Influence of Dissolved Organic Carbon, pH, and Microbial Respiration Rates on Mercury Methylation and Demethylation in Lake Water

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Effects of changes in DOC concentrations, pH, and microbial respiration rates on specific rates of mercury methylation and demethylation in lake water were studied using radioisotopic techniques. Increased concentrations of DOC resulted in decreased specific rates of net methylation, possibly as a result of complexation of inorganic mercury with DOC. A reduction in pH from 7.0 to 5.0 had the greatest effect, causing large to moderate increases in net methylation rate at both low and high DOC concentrations (500–2600 μM). Rates of respiration (indicative of general rates of microbial activity), which were insensitive to pH change over the range tested (5.0–7.0), had the smallest effect on net methyl mercury production rates. We propose the following explanations for three situations in which high mercury concentrations are commonly found in fish. (1) in acidified dilute clear-water lakes, high fish mercury concentrations may be a result of enhanced in-lake methylation; (2) in brown-water circumneutral lakes, where in-lake methylation is inhibited by high DOC concentrations, terrestrial inputs of methyl mercury may be most important; and (3) in brown-water, low-pH lakes, both in-lake and terrestrial sources of methyl mercury may contribute to elevated mercury concentrations in fish.

Des techniques radio-isotopiques ont été utilisées pour l'étude des effets de la modification des concentrations du COD, du pH et des taux respiratoires microbiens sur la méthylation et la déméthylation du mercure en milieu lacustre. L'accroissement des concentrations de COD a donné lieu à une baisse des taux spécifiques de la méthylation nette, probablement à cause de la formation de complexes entre le mercure inorganique et le COD. Une baisse du pH, de 7,0 à 5,0, a permis d'obtenir le plus important effet, soit une augmentation, d'importante à moyenne, du taux de méthylation nette, ceci aux concentrations de COD faibles et élevées (500–2 600 μM). Les taux de respiration, indicatifs de l'activité microbienne générale, qui n'ont pas réagi dans la gamme de pH de l'essai (5,0–7,0) sont le facteur qui a eu le moins d'effet sur les taux de production nets de méthyl mercure. Les auteurs proposent une explication pour les trois situations où des concentrations élevées de mercure sont généralement notées chez le poisson : 1) dans les lacs à eau limpide diluée acidifiée, les concentrations élevées de mercure chez le poisson s'expliquent par un accroissement de la méthylation dans le lac même; 2) dans les lacs presque neutres à eau brune, où la méthylation est inhibée par les fortes concentrations de COD, ce sont les apports terrestres de méthyl mercure qui sont les plus importants; et 3) dans les lacs de faible pH à eau brune, les concentrations élevées de mercure chez le poisson s'expliquent par des apports de méthyl mercure de sources lacustres et terrestres.

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Because essentially all of the mercury in freshwater fish tissue is methyl mercury (99%, Grieb et al. 1990; >95%, Surma-Aho et al. 1986), it is important to understand the factors that control its production and bioaccumulation in aquatic environments. This understanding is presently incomplete. For example, low pH is correlated with high mercury levels in fish tissue (≥0.5 ppm) in both drainage lakes (Wren and MacCrimmon 1983; Hakanson et al. 1988; Grieb et al. 1990) and seepage lakes (Cope et al. 1990; Grieb et al. 1990). In addition, high mercury concentrations in fish also occur in drainage lakes at both low and high pH levels that have high dissolved organic carbon (DOC) concentrations (as Pt–Co colour: Mannio et al. 1986; Minnesota Pollution Control Agency 1985; Paasivirta et al. 1983). However, even though this positive correlation of fish mercury with DOC is seen in drainage lakes, a negative correlation with DOC is found in seepage lakes (Grieb et al. 1990).

These seemingly inconsistent relationships in fish methyl mercury concentrations might be explained partly by differ-

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TABLE 1. Brief description of the water and sediment trap material sources used in all experiments.

