfate). These data indicate that in situ conditions were maintained during the incubation assays and offer reassurance that the methylation

Fig. 4. ARISA profiles generated from microbial communities before (solid line) and after (shaded line) incubation for 25 h in Devils Lake. Sample depth and average Sorenson's index (S_{inc}) for comparisons between the initial time point and the replicate profiles from the incubated samples are indicated on each panel along with the Sorenson's index for comparisons between the replicate profiles (S_{rep}). The Sorenson's index of similarity indicates the percentage of peaks in common between samples. Variability between ARISA profiles from different time points does not exceed the variation between replicate profiles.



data accurately reflect the in situ methylation activity. However, there is still some uncertainty concerning the bioavailability of the added Hg and the rates presented are perhaps best considered as the in situ methylation potential (Hintelmann et al. 2000).

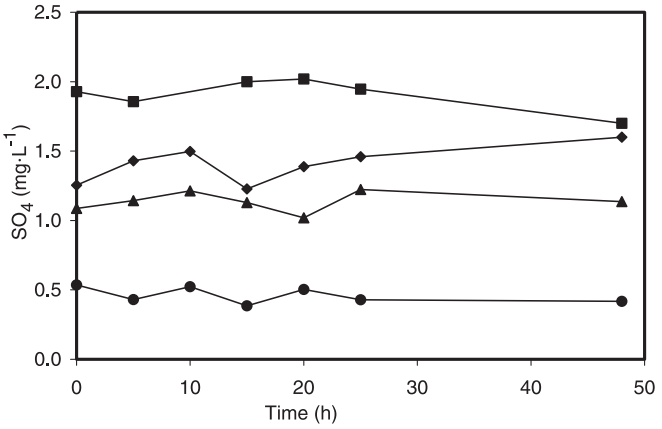
Water column methylation and demethylation assays

The depths sampled at Pallette Lake for Hg methylation and demethylation assays were 8.5, 13.0, 14.0, 15.0, 16.0, and 17.5 m. These depths were chosen because they coincided with peaks in chlorophyll fluorescence and beam attenuation. No methylation activity was detected at 8.5 and 13.0 m. The water at 8.5 m was above the oxycline and 13.0 m was right at the zone of rapid oxygen depletion.

Methylation activity was detected at 14.0, 15.0, 16.0, and 17.5 m in Pallette Lake (Fig. 1). A peak in methylation activity (4.3%·day^{-1}) was detected at 14.0 m ($F_{[3,16]} = 11.65$, $p = 0.0003$). The peak coincided with the appearance of sulfide (0.9 µmol·L^{-1}) in the water column, which continued to increase with depth (14.6 µmol·L^{-1} at 17.5 m). Also, at the depth of peak methylation activity (14.0 m), MeHgT increased by almost one order of magnitude compared with the 10.0-m stratum (the closest overlying depth at which ambient MeHgT was measured). The lower methylation potentials at 15.0, 16.0, and 17.5 m were statistically indistinguishable and averaged 1.7 ± 0.5%·day^{-1} ($F_{[2,12]} = 2.23$, $p = 0.150$). The MeHgT concentration approximately doubled below 14.0 m (Table 1), seemingly contradicting the measured methylation rates at these depths. However, as discussed below, a possible explanation for the increasing buildup of MeHg with increasing depth below 14.0 m is that methylation started and peaked earlier at the lower depths. The sediment could be an additional source of MeHg, although our modeling work suggests that a sediment source would not be necessary to explain the observed MeHg levels in the hypolimnion (see following section).

At Devils Lake, the depths chosen for methylation and demethylation assays were: 3.0, 4.5, 4.9, 5.5, and 6.0 m. These depths were chosen because they corresponded to peaks in chlorophyll fluorescence, beam attenuation, and anoxic water. Methylation activity was detected at all of the depths sampled (Fig. 2). The lowest methylation potential was at 3.0 m, which corresponded to the transition zone between oxic and anoxic conditions (DO = 3.53 mg·L^{-1}). Two distinct peaks in methylation activity were detected at 4.5 and 6.0 m ($F_{[4,19]} = 7.02$, $p = 0.001$). The two peaks were of very similar size (4.5 m: 9.4%·day^{-1}; 6.0 m: 9.3%·day^{-1}) compared with the potentials detected at the other sample

Fig. 5. Stability of sulfate concentrations in incubation bottles during in vitro methylation and demethylation assays in Devils Lake. Squares, 3.0 m; diamonds, 4.5 m; triangles, 4.9 m; circles, 5.5 m.

