

THE SEASONAL AND SPATIAL VARIATIONS OF  
MERCURY METHYLATION AND METHYLMERCURY DECOMPOSITION  
IN AN OLIGOTROPHIC NORTHERN WISCONSIN LAKE

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## ABSTRACT

The formation of methylmercury represents a potentially serious problem in aquatic ecosystems because of its toxicity and accumulation in aquatic biota. Elevated levels of mercury in edible fish have recently been reported for several lakes in Northern Wisconsin. The majority of mercury in fish tissue is in the methylated form, suggesting that biological mercury methylation is a key process regulating mercury levels in fish. Numerous studies have examined mercury methylation in aquatic ecosystems but most have addressed only net rates of methylmercury production. The net amount of methylmercury produced in the aquatic environment is dependent on both methylation and demethylation. Recent studies have examined the effects of pH on mercury methylation and demethylation in several lakes in Canada and Northern Wisconsin. However, little else is known about the relative rates of these simultaneous processes in aquatic ecosystems.

The purpose of this study was to examine where and when the potential for methylation was the greatest in Lake Clara; to examine the products of microbial demethylation; and to examine the effects of acidification on methylation and demethylation. Microbial mercury methylation and methylmercury decomposition were examined in Lake Clara, an oligotrophic Northern Wisconsin seepage lake, using

$^{203}\text{Hg}(\text{NO}_3)_2$  and  $^{14}\text{CH}_3\text{HgI}$ , respectively. The primary sites for mercury methylation in Lake Clara were in the surficial sediments of the backwater area and the littoral-profunda transitional zone. Mercury methylation in surficial sediments was greatest from mid-July through September. The potential for methylation in the water column and attached microbial communities was low; whereas, demethylation activity was substantially greater. Thus, the net rate of methylmercury production may be significantly affected by demethylation activity.

The production of  $^{14}\text{CO}_2$  from  $^{14}\text{CH}_3\text{HgI}$  suggests that an oxidative demethylating enzyme may exist. Acidification decreased methylation activity in surficial sediments, while demethylation activity remained relatively constant until pH 4.5, suggesting that sustained demethylation activity may play a role in the decreased net methylation activity in acidified sediments.

These data suggest that the surface sediments in Lake Clara have the greatest potential for mercury methylation and that methylation activity in surface sediments was greatest during late summer. These data also suggest that an oxidative demethylating enzyme may exist and that demethylation activity may play a role in decreased methylation in acidified sediments.

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## INTRODUCTION

Mercury has been used extensively by man throughout history. Early Greek and Far Eastern civilizations used elemental mercury for medicinal purposes. Mercuric oxide was used by the 18th Century English to make felt hats. Modern applications of mercury include thermometers, disinfectants, slimicides, pesticides, chemical catalysts, and "long-life" alkaline batteries. The use of mercury has, however, not been without tragedy. Highly toxic mercury compounds have caused death and illness in Canada, Iraq, and Japan (D'Itri and D'Itri, 1977; Irukayama, 1967). Contamination of edible fish has led the U.S. Food and Drug Administration to establish a consumption health standard for mercury (1 ppm methylmercury). Although environmental pollution with mercury has been studied intensively in the last decade, much remains to be learned about its transformations in the aquatic ecosystem.

Elevated levels of mercury in edible fish have recently been reported for several lakes in Northern Wisconsin (WDNR PUBL FM-301 85). These lakes do not have a direct anthropogenic input of mercury; therefore, it is important to more completely understand the factors affecting methylmercury formation in the aquatic environment.

I examined mercury methylation and demethylation in Lake Clara, a Northern Wisconsin oligotrophic lake. Lake Clara is

one of several lakes in Northern Wisconsin with a fish advisory from the Wisconsin Department of Natural Resources due to mercury contamination of fish. It is also the subject of several studies dealing with mercury transformations and contamination. The objectives of this study were:

1. examine the spatial and seasonal variations in mercury methylation and degradation to determine where and when the greatest potential for methylation exists;
2. to determine if increases or decreases in demethylation activity affects net methylmercury production;
3. to determine the gaseous products of microbial degradation of methylmercury in the aquatic environment;
4. to determine the effects of acidification on methylation and demethylation in surficial sediments.

This is the first study to examine the spatial and seasonal variations of methylmercury production and degradation, and to identify the products of microbial demethylation in the aquatic environment.

This thesis contains four chapters and two appendices. Chapter 1 is a literature review concerning the mercury cycle and the effects of acidification on mercury transformations.

Chapter 2 (Seasonal and spatial variations of mercury methylation and demethylation in an oligotrophic lake) and Chapter 3 (The products of microbial methylmercury degradation) are manuscripts to be submitted for publication. Chapter 3 was written as a note and is formatted to note style. Chapter 4 is a general discussion of the results. Appendix A contains details of methods not discussed in Chapters 2 and 3. Appendix B contains additional results not included in Chapters 2 and 3.

## CHAPTER 1: LITERATURE REVIEW

Sources of Mercury. Although the majority of mercury in aquatic environments originates from natural sources (National Research Council, 1978), anthropogenic input may be important in localized situations. Since 1973, global production of mercury-containing products has declined (Moore and Ramamoorthy, 1984), however, large amounts of mercury have been introduced through the manufacturing of chlorine, paper products, plastics, and electronic components (Andren and Nriagu, 1979). The use of organomercurials in personal products, insecticides, fungicides, and disinfectants have also released significant amounts of mercury into the environment (Wood, 1971). Atmospheric mercury introduced by the burning of coal (Evans et al., 1980; Haines, 1981) and

oil (Summers and Silver, 1978) can enter the aquatic environment in precipitation or dry deposition. The burning of fossil fuels and incineration of municipal sludges is expected to increase the mercury burden in air over time (Moore and Ramamoorthy, 1984).

Toxicity of Mercury. Mercury and organomercurial compounds are highly toxic. Methylmercury is 10 to 100 times more toxic than inorganic mercury (Friberg and Vostal, 1972). Methylmercury accumulates in aquatic organisms because of its lipid-solubility and slow rate of excretion (Jernelov and Lann, 1971). Methylmercury persists in tissues of aquatic biota and is biomagnified in higher organisms of aquatic food chains (Huckabee et al., 1979). Mercury concentrations in higher organisms can be as much as  $10^5$  times higher than the concentration in the water.

In humans, methylmercury affects the central nervous system (Clarkson et al., 1984). Symptoms of mercury intoxication include parathesia, contraction of vision, loss of coordination, and impaired speech (D'Itri and D'Itri, 1979). Prolonged exposure to mercury can result in death. Methylmercury is most toxic to the developing embryo/fetus (Mottet et al., 1985). Methylmercury may cross the placenta and cause mental retardation and severe nervous disorders in infants (Clarkson et al., 1984; Mottet et al., 1985)

## Mercury Cycle

Chemistry of Mercury. Mercury can exist in both inorganic and organic forms. Inorganic mercury can exist in three valence states: elemental mercury,  $\text{Hg}^0$ ; mercurous ion,  $\text{Hg(I)}$ ; and mercuric ion,  $\text{Hg(II)}$ .  $\text{Hg(II)}$  is the most common dissolved mercury species.  $\text{Hg(II)}$  readily forms inorganic salts, and may form ionic or covalent bonds with organic compounds (Jernelov and Martin, 1980).

Mercury may enter the aquatic environment bound to inorganic ligands, organic ligands, or associated with suspended solids (Moore and Ramamoorthy, 1984). After mercury has entered an aquatic ecosystem, the majority is bound or adsorbed onto sediment or suspended particles (Kudo et al., 1977). Organic mercury compounds may be degraded to inorganic forms (Spangler et al., 1973a). Under reducing conditions in the sediments, mercury can be effectively immobilized by the formation of  $\text{HgS}$  (Gavis and Ferguson, 1972).

Methylation.  $\text{Hg(II)}$  may be methylated to form monomethylmercury and further methylated to dimethylmercury. Monomethylmercury is, however, the major species found in aquatic ecosystems at neutral or acidic pH values (Jernelov and Martin, 1980). The methylation of mercury can occur biologically or chemically (Summers and Silver, 1978),

although abiological mechanisms appear to be insignificant under natural conditions (Berman and Bartha, 1986a; Blum and Bartha, 1980; Jensen and Jernelov, 1969).

Several mechanisms for the biological methylation of mercury have been proposed. Three major coenzymes are known to be involved in methyl transfer: N<sup>5</sup>-methyltetrahydrofolate derivatives; S-adenosylmethionine; and methylcobalamin (Robinson and Tuovinen, 1984). Methylcobalamin is believed to be responsible for the majority of methylation of inorganic Hg because it is the only agent capable of transferring carbanion methyl groups (Wood et al., 1978). Although Tonomura et al. (1971) and Hamdy and Noyes (1975) demonstrated that methylcobalamin stimulated methylation in bacteria capable of forming methylmercury; other methylation mechanisms must also exist. Escherichia coli is able to methylate mercury in the absence of methylcobalamin, although the mechanism is unknown (Silver et al., 1976). Neurospora crassa also methylates mercury although it does not use methylcobalamin in its metabolism (Landner, 1971). Landner suggested that the methylation in N. crassa might be an "incorrect" synthesis of methionine.

Wood et al. (1968) demonstrated methylation of mercury by cell-free extracts of methane-producing bacteria, suggesting that methanogens may be agents of mercury methylation. However, other researchers (Compeau and Bartha,

1985; McBride and Edwards, 1977; Winfrey, 1985) demonstrated that methane-producing bacteria were not responsible for mercury methylation in sediments. Instead of methanogens, sulfate-reducing bacteria appear as likely methylators of mercury in anoxic sediments (Compeau and Bartha, 1985; Winfrey, 1985). Molybdate, an inhibitor of sulfate reduction, suppressed mercury methylation (Compeau and Bartha, 1985; Winfrey, 1985), whereas, the addition of sulfate stimulated mercury methylation (Winfrey, 1985). Compeau and Bartha (1985) isolated a Desulfovibrio desulfuricans culture that vigorously methylated mercury. Mercury in sediments therefore does not appear to be precipitated as HgS in the presence of active sulfate reduction and is still available for methylation (Winfrey, 1985).

Mercury methylation occurs primarily in the sediments (Callister and Winfrey, 1986; Furutani and Rudd, 1980; Steffan, 1984) and to a lesser extent in the water column (Callister and Winfrey, 1986; Furutani and Rudd, 1980; Steffan, 1984). Mercury methylation has also been observed in fish intestines (Rudd et al., 1980), marine sediments (Blum and Bartha, 1980), and sewage sludge (Bisongi and Lawrence, 1975). Microorganisms can methylate mercury under both anaerobic and aerobic conditions although anaerobic conditions are more favorable to methylmercury production

(Callister and Winfrey, 1986; Compeau and Bartha, 1984; Olson and Cooper, 1976).

Demethylation. Methylmercury formed in aquatic environments can be biologically demethylated (Billen et al., 1974; Spangler et al., 1973b). Abiological demethylation appears unlikely because of the lack of activity in sterilized samples (Spangler et al., 1973a). The major products of demethylation are inorganic mercuric ion and  $\text{CH}_4$  (Spangler et al., 1973a). Kozak and Forsberg (1979) also observed  $\text{CO}_2$  as a product of demethylation; however, it was not determined if  $\text{CO}_2$  was a direct product of demethylation. Concurrent production and degradation of methylmercury suggests that an equilibrium is reached between the two processes in the environment (Billen et al., 1974; Jernelev and Martin, 1980).

Methylmercury is toxic to microorganisms; therefore, demethylation is a resistance mechanism to methylmercury toxicity. Demethylation is believed to result from the cleavage of the carbon-mercury linkage by the organomercurial lyase, resulting in  $\text{Hg(II)}$  and  $\text{CH}_4$  (Robinson and Tuovinen, 1984). Bacterial degradation of methylmercury has been demonstrated with both mixed and pure cultures from lake sediment (Furukawa et al., 1969; Spangler et al., 1973a; Spangler et al., 1973b; Tonomura et al., 1968). Microbial demethylation activity has also been reported in the rumen

(Kozak and Forsberg, 1979), human feces (Edwards and McBride, 1975), and sediment (Billen et al., 1974; Ramlal et al., 1985). Demethylation occurs in both aerobic and anaerobic environments (Ramlal et al., 1986; Xun et al., in press). However, Ramlal et al. (1986) and Compeau and Bartha (1984) reported greater demethylation under aerobic conditions.

Volatilization. The volatilization of mercury occurs both biologically (Colwell et al., 1976; Colwell et al., 1977; Nelson and Colwell, 1975; Olson et al., 1979; Spangler et al., 1973b) and chemically (Ramamoorthy et al., 1983; Steffan, 1984). Hg(II) in the environment or produced by the organomercurial lyase may be reduced to form volatile elemental mercury. Microbial reduction of mercuric ion is an enzymatic process carried out by mercuric reductase (Robinson and Tuovinen, 1984).

Winfrey et al. (1986) reported that mercury volatilization in aquatic habitats was largely due to an abiological reduction of Hg(II) despite the presence of large numbers of mercury-resistant bacteria. Rapid rates of abiotic volatilization of mercury have been observed in sewage sludge (Bisongi and Lawrence, 1975) and culture media (Vaituzis et al., 1975). Toribara et al. (1970) suggested that almost any reducing substance could convert  $\text{Hg}^{2+}$  to  $\text{Hg}_2^{2+}$ . The  $\text{Hg}_2^{2+}$  may then dissociate to form  $\text{Hg}^{2+}$  and volatile  $\text{Hg}^0$  ( $\text{Hg}_2^{2+} \rightarrow \text{Hg}^{2+} + \text{Hg}^0$ ).

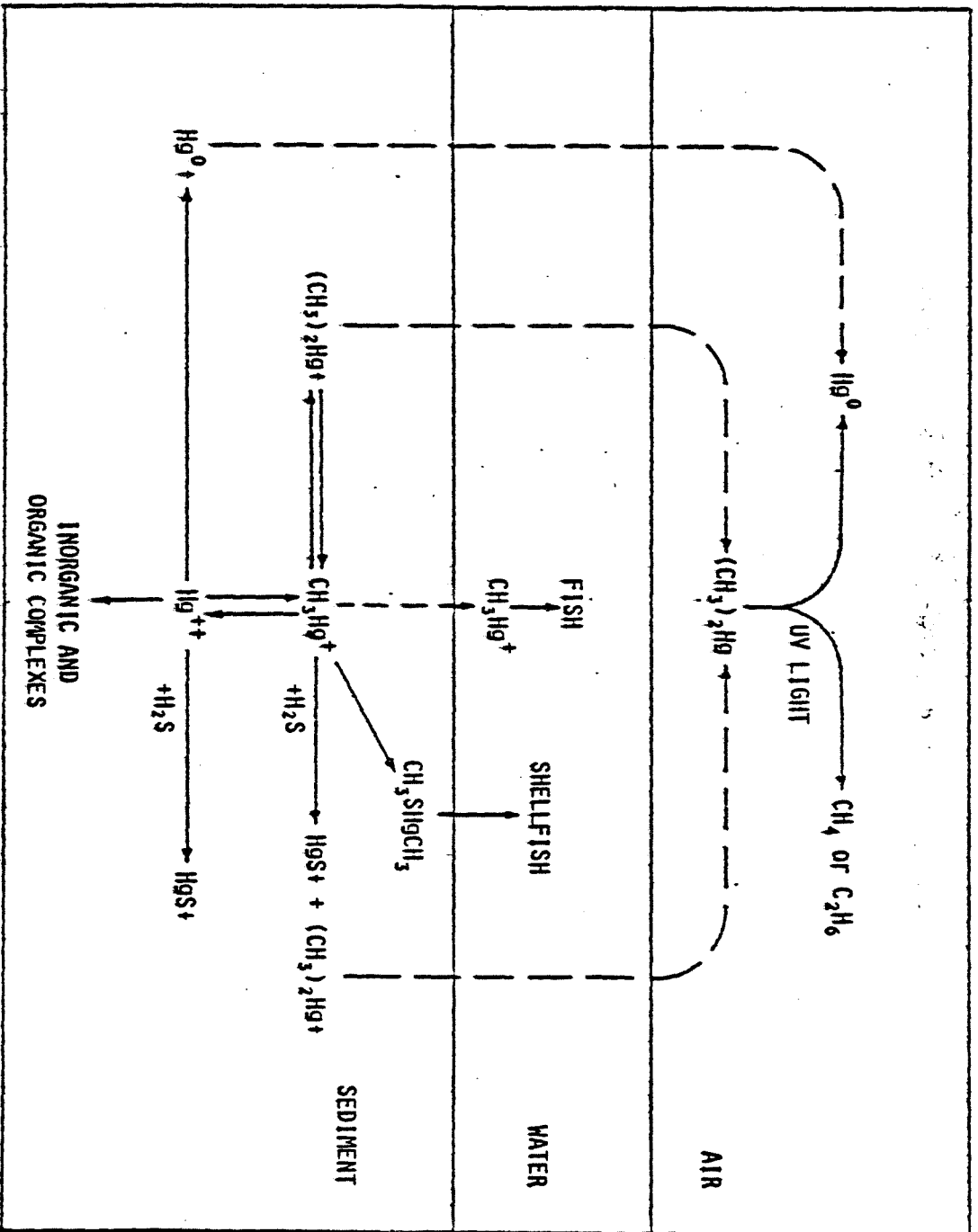
A summary of the steps involved in the mercury cycle are presented in Figure 1-1.

### Lake Acidification and the Effect of Acidification on Mercury Transformations

The release of large amounts of industrially produced sulfuric and nitric oxides into the atmosphere results in acidic precipitation. Precipitation with a pH value lower than 5.6 is considered acidic (Haines, 1981). Large regions of Europe and North America are receiving precipitation with pH values below 4.7, and adverse effects on aquatic biota have been observed (Haines, 1981).

Acidic deposition results in acidification of water of lakes and streams with a low buffering capacity. The susceptibility of a lake to acidic deposition is primarily based on its alkalinity and the type of watershed (Dillon et al., 1984). Bicarbonate supplied by the watershed is the major component of the acid-neutralizing capacity in most lakes (Dillon et al., 1984). Lakes with watersheds low in carbonate minerals tend to be acid-susceptible. When the acid-neutralizing capability of a lake cannot assimilate acidic deposition fast enough, the alkalinity and pH of the water decline.

FIG. 1-1. Mercury cycle in the aquatic environment



Acidified lakes generally have a much higher waterborne metal content than do similar non-acidic lakes (Haines, 1981). Acidic precipitation on watershed soils may mobilize aluminum and manganese and carry them to lakes by runoff and groundwater (Cronan et al., 1978). Mercury and other trace metal content of acidic precipitation is higher than that of unacidified precipitation (Beamish and Van Loon, 1977; Tomlinson et al., 1980).

