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The methylmercury cycle in Little Rock Lake during experimental acidification and recovery

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Abstract

The cycle of waterborne methylmercury (meHg) in Little Rock Lake is characterized by a period of accumulation during summertime (when the lake is warm and open to the atmosphere) and a period of decline during winter (when the lake is sealed by ice). We followed this cycle for 16 yr, during which time the lake was acidified with H_2SO_4 and then allowed to recover naturally as part of a long-term field experiment on acidic rain. Mass balance was used to quantify meHg sources and sinks during acidification and recovery. Although year-to-year variability in the summertime accumulation of meHg was high during both acidified and de-acidified years (C.V. = 0.7 and 0.5, respectively), on average 65% more meHg accumulated in the water column during acidification. Most of the meHg mass accumulated in the anoxic hypolimnion (>70%), even though the hypolimnion constituted <5% of the lake volume. In hypolimnetic waters, we observed a direct correlation between the maximum meHg concentration and the sulfate deficit for each year (r^2 = 0.5–0.9) and a direct correlation between meHg and sulfide concentrations (r^2 = 0.7). Sulfide was directly related to dissolved organic carbon at concentrations between 300 and 600 μmol L^{-1} carbon (C). Seasonal changes in waterborne $Hg^{(II)}$, meHg, and sulfate reduction covaried with the atmospheric deposition of $Hg^{(II)}$ and SO_4^{2-}. Across all years, the interaction term [$SO_4^{2-} \times Hg^{(II)}$] explained 70% of the variation in the meHg accumulation rate during summer. These results indicate that meHg production was co-mediated by several simultaneously occurring processes that affect the supply of $Hg^{(II)}$ substrate to the anoxic hypolimnion and the activity of methylating bacteria that are present there. They imply that meHg levels in lakes may respond to future changes in atmospheric Hg deposition in a rapid but complex way, modulated by environmental variables that can interact synergistically with $Hg^{(II)}$ supply. Such variables include sulfate in acid rain, organic carbon in terrestrial runoff, and temperature.

Acid rain and methylmercury (meHg) contamination emerged as limnological issues during the 1970s, but it was not until the late 1980s that both were linked to atmospheric deposition and widespread human perturbation of the sulfur and mercury cycles. A direct connection between acid deposition and the aquatic meHg cycle was first indicated by early synoptic surveys that documented strong negative correlations between lake water pH and the concentration of meHg in fish (Spry and Wiener 1991; Wiener and Spry 1996). Today, pH remains the strongest environmental correlate of fish meHg in northern Wisconsin lakes, explaining 60–70% of the variability in standardized fish (Watras et al. 1998).

The negative relationship between fish meHg and pH is likely the result of several factors that covary with acidification rather than the direct effect of pH per se. Since the supply of meHg to the lower food web may ultimately determine levels in fish (after the influence of diet and growth rate is removed, e.g., Wiener et al. 2003), factors affecting rates of methylation are clearly important. One such factor is the loading of SO_4^{2-}, the dominant anion in acidic rain. Many studies show that sulfate-reducing bacteria (SRB) are the principal methylators of inorganic mercury [$Hg^{(II)}$] in aquatic ecosystems, and SRB activity can affect meHg production in a variety of ways (Ullrich et al. 2001). Another factor is the loading of $Hg^{(II)}$, which may be co-transported atmospherically with SO_2 in combustion gases. Several biogeochemical mechanisms have been proposed to explain how pH, SO_4^{2-}, $Hg^{(II)}$, and meHg might be related causally to observed patterns of fish contamination in otherwise pristine northern lakes (Table 1). However, these mechanisms are neither mutually exclusive nor independent, so it has

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Table 1. Hypothetical mechanisms potentially linking acid deposition to the enhanced accumulation of meHg in freshwaters.

Category	Hypothetical mechanism	References
Biochemical	Sulfate stimulates the physiological activity of sulfate-reducing bacteria (SRB), which enzymatically catalyze the methylation of $Hg^{(II)}$ (SRB metabolic activity limits methylation).	Compeau and Bartha 1985; Gilmour and Henry 1991; Gilmour et al. 1992; Choi et al. 1994; King et al. 1999, 2000
Geochemical	Low pH increases the pool of $Hg^{(II)}$ available for methylation by decreasing the evasion of Hg^0 across the air–water interface ($Hg^{(II)}$ concentration limits methylation).	Brosset 1987; Winfrey and Rudd 1990; Fitzgerald et al. 1991
	Low pH favors the formation of neutrally charged meHgCl species, which are passively transported across the cell membrane of phytoplankton, increasing bioaccumulation at the base of aquatic food chains (meHg speciation limits bioaccumulation).	Mason et al. 1996
	Sulfide production by SRB governs the speciation and bioavailability of $Hg^{(II)}$ to methylating microbes in anoxic environments ($Hg^{(II)}$ speciation limits methylation).	Hudson et al. 1994; Jay et al. 2002; Benoit et al. 2003
Ecological	Low pH decreases biological productivity in lakes, which in turn decreases the biodilution of meHg in aquatic food webs (growth rates control biotic meHg concentrations).	Meili 1994

been difficult to establish their relative importance under natural conditions.

Wetland runoff is another factor that can influence pH and meHg in northern lakes. Wetlands export organic acids, SO_4^{2-}, $Hg^{(II)}$, and meHg to receiving waters, affecting ambient pH, $Hg^{(II)}$, and meHg concentrations. The strongest correlate of waterborne $Hg^{(II)}$ and meHg in northern Wisconsin is dissolved organic carbon (DOC) of wetland origin, which explains >80% of the variability among lakes in the region (Watras et al. 1998). It has been proposed that the export of meHg from inundated wetlands is the direct cause of elevated meHg in dystrophic northern lakes (Rudd 1995; St. Louis et al. 1996), but there is also evidence that the strong correlation between meHg and DOC arises indirectly from some other property of wetland runoff that enhances net methylation or mercury retention within the lakes themselves (Eckley et al. 2005; Watras et al. 2005).

Fig. 1. Aerial photograph of Little Rock Lake in the Northern Highland Lake District of Wisconsin (Vilas County). Treatment basin (T) was acidified experimentally from 1985 to 1991. Reference basin (R) not acidified. Impermeable curtain divides the two basins.

In this article we examine the internal cycling of meHg in Little Rock Lake (LRL), a precipitation-dominated seepage lake in northern Wisconsin that was experimentally acidified with H_2SO_4 and then allowed to recover naturally, all over the course of 20 yr (Fig. 1; Brezonik et al. 1993; Frost et al. 1999). This clear-water lake receives little wetland runoff, and the concentration of waterborne meHg follows a well-defined annual cycle, increasing during summer, when the lake is warm and open to atmospheric deposition and declining during winter, when the lake is ice covered (Fig. 2). Concentrations of waterborne Hg and meHg have been shown to generally track the annual cycle of atmospheric Hg deposition and to follow multiyear trends in depositional loading, which indicates that lakes like LRL are highly sensitive to external Hg inputs (Watras et al. 2000, 2002).

This article focuses on the accumulation phase of the annual meHg cycle, and the main objective is to determine how rates of meHg accumulation in the lake as a whole were affected during experimental acidification and recovery.

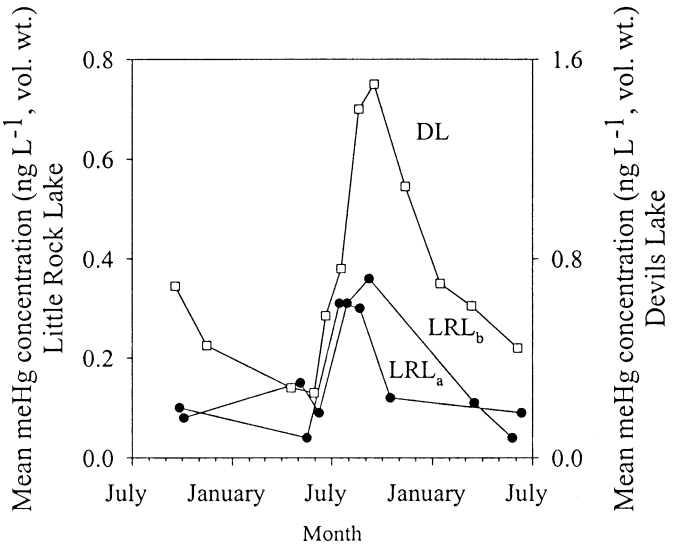

Fig. 2. Annual cycle of waterborne meHg in Little Rock Lake (filled circles, LRL_a, 1991; LRL_b, 1994) and in a dystrophic, drainage lake (Devils Lake, DL, open squares, 2002). Data are volume-weighted average concentrations for the entire lake gleaned from Watras et al. (1994, 2005).

