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THE INFLUENCE OF DISSOLVED ORGANIC CARBON ON METHYL MERCURY PRODUCTION AND SEDIMENT-WATER PARTITIONING IN PRECAMBRIAN SHIELD LAKES

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In Partial Fulfillment

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Master of Science

By

Brenda Margaret Miskimmin

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ABSTRACT

Specific rates of bacterial methylation and demethylation at a range of dissolved organic carbon (DOC) concentrations were examined following reports of elevated fish mercury concentrations in remote high-DOC lakes. Water and sediments from the Experimental Lakes Area, northwestern Ontario were utilized to study the production of methyl mercury from $^{203}HgCl$, the demethylation of $^{14}CH_3HgI$ and microbial respiration. Mercury specific rates of Hg methylation in aerobic water decreased with increasing DOC, while demethylation and respiration increased with increasing DOC. Methylation in water was higher at pH 5 than at pH 7 while demethylation showed the opposite trend. At both pH's, DOC suppressed methylation but enhanced respiration.

Methylation and sediment-water partitioning experiments were undertaken to determine whether DOC affected methylmercury solubility. In methylation experiments using sediments with overlying water with high and low DOC concentrations, significantly more $CH_3-^{203}Hg$ remained in overlying high DOC water than low DOC. K_d experiments supported DOC-dependent solubility of [14-C] methylmercury.

Increased microbial respiration has been found by others to stimulate methylmercury production in some circumstances. When fresh sediment trap material (particulate organic carbon, POC) was added to water samples, specific methylation rates decreased by 30% or more even though respiration and demethylation were stimulated. With sediment samples, respiration, and not DOC, appeared to influence potential methylmercury production (M/D).

From these experiments, Hg availability appears to regulate methylation in the water column while respiration may be more important in sediments. High fish methylmercury concentrations in Precambrian Shield lakes may be partly explained by DOC solubilization of CH_3Hg^+ and enhanced methylation in water at low pH.

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BACKGROUND

The sources, movement, transformations and sinks of mercury in the aquatic environment are important properties to understand because of this metal's potential toxic effect on aquatic organisms and fish consumers. Methylmercury is the most toxic of the mercury species, is formed mainly by microorganisms, and is readily bioaccumulated both directly from water (by fish and other aquatic organisms) and by ingestion of contaminated organisms (by fish, birds or humans)[1]. The complexity of the behaviour of mercury in the environment is manifested by the fact that after two decades of directed research, there are still numerous gaps in our understanding of the biogeochemistry of this metal.

This thesis considers how dissolved organic carbon (DOC) affects methylmercury formation, partitioning and microbial respiration in Canadian Shield lakes to help understand why fish in many high DOC lakes have high mercury concentrations. The following introduction summarizes the toxic effects of methylmercury, mercury sources, the chemistry and cycling of mercury in the environment, bacterial methylation and demethylation, bioaccumulation by fish, and also examines the nature of DOC in aquatic environments.

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[1] Each of these will be discussed in more detail.

Toxic Effects of Methylmercury to Humans

Mercury compounds have no known metabolic function and research has not found any threshold level below which there are no toxic effects to humans (Summers 1986; Harriss and Hohenemser 1978). Methylmercury is considered to be a potent neurotoxin. In extreme cases such as in Minimata, Japan, where highly contaminated shellfish (10-35 ug/g) were consumed, 46 deaths occurred, in addition to a variety of sublethal clinical symptoms (Table 1; Chang 1979). Chronic effects including motor and speech disturbances, mental retardation, seizures and chromosome breakages may be passed on congenitally and teratogenically (Khera 1979).

In a case of methylmercury poisoning in Iraq in 1972, where contaminated barley and wheat were ingested, chronic effects became evident at concentrations in blood of <100 ng ml^{-1}, visual and hearing impairment occurred at 500-1,000 ng ml^{-1} and death was common at >3,000 ng ml^{-1} (Takizawa 1979).

In Canada, the English-Wabigoon River system of northwestern Ontario became heavily polluted with mercury when 9,000-11,000 kg of mercury was discharged from a chlorine-alkali plant in the 1960's (Armstrong and Hamilton 1973). The symptoms experienced by members of the Grassy Narrows and White Dog Indian Bands were attributed to the consumption of fish containing elevated methylmercury concentrations (Clarkson 1976; Wheatley 1979), although it

Table 1: Frequency of clinical signs and symptoms in Minimata Disease where humans consumed shellfish containing 10-35 ug/g mercury.

Symptom or sign	Frequency (%)
Constriction of visual fields	100
Sensory disturbance	100
Ataxia	94
Impairment of speech	88
Impairment of hearing	85
Impairment of gait	82
Tremor	76
Mental disturbance	71
Exaggerated tendon reflexes	38
Hypersalivation	24
Hyperhydrosis	24
Muscular rigidity	21
Ballism	15
Chorea	15
Pathologic reflexes	12
Athetosis	9
Contractures	9

Ref: Chang 1979.

was inorganic mercury which was discharged from the local industry.

Mercury is one of only a few metals which may be methylated by bacteria in the environment (with As, Cd, Pb, and metalloids Se, Sn and Te; Summers and Silver 1978). The conversion of mercury to a species which is 100 times more toxic than the inorganic form (Robinson and Tuovinen 1984), may lead to the situations outlined above in extreme cases. Because of the potential danger to human health, the currently recommended guideline for fish consumption is 0.5 ug Hg g^{-1} (WHO 1976; IJC 1977).

Sources

The sources of mercury are both natural and anthropogenic. Various amounts of the metal are found in rocks and surface minerals in all parts of the world. The most abundant mercury-containing ores are cinnabar (red HgS), metacinnabar (black HgS) and Livingstonite ($HgSb_4S_7$) (D'Itri 1972). The volatile nature of elemental mercury makes degassing of the earth's mantle (1.78×10^{10} g yr^{-1}), oceans (90×10^{8} g yr^{-1}) and volcanic emissions (2×10^{7} g yr^{-1}) important natural sources of mercury to the atmosphere (U.S. National Academy of Sciences 1977). Rocks and soils commonly have concentrations of total Hg between 5 and 1000 ug kg^{-1} with high averages near towns or in rice soils and the lowest averages in cultivated chernozemic soils of the Canadian

prairie provinces (Anderson 1979). Weathering, leaching and runoff of mercury from soils contribute to the mercury in receiving waters.

The industrial point sources of mercury originate from chlor-alkali plants, agriculture and pulp and paper industries (disinfectants, catalysts and fungicidal agents). Mercury from these types of sources were responsible for the methylmercury poisoning episodes described above. This direct discharge to aquatic systems has been largely curtailed in recent years as a result of mercury poisoning episodes. Sewage treatment plants are another large point-source of mercury in that raw sewage contains an average of 2 ppb of mercury (Matheson 1979). Mercury can volatilize from the sewage, or if not removed by the treatment process, be discharged to water bodies.

The main non-point anthropogenic source of mercury is fossil fuel burning (Robinson and Tuovinen 1984). Over 3,000 tons of mercury per year are released into the environment from coal burning and an estimated 10,000 to 60,000 tons are released from crude oils (Joensuu 1971). The atmosphere plays an important role in the global circulation of mercury (Andren and Nriagu 1979). Since mercury emitted to the atmosphere must return to the earth by one or more of several mechanisms (Matheson 1979), it is not surprising that recent studies have suggested that atmospheric sources are important in contributing mercury to many surface waters (Evans 1986;

Rada et al 1989). Methylmercury may be deposited directly into lakes from the atmosphere in precipitation (Fitzgerald et al 1989). It may also originate from within-lake or lake catchment methylation of deposited or naturally occurring Hg^{2+} (Winfrey and Rudd, in press).

Chemistry

Elemental mercury is a heavy (density 13.5 g ml^{-1}), silver-white liquid at room temperature (Andren and Nriagu 1979). It may be found in the environment in this form but occurs usually as inorganic salts which have much lower vapour pressures than elemental mercury (Hg^{o}). Some physical /chemical properties of mercury are listed in Table 2. Mercury may be found in the 2+ (mercuric) or 1+ (mercurous) oxidation state, and as $HgCl_2$, Hg_2Cl_2 or HgS. Mercurous ions may combine to form the polymer, Hg_2^{2+} (Andren and Nriagu 1979). These forms exist in equilibrium by chemical dismutation (Moser and Voight 1957):

$$Hg_2^{2+} \quad <====> \quad Hg^{o} + Hg^{2+}.$$

The bond with sulphide (to form cinnabar) is particularly notable because it is almost completely water insoluble (K_s = 5 X 10^{-9} - 10^{-6}; Benes and Havlik 1979).

Among the organomercurials, monomethylmercury (CH_3Hg^{+}) also forms extremely stable complexes with anionic sulphur ligands (Carty and Malone 1979). Methylmercury's solubility in lipids (and its attraction to S-groups in proteins) and

Table 2: Some Physical and Chemical Properties of Mercury

Atomic number	80
Atomic weight	200.59
Freezing Point (^{o}C)	-38.87
Boiling point (^{o}C)	356.9
Solubility:	
Hg^{o}: g/100 g water at 25^{o}C	6×10^{-6}
$HgCl_2$: g/100 g water at 20^{o}C	6.6
Surface tension (dynes/cm)	480
Strengths of chemical bonds	
Hg-Hg (kcal mol^{-1})	4.1
Hg-C	25
Hg-I	9
Hg-S	40
Hg-K	2
Electrode potentials (V)	
$Hg_2^{2+} + 2e \rightarrow 2Hg(l)$	0.792
$2Hg^{2+} + 2e \rightarrow Hg^{2+}$	0.907
$Hg_2Cl_2 + 2e \rightarrow 2Hg(l)+2Cl^{-}$	0.268
$Hg^{2+} + 2e \rightarrow Hg(l)$	0.854

Ref: Andren and Nriagu 1979; Carty and Malone 1979.

inactivation of sulfhydryl-dependent processes account for its toxicity to organisms. Monomethylmercury is the form usually found in fish muscle. Dimethylmercury (CH_3HgCH_3) may also be formed in lakes at neutral and alkaline pH, however, its volatility and chemical instability makes it more likely to be transferred to the atmosphere than to aquatic biota (Benes and Havlik 1979).

Mercury Cycling in Freshwater Systems

A simplified schematic of the transformations and movement of mercury in a lake ecosystem is depicted in Figure 1. The exact quantities found in each "compartment" is dependent upon biological and limnological characteristics of the individual lake, the geology of its watershed and atmospheric and terrestrial inputs.

The species found are dependent upon the chemistry of the lake. However, it is certain that in excess of 85% of mercury found in fish is methylmercury (Huckabee 1979). Metallic mercury (Hg^O) is very insoluble in water ($K_s = 10^{-7}$); so while it is the dominant form of mercury in the atmosphere (Lindqvist and Rodhe 1985; Slemr et al 1985), it may be oxidized to Hg^{2+} by a variety of photocatalytic reactions (Brosset 1987; Iverfeldt and Lindqvist 1986). Inorganic Hg^{2+} is the only form known to become methylated and is the primary form found in fresh and marine waters and sediments. It can be methylated by various methyl donors,

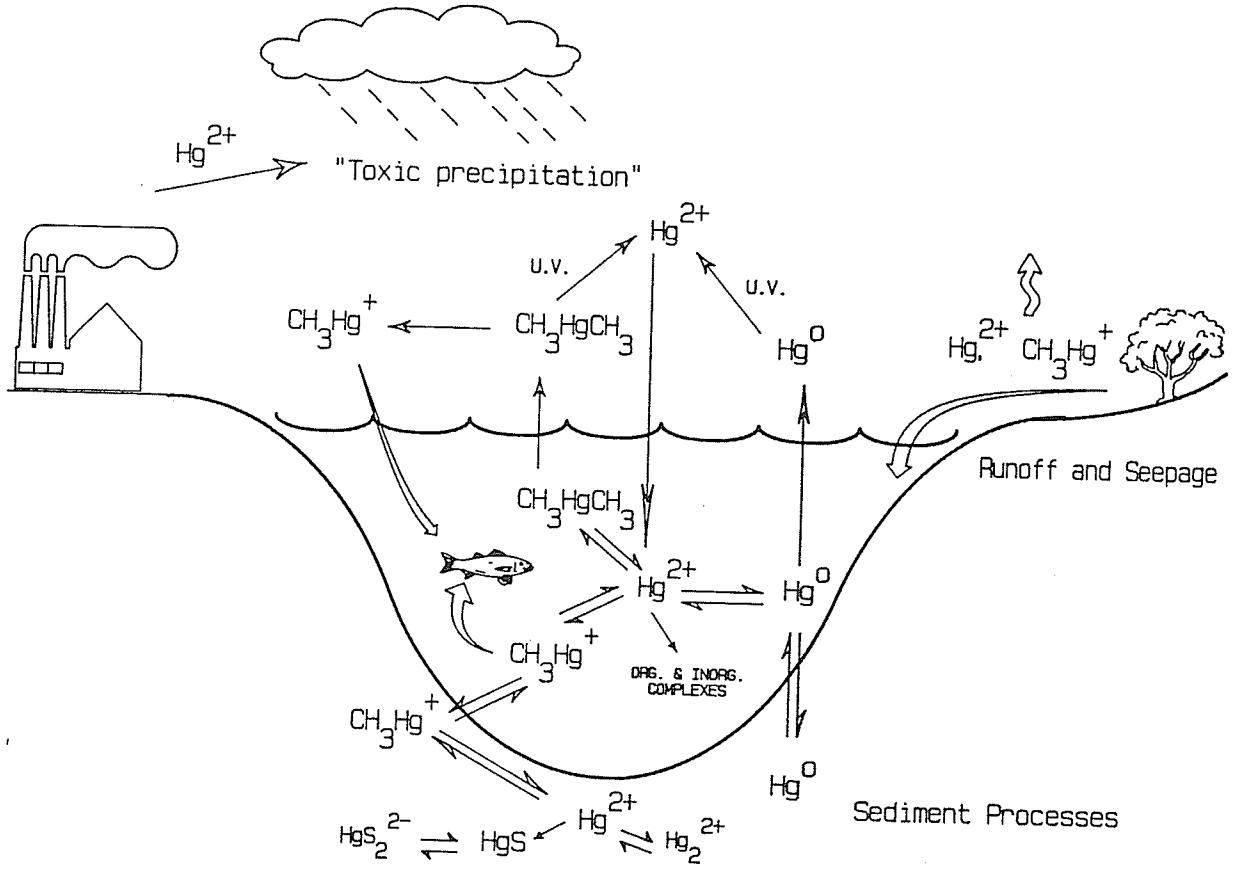

Figure 1: A simplified schematic of the Mercury Cycle

hence a small percentage of the total mercury in water and sediments is CH_3Hg^+ (Kudo et al 1982; Benes and Havlik 1979).

Because sediments below the interface are usually anaerobic, the formation of HgS is promoted when sulphide is available (Ramlal et al 1985).

Hg^{2+} may be complexed with organics such as dissolved organic carbon and other organic and inorganic substances. Organic complexes of mercury with low or high molecular weight substances may form a large part of the dissolved mercury pool depending on the concentration and nature of the dissolved organics (Benes and Havlik 1979). This can affect its availability for methylation (see Chapter I).

Mercury Methylation

Jensen and Jernelov (1967) were the first to demonstrate that microorganisms in natural lake sediments could methylate inorganic mercury. Microbial methylation has since been shown to occur in soils (Beckert et al 1974), water (Furutani and Rudd 1980) and fish intestines (Rudd et al 1980).

The mechanism of methylation is currently unclear but may involve the nonenzymatic transfer of methyl groups from methylcobalamin to Hg^{2+} (DeSimone et al 1973; Robinson and Tuovinen 1984). The major coenzymes involved in methyl transfer reactions in cells are N5-methyltetrahydrofolate derivatives, S-adenosylmethionine and methylcobalamin (vitamin B_{12}). Methylcobalamin is thought to be responsible

for methylation of inorganic Hg^{2+} salts because it is the only agent capable of transferring carbanion methyl groups (Bertilsson and Neujahr 1971; DeSimone et al 1973). The overall reaction is may proceed by the electrophilic attack of the mercuric ion on the carbanion species which is stablized by the cobalt atom, as follows:

$$Hg^{2+} \xrightarrow{CH_3B_{12}} CH_3Hg^{+} \xrightarrow{CH_3B_{12}} (CH_3)_2Hg.$$

Enzymatic transfer of CH_3 to Hg^{2+} has also been proposed in view of the fact that bacteria which do not have a methylcobalamin metabolism can methylate mercury (Landner 1971).

Abiotic or chemical mercury methylation has been shown to occur but is generally thought to be of minor import in the aquatic environment compared to biological methylation (Berman and Bartha 1986). For instance, Akagi and Takabatake (1973) reported photomethylation by irradiation with ultraviolet light, and methylation in the dark with methanol, acetic acid and propionic acid as methyl donors. Nagase et al (1984) found that humic material methylated mercury when using very high concentrations and temperatures (8.3-332 X 10^3 uM humic compound, 70°C). Lee et al (1985) found abiotic methylation using 171-285 mg DOC L^{-1} (14-23.7 X 10^3 uM), 20 mg L^{-1} inorganic mercury, and adding various metal ions. While significant abiotic methylation has not yet been demonstrated under natural conditions, its potential

importance in certain circumstances (such as water associated with soils) can not be dismissed.

Mercury Demethylation

The actual concentration of methylmercury in aquatic ecosystems is the net result of two reactions, mercury methylation (CH_3Hg^+ production) and methylmercury decomposition or demethylation. Of these "detoxification" processes by bacteria, demethylation is the better understood of the two (Winfrey and Rudd in press).

Demethylation results from the cleavage of the carbon-mercury linkage followed by the reduction of Hg^{2+} to Hg^o (Robinson and Tuovinen 1984; Summers and Silver 1978). The first reaction is mediated by the organomercurial lyase enzyme as follows:

$$CH_3Hg^+ \xrightarrow{NADPH \;\backslash\; NADP^+} CH_4 + Hg^{2+}.$$

The reduction is by the mercuric reductase enzyme:

$$Hg^{2+} + H^+ \xrightarrow{NADPH \;\backslash\; NADP^+} Hg^o + 2H$$

to form the volatile elemental mercury.