An increase in solubility of Al, Mg, Zn, and Fe has been observed to occur with a reduction in lake pH (Schindler et al., 1980). Wood (1980) theorized that mercury solubility would increase at acidic pH values, however, research indicates decreased solubility (Ramlal et al., 1985; Schindler et al., 1980). Therefore, lake acidification would not be expected to mobilize mercury from the sediments.

The effect of pH on methylation and demethylation has recently been studied. A decrease in methylation was observed in acidified surficial sediments (Ramlal et al., 1985; Steffan, 1984). In contrast, Xun et al. (in press) observed increased methylation rates in acidified lake water.

Ramlal et al. (1985) observed that the rate of demethylation decreased to a lesser extent than methylation in acidified lake sediment. In a similar study, Xun et al., (in press) observed that both increases and decreases in the ambient pH reduced demethylation. However, the pH-induced

changes in the rate of demethylation in the water column and at the sediment-water surface were smaller than for methylation.

Elevated levels of mercury occur in fish from naturally acidic and acidified lakes in Northern Wisconsin (Wiener, 1983), Maine (Akielaszek and Haines, 1981), Canada (McLean et al., 1980; Wren and MacCrimmon, 1983; MacCrimmon et al., 1983), and Sweden (Bjorklund et al., 1984; Lindqvist et al., 1984). Elevated levels of mercury in fish (Lindqvist et al., 1984; Wiener, 1983) and epiphytic algae (Stokes et al., 1983; Stokes et al., 1985) are inversely correlated with pH.

Several potential mechanisms for increased mercury content in fish have been proposed. The increased mercury content in fish may be due to increased uptake at acidic pHs, increased mercury transport to aquatic ecosystems by acidic precipitation, or greater methylmercury production at lower pHs.

The direct uptake of waterborne methylmercury by fish occurs almost entirely across the gills (Olson et al., 1973). The efficiency of this direct uptake is apparently enhanced in waters with low pH and low calcium concentration, because of increased gill permeability under these conditions (McDonald, 1983; Rodgers and Beamish, 1983)

Although methylmercury is the major form of mercury found in organisms (Huckabee et al., 1979), the increased

levels are not associated with increased methylation in sediments. A decrease in mercury methylation occurs in acidified surficial sediments (Ramlal et al., 1985; Steffan, 1984). In contrast, Xun et al. (in press) predicted that atmospheric acidification of lakes would increase the net rate of methylmercury production in the water column.

None of the above mechanisms completely explains why biota of acidic lakes contain elevated mercury concentrations. Although elevated mercury levels are found in fish of several acidic Northern Wisconsin lakes, the increased levels are not explained by increased methylation in acidified sediments. Therefore, mechanisms other than increased mercury methylation must account for enhanced mercury accumulation in biota of low pH lakes. Further research on the factors affecting methylmercury formation in the aquatic environment is needed.

## CHAPTER 2: THE SEASONAL AND SPATIAL VARIATIONS OF MERCURY METHYLATION AND DEMETHYLATION IN AN OLIGOTROPHIC LAKE

The formation of methylmercury represents a potentially serious problem in aquatic ecosystems because of its toxicity and accumulation in aquatic biota. The majority of mercury in fish tissue is in the methylated form (Huckabee et al., 1979), suggesting that methylation of inorganic mercury is a key process regulating mercury levels in fish. Numerous studies have examined mercury methylation in aquatic ecosystems but most have addressed only net rates of methylmercury production. The net amount of methylmercury produced in the aquatic environment is dependent on both methylation and demethylation (Jensen and Martin, 1980). Rudd and co-workers (Ramlal et al., 1985; Ramlal et al., 1986; Xun et al., in press) have recently examined the effects of pH on methylation and demethylation in the water column and sediments. However, little else is known about the relative rates of these simultaneous processes in aquatic ecosystems.

Methylation of mercury can occur biologically or abiologically (Summers and Silver, 1978). Abiological mechanisms of methylation appear to be insignificant under natural conditions (Berman and Bartha, 1986; Blum and Bartha, 1984; Jensen and Jernelov, 1969). Microorganisms can

methylate mercury under both aerobic or anaerobic conditions although anaerobic conditions are more favorable to methylmercury production (Callister and Winfrey, 1986; Compeau and Bartha, 1984; Olson and Cooper, 1976).

Mercury methylation occurs primarily in sediments (Callister and Winfrey, 1986; Furutani and Rudd, 1980; Wright and Hamilton, 1982) and to a lesser extent in the water column (Callister and Winfrey, 1986; Furutani and Rudd, 1980; Kudo et al., 1978). Callister and Winfrey (1986) reported that methylation activity was greatest in surface sediments and decreased with sediment depth. Callister and Winfrey (1986) also reported that methylation in mercury-contaminated Upper Wisconsin River sediments was low in early summer, greatest in late summer, and declined sharply in the fall. Ramlal et al. (1985) observed a decrease in mercury methylation with a reduction of sediment pH. However, Xun et al. (in press) observed increased methylation in acidified lake water.

Methylmercury formed in the aquatic environment can be biologically demethylated (Billen et al., 1974; Spangler et al., 1973b). Abiological demethylation appears unlikely because of the lack of activity in sterilized samples (Spangler et al., 1973b). Demethylation can occur in both aerobic and anaerobic conditions (Furukawa et al., 1968; Spangler et al., 1973a; Spangler et al., 1973b, Ramlal et al., 1986; Xun et al., in press) although Compeau and Bartha

(1984) and Ramlal et al. (1986) reported greater demethylation under aerobic conditions. Ramlal et al. (1985) and Xun et al. (in press) reported that a reduction in the ambient pH decreased demethylation but that the pH-induced decrease was less than for methylation.

Elevated mercury concentrations have recently been reported in remote acidic lakes in Northern Wisconsin (Wiener, 1983), Northeastern U.S. (Akielaszek and Haines, 1981), Canada (MacCrimmon et al., 1983; Wren and MacCrimmon, 1983), and Scandanavia (Bjorklund et al., 1984; Lindqvist et al., 1984). The majority of these systems do not have a direct anthropogenic input of mercury. Therefore, lake acidification affects the bioavailability of methylmercury. However, the mechanism of methylmercury formation and uptake by aquatic biota is not clearly understood.

The majority of studies conducted on the simultaneous processes of methylation and demethylation have focused on the effects of salinity and redox potential (Compeau and Bartha, 1984) or pH (Ramlal et al., 1985; Xun et al., in press). Despite the potential mercury problem in many remote acidic or potentially acidic lakes, the spatial and seasonal variations of mercury methylation and demethylation are unknown.

The purpose of this study was to compare both methylation and demethylation activity in order to evaluate the importance of demethylation in regulating net

methylmercury production. We examined the spatial and seasonal variations in methylmercury production and degradation in Lake Clara, an acid-susceptible oligotrophic seepage lake. Lake Clara is one of several lakes with a fish advisory from the Wisconsin Department of Natural Resources due to the mercury contamination of fish (WDNR PUBL FM-301 85). We observed that the greatest potential for mercury methylation in Lake Clara occurred in surficial sediments and that methylation in surficial sediments was greatest from mid-July through September.

#### METHODS AND MATERIALS

Study Area. Lake Clara is an oligotrophic, acid-susceptible seepage lake (Eilers et al., 1983) located in Lincoln County, Wisconsin (Fig. 2-1, 2-2). The lake has a maximum depth of 11.4 m and contains highly organic flocculent sediments. The southern portion of the lake is shallow and supports heavy macrophytic vegetation during the summer. Additional chemical and physical characteristics of Lake Clara are provided in Table 2-1.

Sample Collection. Surficial sediments from sites 3 and 7 (Fig. 2-2) were collected for the seasonal study from April, 1985 to April, 1986. Samples were collected at approximately two week intervals from spring to fall turnover and once during ice cover. Samples for the spatial study

were collected on July 22, 1985. Samples collected included: water (0.5, 3, 6, 8, 10 m at site 7), surficial sediments along a lake transect (sites 1-7, Fig. 2-2), sediment cores (sites 3 and 7), and periphyton samples from a variety of surfaces in the littoral zone.

Water and flocculent surficial sediments were collected with a peristaltic pump (Horizon Ecology Co.). A T-shaped sample inlet was used to obtain samples from a horizontal stratum (Winfrey and Zeikus, 1979). More consolidated surficial sediments were sampled with an Eckman dredge. A sediment core was taken from the profundal site (site 7) by SCUBA divers using an 8-in diam PVC pipe with horizontal slots every 5-10 cm. The profundal core was sectioned by inserting plexiglass plates into the slots while it was still embedded in the sediment. Littoral sediment cores were collected as described by Callister and Winfrey (1986) with 50-mL open-end syringes and sealed with butyl rubber stoppers. Littoral cores were anaerobically sectioned and homogenized by adding 10 mL of sterile, anoxic lake water to 15 mL of sediment (Callister and Winfrey, 1986). All sediments were transferred to and stored in acid-washed glass bottles using strict anaerobic technique modified for field use (Winfrey and Zeikus, 1977). Water samples were stored in acid-washed 1-L polyethylene bottles.

Periphyton samples were collected by aseptically scraping a 16 cm<sup>2</sup> area of substrate (Geesey et al., 1978)

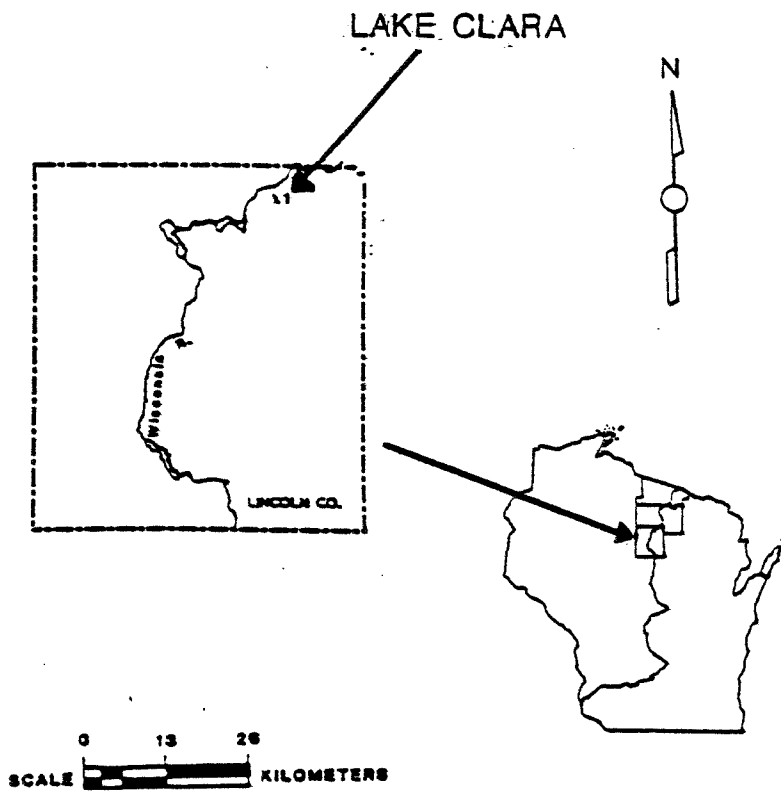


FIG. 2-1. Location of Lake Clara in Lincoln County, Wisconsin.

FIG. 2-2. Morphometric map of Lake Clara. Depth contours in meters. Stars represent the location of sampling sites along a transect. Sites 1 and 2 were in a shallow macrophyte bed. Sites 3-7 were in the open lake.

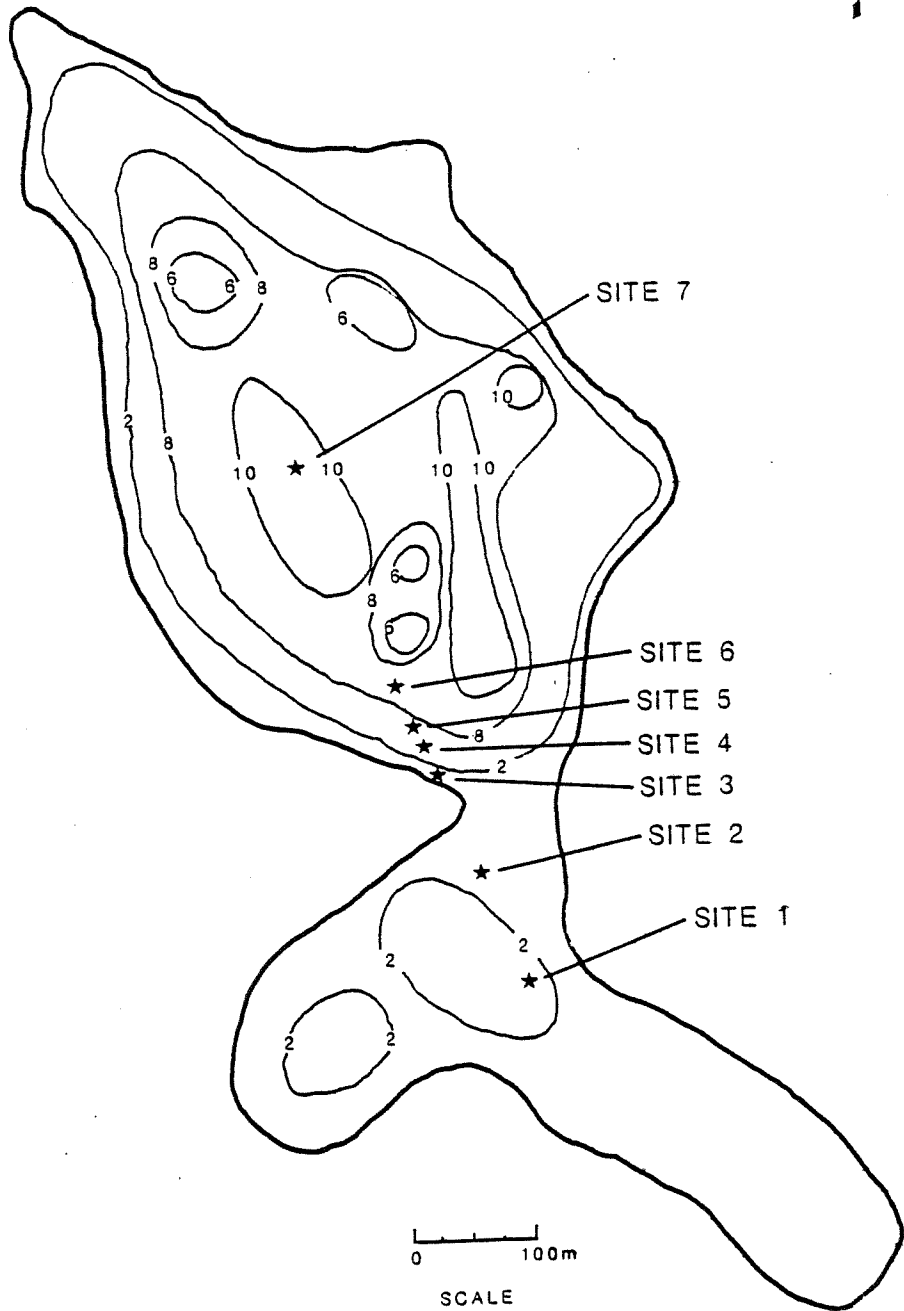


TABLE 2-1. Chemical and Physical characteristics of Lake Clara

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Surface area (ha)	34.1
Maximum depth (m)	11.4
Mean depth (m)	5.1
pH <sup>a</sup>	6.5 ± 0.3
Alkalinity (ueq/L) <sup>b</sup>	35.9 ± 5.7
Secchi disk transparency (m) <sup>c</sup>	3.6 ± 0.6

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<sup>a</sup> mean surface water (0.5 m) pH ± standard deviation during sampling period (n = 15).

<sup>b</sup> mean surface water (0.5 m) alkalinity ± standard deviation during sampling period (n = 15).

<sup>c</sup> mean ± standard deviation during sampling period.

into sterile acid-washed, 60-mL BOD bottles (Wheaton) containing 15 mL of filter-sterilized lake water. All samples were transported to the laboratory on ice and processed within 24 h of collection.

Chemical Analyses. Field pH was measured with a Fisher model 107 pH meter. Dissolved oxygen and temperature were measured with a Yellow Springs Instrument model 54A oxygen meter. Alkalinity of flocculent surficial sediments and surface water was determined by two-end point titration with 0.01N HCl (APHA, 1981). A Fisher model 701 pH meter and an Ingold pH electrode (number 6015-03-K7) were used for alkalinity determinations. Sediment dry weight was determined by drying at 105 C. Organic content (volatile residue) was determined by combustion at 550 C (APHA, 1981).

Methylation Assay. Mercury methylation activity was determined by incubating 15 mL of water, sediment, or periphyton sample with 1.0 ug  $^{203}\text{Hg}(\text{II})$  (3.05 to 8.66 uCi as  $^{203}\text{Hg}(\text{NO}_3)_2$ , New England Nuclear) in acid-washed, 60-mL BOD bottles. Samples were incubated for 24 h and terminated with the addition of 1.0 mL of 6N HCl. Methylation assays for water and periphyton samples were setup and incubated aerobically. Methylation assays for sediments were setup and incubated using strict anaerobic technique (Hungate, 1969). Samples for seasonal methylation assays were incubated at both in situ and constant (15 C) temperatures. Samples for

spatial methylation assays were incubated only at in situ temperatures.

Methylmercury formed during incubation was extracted by the method of Furutani and Rudd (1980) as modified by Steffan (1984). After incubation was terminated 0.5M  $\text{CuSO}_4$  (2.0 mL) and 3.0M NaBr in 11%  $\text{H}_2\text{SO}_4$  (5.0 mL) were added to each sample and vortexed for 1 min. Flocculent sediments and water samples were transferred to 125-mL glass separatory funnels containing 25 mL of toluene. Separatory funnels were shaken for 3 min, and the toluene layer was removed and centrifuged at  $1000 \times g$  for 10 min. Following centrifugation, the toluene layer was removed and dried twice with 1 g anhydrous sodium sulfate. After drying, the toluene layer was further extracted as described by Furutani and Rudd (1980).

Sediments with a high sand content were centrifuged after the addition of  $\text{CuSO}_4$  and NaBr, and the supernatant was extracted as described above. The radioactivity in a 500  $\mu\text{L}$  aliquot of the final benzene extract was quantified by scintillation counting in 4 mL of 4a20 scintillation cocktail (Research Products International Co.).

Demethylation Assay. Demethylation activity was determined for the spatial study by incubating 30 mL of water, sediment, or periphyton sample with 1.0  $\mu\text{g}$  of Hg(II) (as  $^{14}\text{CH}_3\text{HgI}$ , Amersham Corp.) in stoppered 125-mL acid-washed, glass bottles. Samples for the seasonal study were incubated with 0.26-1.15  $\mu\text{g}$  Hg(II) (as  $^{14}\text{CH}_3\text{HgI}$ ).

Samples were incubated for 24 h and terminated by injecting 2 mL of 6N HCl through the butyl rubber stoppers with a syringe. Samples were incubated at the conditions and temperatures as described for the methylation assay.