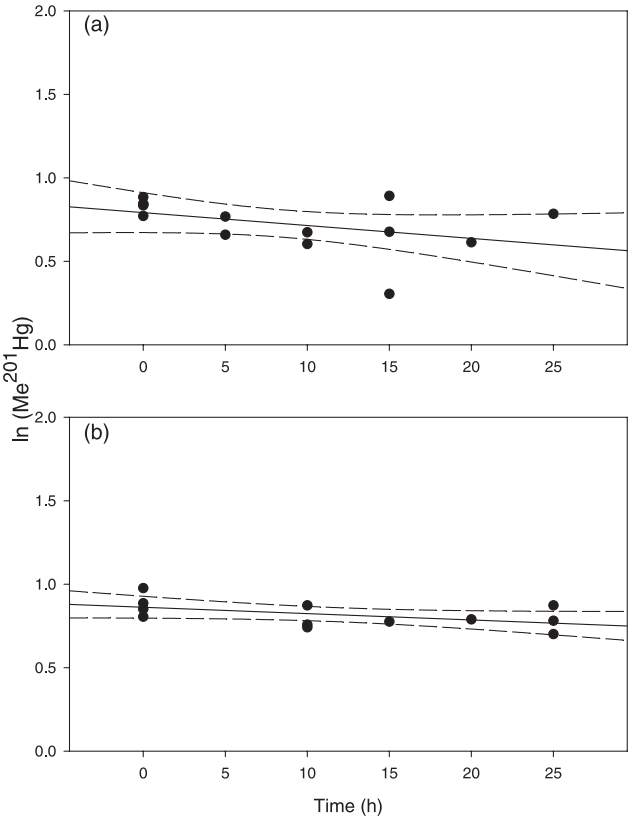


depths (1.2–5.6%·day^{-1}). The peak at 4.5 m corresponded to an increase in sulfide by over an order of magnitude (0.4–5.7 µmol·L^{-1}) and a doubling in the MeHgT concentration compared with 3.0 m. Following the peak at 4.5 m, the methylation potential decreased at 4.9 and 5.5 m before the second peak was detected at 6.0 m. Below 4.5 m, the MeHgT concentration continued to increase between depths sampled but in smaller increments. The concentration of sulfide increased with depth, reaching 30 µmol·L^{-1} at 6.0 m.

The presence of a second peak in methylation potential lower in the water column of equal magnitude to the one below the oxycline has not been observed at other lakes studied (e.g., C.S. Eckley and H. Hintelmann, unpublished data; Watras et al. 1995*a*; Mauro et al. 2002). The DOC content in Devils Lake was highest at 6.0 m (13.6 mg·L^{-1}), which, combined with the presence of sulfate at this depth, may have stimulated the activity of some bacteria, while other bacteria were more favored by the higher sulfate concentrations made available farther up in the water column as the oxycline migrated upwards. It is possible that this second peak is unique to shallow lakes containing high nutrient, DOC, and sulfate concentrations under anoxic conditions. Alternatively, as peaks in methylation activity occur at discrete depths (0.5 m or less thick), it is possible that other studies may have missed this second peak in methylation closer to the sediment, especially since more attention has been paid to sampling the transition zone between oxic and anoxic conditions (C.S. Eckley and H. Hintelmann, unpublished data; Watras et al. 1995*a*). We are presently investigating possible relationships between methylation rates and the presence of various bacteria (O. Regnell et al., unpublished data).

In the demethylation assays, the slope of the regressions of ln[Me^{201}Hg] versus time were negative for both Pallette Lake and Devils Lake, suggesting that demethylation did take place in both hypolimnia, albeit occurring at highly uncertain rates (Fig. 6). Roughly estimated this way, the 90% confidence interval for K_{dm} was 0.001–0.37·day^{-1} in Devils Lake and 0.001–0.18·day^{-1} in Pallette Lake. The role of demethylation is further discussed in the source assessment below.

