THE EFFECT OF ACID STRESS ON METHYLATION AND DEMETHYLATION OF MERCURY IN WHOLE-LAKE ECOSYSTEMS

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Submitted to

The Faculty of Graduate Studies

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In Partial Fulfillment

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Master of Science

By

Luying Xun

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To My Mother and Father

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ABSTRACT

Mercury methylation and demethylation activities in the water column of fresh water lakes are confirmed in this study. When water pH was lowered within the natural water pH ranges from 8.5 to 5.0, methylation increased and demethylation decreased. Methylation rates in water appeared to increase exponentially with an increase in Hg^{2+} concentration while demethylation rates increased linearly with an increase in CH_3Hg^+ concentration. Methylmercury production was cyclic in pattern in water samples due to the presence of a concomitant demethylation activity. Epilimnion waters had higher methylation and demethylation activities than did the hypolimnion waters. Epilimnetic water samples taken from acidified lakes usually had higher methylating activity than circumneutral lakes.

Studies of intact sediment cores indicated that most the mercury in sediment was methylated at the sediment-water interface. Low pH increased methylmercury production in core samples.

The study indicates that the "acid rain" stress combined with the increased loading of mercury arising from polluted precipitation may account for the increased mercury content of fish harvested from "acid rain"-stressed lakes.

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Introduction

High mercury concentrations in fish harvested from acidified waters have been reported from the United States, Norway, and Sweden(Jernelov 1976 , Brouzes et al. 1977, Scheider et al. 1979, Kelso et al. 1982, and Wiener et al 1983) and have been the subject of a number investigations.

The source of both "acid rain" and many airborne trace metals including mercury is believed to be mainly fossil fuel combustion which emanates oxides of sulphur and nitrogen and also metallic mercury. In the atmosphere these compounds will be further oxidized to sulphuric acid, nitric acid and mercuric ion and precipitated with rainfall(NRC 1981 and Joensuu 1971). Acid precipitation, coupled with the enhanced input of mercury from polluted precipitation might be one possible explanation for the increase in mercury content noted in fish taken from acid-stressed, poorly buffered inland waters; however, the reason has been still very poorly understood.

Jackson et al (1980) found that the adsorption of the isotope $^{203}Hg^{2+}$ to organic material in sediments at pH 5.1 was much lower than at near-neutral pH. They also suggested that acidification of a soft-water lake would probably not result in appreciable displacement of Hg^{2+} ions from organic bottom sediments by the action of H^{+} ion. Schindler et al. (1980) demonstrated that acidification could inhibit the transfer of Hg^{2+} from water to bottom sediments. Thus acidification could increase the mercury concentration in the water column by inhibiting the removal of Hg^{2+} from the water. Mercuric ion might be methylated in the water and then accumulated by fish. However the methylation of mercury in the water is poorly understood. Furutani and Rudd(1980) first demonstrated methylation activity in the water column

in a mercury polluted lake. However, of 13 separate tests during the summer of 1979 they only found methylation activity five times. Topping and Davies(1981) reported that methylmercury was formed from inorganic mercury added to see water in large in situ enclosures and found in settlement material. The concentration of methylmercury in the settlement material was positively related to primary production. There have been no subsequent investigations on methylation of mercury in the water column.

Most investigations emphasized methylation activity in sediments to determine whether lowered pH favors the formation of methylmercury. For example, Ramlal(1983) found that methylation rates decreased in sediment samples incubated anaerobically as the pH was lowered. Baker et al.(1983) found that formation of methylmercury in sediment samples incubated aerobically for 2 weeks only occurred in the pH range of 5.5-6.5 and the methylmercury formed in the sample was reduced as pH was progressively lowered by H_2SO_4.

Considering the observations of the effect of pH change on mercury methylation in sediments, it may seem incongruous that fish tend to have increased levels of mercury in lakes which had been stressed by acidic precipitation(Jernolov et al. 1976 and Brouzes et al. 1977).

To gain a comparatively greater insight into the effects of acid stress on methylation and demethylation in the whole lacustrine ecosystem, the activities of methylation and demethylation in sediments, in water columns and at water-sediment interfaces were investigated. In view of the general lack of information concerning these activities in the water column, it was felt necessary to direct our research emphasis on this aspect of the lacustrine environment.

Historical

1.1 Mercury cycle in the environment

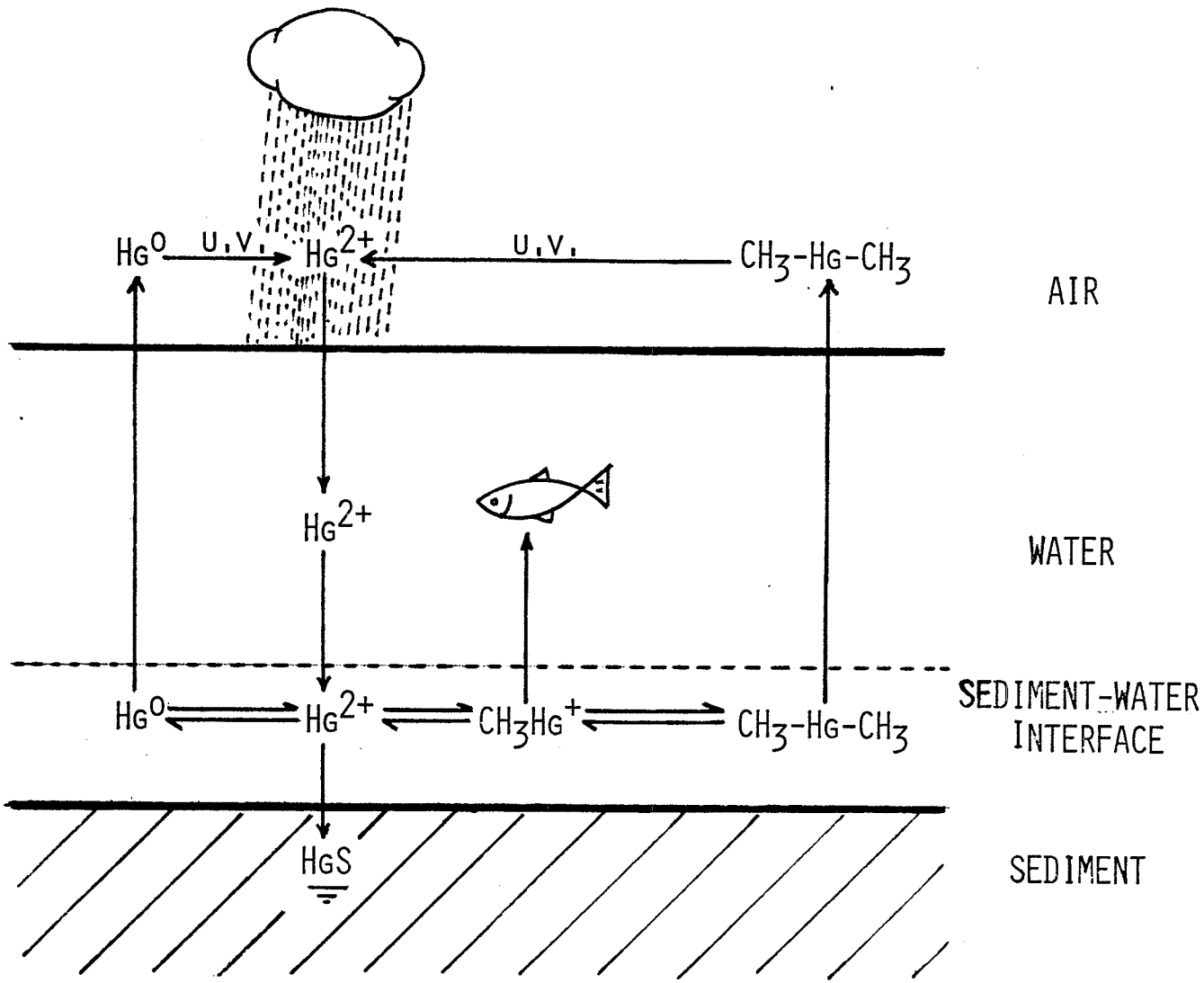
Mercury is a heavy metal existing mainly in five forms in nature, metallic mercury Hg^0, mercurous ion Hg_2^{2+}, mercuric ion Hg^{+2}, methylmercury CH_3Hg^+ and dimethylmercury CH_3HgCH_3. Inorganic mercury exists in equilibrium in water due to chemical dismutation (Moser and Voight 1957):

$$Hg_2^{2+} = Hg^0 + Hg^{2+} \text{----------Eq.1}$$

Hg^0 is the less toxic of the five forms, is volatile and can thus escape from water into the air. Hg^{2+} is more toxic, has a high affinity for sulfhydryl groups in the active sites of enzymes and causes enzyme inactivation(Friberg and Vostal 1972). The most toxic form of mercury for human beings is methylmercury; because it is lipid soluble it can be taken up by fish and accumulated in their bodies due to its long retention time in their fatty tissues. Methylmercury is a potent neurotoxin for human beings(Summers and Silver 1978).

The mercury cycle in the lacustrine ecosystem is mainly driven by bacteria(Fig.1). It is generally considered that the most active site for biotransformation of mercury is at the sediment-water interface(Robinson and Tuovinen 1984). In this interface microbial activity is higher and the mercury concentration is also higher than in the water column(Parks et al. 1976 and Wetzel 1983). The biological conversion of mercuric ion to methylmercury is called methylation with the principal product being methylmercury(Bisogni and Lawrence 1975). The conversion from methylmercury to mercuric ion and then metallic mercury is called demethylation. Both metallic mercury and dimethylmercury have high vapor pressures and can be carried by wind for

Fig. 1 A simplified mercury cycle in lakes.


Hg^0 U,V Hg^{2+} U,V $CH_3-Hg-CH_3$
AIR
Hg^{2+}
WATER
Hg^0 Hg^{2+} CH_3Hg^+ $CH_3-Hg-CH_3$
SEDIMENT-WATER INTERFACE
HgS
SEDIMENT

a long distance(Wolfe et. al. 1973). It has been found that less than 1% of the atmospheric mercury exists as dimethylmercury (Johnson and Braman 1974 and Soldano et. al. 1975). In the air, both metallic mercury and dimethylmercury can be changed into mercuric ion by ultra violet light. Mercuric ion will then return to the earth's surface as rainfall for further cycling(Brosset 1981 and Summers and Silver 1978).

The main form of mercury in both marine and fresh waters is mercuric ion and the main form of mercury in the air is metallic mercury(Colwell et. al. 1976 and Johnson and Braman 1974).

The methylmercury formed by bacteria can be accumulated in fish tissues in three ways; a) by direct absorption of CH_3Hg^{+1} from the water and concentration due to the long retention time in fatty tissues(Wood et. al. 1978), b) by direct formation of methylmercury by intestinal bacteria or by bacteria in the surface slime of fish with subsequent absorption into the fish tissue(Rudd et. al. 1980), or c) the ingestion by fish of preformed methylmercury in their food supply, ie. the methylmercury-producing bacteria are eaten by various larger microscopic forms and the latter are eaten by fish(Nishimuru and Kumagai 1983).

In the anaerobic lake sediments, most mercury is precipitated as mercuric sulfide, which will be sealed in sediment(Fagerstrom and Jernelov 1972).

1.2 Mercury sources

Sources of mercury in the environment are both natural and anthropogenic in origin. In nature, mercury is believed to originate primarily from the weathering of the earth's crust(Weiss, et. al. 1971). Other sources, like volcanism, also provide mercury(Gavis and Ferguson 1972). Mercury released into the environment due to natural degassing of

earth's crust is estimated at $2.5x10^4$ to $5.0x10^5$ tons $year^{-1}$; the total level of mercury in the ocean is estimated at $2x10^8$ tons(Weiss, et.al. 1971).

Anthropogenic source of mercury include those associated with mining and its use in the chlor-alkali process of wood pulping; paint production, in agriculture, pharmaceutical and the paper industries where it is variously used for its disinfectant, fungicidal and catalytic properties. Over 12,500 tons of mercury per year are released into the environment as a result of the direct human use of the metal world wide (D'Itri 1972). Mercury is also released to the atmosphere from smelting processes for other metals, the ores of which may contain mercury, and from the burning of fossil fuels, which release an estimated 10,000 to 60,000 tons of mercury $year^{-1}$(Joensuu 1971 and Summers and Silver 1978). Therefore, human activities are estimated to account for between $2x10^4$ and $7x10^4$ tons of mercury $year^{-1}$ to be released into the atmosphere and water supply, which is much less than that released by natural processes. Although man has had a negligible effect on a global scale by his indiscriminate discharge of mercury into the environment, he has created serious local and regional problems.

1.3 Mercury pollution

Some of the world's, worst pollution episodes were caused by mercury, as in the "Minamata outbreak" of mercury poisoning referred as "Minamata disease"(D'Itrti 1972). Minamata Bay, Kyushu, Japan was contaminated by methylmercury from a vinyl chloride-producing factory upstream from the bay area. Methylmercury was concentrated in the fish and shellfish ,so that a continuous normal diet of the fish resulted in further concentration of methylmercury to neurotoxic levels in the local

human populace(Tsubaki and Irukayama 1977 and Nriagu 1979). Between 1953 and 1970, 120 cases of severe intoxication occurred, and 43 persons died as a result.

The Iraqi episode occured from 1970 to 1972 where about 459 people died and 6,530 people were hospitalized after eating bread made from wheat contaminated by methylmercury fungicide(Bakir et. al. 1973 and Takizawa 1979). Methylmercury pollution has also reported from Sweden and New Mexico(D'Itri and D'Itri 1977).

The final example of mercury pollution reported here is from Canada. In this case, biomethylation was implicated as the source of toxic organic mercury. The Wabigoon-English River system in northwestern Ontario is one of the most severely mercury polluted waterways in the world(Armstrong and Hamilton 1973). The mercury pollution was derived mostly from the effluents of a chlor-alkali plant which operated from 1963 to 1975. From 1963 to 1970, unrestricted quantities of inorganic mercury (approximately 9-11 metric tons in total) were released into the river system. In 1970 the factory was advised to treat its effluents and mercury discharge was reduced by about 99 per cent.

The inorganic mercury in this river was methylated by microorganisms and the methylmercury concentration was found to be very high in fish taken from the river(Armstrong and Hamilton 1973, Furutani and Rudd 1980, Jackson 1979 and Parks 1976). Various sub-lethal health problems have been attributed to the consumption by the local people of fish contaminated with methylmercury(Health and Welfare Canada 1973, Clarkson 1976 and Wheatley 1979). Ever since the potential danger from mercury poisoning became apparent, the local people have been urged to stop eating fish from the river and the Government has been providing

the communities with fish from uncontaminated sources free of charge.

Since methylmercury can be produced from inorganic mercury by microorganisms, its bioaccumulation along the food chain can cause human neurotoxic problems, for this reason mercury pollution has received a great deal of attention recently.

1.4 Biomethylation of mercury

Biomethylation was first demonstrated by Jensen and Jernelov in 1969. They found that lake sediments were capable of converting inorganic mercury salts into methylmercury and the use of autoclaved controls proved that this reaction was catalyzed by microorganisms. Since then many reports about the methylation of mercury by bacteria have been published. The biological methylation of mercury has been demonstrated under anaerobic conditions by bacteria in sediments(Furutani and Rudd 1980 and Jensen and Jernelov 1969), in intestines of humans(Rowland et. al. 1975) and fish (Rudd et. al. 1980).