Products of  $^{14}\text{CH}_3\text{HgI}$  demethylation ( $^{14}\text{CO}_2$  and  $^{14}\text{CH}_4$ ) were gassed for 30 min from the reaction bottles with compressed air (approx. 50 mL/min) by a modification of the method developed by Ramlal et al. (1986) (Fig. 2-3). The  $^{14}\text{CO}_2$  produced during incubation was captured in a series of two vials, containing a 8 mL of a  $^{14}\text{CO}_2$  trapping cocktail ( $^{14}\text{CO}_2\text{UNT-SORB}$ , Research Products International). The  $^{14}\text{CH}_4$  produced during incubation passed through the first series of traps and was oxidized to  $^{14}\text{CO}_2$  in a tube furnace (Sargent Welch) containing hot copper oxide (Curtin Matheson). The  $^{14}\text{CO}_2$  was then trapped in second series of two vials containing  $^{14}\text{CO}_2$  trapping cocktail. The radioactivity in each vial was quantified by liquid scintillation counting.

Although the radioisotopic techniques used in this study did not provide in situ rates of methylation and demethylation, they did provide useful estimates of potential rates.

Quality Assurance. Quality assurance for this study consisted of killed controls, procedural blanks, and standard additions. Killed controls were prepared by autoclaving samples or by the addition of 6N HCl, 6N NaOH, or formalin. Procedural blanks were prepared by the addition of

radioisotope [ $^{203}\text{Hg}(\text{NO}_3)_2$  or  $^{14}\text{CH}_3\text{HgI}$ ] to sterile, deionized water. The procedural blanks were acidified, and the methylation and demethylation assays were conducted as previously described. Methylation and demethylation were not detected in killed controls or procedural blanks (Appendix B, Table B-1).

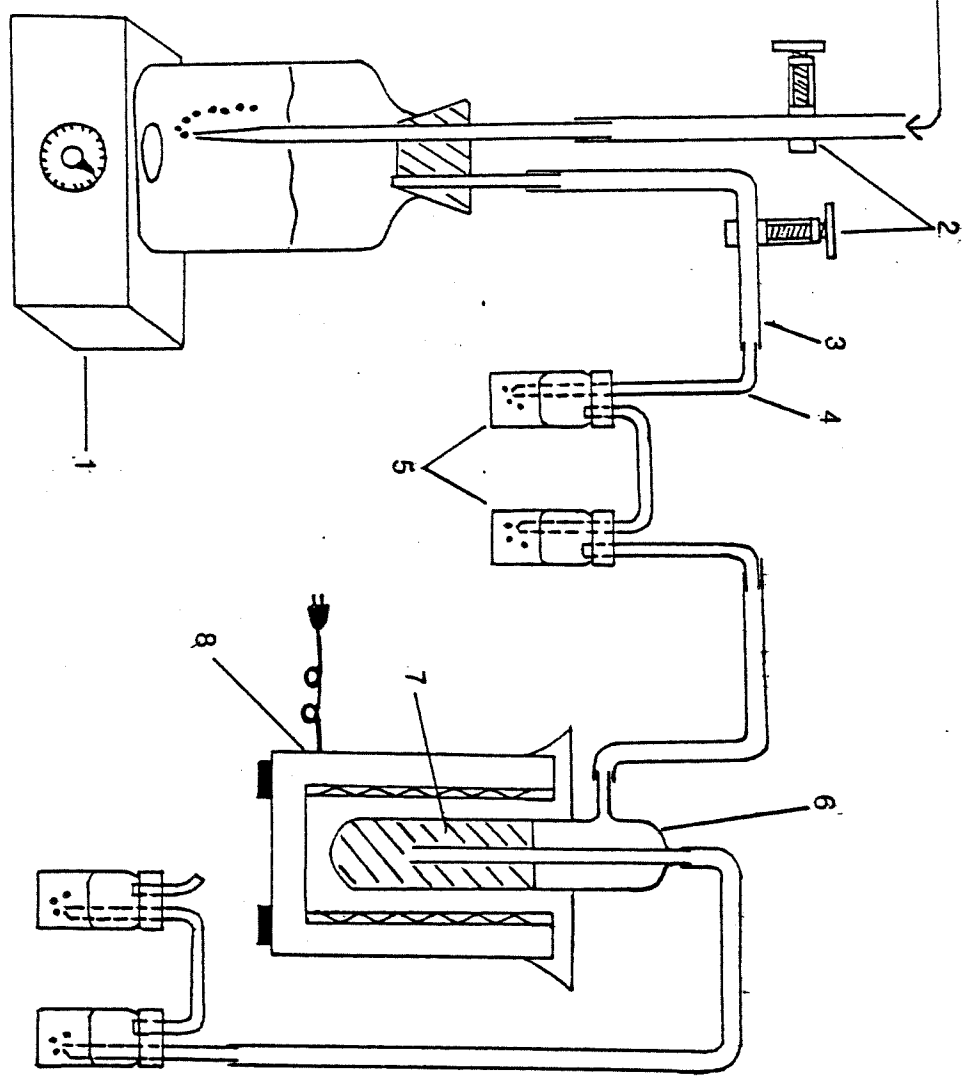
Standard additions for the methylation assay were prepared by adding a known DPM of  $^{14}\text{CH}_3\text{HgI}$  to acidified samples, and the  $^{14}\text{CH}_3\text{HgI}$  extracted as previously described. Extraction efficiency of the methylation assay for highly organic sediments, water, and periphyton samples was essentially 100 % ( $x \pm \text{SE} = 98.3 \pm 1.0$ ). Extraction efficiency for sandy sediments was significantly less than 100 % ( $x \pm \text{SE} = 84.8 \pm 6.2$ ). Methylation data for sandy sediments was corrected accordingly.

The standard additions for the demethylation assay were prepared by adding a known DPM of  $\text{NaH}^{14}\text{CO}_3$  (New England Nuclear Corp.) to samples prior to acidification. Sample bottles were immediately stoppered and acidified with 2 mL of 6N HCl. Acidification lowered the pH sufficiently to convert all  $\text{H}^{14}\text{CO}_3$  to  $^{14}\text{CO}_2$ . The  $^{14}\text{CO}_2$  was gassed from the reaction bottles and captured as previously described. Extraction efficiency of the demethylation assay for all sample types was essentially 100 % ( $x \pm \text{SE} = 104.4 \pm 0.7$ ).

Reagents were periodically checked for radioactive contamination. Radioactivity in 1 mL of each reagent was

FIG. 2-3. Apparatus used to strip and capture  $^{14}\text{C}$  demethylation products from samples. 1) stir plate, 2) tubing clamps, 3) latex tubing, 4) Teflon tubing, 5) scintillation vials, 6) glass tube, 7) copper oxide, 8) tube furnace.

COMPRESSED AIR



quantified by scintillation counting in 8 mL of PCS scintillation cocktail (Amersham Corp.).

All radioisotope counting was done on a Beckman LS 230 liquid scintillation counter. Counts per minute (CPM) were corrected for counter efficiency from previously prepared quench curves using external standardization, and DPM were used in all calculations.

Chemicals and Radioisotopes. All chemicals used were reagent grade except for benzene, which was certified grade. Stock solutions of  $^{203}\text{Hg}(\text{NO}_3)_2$  in 0.5M  $\text{HNO}_3$  (New England Nuclear Corp.) were prepared by dilution in distilled water to yield a final concentration of 0.10 ug Hg/uL. Stock solutions of  $^{14}\text{CH}_3\text{HgI}$  (Amersham Corp.) and  $\text{NaH}^{14}\text{CO}_3$  (New England Nuclear Corp.) were prepared by dilution in distilled water.

Data Analyses. All assays were conducted in triplicate unless specified otherwise. Results of the assays were expressed as the percent of total added mercury methylated or demethylated or as a rate ( $\text{ng Hg}\cdot\text{ml}^{-1}\cdot\text{d}$ ). To enable comparisons between different sampling dates, the seasonal demethylation rates were normalized to 1.0 ug Hg if the amount of added Hg was greater or less than 1.0 ug. The normalized demethylation rates were calculated as follows:

$$\text{normalized rate} = \frac{\text{measured demethylation rate}}{\text{ug of added Hg}}$$

The validity of normalizing data was determined by a previous kinetics experiment (Appendix B, Fig. B-3) in which the amount of  $^{14}\text{CH}_3\text{HgI}$  demethylated in sediments was directly proportional to the concentration of added  $^{14}\text{CH}_3\text{HgI}$  at the concentration range used in these experiments ( $r^2 = 0.996$ ).

Methylation/demethylation ratios (M/D) were calculated by dividing the methylation rate ( $\text{ng Hg}\cdot\text{mL}^{-1}\cdot\text{d}$ ) by the demethylation rate ( $\text{ng Hg}\cdot\text{mL}^{-1}\cdot\text{d}$ ). This ratio is a measure of the relative balance of methylating and demethylating activity and may be used in identifying potential sites of accelerated methylmercury production (Ramlal et al., 1986).

The effects of chemical and physical variables on seasonal methylation and demethylation were evaluated using a stepwise multiple linear regression model. Significant differences in methylation and demethylation in the spatial study were determined using one-way analysis of variance and either Tukey's or Tukey-Kramer multiple comparison tests.

## RESULTS

### Spatial Variations of Methylation and Demethylation

Mercury methylation activity was near background in the water column, was high in the profundal surficial sediment (floc), and decreased with depth in the profundal sediment core (Fig. 2-4A). Active demethylation occurred in the water column but was variable with no visible trends (Fig. 2-4B). Demethylation was greatest in the sediment floc and decreased

slightly with depth (Fig. 2-4B). The M/D ratio was less than 1 in the water column, exhibited a sharp peak in the surficial sediments, and decreased in deeper sediments (Fig. 2-4C). A similar trend in methylation, demethylation, and M/D was observed in the littoral sediment core (Fig. 2-5).

The amount of methylation and demethylation varied in surficial sediments collected along a lake transect (Table 2-2, see Fig. 2-2 for sampling locations). Methylation activity was greatest at site 5 and least at site 7. Demethylation was greatest at sites 3-5 and least in the surficial sediments of the backwater area (sites 1 and 2) and in profundal surficial sediments (site 7). The variation in methylation activity between sites was not highly correlated ( $r^2 < 0.093$ ) to in situ temperature, % carbon, or % dry weight. A significant positive correlation did exist between % carbon and demethylation activity in surficial sediments ( $r^2 = 0.68$ ). The M/D ratio in surficial sediments ranged from 1.4-5.8 (Table 2-2). The M/D ratio was greatest in the surficial sediments of the backwater area and sites 5 and 6.

Methylation in the attached microbial communities was near background, while demethylation was high (Table 2-3). Demethylation activity was not significantly different ( $\alpha = 0.05$ ) between samples. The M/D ratios in the attached communities were less than 0.20 (Table 2-3), suggesting that these populations do not possess great potential for mercury methylation.

FIG. 2-4. Depth distribution of mercury methylation and demethylation at the profundal site (7) (July 22, 1985). Samples were incubated at the in situ temperature at the time of collection. Horizontal bars represent the standard error of the mean.

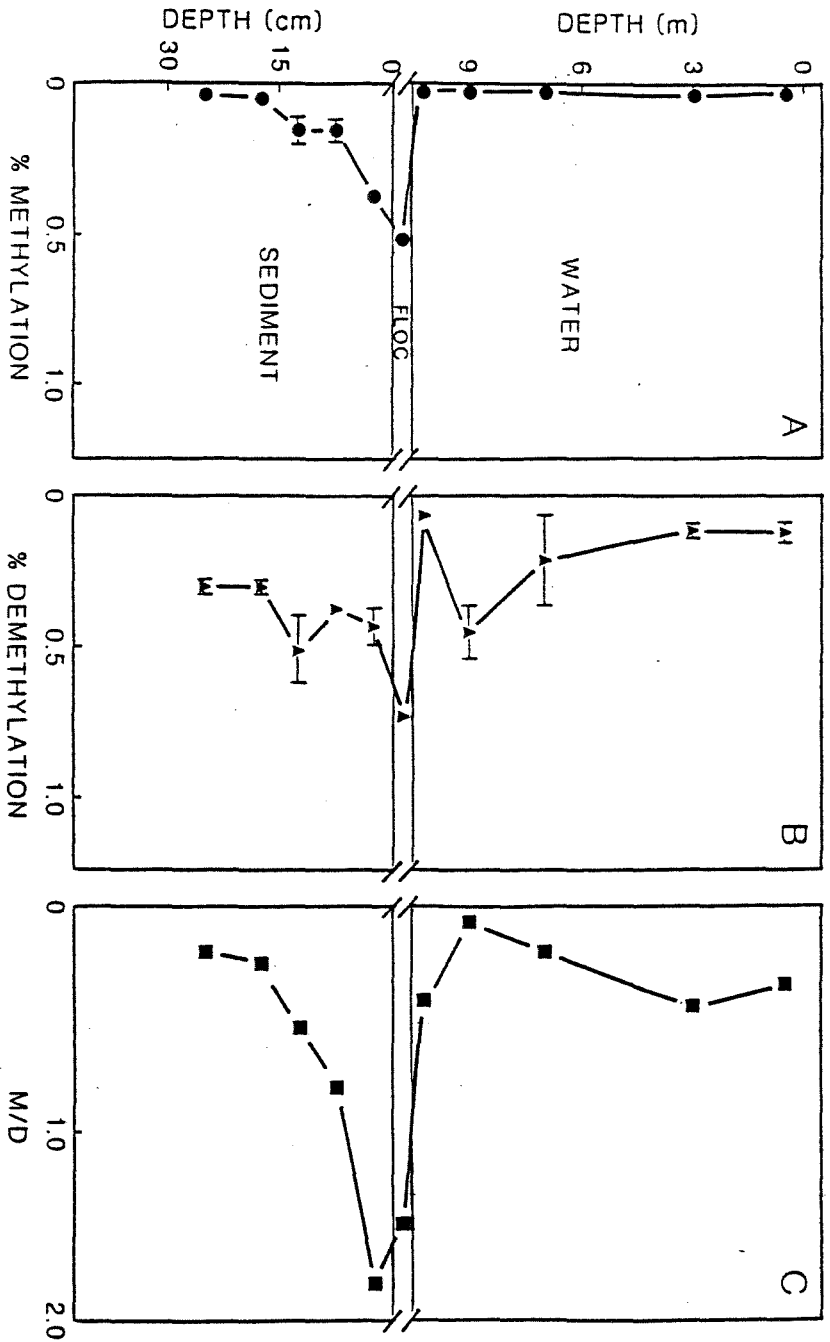


FIG. 2-5. Depth distribution of mercury methylation and demethylation in the littoral sediment core, site 3 (July 22, 1985). Samples were incubated at the in situ temperature (23 C) at the time of collection. Horizontal bars represent the standard error of the mean.

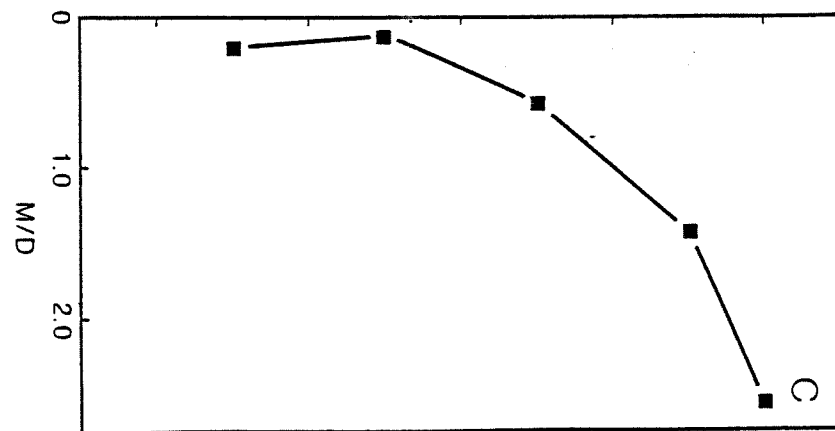
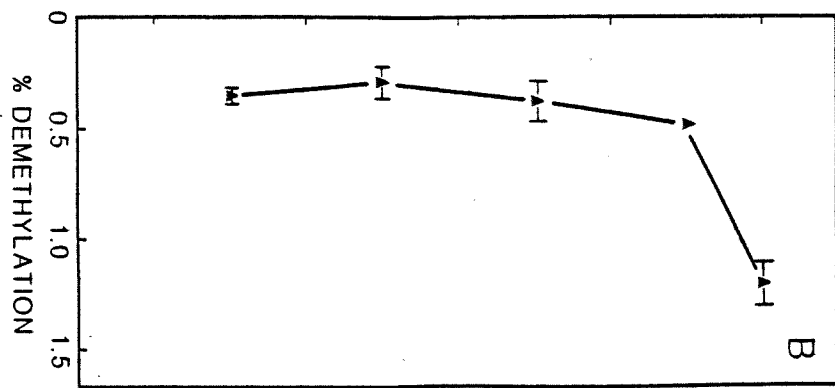
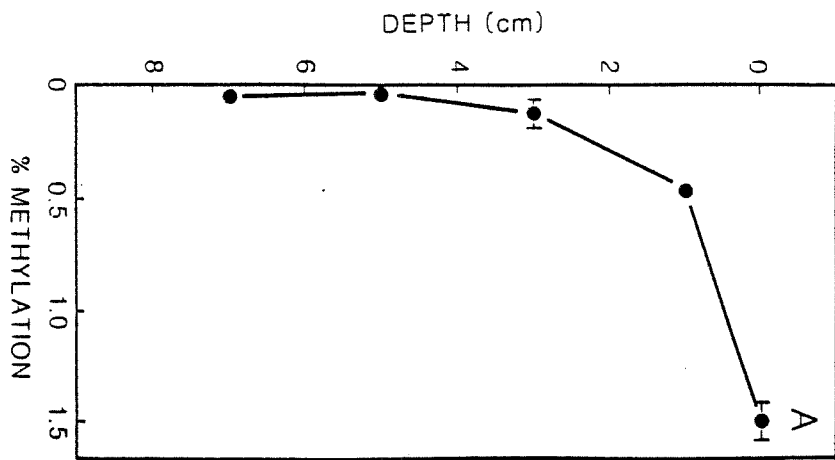


TABLE 2-2. Mercury methylation and demethylation in surficial sediments.\*

Site	Water Depth (m)	% Methylation (avg. $\pm$ SE)**	% Demethylation (avg. $\pm$ SE)**	M/D
1 (0-4 cm)***	2.0	1.16 $\pm$ 0.01 <sup>b</sup>	0.43 $\pm$ 0.00 <sup>a,b</sup>	5.5
2 (0-4 cm)	1.0	1.65 $\pm$ 0.48 <sup>b,c</sup>	0.57 $\pm$ 0.00 <sup>a,b</sup>	5.8
3 (0-1 cm)	1.0	1.27 $\pm$ 0.09 <sup>b</sup>	1.19 $\pm$ 0.10 <sup>c,d</sup>	2.2
4 (0-4 cm)	3.0	1.17 $\pm$ 0.12 <sup>b</sup>	1.57 $\pm$ 0.12 <sup>c,d</sup>	1.6
5 (0-4 cm)	5.0	2.83 $\pm$ 0.23 <sup>d</sup>	1.32 $\pm$ 0.07 <sup>c,d</sup>	4.4
6 (0-7 cm)	7.5	2.46 $\pm$ 0.02 <sup>c,d</sup>	0.92 $\pm$ 0.11 <sup>b,c</sup>	5.3
7 (0-7 cm)	10.5	0.51 $\pm$ 0.03 <sup>a</sup>	0.73 $\pm$ 0.01 <sup>b</sup>	1.4

\* See Fig. 1 for site location. Samples were incubated at in situ temperature (sites 1-5 at 23 C, site 6 at 11 C, site 7 at 5 C).