Source	Z_{max} (m)	pH[a]	Alkalinity (μequiv·L^{-1})[a]	μM DOC	Used for:
Lake 239	30	7.2	165.2	500–580	Experiments 1, 2, and 3
Lake 302N	12	5.3	2.3	500–580	Sediment trap material[b]
Sphagnum bog	—	~4.0	−182.1	>2000	DOC concentrate

[a]Mean surface water values for May–September 1988.
[b]Collected from the hypolimnion.

ences in factors that control in-lake mercury methylation. For example, the fact that methylation rates and the ratio of methylation to demethylation in Precambrian Shield lakes increase with decreasing pH in the water column and surface sediments (Xun et al. 1987) may explain high fish mercury concentrations in many clear-water acidic lakes (Winfrey and Rudd 1990). However, the situation appears to be more complicated in high-DOC lakes where DOC as well as pH may affect the rate of methyl mercury production.

DOC might affect in-lake rates of mercury methylation and demethylation in at least two ways. Firstly, some forms of terrestrial DOC are known sources of decomposable carbon for microbial populations (Tranvik 1988). Inputs of such organic carbon to lakes may stimulate microbial activity and mercury methylation in a manner analogous to that caused by decomposition of carbon in flooded soils and vegetation (Hecky et al. 1987). Secondly, inorganic mercury is bound strongly by DOC, notably humic substances (Kerndorff and Schnitzer 1980; Lodenius et al. 1987; Jackson 1989). This binding may reduce the bioavailability of inorganic mercury to the microbial methylators, decreasing net rates of methyl mercury production.

The purpose of our study was to provide insight into three factors that may interact and control the production of methyl mercury within lakes. The first set of experiments was designed to examine the influence of DOC concentration on mercury methylation and demethylation at natural pH and constant respiration rates. For these experiments, because increasing the DOC concentration stimulated respiration rates, respiration was held constant by overwhelming the decomposition of the natural DOC with addition of another natural substrate (sediment trap material). The objective of the second set of experiments was to observe the influence of different DOC concentrations on specific methylation and demethylation rates, at a uniform pH of 6.0. The third set of experiments examined the effect of changes in both pH and DOC on specific methylation, demethylation, and respiration rates.

Materials and Methods

Study Site

All experiments and sampling were done during the spring and summer of 1988 at the Experimental Lakes Area (ELA), northwestern Ontario, Canada. The area is located in the Precambrian Shield (boreal forest) and has much exposed granite bedrock with minimal soil coverage. The lakes in the area are typically oligotrophic and of low buffering capacity (Brunskill and Schindler 1971). Water samples were taken mainly from Lake 239, a lake that has not been manipulated experimentally. The sample sources are briefly described in Table 1.

Sampling Protocol

Samples of epilimnetic water were taken by hand approximately 10 cm below the surface of Lake 239 with a 2-L Nalgene bottle that had been rinsed twice with sample water. The samples were processed within 4 h after collection. High-DOC water, used for the DOC concentrate, was collected in a pre-rinsed carboy from the outflow of a sphagnum bog flowing into Lake 239. The bog water, and especially the concentrate, was brown in colour, indicating the presence of humic substances.

Sedimenting particles were collected from the hypolimnion of Lake 302N using cylindrical (60 cm × 10 cm in diameter) sediment traps. Particulate matter was removed from the lake every 7–10 d, refrigerated, and used within 1–2 d to equalize decomposition (respiration) rates in some experiments.

TABLE 2. Chemical composition of Lake 239 water amended with cation-exchanged DOC concentrate. Values are approximate composition of "highest [DOC]" for experiment 2.

Chemical	Concentration (μmol·L^{-1})
DOC	3100
Al	2.5
Cu	0.02
Fe	1.3
Mn	0.01
Mg	0.14
Na	190
K	0.15
Cd	0.003
Ca	0.20

Dissolved Organic Carbon (DOC)

The DOC concentrate was obtained by rotary-evaporating the sphagnum bog outflow water at 60°C to concentrate DOC. The concentrate was then passed through a cation-exchange column to replace cations with H^+.

DOC was defined as all forms of organic carbon passing through a glass fibre filter (Whatman GF/C, Fisher, 0.7–1.0 μm pore size) and was not further characterized. The concentrate was dark brown and likely contained high concentrations of humic substances. DOC was by far the dominant solute in the bog water used (Table 2). DOC concentrations were measured in GF/C-filtered sample water, after high-temperature acid persulphate digestion, by infrared detection of CO_2 on a model 700 Carbon Analyzer (OI Corp., Houston TX).