Formation of methylmercury under aerobic conditions has also been demonstrated by soil and sediment organisms(Rogers 1976 and Vonk and Sijpesteijn 1973) and by bacteria in a lacustrine water column(Furutani and Rudd 1980).

Mercury methylation occurs widely in nature. The mechanism of biomethylation involves the nonenzymatic transfer of a methyl group from methylcobalamin to Hg^{2+}(Bertilsson and Neujahr 1971, DeSimone et. al. 1973, Imura et. al. 1971 and Wood 1974) The identity of the mercury methylating agents has been investigated(DeSimone et. al. 1973). Of the main natural methylating agents--(i)S-adenosylme- thionine, (ii) N^5-methyltetrahydrofolate derivatives and (iii) methyl- cobalamin(a derivative of vitamine B12)--only methylcobalamin can transfer its

methyl group with its electrons, that is as a negatively charged methyl carbanion(CH_3^-). It would seem that the transfer of the methyl group to Hg^{2+} has to take place in this form because Hg^{2+} is positively charged. The methylation takes place by the mercuric ion electrophilically attacking the Co-C bond. The methylcobalamine-dependent mercury methylation can take place within cells. Hg^{2+} is taken up in cells by an active transport system mediated by an unspecific enzyme. When Hg^{2+} ions are methylated in the cells, CH_3Hg^+ will leave the cell by diffusion(Wood et. al. 1978). Bacteria also can carry out mercury methylation by excreting methylcobalamin, which reacts with mercury exogenously(Summers and Silver 1978). Enzymatic transfer of the methyl group to mercury has also been proposed based on the fact that some bacteria have not evolved a methylcobalamine metabolism but can methylate mercury(Landner 1971, Vonk and Sijpesteijn 1973).

Although methylmercury is more toxic than inorganic mercury to human beings, methylation is one approach bacteria use for detoxification of mercury. In some cases(Landner 1971 and Hamdy et al 1975), it has been shown that bacteria which are able to methylate mercury can tolerate higher concentration of mercury in their environment. The ecological advantage of B_{12}-dependent biomethylation is best illustrated by B_{12}-independent and B_{12}-dependent strains of Clostridium cochlearium(Pan-Hou and Imura 1982). The B_{12}-independent strain is capable of synthesizing methylcobalamine and methylating mercury, whereas the B_{12}-dependent strain, which is an auxotroph derived from a B_{12}-independent strain by mutation, is incapable of methylating mercury. Both strains transport Hg^{2+} into cells at the same rate but the B_{12}-dependent strain is much more sensitive to Hg^{2+} and is inhibited by

at least a 40-fold lower concentration of Hg^{2+} than the B_{12}-independent strain. This result clearly demonstrated that Clostridium cochlearium uses biomethylation as a mechanism for detoxification, giving the organism a clear advantage in mercury-contaminated systems. Indeed, at comparable concentrations CH_3Hg^+ was no more toxic than Hg^{2+} to marine water microbial communities(Jonas et. al. 1984).

There are several reports on the chemical processes of methylmercury production. Akagi and Takabatake(1973) reported that inorganic mercury was photomethylated by irradiation with ultraviolet light and in the dark by such compounds as methanol, acetic acid and propionic acid as methyl donors. Mercury also can be methylated by concentrated humic acid and fulvic acid extracted from lake sediments(Nagase et. al. 1982). But chemical methylation contributes less to methylmercury production in the environment than biomethylation does, as illustrated by the reduction in methylation following autoclaving of environmental samples(Jensen and Jernelov 1969 and Olson and Cooper 1976).

1.5 Effect of environmental factors on biomethylation

Under anaerobic conditions in human faeces, methylmercury was produced in a cyclic pattern. Methylmercury reached the highest concentration at the 2nd day and then decreased, increasing again at the 10th day(Edwards and McBride 1975). Methylmercury has been shown to be formed from $HgCl_2$ added to lake sediment incubated aerobically. During 50 days of incubation methylmercury appeared in cycles. Periods of methylmercury production were followed by decreases in the amount of methylmercury and concomitant increases in Hg^0--the product of microbial degradation of CH_3Hg^+(Spangler et. al. 1973). Even in pure culture,

Enterobacter aerogense produces methylmercury in a cyclic pattern. Methylmercury formation was observed to be maximum after 3 days incubation, rapidly decreasing after 10 days. However, after 20 days the methylmercury concentration increased again under both aerated and non-aerated conditions(Hamidy et. al. 1975).

A comparison of aerobic and anaerobic methylation of $HgCl_2$ in San Francisco Bay sediments indicated that methylmercury formation was faster and resulted in higher net production under anaerobic conditions. Although in pure culture studies, the production of methylmercury by E.coli and Enterobacter aerogenes were lower under anaerobic condition than under aerobic condition(Hamdy et. al. 1975 and Vonk and Sijpesteijn 1973).

Hydrogen sulfide may be evolved in anoxic sulphur-containing river or lake sediments. The formation of mercuric sulfide, which has an extremely low solubility in water($K_{sp}=10^{-53}$), is very likely to reduce the availability of inorganic mercury for biomethylation(Fagerstrom and Jernelov 1972). Methylmercury is formed from mercuric sulfide by aerobic organic sediments but at much lower rates(100 to 1000 times slower) than those observed for formation from $HgCl_2$(Fagerstrom and Jernelov 1971). Methylmercury was not produced from HgS under anaerobic conditions(Yamada and Tonomura 1972). No methylmercury was formed under anaerobic conditions, presumably because of the stability of mercuric sulfide under the low redox potential prevailing. Under aerobic conditions sulfide is slowly oxidized to sulfate releasing of Hg^{2+} which is methylated. However, in the natural environment, the Hg^{2+} was found to be actively methylated in anaerobic FeS-containing sediment-floc samples(Furutani and Rudd 1980). Probably the geochemical dissociation

of FeS($K_{sp}=10^{-16}$) and the sulfide binding of mercuric ion as HgS($K_{sp}=10^{-53}$) does not occur quickly enough to severely inhibit methylation.

It is also interesting to note that hydrogen sulfide aids the volatilization of mercury(Craig and Bartlett 1978) The reaction is given below:

$$CH_3Hg^+ + HS \longrightarrow (CH_3Hg)_2 + H^+$$
$$\longrightarrow (CH_3)_2Hg + HgS + H^+ \text{-------Eq. 2}$$

The same investigators have also demonstrated that diffusion of H_2S in sediment significantly reduces the concentration of methylmercury in the sample relative to a control.

Microorganisms can methylate mercury both aerobically and anaerobically, thus transferring inorganic mercury into organic mercury which can be accumulated in food chains. The rate of methylation is also dependent upon several other variables including the concentration and availability of Hg^{2+}, composition of the microbial population, pH, temperature, and synergistic or antagonistic effects of chemical and biological processes. For example, methylation rate in sediment increased linearly with increasing in mercury concentration(Rudd et al. 1983). When pH was changed in sediment from 5.5 to 9.5, total methylmercury production was not changed, but the main methylation product was shifted from monomethylmercury to dimethylmercury(Fagerstrom and Jernelov 1972).

1.6 Biodemethylation of mercury

In aquatic ecosystems, methylmercury concentration was found to be in a relative steady-state condition(Parks et. al. 1984 and Wood 1974). This is partially due to the presence of bacteria capable of degrading

methylmercury as well as those able to produce it.

Spangler and colleagues (1973) demonstrated the degradation of methylmercury by lake sediment bacteria. One ml of lake sediment was transferred into 100ml of trypticase soy broth(TSB) containing 25ug of $CH_3{}^{203}HgBr$. $^{203}Hg^0$ was evolved during the growth of the mixed culture as compared to that of uninoculated control containing methylmercury bromide. After 4 days incubation approximately 35% methylmercury was released as Hg^0. They isolated four pure cultures which had the ability to degrade methylmercury from this mixed culture. Bacterial demethylation has also been found in river sediment and in human faeces(Billen et. al. 1974 and Edwards and McBride 1975).

Microbial demethylation requires two enzymes: 1)the organomercurial lyase which break down the mercury-carbon bond:

$$CH_3Hg^+ \longrightarrow CH_4 + Hg^{2+} \text{----------Eq.3}$$

and 2)the mercuric reductase which catalyzes the following reaction:

$$Hg^{2+} + NAD(P)H + H^+ \longrightarrow Hg^0 + NAD(P)^+ + 2H \text{-----Eq.4}$$

The mechanism of demethylation was studied in Pseudomonas sp K62, where it was first reported that a metallic mercury-releasing enzyme present in cell-free extracts was responsible for catalyzing both the reduction of Hg^{2+} and organomercurials to elemental mercury(Furukawa and Tonomura 1972a and 1972b). In subsequent work, however, it has been shown that the original preparation contained the mercuric reductase enzyme and that two separate organomercurial lyase enzymes(designated S-1 and S-2) were present in the cell-free extracts(Tezuka and Tonomura 1976 and 1978).

The S-1 enzyme catalyzes the splitting of carbon-mercury bonds of both aryl- and alkylmercury compounds. This enzyme is capable of catalyzing the decomposition of methylmercury, ethylmercury, phenylmercury and p-hydroxymercuribenzoate(Tezuka and Tonomura 1976). The S-2 enzyme catalyzes the splitting of carbon-mercury linkages of arylmercury compounds. The enzyme is capable of catalyzing the decomposition of phenylmercury and p-hydroxymercuribenozoate, but not methylmercury. Other organomercurial lyase and mercuric reductase have been purified from E.coli K12 strain KJ53-1 carrying the plasmid R831(Fox and Walsh 1982 and Schottel 1978).

Bacteria which contain mercuric reductase and organomercurial lyase are resistant to mercury and organomercurials. The enzymes are coded by genes on plasmids(Izaki 1977, Komura et. al. 1971, Olson et. al. 1979, Schottel et. al. 1974, Summers and Silver 1972). The organomercurial lyase break down the Hg-C bond to release Hg^{2+}(Tezuka et. al. 1976) and the mercuric reductase reduces Hg^{2+} to Hg^{0}(Fox and Walsh 1982 and Summers and Silver 1972), Hg^{0} volatilizes from the growth environment, thereby allowing the bacteria to grow.

More detailed studies have provided information on the tolerance range of mercury-resistant plasmid-bearing bacteria(Hg^{r}) to mercury and organomercury compounds. The Hg^{r} plasmids fall into "narrow-" and "broad-spectrum" classes of resistance. The "narrow-spectrum" plasmids confer the ability to volatilize mercury only from inorganic mercury, the "broad-spectrum" plasmids confer the ability to volatilize mercury from both inorganic and organic compouds(Robinson and Tuovinen 1984).

A methylmercury-decomposing strain of Clostridium contains a plasmid. When the plasmid is cured by the treatment with acridine dye, the bacterium shows the ability to methylate mercury. The authors suggested a possible role of plasmids in controlling the mercury biotransformation in the two opposite directions in a single bacteria: methylation in the absence of the plasmid and demethylation in the presence of it(Pan-Hou et. al. 1980).

Both mercuric reductase and organomercurial lyase have been demonstrated to be inducible in nature(Clark et. al. 1977, Foster and Nakahara 1979, Foster et. al. 1979, Furukawa and Tonomura 1969 and 1972b, Schottel et. al. 1974 and Summers and Silver 1972). In "broad-spectrum" mercury-resistant strains, both mercuric reductase and organomercurial lyase were inducible by either inorganic mercury or organic mercury. However, induction was not strictly coordinate(Clark et. al. 1977 and Weiss et. al. 1977).

Genetic analysis revealed the existence of a mer operon, which contains at least four genes: mer A, which encodes the mercuric reductase enzyme; mer B, encoding the organomercurial lyase enzyme in "broad-spectrum" resistant strain; mer T, the gene believed to govern Hg^{2+} uptake; and mer R, which encodes the regulatory protein responsible for the inducibility of the system. The system was suggested under positive control(Dempsey and McIntire 1979, Dempsey and Willetts 1976, Foster and Nakahara 1979, Foster et. al. 1979, Jackson and Summers 1982, Miki et. al. 1978, Summers and Kight-Olliff 1980 and Tanaka et. al. 1976).

The mapping of "broad-resistance" plasmids was accomplished by using a combination of deletion mapping, restriction analysis, cloning of restriction fragments and transposon insertion techniques(Lane and Chandler 1977, Novick et. al. 1979 and Weiss et. al. 1977).The results indicate separate genetic loci for the mer A and mer B genes and the two genes may be transcribed from different promoters but are regulated in the same manner.

To conclude, both biomethylation and biodemethylation widely occur in the environment and both have ecological advantages for the bacteria that catalyze the processes. The processes are influenced by environmental factors both biological and nonbiological.

Materials and Methods

2.1.Sampling locale

The field work was conducted in the Experimental Lakes Area(ELA), northwesten Ontario, during summer 1984. A total of 11 lakes were used for this study, Lakes 114, 223, 227, 239, 302 N(north basin), 302 S(south basin), 303, 305, 373, 382 and 623. The area, maximum depth, degree of eutrophication and average epilimnion pH of the lakes during summer 1984 are given in Table 1.

Lakes 114, 223 and 302 S have received H_2SO_4 additions since 1979, 1976 and 1982 respectively as part of an experimental acidification program. L.302 N has received HNO_3 since 1982 in the same program. The initial pH values of Lakes 114, 223, 302 N,and 320 S were 6.0, 6.7, 6.7, and 6.6 respectively(Cruishunk 1984 and Schindler and Turner 1982).

2.2.Sediment samples collection, manipulation and incubation.

Sediment samples used for measurement of methylation and demethylation were collected at a depth of 2M from L.114, 303 and at 13M from L.302 N by a modified Ekman grab, which sampled 233 cm^2 of surface area(Burton et. al. 1973). The sediment at the water-sediment interface was transferred into 2.5L acid bottles until each bottle was completely filled to minimize exposure to atmospheric oxygen.