\*\* n = 2 for all samples except sites 3 and 7 (n = 3). Letters represent statistical comparisons between means ( $\alpha = 0.05$ ).

\*\*\* Ranges represent the depth of the surficial sediment sampled. A smaller interval was used at site 3 because the sediment was hard packed sand. Larger intervals were used at sites 6 and 7 because the surficial sediments were very flocculent.

TABLE 2-3. Mercury methylation and demethylation in attached microbial communities.\*

Sample	% Methylation (avg. $\pm$ SE)**	% Demethylation (avg. $\pm$ SE)**	M/D
Floating vegetation	0.011 $\pm$ 0.003 <sup>a</sup>	0.62 $\pm$ 0.23 <sup>a</sup>	0.04
Submerged log	0.062 $\pm$ 0.013 <sup>b</sup>	0.71 $\pm$ 0.038 <sup>a</sup>	0.20
Submerged rock	0.015 $\pm$ 0.003 <sup>a</sup>	1.28 $\pm$ 0.010 <sup>a</sup>	0.02
Submerged wooden pier post	0.013 $\pm$ 0.0007 <sup>a</sup>	0.78 $\pm$ 0.19 <sup>a</sup>	0.03
Floating branch	0.019 $\pm$ 0.005 <sup>a</sup>	0.79 $\pm$ 0.18 <sup>a</sup>	0.05

\* Letters represent statistical comparisons between means ( $\alpha = 0.05$ ). All samples were incubated at 23 C.

\*\* n = 2

### Seasonal Variations of Methylation and Demethylation

Fig. 2-7 and 2-8 show the seasonal variations of methylation and demethylation in profundal and littoral surficial sediments, respectively, incubated at in situ temperature.

Spring turnover in 1985 occurred just prior to the first sampling date. Lake Clara was thermally stratified from May until mid-October, 1985. Ice cover was present from December, 1985 until just prior to the last sampling date, at which time the lake was undergoing turnover.

Methylation activity in the surficial sediments increased from spring to late summer and decreased in the fall. The decrease in methylation activity in profundal sediments at the beginning of September, 1985 was due to unknown factors, but was probably not seasonally important. Methylation activity in the profundal sediment floc was positively correlated to in situ temperature although temperature did not account for a substantial percentage of the variation ( $r^2 = 0.298$ ). Seasonal methylation activity lacked significant correlation with other variables. Demethylation increased from early summer to mid-summer and then declined (Figs. 2-6B, 2-7B). Seasonal demethylation activity lacked significant correlation with measured variables. The M/D ratio in the surficial sediments increased from mid to late summer, and decreased in the fall (Fig. 2-6C, 2-7C).

Figures 2-8 and 2-9 show the seasonal variations of methylation and demethylation in profundal and littoral surficial sediments, respectively, incubated at constant temperature (15 C). Methylation fluctuated but did not show apparent trends (Fig. 2-8A, 2-9A). Demethylation increased in early summer, was greatest in mid-summer, and then declined (Fig. 2-8B, 2-9B). The M/D ratio also fluctuated and did not show apparent seasonal trends (Fig. 2-8C, 2-9C).

Although seasonal trends in methylation in sediments incubated at in situ and constant temperature were not similar, seasonal trends in demethylation in sediments were similar between the two temperature treatments.

#### DISCUSSION

These results provide important information for evaluating the potential for mercury methylation and mercury accumulation in the aquatic environment. Because aquatic organisms accumulate the majority of their mercury-burden as methylmercury, it is important to examine where and when the greatest potential for mercury methylation exists.

The greatest potential for mercury methylation in Lake Clara existed in the surficial sediments of the densely vegetated backwater area and the littoral-profundal transitional zone. This is of particular importance because these areas are important as the spawning and feeding regions of many lake fish (Lagler et al., 1977). The variation in

FIG. 2-6. Seasonal variations of mercury methylation and demethylation at the profundal site (7). Numbers above data points represent the incubation (in situ) temperatures. M/D values are a comparison of the methylation and demethylation rates. Vertical bars represent the standard error of the mean.

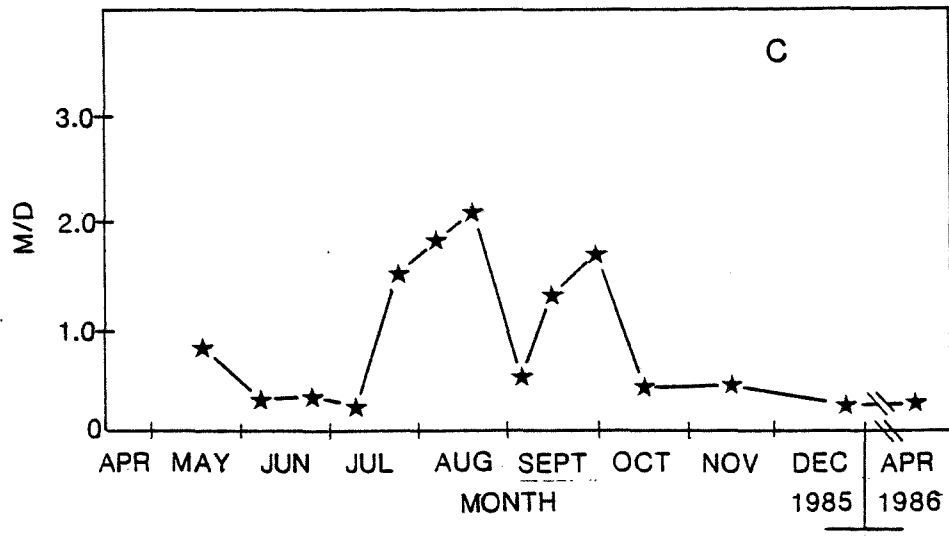
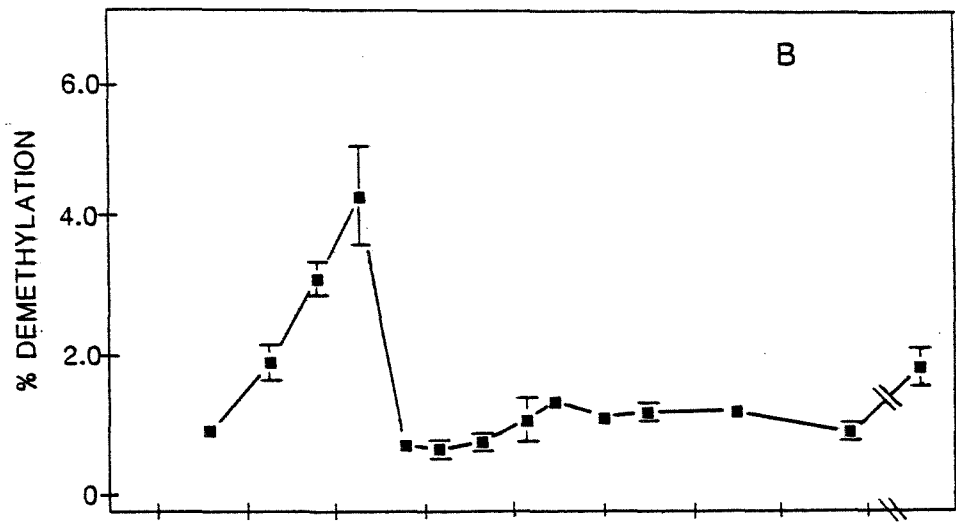
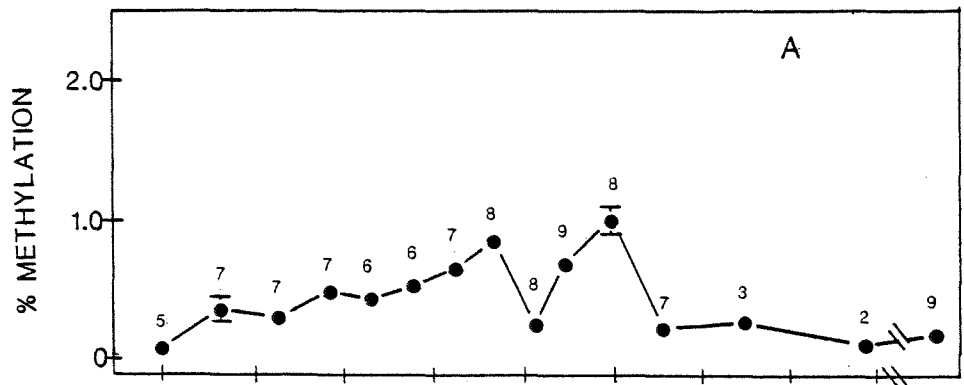


FIG. 2-7. Seasonal variations of mercury methylation and demethylation at the littoral site (3). Numbers above data points represent the incubation (in situ) temperatures. Vertical bars represent the standard error of the mean.

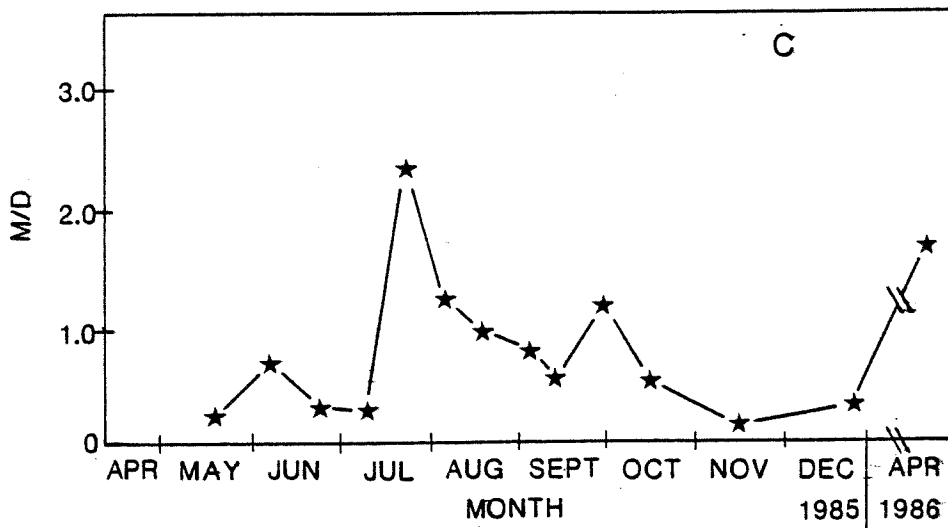
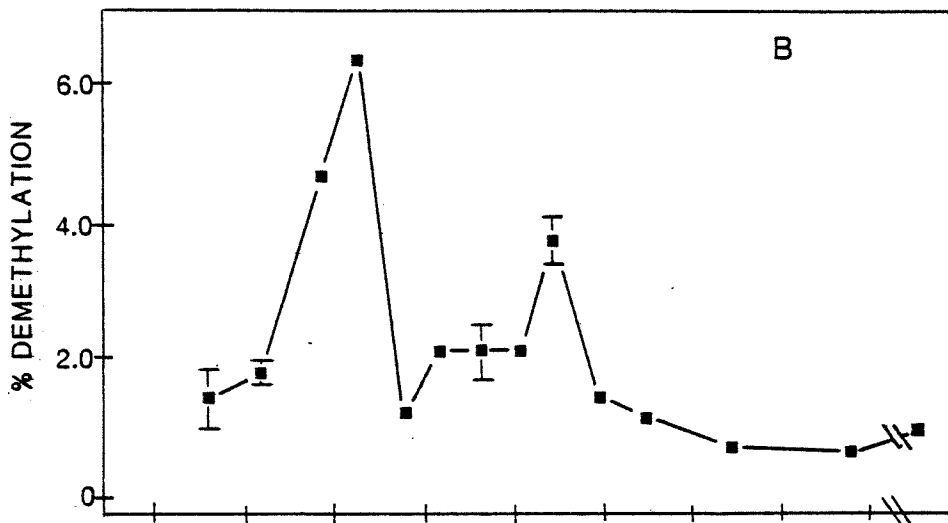
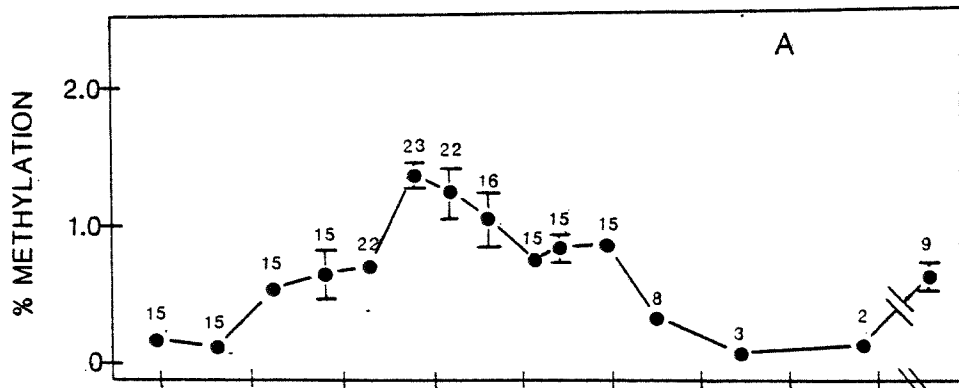


FIG. 2-8. Seasonal variations of mercury methylation and demethylation in profundal surficial sediments (site 7) incubated at constant temperature (15 C). Vertical bars represent the standard error of the mean.

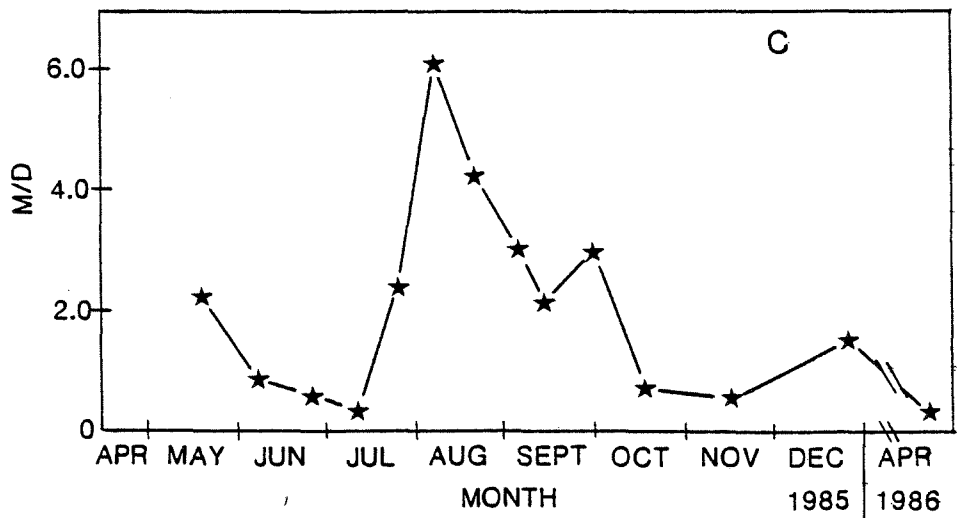
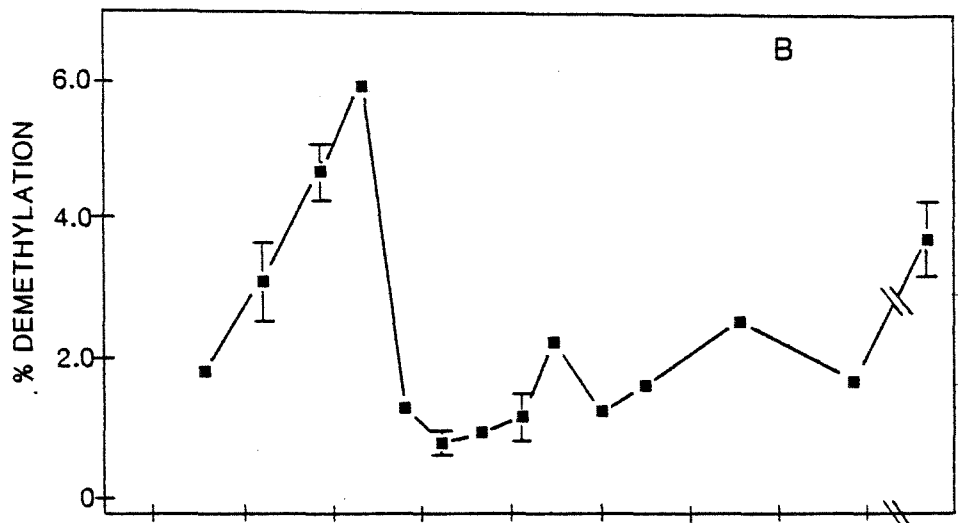
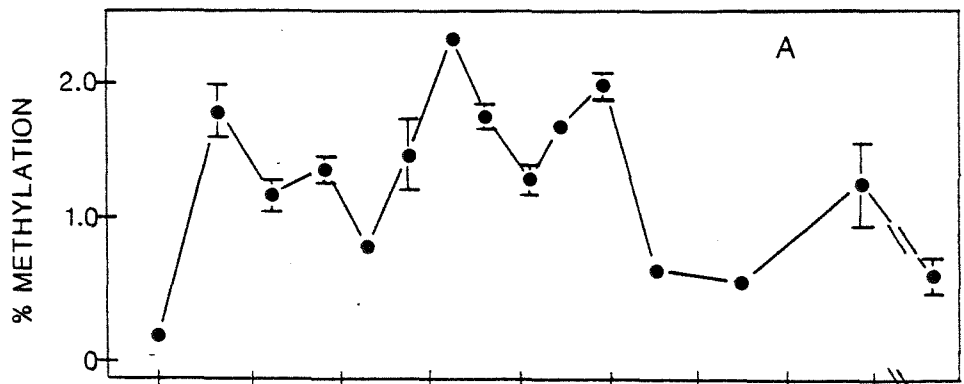
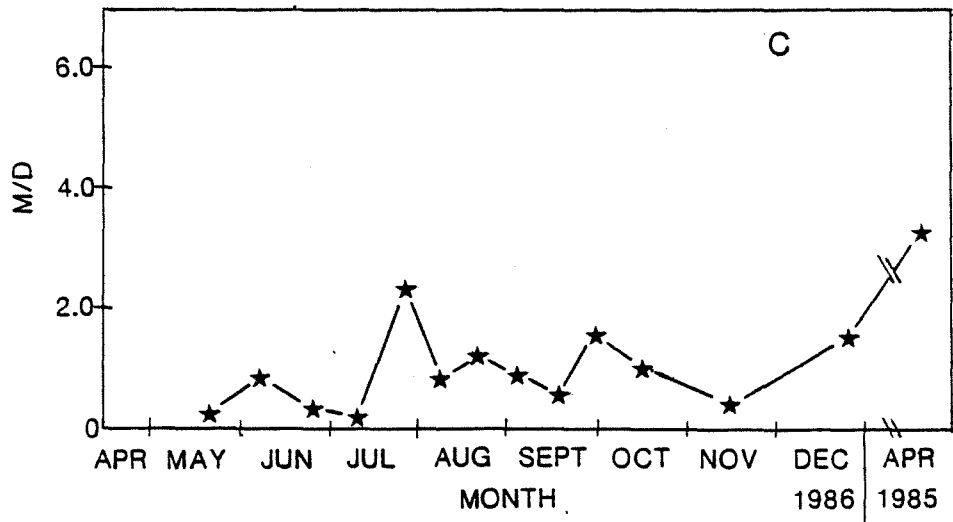
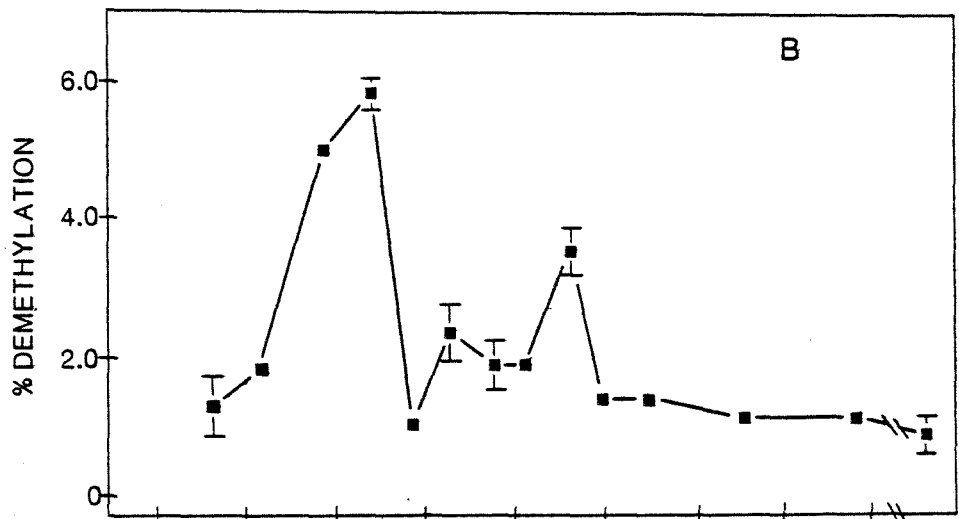
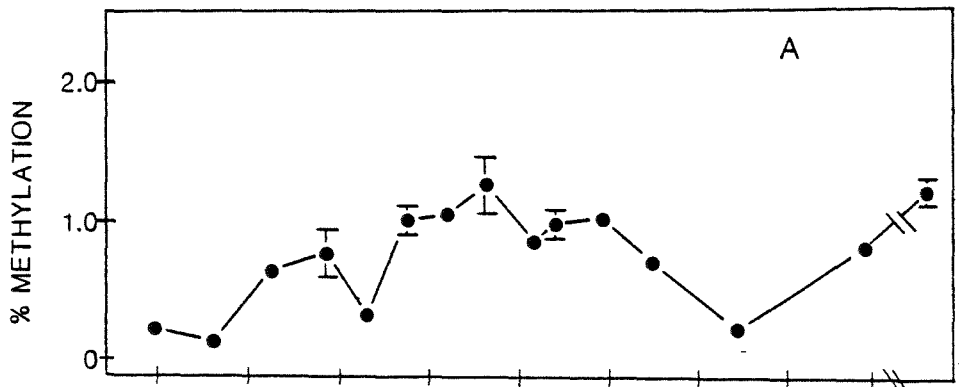