Fig. 6. Demethylation time series for Devils Lake and Pallette Lake pooling available data for all depths in each lake. Regression analysis indicates that slopes are significantly different from zero at a probability level of 0.13 ((*a*) Devils Lake) and 0.09 ((*b*) Pallette Lake). The 90% confidence interval (broken lines) bounding K_{dm} for Devils Lake extended from 0.02 to –0.39·day^{-1}; for Pallette Lake, it extended from 0.00 to –0.18·day^{-1}.



K_m and the buildup of hypolimnetic MeHg

A simple iterative model was used to test whether the experimentally measured rates of water column methylation were sufficient to account for the accumulation of MeHg in the hypolimnion during summer. The modeling was not designed to take into consideration all of the sources of MeHg but simply to put the measured K_m values in perspective, thus relying on several simplifications to meet our objective.

Iteration, using daily time steps, started with the measured ambient concentration of MeHg during spring assuming well-mixed conditions in both lakes (Table 2) and ended with those measured in the fall of 2002 (mid-September) (Table 3) representing a period of 90 days. To match the observed accumulation of Hg at each depth, the model included an incremental (daily) addition of Hg. This daily addition was estimated for each depth as

$$(2) \qquad Hg_{input} = (Hg_{end} - Hg_{start})/90$$

The source of the "new" Hg input could be from settling epilimnetic particles (rainfall or streamflow) and (or) diffusion from the bottom sediments. The speciation of the Hg_{input} was assumed to be the same as that in the epilimnetic waterborne pool (i.e., 88% $Hg^{(II)}$ and 12% MeHg in Devils Lake based on the mean of five monthly epilimnetic samples

Table 2. Data indicating that the water columns of both lakes were mixed during the spring.

Lake	Date	Depth (m)	HgT ($ng \cdot L^{-1}$)	MeHgT ($ng \cdot L^{-1}$)	DO ($mg \cdot L^{-1}$)	Temperature (°C)
Devils	30 May 2002	1.1	3.95	0.28	8.6	19.1
Devils	30 May 2002	2.0	4.03	0.20	9.3	14.4
Devils	30 May 2002	5.0	4.25	0.32	5.6	8.0
Pallette	21 March 1990	2.0	0.67	0.067	9.83	4.8
Pallette	21 March 1990	10.0	1.36	0.175	3.38	4.0
Pallette	21 March 1990	18.0	2.54	1.006	0.00	4.5
Pallette	26 April 1990	0.3	0.43	0.040	9.95	14.4
Pallette	26 April 1990	2.0	0.47	0.030	9.8	14.0
Pallette	26 April 1990	6.0	1.11	0.060	9.55	8.4
Pallette	26 April 1990	8.0	0.61	0.030	7.81	7.0
Pallette	26 April 1990	10.0	0.69	0.160	7.46	6.1
Pallette	26 April 1990	12.0	0.47	0.080	6.77	5.8
Pallette	26 April 1990	14.0	0.67	0.080	7.21	5.5
Pallette	26 April 1990	16.0	0.82	na	6.12	5.4

Note: Devils Lake was measured during turnover, whereas Pallette Lake was measured before and after turnover (as indicated by the large decrease in HgT and MeHgT below a depth of 10 m). The HgT and MeHgT values measured in April and May were used to calculate initial concentrations in the modeling work. na, not available.

collected between May and September 2002 and 90% $Hg^{(II)}$ and 10% MeHg in Pallette Lake based on 41 epilimnetic samples (≤12.0 m) from eight sample dates between April and September 1998–2002). These values were then entered into eqs. 3 and 4, which were used to calculate the daily changes in Hg species for each depth in the absence of any demethylation (i.e., $K_{dm} = 0$).

$$(3) \qquad H_t^{(II)} = Hg_{t-1}^{(II)} + Hg_{input}^{(II)} - K_m Hg_{t-1}^{(II)}$$

$$(4) \qquad MeHg_t = MeHg_{t-1} + MeHg_{input} + K_m Hg_{t-1}^{(II)}$$

where t represents successive days of the 90-day iteration. Iteration over the 90-day period showed that without any demethylation, the measured methylation rates (K_m) overestimated the accumulation of MeHg by 207% in Pallette Lake and by 177% in Devils Lake. This overestimation of MeHg indicates the importance of demethylation in lake water and the need to incorporate a demethylation constant (K_{dm}), in the model. We calculated the values of K_{dm} needed to match the measured ambient concentrations at each depth after 90 days using eqs. 5 and 6:

$$(5) \qquad MeHg_t = K_m Hg_{t-1}^{(II)} + MeHg_{input} + MeHg_{t-1} e^{-K_{dm}}$$

$$(6) \qquad Hg_t^{(II)} = Hg_{t-1}^{(II)} - K_m Hg_{t-1}^{(II)} + MeHg_{t-1}(1 - e^{-K_{dm}}) + Hg_{input}^{(II)}$$