Several bottles of sediment from each sampling site were pooled and mixed well under a deoxygenated nitrogen atmosphere(Hungate 1969) to ensure homogeneity. One liter subsamples were dispensed under oxygen-free nitrogen into 2.5L glass bottles. After addition of sediment, the gas phase of each container was flushed with nitrogen for a few minutes. The bottles were sealed with two-hole silicone stoppers

Table 1 Characteristics of 11 lakes in ELA

Lake	*Lake Surface area (hectares)	*Maximum depth (meter)	pH mean	**Degree of eutrophication
114	12.1	5.0	5.6	oligotrophic
223	30.2	11.2	5.3	oligotrophic
227	5.0	10.0	8.5	eutrophic
239	56.1	30.4	7.0	oligotrophic
302 N	12.8	13.8	6.5	oligotrophic
302 S	10.9	10.6	5.5	oligotrophic
303	9.9	2.5	8.0	oligotrophic
305	52.0	32.7	7.0	oligotrophic
373	28.3	>16.4	7.0	oligotrophic
382	37.3	11.9	7.0	oligotrophic
623	>30.0	>16.4	7.0	oligotrophic

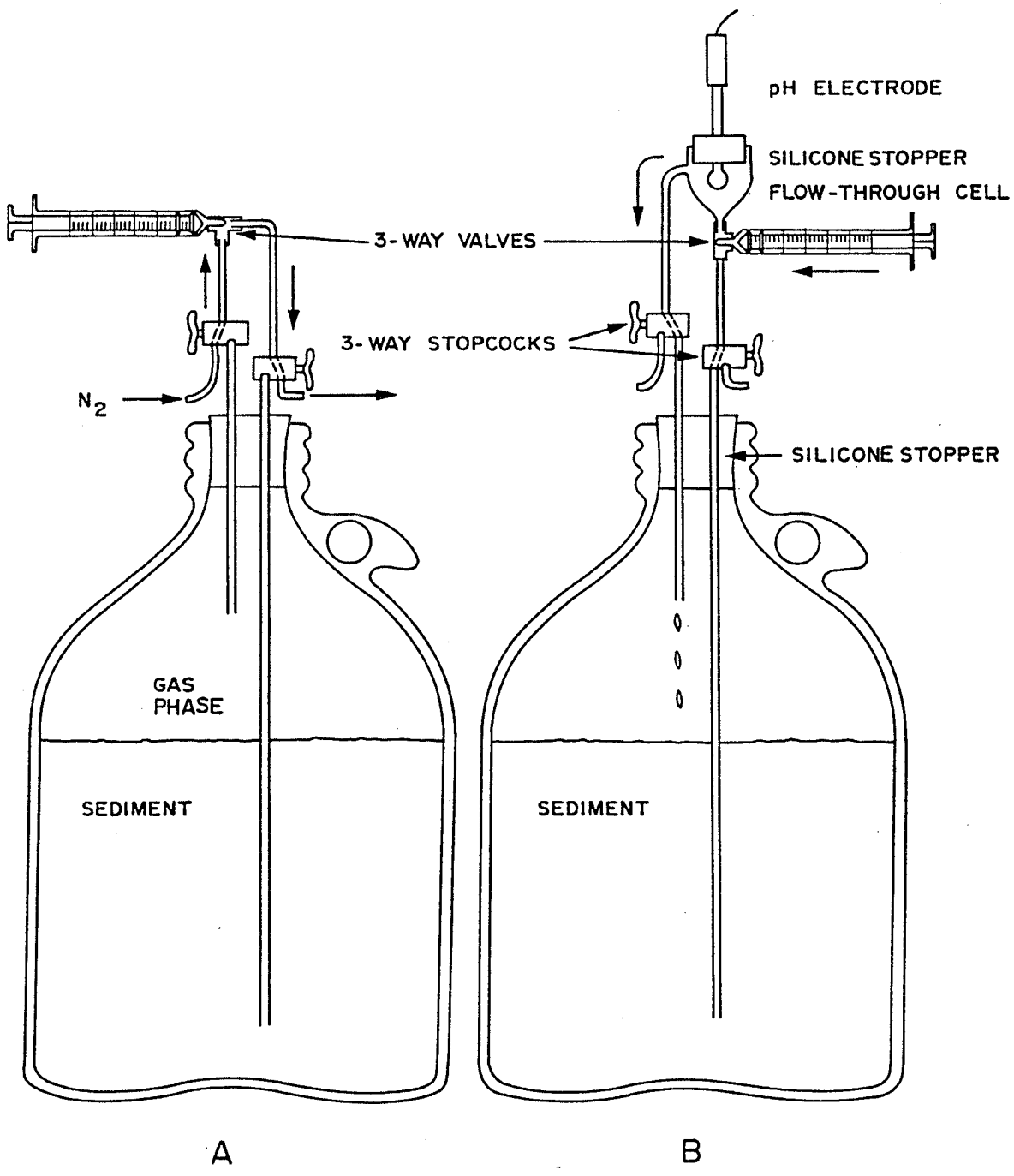
* Cleugh and Hauser 1971 and Brunskill and Schindler 1971.
** Schindler, Pers. comm.

fitted with 3-way glass stopcocks(Fig.2). One stopcock was used to sample the gas phase and the other for sediment. The samples were incubated in the dark at room temperature. Interior pressure was monitored by attaching a (5 lb.)pressure gauge to the gas port. A slight positive pressure was maintained to reduce the possibility of oxygen invasion into the containers. When a large amount of sediment was removed, a measured amount of nitrogen was added to re-establish a slightly positive pressure(1lb.). Sediment pH was adjusted by addition of acid or NaOH, and measured with a flow-through cell equipped with a glass combination pH electrode. After flushing with nitrogen, sediment was circulated through the cell and the pH was measured. Between readings, the electrode was rinsed with distilled water and re-calibrated.

For methylation and demethylation assays sediment was withdrawn anaerobically from the sediment port with 50ml plastic syringes and added to incubation bottles. For methylation experiments 100ml sediment was anaerobically added to 300ml BOD bottles and 2uCi of $^{203}HgCl_2$(1uCi/1ug Hg) was added to each sample. The top was water-sealed. Each test used 3 incubation bottles: duplicate tests and a control. The control sample was "fixed" before incubation by addition of 1ml of 4N HCl. After incubation for 12 hours at room temperature(22 ± 2 ^{o}C) in the dark, the reaction was terminated by addition of 1ml HCl and the methylmercury produced in the sample was measured.

For demethylation, 50 ml sediment was anaerobically added to each 125 ml reagent bottle and 14000 dpm of $^{14}CH_3HgI$(10,000dpm/0.1ug) was added. The incubation bottle was sealed with a 2-hole, No.2 rubber stopper(Ramlal et al. in prep.). A piece of 4mm diameter glass tubing

Fig. 2 Modified 2.5L acid bottles used to adjust pH of sediments.


pH ELECTRODE
SILICONE STOPPER
FLOW-THROUGH CELL
3-WAY VALVES
3-WAY STOPCOCKS
N_2
SILICONE STOPPER
GAS PHASE
SEDIMENT
SEDIMENT
A
B

was inserted through each of the holes(Fig.3). One piece was flush with the bottom of the stopper and the other extended approximately 1 cm past the stopper bottom and was connected to a disposable glass pipette with a short length of Tygon tubing. The disposable glass pipette extended into the sediment. Each test used 3 incubation bottles: duplicate tests and a control. The control was "fixed" before incubation. The samples were incubated at room temperature(22 ± 2 ^{o}C) in the dark for 12 hours. After termination by addition of 1ml of 3M H_2SO_4, demethylation activity was measured.

2.3.Measurement of methylation

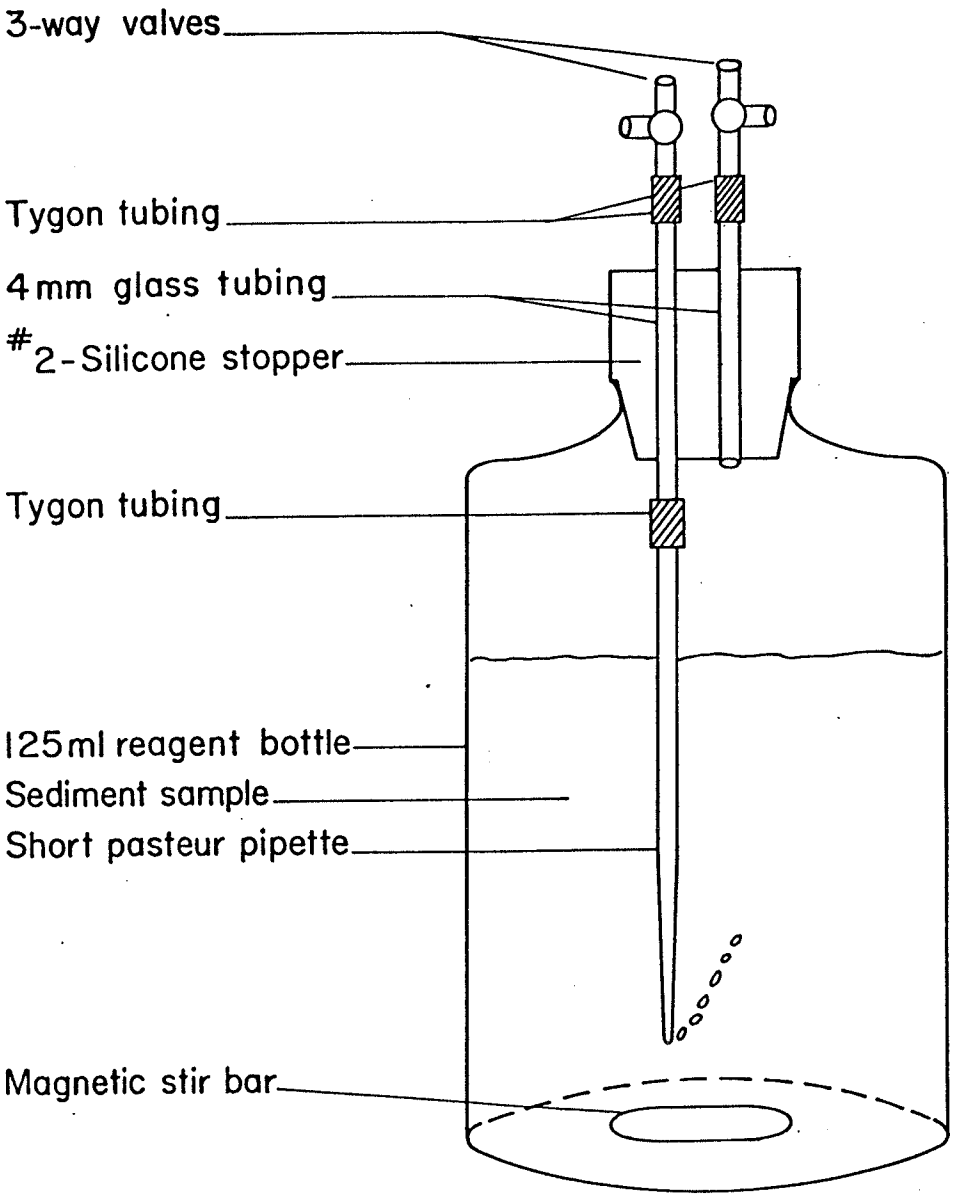
The radioactive assay method developed by Furutani and Rudd(1980) was used to measure the methylation activity in these lakes. Inorganic mercury as mercury-203 mercuric chloride was added to the samples at the rate of 1uCi per 150ml water sample. After incubation for 24hours or for variable periods as decided by experimental design,the mercury-203 methyl mercury was extracted by the procedure as follows:

1) 2ml 0.5M $CuSO_4$ solution and 10ml 3M NaBr in 11% H_2SO_4 were added to each sample. The sediment sample was shaken by hand for 3 minutes then held until the floc had settled.

2) 60-90ml of the aqueous phase of each sediment sample or 162ml of each water sample was transferred to a 250ml separatory funnel and 20ml of scintanalyzed toluene(Fisher) was added. The samples were shaken for 3 minutes and held until the aqueous and organic phases had separated.

3) The aqueous phase was removed, and the remaining toluene phase was partially dried with 1.0g anhydrous Na_2SO_4. The toluene was decanted into a 50ml Erlenmeyer flask and further dried by 0.5g

Fig. 3 Apparatus used for sediment demethylation measurement.


3-way valves
Tygon tubing
4mm glass tubing
#2-Silicone stopper
Tygon tubing
125ml reagent bottle
Sediment sample
Short pasteur pipette
Magnetic stir bar

anhydrous Na_2SO_4.

4) 10ml dried toluene was placed in an acid washed 20ml screw-cap test tube to which 5ml 0.0025M sodium thiosulphate in 20% ethanol were added. The tube was shaken for three minutes and stored until the aqueous and organic phases had separated.

5) 3ml of the lower aqueous phase was pipetted into a 5ml glass stoppered test tube to which 1ml of 3M KI and 1ml scintillation grade benzene(Fisher) were added and shaken for 3 minutes.

6) A 600ul portion of the extracted $CH_3{}^{203}HgI$ concentrated in the benzene phase was assayed for radioactivity by liquid scintillation counting in plastic minivials(Fisher) containing 5 ml of Scinti-Verse I cocktail(Fisher).

Samples were counted in a Beckman 7000 Liquid Scintillation Counter for 10 minutes. The extraction efficiency was 97 to 100%(Furutani et. al. 1980).

2.4.Measurement of demethylation

The radioactive method developed by Ramlal et. al.(in prep.) with a little modification was used to measure demethylation activity. Since the process of demethylation produces methane from methylmercury, the amount of methane produced was used for demethylation measurement. $^{14}CH_3HgI$(Amersham) was added to the samples at the rate of 28,000 DPM pre 150ml water sample. After incubation for 24 hours or for variable periods as decided by the experimental design, the samples were stripped by bubbling with oxygen or compressed air(50ml/min) for 1 hour, while the sample was stirred. The gas stripped from the sample passed through a dimethylmercury trap(100g KBr and 15g HgBr in 1L distilled water, Spangler et.al. 1973) and then passed through a 50cm Vycor tube(Fisher)

packed with copper oxide and heated in a tube furnance at 450^{0}C. The $^{14}CH_4$ produced from $^{14}CH_3HgI$ was combusted to $^{14}CO_2$ and then collected in a CO_2 trap consisting of 10ml Scinti-Verse I, 2ml CO_2 MET(Amersham) and 2ml ethanol in a glass scintillation vial(Fisher). The samples were counted in a Beckman 7000 Liquid Scintillation Counter for 10 minutes.

2.5. Water sample collection, manipulation and incubation.

Samples of epilimnetic water from all the lakes were taken approximately 10 cm below the surface by submerging an 8-liter polyethylene bottle. The bottles were rinsed with the sampling water twice before samples were taken. Samples were returned to the field lab immediately and approximately 1.2 L of each sample was transferred to a 2-L Erlenmeyer flask, and received the necessary amount of H_2SO_4 or NaOH to adjust the pH as appropriate. An 150 ml subsample from each pH-adjusted sample was then transferred to an acid-washed BOD bottle for methylation or demethylation test. Usually 1uCi of $^{203}HgCl_2$(1uCi/1ug Hg) was added to 150ml water samples for methylation measurements and 28000DPM of $^{14}CH_3HgI$(28000dpm/0.28ug Hg) was added to 150ml water samples for demethylation measurements. Samples were incubated at 22$\pm$2 ^{0}C in the dark for 1 day or decided by particular experimental design.

The incubation bottles used in demethylation experiments were different from those used for sediment samples. In the latter case, BOD bottles capped by ordinary water-sealed BOD bottle stoppers were used. To terminate the experiment, samples were cooled at -10 ^{0}C for 1 minute then the stoppers were removed, acid added and stoppers replaced as soon as possible. The stoppers should be kept wet all the time to prevent gas from escaping. Samples were then stored at 4 ^{0}C. When stripping, BOD

stoppers were replaced by the rubber stoppers as used for sediment samples. Though some methane produced by demethylation could escape from the incubation bottle in the process, it was probably quite small and unlikely to invalidate the method for comparing the effect of pH change on demethylation activities.