Since internal production is known to be the major source of meHg in seepage lakes like LRL (Rudd 1995), rates of accumulation provide a reasonable measure of net in-lake Hg methylation. The relationships between net methylation and factors that varied with acidification (i.e., pH, SO_4^{2-}, H_2S_T (total sulfide), DOC, iron [Fe], and manganese [Mn] are of particular interest since they potentially provide insight into the biogeochemical mechanisms underlying fish contamination.

Materials and methods

Study site—LRL is a small (0.2 km^2), precipitation-dominated, mesotrophic seepage lake situated in an undisturbed, forested watershed in north–central Wisconsin (46°N, 89°W). In 1984, the lake was separated into two basins by stretching a flexible barrier across a narrows (Fig. 1). One basin of the lake was gradually acidified from pH 6.1 to pH 4.7 by mixing H_2SO_4 into surface waters over a 6-yr period. During the acidification, sulfate loading to the treatment basin increased fourfold over background levels (Brezonik et al. 1993). The other basin of the lake served as an untreated reference. In 1991, experimental acidification ceased and the treatment basin was allowed to recover naturally. The time course of acidification and de-acidification is shown graphically on Fig. 3A. During acidification, meHg concentrations in phytoplankton, zooplankton, and fish increased significantly (Frost et al. 1999).

Previous studies have established that the major source of $Hg^{(II)}$ to LRL is atmospheric deposition, and the major source of meHg is internal production (Watras et al. 1994). The major sink for $Hg^{(II)}$ was shown to be sedimentation, and the residence time of atmospherically deposited $Hg^{(II)}$ in the water column was estimated to be roughly 150 d. Demethylation is considered the major sink for meHg over an annual timescale (Hudson et al. 1994). Although meHg fluxes through the food web are uncertain, the annual production and destruction of meHg seem to roughly balance, because waterborne meHg has historically returned to low levels after fall mixis and because sediment meHg has typically constituted $\leq$1% of the sediment Hg_T (Watras et al. 1998). For this reason, we focus on the period between spring mixis and peak summer stratification, when meHg accumulates in the water column.

Water-column sampling and analysis—Sampling for waterborne mercury species and ancillary analytes began in 1988, near the peak of experimental acidification. All aqueous mercury samples were collected, handled, and analyzed using ultraclean techniques, as described in U.S. Environmental Protection Agency (U.S. EPA) Method 1669 (USEPA 1996). Water was pumped from various depths at a sampling station over the deepest part of each basin and was collected into rigorously cleaned and blanked bottles that were hermetically sealed and double-bagged prior to placement on ice in a dark cooler. Samples were preserved with clean HCl (to 0.5% v:v) and stored in the clean-lab facility at the University of Wisconsin (UW)—Trout Lake Station prior to analysis.

Samples were analyzed for total waterborne mercury (Hg_T) and meHg, following the purge-trap/CVAFS technique of Bloom and Fitzgerald (1988), as described in U.S. EPA Method 1631 (USEPA 2002) and the ethylation/GC/CVAFS procedure of Bloom (1989) as modified by Liang et al. (1994) and described in U.S. EPA Method 1630 (USEPA 2001). Inorganic mercury [$Hg^{(II)}$] was estimated as the difference between Hg_T and meHg. Since total gaseous Hg (TGM) has been shown to constitute <1% of the waterborne Hg_T in LRL (Fitzgerald et al. 1991), concentrations of TGM were not routinely determined. Particulate forms of Hg and meHg reported here were determined directly using the dual-filtration method (Morrison and Watras 1999). Method detection limits in the Trout Lake lab were 0.05 ng Hg_T L^{-1} (estimated from the pooled variance of method blanks, n = 225) and 0.03 ng meHg L^{-1} (pooled variance of distillation blanks, n = 123). Ongoing precision was 99.7% ± 6.3% for Hg_T (mean ± SD, n = 707) and 100.6% ± 10.2% for meHg (n = 570). Lab duplicate and field duplicate relative percent difference between duplicates (RPDs) averaged 5% for Hg_T (n = 171 and 36, respectively) and 11% (n = 195) and 8% (n = 36) for meHg. Matrix spike recoveries averaged 95% ± 7% for Hg_T (n = 111) and 96% ± 11% for meHg (n = 118), with spikes of <1 ng L^{-1}, final concentration.

Ancillary solutes and physical variables in the water column, including pH, total sulfide (H_2S_T), DOC, dissolved oxygen (DO), SO_4^{2-}, Fe, Mn, and suspended particulate matter (SPM), were determined following the methods described by Brezonik et al. (1993), Watras et al. (1998), and the UW–Madison/NSF-Long-Term Ecological Research protocols (http://lter.limnology.wisc.edu). Samples for pH determination were collected in 20-mL plastic scintillation vials with displacement caps to limit gas exchange during transport to the lab. pH was measured in the closed cell using a glass combination electrode designed for low–ionic strength waters. A low-conductivity U.S. EPA Survey Sample was used

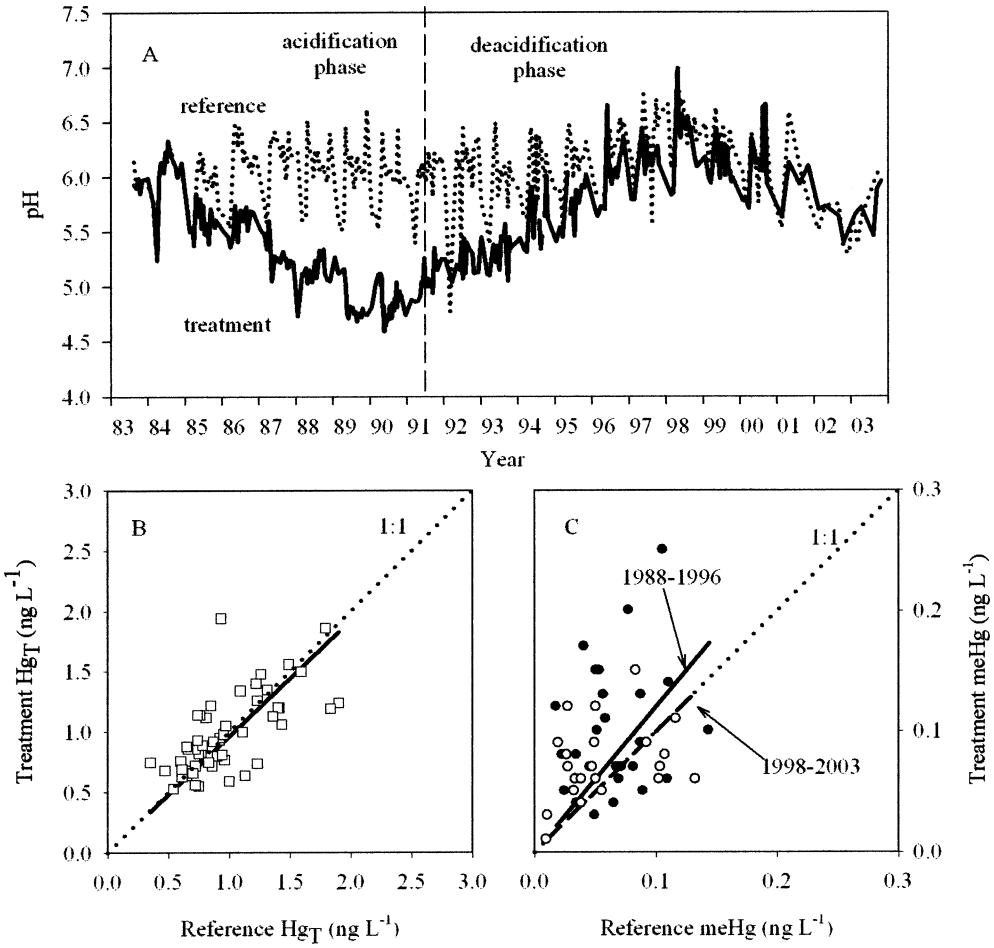

Fig. 3. Comparison of the treatment and reference basins of Little Rock Lake during acidification and recovery. (A) pH of surface waters: treatment basin (solid line) and reference basin (dotted line); (B) concentrations of Hg_T in surface waters, 1988–2003; (C) concentrations of meHg in surface waters, 1988–1996 (treatment basin relatively acidic; filled circles and solid line) and 1998–2003 (pH of two basins indistinguishable; open circles, dashed line).

as the pH standard. H_2S_T samples were collected in 60-mL glass serum bottles using the overflow technique to maintain in situ redox conditions. The bottles were sealed with Teflon septa. Total dissolved sulfide (DL: 0.1 μmol L^{-1}) was determined using an Orion Model 94-16BN silver/sulfide electrode with an Orion double-junction reference after preserving the samples with sulfide anti-oxidation buffer (SAOB), as described by Van Gemerden (1987). DOC was determined on samples filtered through precombusted, 0.4-μm glass-fiber filters (using a pre-cleaned all-glass syringe) into acid-washed, precombusted glass vials with Teflon-lined screw caps. The filtered samples were wet-oxidized with sodium persulfite, heated to 200°C, and the liberated CO_2 was measured with an NDIR detector. DO was measured using the Winkler technique or with an in situ probe. SO_4^{2-} was determined by ion chromatography using U.S. EPA Method 300.0. Fe and Mn were determined using inductively coupled plasma emission spectroscopy (ICP) following SM 3120B. SPM was determined on oven-dried (60°C), preweighed, 0.4-μm polycarbonate membrane filters using a Cahn C-31 microbalance.