The degradation of organic mercury compounds was first detected in the Pseudomonas sp. K62 soil isolate (Furukawa et al 1969) when a phenylmercuric compound was shown to be degraded to Hg^o and benzene by using ^{203}Hg or ^{14}C-labelled phenylmercuric acetate. The products of the reaction were

separated by adsorption to activated carbon and eluted with toluene, indicating the cleavage of the carbon-mercury bond. In similar experiments using methylmercury, the end-products methane and elemental mercury were found (as shown in the above reactions; Furukawa et al 1969; Robinson and Tuovinen 1984). In addition to methane, CO_2 has been found to be produced from demethylation and recovery of both is done in the ^{14}C demethylation procedure used in this thesis (Rudd pers. comm.; Ramlal et al 1986). Extensive reviews as to the biochemistry and genetics of the enzymes and plasmids involved in mercury demethylation are given by Summers and Silver (1978), Robinson and Tuovinen (1984), Summers (1986) and Foster (1987).

The process of demethylation is not necessarily altered in the same way as methylation in response to a given variable in an ecosytem. The effect of changing a variable (such as increasing DOC or pH) must be studied for methylation and demethylation, using one of the available methods (Furutani and Rudd 1980; Ramlal et al 1986), to predict the combined effect of each process on overall methylmercury production.

Mercury Bioaccumulation by Fish

Methylmercury is absorbed by fish directly from the water, primarily across the gills, and also across the gastrointestinal tract from food (Rodgers and Beamish 1981;

1982). Methylmercury absorbed by fish by either pathway is transferred through the body via its blood until it is deposited in tissues, or removed by the liver and spleen (Windom and Kendall 1979). Since methylmercury uptake in fish is very efficient and rapid in relation to depuration, bioaccumulation occurs throughout the life of a fish. As a result of this, the largest and oldest members of a population typically have the highest concentrations of mercury[2] (MacCrimmon et al 1983).

There are numerous biotic and abiotic paramaters which influence the bioaccumulation of methylmercury by fish. Methyl Hg uptake rate has been found to increase with increasing growth rate, metabolic rate (Rodgers and Beamish 1981), fish body size (DeFreitas and Hart 1975), water temperature (Reinert et al 1974), and watershed area/lake area (Suns et al 1987). Uptake has been found to increase with decreasing calcium (McFarlane and Franzin 1980; Wren and MacCrimmon 1983), alkalinity (Schneider et al 1979), water hardness (Rodgers 1982) and pH (Jernelov et al 1975; Suns et al 1980; Hakanson 1980). The nature of this contaminant and the complexity of the interrelationships in lakes among the various parameters (often covarying) mentioned has made it extremely difficult to isolate the relative influences of each.

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[2] Whenever mercury in fish is discussed, it is assumed to be methyl Hg because this is the major form found in fish muscle (Huckabee 1979).

Of all of the factors which were found in the laboratory or otherwise to affect mercury bioaccumulation by fish, large data sets have recently highlighted two lake factors which most often are correlated with elevated fish mercury concentrations. These are pH and DOC. High fish mercury concentrations have been reported in remote low pH lakes (Bjorkland et al 1984; Lindqvist et al 1984; Wiener 1983) as well as drainage lakes with high dissolved organic carbon concentrations (Mannio et al 1986; Minnesota Pollution Control Agency 1985; Paasivirta et al 1983; Surma-Aho et al 1986).

Low pH (and associated low Ca^{+}) may affect fish directly by increasing gill permeability (Rodgers and Beamish 1983). It also desorbs mercury from particles which may increase the potential methylation rates in the water column and surface sediments (Xun et al 1987). Further, low pH promotes the formation of monomethylmercury rather than the more volatile dimethylmercury which is produced at higher pH. Additional discussion of the influence of pH on methylmercury formation may be found in Chapter I and is reviewed by Winfrey and Rudd (in press).

Dissolved Organic Carbon

Dissolved organic carbon, or DOC, is found in soils, peats and water. It is a mixture of plant and animal products in various stages of decomposition, biologically

and/or chemically synthesized substances, as well as microorganisms (Choudhry 1984). DOC is usually divided into two groups (i) humic substances, and (ii) nonhumic substances. Humic substances include humic acids, which are soluble in dilute base, but are precipitated by acidification to pH 2.0, and fulvic acids, which are soluble in both acid and base. The third component of humic substances, humin, is insoluble in acid or base in that it firmly binds with inorganic particles (Choudhry 1984).

Nonhumic substances are made up of simple compounds of known structures such as carbohydrates, proteins, peptides, amino acids, fats, waxes, resins, pigments and other low-molecular-weight organic substances. Nonhumic substances are easily degraded by bacteria in comparison to humic substances, which have been called refractory (Wetzel 1983) although decomposition of some part of the DOC has certainly been demonstrated (Sederholm et al 1973; Tranvik 1988).

DOC may originate from within a lake as extracellular products of plants, animals and microbial metabolism, or allochthonously from terrestrial soils and plants (Wetzel 1983). The occurrence of significant amounts of natural organic acids (humic substances) imparts a yellowish or brownish stain to water and can result in waters of naturally low pH (Oliver et al 1983; Brakke et al 1987). Brown-water or dystrophic lakes often occur in bog environments. They also may occur when water residence times are short, that is,

when large amounts of DOC enter the lake from the surrounding watershed due to high inflow rates. Terrestrial runoff of high-DOC water may be important in transporting contaminants, such as mercury, into the water column of lakes (Lee and Hultberg submitted).

Dissolved organic carbon has been found to reduce the uptake of inorganic mercury to fish (Oh et al 1986). Mercury is tightly bound by humic substances (Kerndorff and Schnitzer 1980) which may reduce its transport across tissue boundaries. No published studies to date have examined the effect of DOC on methylmercury uptake to fish or other organisms.

The possible contribution of humic substances to abiotic mercury methylation was discussed above. An accounting of other aspects of humic substances including interactions with environmental chemicals is given by Choudhry (1984).

PREFACE

This research component of this thesis is organized as individual manuscripts which make up three chapters. Chapter I examines the influence of DOC, pH and respiration on biological mercury methylation and demethylation in water. Chapter II is the first known report of the effect of DOC on the sediment-water partitioning of **methylmercury.** Chapter III represents other experiments done at the Experimental Lakes Area, aimed at exploring the natural variability of lakes in terms of methylation, demethylation and respiration.

CHAPTER I

THE SHORT-TERM INFLUENCE OF DISSOLVED ORGANIC CARBON, pH AND RESPIRATION ON MERCURY METHYLATION AND DEMETHYLATION IN LAKE WATER

INTRODUCTION

Although all geologically-derived mercury and most culturally distributed mercury is inorganic, eighty-five percent or more of the mercury found in fish tissue is methylmercury (Huckabee et al 1979). Thus, the microbial transformation of mercury to methylmercury (CH_3Hg^+) and the factors which affect it, are important in understanding the patterns of CH_3Hg^+ dynamics in aquatic ecosystems. High CH_3Hg^+ in fish (in excess of 0.5 ppm) has been reported in remote low pH lakes (Bjorkland et al 1984; Lindqvist et al 1984; Wiener 1983) as well as in remote drainage lakes with high dissolved organic carbon concentrations (Mannio et al 1986; Minnesota Pollution Control Agency 1985; Paasivirta et al 1983; Surma-Aho et al 1986). I have investigated the effects of DOC and pH on mercury methylation and demethylation. I have looked at both direct effects on the methylation and demethylation processes and indirect effects caused by changes in overall microbial activity.

Previous studies in Precambrian Shield lakes have shown that the balance of specific methylation and demethylation decreases with decreasing pH in sub-surface sediments (Ramlal et al 1985; Steffan et al 1988) but increases with decreasing pH in the water column and surface sediments (Xun et al 1987). Thus the changing balance of methylation and demethylation in the water and at the sediment surface help

to explain high fish mercury concentrations in many low pH lakes (Winfrey and Rudd in press).

DOC concentrations have been positively correlated with planktonic bacterial activity and biomass in a study of Precambrian Shield lakes (Tranvik 1988). It has also been demonstrated that net mercury methylation can increase in response to increased microbial respiration rates in sediments (Furutani and Rudd 1980; Wright et al 1982; Hecky et al 1987). Rudd and Turner (1983) concluded that stimulation of microbial respiration was the primary factor in increasing CH_3Hg^+ in fish in enclosures while pH was a modifying factor. Dissolved organic carbon (DOC) may act as a source of decomposable carbon for bacteria (Tranvik 1988), therefore its interaction with bacterial methylators may involve effects such as changes in respiration as well as in complexation.

The complexation by DOC of mercury compounds may affect the biological availability of this metal. Inorganic mercury has been shown to bind strongly with dissolved organic carbon, notably humic substances (Kerndorff and Schnitzer 1980; Lodenius et al 1987). DOC binding could reduce methylation by rendering it less available for methylation. Inorganic mercury bioavailability to fish was demonstrated to be reduced in the presence of DOC (Oh et al 1986). Similar uptake studies with DOC and CH_3Hg^+ have not been done. If binding with organic matter reduces

availability of inorganic mercury to bacteria for methylation, DOC complexes with mercury should indirectly reduce CH_3Hg^+ accumulation in fish. Such an effect would mean that high DOC drainage lakes with elevated fish mercury concentrations can not be explained by within-lake stimulation of microbial methylation but by some other factor or factors.

If binding of mercury with DOC is a more important influence than DOC-induced respiration, net methylation rates should be reduced with increasing DOC. If stimulation of respiration is more important, then mercury methylation should follow respiration regardless of a change in DOC concentration. The purpose of this study was to examine separately how dissolved organic carbon concentrations, respiration rates, and pH affected specific rates of microbial mercury methylation and demethylation in lake water samples.

MATERIALS AND METHODS

Study Site

All experiments and sampling were done at the Experimental Lakes Area (ELA), northwestern Ontario, Canada, during the spring and summer of 1988. The area is located on the Precambrian Shield, with a high proportion of granite bedrock exposure and minimal soil coverage. The lakes are typically oligotrophic and of low buffering capacity

(Brunskill and Schindler 1971). Water samples were taken from oligotrophic Lake 239 and the northeast inflow to Lake 239. This lake has a maximum depth of 30 m and a circumneutral mean pH. The northeast inflow water used as a source of DOC was the drainage of a low pH (4.0) sphagnum bog.

Sampling Protocol

Samples of epilimnetic water were taken by hand approximately 10 cm below the surface of Lake 239 with a 2-litre Nalgene bottle which had been twice rinsed with sample water. The bottle was returned to the laboratory and the sample used within four hours of sampling. High-DOC bog water was collected from a weir outflow directly into a large carboy.

Sediment trap material was collected over a week to ten days in Lake 302N with a trap consisting of two upright cylinders (height=six times the diameter: ~60 X 10 cm) held in place by a small anchor and a submerged buoy. Traps were placed in the hypolimnion about one metre from the lake bottom. Overlying water was siphoned from the trap leaving a slurry of fresh particulate matter which was used in some experiments to enhance respiration.

Specific Rates of Mercury Methylation and Demethylation

The term "specific rates" when referring to methylation and demethylation means the rates in the context of the radioisotopic methods used, ie. the percent of the isotope added which has been methylated/demethylated. Units are percent L^{-1} hr^{-1} expressed per unit weight of added Hg. Specific rates of mercury methylation and demethylation were measured using the radiochemical methods of Furutani and Rudd (1980) and Ramlal et al (1986), respectively. The methylation method involves the addition of $^{203}HgCl_2$ and extracting any alkylated $^{203}Hg^+$ produced over a 24-hour period. The demethylation method consists of quantifying the volatile $^{14}CO_2$ and $^{14}CH_4$ produced in the microbial degradation of $^{14}CH_3HgI$ over the same incubation period. Because ambient mercury concentrations are overwhelmed by the radioisotope and carrier mercury, the methods give rates **specific** to the amount added rather than an in situ rate. In both methods the amount of mercury added is kept constant, thus, the effect of other variables on the specific rates of methylation and demethylation (and their ratio) can be studied.

Specific rates of mercury methylation were determined by adding 1.0 or 2.0 ug of Hg(II) [1.0-2.0 uCi as $^{203}HgCl_2$, New England Nuclear Corp.] to 100 mLs of water as described above. Two or three replicate samples plus one acid-killed control were incubated for 24 hours at 20 C +/- 2 C, then

were killed with 1.0 mL of 4N HCl. The method establishes a specific mercury methylation rate, that is, $CH_3{}^{203}Hg^+$ production minus degradation. $CH_3{}^{203}Hg^+$ extraction efficiency is close to 100% even in very highly organic matrices such as sediments and fish tissue. Thus, the range of DOC concentrations in these experiments should not have affected the extraction efficiency.

Specific rates of demethylation were measured by the addition of 0.2 ug Hg(II) [as 2 uCi ^{14}C-methyl mercuric iodide, Amersham Laboratories] to 100 mLs of sample. The sample numbers and experiment termination were the same as for methylation, although the 4N HCl was added by injection through the silicone stoppers. The $^{14}CO_2$ produced was trapped in a scintillation vial containing 10 mLs of scintillation fluor (ACS, Amersham), 2 mLs of methanol and 2 mLs of Protosol (New England Nuclear). The use of the tissue solubilizer (Protosol) required storage of the vials in the dark for at least 48 hours until background chemiluminescence had diminished. The method quantifies gross specific $^{14}CH_3Hg^+$ degraded, and does not account for inorganic mercury converted back to CH_3Hg^+. Both methylation and demethylation measurements are done using Hg^{2+} or CH_3Hg^+ which are elevated over in situ concentrations. The reason for this is that the biologically available in situ concentrations are unknowable, and so measurements are made

comparable by using the same artificial concentrations in all incubations.

Respiration

Respiration rates were measured by incubating 50 mLs of sample water in glass syringes at the same temperature and the same treatment as for methylation experiments. Experiments which included sediment trap material were incubated for 18 hours while all others were incubated 24 hours. Initial and final dissolved inorganic carbon (DIC) concentrations were measured by injection of 0.5 mL water samples into an infrared spectrophotometer (Stainton et al 1977). Oxygen consumption rates were quantified using a scaled-down Winkler technique (A.P.H.A. 1971) on a 10 mLs aliquot of the sample and using phenyl arsine oxide (Hach Chemical Co.) as the titrant in place of sodium thiosulphate. All measurements were done in duplicate.

Dissolved Organic Carbon (DOC)

The DOC concentrate was obtained by roto-evaporating L.239 northeast inflow water (bog runoff) at 60^{o}C to concentrate dissolved organic carbon. The concentrate was then passed through a cation exchange column to replace cations with H^{+}. The pH after this step was <4.0 and was adjusted with dilute NaOH and/or HCl at the time of each experiment.

DOC was defined as the crude measure of all dissolved organic carbon (including associated bacteria) passing through a glass fibre filter (Whatman GF/C, Fisher, 0.7-1.0 um pore size) and was not further characterized. It is the predominant solute found in the bog water used as evidenced by the measurement of other ions in the concentrate (Table 1).

Sample water was passed through the GF/C filter before DOC analysis. Dissolved organic carbon concentrations were measured using a high-temperature acid persulphate digestion followed by infrared detection of CO_2 on a Model 700 Carbon Analyzer (OI Corp., Houston TX).

Experimental Design

All the DOC concentrations tested were obtained either by using lake and bog water directly or by diluting the DOC concentrate with L.239 water. The experiments are summarized in Table 2 and detailed in the following paragraphs.

The first set of experiments was designed to examine the influence of three different DOC concentrations, at their natural pH, and at constant respiration rates on mercury methylation and demethylation. Because increasing the DOC concentration stimulated respiration rates, respiration was held constant by overwhelming the DOC with large amounts of natural substrate. This was done by adding equal quantities of a slurry of sediment trap material (6.0 mg dry weight) to

Ch. I, Table 1: Chemical Composition of DOC Concentrate

UNITS	DOC	Al	Cu	Fe	Mn
$ug.L^{-1}$	312000	572	13	618	5.0
$umol.L^{-1}$	26000	21	0.2	11	0.09
uM in "HIGH"	**3100**	**2.5**	**0.02**	**1.3**	**0.01**

	Mg	Na	K	Cd
$ug.L^{-1}$	28.0	37200	51	2.5
$umol.L^{-1}$	1.15	1618	1.3	0.02
uM in "HIGH"	**0.14**	**190**	**0.15**	**0.003**

Note: Boldfaced values are approximate composition of "highest [DOC]" for Experiment 2.

Ch. I, Table 2: Description of Experimental Design

Number	Objective	Design
1	Constant respiration; low, mid-range, high DOC; Methylation, demethylation; Natural pH.	Bog outflow water diluted with L239 water + 6 mg ST[3].
2	Influence of increasing [DOC] on M, D; Respiration not constant.	4 [DOC] achieved by diluting concentrate with L239; pH=6.0 +/- 0.2.
3	pH v DOC effect; How each effects M, D and respiration.	High & low [DOC] @ pH 5 and 7. 2 [DOC] achieved by diluting concentrate with L239.

[3] ST = sediment trap material

each 100 mL sample. Lake 239 epilimnion water was the low DOC treatment (500 uM), L.239 northeast inflow water was high (2600 uM) and a mixture of the two represented the mid-range of the DOC concentrations (1150 uM). pH was not adjusted.

The objective of the second set of experiments was to observe the influence of increasing DOC concentrations on specific methylation and demethylation rates. No sediment trap material was added so respiration rates were the natural rates for the water used. The lowest DOC concentration was L.239 epilimnion water (560 uM DOC). All other DOC concentrations were derived by dilution of the DOC concentrate with L.239 water to achieve 760, 1600 and 3100 uM DOC. The pH of each dilution was adjusted to 6.0 +/- 0.2.

The third part of the study examined the effect of changes in both pH and DOC on both specific methylation and respiration rates. The experiments consisted of high (2600 uM) and low (530 uM) DOC concentrations. The low DOC concentration was again L.239 epilimnion water and the higher DOC concentration was a dilution with L.239 water of the DOC concentrate. The L.239 epilimnion water was used at its natural pH and DOC concentration ("low DOC, pH 7.0") while each of the remaining three treatments was adjusted in bulk to a pH of 5.0 or 7.0 with dilute HCl and/or NaOH for use in all parts of the experiment.