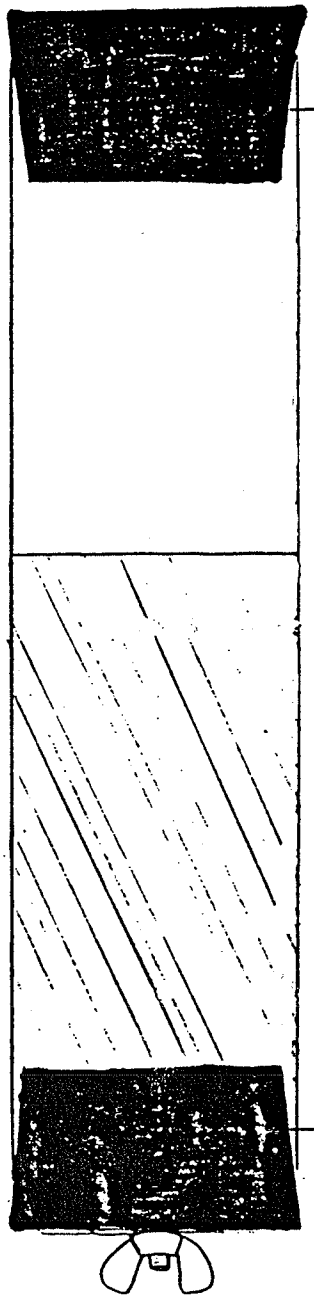
2.6.Water profile sample collection, manipulation and "in situ" incubation

Water profile samples were taken by a field peristaltic pump and transferred into acid-washed BOD bottles; overflow was permitted to minimize atmospheric oxygen contamination. 2uCi of $^{203}HgCl$(1uCi/1ug Hg) or 56000DPM of $^{14}CH_3HgI$(10,000/0.1ugHg) was added directly into the seperate bottles for methylation or demethylation assays. The bottles were put back into the lake at the depth where the samples were taken. Methylation and demethylation activities were stopped by addition of 1ml concentrated HCl or concentrated H_2SO_4 respectively after incubation bottles were retrieved. Each test used 3 incubation bottles: 2 duplicate tests and a control. After termination, 150 ml of subsamples were used for methylation and demethylation measurements. In order to retain maximium methane in the water phase during the demethylation experiments, incubation bottles were brought back to the field lab and stored at 4 ^{0}C. When stripping the sample, we poured out 150ml water and the remaining 150 ml subsample was used for demethylation measurement.

2.7. Core sample collection, manipulation and incubation

The core tube was constructed from Plexiglas tubing(ID 5 cm) cut into 20 cm lengths(Fig.4). The core was sealed with a No. 11 rubber stopper at the top and a No. 10 rubber stopper at the bottom. The No. 10 stopper was modified by drilling a hole through the center of the

Fig. 4 Plexiglas tubing with intact sediment core.


No. 11 RUBBER STOPPER
SURFACE WATER
PLEXIGLAS
TUBING
SEDIMENT
No. 10 RUBBER STOPPER

stopper and layering two washers, a 4 cm and a 2 cm washer on either side of the stopper held securely with a bolt and wing nut. Tightening or loosening of the wing nut adjusted the diameter of the stopper to facilitate tightening or loosening of the stopper in the core tube. Intact cores consisted of 8-11 cm of sediment with 9-12 cm of the water above and without air bubbles. The cores were taken from epilimnetic sediments by diver and returned to the field lab submerged in lake water to eliminate problems of leakage of pore water through the bottom of the core. Methylation experiments were initiated by adding 2 uCi of $^{203}HgCl_2$ (1uCi/1ug Hg) to the overlying water and replacing the stopper. The pH of overlying water was adjusted by addition of H_2SO_4 or HCl. The cores were incubated at lab temperatures equivalent to those prevailing at "in situ" locations.

After incubation for 1 day, the top stopper was removed, the water phase(~ 180-240 ml)was pipetted into a BOD bottle for methylmercury measurement. After all of the overlying water was removed from the core tube, a second empty core tube was placed on the top of the core tube, the sediment core in the first tube was forced up into the top tube to the desired depth by loosening the wing nut on the bottom stopper and applying an upward pressure on the stopper. The sediment segment was then sliced with a stainless steel knife whose blade was wider than the diameter of the core tube by slipping the knife between the two tubes. The top core tube was then lifted with the knife serving as a temporary "bottom". The sediment segment was transferred into a BOD bottle with 100ml water for methylmercury measurement.

2.8.Calculations

2.8.1. Methylation rates in sediment

The calculation for determining the rate of methylation as ng Hg/g dry weight sediment/hour was as follows:

$$Rm=(s-b)(d)(a)/(w)/(t)\text{----------Eq. 5}$$

where:

Rm= rate of methylation as ng Hg/g dry wt. sediment/hour

s= sample activity as dpm

b= control activity as dpm

d= dilution factor from extraction procedure

a= ng Hg per dpm added

w= sample dry weight in grams by drying at 60 ^{o}C

t= incubation period in hours

The projected isotope addition in sediment was 1uCi/1ug Hg/g dry wt sediment.

2.8.2. Methylation rates in water

The calculation for determining the rate of methylation as ng Hg/L water/actual incubation time was as follows:

$$Rm=(s-b)(d)(a)(g)\text{ -----Eq. 6}$$

where:

Rm=rate of methylation as ng Hg/L water/actual incubation time

s= sample activity as dpm

b= control activity as dpm

d= dilution factor from extraction procedure

a= ng Hg per dpm add

g= 1000/150 (factor converting rate/150ml to rate/L)

The projected isotope addition in water was 1uCi/1ug Hg/150ml.

During the investigation, the incubation periods were changed from 24 to 6 hours because the methylation rate was not linear within one day. The actual incubation time was used as the basis for calculation. The same situation provided when calculating for demethylation activities in water.

2.8.3. Demethylation rates in sediment

The calculation for determining the rate of demethylation as ng Hg/g dry wt. sediment/hour was as follows:

$$Rd=(s-b)(e)/w/h \text{----------Eq. 7}$$

where:

Rd= rate of demethylation as ng Hg/g/h.

s= sample activity as dpm

b= control activity as dpm

e= ng Hg per dpm as CH_3Hg^+ added

w= dry weight of sample in grams

h= incubation period in hours

The projected isotope addition in sediment was 14000dpm/o.14ug Hg/g dry wt sediment.

2.8.4. Demethylation rates in water

The calculation for determining the rate of demethylation as ng Hg/L/actual incubation time was as follows:

$$Rd=(s-b)(e)(g) \text{----------Eq. 8}$$

where:

Rd= rate of demethylation as ng Hg/L /actual incubation time

s= sample activity as dpm

b= control activity as dpm

e= ng Hg per dpm as CH_3Hg^+ added

g= 1000/150 (factor converting rate/150ml to rate/L)

The projected isotope addition in water was 28000dpm/0.28ug Hg/150ml water.

Results

3.1.Sediment experiments

Several series of replicate sediment samples were collected from Lakes 303, 114 and 302 N for examination. Lake 303 and 114 sediments were sampled at a depth of 2 M and from L.302 N at a depth of 13 M. The pH of these sediment samples after suspension in modified 2.5 L acid bottles was adjusted where appropriate by addition of sulphuric acid, nitric acid or sodium hydroxide(Furutani et. al. 1984). Methylation and demethylation activities were monitored under anaerobic conditions and measured after 12 hours incubation.

Results for all sediments examined showed that as the pH was lowered the rate of methylation decreased rapidly, while demethylation rates decreased slowly . Table 2 presents the results from L.303 sediments sampled on May 21, 1984. Here, methylation rates decreased from 0.387(ng Hg/g dry wt. sediment/hs.) to <0.001 through a pH change from 5.9 to 4.9 while demethylation rates decreased from 0.181(ng Hg/g.dry wt. sediment/hs.) to 0.062 over the same pH decrease. Table 3 provides similar results derived from L.303 for samples taken on July 13th.

Tables 4 to 6 provide data from L.114 sediments for a series of samples taken from May 23 to June 9 . Decreases in rates of methylation and demethylation followed the trend reported in Table 3 for L.303. The results from L.302 N sediment experiments also showed that rates of methylation and demethylation decreased when the pH was lowered(Table 7).

Table 2 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.303 sediments sampled on May 21, 1984

pH	*Rm ng/g dr. sed./h	*Rd ng/g dr. sed./h
5.9(untreated)	0.387 ± 0.024	0.181 ± 0.05
4.9(H_2SO_4)	<0.001	0.069 ± 0.016

Table 3 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.303 sediments sampled on June 13, 1984

pH	*Rm ng/g dr. sed./h	*Rd ng/g dr. sed./h
6.0(NaOH)	0.584 ± 0.012	0.112 ± 0.002
5.6(untreated)	0.379 ± 0.021	0.082 ± 0.001
5.0(H_2SO_4)	0.251 ± 0.071	0.069 ± 0.0007
5.3(HNO_3)	<0.001	0.038 ± 0.004

* mean rate of wto samples with range

Table 4 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.114 sediments sampled on May 23, 1984

pH	*Rm ng/g dr. sed./h	*Rd ng/g dr. sed./h
5.9(untreated)	0.116 ± 0.018	0.061 ± 0.007
5.6(H_2SO_4)	0.105 ± 0.031	0.057 ± 0.005
4.9(H_2SO_4)	0.039 ± 0.014	0.045 ± 0.003

Table 5 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.114 sediments sampled on June 8, 1984 and incubated aerobically**

pH	*Rm ng/g dr. sed./h	*Rd ng/g dr. sed./h
6.4(untreated)	<0.001	0.058 ± 0.003
5.3(H_2SO_4)	<0.001	0.042 ± 0.004

**only aerobic incubation indicated

Table 6 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.114 sediments sampled on June 9, 1984

pH	*Rm ng/g dr. sed./h	*Rd ng/g dr. sed./h
7.1(NaOH)	1.041 ± 0.422	0.070 ± 0.007
6.3(untreated)	0.444 ± 0.167	0.121 ± 0.006
5.7(H_2SO_4)	0.135 ± 0.134	0.119 ± 0.012
5.1(H_2SO_4)	0.006 ± 0.001	0.099 ± 0.011
5.7(HNO_3)	0.022 ± 0.012	0.066 ± 0.011
5.1(HNO_3)	<0.001	0.048 ± 0.003

* mean rate of two samples with range

Table 7 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.302 N sediments sampled on June 6, 1984

pH	*Rm ng/g dr. sed./h	*Rd ng/g gr. sed./h
6.6(NaOH)	1.438 ±0.118	0.184 ±0.007
6.2(untreated)	0.334 ±0.065	0.177 ±0.002
5.6(H_2SO_4)	0.141 ±0.016	0.126 ±0.010
5.2(H_2SO_4)	0.171 ±0.052	0.067 ±0.016
5.3(HNO_3)	<0.001	0.033 ±0.003
6.2(with air)	0.174 ±0.038	0.088 ±0.011

* mean rate of two samples with range

When pH was raised by addition of NaOH, methylation rates increased in the experiments reported in Tables 3, 6 and 7. However, demethylation rates increased in data shown in Table 3 and 7, but decreased in that of Table 6.

When HNO_3 was used to acidify sediment samples, the rates of decreases in methylation and demethylation activities were more pronounced as reported in Tables 3, 6 and 7.

Effects of the presence of O_2 on these activities in fresh water sediments were examined in experiments where anaerobic incubation conditions were not provided. Data in Tables 5, 6, and 7 clearly show that methylation and demethylation activities were higher under anaerobic condition(Tables 6 and 7) than under aerobic environment(Tables 5 and 7).

3.2. Effect of pH on rates of methylation and demethylation in epilimnion water samples

Mercury methylation and demethylation were consistently found in the water columns of ELA lakes during the investigations in the summer of 1984. The pH of epilimnion water samples collected from L.239 were lowered where appropriate by addition of sulphuric acid. The highest pH as indicated in figures and tables was the lake epilimnion pH. Methylation and demethylation activities were measured after 1 day of incubation. The results for July 10 and July 18,1984 are shown in Figs.5 and 6. Methylation rates increased as pH was lowered, but demethylation rates decreased over the same pH range. Fig. 7 and 8 indicated the results from L.239 for 12 hours incubation. The results showed the same trend as those for 24 hours incubation.

Fig. 5 Effect of pH on rate of methylation for L.239 epilimnion water sampled on July 10 and July 18. Initial pH (untreated sample), 7.1. Incubation time, 24hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

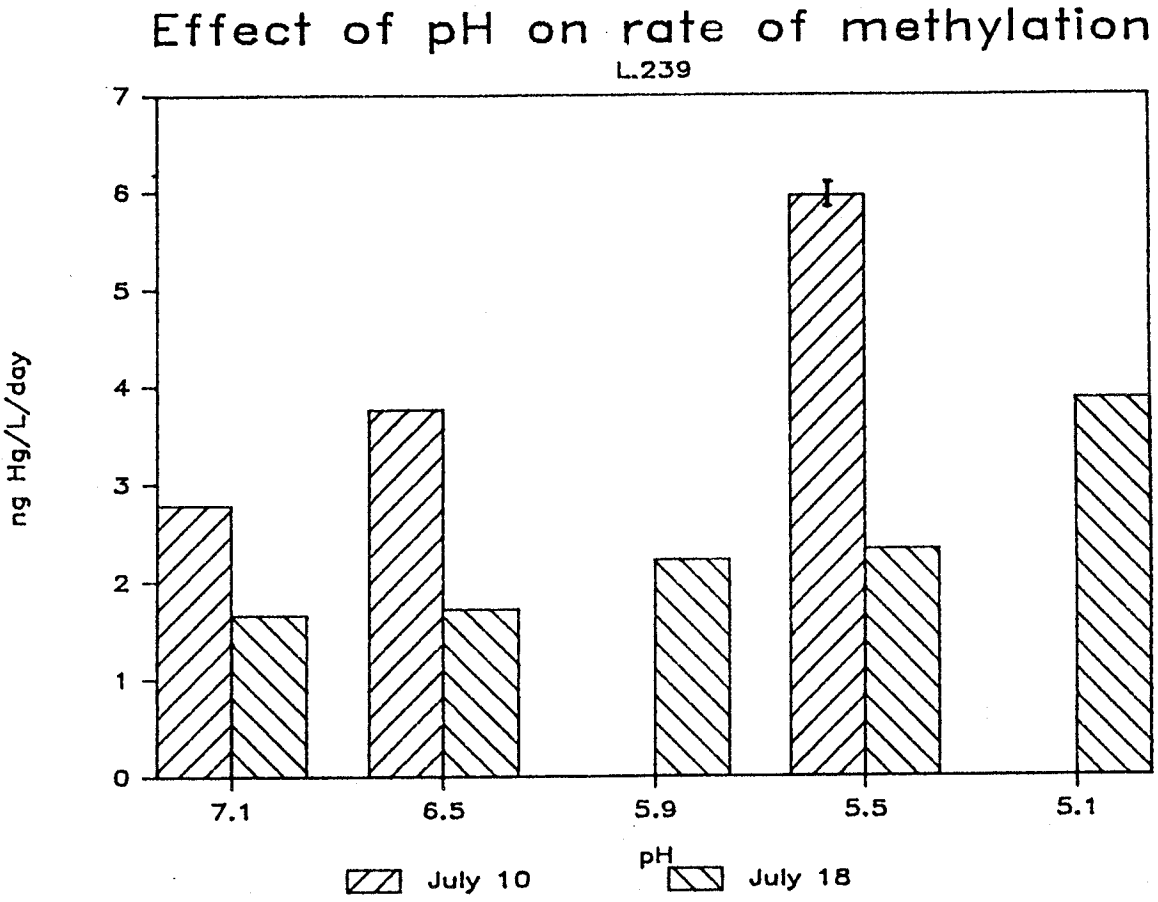

Effect of pH on rate of methylation
L.239
ng Hg/L/day
0
1
2
3
4
5
6
7
7.1
6.5
5.9
5.5
5.1
pH
July 10
July 18