Water budgets—The hydrology of LRL during the preacidified and the acidification periods (1984–1990) was described by Rose (1993), who constructed water budgets for both basins during each of these 7 yr. For the treatment basin, these annual budgets indicated that direct precipitation dominated inputs [91% ± 2% (SD)] and evaporation dominated outputs [59% ± 4% (SD)]. The remaining hydrologic input and outflow were attributed to interbasin transfer [9% ± 2% (SD)] and groundwater recharge [41% ± 4% (SD)]. Given the consistency of results over a 7-yr period that included both high water and drought years, the mean values and errors were extrapolated to construct water budgets for later years.

Precipitation sampling—Precipitation volume was measured during all years at the nearby U.S. NADP monitoring site on Trout Lake (Site No. WI36, http://nadp.sws.uiuc.edu/nadp). Bulk atmospheric Hg deposition samplers co-located at this site operated continuously with weekly collections for the time period extending from 1994 to 2004. Sampling and analytical protocols followed those of Morrison et al. (1995).

Based on NADP/MDN data for the Trout Lake site during 2002 and 2003, the atmospheric deposition of meHg was estimated to be 1.3% of the atmospheric Hg_T deposition (http://nadp.sws.uiuc.edu/mdn). This estimate agrees well with an earlier figure of 1.5% derived from event sampling at LRL (Fitzgerald et al. 1991).

Mass balance calculations—Seasonal mass balances (spring overturn to peak summer stratification) for waterborne mercury were constructed for the acidified (1990–1996) and deacidified (1998–2003) time periods in the treatment basin. Each term in the two mass balances was calculated as the mean ± 1 SD for the 6 or 7 yr of interest. Errors were propagated assuming that they were randomly distributed across years. The governing expression was

$$\Delta S_{Hg} = (P_{Hg} + I_{Hg}) - (G_{out\text{-}Hg} + L_{Hg}) + M_{net\text{-}Hg}$$

where ΔS_{Hg} is the change in waterborne mercury mass (storage), P_{Hg} is the mass deposited atmospherically, I_{Hg} is the mass delivered from the reference basin via interbasin transfer, $G_{out\text{-}Hg}$ is the mass lost to groundwater, L_{Hg} is the mass lost by all other processes (i.e., sedimentation and evasion), and M_{net} is the waterborne mass attributable to net methylation (regardless of whether the meHg is produced in the water column or in sediments). Separate mass balances were constructed for Hg_T, $Hg^{(II)}$, and meHg during both acidified and deacidified years.

Change in storage (ΔS_{Hg}) was estimated as the difference between the waterborne mass during mixis in early spring and the mass during stratification in late summer. Whole lake masses were computed from vertical concentration profiles and lake hypsometry, according to $\Sigma c_i \cdot v_i$, for i = 1 to n, where c_i and v_i represent the concentration and volume for a given depth stratum (i). Atmospheric deposition (P_{Hg}) was the product of the volume-weighted average Hg concentration in bulk deposition times the amount of precipitation falling directly on the lake. Interbasin transfer (I_{Hg}) was the product of the mean mercury concentration in the reference basin times the volume of water transferred. Groundwater output ($G_{out\text{-}Hg}$) was the product of groundwater outflow volume times the concentration of mercury in the epilimnion (assuming no profundal recharge). Interbasin transfer and groundwater fluxes were seasonally prorated from the annual hydrologic budgets reported by Rose (1993). The residual loss term L_{Hg} was estimated by difference for Hg_T ($L_{Hg} = P_{Hg} + I_{Hg} - G_{out\text{-}Hg} - \Delta S_{Hg}$). In the case of meHg, L was set to 1% of L_{HgT}, since meHg constitutes roughly 1% of the Hg_T in lake sediments but it does not evade across the air–water interface to a significant degree (Hudson et al. 1994; Ullrich et al. 2001). Note that the residual loss term for meHg constitutes a lower limit, since demethylation at the sediment surface is not considered. M_{net} was calculated according to: $M_{net} = \Delta S_{meHg} + G_{out\text{-}meHg} + L_{meHg} - P_{meHg} - I_{meHg}$. This expression provides a conservative estimate of net methylation, because L_{meHg} likely underestimates the gross flux of meHg to sediments. By definition, M_{net} includes demethylation in the water column. Note that M_{net} has a value of zero in the mass balance for Hg_T, but it may have a negative value in the mass balance for $Hg^{(II)}$, since $Hg^{(II)}$ is transformed to meHg during methylation.

Results and discussion

Epilimnetic pH and meHg—During the experimental acidification, target pH levels were set a priori at steps of 0.5 pH units, and each target pH was maintained for 2 yr, yielding the stair-step pattern of pH decline evident on Fig. 3A. After acid additions were stopped in 1991, recovery from acidification took roughly 6 yr. The pH of both basins has been similar since 1997, the year which here demarks acidified and deacidified years.

Epilimnetic concentrations of Hg_T in the treatment basin were indistinguishable from those in the reference basin during the full time period ranging from 1988 to 2004, which indicates that there was no net change in the input–output balance for epilimnetic Hg_T as a result of acidification (Fig. 3B). This finding differs from observations for several other metals, such as aluminum, Fe, and Mn, whose epilimnetic concentrations increased with acidification, presumably as a result of the dissolution of particulate phases and/or cation exchange with sediments (Brezonik et al. 1993; Frost et al. 1999).

In contrast to Hg_T, epilimnetic concentrations of meHg were higher in the acidified basin when compared to the reference basin. Simple regression analysis of the two data sets plotted on Fig. 3C (1988–1996 and 1998–2003) yielded slopes of 1.2 for the acidified years and 1.0 for the deacidified years. Paired *t*-tests confirmed that mean meHg concentrations in the treatment and reference epilimnia were significantly different during the acidified years (0.09 and 0.07 ng L^{-1}, respectively; $p = 0.01$); but after 1998 they were statistically indistinguishable.

Hypolimnetic meHg—In the treatment hypolimnion, summertime concentrations of waterborne meHg and Hg_T were elevated compared to the epilimnion in all years. During acidified summers, mean hypolimnetic concentrations ranged from 5 to 10 ng meHg L^{-1} and from 10 to 20 ng Hg_T L^{-1} (Fig. 4). The highest concentration of meHg observed in the hypolimnion was 30 ng L^{-1} at a depth of 9 m in August 1990, at the peak of acidification. Concentrations of meHg and $Hg^{(II)}$ were strongly seasonal in both the epilimnion and hypolimnion throughout the study period, increasing during summer and declining during winter. The summer increase was greatest for meHg in the hypolimnion, especially during acidified years (Fig. 4C). MeHg constituted up to 90% of the Hg_T in the hypolimnion, averaging 70% of the Hg_T during peak stratification from 1988 to 1997. During the deacidified years (1998–2004), hypolimnetic meHg decreased to 57% of the peak summer Hg_T, on average. Across all years, meHg accumulated in the hypolimnion at a rate almost two orders of magnitude higher than in the rest of the water column (Table 2).

MeHg and sulfate—Over the long term, waterborne meHg in the treatment basin increased and declined with sulfate. However, there was substantial year-to-year variability, especially during the acidified period (Fig. 5). Possibly, high sulfate levels increased the potential for sulfate reduction and thereby the potential for meHg production. However, as discussed below, sulfate reduction rates may be limited by fac-

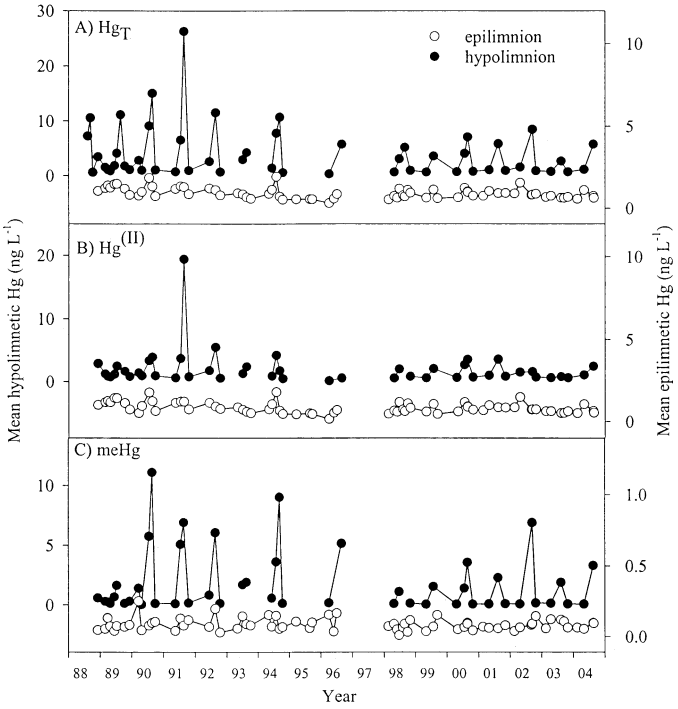

Fig. 4. Annual cycles of waterborne Hg_T, $Hg^{(II)}$, and meHg in the epilimnion and hypolimnion of the treatment basin over 16 yr. Epilimnetic data are means of samples ≤4 m in depth on dates when both meHg and Hg_T were measured (one outlier omitted). Hypolimnetic data are means for depths ≥8 m. $Hg^{(II)} = Hg_T -$ meHg.

tors other than sulfate concentration, and some of these factors evidently show strong year-to-year variability. At low sulfate levels, the influence of other factors may be overridden by sulfate limitation itself. Hence, less interannual variability would be expected during the deacidified years, as was observed (Fig. 5).