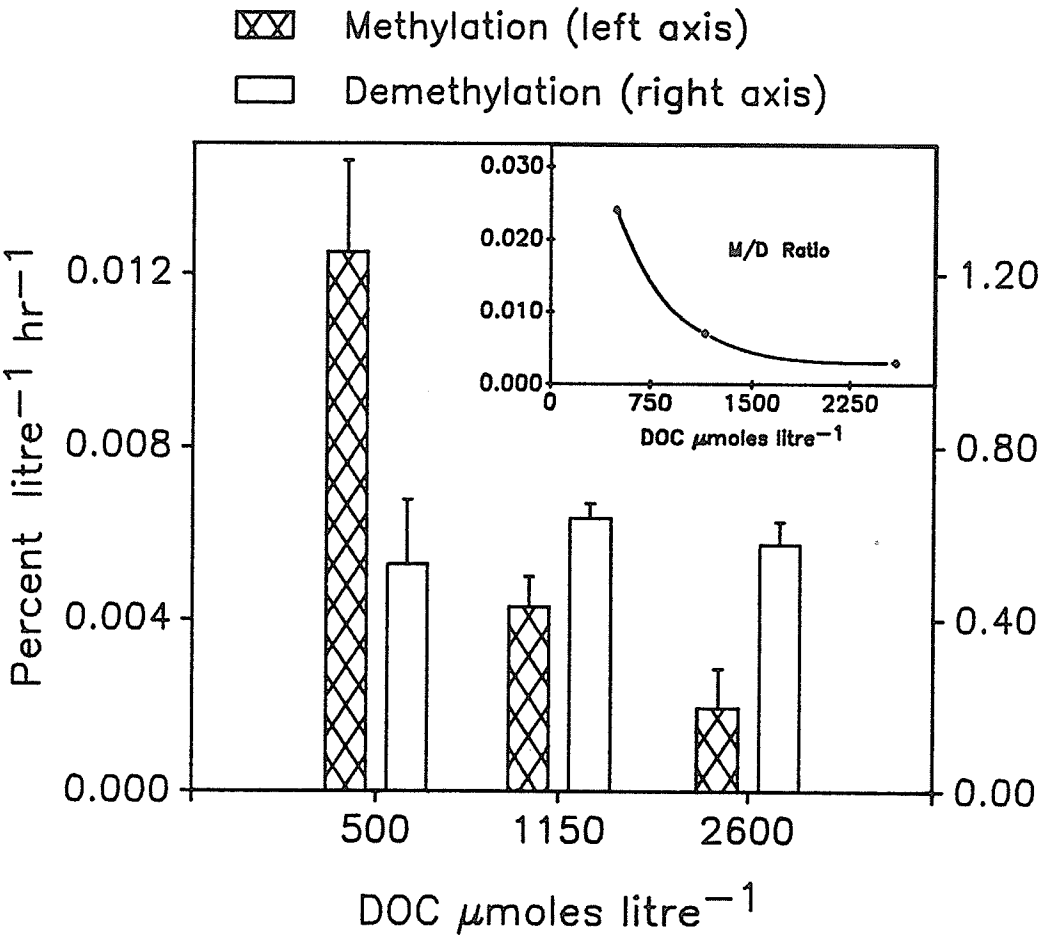
For each of the three types of experiments, methylation, demethylation and respiration measurements were made as

described previously. Each experiment was repeated to determine reproducibility of results. Results of the radioisotopic assays were reported as the percentage of total isotope added which was methylated or demethylated. Significant differences ($P=<0.05$) among treatments were evaluated by analysis of variance (ANOVA).

RESULTS

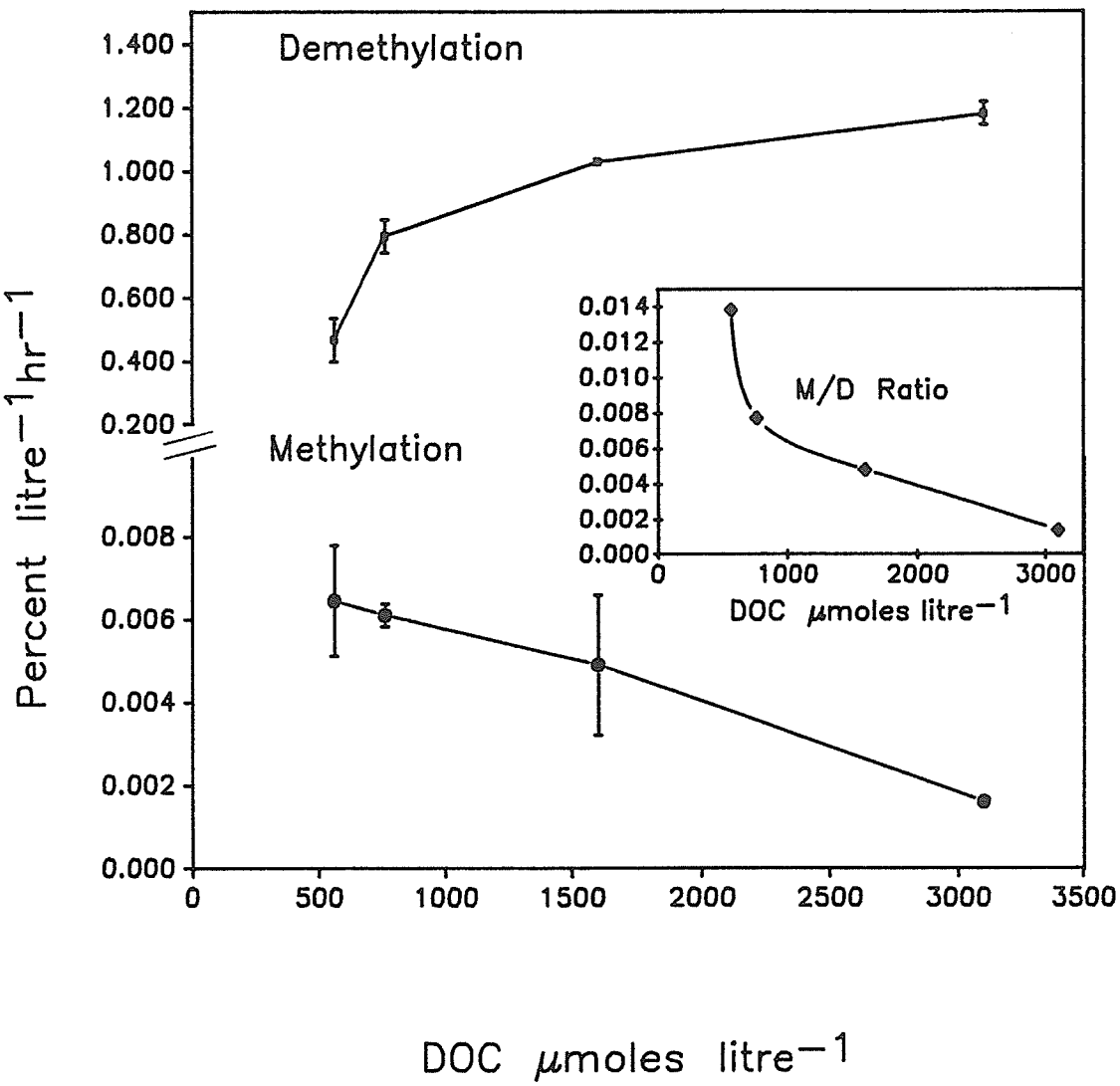
In the constant respiration (sediment trap material addition) experiments, measured respiration was the same and showed no trend with DOC concentration. DIC production averaged 1.24 +/- 0.90 umoles L^{-1} h^{-1} and O_2 consumption averaged 1.04 +/- 0.19 umoles L^{-1} h^{-1}. There were two- to three-fold decreases in the rates of methylation at the higher DOC concentrations, whereas there was no detectable difference between rates of demethylation at each treatment (Figure 1).

In the second set of experiments, where DOC was varied and respiration was not constant (no sediment trap material added), DIC production increased from approximately 0.08 umoles L^{-1} h^{-1} in 560 uM DOC to 0.80 umoles L^{-1} h^{-1} at the higher concentrations. O_2 consumption increased from undetectable in 560 uM DOC to approximately 1.2 umoles L^{-1} h^{-1} in higher DOC treatments. Specific methylation rates decreased consistently with increasing DOC concentration (Figure 2). The methylation rate was significantly lower at



Ch. I,
Figure 1 Rates of methylation and demethylation with DOC concentration.
Additions of sediment trap material to all samples held respiration constant among each. Standard deviation shown.

Inset: Ratio of rates, M/D. JUNE 4/88.



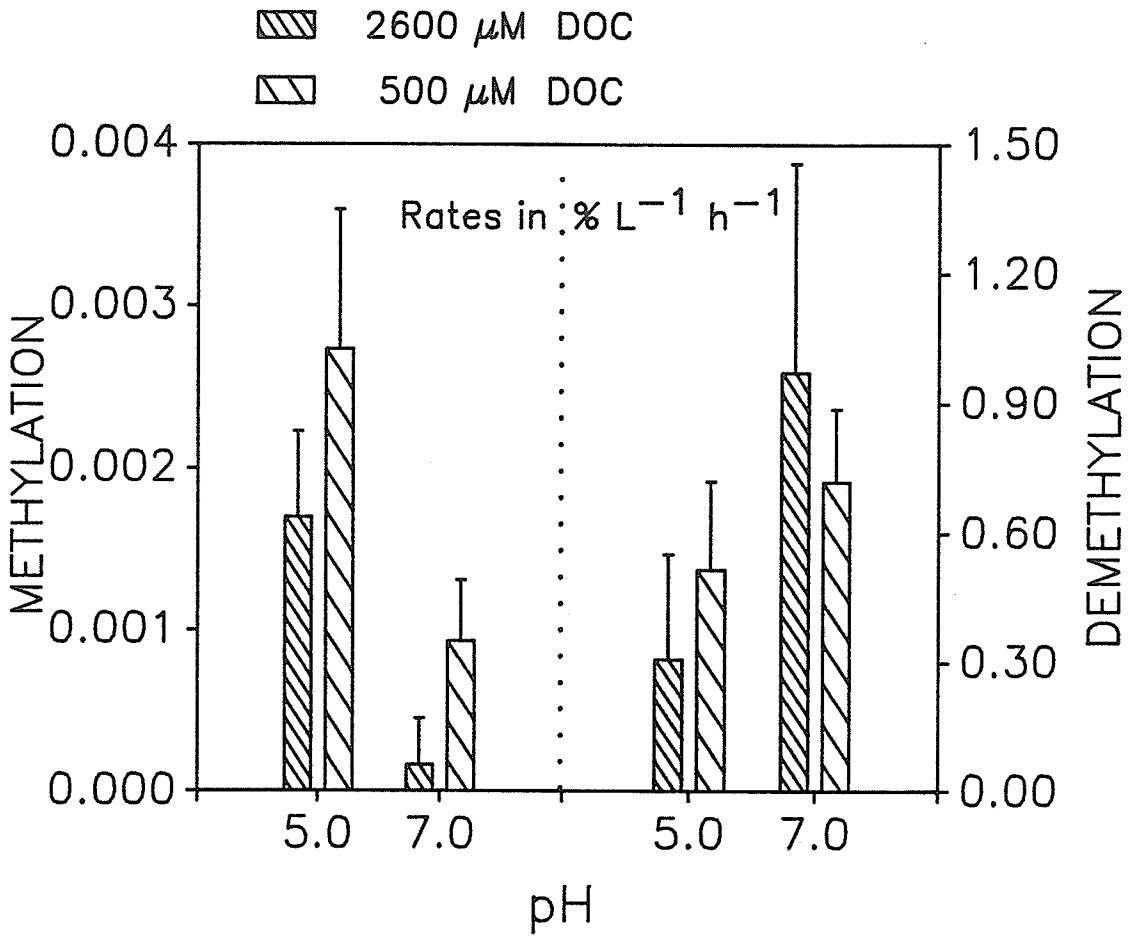
Ch. I,
Figure 2 Rates of methylation and demethylation with increasing DOC concentration. Respiration increased between the lowest DOC concentration and all others. Standard deviation shown.

Inset: Ratio of rates, M/D.
AUGUST 11/88.

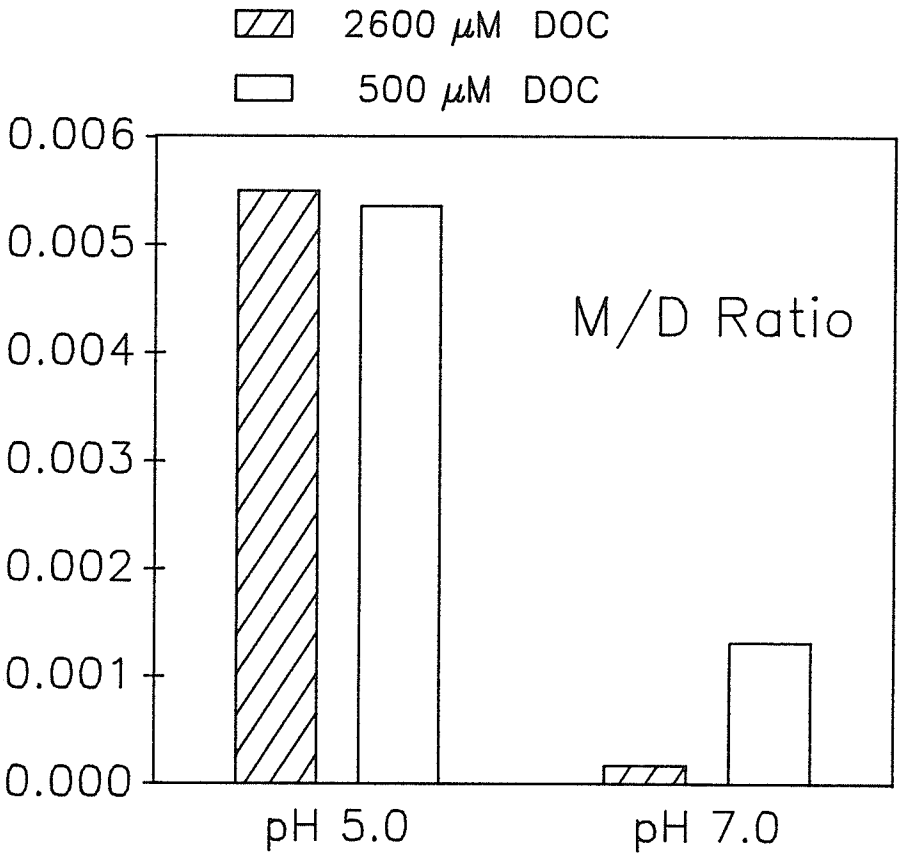
the highest DOC concentration (3100 uM) than at all of the other DOC concentrations. Also, methylation rates were similar to these obtained in the experiments where respiration was higher due to sediment trap additions (Figs. 1, 2). Demethylation rates increased significantly with each increase in DOC concentration. The largest increase in demethylation was between 560 uM and 760 uM, which also corresponded to the only significant increase in respiration rate.

It should be noted that in both of the above experiments the M/D ratio (rate of methylation/rate of demethylation), which is an indication of the relative potential for net CH_3Hg^+ production, is clearly highest at the lowest DOC concentration and decreases with increasing DOC. This occurred whether or not sediment trap material was present, ie. M/D was primarily controlled by DOC concentration rather than by rates of respiration (Figure 1 and 2 insets).

When both pH and DOC were adjusted, methylation and demethylation were influenced more by pH, whereas respiration was influenced more by DOC. Methylation was higher at pH 5.0 than at 7.0, but was suppressed by high DOC at both pH levels (Figure 3). Demethylation rates were higher at pH 7.0 than at pH 5.0 but did not follow a consistent pattern with respect to DOC concentrations (Figure 3). M/D ratios indicate a higher net potential for CH_3Hg^+ production at pH 5.0 than at pH 7.0 regardless of DOC concentration (Fig. 4).



Ch. I,
Figure 3 Rates of methylation and demethylation at 2600 uM and 500 uM DOC. Rates are for pH 5.0 and 7.0. Standard deviation shown. AUGUST 23/88.

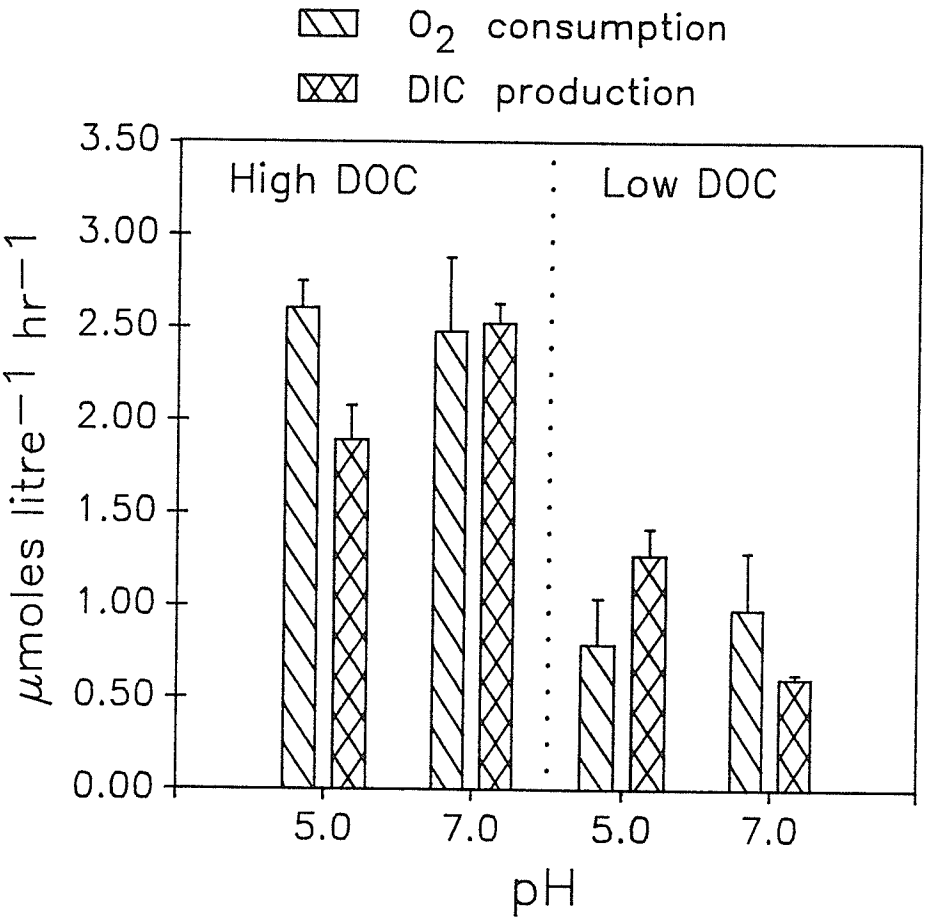


Ch. I,
Figure 4 Ratio of methylation to demethylation (M/D) at 2600 uM and 500 uM, pH 5.0 and 7.0. AUGUST 23/88.

Respiration (O_2 consumption, DIC production) was significantly higher in the high DOC (2600 uM) treatments than in the low DOC (530 uM) treatments regardless of pH (Figure 5). There was no difference in respiration between pH 5.0 and pH 7.0 for any one DOC concentration.