FIG. 2-9. Seasonal variations of mercury methylation and demethylation in littoral surficial sediments (site 3) incubated at constant temperature (15 C). Vertical bars represent the standard error of the mean.



methylation between different sites is also very important because it implies that the conditions at certain sites may be more favorable for methylating microorganisms, thus increasing net methylmercury production.

The net rate of mercury methylation was greatest in surficial sediments of a profundal sediment core. Callister and Winfrey (1986) also observed that methylation activity was greatest in surface sediments of a sediment core from a mercury-contaminated site in the Upper Wisconsin River. The surficial maximum is of particular importance because surface sediments have the greatest potential to release methylmercury into the water column. The decrease in methylation activity with depth in the Lake Clara sediment core correlates well with the known depth distribution of overall microbial activity in sediments (Winfrey, 1984). This implies that the greatest potential for mercury methylation may occur wherever microbial activity is high.

At the sediment surface, we observed both high M/D ratios as well as high rates of methylation and demethylation. Thus, the surficial peak in net methylmercury production did not appear to be due to decreased demethylation activity, but rather to an increase in methylation relative to demethylation. This implies that the actual methylmercury production at the sediment surface may be greater but is possibly reduced due to a high potential for methylmercury degradation. Environmental conditions that

decrease demethylation would increase net methylmercury production at the sediment surface, increasing the potential for release of methylmercury into the water column.

Net methylmercury production in the water column and attached microbial communities was low. The low rate of methylmercury production in the water column may in part be due to the small number of microorganisms (Atlas and Bartha, 1981) and low nutrient concentrations. Addition of organic materials stimulates mercury methylation, suggesting the process is nutrient limited (Akagi et al., 1979; Callister and Winfrey, 1986; Wright and Hamilton, 1982). Aerobic conditions decrease methylation (Callister and Winfrey, 1986; Compeau and Bartha, 1984) and may have contributed to the low methylation activity in the water column. The low M/D ratios suggest that demethylation may play a role in low net methylmercury production in the water column.

Attached bacterial populations represent important components of microbial biomass and activity in aquatic environments (Costerton and Geesey, 1979; Fletcher and Marshall, 1983). Because of the high microbial activity in attached communities, mercury methylation would also be expected to be high. However, mercury methylation in the attached microbial communities of Lake Clara was very low. Similar results were reported by Steffan (1984) in attached communities colonizing artificial substrates. In contrast to the water column, demethylation activity in the attached

communities was high and may have contributed to the low net methylmercury production. Aerobic conditions increase demethylation (Compeau and Bartha, 1984; Ramlal et al., 1986) and may have favored demethylating organisms over methylating organisms in the attached communities.

Compeau and Bartha (1985) and Winfrey (1985) have suggested that sulfate-reducing bacteria are important agents of mercury methylation. Because sulfate-reducing bacteria are strict anaerobes inhibited by aerobic conditions, they would not be expected in attached communities. Their absence may also explain why methylation activity was low in the attached communities.

The greatest potential for methylation in surficial sediments occurred during a period from mid-July through September. In situ temperature was the only environmental variable that showed significant positive correlation to seasonal methylation in profundal surficial sediments. In situ temperature, however, only accounted for a small percentage of the seasonal variation in mercury methylation. Callister and Winfrey (1986) also observed that a seasonal peak in methylation in Wisconsin River sediments was not due solely to an increase in temperature. Thus, other factors must influence mercury methylation rates in surficial sediments. Other possible factors may be demethylation activity and oxic conditions.

The seasonal peak in methylation activity in surficial

sediments incubated at the in situ temperature corresponded to a substantial decrease in demethylation activity. This implies that increased methylation activity may be due to decreased demethylation activity rather than an increase in the actual methylation rate.

Seasonal variations of mercury methylation in surficial sediments may have been influenced by the oxic condition of the overlying water. The seasonal peak in methylation in profundal surficial sediments coincided with a complete depletion of dissolved oxygen in the lower hypolimnion. The decline in methylation activity in October also coincided with the reaeration of the hypolimnion during fall turnover.

In conclusion, these results suggest that the primary site for mercury methylation in Lake Clara occurred in surficial sediments and that methylation in surficial sediments was greatest from mid-July through September. These results also show that the net rate of methylmercury production may be significantly affected by demethylation. In addition, the simultaneous measurement of mercury methylation and demethylation is useful in determining why the potential for mercury methylation varies in aquatic habitats.

## CHAPTER 3: THE PRODUCTS OF MICROBIAL METHYLMERCURY DEGRADATION

The production of methylmercury is of concern because of its increased toxicity relative to inorganic mercury and its ability to accumulate in aquatic biota. The concentration of methylmercury in aquatic ecosystems is dependent on the concurrent processes of production and degradation (Jernelov and Martin, 1980). Thus an equilibrium between production and degradation would be expected in the aquatic environment (Billen et al., 1974). The formation of methylmercury and its subsequent degradation are primarily the result of bacterial processes (Spangler et al., 1973a). Methylmercury degradation is an enzymatic detoxification mechanism that results in the formation of inorganic mercuric ion from methylmercury (Robinson and Tuovinen, 1984). Mercuric ion is then enzymatically reduced to  $\text{Hg}^0$  which is volatile and released from the cells and ecosystem (Colwell et al., 1976; Schottel et al., 1974).

Whether  $\text{CH}_4$ ,  $\text{CO}_2$ , or both are produced as result of demethylation is controversial. The purified organomercurial lyase isolated from mercury-resistant bacteria breaks the carbon-mercury bond of methylmercury, thus producing methane (Colwell et al., 1976; Olson et al., 1979; Schottel, 1978; Tezuka and Tonomura, 1976). Methane as the only product of demethylation has been observed in cellular extracts (Tezuka

and Tonomura, 1976), pure cultures (Tonomura et al., 1971), and lake sediments (Spangler et al., 1973a). Kozak and Forsberg (1979) observed  $^{14}\text{CO}_2$  as well as  $^{14}\text{CH}_4$  produced from  $^{14}\text{CH}_3\text{HgCl}$  in the rumen. Edwards and McBride (1975) observed demethylation of  $^{14}\text{CH}_3\text{HgCl}$  without  $^{14}\text{CH}_4$  formation in human feces, although, they did not look for  $^{14}\text{CO}_2$ . The formation of  $^{14}\text{CO}_2$  from  $^{14}\text{CH}_3\text{HgCl}$  may have resulted from either an oxidative demethylation process or the oxidation of the  $^{14}\text{CH}_4$ .

In this study, we examined the gaseous products of methylmercury decomposition in the sediment, water, attached microbial communities, and bacterial isolates from Lake Clara, a 33.2-ha oligotrophic seepage lake in Northern Wisconsin. Lake Clara is one of several lakes with a fish advisory from the Wisconsin Department of Natural Resources due to mercury contamination of fish (WDNR PUBL FM-301 85). It is also the subject of several studies related to mercury contamination (Korthals and Winfrey, in prep.; Steffan and Winfrey, submitted; Steffan et al., in preparation; Wiener, 1983; Winfrey, 1986).

Water samples and flocculent surficial sediments were collected with a peristaltic pump (Steffan et al., in preparation). More consolidated sediments were sampled with an Eckman dredge. A profundal sediment core was taken by SCUBA divers using an 8-in diam PVC pipe. Sediments were collected and stored using strict anaerobic technique

modified for field use (Winfrey and Ziekus, 1977).

Periphyton samples were collected by aseptically scraping a  $16 \text{ cm}^2$  area of substrate (Geesey et al., 1978) into sterile, 60-mL BOD bottles (Wheaton) containing 15 mL of filter-sterilized lake water.

Mercury resistant ( $\text{Hg}^r$ ) colonies were isolated from lake sediment on R2A medium (Reasoner and Geldreich, 1985) containing 25 ppm Hg. Mercury (as  $\text{HgCl}_2$ , Morton Thiokol, Inc.) was added from a sterile stock solution after autoclaving the media to prevent loss of mercury due to volatilization.

Demethylation activity was determined by dispensing 30 mL of water, sediment, periphyton, or culture into stoppered 125-mL acid-washed, glass bottles. Environmental samples were incubated with 1.0 ug Hg(II) (as  $^{14}\text{CH}_3\text{HgI}$ , Amersham Corp.) for 24 h at in situ temperature. Sediments were processed and incubated using strict anaerobic technique (Hungate, 1969).  $\text{Hg}^r$  broth cultures were incubated with 1.4 ug Hg(II) (as  $^{14}\text{CH}_3\text{HgI}$ ) for 48 h at room temperature. Incubations for all assays were terminated by injecting the sample bottles with 2 mL of 6N HCl.

The gaseous products of demethylation ( $^{14}\text{CO}_2$  and  $^{14}\text{CH}_4$ ) were trapped by a modification of the method developed by Ramlal et al. (1985). The  $^{14}\text{CO}_2$  produced during incubation was gassed for 30 min from the reaction bottles and trapped in a series of two vials containing 8 mL of a  $^{14}\text{CO}_2$  trapping

scintillation cocktail ( $^{14}\text{CO}_2$ UNT-SORB, Research Products International). The  $^{14}\text{CH}_4$  produced during incubation passed through the first series of traps and was oxidized to  $^{14}\text{CO}_2$  in a tube furnace (Sargent Welch) containing hot copper oxide (Curtin Matheson). The  $^{14}\text{CO}_2$  from the hot copper oxide was then trapped in a second series of two  $^{14}\text{CO}_2$  trapping vials. The radioactivity in each vial was quantified on a Beckman LS model 230 liquid scintillation counter. All data was corrected for counter efficiency by external standardization using a previously prepared quench curve.

Quality assurance for this study consisted of killed controls, procedural blanks, and standard additions. Killed controls were prepared by autoclaving samples or by the addition of 2 mL of 6N HCl, 6N NaOH, or formalin. Procedural blanks were prepared by the addition of  $^{14}\text{CH}_3\text{HgI}$  to sterile, deionized water. The procedural blanks were acidified and the demethylation assay conducted as previously described. Significant demethylation activity was not detected in killed controls or procedural blanks. Standard additions were prepared by adding a known DPM of  $\text{NaH}^{14}\text{CO}_3$  (New England Nuclear Corp.) to samples prior to acidification. Sample bottles were immediately stoppered and acidified with 2 mL of 6N HCl. Acidification lowered the pH sufficiently to convert all  $\text{H}^{14}\text{CO}_3$  to  $^{14}\text{CO}_2$ . Extraction efficiency for all sample types was essentially 100 % ( $x \pm \text{SE} = 104.4 \pm 0.7$ ).

Demethylation data was reported as DPM  $^{14}\text{CO}_2$  or  $^{14}\text{CH}_4$ .

The relative amount of  $^{14}\text{CO}_2$  produced was indicated by calculating a respiratory index (RI),  $^{14}\text{CO}_2 / (^{14}\text{CO}_2 + ^{14}\text{CH}_4)$  (Winfrey and Zeikus, 1979). A respiratory index is commonly used to report the products of anaerobic respiration. The respiratory index is used to determine if a process is oxidative or non-oxidative. A respiratory index value of 1 indicates the  $^{14}\text{CH}_3\text{HgI}$  was only degraded to  $^{14}\text{CO}_2$ , and values less than 1 indicate increasingly greater amounts of  $^{14}\text{CH}_4$  produced from  $^{14}\text{CH}_3\text{HgI}$ . Significant differences in the products of demethylation between sites and different depths were determined using one-way analysis of variance and either Tukey's or Tukey-Kramer multiple comparison tests.

In a vertical profile of Lake Clara at the profundal site, the amount of  $^{14}\text{CH}_3\text{HgI}$  demethylated as  $^{14}\text{CO}_2$  did not differ significantly ( $p = 0.076$ ) at various depths in the water column (Fig. 3A). In the sediments, the amount of  $^{14}\text{CO}_2$  was greatest in the surficial floc, and decreased with depth in deeper sediments (Fig. 3-1A).

The amount of  $^{14}\text{CH}_3\text{HgI}$  demethylated as  $^{14}\text{CH}_4$  also did not differ significantly ( $p = 0.34$ ) at various in the water column (Fig. 3-1B). The amount of  $^{14}\text{CH}_4$  in the sediments remained constant without an apparent trend (Fig. 3-1B).

The RI in the water column was less than 0.5 at the thermocline and upper hypolimnion, but was equal to or greater than 0.5 at other sampled depths (Fig. 3-1C). The RI values in the sediments were greater than 0.6 (Fig. 3-1C).

These results suggest that the products from the degradation of  $^{14}\text{CH}_3\text{HgI}$  were variable in the water column, and that  $^{14}\text{CO}_2$  was the primary product of demethylation in the sediments.

In surficial sediments collected along a transect, the amount of  $^{14}\text{CO}_2$  produced from  $^{14}\text{CH}_3\text{HgI}$  was greater than the amount of  $^{14}\text{CH}_4$  (Table 3-1). The RI was greater than 0.7 at all sites (Table 3-1), suggesting that  $^{14}\text{CO}_2$  was the primary product.

In attached microbial communities, the relative amount of  $^{14}\text{CH}_4$  (RI < 0.57) was significantly higher than in sediment (RI > 0.7). In all but one case the RI in the attached communities (Table 3-2) indicated nearly equal amounts of  $^{14}\text{CH}_4$  and  $^{14}\text{CO}_2$  (RI = 0.48 - 0.56).

The products of demethylation in  $\text{Hg}^r$  cultures were determined to find out whether the products of demethylation in environmental samples were similar to the products from bacterial isolates from Lake Clara sediment. The amount of  $^{14}\text{CO}_2$  from the two dominant  $\text{Hg}^r$  bacterial cultures incubated anaerobically was greater than  $^{14}\text{CH}_4$  produced from the degradation of  $^{14}\text{CH}_3\text{HgI}$  (RI > 0.85, Table 3-3). This suggests that the products of demethylation in environmental samples incubated anaerobically may be consistent with those from  $\text{Hg}^r$  bacterial isolates.

FIG. 3-1. Depth distribution of the products of demethylation at the profundal site (July 22, 1985). Horizontal bars represent the standard error of the mean.