The K_{dm} values calculated from this procedure averaged $0.034 \cdot day^{-1}$ in the hypolimnion of Pallette Lake and $0.055 \cdot day^{-1}$ in Devils Lake (Table 3). The K_{dm} values were also measured directly using stable isotopes; however, owing to difficulties in detecting the small decreases in the added isotope over 25-h incubations, they have an associated 90% confidence interval ranging from 0.02 to $0.39 \cdot day^{-1}$ in Devils Lake and from 0.001 to $0.18 \cdot day^{-1}$ in Pallette Lake (Fig. 6). The utility of using eqs. 5 and 6 to calculate K_{dm} is that it provides a more precise measure of demethylation fitted to the measured ambient MeHg concentrations at each depth. At all depths in both lakes, the calculated K_{dm} values fell within the 90% confidence interval of the measured demethylation constants. These values for K_{dm} yielded half-lives for hypolimnetic MeHg ranging from 1 to 8 weeks. These results indicated that in situ methylation and demethylation could account for the hypolimnetic accumulation of MeHg at each depth in both lakes during summer. In general, K_{dm} varied directly with K_m, a result that is consistent with the hypothesis that methylation and demethylation in the low-light environment of anoxic hypolimnia are both biologically driven, perhaps by the same microbes (e.g., Marvin-Dipasquale and Oremland 1998; Pak and Bartha 1998).

We note that our modeling exercise assumes that K_m and K_{dm} were constant over the course of the summer, an assumption that we consider unlikely. It seems more likely that Hg methylation begins in sediments and evolves into the water column as summer progresses. Migration into the water column brings sulfate-reducing bacteria and other microbes closer to the source of important substrates (like sulfate) that have significant atmospheric sources. Proximity to epilimnetic sources of sulfate may confer an advantage to some sulfate-reducing bacteria that compete with methanogens for electron donors (Lovley and Klug 1986). In Devils Lake and Pallette Lake, anoxic conditions develop in June at the sediment–water interface, which is presumably where the zone of highest methylation potential would occur (C.S. Eckley and H. Hintelmann, unpublished data). In August, the zone of highest methylation potential (just below the oxycline) was detected at 14.0 m in Pallette Lake and 4.5 m in Devils Lake. Assuming that anoxia developed at a steady rate, the zone of highest methylation potential migrated roughly $1 \text{ m} \cdot \text{month}^{-1}$ up into the water column during the summer.

Our findings do not preclude the importance of methylation and demethylation in littoral sediments. Although the concentration of MeHg in surface waters is low and relatively constant over the summer, this apparent constancy may simply reflect much higher turnover driven by elevated

Table 3. Rates of methylation, demethylation, and net MeHg accumulation in the hypolimnetic waters of Pallette Lake and Devils Lake during summer stratification.