The DOC concentrate was not heat-sterilized because this would have chemically altered the DOC and destroyed the natural flora. The intent of our experiments was to simulate the natural environment, where high-DOC runoff mixes with lower DOC lake water.

Experimental Design

DOC concentrations in these experiments were obtained either by using lake and bog water directly or by diluting the

TABLE 3. Description of Experimental design.

Experiment	Objective	Design
1	Influence of DOC on methylation and demethylation. Natural pH, and at constant respiration rate	3 DOC concentrations used. DOC from bog outflow water diluted with lake water. Respiration held constant with 6 mg sediment trap (ST) material. Treatments: DOC 500, 1150, 2600 μM; unamended pH 5.8, 5.2, 5.0
2	Influence of DOC on methylation and demethylation. pH uniform, and respiration not constant	4 DOC concentrations used. DOC concentrate diluted with lake water; no ST material added. Treatments: DOC 560, 760, 1600, 3100 μM; pH = 6.0 ± 0.2 in each
3	Influence of DOC and pH on methylation, demethylation, and respiration	2 DOC concentrations and 2 pH levels used. DOC concentrate diluted with lake water; no ST material added. Treatments: DOC 500, 2600 μM; pH adjusted to 5.0 and 7.0 for each [DOC] (4 treatments)

DOC concentrate with lake water. Experimental designs are summarized in Table 3.

Decontaminated glass bottles containing 100-mL samples were incubated for 24 h for methylation and demethylation experiments. For respiration measurements, 50-mL glass syringes containing samples from the same batch solution as for the methylation and demethylation bottles were incubated under identical conditions.

For experiment 1, a slurry of sediment trap material equalized respiration among the three DOC treatments. the samples were composed of 1:1 Lake 239 water and sediment trap material (500 μm DOC), 1:1:2 Lake 239 water, bog water, and sediment trap material (1150 μM DOC), and 1:1 bog water and sediment trap material (2600 μM DOC). The incubation temperature was the same as the lake water temperature at 18°C; pH was not adjusted (Table 3).

For experiment 2, the Lake 239 water was amended with aliquots of DOC concentrate to achieve a natural range of DOC concentrations (Table 3). The lowest DOC concentration of 560 μM was unamended lake water, and the highest of 3100 μM was 1:9 DOC concentrate and lake water. The incubation temperature was 20°C; pH was adjusted to 6.0 with dilute NaOH or HCl.

In experiment 3, four treatments consisted of low and high DOC concentrations and pH levels of 5.0 and 7.0 (Table 3). As in experiment 2, the low DOC concentration was the natural Lake 239 DOC concentration (500 μM), while the higher DOC concentration (2600 μM) was a mixture of DOC concentrate and lake water. Each batch was halved, with the pH of one of each DOC concentration adjusted to 5.0 and the others to 7.0 with dilute NaOH or HCl. The incubation temperature matched the actual lake water temperature for the season at 25°C.

For each of the three sets of experiments, short-term methylation, demethylation, and respiration measurements were made as described below. Differences in sample water sources over time (temperature, bacterial numbers, chemical variables, etc.) disallow making any direct comparisons among rates found in the separate experiments. Only within-experiment comparisons can be made. Significant differences ($P \leq 0.05$) among treatments within an experiment were evaluated by analysis of variance (ANOVA).

Specific Rates of Mercury Methylation and Demethylation

Specific rates of mercury methylation were determined by the radiochemical method of Furutani and Rudd (1980), by adding 1.0 or 2.0 μg of Hg(II) (0.037 or 0.074 MBq as $^{203}HgCl_2$, New England Nuclear Corp.) to 100 mL of water. Briefly, the percent of added mercury that is converted to methyl mercury over a 24-h incubation period is quantified. All glassware was thoroughly cleaned and decontaminated by soaking overnight in a 2% Decon (BDH Laboratories) solution. The final rinse was the sample water that contained the natural microbial flora used in our experiments. The vessels used for sample incubation were 300-mL glass bottles with ground-glass stoppers. Two or three replicate samples plus one acid-killed control were incubated in the dark for 24 h under the conditions previously described and then were killed with 1.0 mL of 4 N HCl. $CH_3{}^{203}Hg^+$ extraction efficiency is essentially 100% even in very highly organic matrices such as sediments and fish tissue (Furutani and Rudd 1980), so it was assumed not to vary over the range of DOC concentrations used here.