Fig. 6 Effect of pH on rate of demethylation for L.239 epilimnion water sampled on July 10 and July 18. Initial pH (untreated sample), 7.1. Incubation time, 24hs. Means of dublicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

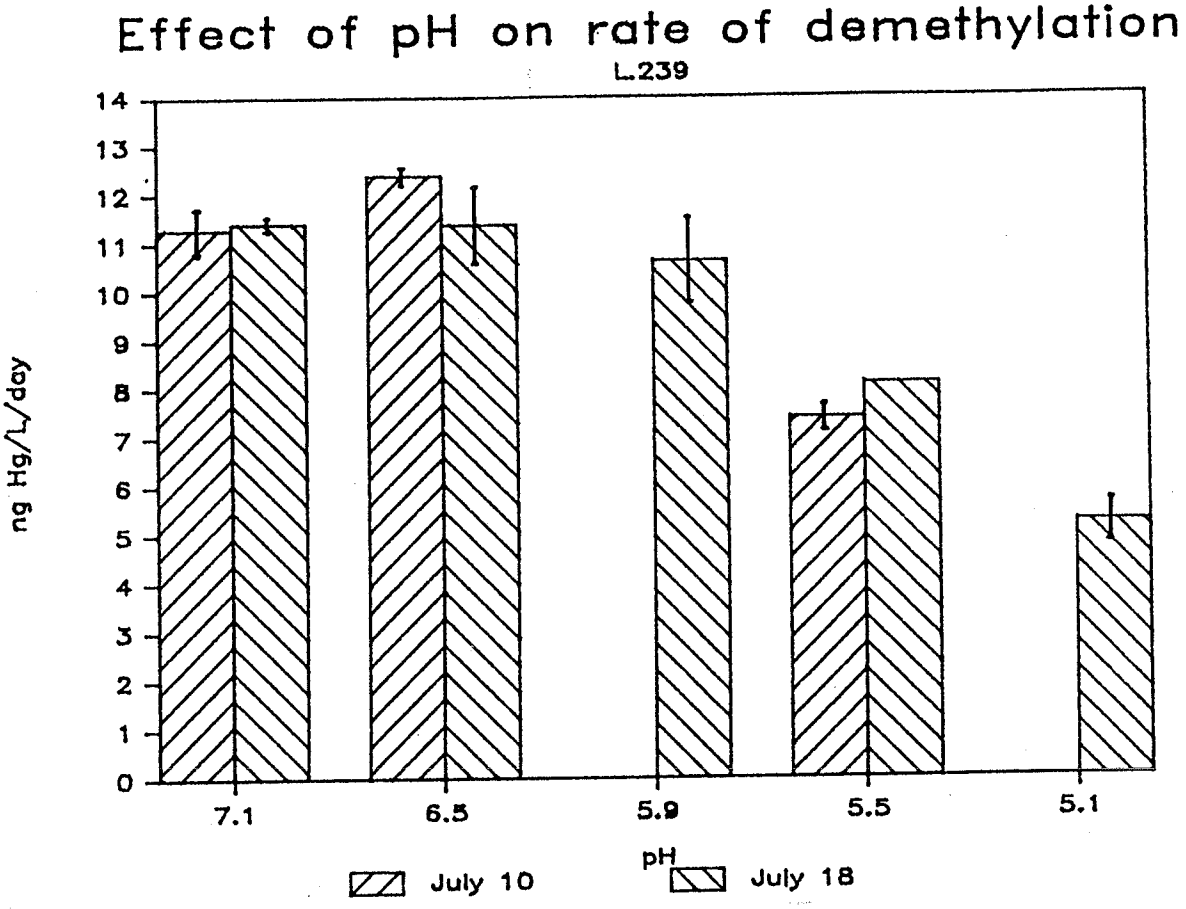

Effect of pH on rate of demethylation
L.239
ng Hg/L/day
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
7.1
6.5
5.9
5.5
5.1
pH
July 10
July 18

fig. 7 Effect of pH on rate of methylation for L.239 epilimnion water sampled on Sept. 11. Initial pH (untreated sample), 6.94. Incubation time, 12 hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

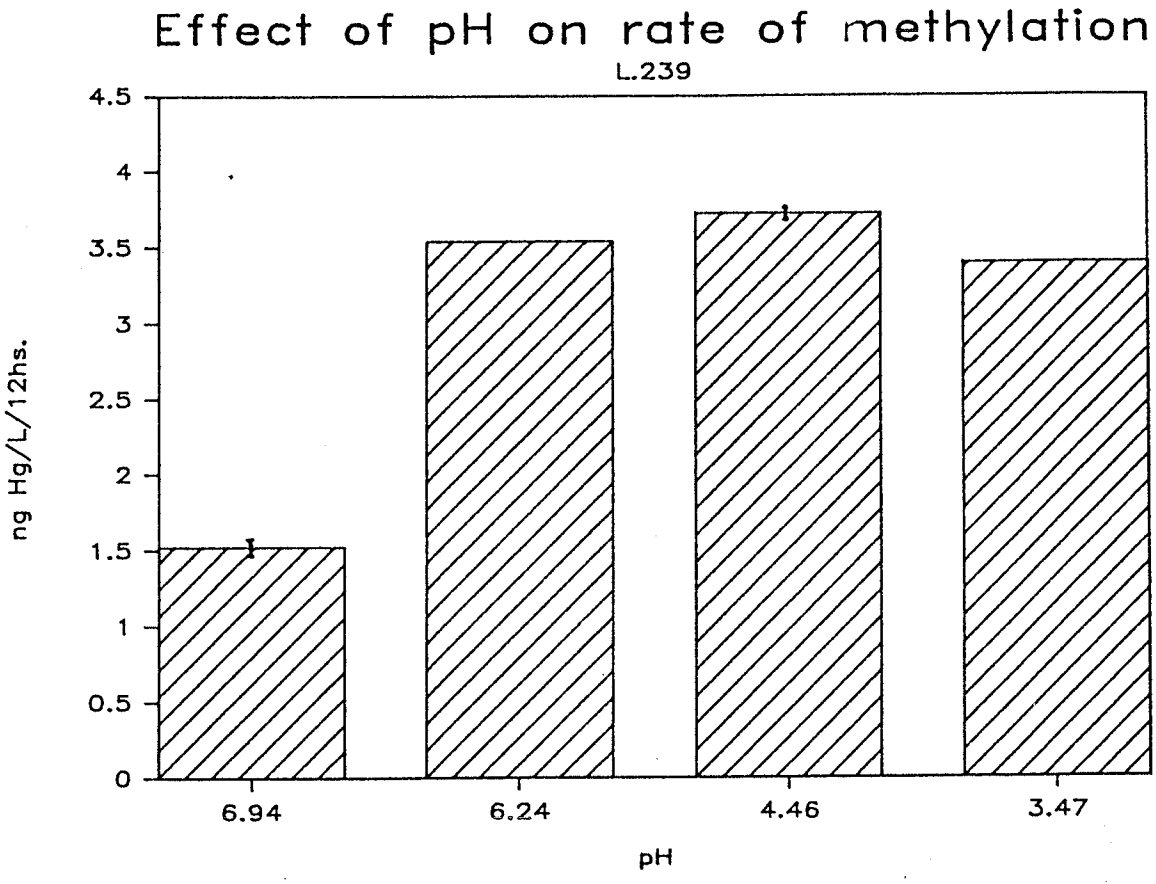
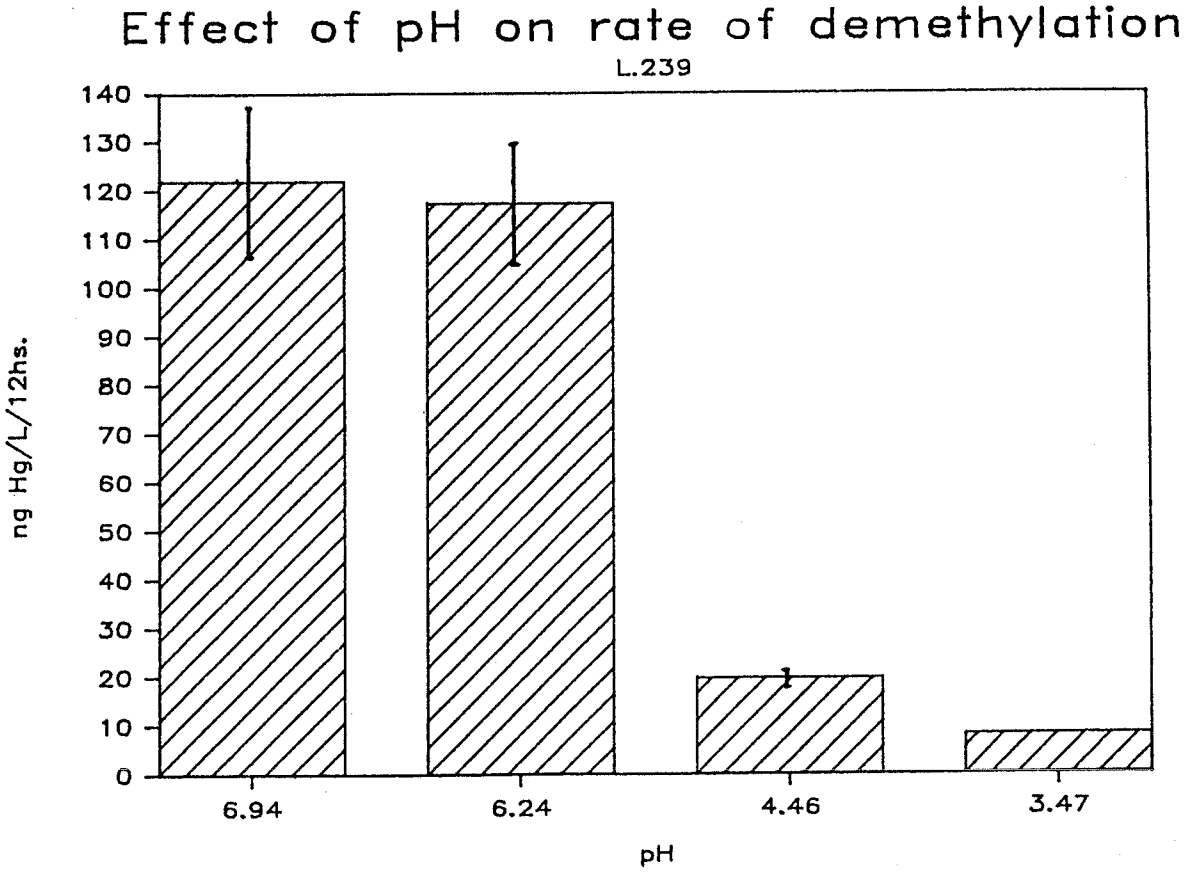

Effect of pH on rate of methylation
L.239
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
ng Hg/L/12hs.
6.94
6.24
4.46
3.47
pH

fig. 8 Effect of pH on rate of demethylation for L.239 epilimnion water sampled on Sept. 11. Initial pH (untreated sample), 6.94. Incubation time, 12 hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.


Effect of pH on rate of demethylation
L.239
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ng Hg/L/12hs.
6.94
6.24
4.46
3.47
pH

Similar experiments were conducted from time to time with epilimnion waters collected from other ELA lakes not involved in the acidification program. Twenty-four hours incubation results from epilimnetic water of other oligotrophic and stratified lakes(L.623 and 382) showed the same trend as those of L.239 after 24 hours of incubation(Table 8 and Fig. 9). The results from L.227, an eutrophic and stratified lake, showed that methylation rate decreased a little then increased but demethylation rate increased a little then decreased when pH were lowered(Table 9). The results from L.303, a shallow and unstratified lake, again showed that methylation rates increased but demethylation rates did not decrease when the pH was lowered(Table 10).

Epilimnion water samples from lakes 114, 302 S and 302 N, to which acid had been added, were also collected for our survey. The pH of the water samples was raised where appropriate by addition of sodium hydroxide. The lowest pH value as indicated in tables and figures was the existing epilimnion pH. Methylation and demethylation activities were measured after 24 hours incubation. Rates of methylation and demethylation decreased when pH was raised, although the rates changed a little at pH range from 5.7 to 7.5(Tables 11 and 12). Figs, 10 and 11 indicate the results from L.302 S for 12 hours incubation. Decreases in methylation and demethylation activities were very clear as pH was raised in this case.

Further investigations on the effect of pH on rates of methylation and demethylation in L.239 epilimnion water were conducted. The incubation periods were increased to 3 or 6 days. The results are shown in Fig. 12 and 13. For 3 day incubations, methylation rates (ng Hg/L/3 days) increased but to a considerably lower extent than those of 1 day

Fig. 9 Effect of pH on rate of demethylation for L.382 epilimnion water sampled on Aug. 10. Initial pH (untreated sample), 7.2. Incubation time, 24 hs. Means of duplicates samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

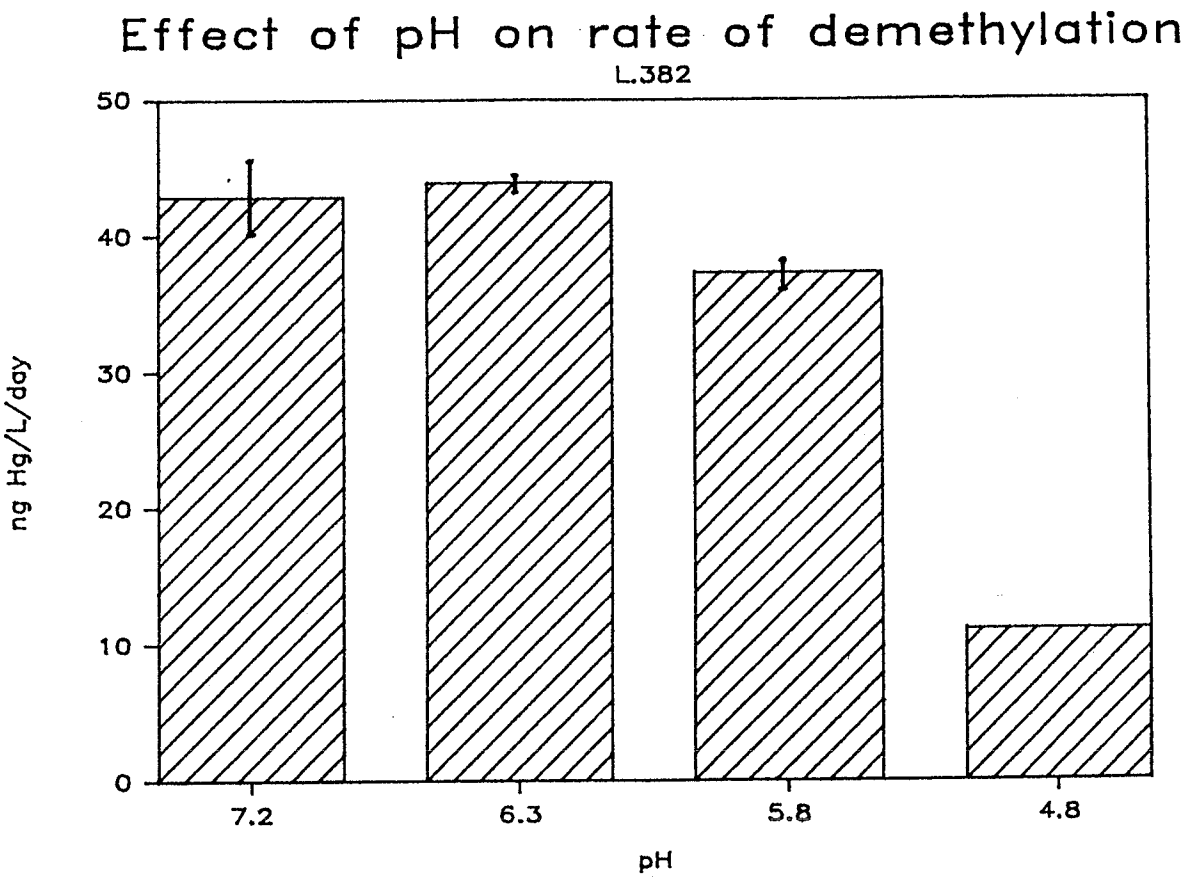

Effect of pH on rate of demethylation
L.382
50
40
30
20
10
0
ng Hg/L/day
7.2
6.3
5.8
4.8
pH