DISCUSSION

The effect of dissolved organic carbon concentrations, pH and respiration on Precambrian Shield water column CH_3Hg^+ production in these laboratory experiments may help to explain variation in fish mercury levels in lakes with different pH's and DOC concentrations. The suppression of specific methylation rates with increasing DOC is contradictory to the observation of high mercury concentrations in fish tissue in high-DOC drainage lakes. However, the stimulation of specific methylation at low pH means that this process may contribute to fish methylmercury concentrations even in high DOC lakes. DOC appeared to reduce the availability of inorganic mercury to methylating bacteria even though a portion of the DOC was being decomposed. Changes in respiration rates caused by differences in the amount of DOC present for decomposition had the least effect on relative potential methylmercury production as measured by the M/D ratio (Figure 2 inset). However, the importance of pH in methylmercury production



Ch. I,
Figure 5 Rates of respiration -- oxygen consumption and dissolved inorganic carbon production at 2600 uM and 500 uM DOC in water at pH 5.0 and 7.0. Standard deviation shown. AUGUST 23/88.

regardless of DOC concentration (Figure 3 and 4) suggests that water column methylation may be of consequence in lakes of low pH. This finding of higher CH_3Hg^+ production with reduced pH agrees with the work of Xun et al (1987) and may further help to explain elevated mercury concentrations in fish in acidified lakes.

Some authors have found abiological methylation in the presence of high concentrations of fulvic and humic substances and suggested its possible importance in contributing to CH_3Hg^+ production (Nagase et al 1984; Lee et al 1985). While this study did not specifically examine this question, it would appear that such a mechanism was negligible, if it occurred. If abiological mercury transformations contributed importantly to methylation at the natural levels of DOC used in this study, higher methylation rates would have been detected in the high DOC treatments. The fact that the low DOC treatments gave higher methylation rates is a strong suggestion that biological methylation (apparently regulated by Hg availability) was the dominant mechanism under the conditions of this study. This is in agreement with the conclusions of Berman and Bartha (1986) that the environmental significance of abiotic methylation in sediments was minor in comparison to biological methylation.

While DOC originating from terrestrial sources such as bogs is often considered refractory, it has been known for many years that some portion of it is apparently available

for decomposition (Sederholm et al 1973). In recent studies, Tranvik (1988) and Tranvik and Hofle (1987), found that a substantial fraction of the total DOC pool was available for degradation. Because the above studies were also done in Precambrian shield watersheds, the DOC may have been similar in composition, resulting in analogous findings in terms of bacterial activity. My short-term experiments suggest that even though microbial decomposition was higher at higher DOC concentrations, the increased respiration was unimportant in comparison to the binding of inorganic mercury to DOC which resulted in a decrease in M/D ratios.

It was not surprising that respiration was unaffected by pH. Lake acidification to pH 5.0 did not significantly influence rates of microbial decomposition of organic carbon in Lake 223 at the Experimental Lakes Area (Kelly et al 1984) and the short-term laboratory experiments presented here showed the same lack of influence (Fig. 5). Thus, it seems unlikely that an effect related to overall respiration rates is important in explaining the relationship between low pH and high fish mercury levels. Also, the increased respiration that occurred at increased DOC concentrations did not result in higher specific methylation rates. Aside from the influence of respiration on demethylation rates, pH and DOC concentrations (in the short term) were more important than respiration rates in water column methylmercury production.

Low pH has often been cited as the most important factor in predicting high fish mercury concentrations in lakes (Bjorkland et al 1984; Lindqvist et al 1984; Wiener 1983), while high DOC has been the second factor of consequence. McMurtry et al (1989) found that acidity (and water hardness) correlated with mercury concentrations in smallmouth bass and that DOC correlated with mercury concentrations in lake trout.

In examining both pH and DOC effects concurrently, a likely explanation for the observation of Xun et al (1987) that reduced pH in lake water and at the sediment-water interface increases rates of net methyl mercury production is suggested. It is known that reduction in pH changes the character of DOC by increasing protonation of anionic moieties and desorbing metals (Davis et al 1985). This may explain the reduced binding capacity of DOC for inorganic mercury (Hg^{2+}, Hg^{+}) in acidified water. Increased methylation at low pH in lake water may be explained by reduced binding of inorganic mercury to DOC making it more available for methylation. A similar mechanism may occur at the sediment-water interface. The pH of sediments is usually only lowered at the interface of acidified lakes because of microbial acid consumption below the interface (Kelly et al 1984). This DOC binding mechanism could explain the pH-related increase of methylation that has been observed in acidified lake water samples.

In contrast to water column experiments, specific mercury methylation in sediments has been found by others to be related to respiration rates. Furutani and Rudd (1980) found a linear relationship between overall sediment microbial activity and methylation rates using tryptic soy broth as a bacterial substrate. Similarly, Hecky et al (1987) found that stimulation of mercury methylating bacteria by flooding of organic reservoir sediments was primarily responsible for increased net methylation because demethylation was relatively unaffected. These sediment methylation studies found the opposite to my water methylation results perhaps because of differences in microbial populations, organic carbon substrates, water chemistry and differences of binding of mercury in the ecosystems. Respiration rates in the sediments were much higher than in water, and the magnitude of change in respiration rates in the studies cited were greater. It is also not surprising that the most important rate-controlling variable might be different in low alkalinity lake samples as compared to artificial reservoir samples.

The constant demethylation and respiration (Figure 1) and increased demethylation with increasing DOC concentration (Figure 2) does suggest a possibly important contribution of planktonic bacteria to the breakdown of organic mercury compounds. Larsson et al (1988) found that degradation of a number of chlorinated organic contaminants increased in lakes

of increasing humic concentrations. Methylmercury is not as strongly attracted to negative ligands as inorganic mercury is (Carty and Malone 1979) which may make DOC-bound CH_3Hg^+ more available to bacteria than inorganic mercury. This difference in binding strength could explain why methylation was influenced more by availability of $^{203}Hg^{2+}$ than by respiration rates and why demethylation was influenced more by respiration than $^{14}CH_3Hg^+$ availability. The importance of the demethylation process in attenuating CH_3Hg^+ accumulation in lakes can only be appreciated by measuring methylation and demethylation rates independently.

The bioavailability of methylmercury for demethylation is unlike other organic contaminants (PAHs, McCarthy and Jiminez 1985; PCBs, Landrum et al 1987; a dioxin, Servos and Muir 1989) as well as inorganic mercury (Oh et al 1986) which have exhibited reduced availability to uptake by various fish and invertebrates in the presence of dissolved organic matter. The reduced uptake of the compounds was attributed to binding with DOC. It is not known whether methylmercury is as available to higher organisms when bound to DOC as it apparently is to demethylating bacteria.

It should be noted that while my short incubation period (24 hour) may be a good time-frame to maintain natural bacterial activities, the in situ (long-term) effect of DOC on specific methylation and demethylation might not be predictable from these experiments. I compared a range of

DOC concentrations and all of the DOC had a similar character unless pH was adjusted. Longer term DOC degradation may change the character of these molecules which may cause changes in the availability of the various forms of mercury.

If it is true that DOC inhibits methylation in brown-water lakes but not in clear-water lakes, this suggests that there may be a fundamental difference in the origin of methyl mercury in brown-water as compared to clear-water lakes. Methylmercury in brown-water lakes may largely originate from terrestrial sources as reported by Lee and Hultberg (submitted) and speculated by others (Mannio et al 1986; Simola and Lodenius 1982; Surma-Aho et al 1986), while methylmercury in clear-water lakes may come mostly from in-lake production. This could explain why it has been difficult to determine whether pH or DOC content are primarily responsible for elevated mercury in fish in low pH lakes. A consistent scenario may be unrealistic when comparing fish mercury from low pH clear-water lakes and low pH brown-water lakes because the site of methylmercury production could be terrestrial in one case and in-lake in the other.

CHAPTER II

METHYLMERCURY FORMATION AND SEDIMENT-WATER PARTITIONING AS AFFECTED BY NATURAL LEVELS OF DISSOLVED ORGANIC CARBON

INTRODUCTION

Over the past several years, there has been considerable effort made to establish the most important source of methylmercury in Precambrian Shield lakes that are distant from direct cultural influences. Data sets have reflected unexpectedly high mercury concentrations in fish tissue in remote low pH lakes (>0.5 ppm; Bjorkland et al 1984; Lindqvist et al 1984; Wiener 1983) as well as remote drainage lakes with high dissolved organic carbon (DOC) concentrations (Mannio et al 1986; Minnesota Pollution Control Agency 1985; Paasivirta et al 1983; Surma-Aho et al 1986). One recent report of Ontario lakes found lake trout mercury concentrations to be positively correlated with DOC concentration, while smallmouth bass mercury concentrations were correlated with acidity (McMurtry et al 1989). Probable reasons that fish-methylmercury concentrations are high in remote high-DOC lakes are outlined by Winfrey and Rudd (in press) but have not been fully established to date.

Methylmercury is produced biologically from inorganic mercury by microorganisms in soils, sediments and water and is produced to a much lesser extent by chemical methylation (Berman and Bartha 1986; Korthals and Winfrey 1986). The transport of methylmercury produced in lake surface sediments or the terrestrial environment into lake water might be mediated by binding to DOC.

It is methylmercury in the dissolved phase which is potentially available for bioaccumulation by food web organisms and fish. Thus, any characteristics which enhance both the production and solubility of methylmercury must be studied to more fully understand the mercury problem. Only one study has examined the partitioning of methylmercury in aquatic systems, finding that as water pH decreased, adsorption of methylmercury to sediments also decreased (Miller and Akagi 1979). This paper examines the role of DOC in sediment-biological [^{203}Hg] methylmercury formation and [^{14}C]methylmercury partitioning (distribution) between lake sediments and water.

MATERIALS AND METHODS

Two types of experiments were performed. The first measured specific mercury methylation in a sediment-water mixture in which the water used had a range of three DOC concentrations. Methylmercury produced from the $^{203}HgCl_2$ added was extracted separately from the water and the sediments. The second was a sediment-water partitioning experiment which measured the distribution (K_d) of $^{14}CH_3Hg^+$ added. Five different DOC concentrations were used to determine the influence of DOC on K_d.

Sediment Sampling

Sediments for the methylation and partitioning experiments were obtained from 4 m depth in East Bay of Lake 239, an oligotrophic Canadian Shield lake at the Experimental Lakes Area, northwestern Ontario (Brunskill and Schindler 1971). An Ekman dredge was used for sampling sediments, of which only the surface 2-3 cm were immediately transferred to glass bottles. The bottles were completely filled to exclude air. The sediments were refrigerated for up to one month until used in experiments or to determine in situ Hg concentrations.

Water Preparation

All the DOC concentrations tested were obtained by diluting a DOC concentrate with distilled water. The DOC concentrate was obtained by roto-evaporating L239 northeast inflow water (bog runoff) at 60^{o}C to concentrate the dissolved organic carbon. The concentrate was passed through a cation exchange column to remove any free cations remaining after the roto-evaporation process. The lowest DOC concentration used for experimentation was distilled water that contained only DOC present in the sediment porewater of added sediments.

Specific Rates of Mercury Methylation

Specific rates of mercury methylation were determined using a modification of the method of Furutani and Rudd (1980) which involves the addition of $^{203}HgCl_2$ to water or sediment samples and extracting any alkylated $^{203}Hg^{+}$ produced over a 24-hour period. 1.0 ug of Hg^{+2} [1.0 uCi as $^{203}HgCl_2$, New England Nuclear Corp.] was added to 125 mL glass bottles containing 15 mL of sediment mixed with 70 mL of water at one of three DOC concentrations. The pH of all DOC dilutions were adjusted to 6.0 +/- 0.1 with dilute HCl or NaOH prior to the addition of sediments. The bottles were tightly capped and vigorously shaken to distribute the Hg^{2+}. Duplicate samples plus one acid-killed (using 2 mL 4N HCl) control were incubated for 24 hours at 22^{o} C +/- 2^{o} C. After incubation, the samples were shaken for 15 seconds to disperse $CH_3{}^{203}Hg^{+}$ produced in the sediments, then each bottle was centrifuged @1800 g (3200rpm) for 15 minutes. A known amount of supernatant (most of it) was transferred to a 125 mL separatory funnel through a 54 um mesh[4] in a small glass funnel. Methylmercury could then be extracted from the water and sediments independently. The supernatant in the separatory funnels and sediments in the bottles were killed with 2.0 mL of 4N HCl. Fifty mL of distilled water was added to the sediment samples before any further reagents were

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[4] Captured any large particles that may have dislodged from the "pellet".

introduced. The remaining extraction steps are described in Furutani and Rudd (1980).

It was not possible to separate water from sediments for demethylation experiments because the method of Ramlal et al (1986) involves quantifying the yield of gaseous byproducts of the microbial degradation of $^{14}CH_3Hg^+$. Therefore, the demethylation procedure was excluded.

Results of the assays were reported as the percentage of total activity of the isotope added which was methylated. Significant differences ($P=<0.05$) between treatments were evaluated by analyses of variance (ANOVA).

Partitioning (K_d) Experiments

The DOC-dependent distribution of methylmercury was determined by a modification of the suspension technique described by Nyffeler et al (1984). Both DOC and methyl mercury concentrations were used at levels found naturally in Precambrian Shield lakes in Ontario (ELA region, Dept. Fisheries and Oceans, M. Stainton pers. comm.). A gradient of DOC concentrations was obtained by dilution of the concentrate. The pH of each dilution was adjusted to an equal value (6.2) before sediment addition. 0.002 ug methylmercury ($^{14}CH_3HgI$; 9.2 X 10^{-6} umoles Hg) was added to 10 mL of each DOC dilution in duplicate 125 mL flasks. The solution was then equilibrated by rotating at 160 rpm in a mechanical shaker for 1.5 hours at 28^{o}C. From a suspension

of sediment particles, 500 ul of water containing 15 mg of sediments was added to the equilibrated radioactive water samples. The sediment-water mixture was returned to the shaker (160 rpm) which functioned to keep the sediment in suspension. At four separate time intervals ranging from 2 hours to 7 days, two flasks of each DOC concentration were removed and the contents filtered through a Nucleopore filter of 0.4 um pore size and 25 mm diameter. Filters were dissolved in scintillation vials with 1.0 mL ethyl acetate and counted after the addition of 14 mL scintillation fluor (ACS, Amersham). Filtered water was subsampled, diluted with fluor and counted.

The filtering efficiency was checked by using two stacked filters on each of the first set of samples. This revealed that activity on the second of the two filters was undetectable, therefore, for subsequent samples only one filter was used for each. pH was measured after three days of equilibration on a complete set of samples that did not contain $^{14}CH_3Hg^+$.

The partition coefficient K_d was calculated using the equation

$K_d = Cs / Cw$ where

Cs = $^{14}CH_3Hg^+$ concentration in sediments (ng kg^{-1} dry sediments)

Cw = $^{14}CH_3Hg^+$ concentration in water (ng kg^{-1}).

Dissolved Organic Carbon Measurement

This measurement was made on a set of samples from each experiment that did not contain $^{14}CH_3Hg^+$ but were otherwise identical to the experimental treatments. Analyses for DOC as described by Stainton et al (1977) were done on filtered (Whatman GF/C, Fisher) sample water after 24 hours of incubation (for methylation experiments) or after 3 days' rotation (for partitioning experiment).

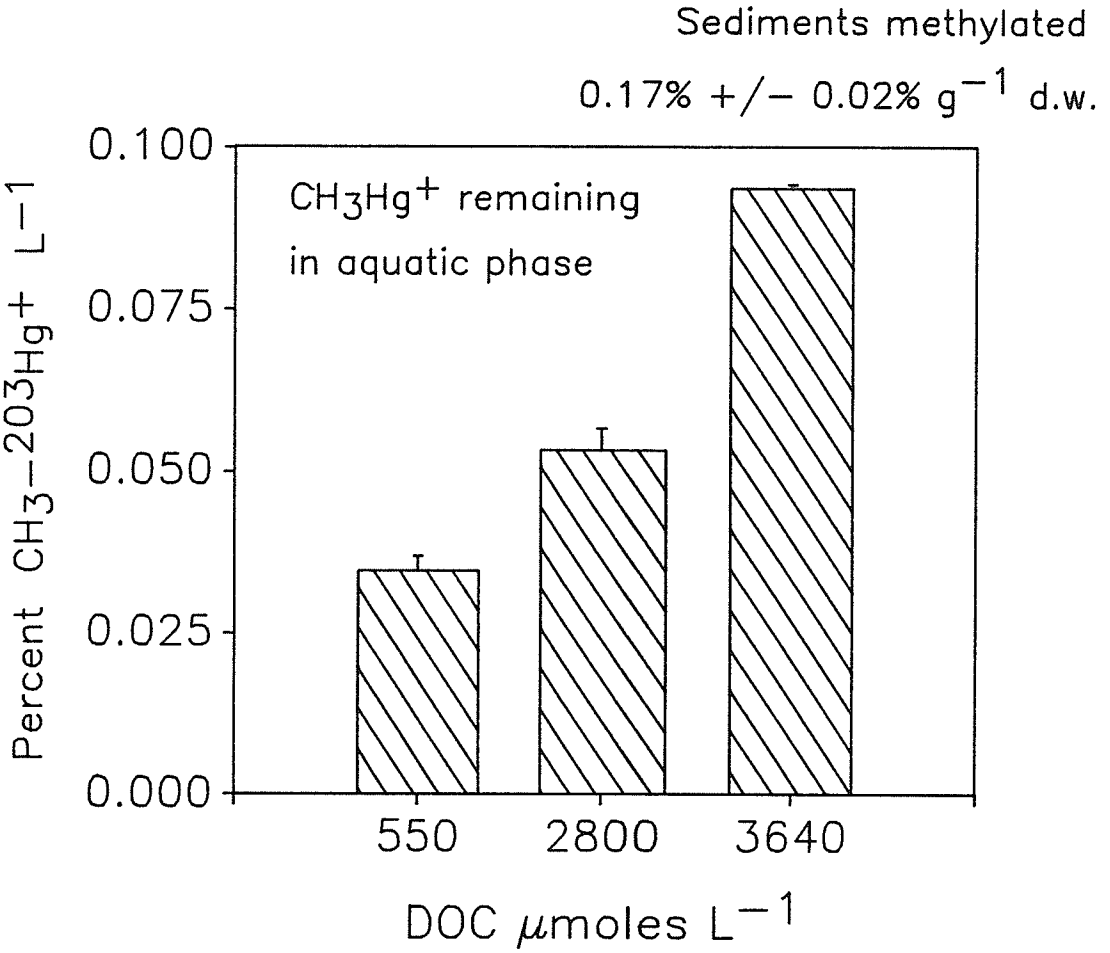
RESULTS

Specific Mercury Methylation

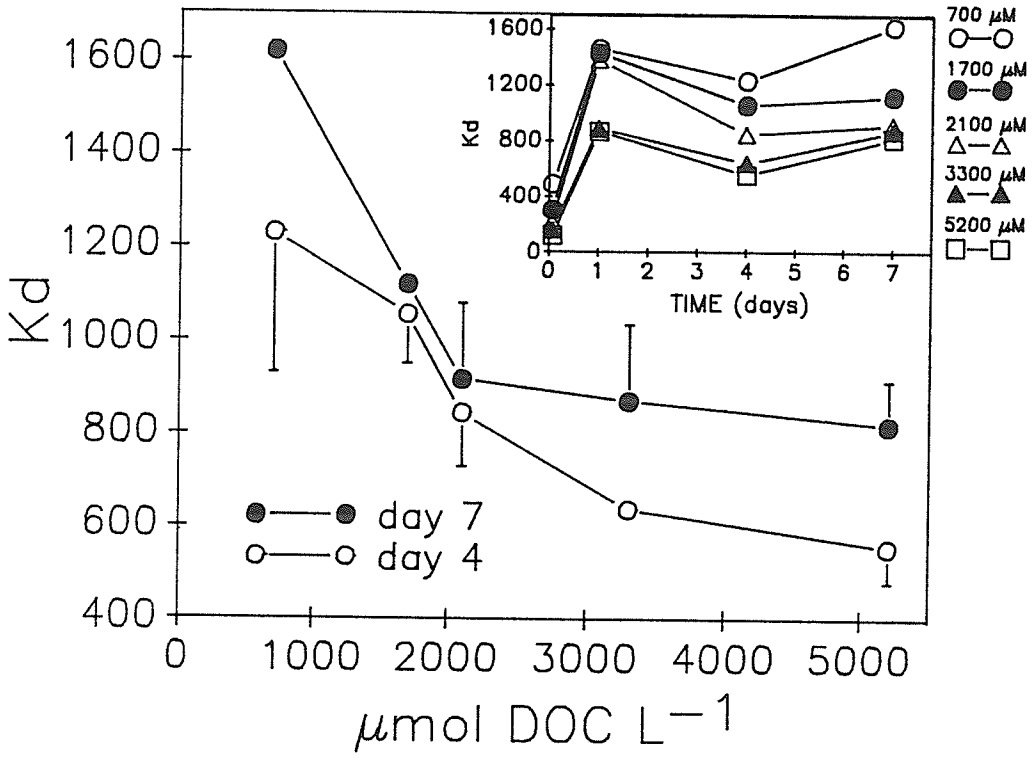
Similar amounts of $CH_3{}^{203}Hg^+$ was produced by the 15 mL of sediments in each of the three DOC concentrations (0.17% +/- 0.02% methylated g^{-1} d.w. d^{-1}). However, for each increasing DOC concentration, the 70 mL of overlying water contained significantly more $CH_3{}^{203}Hg^+$ (Figure 1). That is, at higher DOC concentrations, the methylmercury produced was less likely to return to the disturbed (shaken for 15 seconds) sediments.