Specific rates of demethylation were measured by addition of 0.2 μg Hg(II) (7.4×10^{-5} MBq as ^{14}C-labelled methyl mercuric iodide, Amersham Laboratories) to 100 mL of sample and recovery of volatile ^{14}C ($^{14}CO_2$ and $^{14}CH_4$, M. R. Winfrey, University of Wisconsin, La Crosse, WI, pers. comm.). The original procedure was fully described by Ramlal et al. (1986), who determined that the method recovers 99.7% of the volatilized ^{14}C. The sample replicates, experimental conditions, and time of incubation were the same as for methylation, except that the 4 N HCl added to terminate was added by injection through the stoppers so as to prevent escape of ^{14}C gases.

The term "specific rates," when referring to methylation and demethylation, means the percent of the isotope added that was methylated or demethylated. The $^{14}CH_4Hg^+$ is more available to the microbes than the $^{203}Hg^{2+}$, so a greater percentage of the labelled substrate is typically demethylated than methylated (Ramlal et al. 1986). The rate of methylation/rate of demethylation (*M/D*) ratios were <1 and are meaningful in a comparative rather than an absolute sense. Because ambient mercury or methyl mercury concentrations were overwhelmed by the radioisotope and carrier, our methods provided rates specific to the amount added rather than an in situ rate. Within each methylation and demethylation experiment, the amount of mercury added was kept constant. Thus, the effect of other variables on the specific rates of methylation and demethylation (and their ratio) could be studied.

Respiration

Respiration rates were measured by incubating duplicate 50-mL water samples in glass syringes from the same treatment batch under the same incubation conditions as for methylation experiments. In experiments that included sediment trap material (experiment 1), samples were incubated in the dark for 18 h at 18°C, while those without particulates were incubated 24 h at either 20°C (experiment 2) or 25°C (experiment 3). Initial

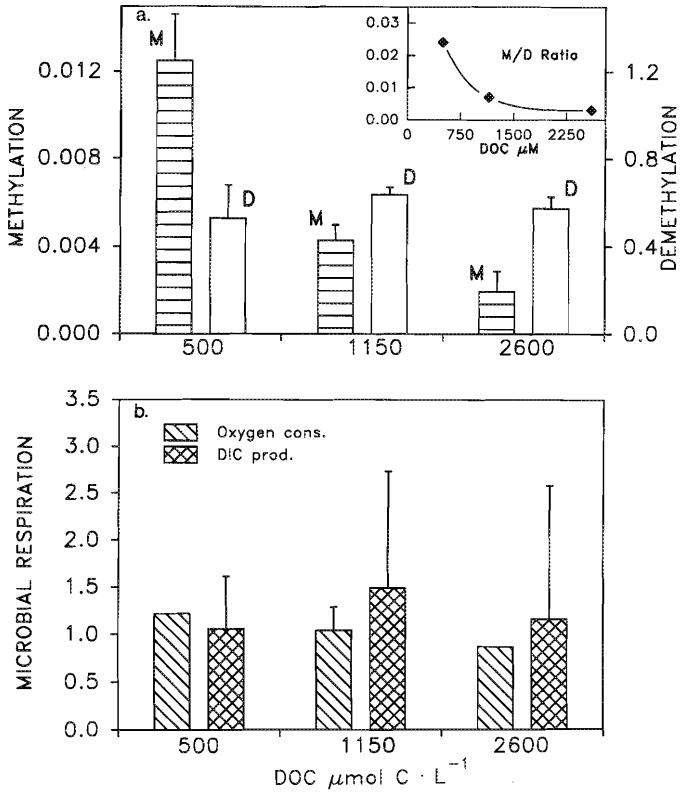

FIG. 1. (a) Rates of methylation (*M*, left axis) and demethylation (*D*, right axis) with DOC concentration. Units are $\%\cdot L^{-1}\cdot h^{-1}$. Standard deviation shown. Inset: ratio of rates, *M/D*. (b) Rates of microbial respiration with DOC concentration. Units are $\mu mol\cdot L^{-1}\cdot h^{-1}$. Additions of sediment trap material to all samples held respiration approximately constant for all DOC treatments.