Table 8 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.623 and L.382 epilimnion waters sampled on Aug. 8 & 19, 1984

Date Lake	pH	*Rm ng Hg/L/d	*Rd ng HgL/d
Aug. 8 L.623	7.1(untreated)	7.712 ± 0.051	38.41 ± 0.20
	6.4	9.959 ± 0.110	34.61 ± 1.67
	5.5	12.003 ± 0.028	31.39 ± 1.40
	4.6	14.828 ± 0.016	18.70 ± 0.30
Aug. 19 L.382	6.9(untreated)	5.333 ± 0.028	30.17 ± 0.63
	5.8	7.299 ± 0.208	18.85 ± 0.33

Table 9 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.227 epilimnion water sampled on July 11, 1984

pH	*Rm ng Hg/L/d	*Rd ng Hg/L/d
8.4(untreated)	0.821 ± 0.039	15.37 ± 0.20
7.0	0.690 ± 0.034	16.44 ± 0.10
6.0	1.567 ± 0.018	12.23 ± 0.13

Table 10 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.303 epilimnion water sampled on July 8 & 19, 1984

Date	pH	*Rm ng Hg/L/d	*Rd ng Hg/L/d
July 8	6.6(untreated)	1.489 ± 0.056	10.72 ± 0.33
	6.0	2.152 ± 0.033	10.17 ± 2.27
	5.4	2.811 ± 0.063	13.91 ± 0.13
July 19	8.3(untreated)	3.085 ± 0.100	12.39 ± 2.13
	6.1	3.893 ± 0.052	14.05 ± 1.47
	5.1	3.276 ± 0.009	13.25 ± 1.83

* mean rate of two samples with range

Table 11 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.302 N & S epilimnion waters sampled on Aug. 21, 1984

Date Lake	pH	*Rm ng Hg/L/d	*Rd ng Hg/L/d
302 N	7.0	9.559 ± 0.008	28.30 ± 1.23
	6.3(untreated)	9.695 ± 0.232	24.98 ± 2.70
302 S	8.7	14.520 ± 0.236	16.48 ± 1.00
	7.5	20.762 ± 0.441	26.71 ± 0.70
	5.7(untreated)	23.228 ± 0.618	29.12 ± 2.37

Table 12 Effect of pH on rates of methylation(Rm) and demethylation(Rd) for L.114 epilimnion water sampled on Aug. 23, 1984

pH	*Rm ng Hg/L/d	*Rd ng Hg/L/d
7.0	2.158 ± 0.129	15.06 ± 0.10
6.1	8.489 ± 0.220	26.17 ± 0.30
5.6(untreated)	9.148 ± 0.291	26.79 ± 0.20

* mean rate of two samples with range

Fig. 10 Effect of pH on rate of methylation for L.302 S epilimnion water sampled on Sept. 12. Initial pH (untreated sample), 5.56. Incubation time, 12 hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

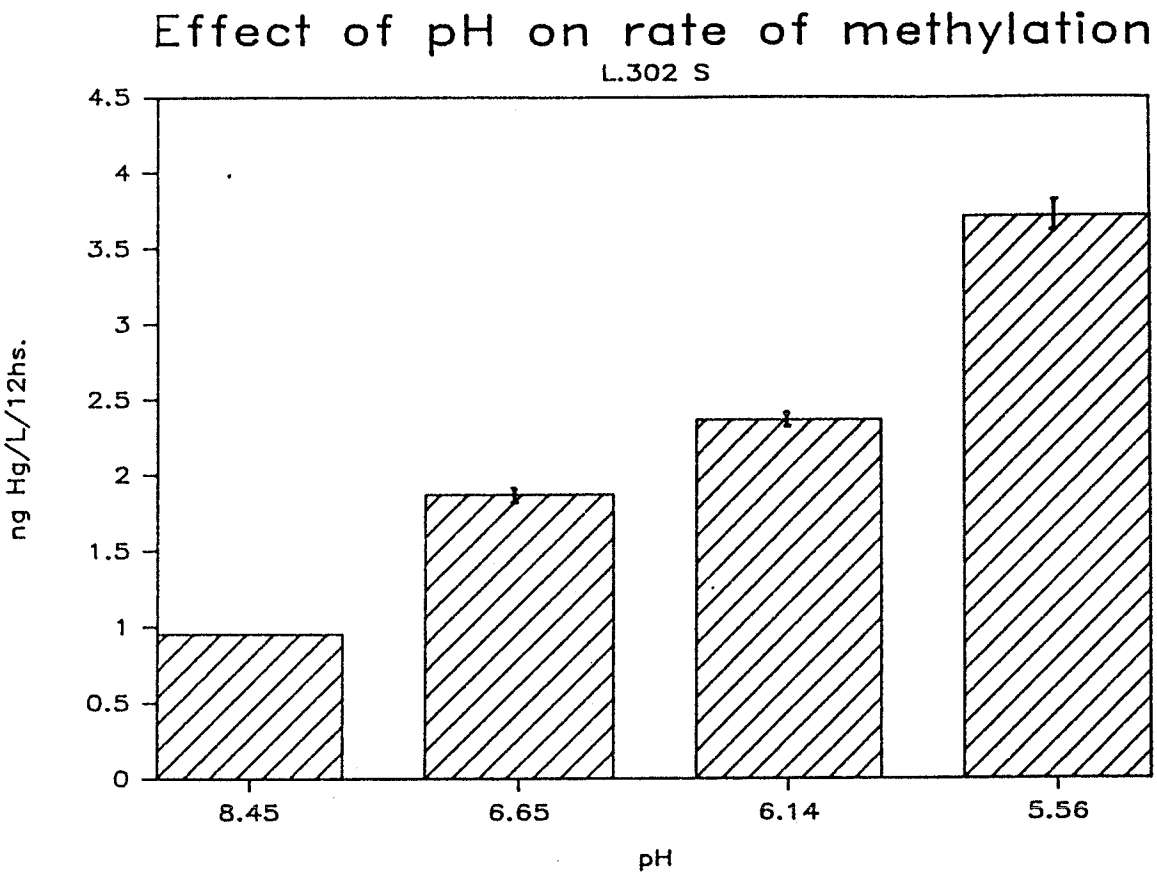

Effect of pH on rate of methylation
L.302 S
ng Hg/L/12hs.
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
8.45
6.65
6.14
5.56
pH

Fig. 11 Effect of pH on rate of demethylation for L.302 S epilimnion water sampled on Sept. 12. Initial pH (untreated sample), 5.56. Incubation time, 12 hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences.

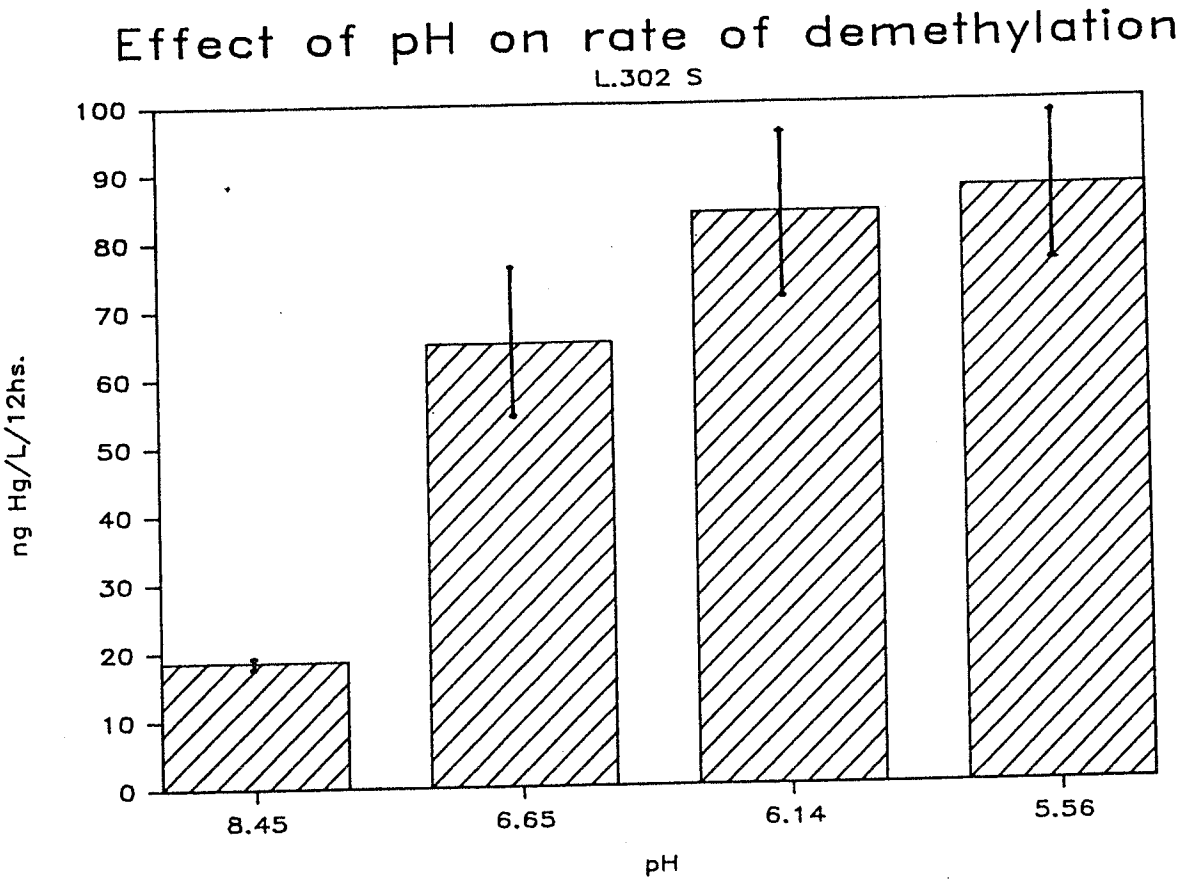

Effect of pH on rate of demethylation
L.302 S
ng Hg/L/12hs.
100
90
80
70
60
50
40
30
20
10
0
8.45
6.65
6.14
5.56
pH

Fig. 12 Effect of pH on rate of methylation for L.239 epilimnion water. 3 days incubation started on July 13. 6 days incubation started on July 25. Initial pH (untreated sample), 7.1. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

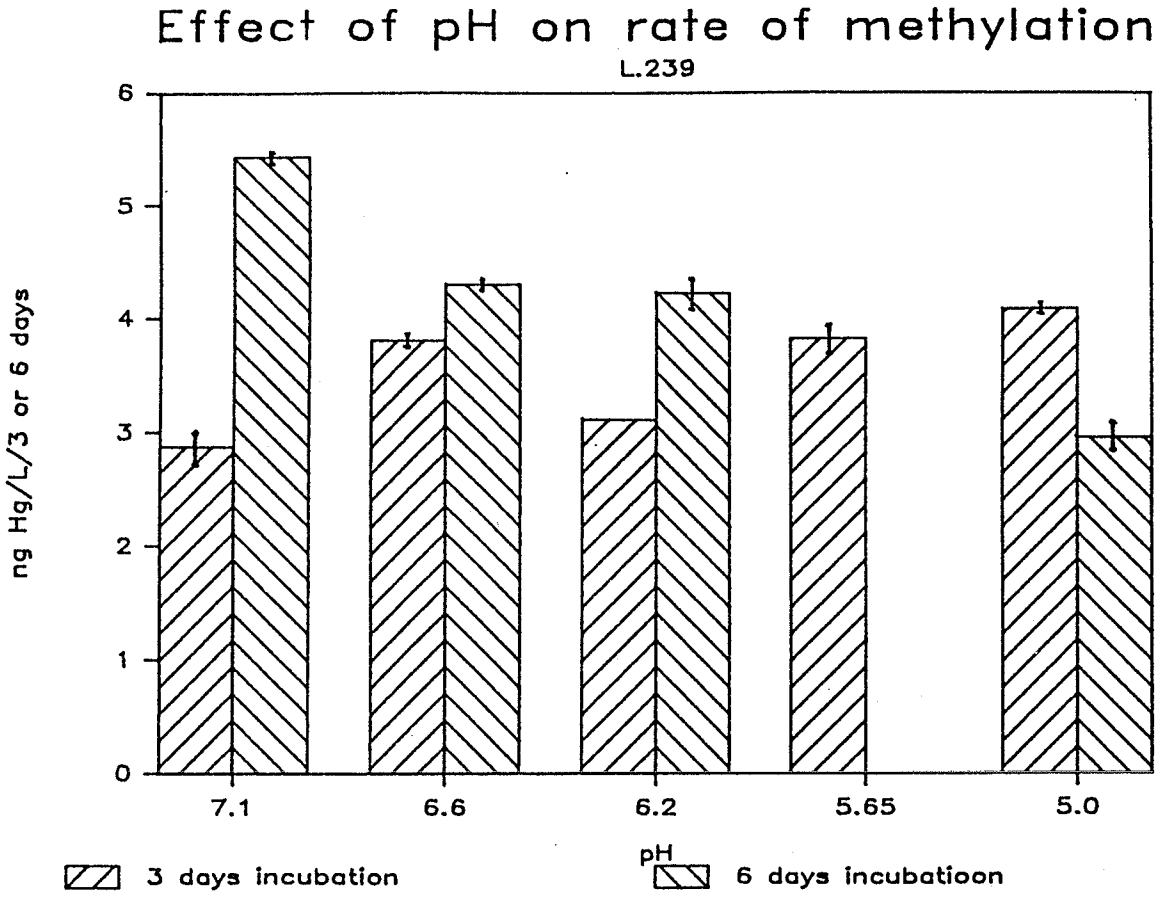
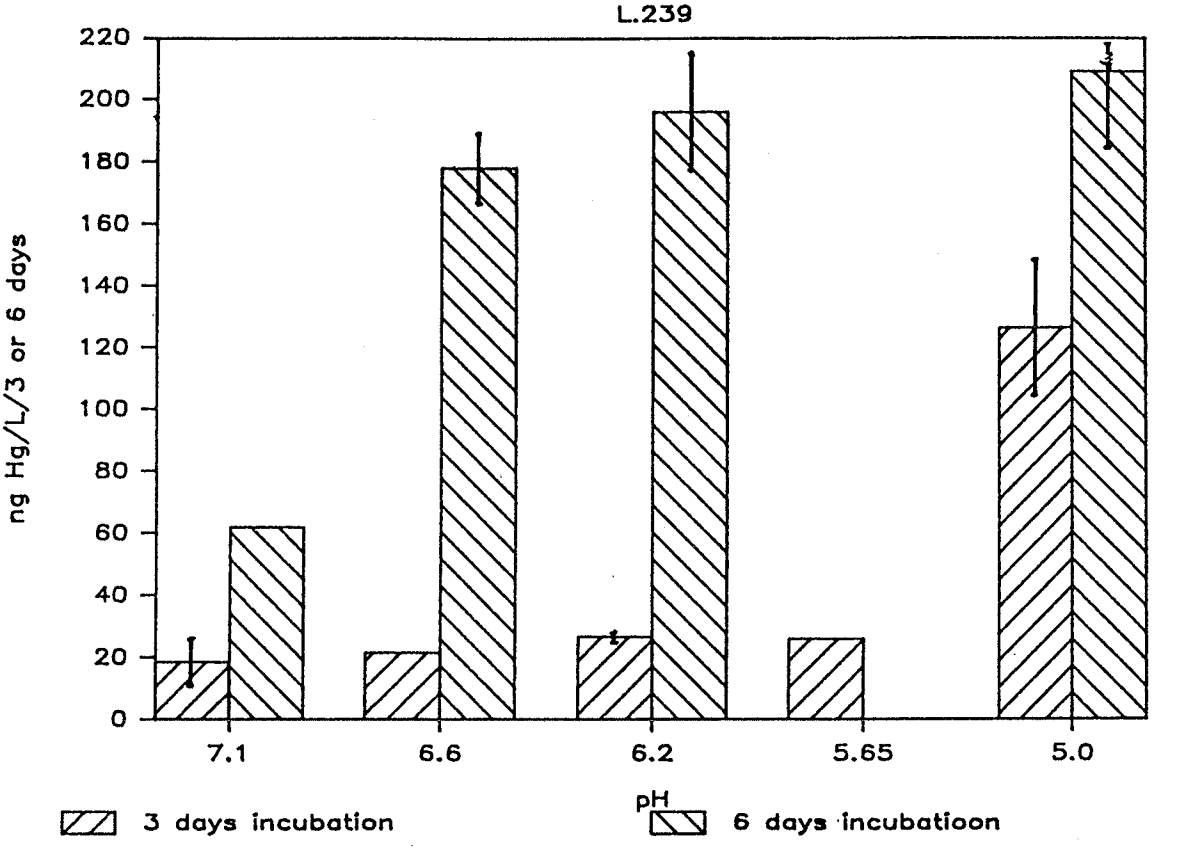