During acidification, the loading of SO_4^{2-} to the treatment basin was artificially elevated by a more than fourfold measure, and the epilimnetic concentration of SO_4^{2-} increased in rough proportion to loading (Fig. 5A). When sulfate was elevated, the summertime meHg accumulation rate for the whole lake was, on average, >75% higher than during deacidified years (Table 2). Almost all of the meHg potentially attributable to elevated sulfate accumulated in the hypolimnion (Table 2).

The annual SO_4^{2-} cycle in LRL mirrored the cycle of meHg, with large SO_4^{2-} decreases occurring in the hypolimnion during summer as a result of sulfate reduction and rebounds during winter presumably due to sulfide oxidation (Fig. 6A; Urban and Monte 2001; Urban et al. 2001). Sulfate deficits in the hypolimnion during summer were accompanied by changes in hypolimnetic pH and alkalinity that characterize sulfate-reducing zones (Fig. 6B; Morel 1983). Prior to acidification, hypolimnetic alkalinity (ANC) averaged 250 μeq L^{-1} during summer stratification (compared to <30 μeq L^{-1} in the epilimnion), but ANC reached 880 μeq L^{-1} at the peak of acidification (Brezonik et al. 1993). Thus, microbial processes buffered the acid–base status of the hypolimnion during summer, maintaining the ambient pH at 6.0–6.5 despite the low pH of upper waters.

Table 2. Accumulation of meHg in the treatment basin of Little Rock Lake between spring and summer during acidified and de-acidified years.

	mgHe Accumulation					
	Whole lake*		Epi+metalimnion		Hypolimnion†	
Year	Mass (mg)	Rate (pg L^{-1} d^{-1})	Mass (mg)	Rate (pg L^{-1} d^{-1})	Mass (mg)	Rate (pg L^{-1} d^{-1})
Acidified						
1990	179	4.0	13	0.3	166	84
1991‡	80	1.9	2	0	78	43
1992‡	211	5.1	140	3.5	70	39
1993	35	0.9	12	0.3	23	13
1994	79	1.7	−30	−0.7	109	53
1995	77	1.7	27	0.6	50	26
1996	33	0.6	−32	−0.6	66	28
Mean±SD	99±69	2.3±1.6	19±58	0.5±1.4	80±46	41±23
De-acidified						
1998	52	1.1	17	0.4	35	17
1999	49	1.6	24	0.8	25	18
2000	85	1.8	29	0.6	56	28
2001	41	1.0	4	0.1	37	21
2002§	111	2.1	23	0.4	89	38
2003	19	0.4	−1	0	20	11
Mean±SD	60±33	1.3±0.6	16±12	0.4±0.3	44±25	22±10

* Whole lake area = 9.8×10^4 m^2; volume = 3.8×10^8 L.
† Hypolimnion area = 1.3×10^4 m^2; volume = 1.6×10^7 L, defined as depth >7.5 m.
‡ Concentration during spring mixis estimated rather than measured.
§ Littoral sediments resuspended during woody biomass removal experiment.

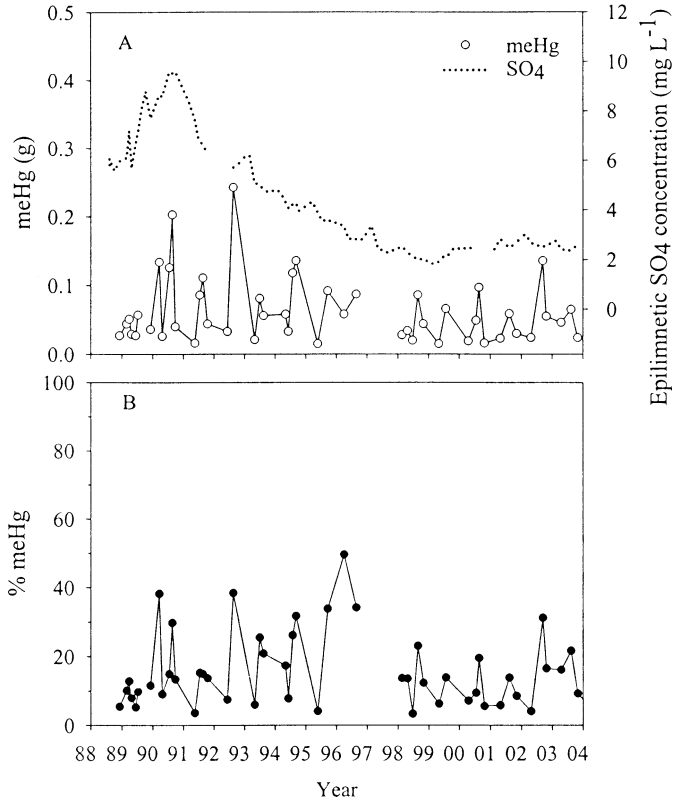

Fig. 5. Annual cycles of meHg over 16 yr expressed as (A) the total mass in the water column and (B) the percentage of waterborne Hg_T that was meHg. Dotted line in (A) shows the concentration of sulfate in the epilimnion over the same time period.

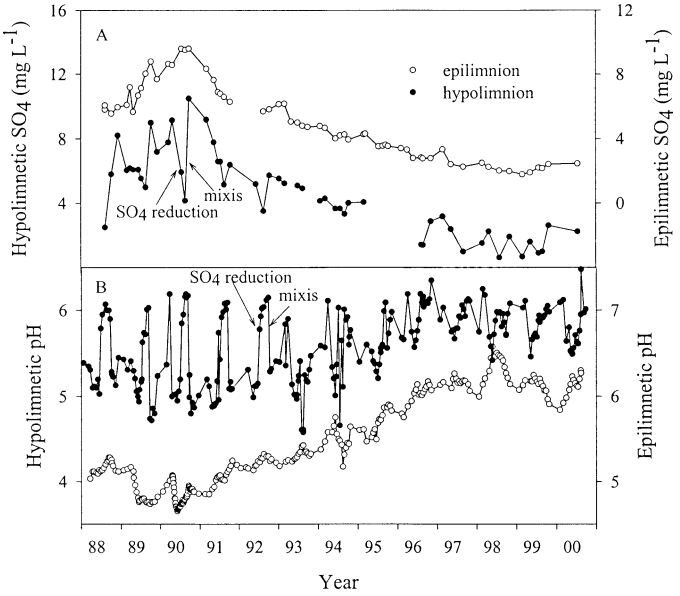

Fig. 6. Annual cycles of (A) SO_4^{-2} and (B) acidity in the epilimnion and hypolimnion of the treatment basin over time. (A) epilimnetic mean, ≤4 m; hypolimnetic mean, ≥8 m). (B) epilimnetic value, <1 m; hypolimnetic value, >9 m.

Vertical profiles through the water column during summer indicate that meHg accumulation, sulfate reduction, and sulfide generation followed similar time courses in the anoxic hypolimnion (Fig. 7A–C). DOC increased concurrently, presumably because of the remineralization of POC from upper waters (Fig. 7E). The pattern of pH change was more complex, and it likely reflects the varied metabolism of stratified microbial assemblages across the O/A boundary (Fig. 7D). The pH increase at the bottom of the profile presumably resulted from the microbial reduction of sulfate, which depletes hydrogen ions. Concentrations of Fe and Mn also increased in the hypolimnion as the redox potential declined during summer (Fig. 7F,G). Mn concentrations increased prior to detectable sulfide production or meHg accumulation. Fe dissolution followed later in summer, as observed in many northern lakes (Wetzel 2001). Although the timing of meHg and Fe accumulation in the hypolimnion was similar, several lines of evidence indicate that meHg accumulation did not result directly from the reductive dissolution of Fe or Mn (*see following*).