Partitioning (K_d) Experiments

Solubility of $^{14}CH_3Hg^+$ increased with increasing DOC concentration at days 4 and 7 (Figure 2). K_d decreased most notably from the lowest DOC concentration (700 uM) to the mid-range concentration (2100 uM) which are well within the natural levels of DOC found in Precambrian Shield lakes and



Ch. II, Figure 1 Percent [^{203}Hg] methylmercury found in water overlying sediments. Sediment-water mixture was incubated for 24 hours at room temperature. Values are percent of $^{203}HgCl_2$ added to sediment-water mixture which was methylated by the mixture and extracted from the water. Standard error bars shown. NOVEMBER 24/88.



Ch. II,
Figure 2 Partition coefficients (Kd) for $^{14}CH_3Hg^+$ at days 4 and 7 in sediment-water solutions with increasing DOC concentration. Standard error bars shown.

Inset depicts Kd over 7 days at five DOC concentrations. DECEMBER 5/88.

their watersheds which may be 8 mM or higher in the ELA region (M. Stainton pers comm).

Over time with a range of five DOC concentrations tested, $^{14}CH_3Hg^+$ was more likely to be bound by sediments when water concentrations of DOC were lower (Figure 2 inset). The most dramatic increase in K_d for all DOC concentrations occurred between 2 and 24 hours following sediment introduction, after which the $^{14}CH_3Hg^+$ distribution at each concentration effectively stabilized. It should be noted that about 80% of the methylmercury added was lost either due to volatilization, adsorption to the flasks or demethylation and that this loss occurred more slowly with increasing DOC concentration (data not shown). These losses occurred within the first 24 hours. The volatilization losses were likely due to the sample pH, small sample volumes and exposure to air caused by the mechanical rotation. The pH of the samples was 6.5 +/- 0.2 after 3 days of equilibration.

DISCUSSION

Dissolved organic carbon may be an important factor regulating the distribution of methylmercury between sediments and water. Since this was true whether the methylmercury was produced from $^{203}HgCl_2$ (Figure 1) or added as $^{14}CH_3Hg^+$ (Figure 2), it would appear that DOC-"solubilization" may occur in situations either where methylation processes are important or where methylmercury is

introduced directly to aquatic systems from outside sources. Uptake by aquatic organisms of other organic contaminants (PAHs, McCarthy and Jiminez 1985; PCBs, Landrum et al 1987; a dioxin, Servos and Muir 1989) and inorganic mercury (Oh et al 1986) is reduced in the presence of DOC. The extent to which DOC-bound methylmercury is available for bioaccumulation is unknown.

For years, it was speculated that one of the reasons for high methylmercury concentrations in fish in brown-water lakes was mercury transport to lakes from terrestrial environments via DOC or humic substances (Simola and Lodenius 1982; Mannio et al 1986; Surma-Aho et al 1986; Lodenius et al 1987). My study did not involve the use of terrestrial soils, but for the sediments used, DOC enhanced the solubility of methylmercury. The findings of this study support the possibility that methylmercury produced in sediments is more likely to be transported from sediments if associated water has a high DOC concentration. Only more detailed studies using a variety of sediments and soils, and measuring in situ concentrations will strengthen the current knowledge of methylmercury movement in aquatic systems.

An examination of the K_d values indicates that methylmercury is very soluble by comparison with other organic contaminants (DDT, PCB, lindane; Chiou et al 1986) and metals (^{59}Fe, ^{65}Zn, ^{60}Co, ^{75}Se; Hesslein 1987). While it is acknowledged that the organic composition of the

sediments used in partitioning experiments can make large differences in K_d values (Chiou 1981), my results are within the range found by Akagi et al (1979), who studied methylmercury with a variety of sediments and reported K_d values of 170 for sand, 760 for silt/woodchips and 4200 for woodchips. The fact that methylmercury is water soluble as well as so readily bioaccumulated makes understanding any factor which increases its solubility very important.

The tendency of $^{14}CH_3Hg^+$ to be more readily bound to sediments at the lower DOC concentrations (Figure 2), suggests that water methylmercury concentrations may be expected to be higher in brown-water lakes than in clear-water lakes. This is an unconfirmed hypothesis because it was only recently that methods to detect natural levels of methylmercury in water were developed (Lee 1987; Bloom 1989). Corroboration with in situ measurements would further support the previously mentioned reports of high fish methylmercury in high-DOC lakes.

Low DOC Precambrian Shield lakes usually have long water residence times and may be anthropogenically acidified, whereas high-DOC lakes are relatively fast-flushing and are often of naturally low pH (due to organic acids; Oliver et al 1983; Brakke et al 1987). These characteristics are important because it has been suggested that in short water residence time lakes, terrestrial inputs of mercury may be most important, but in slower flushing lakes, in-lake

methylmercury production likely predominates (Winfrey and Rudd, in press). The specific methylation study reported in Chapter I, together with this study support these propositions. I would hypothesize that the surrounding watershed may be very important in introducing methylmercury to fast-flushing high-DOC lakes, and once in the water column, DOC-bound methylmercury may be resistant to entering lake sediments.

CHAPTER III

Additional Studies at the Experimental Lakes Area

1) Methylation in water with increasing Hg concentration,
2) Cores: Shallow vs Deep sediments, and
3) Sediment trap, Water and Sediment Studies in Lakes with Low and High DOC Concentrations

INTRODUCTION

This chapter further explores the influence of DOC and microbial respiration on mercury methylation and demethylation in both sediments and water. The difference between these and previous experiments is that these were done on lakes of naturally differing DOC concentration rather than by using a DOC concentrate to create a range of values. Use of the concentrate allowed for the control and consistency of important variables. This study tests hypotheses concerning DOC concentration and respiration effects in situations as close as possible to in situ, using lake waters and sediments in their natural state. The disadvantage is that many variables can not be controlled. However, relevant factors were measured and will be discussed. Also, most of the lakes in the Experimental Lakes Area are similar in that all are dilute softwater lakes in granite basins, and have similar productivities. Thus, the comparisons were assumed to be valid based upon measuring pH and DOC which have been cited as the most useful factors in predicting fish mercury concentrations in Precambrian Shield lakes (refer to Chapters I and II).

In addition to examining natural variability of DOC and respiration levels, the effect of increasing Hg^{2+} concentration on water column methylation and differences in respiration and methylmercury production in shallow and deep sediments of two lakes is examined.

MATERIALS AND METHODS

Methylation in Water with Increasing Hg^{2+} Concentration

Water was collected from the epilimnion of Lake 302S (pH 4.5) for use in methylation experiments. Duplicate 100 mL water samples plus one acid-killed control, in stoppered glass bottles, were used for each Hg concentration. Five concentrations of inorganic mercury were created using equal volumes of $^{203}HgCl_2$ (1.06 uCi/1.01 ug Hg/100 uL) and increasing volumes of "cold" $HgCl_2$ (1100 ug mL^{-1}). Thus, the radioisotopic activity of each was the same, with cold $HgCl_2$ varied to achieve experimental Hg concentrations[5] (ug L^{-1}) of 5, 10, 32, 54 and 120.

All samples were incubated at 26°C for 24 hours, and terminated with 1 mL of 4N HCl. Four grams of DOWEX (Dow Chemical Co)[6] were added to each to scavenge inorganic Hg prior to CH_3Hg^+ extraction. Methylmercury ($CH_3{}^{203}Hg$) produced was extracted using the method of Furutani and Rudd (1980). Total nanograms L^{-1} of mercury methylated was calculated using proportions as follows:

$$\frac{\text{Total Hg added (ng)}}{\text{DPM added}} = \frac{x \quad \text{ng Hg (as } CH_3{}^{203}Hg)}{\text{DPM in MeHg fraction}}$$

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5 10 ug L^{-1} was concentration used in most of previous experiments.

6 DOWEX, is an anion exchange resin, 50-100 mesh, Cl^- form, Bio-Rad Laboratories.

For example, for 54 ug L^{-1}, the 100 mL samples contain 5400 ng, and if the sample count was 102.5 DPM,

$$\frac{5400 \times (102.5 \text{ dpm} - 59 \text{ bkgd dpm})}{2220000 \text{ DPM}} = 0.106 \text{ ng/100 mL} = 1.06 \text{ ng } L^{-1} \text{ methylated.}$$

Cores: Shallow vs Deep sediments

Three sediment cores were taken from two sites in each of Lake 239 and Lake 305. In L239 the samples were taken from 4 m depth in East Bay and 10 m depth in the lake, and in L305 from 3 m (sandy) and 17 m depths. The cores (5 cm dia X 15-20 cm ht) were taken from an Ekman dredge which was gently lowered to the sediments keeping the sediment-water interface as undisturbed as possible. About 5-7 cm of water above 7-10 cm of sediments was sampled in the cores. The stoppered cores were returned to the lab in a bucket partly filled with in situ temperature water. The top rubber stoppers (#11) were removed, the cores were topped up with lake water and the bucket was placed in an incubator (13°C) on a mechanical rotator[7] to equilibrate for 4 - 6 hours.

Respiration (CO_2 production, O_2 consumption) measurements were made by sampling the water column of the cores before and after 12 hours (L239) or 16 hours (L305) of incubation at 13°C. Before sealing, each core was gently stirred and 10 mL and 1 mL glass syringe samples were removed from each. The top stoppers were again used to seal the

[7] To simulate turbulent water movement.

cores, carefully ensuring that no air bubbles remained and the cores were returned to the rotator for the incubation period. The method of measuring total CO_2 or DIC (0.5 mL samples) and O_2 (10 mL "mini Winklers") is described in Chapter I of this thesis. After the incubation period, the core stoppers were removed, and final DIC and O_2 were sampled and measured in the same manner. There were three cores per site for respiration.

The surface 2-3 cm of sediments in the cores were then sectioned and used for specific methylation and demethylation experiments. To section the cores, the bottom stopper (#10) was loosened by untightening the wing nut (mounted on a bolt inserted through the stopper, which causes the rubber to expand when tight). The stopper was then pushed slowly up through the core using a wooden rod. A spare piece of core tubing (6 - 8 cm ht) was held over the core to contain sediments which are forced through, and a thin aluminum blade (10 X 10 cm) was inserted between the two core tubes. The surface sediments from each site were placed together in clean glass beakers, making a batch per site. Each batch was stirred for about thirty seconds to ensure that the sediments were uniformly mixed to produce replicate samples for the methylation and demethylation experiments.

Twenty mL of sediments from each of the shallow and deep sites were added to duplicate 30 mL glass centrifuge tubes for methylation experiments and to 35 mL duplicate stoppered

glass bottles for demethylation experiments. A killed control sample for each was also used. To measure specific methylation, 2.0 uCi $^{203}HgCl_2$ (2.5 ug Hg/100 uL) was added to each centrifuge tube. For specific demethylation, 0.02 uCi $^{14}CH_3HgI$ (0.2 ug Hg/100 uL) was added to each bottle. The centrifuge tubes and bottles were stoppered and shaken vigorously to mix the radioisotopes throughout the samples.

The methylation and demethylation samples were incubated at 13°C (+/- 1°C) for 24 hours and terminated with 1 mL of 4N HCl. The methods of Furutani and Rudd (1980) and Ramlal et al (1986) were used for methylmercury extraction and demethylation, respectively. Some variation on the methylation procedure was made in that 1 mL $CuSO_4$, 5 mL NaBr and 14 mL toluene[8] were used because of the small volume of sediment sample. Also, after the addition of HCl, $CuSO_4$ and NaBr reagents, the samples were centrifuged at 5900 g (7000 rpm) for 20 minutes to allow for easy separation of about 17 mL of the supernatant.

Sediment trap (ST), Water and Sediment Studies in Lakes with Low and High DOC Concentrations

This study was designed to determine whether the relationships which were found in laboratory manipulations of

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8 The original method intended for larger sediment volumes requires 2 mL $CuSO_4$, 10 mL NaBr and 20 mL toluene.

DOC would also be found in lakes of different DOC concentrations. Often DOC was not the only variable.

(i) With and Without Sediment Trap material - L224 and L225

Epilimnetic water was collected in 2L Nalgene bottles from Lakes 224 (270 uM DOC) and 225 (870 uM DOC) and fresh sediment trap (ST) material was collected from Lake 240 as described in Chapter I. The pH of each water sample was measured with an electronic pH meter.

Methylation and demethylation experiments were performed on duplicate water samples with and without ST material. The samples for specific methylaton contained either 150 mL of lake water or 140 mL of lake water plus 10 mL (~5 mg dry weight) ST material. 1.14 uCi of $^{203}HgCl_2$ (1.42 ug Hg/100 uL) was added to each sample. Specific demethylation samples were 100 mL total volume with the ST additions made in the same proportions as in the methylation samples. 0.01 uCi $^{14}CH_3HgI$ (0.2 ug Hg/100 uL) was added to each and the controls were killed with 1 mL of 4N HCl. All samples were incubated for 24 hours at 19^{o}C. The remaining steps in the methylation and demethylation procedures are described by Furutani and Rudd (1980) and Ramlal et al (1986), respectively.

Respiration measurements were made only on samples to which ST material had been added because unamended water samples at this time of year showed undetectable respiration in these lakes (later in the summer they were detectable). A

total of 66 mL of unlabelled (ie. no radioisotope) sample in large glass syringes contained proportionately the same amount of ST material as the above-described samples. After the initial O_2 and CO_2 samples were taken, each syringe was brought to a volume of 50 mL and sealed[9]. The glass syringes containing the samples were incubated for 24 hours at 19°C.

(ii) Water from 4 lakes: L304, L240, L225, L224

Lakes 304 and 225 are small headwater lakes with relatively high DOC concentrations (780 and 810 uM respectively, at the time). Lakes 240 and 224 are low-DOC (480 and 260 uM respectively, at the time), slightly larger lakes which receive inflow waters from L304 and L225, respectively.

Two litres of epilimnetic water were collected from each lake as described previously. The samples were returned to the laboratory and equilibrated to a uniform temperature of 25°C. The pH of each was measured with an electronic pH meter. Triplicate (plus one control) 100 mL water samples from each lake were used for methylation and demethylation experiments. 1.06 uCi of $^{203}HgCl_2$ (1.01 ug Hg/100 uL) was added to each methylation sample and 0.009 uCi $^{14}CH_3HgI$ (0.2 ug Hg/100 uL) was added to each demethylation sample. The controls were killed with 1 mL of 4N HCl and the samples were

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[9] Method described in full in Chapter I.

incubated for 24 hours at 25°C. Duplicate samples were used for respiration measurements[10] which were incubated for the same time period and at the same temperature as methylation and demethylation experiments.

(iii) Surface sediments of cores - 4 lakes.

Three sediment cores were taken from each of the same four lakes as for the water column experiments described above. An Ekman dredge was used at the deepest part of each of Lakes 304, 240, 225 and 224. All other procedures for core collection, respiration, slicing for methylation and demethylation experiments are exactly as described in the section entitled "Cores: Shallow vs Deep Sediments". For methylation, 0.78 uCi $^{203}HgCl_2$ (1.01 ug Hg/100 uL) was added to each sample, and for demethylation, 0.022 uCi $^{14}CH_3HgI$ (0.2 ug/100 uL) was used. Samples for respiration measurements (O_2 consumption only[11]) were incubated for 15 hours; methylation and demethylation samples were incubated for 24 hours. All samples were incubated at the in situ temperature of 10°C. The pH of the sediments used was measured with an electronic pH meter.

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[10] Methods for methylation, demethylation and respiration are as described previous experiment.