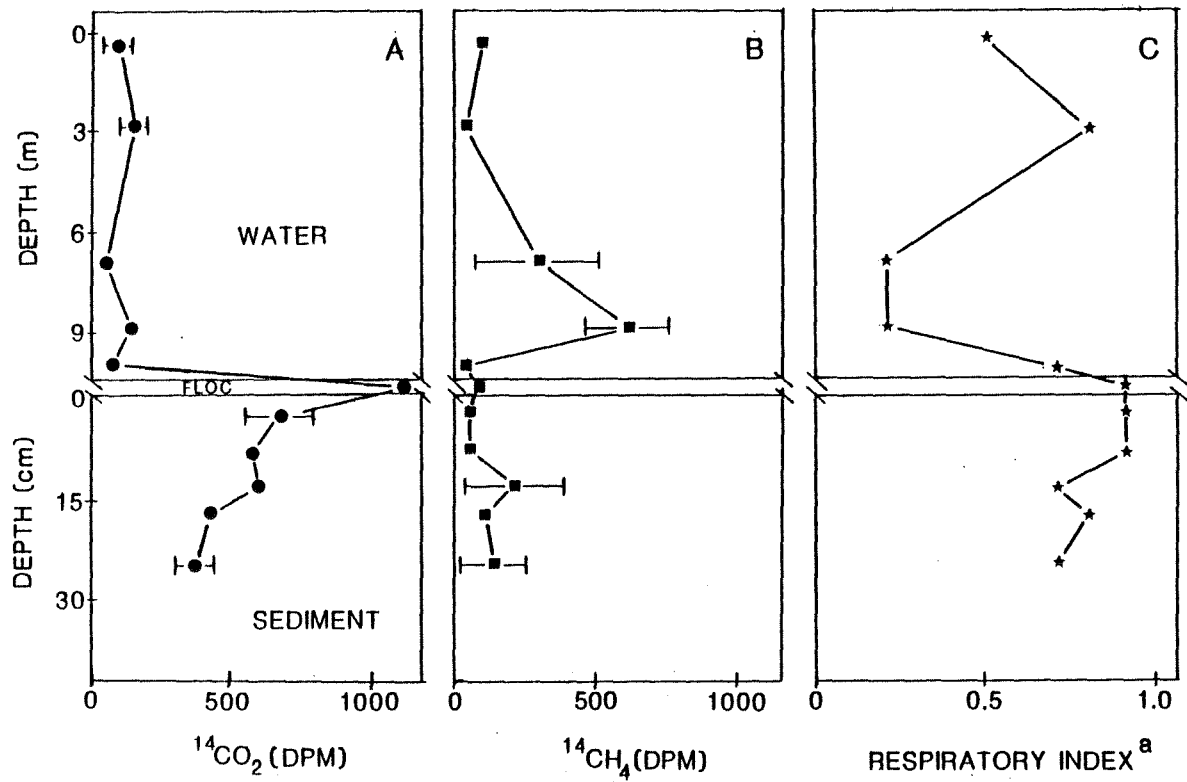


TABLE 3-1. Products of demethylation in surficial sediments collected along a transect (July 22, 1985).

site <sup>***</sup>	water depth (m)	<sup>14</sup> CO <sub>2</sub> (DPM)	<sup>14</sup> CH <sub>4</sub> (DPM)	RI <sup>c</sup>
1 (0-4 cm)	2	488 ± 3 <sup>**</sup>	195 ± 12	0.71
2 (0-4 cm)	1	798 ± 84 <sup>**</sup>	98 ± 75	0.89
3 (0-1 cm)	1	1711 ± 73 <sup>*</sup>	191 ± 89	0.90
4 (0-4 cm)	3	2278 ± 148 <sup>**</sup>	234 ± 45	0.91
5 (0-4 cm)	5	1937 ± 127 <sup>**</sup>	169 ± 16	0.92
6 (0-7 cm)	7.5	1291 ± 42 <sup>**</sup>	171 ± 131	0.88
7 (0-7 cm)	10.5	1054 ± 22 <sup>*</sup>	76 ± 22	0.93

\* mean ± SE (n = 3)

\*\* mean ± SE (n = 2)

\*\*\* Ranges represent the depth of the surficial sediment sampled. A smaller interval was site 3 because the sediments were hard packed sand. Larger intervals were used at sites 6 and 7 because the surficial sediment was very flocculent.

TABLE 3-2. Products of demethylation in the attached microbial communities (July 22, 1985).

sample	$^{14}\text{CO}_2$ (DPM)	$^{14}\text{CH}_4$ (DPM)	RI
floating lily pad root	469 + 41a	516 + 330a	0.48
submerged birch log	639 $\pm$ 15	500 $\pm$ 82	0.56
submerged rock	677 $\pm$ 49	1365 $\pm$ 28	0.33
submerged wooden pier post	603 $\pm$ 94	643 $\pm$ 216	0.48
floating branch	682 $\pm$ 174	578 $\pm$ 470	0.54

a mean  $\pm$  SE (n = 2)

TABLE 3-3. Products of demethylation in Hg<sup>r</sup> bacterial isolates.

isolate	<sup>14</sup> CO <sub>2</sub> (DPM)	<sup>14</sup> CH <sub>4</sub> (DPM)	RI <sup>b</sup>
strain A	498 ± 24 <sup>a</sup>	79 ± 61	0.86
strain B	419 ± 79 <sup>a</sup>	24 ± 6	0.95

<sup>a</sup> mean ± SE (n = 3)

<sup>b</sup> respiratory index

The detoxification of organomercurials is believed to result from the cleavage of the carbon-mercury linkage by the organomercurial lyase followed by the reduction of inorganic Hg(II) to volatile Hg<sup>0</sup> by the mercuric reductase (Colwell et al., 1976; Tezuka and Tonomura, 1976; Schottel, 1978). This bacterial resistance mechanism has been reported to degrade methylmercury to Hg<sup>0</sup> and methane (Spangler et al., 1973a; Tezuka and Tonomura, 1976; Tonomura et al., 1971).

The production of <sup>14</sup>CO<sub>2</sub> from <sup>14</sup>CH<sub>3</sub>HgI may indicate an oxidative enzyme is involved in the demethylation process or that the <sup>14</sup>CH<sub>4</sub> produced by demethylation can be used as a bacterial substrate, thus producing <sup>14</sup>CO<sub>2</sub>. Methane oxidation is primarily an aerobic process requiring an oxygenase (Higgins et al., 1981). Isotopic experiments have demonstrated the occurrence of anaerobic methane oxidation in sediment and water (Zehnder and Brock, 1980). However, the amount of anaerobic methane oxidation was very minute in comparison to the large pool of methane in anaerobic environments. In anaerobic Lake Clara samples, if demethylation of <sup>14</sup>CH<sub>3</sub>HgI was a direct cleavage that resulted in the production of <sup>14</sup>CH<sub>4</sub>, the amount of <sup>14</sup>CH<sub>4</sub> would be very small in comparison to the large pool of unlabelled methane. Thus, in order to produce the amount of <sup>14</sup>CO<sub>2</sub> observed in anaerobic samples, a tremendous amount of anaerobic methane oxidation must occur. Because anaerobic methane oxidation is very minimal, this suggests that demethylation in anaerobic

samples was an oxidative process.

In the samples incubated aerobically, it was not determined if the  $^{14}\text{CO}_2$  was the result of oxidative demethylation or whether it was the result of methane oxidation. Controls with  $^{14}\text{CH}_4$  added are required to determine if the amount of  $^{14}\text{CO}_2$  could have resulted from  $^{14}\text{CH}_4$  oxidation.

In conclusion, these results indicate that oxidative demethylation may be occurring or that the methane from methylmercury may be used as a bacterial substrate. If oxidative demethylation is occurring, an enzyme other than the organomercurial lyase is involved in the demethylation process. This indicates that an unknown mechanism of demethylation may exist in the environment. If an oxidative demethylating enzyme does exist, further research is needed to isolate and characterize the enzyme involved. Additional experiments also are required to distinguish between oxidative demethylation and the oxidation of methane from methylmercury.

These results also indicate that there is a difference in the products of demethylation in different aquatic habitats. These results suggest that  $^{14}\text{CO}_2$  was the primary product of demethylation in anaerobic habitats (high RI), and that less was produced aerobically (low RI).

## CHAPTER 4: DISCUSSION AND CONCLUSIONS

The primary site for mercury methylation in Lake Clara occurred in the surficial sediments of the densely vegetated backwater area and of the littoral-profundal transitional zone. This is of particular importance because these areas are important as the spawning and feeding regions of many lake fish (Lagler et al., 1977).

Methylation activity in a profundal sediment core was greatest at the surface and decreased with depth. Callister and Winfrey (1986) also observed that methylation activity was greatest in surface sediments of a sediment core from a mercury-contaminated site in the Upper Wisconsin River. The surficial maximum is of particular importance because surface sediments have the greatest potential to release methylmercury into the water column.

Net methylmercury production in the water column and attached microbial communities was low. The low rate of methylmercury production in the water column may in part be due to low microbial activity and low nutrient concentrations. Addition of organic materials significantly stimulates mercury methylation, suggesting the process is nutrient limited (Akagi et al., 1979; Callister and Winfrey, 1986; Wright and Hamilton, 1982). Aerobic conditions decrease methylation (Callister and Winfrey, 1986; Compeau and Bartha, 1984) and may have contributed to the low

methylation activity in the water column and attached microbial communities. The low M/D ratios ( $< 0.6$ ) in the water column and attached communities suggest that demethylation may play a role in the low net methylmercury production in those areas.

The composition of the microbial population may have also affected mercury methylation in the attached communities. Compeau and Bartha (1985) and Winfrey (1985) have suggested that sulfate-reducing bacteria are important agents of mercury methylation. Because sulfate-reducing bacteria are strict anaerobes inhibited by aerobic conditions, they would not be expected in attached communities. Their absence may also explain why methylation activity was low in the attached communities.

The greatest potential for methylation in surficial sediments occurred during a period from mid-July through September. In situ temperature was the only environmental variable that was significantly correlated to seasonal methylation in profundal surficial sediments. In situ temperature, however, only accounted for a small percentage of the seasonal variation in mercury methylation. Callister and Winfrey (1986) also observed that a seasonal peak in methylation in Wisconsin River sediments was not due solely to an increase in temperature. This suggests that other factors must influence the rate of mercury methylation in surficial sediments. Other potential factors may be

demethylation activity and oxic conditions.

The seasonal peak in methylation activity in surficial sediments incubated at the in situ temperature corresponded to a substantial decrease in demethylation activity. This suggests that increased methylation activity may be due to decreased demethylation rather than an increase in the actual methylation rate.

Seasonal variation of mercury methylation in surficial sediments may have been influenced by the oxic condition of the hypolimnion. The seasonal peak in methylation in profundal surficial sediments coincided with a complete depletion of dissolved oxygen in the lower hypolimnion. The decline in methylation activity in October also coincided with the reaeration of the hypolimnion during fall turnover.

The formation of methylmercury and its subsequent degradation are primarily the result of bacterial processes (Spangler et al., 1973a). Methylmercury degradation is an enzymatic detoxification mechanism which results in the formation of inorganic mercuric ion from methylmercury (Robinson and Tuovinen, 1984). Mercuric ion is then reduced by a second enzyme to  $\text{Hg}^0$  which is volatile and released from the cells and ecosystem (Colwell et al., 1976; Schottel et al., 1974). The purified organomercurial lyase isolated from mercury-resistant bacteria breaks the carbon-mercury bond of methylmercury, thus producing methane (Colwell et al., 1976; Olson et al., 1979; Schottel, 1978; Tezuka and Tonomura,

1976).

The production of  $^{14}\text{CO}_2$  from  $^{14}\text{CH}_3\text{HgI}$  in this study may indicate an oxidative enzyme is involved in the demethylation process or that the  $^{14}\text{CH}_4$  produced by demethylation can be used as a bacterial substrate, thus producing  $^{14}\text{CO}_2$ .

Bacterial methane oxidation is primarily an aerobic process requiring an oxygenase. Isotopic experiments have demonstrated the occurrence of anaerobic methane oxidation in sediment and water (Zehnder and Brock, 1980). However, the amount of anaerobic methane oxidation was very minute in comparison to the large pool of methane in anaerobic environments. In anaerobic Lake Clara samples, if demethylation of  $^{14}\text{CH}_3\text{HgI}$  was a direct cleavage that resulted in the production of  $^{14}\text{CH}_4$ , the amount of  $^{14}\text{CH}_4$  would be very small in comparison to the large pool of unlabelled methane. Thus, in order to produce the amount of  $^{14}\text{CO}_2$  observed in anaerobic samples, a tremendous amount of anaerobic methane oxidation must occur. Because anaerobic methane oxidation is minimal, this suggests that demethylation in anaerobic samples was an oxidative process.

Elevated levels of mercury have been reported in edible fish from naturally acidic or acidified lakes in Northern Wisconsin (Wiener, 1983), Maine (Akielaszek and Haines, 1981), Canada (MacCrimmon et al. 1983; Wren and MacCrimmon, 1983), and Sweden (Bjorklund et al., 1984; Lindqvist et al., 1984). Although methylmercury is the major form of mercury

in fish (Huckabee et al., 1979), the increased levels are not associated with increased methylation in sediments. A decrease in mercury methylation has been observed in acidified sediments (Ramlal et al., 1985; Steffan, 1984). In contrast, Xun et al. (in press) predicted that atmospheric acidification would increase the net rate of methylation in the water column.

Ramlal et al. (1985) observed that the rate of demethylation decreased to a lesser extent than methylation in acidified lake sediment. In a similar study, Xun et al. (in press) reported that in the water column and surface sediments experimental acidification reduced demethylation. However, the pH-induced changes in the rate of demethylation were smaller than for methylation.

In this study, acidification of surface sediments decreased methylation activity, while demethylation remained relatively constant until pH 4.5. These results suggest that sustained demethylation activity may play a role in the observed decrease in methylation activity in experimentally acidified surface sediments.

Although the effects of seasonal variables on demethylation were examined in this study, no variable was significantly correlated to demethylation activity. Additional research is required to identify the environmental variable(s) affecting demethylation activity in aquatic ecosystems. This study has shown that demethylation activity

may have a role in regulating net methylmercury production. Therefore, factors which influence demethylation activity may increase or decrease the net rate of methylmercury production.

This study also identified were the greatest potential for mercury methylation occurred in Lake Clara; however, the microbial populations responsible for methylation and demethylation were not identified. Additional research is required to characterize the microbial populations responsible for methylation and demethylation in different aquatic habitats, especially the role of sulfate-reducers in mercury methylation. Conditions which favor a certain microbial population may increase or decrease net methylmercury production.

Although the production of  $^{14}\text{CO}_2$  from  $^{14}\text{CH}_3\text{HgI}$  may indicate an oxidative demethylating enzyme exists, controls with  $^{14}\text{CH}_4$  added are needed to determine if the amount of  $^{14}\text{CO}_2$  observed in samples could have resulted from  $^{14}\text{CH}_4$  oxidation. If an oxidative enzyme does exist additional research is needed to isolate and characterize the enzyme involved.

The following conclusions have been derived from the results of this study:

- 1) the primary site for mercury methylation in Lake Clara occurs in the surficial sediments of the backwater area and the littoral-profundal transitional zone;

- 2) net mercury methylation in Lake Clara surficial sediments is greatest during a period from mid-July through September;
- 3) net methylmercury production may be significantly affected by demethylation activity, as well as, temperature and oxic conditions;
- 4) an enzyme may exist that oxidatively degrades methylmercury;
- 5) there is a difference in the products of demethylation in different aquatic habitats;
- 6) sustained demethylation activity may play a role in the decrease in the net rate of mercury methylation in experimentally acidified sediment.

## LITERATURE CITED

- Akagi, H., D. C. Mortimer, and D. R. Miller. 1979. Mercury methylation and partition in aquatic systems. *Bull. Environ. Contam. Toxicol.* 23:372-376.
- Akielaszek, J. J., and T. A. Haines. 1981. Mercury in the muscle tissue of fish from three northern Maine lakes. *Bull. Environ. Contam. Toxicol.* 27:201-208.
- American Public Health Association. 1981. Standard methods for the analysis of water and waste water. 15th ed. American Public Health Association. Washington, D. C.
- Andren, A. W., and J. O. Nriagu. 1979. The global cycle of mercury. In J. O. Nriagu (ed.). *The biogeochemistry of mercury in the environment.* Elsevier/North-Holland Biomedical Press, New York.
- Atlas, R. M. and R. Bartha. 1981. *Microbial ecology: fundamentals and applications.* Addison-Wesley Publishers.
- Beamish, R., and J. Van Loon. 1977. Precipitation loading of acid and heavy metals to a small acid lake near Sudbury, Ontario. *J. Fish. Res. Board Can.* 34:649-658.
- Berman, M. and R. Bartha. 1986a. Levels of chemical versus biological methylation of mercury in sediments. *Bull. Environ. Contam. Toxicol.* 36:401-404.
- Billen, G., C. Joiris, and R. Wollast. 1974. A bacterial methyl-mercury mineralizing activity in river sediments. *Water Res.* 8:219-225.
- Bisogni, J. J., and A. W. Lawrence. 1975. Kinetics of mercury methylation in aerobic and anaerobic aquatic environments. *J. Water Pollut. Control Fed.* 47:135-152.
- Bjorklund, I., H. Borg and K. Johansson. 1984. Mercury in Swedish lakes - its regional distribution and causes. *Ambio.* 13:119-121.
- Blum, J. E., and R. Bartha. 1980. Effect of salinity on methylation of mercury. *Bull. Environ. Contam. Toxicol.* 25:404-408.
- Callister and Winfrey, 1986. Microbial methylation of mercury in Upper Wisconsin River sediments. *Water Air Soil Pollut.* 29:(in press).
- Clarkson, T. W., R. Hamaday and L. Amin-Zaki. 1984. Mercury. p. 285-309. In J. O. Nriagu (ed.). *Changing metal cycles and human health.* Springer-Verlag. New York.

- Colwell, R. R., G. S. Sayler, and J. P. Nelson Jr. 1975. Role of bacteria in bioaccumulation of mercury in the oyster. *Appl. Microbiol.* 30:91-96.
- Colwell, R. R., S. G. Berk, G. S. Sayler, J. D. Nelson, and J. M. Esser. 1977. Mobilization of mercury by aquatic microorganisms. *Symp. Proc. Int. Conf. Heavy Metals.* 2:832-843.
- Colwell, R. R., G. S. Sayler, J. D. Nelson, and A. Justice. 1976. Microbial mobilization of mercury in the aquatic environment. pp. 473-487. In J. O. Nriagu (ed.). *Environmental Biogeochemistry* Vol. 2. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.
- Compeau, G., and R. Bartha. 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. *Appl. Environ. Microbiol.* 48:1203-1207.
- Compeau, G. C., and R. Bartha. 1985. Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediments. *Environ. Microbiol.* 50:498-502.
- Costerton, J. W., and G. G. Geesey. 1979. Microbial contamination of surfaces. *Sur. Contam.* 1:211-221.
- Cronan, C., W. Reiners, R. Reynolds, and G. Lang. 1978. Forest floor leaching: contributions from mineral, organic, and carbonic acids in New Hampshire subalpine forests. *Science.* 200:309-311.
- D'Itri, P. A., and F. M. D'Itri. 1977. *Mercury contamination: a human tragedy.* John Wiley and Sons. New York.
- Dillon, P. J., N. D. Yan, and H. H. Harvey. 1984. Acid deposition: effects on aquatic ecosystems. *CRC Crit. Rev. Environ. Control.* 13:167-194.
- Edwards, T., and B. C. McBride. 1975. Biosynthesis and degradation of methylmercury in human faeces. *Nature (London).* 253:462-464.
- Eilers, J., G. Glass, K. Webster, and J. A. Rogalla. 1983. Hydrologic control of lake susceptibility to acidification. *Can. J. Fish. Aquat. Sci.* 40:1896-1904.
- Evans, D. W., J. M. Wiener, and S. H. Horton. 1980. Trace element input from a coal burning power plant to adjacent terrestrial and aquatic environments. *APCA Journal* 30:567-575.
- Fletcher, M., and K. C. Marshall. 1982. Are solid surfaces of ecological significance to aquatic bacteria? *Adv. Microbiol. Ecol.* 6:199-236.
- Friberg, L., and J. Vostal. 1972. *Mercury in the environment.* CRC Press, Cleveland.