Across years, meHg accumulation in the hypolimnion was strongly correlated with the hypolimnetic sulfate deficit, one indicator of the intensity of sulfate reduction (Fig. 8). Hypolimnetic meHg was also directly related to the concentration of sulfide, a by-product of sulfate reduction (Fig. 9). A similar relationship was observed for $Hg^{(II)}$. These results indicate that sulfide concentrations exceeding 30 μmol L^{-1} did not inhibit methylation or promote the precipitation of $Hg^{(II)}$, in contrast to the findings of some studies (Benoit et

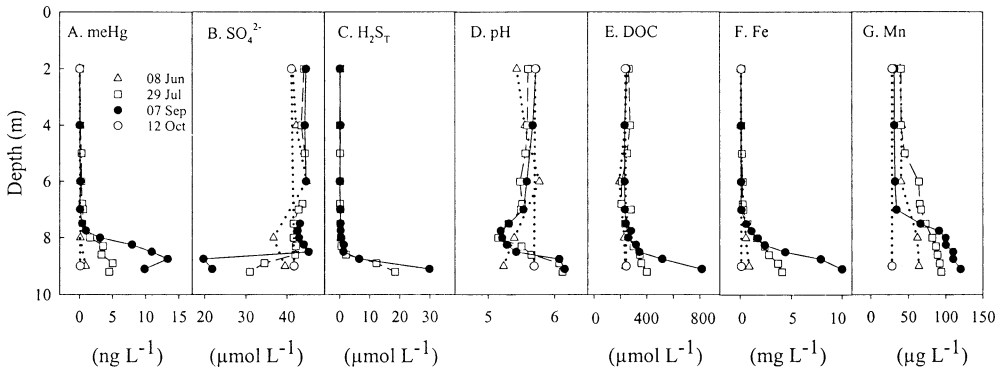

Fig. 7. Seasonal change in the vertical distribution of meHg, SO_4^{-2}, total sulfide, pH, DOC, Fe, and Mn in the water column of the treatment basin (1994).

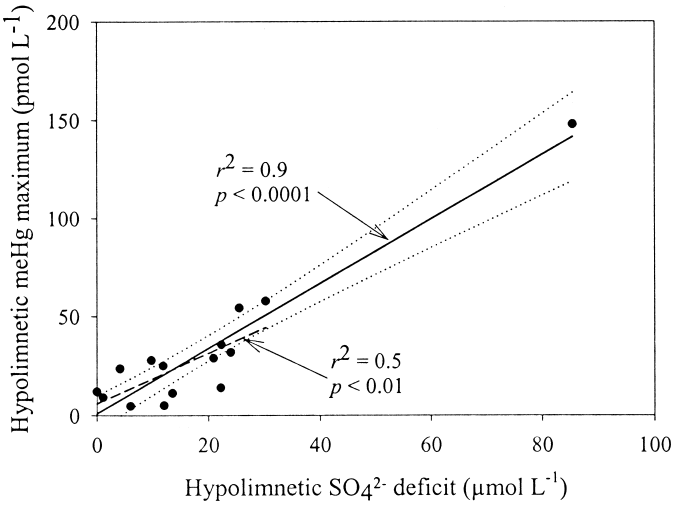

Fig. 8. Relationship between the maximum observed concentration of meHg in the hypolimnion and the magnitude of the sulfate deficit (1989–2000). (SO_4^{-2} deficit = mean epilimnetic SO_4^{-2} concentration − minimum hypolimnetic SO_4^{-2} concentration.) Regressions fit with and without highest value.

al. 2003) but in agreement with those of others (King et al. 2001). This may reflect the low mass of reactive solid in the water column (which can serve as a sink for $Hg^{(II)}$ in sediments) and/or the ambient pH of the LRL hypolimnion (pH < 6.5), which would favor neutrally charged Hg-S species as sulfide concentrations increased (Hudson et al. 1994; Morel et al. 1998). The continuous loading of ''new'' $Hg^{(II)}$ and SO_4^{2-} into the water column via atmospheric deposition may also be an important factor in the hypolimnion, maintaining substrate levels for SO_4^{2-} reduction and methylation throughout summer.

Atmospheric deposition and meHg accumulation—The atmospheric deposition of $Hg^{(II)}$ and SO_4^{2-} are known to be strongly seasonal in northern Wisconsin, with maximal rates during summer (Fig. 10A; Watras et al. 2002). Manual sulfate additions during the experimental acidification followed a similar annual cycle, beginning with ice-out in spring and ending with ice-on in fall each year. As the atmospheric loading of $Hg^{(II)}$ and SO_4^{2-} to LRL increased during summer, $Hg^{(II)}$ built up in the epilimnion and sulfate reduction intensified in the hypolimnion (Fig. 10C). MeHg accumulated in the anoxic hypolimnion over a similar seasonal timescale (Fig. 10B). The synchrony between external loads of $Hg^{(II)}$ and SO_4^{2-} and internal concentrations of $Hg^{(II)}$ and meHg indicates a tight biogeochemical connection among atmospheric deposition, sulfate reduction, and mercury methylation. However, interannual variability in external loading is not sufficient, by itself, to explain the large interannual variation in meHg accumulation evident on Fig. 5.

MeHg and DOC—In addition to an adequate supply of sulfate and $Hg^{(II)}$ substrate, mercury methylation by SRB also depends on an adequate supply of organic carbon to support microbial energy metabolism and growth. There is evidence that mercury methylation in the hypolimnion was at times

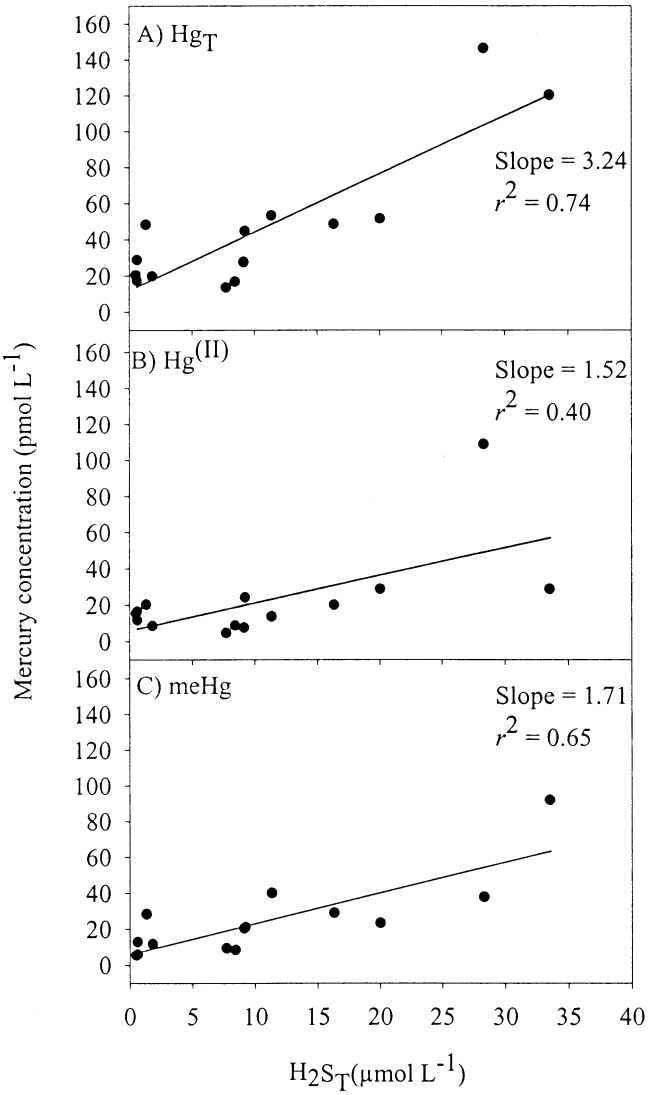

Fig. 9. Relationship between the concentrations of waterborne mercury and total sulfide in the hypolimnion. Concentrations are volume-weighted averages for the hypolimnion on all dates when H_2S_T was greater than 0.5 μmol L^{-1} (1990–2003).

limited by the supply of organic carbon. Using sulfide concentration as a proxy for the intensity of SRB activity and total DOC as a proxy for available carbon, there was an apparent threshold concentration of carbon below which sulfate reduction did not occur during summer anoxia (<300 μmol L^{-1} carbon [C]) (Fig. 11). As the DOC concentration increased from 300 μmol L^{-1} C to 600 μmol L^{-1} C, sulfate reduction increased in proportion to DOC, which indicates that SRB activity was limited by carbon availability. Above 600 μmol L^{-1} C, sulfate reduction was independent of DOC, presumably because of control by another limiting factor, such as sulfate supply. We note that 300 μmol L^{-1} C is roughly the concentration of DOC in the epilimnion, where it is largely composed of terrestrial humic matter. The excess DOC that builds up in the hypolimnion during summer presumably originates from the remineralization of detrital carbon that rains in from the epilimnion and metalimnion. Al-