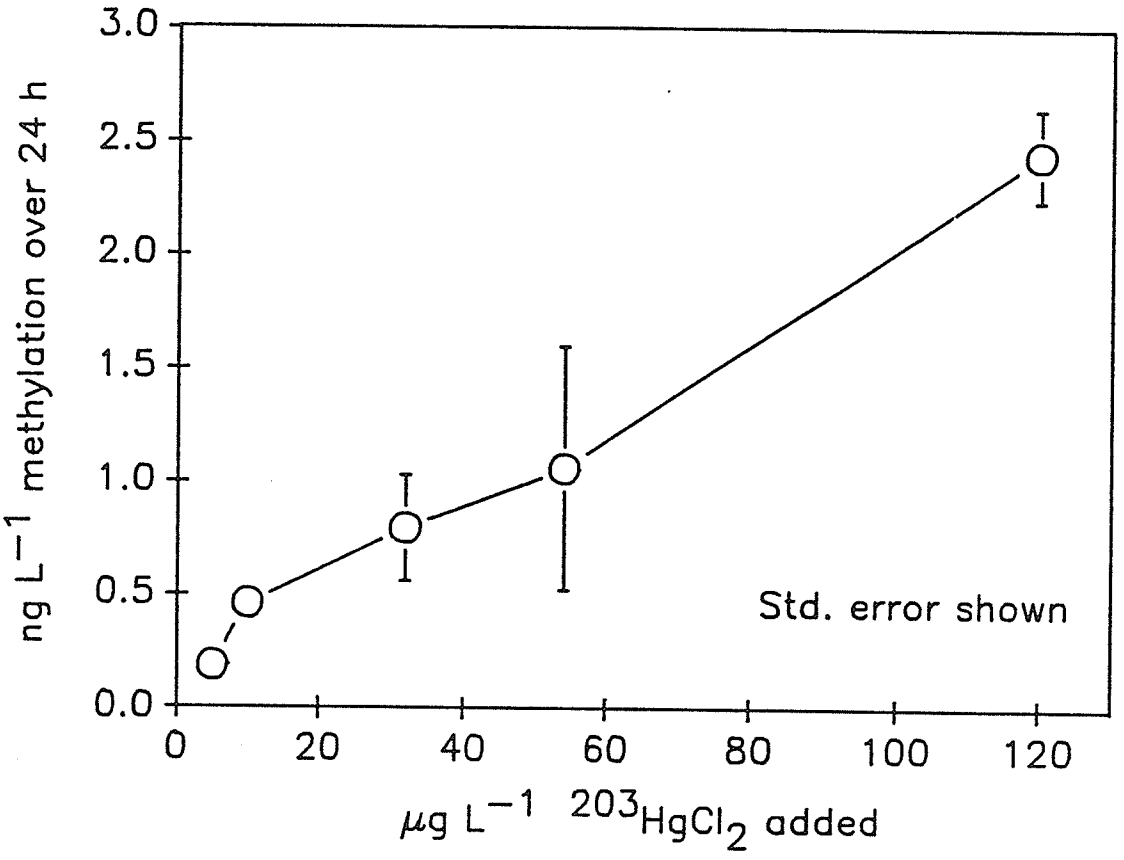
[11] I.R. spectrophotometer for CO_2 analyses was unavailable.

RESULTS AND DISCUSSION

Methylation in Water with Increasing Mercury Concentration

Specific mercury methylation in Lake 302S water increased (close to linearly) with increasing mercuric chloride concentration (Figure 1). Apparently, even the highest mercury concentration was not toxic to the methylating bacteria. It was assumed that the non-radioactive mercury was methylated proportionally to the radioactive mercury added. This should be true because both radioactive and non-radioactive Hg were added in the same chemical form ($HgCl_2$) at the same time.

The second of these assumptions (that bioavailable mercury increased with increasing concentration) was considered by Rudd et al (1983) as an explanation for their direct relationship of mercury methylation to mercury concentrations in sediments (0.04 - 10 ug Hg g^{-1}). Xun et al (1987) felt that the proportion of bioavailable mercury increased in water at higher Hg concentrations due to saturation of available binding sites. In fact, a greater than linear increase in L302S water column methylation with increasing Hg^{2+} concentration was found by Xun et al (1987). This may be expected if the natural Hg pool was significant in relation to their Hg additions. Their lowest Hg concentration was 3.5 ug L^{-1}, whereas mine was 5.0 ug L^{-1}.



Ch. III, Figure 1 Rate of methylation in L302S epilimnion water with increasing $^{203}Hg^{+}$ concentration. Incubation of duplicate samples at 26°C, 24 hours. SEPT 20/88.

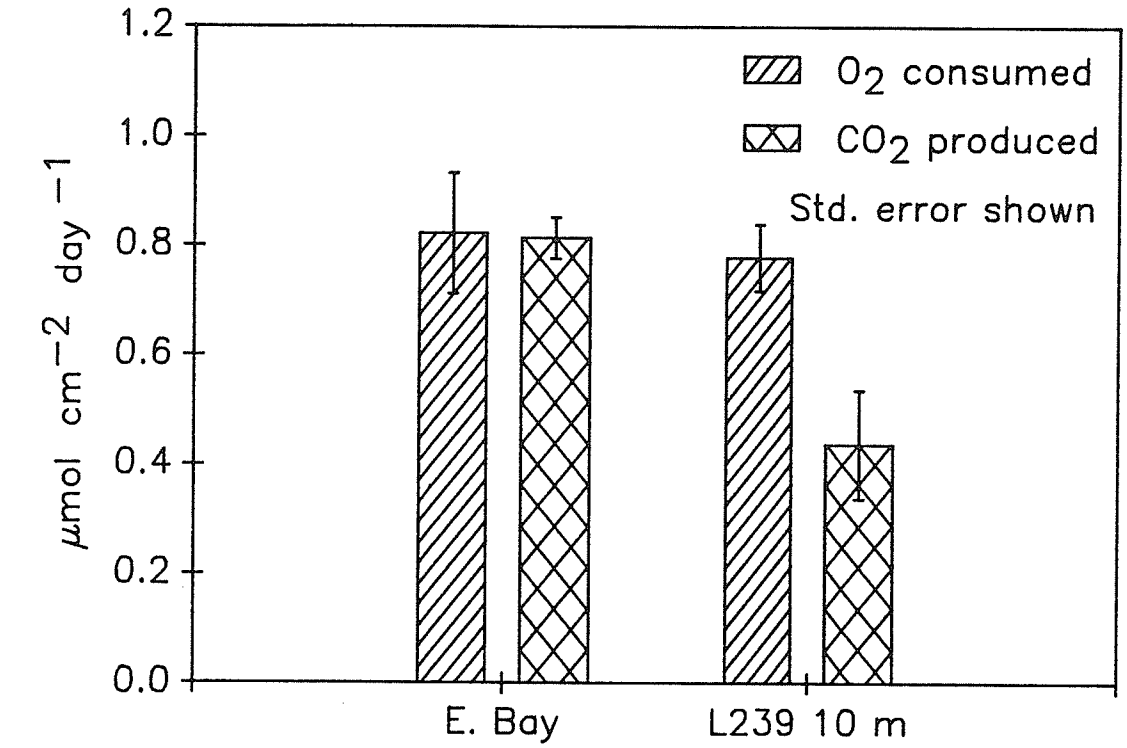
It is highly unlikely that the lake water used had Hg concentrations as high as these, therefore, differences between our results cannot be explained this way. Also, even though their maximum Hg^{2+} concentration was only half of the maximum used in my experiment, they found much higher rates of methylation (eg. 30 ng L^{-1} day^{-1} for 13 ug Hg^{2+} L^{-1} added) at each given concentration. These higher rates may perhaps be explained by the shorter incubation time used by Xun (12 h: 1 day=2X this rate; if there is a slowdown later in the incubation), any differences in the lake water between 1984 and 1988, and/or slight binding of methylmercury by the DOWEX resin used to scavenge Hg^{2+} (may occur; data not shown).

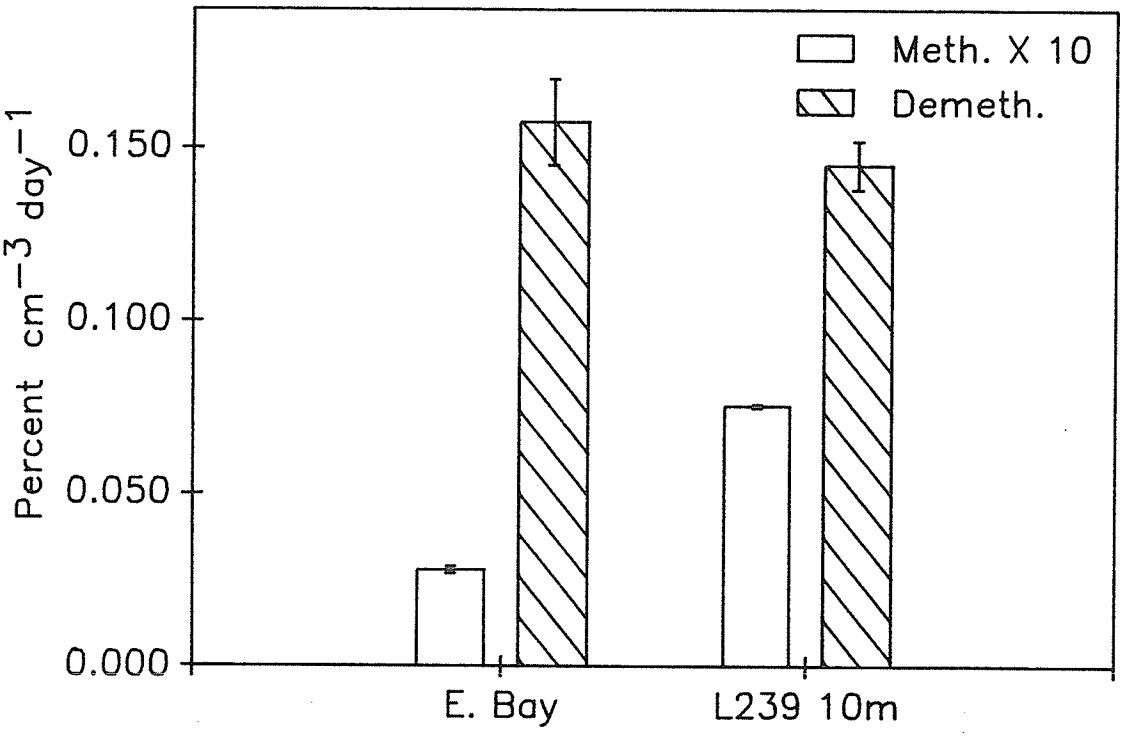
Cores: Shallow vs Deep sediments

In Lake 239, oxygen consumption rates were about the same in the shallow East Bay sediments as in the 10 m depth sediments of the main lake (Figure 2a). DIC production was almost twice as high in East Bay sediments as in the deeper sediments of L.239 (Figure 2a). The same relationship occurred with respiration in Lake 305 sediments, where O_2 consumption rates were not different but CO_2 production rates were higher in the shallow sediments (Figure 3a). This has been observed in other Shield lakes (C. Kelly and J. Rudd, pers. comm). The higher DIC production shows a higher community respiration rates from all other types of

Ch. III,
Figure 2a Respiration of surface sediments in cores taken from shallow (East Bay) and deep sites (10m of L239). Incubation of duplicate samples at 13°C for 12 hours. MAY 22/88.

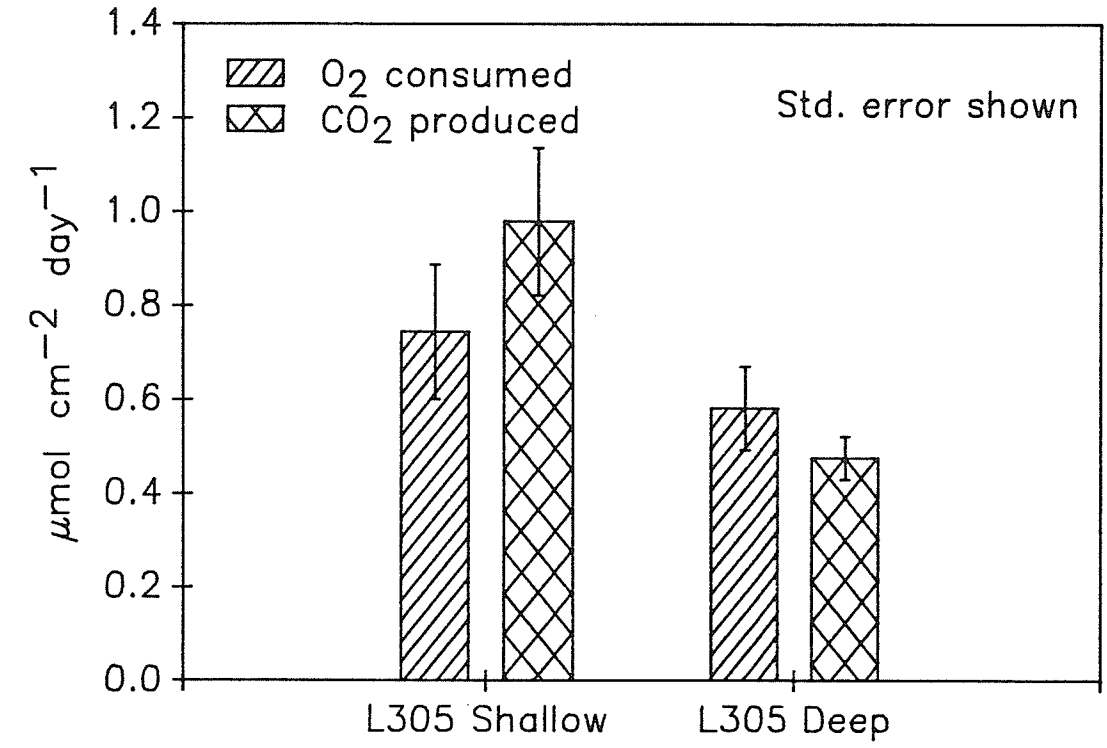
Figure 2b Specific rates of methylation and demethylation of surface sediments taken from cores of shallow (East Bay) and deep sites (10m of L239). Incubation of duplicate samples at 13°C for 24 hours. MAY 22/88.

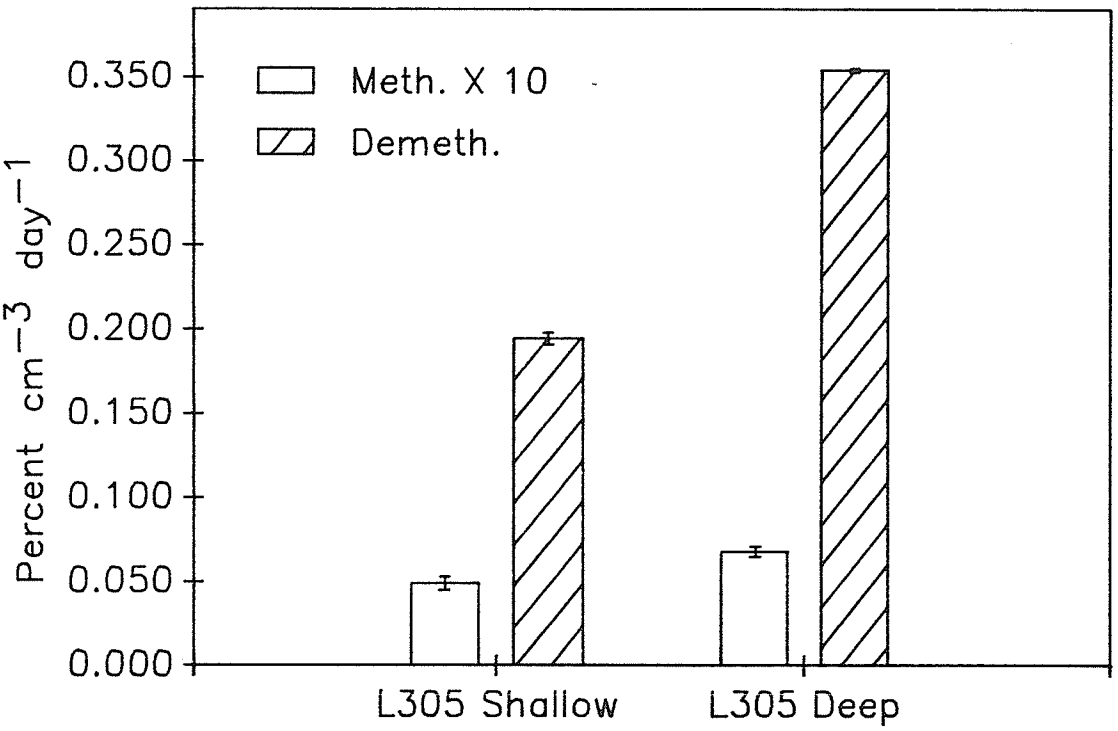

1.2
1.0
0.8
0.6
0.4
0.2
0.0
µmol cm^{-2} day^{-1}
O_2 consumed
CO_2 produced
Std. error shown
E. Bay
L239 10 m


0.150
0.100
0.050
0.000
Percent cm^{-3} day^{-1}
Meth. X 10
Demeth.
E. Bay
L239 10m

Ch. III,
Figure 3a Respiration of surface sediments in cores taken from a shallow (3m) and deep site (17m) of L305. Incubation of duplicate samples at 13°C for 16 hours. MAY 24/88.

Figure 3b Specific rates of methylation and demethylation of surface sediments taken from cores of a shallow (3m) and deep site (17m) of L305. Incubation of duplicate samples at 13°C for 24 hours. MAY 24/88.


1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
μmol cm^{-2} day^{-1}
O_2 consumed
CO_2 produced
Std. error shown
L305 Shallow
L305 Deep


0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Percent cm^{-3} day^{-1}
Meth. X 10
Demeth.
L305 Shallow
L305 Deep

respiration (including aerobic and anaerobic). O_2 uptake ismore ambiguous and could include some chemical uptake due to disruption of anaerobic sediments.

Methylation rates did not follow respiration, but rather were higher in the deeper sediments of both lakes, especially in L239 (Figures 2b, 3b). This study and the results reported in Chapter I indicate that respiration was not the only factor regulating CH_3Hg^+ production. Rudd and Turner (1983) concluded that stimulation of sediment microbial respiration was the primary factor in increasing CH_3Hg^+ in fish in enclosures. While this may be true for a single sediment, the variety of possible binding surfaces in different sediments may cause mercury availability to be a complicating factor in predicting methylation activity from respiration activity.

Demethylation, which was shown to follow water column respiration in Chapter I, did not always do so in these sediments. Demethylation rates were about the same (as was O_2 consumption, but not DIC production) in the shallow sediments and deeper sediments of Lake 239, but were about two times higher in the deep sediments (17 m) than the 3 m (sandy) sediments of Lake 305. (Figures 2b, 3b). Hecky et al (1987), in reservoir studies at Southern Indian Lake, concluded that stimulation of microbial respiration by flooding of organic material did not affect demethylation. Similarly, Ramlal et al (1987) did not see a trend in

demethylation in response to increased respiration. With the lack of agreement as to the effect of respiration on methylation and demethylation, more studies into this as well as the factors governing the "bioavailable" fraction of the various forms of mercury are required.