- Furukawa, K., T. Suzuki, and K. Tonomura. 1969. Decomposition of organic mercurial compounds by mercury resistant bacteria. *Agr. Biol. Chem.* 33:128-130.
- Furutani, A., and J. W. M. Rudd. 1980. Measurement of mercury methylation in lake water and sediment samples. *Appl. Environ. Microbiol.* 40:770-776.
- Gavis, J., and J. F. Ferguson. 1972. The cycling of mercury through the environment. *Water Res.* 6:989-1008.
- Geesey, G. G., R. Mutch, J. W. Costerton, and R. B. Green. 1978. Sessile bacteria: an important component of the microbial population in small mountain streams. *Limnol. Oceanogr.* 23:1214-1223.
- Haines, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. *Trans. Amer. Fish. Soc.* 110:669-707.
- Hamdy, M. K., and O. R. Noyes. 1975. Formation of methylmercury by bacteria. *Appl. Microbiol.* 30:424-432.
- Higgins, I. J., D. J. Best, R. C. Hammond, and D. Scott. 1981. Methane-oxidizing microorganisms. *Microbiol. Rev.* 45:556-590.
- Huckabee, J. W., S. A. Janzen, B. G. Baylock, Y. Talmi, and J. J. Beduchamp. 1978. Methylated mercury in brook trout (*Salvelinus fontinalis*): Absence of an in vivo methylating process. *Trans. Am. Fish. Soc.* 107:848-852.
- Huckabee, J. W., J. W. Elwood, and S. G. Hildebrand. 1979. Accumulation of mercury in freshwater biota. p. 277-302. In J. O. Nriagu (ed). *The biogeochemistry of mercury in the environment.* Elsevier/North-Holland Biomedical Press. New York.
- Hungate, R. E. 1969. A roll tube method for cultivation of strict anaerobes. *Methods Microbiol.* 3B:117-137.
- Irukayama, K. 1967. The pollution of Minamata Bay and Minamata disease. Proceeding of the Third International Conference on Advances in Water Pollution Research, Vol. 3, p. 153. Water Pollution Control Federation, Washington, D.C.
- Jensen, S., and A. Jernelov. 1969. Biological methylation of mercury in aquatic organisms. *Nature (London).* 223:753-754.
- Jernelov, A., and H. Lann. 1971. Mercury accumulation in food chains. *OIKOS.* 22:403-406.
- Jernelov, A., and A. L. Martin. 1980. Mercury in freshwater systems. Swedish Water and Air Pollution Research Institute. Publication B 550.

- Kozak, S., and C. W. Forsberg. 1979. Transformation of mercuric chloride and methylmercury by rumen microflora. *Appl. Environ. Microbiol.* 38:626-636.
- Kudo, A., H. Akagi, D. C. Mortimer, and D. R. Miller. 1977a. Equilibrium concentrations of methylmercury in Ottawa River sediments. *Nature (London)* 270:419-420.
- Lagler, K. F., J. F. Bardach, R. R. Miller, and D. R. May Passino. 1977. *Ichthyology*, 2nd ed. John Wiley and Sons.
- Landner, L. 1971. Biochemical model for the biological methylation of mercury suggested from methylation studies in vivo with Neurospora crassa. *Nature (London)*. 230:452-453.
- Lindqvist, O., A. Jernelov, K. Johansson, and H. Rodhe. 1984. Mercury in the Swedish environment. Global and local sources. National Swedish Environment Protection Board. Report snv pm 1816.
- MacCrimmon, H. R., C. D. Wren, and B. L. Gots. 1983. Mercury uptake by lake trout, Salvelinus namaycush, relative to age, growth, and diet in Tadenac Lake with comparative data from other Precambrian Shield lakes. *Can. J. Fish. Aquat. Sci.* 40:114-120.
- McBride, B. C., and T. L. Edwards. 1977. Role of methanogenic bacteria in the alkylation of arsenic and mercury. pp. 1-19. In: H. Drucker and R. E. Wildung (ed.). *Biological Implications of Metals in the Environment*. Energy Research and Development Administration. Washington, D. C.
- McDonald, D. G. 1983. The effects of H<sup>+</sup> upon the gills of freshwater fish. *Can. J. Zool.* 61:691-703.
- McLean, R. A. N., M. O. Farkas, and D. M. Findlay. 1980. Determination of mercury in natural waters. Sampling and analysis problems. pp. 151-173. In T. Y. Toribara, M. W. Miller, and R. E. Morrow (eds.). *Polluted Rain*. Plenum Press, New York.
- Moore, J. W. and S. Ramamoorthy. 1984. Mercury in heavy metals in natural waters: applied monitoring and impact assessment. Springer-Verlag. 125-160.
- Mottet, N. K., Cheng-Mei Shaw, and T. M. Burbacher. 1985. Health risks from increases in methylmercury exposure. *Environmental Health Perspectives*. 63:133-140.
- National Research Council. 1977. An assessment of mercury in the environment. National Academy of Sciences. Washington, D. C.
- Nelson, J. D., and Colwell, R. R. 1975. The ecology of mercury-resistant bacteria in Chesapeake Bay. *J. Microbiol. Ecol.* 1:191-218.

- Olson, B., T. Barkay, D. Nies, J. M. Bellama, and R. R. Colwell. 1979b. Plasmid mediation of mercury volatilization and methylation by estuarine bacteria. *Dev. Ind. Microbiol.* 20:275-284.
- Olson, B. H. and R. C. Cooper. 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. *Water Res.* 10:113-116.
- Olson, K. R., H. L. Bergman, and P. O. From. 1973. Uptake of methyl mercuric chloride and mercuric chloride by trout: a study of uptake pathways into the whole animal and uptake by erythrocytes in vitro. *J. Fish. Res. Board Can.* 30:1293-1299.
- Ramamoorthy, S., T. C. Cheng, and D. J. Kushner. 1983. Mercury speciation in water. *Can. J. Fish. Aquat. Sci.* 40:85-89.
- Ramlal, P. S., J. W. M. Rudd, and R. E. Hecky. 1986. Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. *Appl. Environ. Microbiol.* 51:110-114.
- Ramlal, P. S., J. W. M. Rudd, A. Furutani, and L. Xun. 1985. The effect of pH on methylmercury production and decomposition in lake sediments. *Can. J. Fish. Aquat. Sci.* 42:685-692.
- Reasoner, D. J., and E. E. Geldreich. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* 49:1-7.
- Robinson, J. B., and O. H. Touvinen. 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical and genetic analysis. *Microbiol. Rev.* 48:95-124.
- Rodgers, D. W., and F. W. H. Beamish. 1983. Water quality modifies uptake of waterborne methylmercury by rainbow trout, Salmo gairdneri. *Can. J. Fish. Aquat. Sci.* 40:824-828.
- Rudd, J. W. M., A. Furutani, and M. A. Turner 1980. Mercury methylation by fish intestinal contents. *Appl. Environ. Microbiol.* 40:777-782.
- Schindler, D. W., R. H. Hesslein, R. Wagemann, and W. S. Broecker. 1980. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. *Can. J. Fish. Aquat. Sci.* 37:373-377.
- Schottel, J. L., A. Mandal, D. Clark, and S. Silver. 1974. Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. *Nature (London)*. 251:335-337.

- Schottel, J. L. 1978. The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of Escherichia coli. J. Biol. Chem. 253:4341-4349.
- Silver, S., J. Schottel, and A. Weiss. 1976. Bacterial resistance to toxic metals determined by extrachromosomal R factors. pp. 899-917. In S. M. Sharpley and A. M. Kaplan (ed.). Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers Ltd., London.
- Spangler, W. J., J. L. Spigarelli, J. M. Rose, and H. M. Miller. 1973a. Methylmercury: bacterial degradation in lake sediments. Science. 180:192-193.
- Spangler, W. J., J. L. Spigarelli, J. M. Rose, R. S. Flippin, and H. H. Miller. 1973b. Degradation of methylmercury by bacteria isolated from environmental samples. Appl. Microbiol. 25:488-493.
- Steffan, R. J. 1984. Effect of acidification on mercury methylation and volatilization in an oligotrophic Northern Wisconsin lake. Masters thesis. University of Wisconsin-La Crosse.
- Stokes, P. M., S. I. Dreier, M. O. Farkas, and R. A. N. McLean. 1983. Mercury accumulation by filamentous algae: a promising biological monitoring system for methylmercury in acid stressed lakes. Environ. Pollut. B. 5:255-271.
- Stokes, P. M., R.C. Bailey, and G. R. Groulx. 1985. Effects of acidification on metal availability to aquatic biota with special reference to filamentous algae. Environmental Health Perspectives. 63:79-87.
- Summers, A. O., and S. Silver. 1978. Microbial transformations of metals. Ann. Rev. Microbiol. 36:637-672.
- Tezuka, T., and K. Tonomura. 1976. Purification and properties of an enzyme catalyzing the splitting of carbon-mercury linkages from mercury resistant Pseudomonas K-62 strain. J. Biochem. 80:79-87.
- Tomlinson, G. H., R. J. P. Brouzes, R. A. N. McLean, and J. Kadlecek. 1980. The link between acid precipitation, poorly buffered waters, mercury and fish. pp. 134-137. In D. Drablus and A. Tollan (ed.). Ecological impact of acid precipitation. SNSF Project, Norwegian Interdisciplinary Research Programme. Oslo.
- Tonomura, K., T. Nakagomi, F. Futai, and K. Maeda. 1968. Studies on the action of mercury-resistant microorganisms on mercurials. J. Ferment. Technol. 46:506-512.
- Tonomura, K., F. Furukawa, and M. Yamada. 1971. Microbial conversion of mercury compounds. pp. 115-133. In F. Matsumura, G. Mallory, and T. Misato (ed.). Environmental Toxicology of Pesticides. Proceedings of the US-Japan Seminar. EOISO, Japan, Oct. 1971.

- Toribara, T. Y., C. P. Shields, and L. Koval. 1970. Behaviour of dilute solutions of mercury. *Talanta*. 17:1025-1028.
- Vaituzis, Z., J. D. Nelson, Jr., L. W. Wah, and R. R. Colwell. 1975. Effects of mercuric chloride on growth and morphology of selected strains of mercury resistant bacteria. *Appl. Microbiol.* 29:275-286.
- Wiener, J. E. 1983. Comparative analyses of fish populations in naturally acidic and circumneutral lakes in Northern Wisconsin. U. S. Fish and Wildlife Service. Eastern Energy and Land Use Team, FWS/OBS-80/40.16.
- Winfrey, M. R. 1984. The microbial production of methane, pp. 153-219. In R. M. Atlas (ed.). *Petroleum Microbiology*. MacMillan, New York.
- Winfrey, M. R., and J. G. Zeikus. 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. *Appl. Environ. Microbiol.* 33:275-281.
- Winfrey, M. R. and J. G. Zeikus. 1979. Anaerobic metabolism of immediate methane precursors in Lake Mendota. *Appl. Environ. Microbiol.* 37:213-221.
- Winfrey, M. R.. 1985. The role of methane-producing and sulfate-reducing bacteria in mercury methylation. Abstracts of the Annual Meeting of the American Society for Microbiology. 85:271.
- Winfrey, M. R., W. J. Pekarske and R. J. Steffan. 1986. The importance of microbial and abiological volatilization of mercury in an oligotrophic northern Wisconsin lake. Abstracts of the Annual Meeting of the American Society for Microbiology. 86:304.
- Wood, J. M. 1971. Environmental pollution by mercury. *Adv. Environ. Sci. Technol.* 2:39-56.
- Wood, J. M. 1980. The role of pH and oxidation-reduction potentials in the mobilization of heavy metals. pp. 223-236. In, T. Y. Toribara, M. W. Miller and R. E. Morrow (eds). *Polluted Rain*. Plenum Press, New York.
- Wood, J. M., F. S. Kennedy and C. G. Rosen. 1968. Synthesis of methyl mercury compounds by extracts of a methanogenic bacterium. *Nature (London)* 220:173-174.
- Wood, J. M., A. Cheh, L. J. Dizikes, W. P. Ridley, S. Rakow, and J. R. Lakowicz. 1978. Mechanisms for the biomethylation of metals and metalloids. *Federation Proc.* 37:16-21.
- Wren, C. D., H. R. MacCrimmon, and B. R. Loescher. 1983. Examination of bioaccumulation and biomagnification of metals in a Precambrian Shield lake. *Water, Air, Soil Pollut.* 19:277-291.

Wright, D. R., and R. D. Hamilton. 1982. Release of mercury from sediments: effects of mercury concentration low temperature and nutrient addition. *Can. J. Fish. Aquat. Sci.* 39:1459-1466.

Xun, L., N. E. R. Campbell, and J. W. M. Rudd. In press. Measurements of mercury methylation and demethylation as a function of pH in the water column and surface sediments of acidified and circumneutral lakes.

Zehnder, A. J. B., and T. D. Brock. 1980. Anaerobic methane oxidation: occurrence and ecology. *Appl. Environ. Microbiol.* 39:194-204.

## APPENDIX A: ADDITIONAL METHODS

### Field Sampling

Flocculent sediments and water were collected with a peristaltic pump (Horizon Ecology Co.). The peristaltic pump was equipped with a T-shaped inlet so samples were collected horizontally, minimizing vertical mixing (Winfrey and Zeikus, 1979). For surficial sediment sampling, the tubing was slowly lowered to the sediment-water interface until small amounts of flocculent sediment appeared in the pump effluent (Steffan, 1984). The tubing was then lowered 5-10 cm to obtain the surficial sediment. It was necessary to move the suspension rope back and forth horizontally while pumping in order to maintain continuous collection of surficial sediment. Sediments were pumped into  $N_2$ -gassed, acid-washed glass bottles under a continuous stream of  $N_2$  and sealed with butyl rubber stoppers. Consolidated sediments were sampled with an Eckman dredge, homogenized in a dishpan and stored in  $N_2$ -gassed, acid-washed glass bottles.

Subsurface water samples were pumped directly into 1-L acid-washed, polyethylene bottles until 1 in from the top to prevent anoxia and allow mixing. Surface water was collected by submerging inverted polyethylene bottles to a depth of approximately 0.5 m. Bottles were filled with water by turning upright, rinsed twice, and refilled.

Periphytic samples were collected by aseptically scraping a 16 cm<sup>2</sup> area of substrate (Geesey et al., 1978). A piece of sterile aluminum foil with a square removed (16 cm<sup>2</sup>) was laid over the substrate and the surface scraped clean with a sterile spatula (Geesey et al., 1978). The material was transferred to sterile, acid-washed BOD bottles (Wheaton) containing 15 mL of filter-sterilized lake water. Lake water was sterilized by filtering through a prefilter (Millipore, type AP) and a 0.45 um Metricel membrane filter (Gelman Sciences, Inc.) in an Swinnex assembly (Millipore) attached to a 50 mL syringe. All samples were transported on ice and processed within 24 h of collection.

Dissolved oxygen and temperature measurements were taken at 1-m intervals in the water column with a Yellow Springs Instrument model 54A dissolved oxygen meter. A plastic-coated lead weight was suspended from the DO probe to maintain a vertical cable position. Field pH readings were recorded for surface water and profundal sediment floc with a Fisher model 107 pH meter.

#### Experimental Acidification and Sulfate Addition

Sediments were acidified to pH values ranging from 5.5-2.5 with 0.5N H<sub>2</sub>SO<sub>4</sub>. After acidification, the sediments were stirred and allowed to equilibrate for 2 h. The pH was remeasured and the sediments reacidified if necessary. The

ambient sediment pH (6.2) was increased to pH 7 and 8 with 6N NaOH, allowed to equilibrate for 2 h with stirring, and the pH remeasured. Sulfate control samples for demethylation were prepared by adding 0.25M Na<sub>2</sub>SO<sub>4</sub> to samples in an equimolar amount to the sulfate added from acidification. Aliquants of pH-adjusted sediment were removed from the preparation bottles for the methylation and demethylation assays. Sediments were processed and incubated using strict anaerobic technique (Hungate, 1969). Sediment samples were delivered under a stream of O<sub>2</sub>-free N<sub>2</sub> to sample bottles gassed with N<sub>2</sub>. Samples were incubated for 24 h at 15 C. Methylation and demethylation assays were conducted as described in Chapter 2.

### Demethylation Assay

The demethylation assay was conducted as basically described in Chapter 2. Water and flocculent sediments were delivered to sample bottles with a 20-mL glass syringe (MULTIFIT, Becton-Dickinson). Sandy sediments were delivered to sample bottles with a 50-mL plastic syringe. A tube attached to the plastic syringe was placed in the sediment bottle after vigorously agitating the bottle, and the plunger of the syringe pulled back and forth until the syringe contained a homogenate of sandy sediment. The sandy sediment was then quickly delivered to the sample bottles to prevent

settling of the sand.

The  $^{14}\text{CH}_3\text{HgI}$  was then added to the reaction bottles, containing 30 mL of sample and a stir bar. The bottles were stoppered while still gassing with  $\text{N}_2$ , and the gassing needle was removed by placing pressure on the stopper and pulling the needle out. A small amount of distilled water was placed on the bottle rim and the stopper pushed down as far as possible. The longer glass tube should be below the sample surface but not in contact with the bottom. The sample bottles were then vortexed for 1 min. The clamps were checked after vortexing to make sure none had popped open.

The  $^{14}\text{CH}_3\text{HgI}$  was added to the samples at a concentration of 1 ug Hg. The isotope, a suspension in a screw-capped tube, was kept in an ultracold freezer until needed. At the time needed, the suspension was thawed, vortexed, and centrifuged at  $500 \times g$  for 1 min. An aliquant of the supernatant was removed and 10 uL of the aliquant counted in PCS scintillation cocktail (in triplicate). The average mercury concentration in 10 uL of supernatant was calculated and the volume added to samples adjusted so as to add 1 ug Hg. The volume of the original aliquant of supernatant removed was then replaced by an equal volume of distilled water and the isotope refrozen.

Incubations were terminated by injecting samples with 2 mL of 6N HCl. The needle (21 gauge, 1.25 in) was inserted

into the stopper at the bottle rim parallel to the glass tubing. The acidified samples were then vortexed for 1 min.