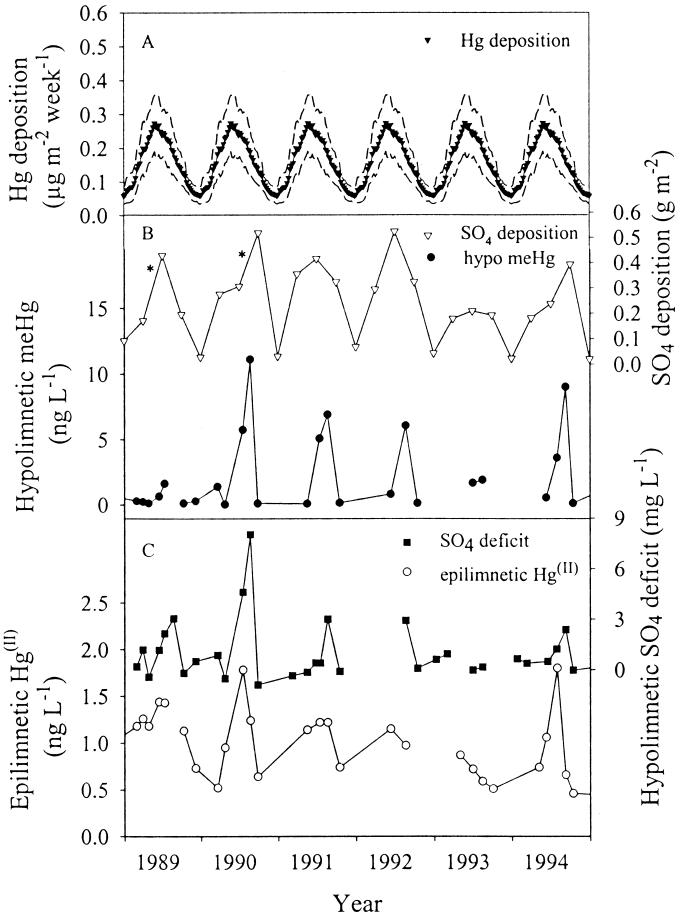

Fig. 10. Annual cycle of meHg accumulation in the hypolimnion of LRL relative to the annual cycles of (A) atmospheric Hg deposition, (B) atmospheric SO_4^{-2} deposition, (C) epilimnetic $Hg^{(II)}$ and the hypolimnetic SO_4^{-2} deficit. Atmospheric Hg deposition data are the average weekly bulk Hg deposition at MDN site WI36 with SD (dashed line) for the period 1994–2004 (Watras et al. 2000). SO_4^{-2} deposition is the total for successive 3-month periods in each individual year (http://nadp.sws.uiuc.edu/ntn). * During 1989 and 1990, atmospheric SO_4^{-2} deposition was supplemented by experimental SO_4^{-2} additions (Urban et al. 2001).

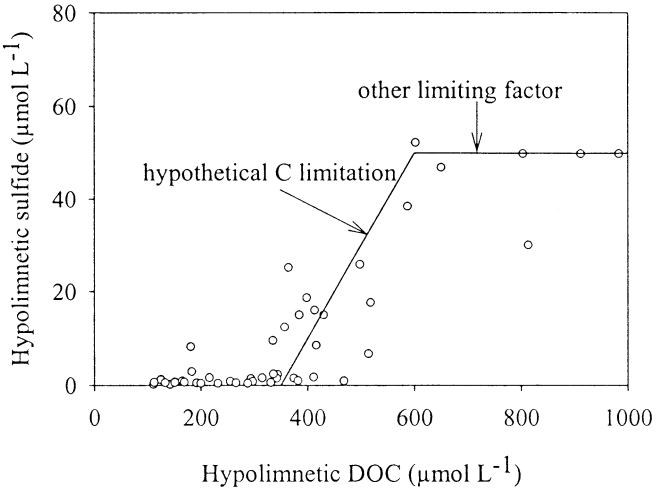

Fig. 11. Relationship between sulfide (proxy for SRB activity) and dissolved organic carbon (DOC) in the hypolimnion of the treatment basin during summer anoxia, 1990–2000.

though this interpretation of Fig. 11 is speculative and does not fully explain the interannual variability evident on Figs. 4 and 5, it reemphasizes the notion that SRB activity and $Hg^{(II)}$ methylation are subject to simultaneous control by several factors that regulate the abundance, community composition, and metabolic activity of SRBs as well as the availability of $Hg^{(II)}$ substrate (Hudson et al. 1994; King et al. 2001; Benoit et al. 2003).

Alternative sources of meHg in the hypolimnion—The observation that meHg and Hg_T tend to accumulate in regions of the hypolimnion below the O/A boundary has prompted mechanistic explanations involving several geochemical processes other than in situ methylation. Hypothetically, the accumulation of meHg in anoxic hypolimnia might result from the downward transport of meHg-laden oxyhydroxides of Fe and Mn from the epilimnion and their subsequent dissolution in deeper anoxic waters (sensu Morel et al. 1998). In this scenario, the source of meHg could be littoral sediments or epilimnetic waters (Ramlal 1993). As Fe and Mn diffuse upward from the anoxic hypolimnion and cross the O/A boundary, they form precipitates that could scavenge meHg in upper waters and transport it down into the anoxic hypolimnion, where the precipitates redissolve and release meHg. An alternative (or additional) downward transport mechanism might involve the settling and decomposition of meHg-laden plankton from the epilimnion (sensu Sellers et al. 2001). It is also possible that meHg associated with Fe and Mn aggregates on the surface of profundal sediments might migrate upward as Fe and Mn dissolve and diffuse into hypolimnetic waters when the redox potential drops. Data from LRL provide some insights into the potential importance of these alternative mechanisms.

Observations from the treatment basin indicate that the redox cycling of Mn did not cause the hypolimnetic accumulation of meHg. When the epilimnetic pH was >5, the spatial and temporal patterns of Mn and meHg enrichment in the hypolimnion were distinctly different (Figs. 7G, 12B). More tellingly, when the epilimnetic pH was <5, the concentration of Mn was uniformly high throughout the water column as a result of the pH-dependence of Mn solubility, and despite the uniform distribution of Mn, meHg accumulated to high concentrations below the O/A boundary during summer stratification (Fig. 12A).

The timing of Fe and meHg enrichment in the hypolimnion were more similar during summer stratification; and like meHg, the intensity of hypolimnetic Fe enrichment was highest during the acidified years (Figs. 7F, 13A; Brezonik et al. 1993). However, the vertical distributions of Fe and meHg were distinctly different during summer and winter anoxia, which indicates that the two cycles were not strongly coupled (Fig. 13B). As both summer and winter anoxia intensified, the concentration of waterborne Fe increased progressively in the hypolimnion, but during winter anoxia, there was a progressive decline in hypolimnetic meHg, presumably because of settling and demethylation.

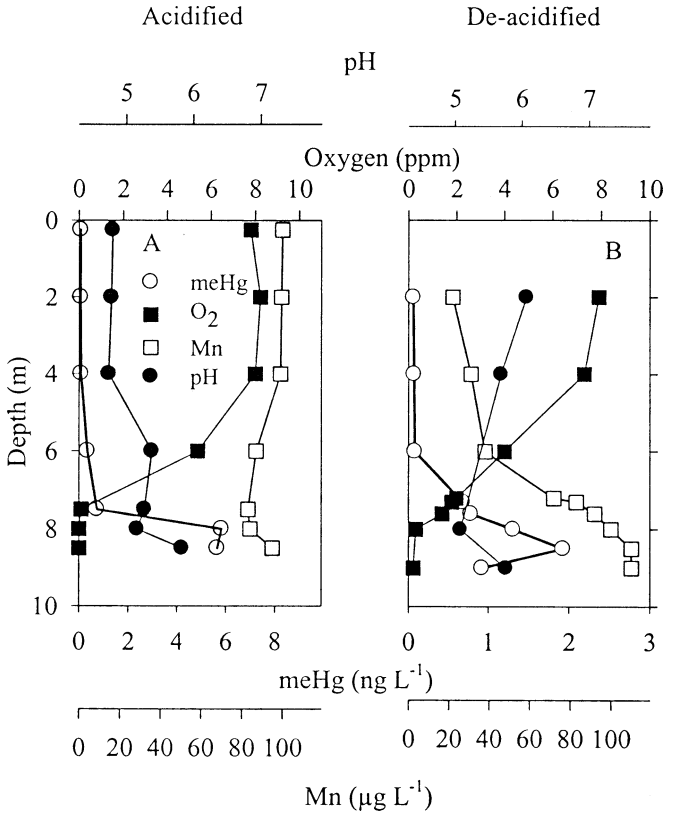

Fig. 12. Vertical distribution of waterborne meHg in the treatment basin relative to Mn during summer stratification at (A) the height of acidification and (B) after deacidification.