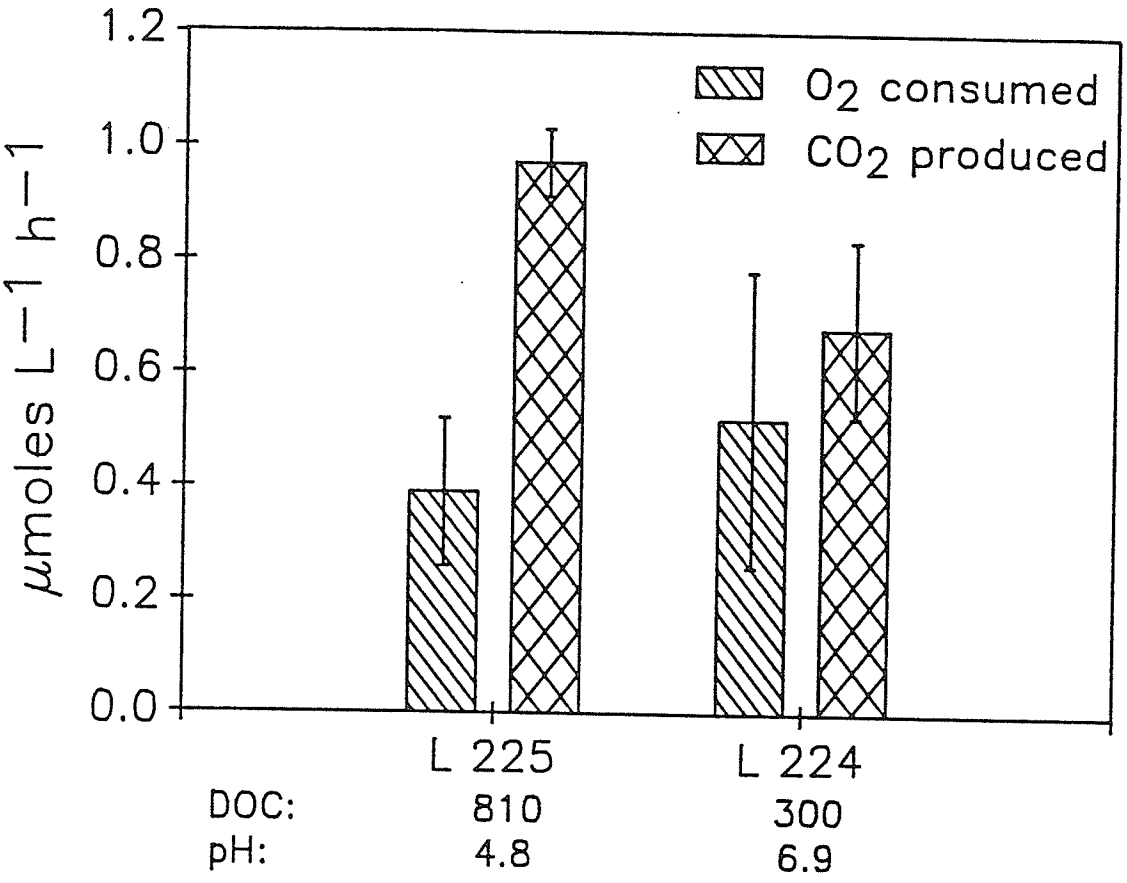
Sediment trap, Water and Sediment Studies in Lakes with Low and High DOC Concentrations

Relationships which were found in laboratory manipulations of DOC were not always found in lakes of different DOC concentrations. In the water column experiments, there was sometimes an important difference between the lakes: pH. The affect of pH on water column and surface sediment methylation was discussed in Chapter I.

(i) With and Without Sediment Trap material - L224 and L225

Respiration measurements in samples with ST material indicated no difference in O_2 consumption rates in samples from Lakes 225 and 224 (Figure 4). CO_2 production and DOC were higher and pH was much lower in L225 than in L224 (Figure 4).

Methylation and demethylation experiments were done in lake water samples with and without the addition of ST material. The use of ST material caused a suppression of methylation rates in both lake waters (Figure 5a). This occurred despite the increased respiration which results from the addition of ST material. Apparently, the reduced Hg availability due to binding of Hg^{2+} by the particles overcame



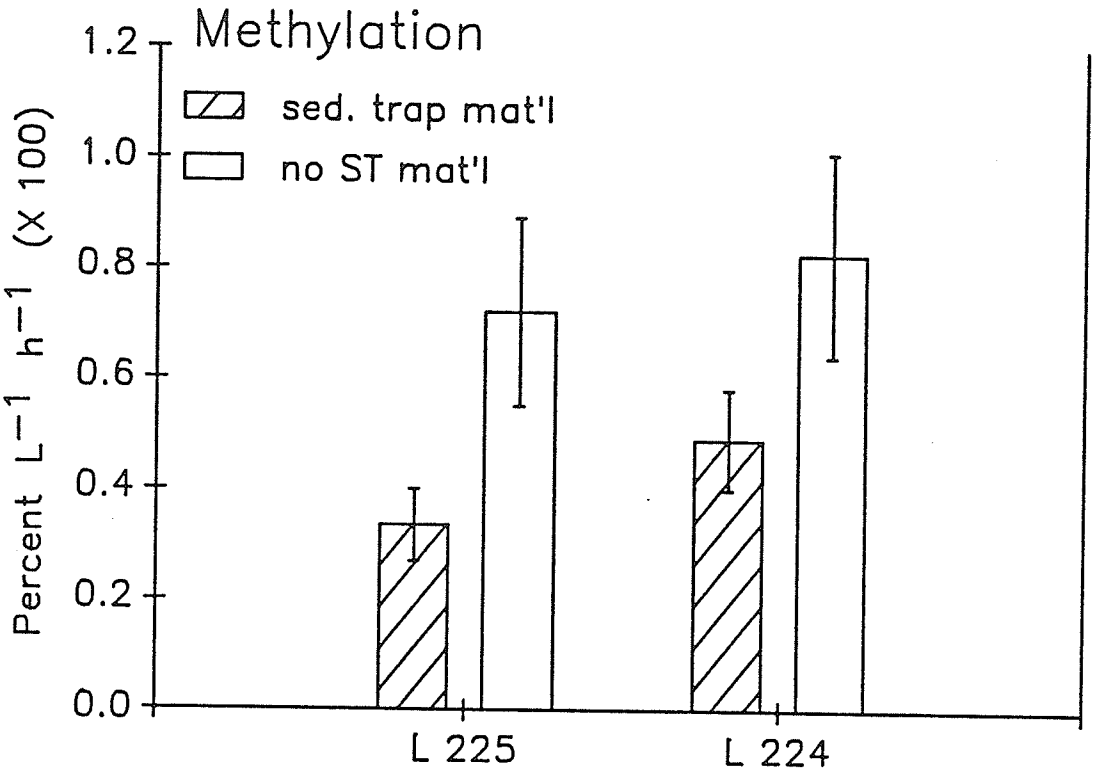
Ch. III,
Figure 4 Water column respiration using Lake 225 and Lake 224 epilimnetic water with 5 mg sediment trap material. Incubation of duplicate samples at 19°C for 24 hours. DOC concentrations and pH values given. JULY 19/88.

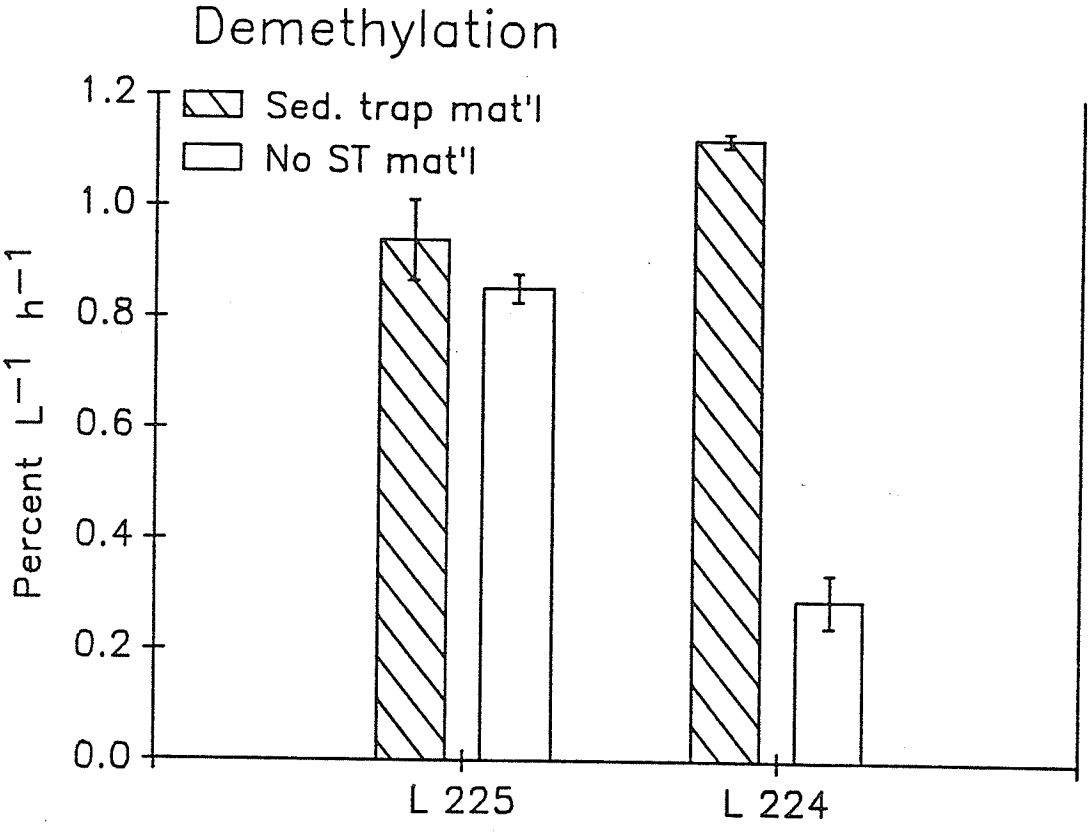
Ch. III,
Figure 5a Specific rates of methylation in Lake 225 and Lake 224 epilimnetic water with and without 5 mg sediment trap material. Incubation of duplicate samples at 19°C for 24 hours. JULY 19/88.

Figure 5b Specific rates of demethylation in Lake 225 and Lake 224 epilimnetic water with and without 5 mg sediment trap material. Incubation of duplicate samples at 19°C for 24 hours. JULY 19/88.

Note: L 225, pH 4.9, DOC 810.

L 224, pH 6.9, DOC 260.


Methylation
sed. trap mat'l
no ST mat'l
Percent L⁻¹ h⁻¹ (X 100)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
L 225
L 224


Demethylation
Sed. trap mat'l
No ST mat'l
Percent L⁻¹ h⁻¹
1.2
1.0
0.8
0.6
0.4
0.2
0.0
L 225
L 224

the effect of high respiration rates. This particulate organic carbon (POC) binding may be considered analagous to the DOC binding which reduced methylation in the experiments in Chapter I. Servos et al (1989) noted that any increase in POC or DOC concentrations should cause a shift in equilibrium away from "freely dissolved" hydrophobic contaminants, resulting in a lower concentration that is available to biota.

There was no difference in methylation rates between the lake waters when no ST material was added (Figure 5a). The pH of L225 was much lower but DOC concentrations were 3 to 4 times higher than L224. Since increased DOC may cause a reduction in methylation (Chapter I) and decreased pH causes increased methylation in water (Xun et al 1987; Chapter I), the effects from these two factors may have cancelled each other and resulted in equalizing methylation in Lakes 225 and 224. The lower methylation despite low pH in L225 (with ST material) than L224 (with ST material) is likely due to the combined effect of DOC and POC binding of Hg^{2+}. Sediment trap material caused an increase in demethylation rates in Lake 225 and notably, in Lake 224 where demethylation was about 4 times higher when ST material was included (Figure 5b). This is the same effect that has been seen in the water column whenever respiration is enhanced. Water column demethylation (no ST material) was about 3 times higher in L225 than in L224 in this experiment (Figure 5b). The

contribution of DOC to respiration and demethylation as discussed in Chapter I, and to higher bacterial biomass reported by Tranvik (1988), again appears to have increased demethylation in this case.

(ii) Water from 4 lakes: L304, L240, L225, L224

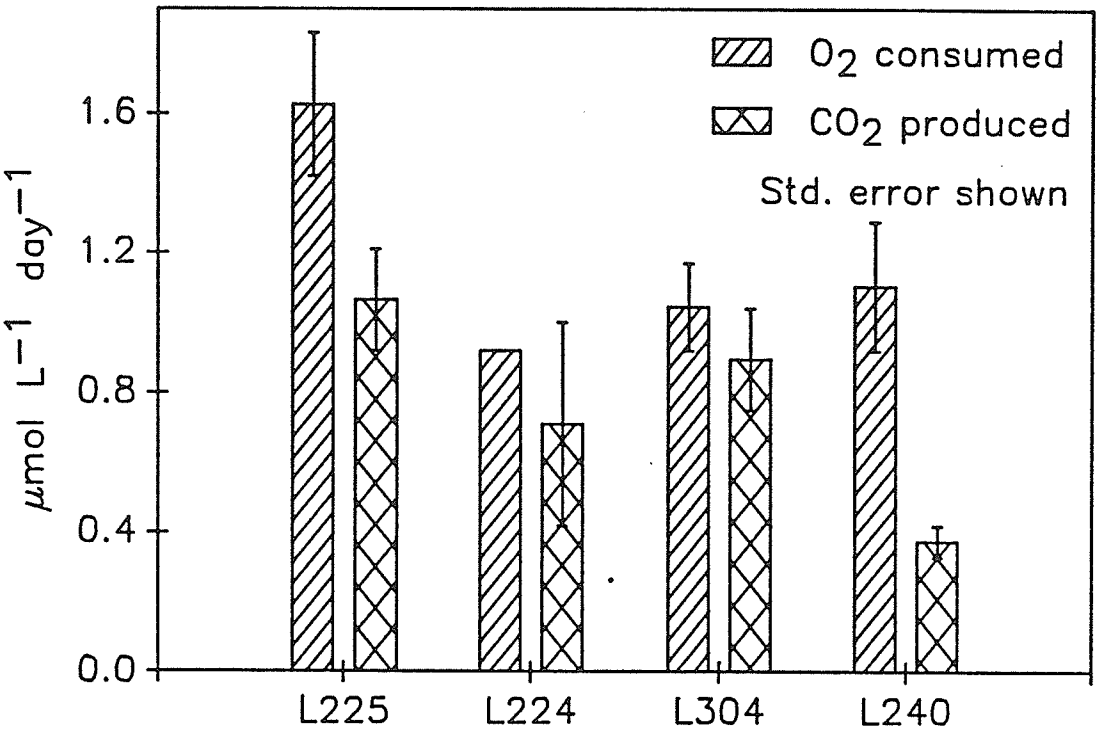
There was no detectable difference in respiration rates in the water from the four lakes except that O_2 consumption was higher in L225 than the others, and CO_2 production in L240 was about one-half of the rates of the others (Figure 6a). While respiratory quotients (RQ, CO_2:O_2) should theoretically be 1.0 and the "generally accepted average" for aerobic respiration is 0.85 (Wetzel 1983), values other than these sometimes occur (this thesis; C. Kelly pers. comm.). In this case, only L240 had an RQ which was much lower than the average.

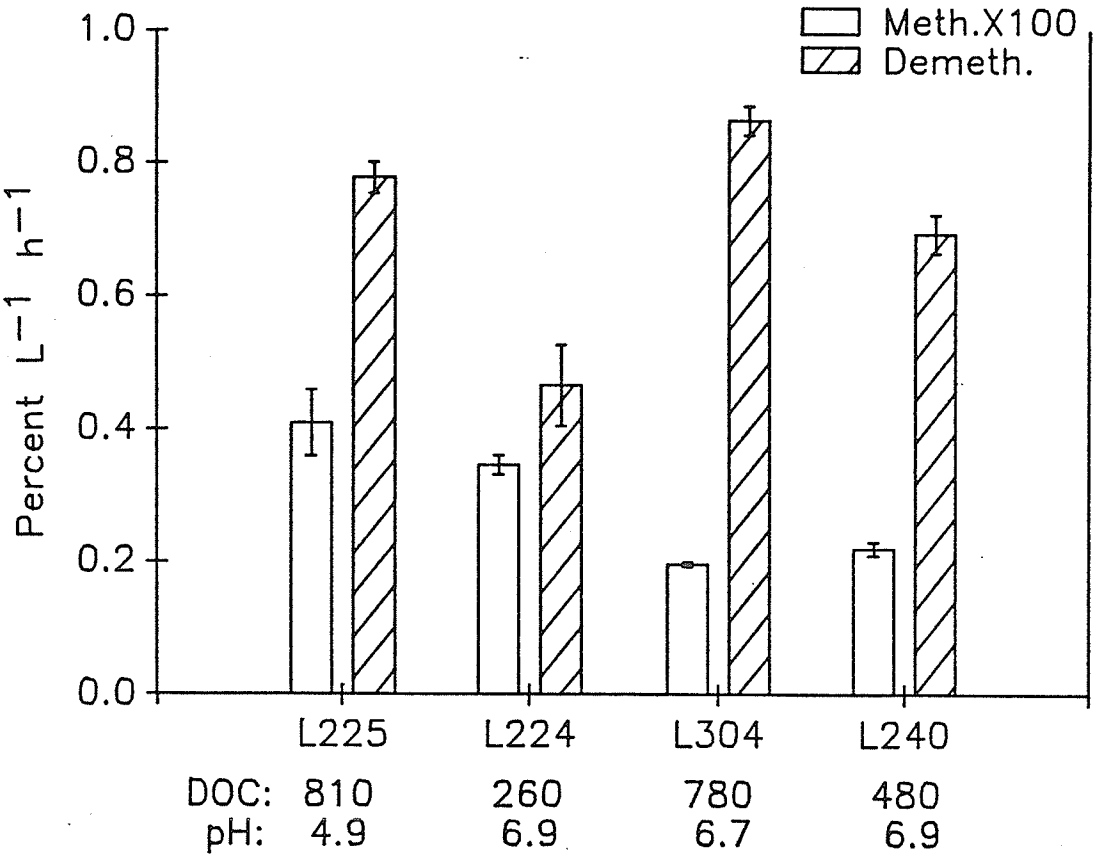
Specific rates of methylation were similar in Lakes 225 and 224 (as in previous experiment, Fig. 5a); the rates were also similar (but lower than L225 and L224) in Lakes 304 and 240 (Figure 6b). Demethylation rates did not follow the same order, but were highest in the lakes with the highest DOC concentrations (L225 and L304), intermediate in the lake with the mid-range DOC concentration (L240) and lowest in the lake with the lowest DOC concentration (L224; Figure 6b).