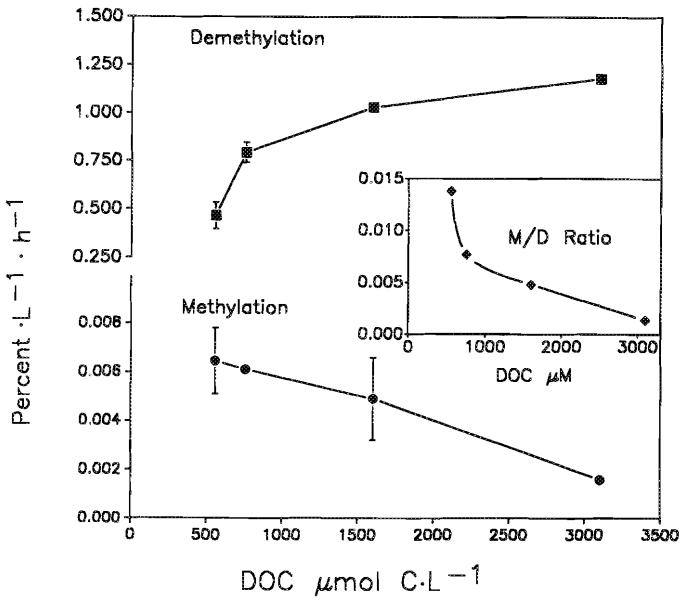

FIG. 2. Rates of methylation and demethylation with increasing DOC concentration. Respiration increased only between the lowest DOC concentration and each of the others. Standard deviation shown. Inset: ratio of rates, *M/D*.

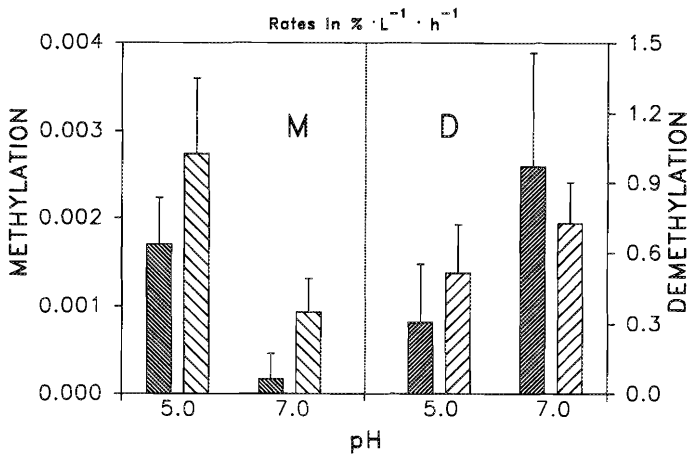

FIG. 3. Rates of methylation and demethylation at 2600 μM (light hatched bars) and 500 μM (heavy hatched bars) DOC. Rates are for pH 5.0 and 7.0. Standard deviation shown.

and final dissolved inorganic carbon (DIC) concentrations were measured with an infrared spectrophotometer on initial and final 0.5-mL aliquots (Stainton et al. 1977) that had been removed from the 50-mL sample syringe. Oxygen consumption rates were quantified using a microscale Winkler technique (American Public Health Association 1972) on initial and final 10-mL aliquots of the sample and using phenylarsine oxide (Hach Chemical Co.) as the titrant in place of sodium thiosulphate.

Results

In experiment 1, there were two- to threefold decreases in rates of methylation at the higher DOC concentrations (Fig. 1a). There were no detectable differences among rates of demethylation for each treatment (Fig. 1a). Respiration rates showed no trend with DOC concentration because of the added sediment trap material (Fig. 1b).