Effect of pH on rate of methylation
L.239
ng Hg/L/3 or 6 days
0
1
2
3
4
5
6
7.1
6.6
6.2
5.65
5.0
pH
3 days incubation
6 days incubatioon

Fig. 13 Effect of pH on rate of demethylation for L.239 epilimnion water. 3 days incubation started on July 13. 6 days incubation started on July 25. Initial pH (untreated sample), 7.1. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

Effect of pH on rate of demethylation



incubation. When pH was lowered, demethylation rates(ng Hg/L/3days) also increased; in fact, they reached their maximium values at the lowest pH value, 5.0. For 6 days incubation , methylation rates(ng Hg/L/6days) decreased when pH was lowered, while demethylation rates(ng Hg/L/6days) increased. The results of 3 day and 6 days incubation experiments were different from those of 24 hours incubation experiments.

3.3. Methylation and demethylation time-course study

When considering the effect of changes in pH on rates of methylation and demethylation in L.239, it seems that their rates apparently changed with changes in incubation time. Within 1 day incubation methylation activities increased and demethylation activities decreased when pH was lowered(Fig.5-8). For 3 days incubation, methylation activities increased slightly but demethylation activities increased very markedly when the pH was lowered. For 6 days incubation methylation activities decreased but demethylation activities increased very markedly when pH was lowered(Fig. 12 and 13).

In order to verify the above observation, a series of time course experiments were done using L.239 and L.302 S epilimnion water samples, the epilimnion pH values being 7.0 and 5.5 respectively. L.239 water samples displayed a very low methylation activity . During the first 12 hours, the amount of methylmercury increased quickly. The rate of increase slowed somewhat over the next 108 hours.By the end of the 5th day's incubation, the methylmercury concentration began to decrease(Fig.14). Demethylation activity showed a long lag, then a sudden increase beginning after 72 hours and persisting for the next 24 hours, to be followed by a somewhat slow rise(Fig.15).

Fig. 14 Methylation time-course for L.239 epilimnion water sampled on July 18. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

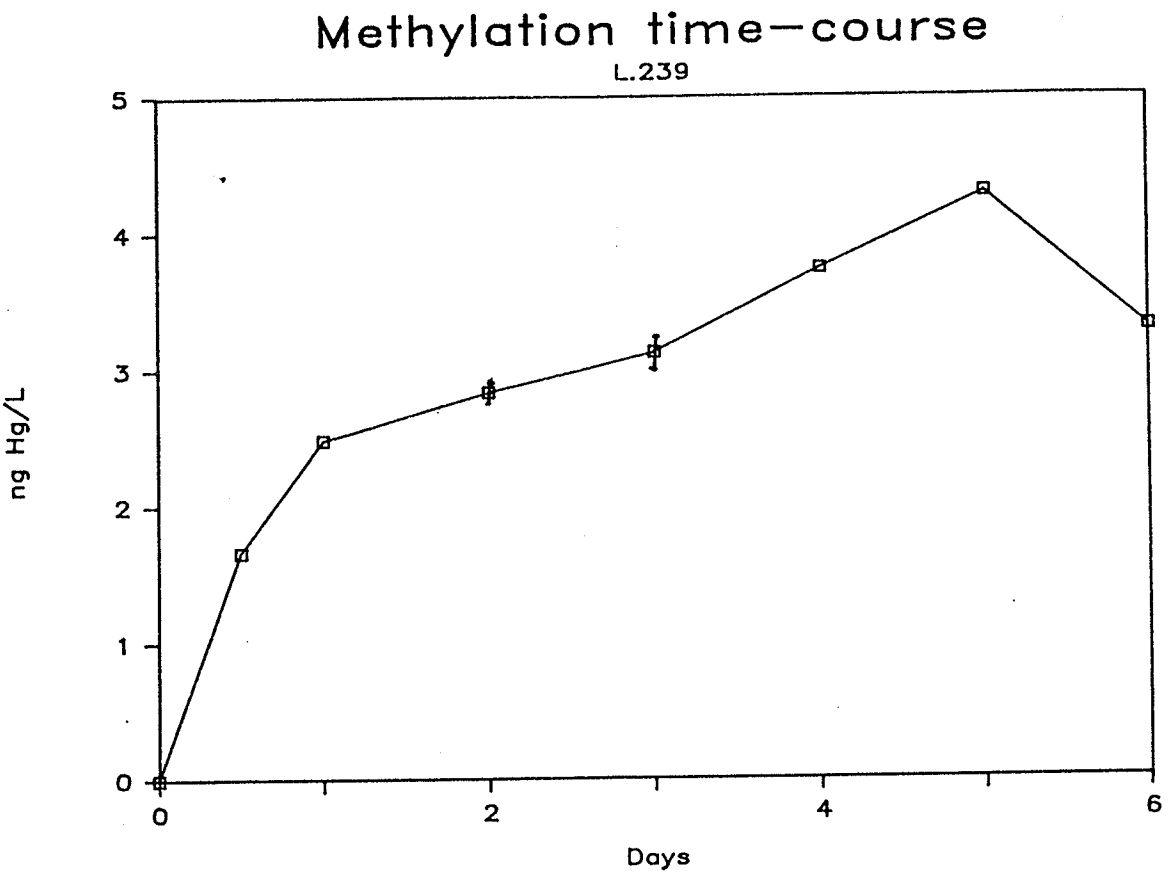
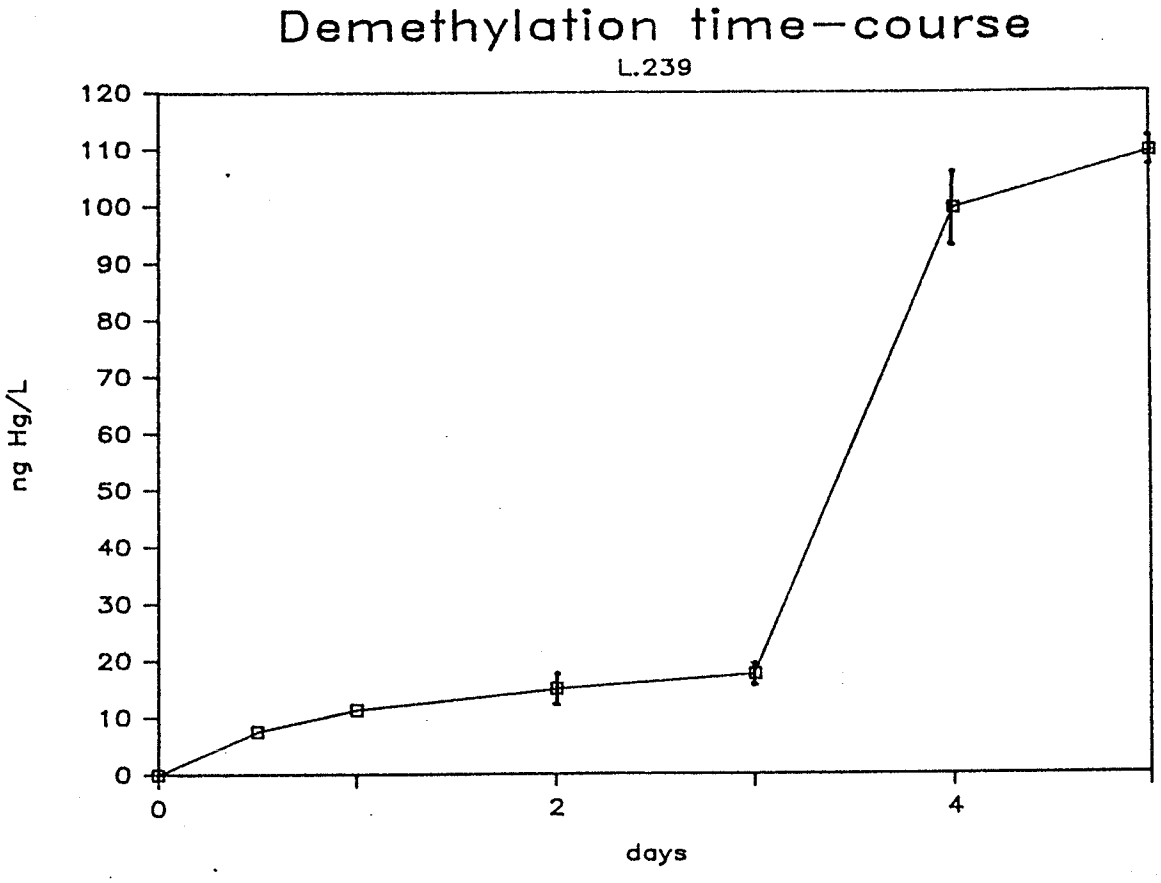

Methylation time-course
L.239
ng Hg/L
5
4
3
2
1
0
0
2
4
6
Days

Fig. 15 Demethylation time course for L.239 epilimnion water sampled on July 18. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.


Demethylation time-course
L.239
120
110
100
90
80
70
60
50
40
30
20
10
0
ng Hg/L
0
2
4
days

On the other hand, L. 302 S water samples displayed a very high methylation activity and methylmercury concentration rose very quickly within 24 hours then gradually increased during next 28 hours, by 96 hours the trend was reversed sharply(Fig.16). During the same period, demethylation activity after an initial slow rise for 24 hours showed a rapid increase over the next 72 hours interval(Fig.17). A longer time course experiment was done using L.302 S water samples to examine the methylation and demethylation activities during incubation periods greater than 96 hours. Methylmercury concentration formed from mercuric ion reached its peak by the end of 48 hours then decreased for the remaining of the 10 day incubation period(Fig.16). Demethylation activity increased very quickly after 48 hours and continued until the 6th day by which time nearly all the added methylmercury had been decomposed(Fig.17). Of the 1.867ug Hg as methylmecury added at the beginning of the experiment, 1.736ug were demethylated by the 6th day of incubation.

Another time course experiment was designed to test methylation and demethylation activities as influenced by pH within a 24 hour time period. Untreated water samples(pH 7.1) and H_2SO_4 acidified samples(pH 5.7) from the L.239 epilimnion were used for the experiment. The production of methylmercury in the methylation experiment and the decomposition product, methane, from the demethylation experiment both increased as the incubation time increased(Fig. 18 and 19). Methylation activity was higher but demethylation activity was lower at pH 5.7 than at pH 7.1. This experiment again showed that methylation rate increased and demethylation rate decreased when pH was lowered over a 24 hours incubation period.

Fig. 16 Methylation time course for L.302 S epilimnion water sampled on Aug. 8 and Aug. 13. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

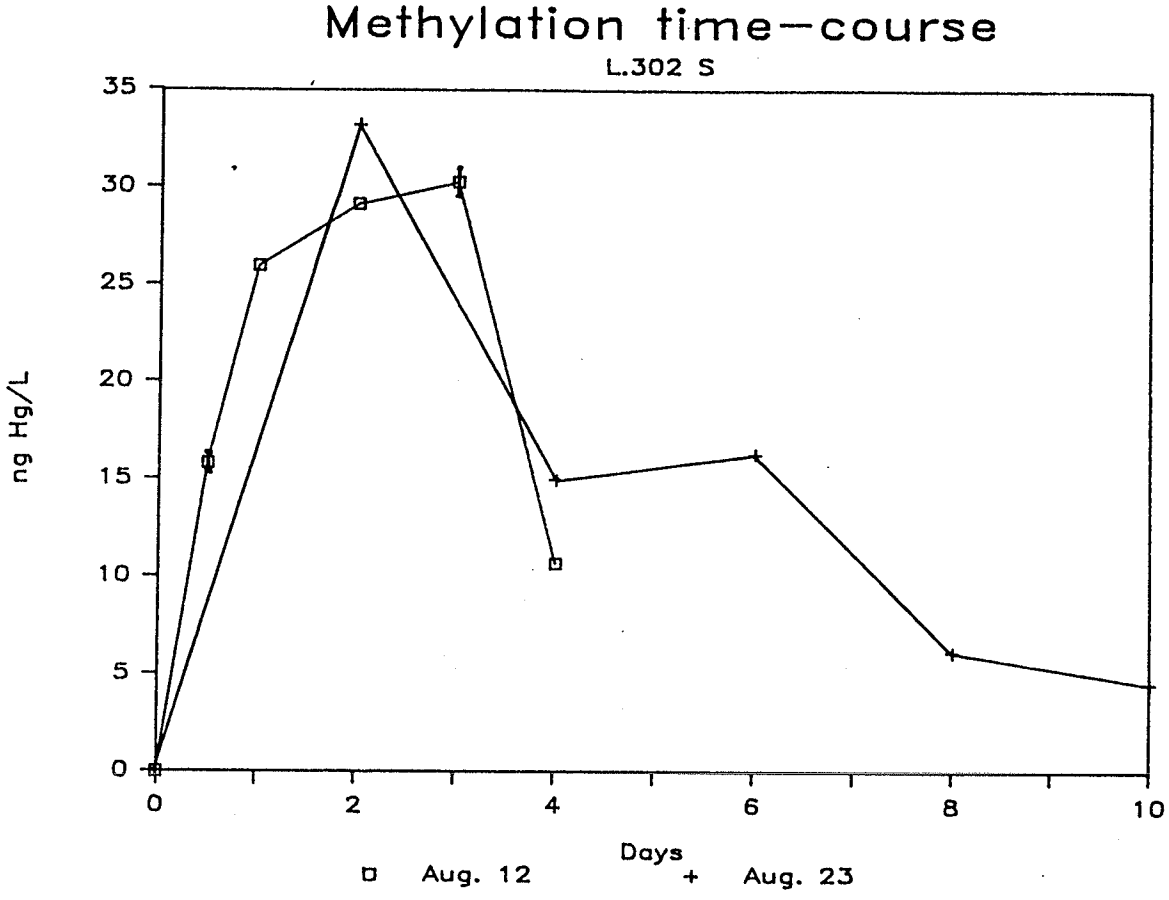

Methylation time-course
L.302 S
35
30
25
20
15
10
5
0
ng Hg/L
0
2
4
6
8
10
Days
Aug. 12
Aug. 23