This study of water from four lakes follows a similar trend to the controlled experiments in Chapter I in which the only variables were DOC concentration and pH. The most

Ch. III,
Figure 6a Water column respiration in epilimnetic water of four lakes. Incubation of duplicate samples at 25°C for 24 hours. SEPT. 5/88.

Figure 6b Specific rates of methylation and demethylation of epilimnetic water of four lakes. Incubation of triplicate samples at 25°C for 24 hours. DOC concentrations and pH values given. SEPT 5/88.


O_2 consumed
CO_2 produced
Std. error shown
μmol L^{-1} day^{-1}
1.6
1.2
0.8
0.4
0.0
L225
L224
L304
L240


Meth.X100
Demeth.
Percent L^{-1} h^{-1}
1.0
0.8
0.6
0.4
0.2
0.0
L225
L224
L304
L240
DOC: 810 260 780 480
pH: 4.9 6.9 6.7 6.9

similar result to the DOC-concentrate experiments was that higher demethylation consistently occurred in the higher DOC lakes. Methylation was higher in the low DOC lakes, as in the previous experiments, with the exception of the pH 4.9 Lake 225 which exhibited relatively high methylation (Fig. 6b). The importance of pH in increasing methylation was demonstrated in Chapter I and appears to remain important when comparing these four natural lakes. This is a significant result because the acidification of the water used in Chapter I was from direct HCl addition and the acidification of L225 is due to long-term inputs of natural organic acids. The organisms in L225 should be well adapted to low pH. It appears that the methylating bacteria active in the Chapter I study required little or no acclimation to HCl pH adjustment. Increased methylation may also occur in naturally low pH water. However, these results indicate that the role of DOC binding in countering the pH effect should not be overlooked.

(iii) Surface sediments of cores - 4 lakes

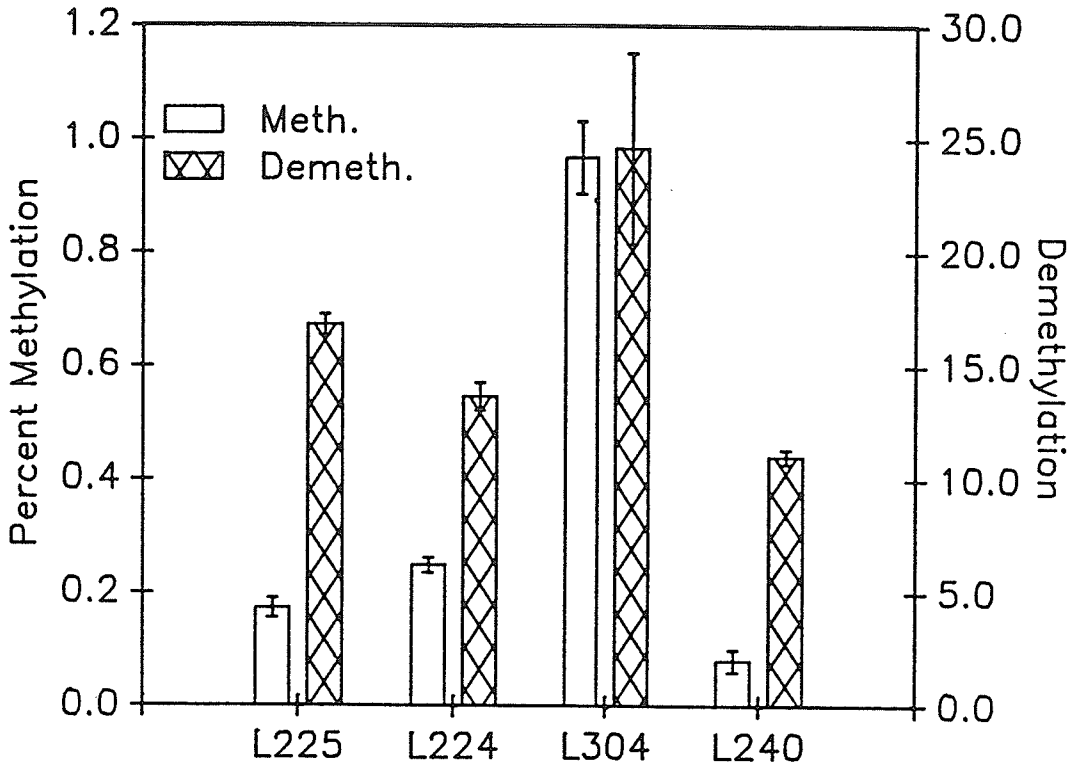
Even though the water column of the four lakes had very different DOC concentrations and pH's (L225), the sediments of these lakes could not be differentiated in this way. The sediment porewaters were all relatively high DOC (L224: 1680 uM, L225: 1960 uM; observation of colour in L304 and L240) and the pH of all sediments was 6.2 +/- 0.1. Variation in

the production of methylmercury was therefore best examined in relation to respiration.

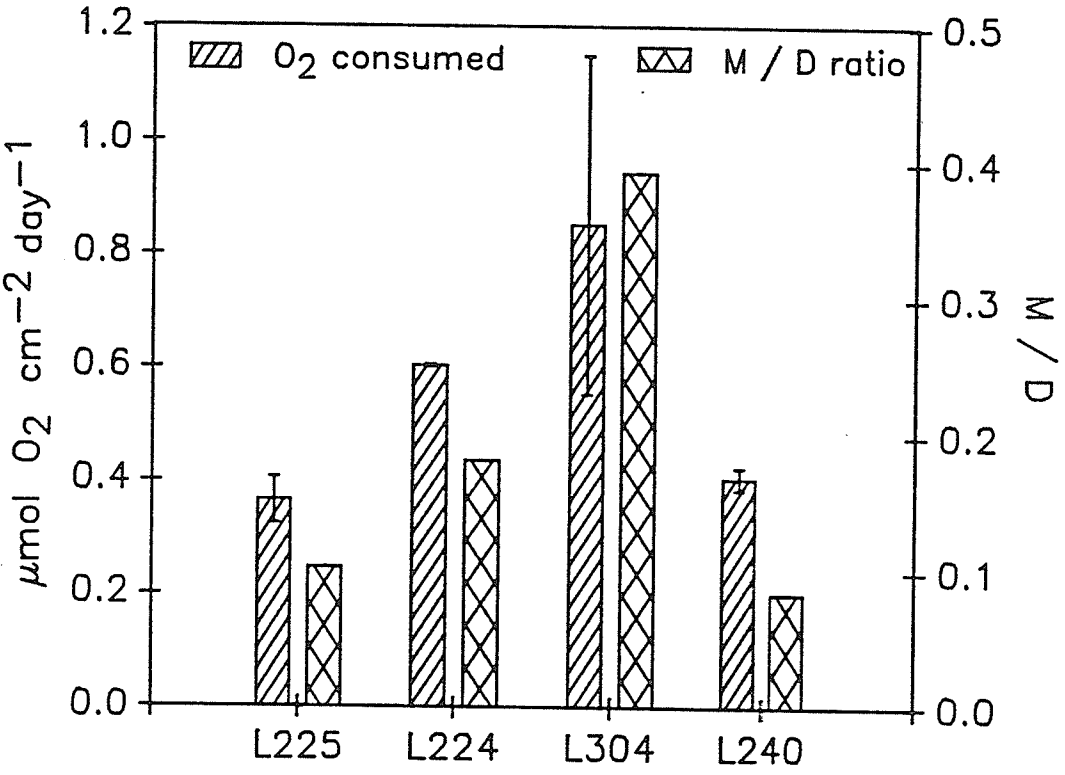
Rates of sediment methylation and demethylation were highest in Lake 304. Rates of sediment methylation followed the order 304 >> 224 > 225 > 240, and demethylation followed the order 304 >> 225 > 224 > 240 (Figure 7).

While demethylation did not follow respiration as it did in the water column experiments, it is notable that the M/D ratio (rate of meth/rate of demeth) increased as respiration (O_2 consumption) increased (Figure 8). The increase in methylmercury production with increased respiration rates is in agreement with the conclusions of others working on sediments (Furutani and Rudd 1980; Wright et al 1982; Hecky et al 1987). This was a relationship not found with water column experiments. A likely explanation is that high sediment microbial respiration and its variability overwhelms differences in $^{203}Hg^{2+}$ binding, whereas respiration in the water column varies less than the $^{203}Hg^{2+}$-DOC binding capacity. In the context of this study, the conclusion is that **availability** was the regulating factor in the water column and **respiration** was usually the regulating factor in sediments.

It should be noted that when a similar experiment was done with surface sediments taken directly from an Ekman dredge, rather than from a sectioned core, the order of methylation and demethylation was not the same as from the



Ch. III, Figure 7 Specific rates of methylation and demethylation in surface sediments taken from cores in four lakes. Incubation of triplicate samples at 10°C for 24 hours. OCTOBER 10/88.



Ch. III, Figure 8 Rates of oxygen consumption and M/D ratio in surface sediments taken from cores in four lakes. Duplicate O_2 consumption samples incubated at $10^{o}C$ for 15 hours. OCTOBER 10/88.

cores used here (data not shown)[1]. This probably reflects the chemical and biological variability which can occur among sites on a lake, as well as the possible disturbance of sediments which can occur when sampling in different ways. Examining these differences was beyond the scope of this study. However, the overall conclusion (noted in the previous paragraph) was still supportable within the context of these experiments.

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1 ie. much depends upon the site and method of sampling surface sediments.

CONCLUSIONS

This study contributes to the current knowledge about mercury methylation, demethylation and methylmercury partitioning in Canadian Shield lakes. In laboratory water sample experiments using the DOC concentrate, mercury methylation was found to be suppressed by DOC and enhanced by reduced pH. The results using lake water with naturally varying DOC concentrations and pH supported the DOC concentrate findings in many cases. An example of this was the equal rates of methylation in L225 (high DOC, pH 4.9) and L224 (low DOC, pH 6.9). Specific demethylation rates almost always increased as DOC increased which further contributed to decreasing the potential for methylmercury production (M/D). Thus, the short-term laboratory experiments are likely applicable to long-term methylation in the water column of lakes.

In general, for water samples, respiration was not important in controlling the potential for methylmercury production (M/D). However, since respiration and demethylation usually both increased as water column DOC concentrations increased, binding of methylmercury by DOC appeared to be less important than binding of Hg^{2+} by DOC. Conversely, availability of inorganic mercury seemed to govern water sample methylation rates. In sediments, M/D ratios often increased when respiration increased, a finding

reported by others. Methylmercury was also solubilized by DOC as shown by methylation and partitioning experiments, however, the long-term fate of these molecules is unknown.

The chapters of this thesis complement one another by suggesting mechanisms for methylmercury production and movement in certain Shield lakes. They also lead to several key hypotheses: 1) a large proportion of mercury species in circumneutral high DOC drainage lakes may originate from the watershed, because they are unlikely to originate within the lake, 2) high DOC, low pH lakes may also be subjected to high (but not as high as low DOC lakes) water column methylation rates, 3) if high DOC, circumneutral lakes are also seepage lakes, fish may not have high methylmercury concentrations.

It is clear that many environmental factors can effect the production, transport and fate of methylmercury in aquatic systems. Only by studying each of these factors individually in the laboratory and in the natural environment (whenever possible) can a more complete understanding of methylmercury dynamics become possible.

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APPENDIX - Methylation & Demethylation Methods

203-METHYLMERCURY EXTRACTION (from Furutani & Rudd, 1980)

Day 1:

Measure 50 mL (or other chosen volume) of sediments; dry at 60 C. - Usually used 15 mL sediments or 100 mL water.
Have ready: 0.5M HCl if necessary to increase volume of stock of radioisotope.

1) Dispense chosen volume of sample to centrifuge tubes or bottles (with sediments, use modified plastic syringe to dispense and 30 mL centrifuge tubes to incubate; water use graduated cylinder to dispense and BOD bottles to incubate).
2) Kill blanks with **1 mL 4N HCl**, stopper and shake.
3) Add **100 uL working stock** (~1 uCi/1 ug Hg/**100 uL**) to each sample, stopper bottles, and shake to mix.
4) Incubate for 24 hours (usually) - keep everything behind Pb shield.

Day 2: (after incubation)

The extraction procedure to remove the ^{203}Hg-methylmercury in the samples was developed by Furutani and Rudd (1980).

1) Add **1 mL of 4N HCl** to kill all samples, seal and shake.
2) Add **~2 mL of 0.5M copper sulphate** and **10 mL of 3M sodium bromide in 11% H2S04** and **50 mls of H20** (if required to dilute sediments), shake for 2 minutes. Centrifuge or allow to bottles to sit to separate sediments and water; need a distinct water layer over sediments.

3) A known volume of the supernatant water is **decanted** into 125 mL separatory funnels containing **20 mL glass distilled toluene.** Using heavy elastics, I secured the stoppers to the sep. funnels and carefully placed them on a "Wrist-action Shaker". Shake 3 minutes (if by hand, or 5 mins. if mechanical) and let stand until aqueous and organic phases separate (at least 1 hr).

4) Draw aqueous phase out of bottom of funnels and discard (down drain with water or in waste container). Dry toluene by adding **~0.5 g of anhydrous sodium sulphate** to funnel. Pour toluene out of top of flask into a 50 mL Erlenmeyer flask containing more (1/2 tsp) sodium sulfate.

5) Remove 10-15 mL of toluene phase with glass pipette, put in test tubes with stoppers containing **5 mL 2.5 mM sodium thiosulphate in 20% ethanol** (this step can also be done in 60 mL sep. funnel rather than t.tubes).

6) Vortex 1 min., remove 3 mL of bottom layer (bubble on way through), and place in smaller test tube (stoppered) containing **1 mL of 3M potassium iodide** and **1 mL of benzene.** Vortex 1 min. and allow to separate.

7) Remove a 500-750 uL aliquot of the benzene (top) phase and add this to **10 mL of scintillation cocktail.** Count at least 10 minutes (to max of 10,000 cpm).

DEMETHYLATION METHOD - Ramlal et al 1986

Specific rates of demethylation were measured by the addition of 0.2 ug Hg(II) [as 2 uCi ^{14}C-methyl mercuric iodide, Amersham Laboratories] to 100 mL water sample or 15 mL sediment sample. For use, crystalline $^{14}CH_3HgI$ was dissolved in distilled, deionized water, with working stock made to the above concentration in 100 uL.

METHOD:

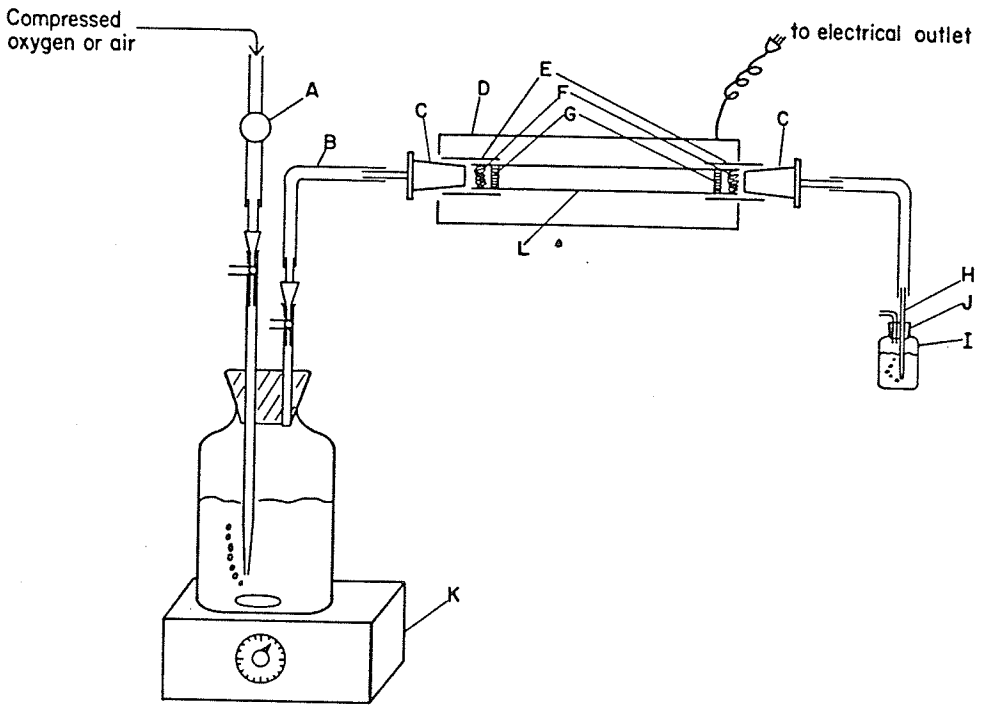
1) Add $^{14}CH_3HgI$ as above noted to 2 + 1 acid-killed samples.

2) Incubate for 24 hours then kill all samples (4N HCl). This was done by injecting acid by syringe with a spinal needle down the opening of a 3-way valve to the port which was submerged. This prevented gas loss and damage to silicone stopper (if injecting directly through the stopper).

3) Volatile ^{14}C produced by demethylation (CH_4 and CO_2) was stripped from the acidified samples as follows:
Samples stirred magnetically and bubbled with air from SCUBA tank (flow rate ~ 50 ml.min^{-1}) for 1 hour. Volatile ^{14}C was passed through a Vycor tube packed with copper oxide beads (which was held in the tube with quartz wool). The tube(s) were heated in a tube furnace at 450^{o}C to oxidize all volatile ^{14}C to $^{14}CO_2$. The $^{14}CO_2$ was collected in a carbon dioxide trap consisting of 10 mL of scintillation fluor (usually ACS, Amersham), 2 mL Protosol (New England Nuclear)

and 2 mL of 100% methanol in a glass scintillation vial. Vials were allowed to sit in the dark for 48 hours after the addition of Protosol to diminish chemiluminescence (this could be done in advance). The ^{14}C activity of the sample was determined with a liquid scintillation counter (usually Beckman 2800). Report percent demethylated.

SCHEMATIC OF THE APPARATUS USED TO STRIP THE ^{14}C END PRODUCTS OF DEMETHYLATION FROM SEDIMENT AND WATER SAMPLES:



A. Gas metering valve (if required); B. flexible hose; C. plastic adapter (or attach hose directly to tube); D. Tube furnace; E. silicone hose (unless vycor tubes extend from furnace); F. steel wool; G. quartz wool; H. disposable glass pipette; I. 20 mL glass scint. vial with CO_2 trap; J. silicone stopper (use on original sample bottle, optional on scint. vial); K. magnetic stir plate; L. Vycor tubing packed with copper oxide.