In the second set of experiments, pH was kept constant, no sediment trap material was added, and DOC was varied. DIC production increased from approximately 0.08 $\mu mol\cdot L^{-1}\cdot h^{-1}$ in 560 μM DOC to 0.8 $\mu mol\cdot L^{-1}\cdot h^{-1}$ at all of the higher concentrations. Oxygen consumption increased from undetectable at 560 μM to approximately 1.2 $\mu mol\cdot L^{-1}\cdot h^{-1}$ at all higher DOC levels. The opposite effect was seen for specific methylation rates. They decreased consistently with increasing DOC concentration (Fig. 2). This was the same methylation trend with DOC concentration as was found in experiment 1, where respiration was uniform due to sediment trap additions (Fig. 1).

The methylation rate was significantly lower at the highest DOC concentration (3100 μM) than at all of the other DOC concentrations. Demethylation rates increased significantly with each increase in DOC concentration. The largest increase in demethylation was between 560 and 760 μM (similar to respiration).

In both of the above experiments the *M/D* ratio, which is an indication of the relative potential for net CH_3Hg^+ production, was clearly highest at the lowest DOC concentration and decreased with increasing DOC. This occurred whether or not sediment trap material was present (Fig. 1a and 2 (inset)).

When both pH and DOC were adjusted (experiment 3), methylation and demethylation were influenced more by pH (Fig. 3 and 4) whereas respiration was influenced more by DOC (Fig. 5). Methylation rates were higher at pH 5.0 than at 7.0, but were suppressed by high DOC at both pH levels. Demethylation rates were higher at pH 7.0 than at pH 5.0 but did not

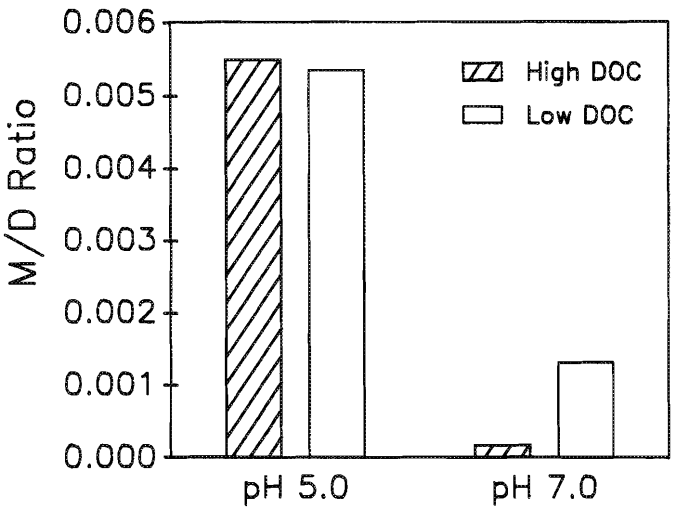

FIG. 4. Ratio of methylation to demethylation (*M/D*) at 2600 μM (hatched bars) and 500 μM (open bars) DOC, pH 5.0 and 7.0

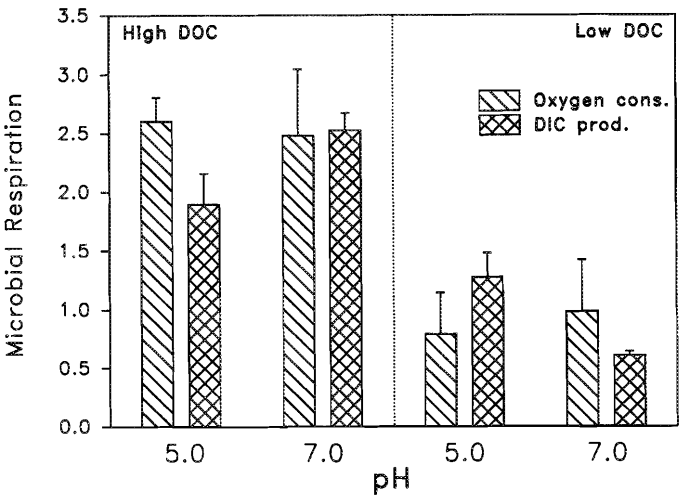

FIG. 5. Rates of microbial respiration (oxygen consumption and D/C production) at 2600 and 500 μM DOC in water at pH 5.0 and 7.0. Standard deviation shown.

follow a consistent pattern with respect to DOC concentrations (Fig. 3). The *M/D* ratios indicated a higher net potential for CH_3Hg^+ production at pH 5.0 than at pH 7.0 regardless of the DOC concentration (Fig. 4).