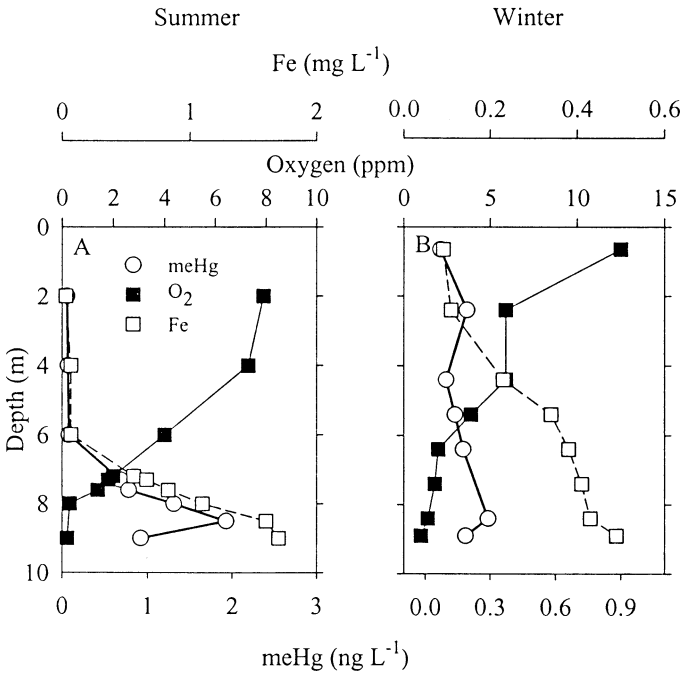

Fig. 13. Vertical distribution of waterborne meHg in the treatment basin relative to Fe during (A) summer stratification and (B) winter stratification.

We conclude that the downward transport of meHg-laden aggregates of Fe and Mn may have delivered some meHg to the anoxic hypolimnion during summer. Similarly, the dissolution of Fe aggregates on the sediment surface may also have released some meHg into hypolimnetic waters. But none of these mechanisms is sufficient to account for the observed pattern of hypolimnetic meHg accumulation in the lake.

We also conclude that spatial changes in the meHg content of plankton are not consistent with the hypothesis that settling plankton are a major source of meHg to the hypolimnion. This hypothesis implies that the meHg content of plankton should decline or remain constant with depth as meHg is released during decomposition in deep water. However, observations in the lake indicate a sharp increase in the meHg content of plankton (μg meHg g^{-1}) in the region of the hypolimnion where meHg accumulates, followed by a decline to minimal values near the sediment–water interface (Fig. 14A). This pattern implies meHg production within a discrete layer of microbes at depth, rather than delivery via settling plankton from above. The near-sediment decline indicates low net methylation rates there. Notably, demethylation seems to be the dominant reaction in profundal sediments (Ullrich et al. 2001).

In situ mercury methylation—Direct evidence of in situ meHg production comes from incubation experiments using both stable and radioisotopes of $Hg^{(II)}$ to measure methyla-

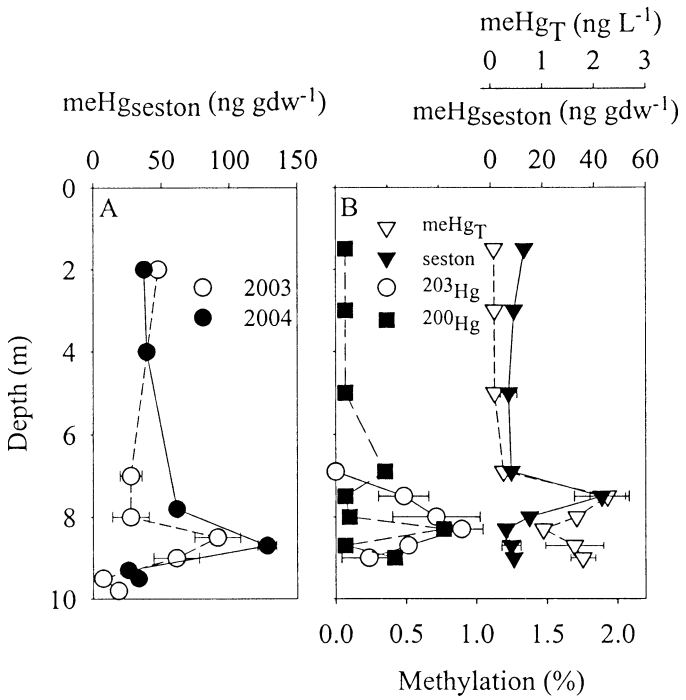

Fig. 14. (A). Changes in the meHg content of plankton with depth during summer stratification in 2003 and 2004 (error bars are range of duplicates). (B) Net Hg methylation potentials, as a percentage of added inorganic ^{200}Hg or ^{203}Hg, during July 1999 (after Mauro et al. 2002). Also shown are the concentrations of waterborne meHg and the meHg content of plankton on the same date.

Table 3. Seasonal mass balances for Hg_T, $Hg^{(II)}$, and $meHg_T$ in the treatment basin of Little Rock Lake during experimentally acidified and de-acidified years. Data are means (±1 SD) for the summertime period, when mercury species accumulate in the anoxic hypolimnion (*see Table 2*). All units are milligrams. Imbalances due to rounding error.

Mass balance term	Acidified years (1990–1996)			De-acidified years (1998–2003)		
	Hg_T	$Hg^{(II)}$	$meHg_T$	Hg_T	$Hg^{(II)}$	$meHg_T$
Δ Storage	170 (±210)	72 (±180)	99 (±69)	84 (±150)	24 (±160)	60 (±33)
Input						
Bulk deposition	510* (±120)	500* (±110)	7* (±2)	370 (±126)	370 (±125)	5 (±2)
Interbasin transfer	2 (±1)	2 (±1)	0.2 (±0.1)	2 (±0)	2 (±0)	0.2 (±0.1)
Methylation (net)	0	*−97*	97 (±68)	0	*−58*	58 (±33)
Loss						
Sedimentation (net) and evasion	330 (±220)	320 (±220)	3 (±2)	280 (±250)	280 (±240)	3 (±2)
Outseepage	13 (±3)	11 (±3)	1 (±1)	11 (±1)	10 (±1)	1.0 (±0.2)

* Data for 1994–1997 (*see Materials and methods*).

tion rates in the water column of LRL. As reported by Mauro et al. (2002), methylation rates were at or below the limit of detection in oxic LRL waters, but they increased significantly in the region of the anoxic hypolimnion where high concentrations of waterborne meHg and sestonic meHg were observed (Fig. 14B). These observations are consistent with findings for other northern lakes, which also demonstrated meHg production in anoxic hypolimnetic waters, sometimes at rates much higher than those typically observed in sediments (ca. 10% d^{-1}) (Matilainen 1995; Watras et al. 1995; Eckley et al. 2005).

Recent studies of the pelagic microbial communities in anoxic hypolimnia support the observations of in situ methylation. DNA analysis of microbial assemblages associated with high methylation rates and high concentrations of meHg in the hypolimnion of one Wisconsin lake indicated an abundance of SRB in the anaerobic water column (Watras et al. 2005). A subsequent study comparing pelagic microbial assemblages across three Wisconsin lakes, including LRL, showed that similar microbial genotypes were positively correlated with meHg concentrations in each anoxic hypolimnion (Kent et al. 2005).

Seasonal mass balances—Although variability between years was high, seasonal mass balances for the treatment basin indicate that, on average, meHg constituted ≥60% of the Hg_T that built up in the lake during summer (Δ storage; Table 3). Net methylation accounted for almost all of this meHg. During acidified summers, net methylation was roughly 70% higher than during deacidified summers. The enhanced methylation may have been driven by two factors. First, atmospheric $Hg^{(II)}$ deposition in the region was about 35% higher during the acidified years, presumably because of emission reductions that occurred in the 1990s (Table 3; Watras et al. 2000). The decline in atmospheric $Hg^{(II)}$ loading was accompanied by a decline in concentrations of Hg_T and meHg in surface waters and fish (Hrabik and Watras 2002). Secondly, the efficiency of $Hg^{(II)}$ methylation was higher during the acidified years. Roughly 20% of the $Hg^{(II)}$ inputs to the lake accumulated as waterborne meHg during acidified summers, compared to 16% in the deacidified period (Table 3). Taken together, greater $Hg^{(II)}$ loading (ca. 35%) and more efficient $Hg^{(II)}$ conversion (ca. 25%) can account for most of the difference in meHg accumulation between acidified and deacidified years (ca. 70%).