The products of demethylation were gassed from the reaction bottles for 30 min (see Fig. 2-3 for assembly). The amber latex tubing was attached to the teflon tubing by sliding it over the teflon tubing and rolling the latex tubing back on itself. After all the amber latex tubing was connected, the clamp on the exit tubing was opened. The samples were gassed with compressed air at approximately 50 mL/min. A pipe cleaner was used on the latex tubing exiting the tube furnace to hold it upright and prevent it from pinching closed. After gassing for the time period, the compressed air was turned off, the clamps were closed, and all latex tubing disconnected from the teflon tubing to prevent siphoning of the scintillation cocktail.

Bristled pipe cleaners were used to clean the glass tubing of the reaction bottles.

#### Regeneration of Copper Oxide

Copper oxide was regenerated when the top 5 cm in the column were reduced to elemental copper (brass colored). Copper oxide was regenerated by flushing the hot copper with oxygen. When the copper began to glow, the flow of oxygen was stopped until the copper oxide cooled. This process was repeated until all the copper in the tube was oxidized as indicated by the black color of copper oxide.

### Media Preparation and Isolation of Hg<sup>r</sup> Bacterial Strains

R2A media was prepared as described by Reasoner and Geldreich (1985). HgCl<sub>2</sub> (Morton Thiokol, Inc.) was added to the media from a sterile stock solution at a final concentration of 25 ppm Hg. HgCl<sub>2</sub> was added after autoclaving the media to prevent loss of mercury due to volatilization. Mercury plates were used within one week of preparation.

Sediment from Lake Clara was serially diluted (1:100) in sterile, phosphate-buffered distilled water, and 0.1 mL of a single dilution series (10<sup>-2</sup>, 10<sup>-4</sup>, 10<sup>-6</sup>) spread on R2A-Hg plates. After incubating for 48 h at room temperature, individual colonies of different morphology were randomly removed from the 10<sup>-5</sup> dilution and streaked for isolation on R2A-Hg plates. Isolated colonies were then transferred to R2A-Hg broth and allowed to grow for 48 h at room temperature.

## APPENDIX B: ADDITIONAL RESULTS

This Appendix contains brief descriptions of results not discussed in Chapters 2 and 3. These additional results are pertinent to this thesis because of their connection with Chapters 2 and 3.

Fig. B-1. The effects of experimental sediment acidification on methylation and demethylation. Vertical bars represent the standard error of the mean (n = 3).

(●) % methylation, (■) % demethylation,

(★) % demethylation in sulfate controls.

<sup>a</sup> 1.725 mmoles  $\text{SO}_4^{=}$  (concentration equimolar to the sulfate concentration in pH 2.5 samples)

<sup>b</sup> 0.188 mmoles  $\text{SO}_4^{=}$  (concentration equimolar to the sulfate concentration in pH 4.25 samples)

<sup>c</sup> 0.131 mmoles  $\text{SO}_4^{=}$  (concentration equimolar to the sulfate concentration in pH 4.50 samples)

<sup>d</sup> 0.075 mmoles  $\text{SO}_4^{=}$  (concentration equimolar to the sulfate concentration in pH 4.75 samples)

Methylation decreased rapidly with a decrease in pH, while demethylation remained constant until approximately pH 4.5. The addition of sulfate did not have a significant effect on (p = 0.043) on demethylation. Steffan (1984) reported that sulfate addition did not have a significant effect on methylation at ambient pH. These results suggest that sustained demethylation activity may play a role in the decreased net methylation activity in acidified surficial sediments.



Fig. B-2. Time course of methylation and demethylation in surficial sediments. Incubations were terminated and the assays conducted at the specified times. Vertical bars represent the standard error of the mean ( $n = 3$ ). (●) % methylation, (■) % demethylation. Demethylation activity increased rapidly during the first 3 h possibly depressing the net rate of methylation during that period. After 3 h the methylation and demethylation activities were approximately linear over the remaining time period ( $r^2 = 0.994$  and  $0.966$ , respectively) which validates the use of only one time point (24 h) in all experiments.

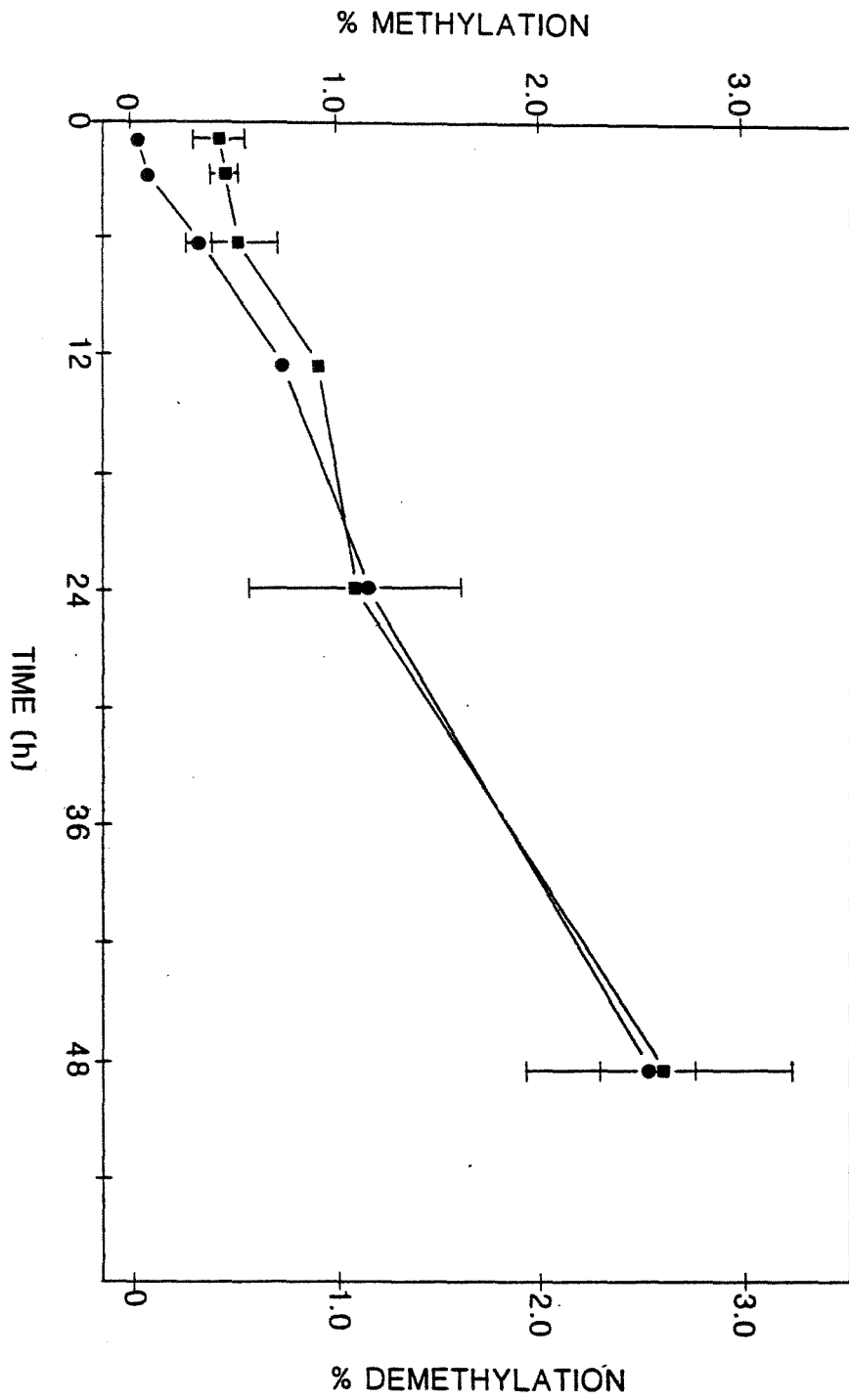


Fig. B-3. The effect of methylmercury concentration on demethylation in profundal surficial sediment. Vertical bars represent the standard error of the mean (n = 3).

The amount of mercury resulting from degradation of methylmercury was directly proportional to the added amount of mercury ( $r^2 = 0.996$ ). Thus, effective comparisons could be made between demethylation activity on different sampling dates. The nanograms of mercury resulting from demethylation of  $^{14}\text{CH}_3\text{HgI}$  was calculated by multiplying the total DPM in traps by the nanograms of added mercury, and then dividing by the DPM in  $^{14}\text{CH}_3\text{HgI}$  stock solution at concentration of added mercury. Normalization was required to eliminate mercury concentration as a variable. Normalized demethylation rates were calculated as described in Chapter 2.

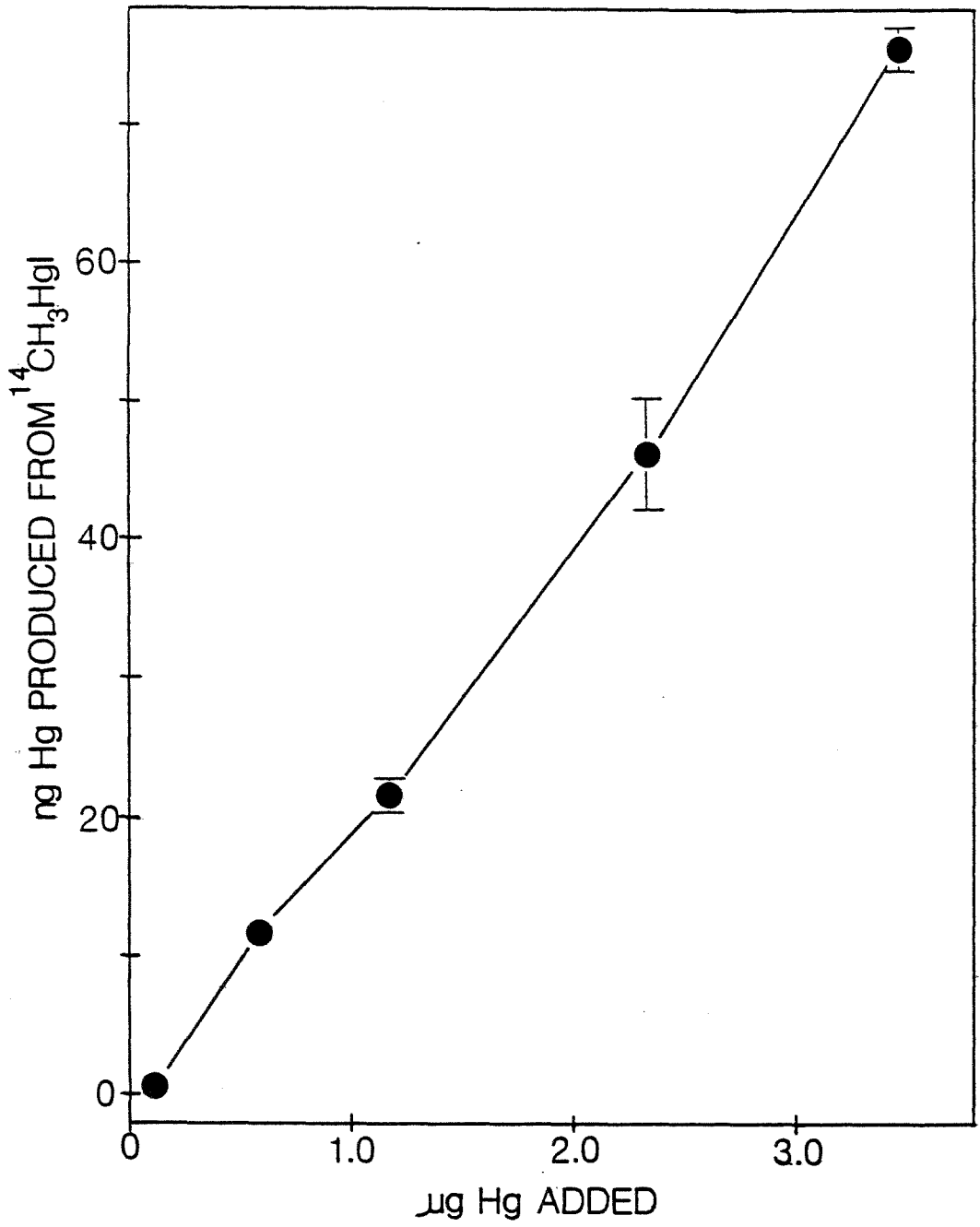


Fig. B-4. Dissolved oxygen and temperature depth profile in the water column of Lake Clara (July 22, 1985).

(●) dissolved oxygen, (■) temperature. Arrows represent the depths at which water samples were collected. This was the lake stratification during the spatial sample collection.

DISSOLVED OXYGEN (ppm)

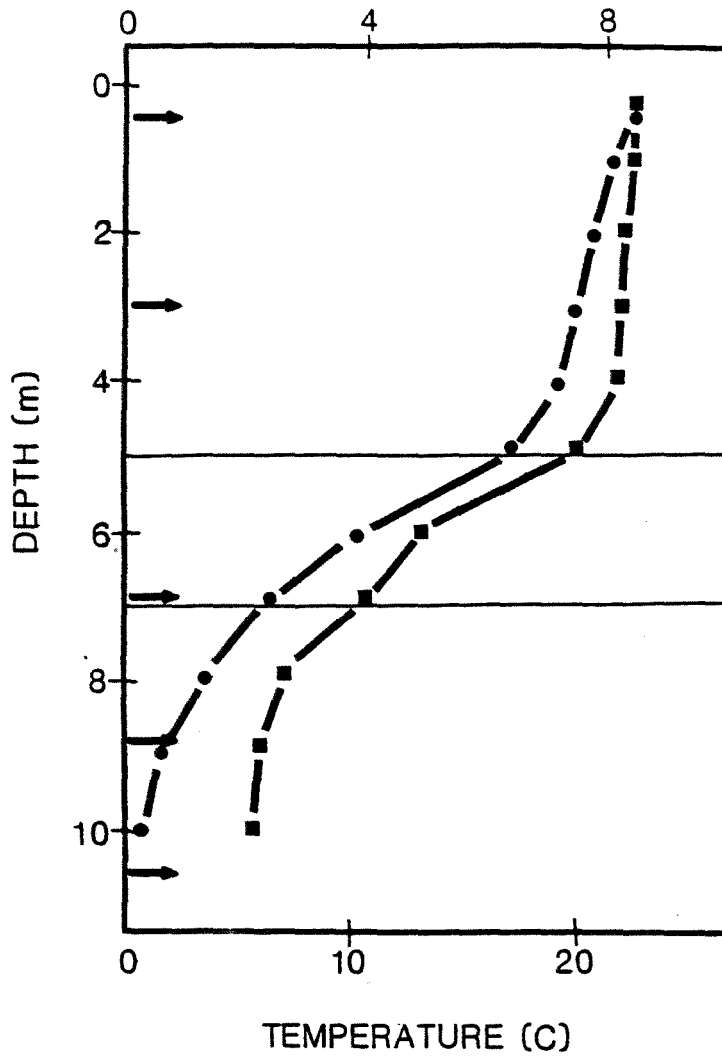


TABLE B-1. Mercury methylation and demethylation  
in procedural blanks and killed sediment samples<sup>a</sup>

treatment	% methylation <sup>b</sup>	% demethylation <sup>b</sup>
no additions	0.52 ± 0.017	2.41 ± 0.13
autoclaved	0.008 ± 0.002	0.053 ± 0.013
HCl killed	0.003 ± 0.001	0.021 ± 0.013
NaOH killed	0.047 ± 0.008	0.018 ± 0.006
formalin	0.020 ± 0.001	0.088 ± 0.026
procedural blank	0.007 ± 0.006	0.055 ± 0.017

<sup>a</sup> surficial profundal sediments collected November 14, 1985

<sup>b</sup> mean ± SE (n = 3)

TABLE B-2. Recovery of standard additions in different sample types for mercury methylation and demethylation assays.

sample	% recovery (meth.) <sup>a</sup>	% recovery (demeth.) <sup>b</sup>
highly organic sediments	98.4 ± 1.4 <sup>c</sup>	105.1 ± 1.1 <sup>c</sup>
sandy sediments	84.8 ± 6.2 <sup>d</sup>	103.1 ± 1.2 <sup>c</sup>
periphyton	96.6 ± 5.6 <sup>c</sup>	-----
surface water	100.0 ± 5.8 <sup>c</sup>	105.0 ± 1.2 <sup>c</sup>

<sup>a</sup> samples collected November 14, 1985.

<sup>b</sup> samples collected August 19, 1985.

<sup>c</sup> mean ± SE (n = 3)

<sup>d</sup> mean ± SE (n = 9)

TABLE B-3. Seasonal variations of methylation, demethylation, and chemical and physical parameters in profundal surficial sediments and lower hypolimnion.

date	% methylation <sup>a</sup>	% demethylation <sup>a</sup>	sediment pH <sup>a</sup>	sediment alkalinity <sup>a</sup> (ueq CaCO <sub>3</sub> /L)	% C <sup>a</sup>	% DW <sup>a</sup>	T (C)	St <sup>b</sup>	anoxic hypolimnion ( $< 0.5$ ppm)
4-27-85	0.06	-----	5.7	364	55.2	1.62	5	3.5	-
5-18-85	0.33	0.84	5.9	1605	44.6	2.10	6	3.3	-
6-6-85	0.24	1.92	5.2	1241	55.0	1.60	7	4.5	-
6-24-85	0.45	3.07	5.7	785	53.4	2.37	7	4.3	-
7-8-85	0.42	4.24	5.8	1569	50.6	1.87	7	4.0	+
7-22-85	0.51	0.73	6.1	1741	50.2	1.30	6	3.5	+
8-5-85	0.64	0.73	6.3	1913	52.2	1.25	6	4.0	+
8-19-85	0.82	0.75	6.2	1054	51.3	1.20	7	3.5	+
9-3-85	0.26	1.13	6.1	617	52.4	0.53	8	3.7	+
9-13-85	0.67	1.40	6.2	1302	50.5	0.57	8	3.9	+
9-29-85	0.95	1.08	6.2	925	55.5	0.80	9	2.3	-
10-14-85	0.21	1.19	6.2	789	55.5	0.80	8	3.0	-
11-14-85	0.24	1.22	6.2	652	33.8	0.80	3	3.5	-
12-23-85	0.08	0.90	6.3	810	81.2	1.33	2	2.7	-
4-21-86	0.16	1.80	5.6	839	60.2	1.10	9	4.0	-

<sup>a</sup> mean (n = 3)

<sup>b</sup> secchi transparency

TABLE B-4. Spatial variations of methylation, demethylation, and physical variables.

site	water depth (m)	% methylation <sup>a</sup>	% demethylation <sup>a</sup>	ambient temperature (C)	% carbon <sup>a</sup>	% dry weight <sup>a</sup>
1	2	1.16	0.43	23	65.0	2.20
2	1	1.65	0.57	23	57.0	2.30
3	1	1.27	1.19	23	0.57	65.0
4	3	1.17	1.57	23	0.72	64.5
5	5	2.83	1.32	23	43.6	1.45
6	7.5	2.46	0.92	11	48.0	1.50
7	10.5	0.51	0.73	6	50.2	1.30

<sup>a</sup> mean (n = 3)