Respiration (oxygen consumption, DIC production) was significantly higher in the high-DOC (2600 μM) treatment than in the low-DOC (500 μM) treatments regardless of pH (Fig. 5). There was no significant difference in short-term respiration between pH 5.0 and pH 7.0 for any one DOC concentration.

Discussion

Among the three factors that we studied, the order of importance in affecting rates of net methyl mercury production was pH > DOC concentration > microbial respiration (Fig. 1–5). The fact that pH had the greatest effect is consistent with a number of fish surveys in which pH was the chemical variable that correlated most significantly with fish mercury (Wren and MacCrimmon 1983; Hakanson et al. 1988; Cope et al 1990).

The mechanism of the pH effect on methylation is unknown. However, the results from our DOC experiments suggest that a DOC-mediated mechanism is possible. Reduction in pH changes the character of DOC by increasing protonation of anionic moieties and thus desorbing metals (Davis et al. 1985). Therefore, the increased methylation at low pH in our experiments with lake water and by Xun et al. (1987) in water and at the sediment–water interface might be explained by reduced binding of inorganic mercury to DOC, making the mercury more available for methylation. Other possible explanations are that low pH favours activity of certain microbial species, or the operation of biochemical pathways that are effective at methylating mercury.

Additions of DOC to our samples caused enhanced microbial respiration (Fig. 5), as has been observed by others (Sederholm et al. 1973; Tranvik 1988; Tranvik and Hofle 1987). Demethylation appeared to follow microbial respiration; it was constant when respiration was constant (experiment 1) and increased when respiration increased (experiment 2). However, any stimulatory effect of increased respiration on demethylation rates or *M/D* was overwhelmed by the large inhibitory effect of DOC on methylation rates and *M/D* (Fig. 2). The inhibitory effect of DOC on methylation and *M/D* (but not on demethylation) was independent of microbial respiration in the experiment where the microbial respiration was held constant by additions of sediment trap material to overwhelm changes in substrate availability at the different DOC concentrations (Fig. 1).

The mechanism by which DOC inhibits biotic methylation of mercury is unknown. The most likely explanation is that the availability of Hg^{2+} was reduced by binding of the free mercury ions to DOC (Kerndorff and Schnitzer 1980; Lodenius et al. 1987; Jackson 1989). Another possibility is that the added DOC inhibited the methylating bacteria even though overall microbial respiration rates were elevated.

Other researchers (Nagase et al. 1982; Lee et al. 1985) have found that DOC stimulates abiotic methylation at very high DOC concentrations (>14 × 10^3 μM). While this may result in substantial methyl mercury production in very high-DOC soil porewater, in our experiments, the fact that methylation rates were inversely proportional to DOC concentration suggests that biotic methylation was predominant in our aquatic samples. This observation is in agreement with the conclusions of Berman and Bartha (1986) and Jackson (1989) that the significance of abiotic methylation in lake sediments is minor in comparison with microbial methylation.

The results of the short-term experiments presented in this paper cannot necessarily be extrapolated to predict long-term effects in lakes with differing pH levels and DOC concentrations. However, these results do suggest several testable hypotheses that might explain high mercury concentrations in fish taken from different types of lakes. On the basis of our experiments, we hypothesize that high mercury concentrations in fish from clear-water, acidified lakes are caused by increased in-lake methylation in the water column and at the sediment–water interface because of decreased binding of Hg^{2+} by DOC at low pH. We hypothesize that in brown-water, low-pH lakes, high fish mercury concentrations may originate from methyl mercury transported from the drainage basin as DOC complexes (as proposed by Lee and Hultberg 1990), as well as from in-lake methylation at low pH and high DOC (Figs. 3 and 4). We further hypothesize that the negative correlation between fish mercury and DOC in seepage lakes found by Grieb et al. (1990) could be explained if most of the methyl mercury in

seepage lakes is produced in situ rather than in the watershed and if the in-lake methylation is reduced, as we have observed, by DOC concentrations.

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