The seasonal mass balances imply that acidification increased the residence time of mercury in the water column during summer. The longer residence time may have been due to lower rates of sedimentation from the hypolimnion. In the sulfidic hypolimnion, particle–water partition coefficients (Kds) for $Hg^{(II)}$ and meHg were two orders of magnitude lower than Kds in oxic upper waters, presumably because sulfide competed effectively with ligands on the surface of settling particles and colloids (Hudson et al. 1994; Watras et al. 1994). Equilibrium models indicate that dissolved CH_3HgSH^0, $Hg(SH)_2^0$, and perhaps HgS^0 predominated in these sulfidic waters (Hudson et al. 1994; Morel et al. 1998; Benoit et al. 1999). Thus, sulfide-related changes in the physical form and chemical speciation of mercury apparently increased the dissolved pool by decreasing the flux to sediments. Assuming that the uptake of neutral $Hg^{(II)}$ species such as $Hg(SH)_2^0$ and HgS^0 by resident SRB was faster than the uptake of charged $Hg^{(II)}$ species, a progressive increase in the waterborne meHg fraction of the dissolved Hg_T pool would be expected as sulfidic conditions developed during summertime (Hudson et al. 1994; Benoit et al. 2003).

As was widely observed in sediments, SRB appear to be the principal methylators of $Hg^{(II)}$ in hypolimnetic freshwaters. By producing sulfide, SRB activity may further accelerate the production and accumulation of meHg in the anoxic water column. High concentrations of meHg and $Hg^{(II)}$ do not usually occur in hypolimnetic waters unless anoxic, sulfidic conditions exist. For example, in oligotrophic Lake Baikal, concentrations of Hg_T and meHg are slightly higher in

Table 4. Correlation between summer meHg accumulation rate and substrates potentially limiting SRB activity and meHg production in LRL between 1990 and 2003.

	Direct correlation	
Substrate	r^2	p
SO_4^{2-}*	0.61	<0.001
$Hg^{(II)}$†	0.29	0.06
$[SO_4^{2-} \times Hg^{(II)}]$	0.70	0.001

* Mean epilimnetic SO_4^{2-} concentration.
† Mass $Hg^{(II)}$ in epi+metalimnion.

surface waters than in the oxic hypolimnion (Leermakers et al. 1996). In Lake Superior, surface- and deep-water concentrations of meHg were similar during August, averaging 5 ± 1 pg L^{-1} and 8 ± 2 pg L^{-1}, respectively (Rolfus et al. 2003). In the much smaller, oligotrophic Crystal Lake in northern Wisconsin, which has an oxic summer hypolimnion but water chemistry otherwise similar to LRL, surface- and deep-water concentrations of meHg were also relatively uniform, at 40 and 50 pg L^{-1}, respectively (Watras et al. unpubl. data).

The wintertime decline in waterborne meHg implies that demethylation is the dominant process after fall overturn (Fig. 2), since there is no evidence of a meHg build-up in sediments. In sediments, meHg constitutes a consistently small fraction of the Hg_T pool (ca. 1%), as is commonly observed in other lakes (Ullrich et al. 2001). The balance between methylation and demethylation apparently resets again each spring as a period of renewed meHg accumulation begins below the oxic/anoxic boundary. Spring methylation activity may start in sediments and migrate into the water column as anaerobic microbes move upward toward the source of limiting nutrients, like sulfate and DOC (Watras et al. 1995; Eckley et al. 2005).

Bioaccumulation may also affect the fate of waterborne meHg after fall overturn in LRL. Slotton et al. (1995) observed seasonal enrichment in zooplankton and young fish during the entrainment of meHg-laden hypolimnetic waters in Davis Creek Reservoir (California), and Herrin et al. (1998) made similar observations in another stratified Wisconsin lake. They concluded that fall overturn shunts meHg into the pelagic food web of lakes that exhibit a hypolimnetic build-up. However, since there is no evidence that food webs became progressively more contaminated over time periods of >1 yr, the ultimate fate of meHg appears to be demethylation as biogenic detritus settles into surficial sediments.

The LRL field data indicate that multiple factors governed net methylation during both acidification and recovery because of their effects on SRB activity and $Hg^{(II)}$ availability. As shown on Table 4, net methylation during summer was directly related to concentrations of SO_4^{2-} and $Hg^{(II)}$ and most strongly correlated with the interaction term, $[SO_4^{2-} \times Hg^{(II)}]$, which accounted for 70% of the variability in the seasonal rate of meHg accumulation over the time period ranging from 1990 to 2003. As observed by Hudson et al. (1994), the direct dependence of methylation on the total concentration of $Hg^{(II)}$ implies that all $Hg^{(II)}$ species were equally available—which could occur if the $Hg^{(II)}$ was transported to an environment in which normal species distinctions vanished, such as an anoxic, sulfidic hypolimnion at pH < 6.5.

$$MMR = MMR_{\max} \cdot \left(\frac{\left[Hg^{(II)} \right]}{k_{Hg^{(II)}} + \left[Hg^{(II)} \right]} \right) \cdot \left(\frac{\left[SO_4{}^{2-} \right]}{k_{SO_4} + \left[SO_4{}^{2-} \right]} \right)$$

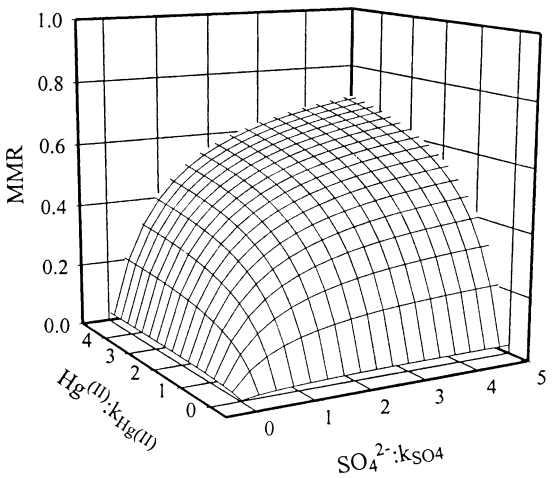

Fig. 15. Theoretical relationship between mercury methylation rate (MMR) and two key substrates (inorganic mercury and sulfate). Response surface follows Michaelis–Menton kinetics (enzymatic control, Ks represent half-saturation concentrations for $Hg^{(II)}$ methylation and sulfate reduction). MMR_{max} governed largely by factors that determine the taxonomic composition and biomass of the SRB community. Based on methylation rate laws of Hudson et al. (1994) and King et al. (1999).

The results in LRL are consistent with several of the hypotheses listed in Table 1. But these results do not indicate that changes in ecosystem productivity played a major role in meHg production or bioaccumulation (cf. Meili 1994). Acidification did not decrease phytoplankton production, biomass, or chlorophyll in the epilimnion of LRL; instead, there was increased phytoplankton production during acidification, particularly in deeper waters, as a result of increased light penetration (Schindler et al. 1991). Thus, the effects of acidification on the meHg cycle appear to be biochemical or geochemical rather than ecological.

The results in LRL are generally consistent with prior modeling studies, which indicate that methylation rates in sediments and water depend on both $Hg^{(II)}$ availability and SRB activity (Hudson et al. 1994; King et al. 1999). The models imply that SRB are the dominant methylators, and they propose that methylation by a given SRB community will respond to the addition of key substrates, like $Hg^{(II)}$ and SO_4^{2-}, according to Michaelis–Menton kinetics. As shown on Fig. 15, the predicted response is quasi–first order at low substrate concentrations, and it approaches saturation as concentrations increase. The generalized rate law specified in Fig. 15 assumes nonlimiting concentrations of electron donor and the substrates needed for biosynthesis. The expression could be rewritten to explicitly show the dependence

on sulfate reduction rates: $MMR = K_m[SRR_{max}][SO_4^{2-}/k_{SO_4^{2-}} + SO_4^{2-}][Hg^{(II)}/k_{Hg^{(II)}} + Hg^{(II)}]$, where K_m is the methylation rate constant (mass $Hg^{(II)}$ methylated per unit sulfate reduced) and SRR is the sulfate reduction rate. Either expression could be expanded to include limitation by other factors, such as electron donor or essential nutrients (e.g., organic carbon, Fig. 11). In any case, the response surface depicted in Fig. 15 illustrates two major points. First, relatively large changes in mercury methylation occur at low substrate concentrations (i.e., above threshold but below saturation). Second, and perhaps more importantly, limiting substrates interact synergistically. Thus, environmental phenomena like acid deposition and mercury deposition have a disproportionately larger effect together than either would have separately.

The results from LRL imply that the response of some lakes to future change in atmospheric Hg deposition may be complicated by concurrent environmental changes that affect microbial community composition or activity (e.g., acid deposition, nutrient subsidies in runoff, or climate change). Policy-makers and natural resource managers need to be cognizant of these potential interactions when assessing the effect of emission controls on natural ecosystems. All aquatic ecosystems may not respond alike, even though they lie within a common airshed. The anoxic hypolimnia of lakes like LRL may be useful models for further study of the biogeochemical links between $Hg^{(II)}$ methylation, anaerobic microbiology, and human-accelerated environmental change.

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