					Initial concentration ($ng \cdot L^{-1}$)		Hg input ($pg \cdot L^{-1} \cdot day^{-1}$)		Final concentration ($ng \cdot L^{-1}$)			Concentration of sulfur species	
Lake	Depth (m)	Measured K_m (day^{-1})	Fitted K_{dm} (day^{-1})	$T_{1/2}$ (days)	MeHg	$Hg^{(II)}$	MeHg	$Hg^{(II)}$	MeHg	$Hg^{(II)}$	MeHg:$Hg^{(II)}$	Sulfate ($\mu mol \cdot L^{-1}$)	Sulfide ($\mu mol \cdot L^{-1}$)
Pallette	14.0	0.043	0.101	7	0.08	0.67	0.2	2.2	0.22	0.75	0.29	22.1	0.9
Pallette	15.0	0.019	0.018	38	0.08	0.67	0.7	6.5	0.62	0.79	0.79	18.8	6.0
Pallette	16.0	0.021	0.015	46	0.08	0.67	0.7	6.7	0.72	0.71	1.02	16.5	13.1
Pallette	17.5	0.011	0.001	—	0.08	0.67	0.8	6.8	0.76	0.68	1.12	15.8	14.6
Devils	1.5	na	na	na	0.30	3.70	na	na	na	na	0.14	29.2	0.3
Devils	3.0	0.012	0.020	34	0.30	3.70	1.4	9.9	1.72	3.30	0.52	31.0	0.4
Devils	4.5	0.094	0.115	6	0.30	3.70	4.6	33.1	3.36	4.04	0.83	23.9	5.7
Devils	4.9	0.057	0.054	13	0.30	3.70	4.2	30.1	3.55	3.53	1.00	21.9	9.1
Devils	5.5	0.027	0.014	51	0.30	3.70	5.9	42.1	4.85	3.47	1.40	15.7	21.2
Devils	6.0	0.093	0.072	10	0.30	3.70	7.5	53.5	5.27	4.21	1.25	9.0	30.1

Note: The K_m was measured directly during 24-h incubations with ^{199}Hg. The K_{dm} was estimated indirectly using an iterative procedure to fit eqs. 3 and 4 to the observed ambient concentrations of MeHg at each depth stratum. Simulation started with observed concentrations of MeHg and $Hg^{(II)}$ in early spring and then MeHg and $Hg^{(II)}$ were incrementally added to the hypolimnion as summer progressed. Also shown is the observed ratio of MeHg:$Hg^{(II)}$ in the lake during late summer and changes in sulfur speciation with depth indicative of sulfate reduction in the water column. na, not available. $T_{1/2}$ is the half-life of MeHg, ln $2/K_{dm}$. MeHg and $Hg^{(II)}$ input is rate at which MeHg and $Hg^{(II)}$ enter the hypolimnion between spring and late summer, presumably via settling from the epilimnion.

K_{dm} in surface waters owing to high light intensities and frequent mixing. However, even if MeHg is produced at high rates in epilimnetic sediments, the presence of an upper oxidized sediment layer may prevent MeHg from reaching the water (Gagnon et al. 1996; Regnell et al. 2001).

We conclude that Hg methylation and demethylation occur in the anoxic water columns of Pallette Lake and Devils Lake at rates sufficient to account for the observed accumulation of MeHg over summer. Both Devils Lake and Pallette Lake had peaks in methylation potential just below the oxycline, which concurs with previous findings for lakes in this region (Watras et al. 1995*a*; Mauro et al. 2002). In both lakes, the sulfide concentration increased below the oxycline, suggesting that sulfate-reducing bacteria are involved in the process of water column Hg methylation, and perhaps demethylation as well. Rates of methylation, demethylation, and MeHg accumulation were all substantially higher in the hypolimnion of the dark-water lake, perhaps because the higher concentrations of DOC and sulfate enhanced Hg methylating and demethylating microbial activity. In addition, lower light intensity might diminish rates of photodemethylation in the dark-water lake, explaining the higher buildup of MeHg in the dark-water lake.

Although wetlands are known to produce MeHg and export it to receiving waters via runoff, the results of this study suggest that the in situ production of MeHg can be a more important process even when wetlands play a dominant hydrologic role. Preliminary results from our ongoing mass balance studies of Devils Lake support this conclusion (C.J. Watras et al., unpublished data). Those data indicate that the stream emanating from the wetland was the major source of water, DOC, and sulfate to the lake during the year of this study. But even though concentrations of MeHg in the stream water were higher than those in the epilimnion of the lake (approaching 1 $ng \cdot L^{-1}$), and even though the wetland exported MeHg at a rate typical for northern wetlands (approximately 0.3 $\mu g \cdot m^{-2}$), the annual input of MeHg attributable to the wetland accounted for <20% of the MeHg accumulating in the entire water column of the lake. Most of the MeHg entering the epilimnion via stream inflow was lost via stream outflow during spring and summer. Thus, the dominant source of MeHg to both Devils Lake and Pallette Lake was in situ production. These findings agree with those of Sellers et al. (2001) who estimated the relative importance of all MeHg sources to be in-lake production >>> inflow from a brown-water lake with riparian wetlands >>> wet deposition > inflow from a clear-water lake or uplands.

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