Fig. 17 Demethylation time course for L.302 S epilimnion water sampled on Aug. 8 and Aug. 13. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

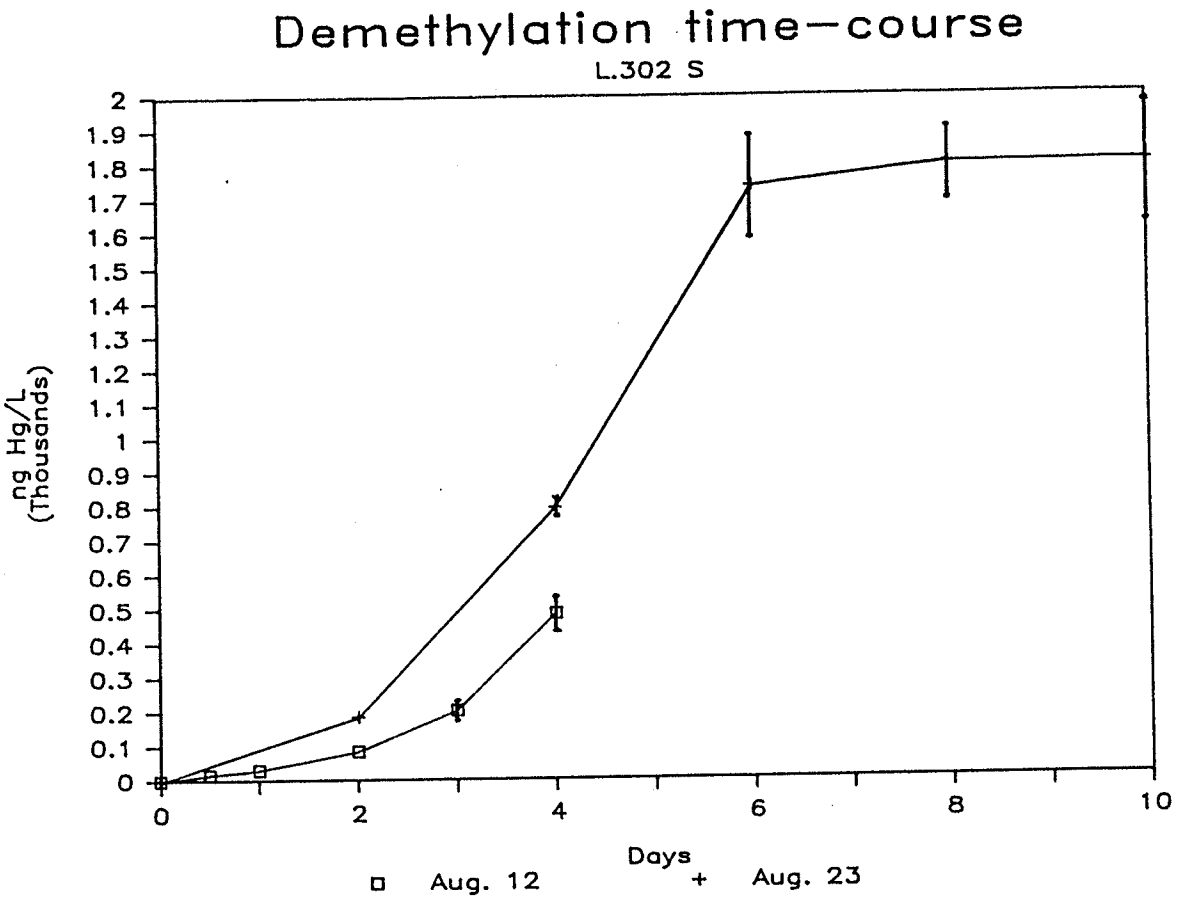

Demethylation time-course
L.302 S
ng Hg/L
(Thousands)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
Days
Aug. 12
Aug. 23

Fig. 18 Effect of pH on methylation time course for L.239 epilimnion water sampled on Aug. 12. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

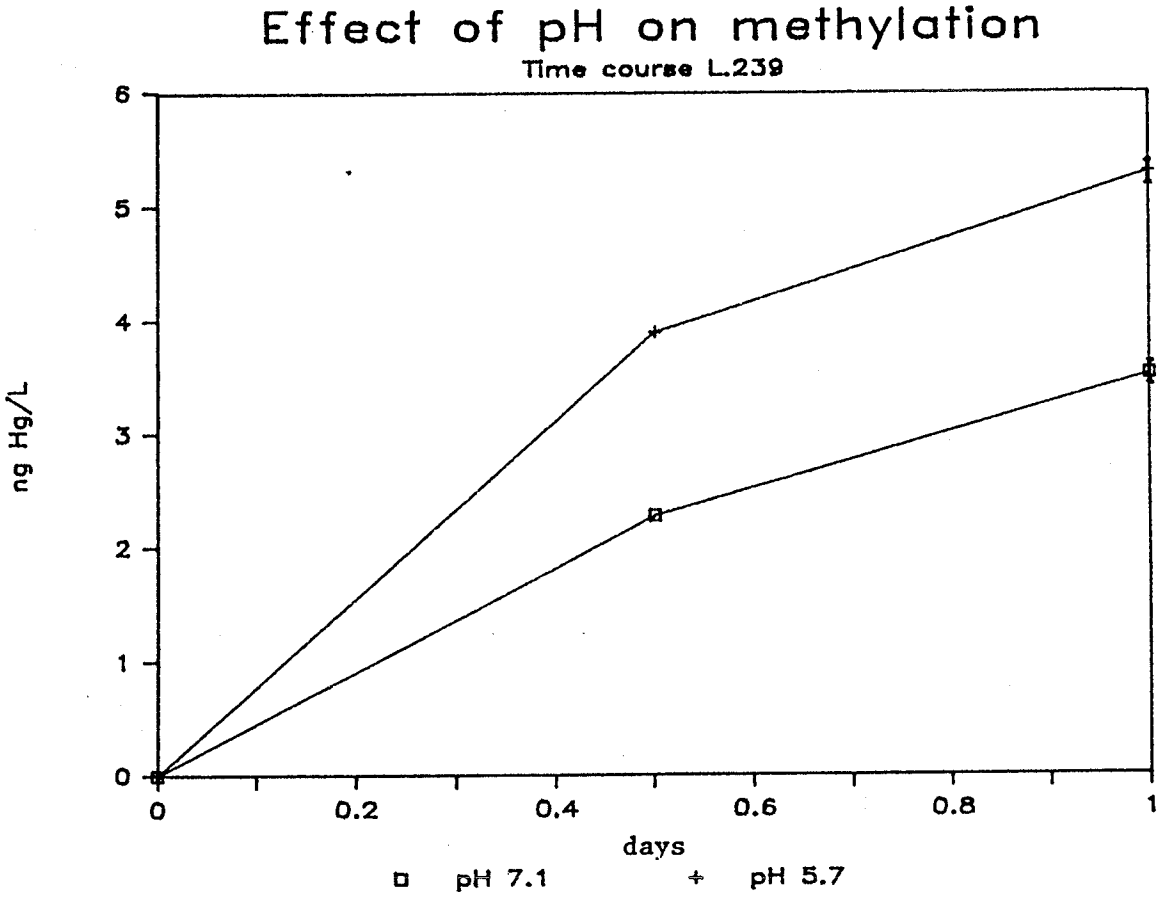
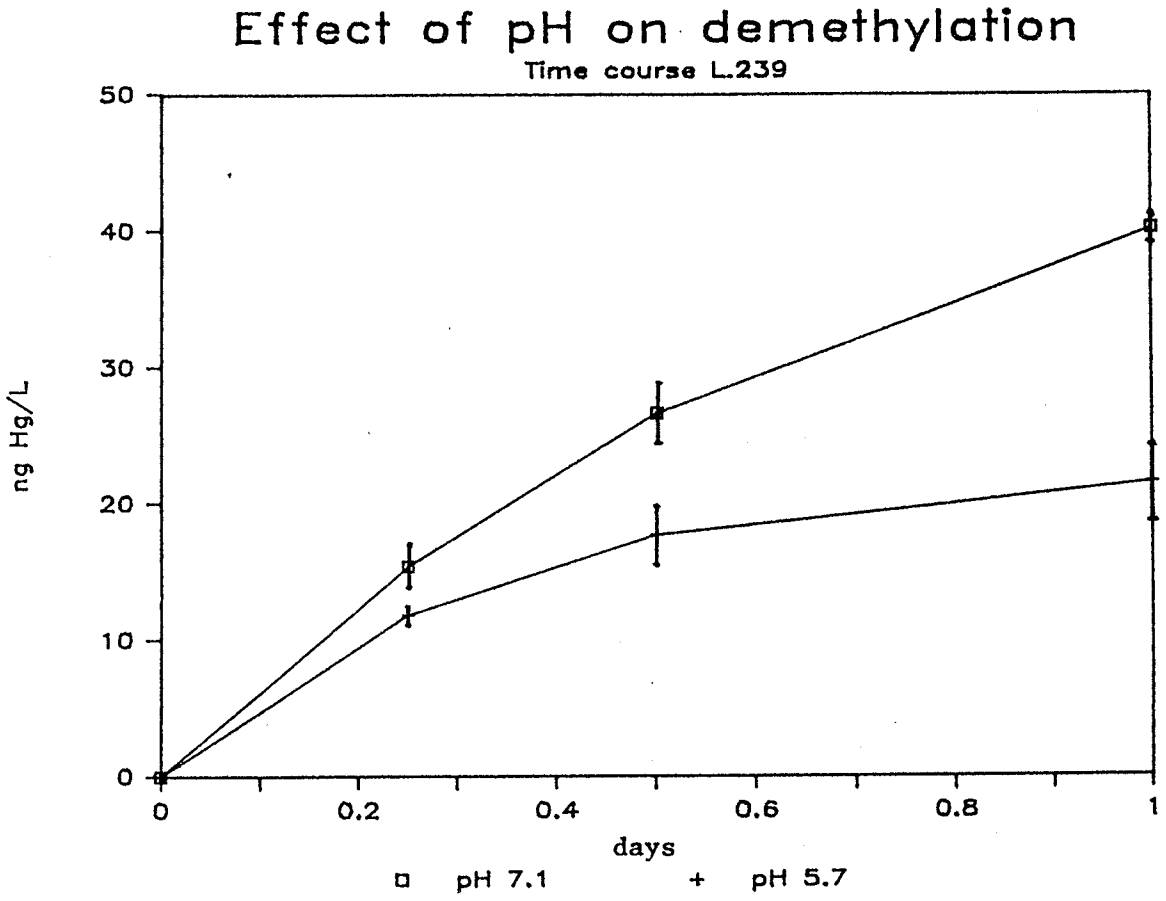

Effect of pH on methylation
Time course L.239
ng Hg/L
6
5
4
3
2
1
0
0
0.2
0.4
0.6
0.8
1
days
pH 7.1
pH 5.7

Fig. 19 Effect of pH on demethylation time course for L.239 epilimnion water sampled on Aug. 12. Means of duplicate samples are plotted, vertical lines show ranges of the differences.


Effect of pH on demethylation
Time course L.239
50
40
30
20
10
0
ng Hg/L
0
0.2
0.4
0.6
0.8
1
days
pH 7.1
pH 5.7

3.4.Comparison of rates of methylation and demethylation in epilimnion water samples of acidified lakes and circumneutral lakes

The effect of long term acidification of whole lakes on activities of methylation and demethylation populations was also investigated during summer of 1984. Two shallow unstratified lakes, L.303 and L.114, having similar degree of eutrophication and sizes were selected for study. As part of the ELA acidification program, L.114 had received addition of H_2SO_4 since 1979 and the surface water pH during the summer of 1984 had a value of 5.5. L.303 has been untreated and serves here as the reference lake for L.114. Measurements on samples taken on July 18 and Aug. 17 from L.114 showed consistently higher methylation activity than for L.303(Table 13). On Aug.17, after the pH of L.303 was raised from 6.6 to 8.5 as a consequence of an algal bloom, the difference in methylation activities was much more obvious; being much higher in L.114 than in L.303. The demethylation rates in L.114 and L.303 were very similar(Table 13).

All the other lakes examined at ELA in this study were both deeper and thermally stratified. L.223 and L.302 S were H_2SO_4-acidified lakes with pH values in the epilimnion waters of about 5.3 and 5.5 respectively. L.302 N is a HNO_3-acidified lake and the pH in its epilimnion water was about 6.5. Lakes 305, 623, 373, 382 and 239 are oligotrophic lakes with epilimnion pH value of about 7.0. L.227,an eutrophic lake,maitained a high epilimnion pH(>8.5) during the summer due to a succesion of algae blooms.

Table 13 Comparison of rates of methylation(Rm) and demethylation(Rd) in acidified L.114 and reference L.303 water samples

Date	Lake	pH	*Rm ng Hg/L/d	*Rd ng Hg/L/d
July 8	303	6.6	1.489 ± 0.057	10.72 ± 0.33
	114	5.9	2.306 ± 0.042	12.63 ± 2.86
Aug. 17	303	8.5	3.156 ± 0.004	43.09 ± 2.40
	114	5.5	18.421 ± 0.165	44.32 ± 1.10

Table 14 Comparison of rates of methylation(Rm) and demethylation(Rd) in acidified L.223, 302 N, 302 S and reference L.239 water samples

Date	Lake	pH	*Rm ng Hg/L/d	*Rd ng Hg/L/d
July 23	239	7.1	3.436 ± 0.100	13.05 ± 1.47
	223	5.3	4.814 ± 0.496	13.43 ± 1.20
	302 N	6.5	6.331 ± 0.029	19.03 ± 2.60
	302 S	5.5	8.555 ± 0.433	16.65 ± 1.03
Aug. 16	239	7.0	4.655 ± 0.043	42.55 ± 1.33
	223	5.4	8.903 ± 0.051	40.52 ± 0.10
	302 N	6.5	9.524 ± 0.071	47.49 ± 7.93
	302 S	5.5	21.063 ± 0.606	44.35 ± 1.63

Table 15 Rates of methylation(Rm) and demethylation(Rd) in circumneutral Lakes 305, 623, 373, 382,239 and eutrophic L.227

Date	Lake	pH	*Rm ng Hg/L/d	*Rd ng Hg/L/d
July 9	305	6.9	3.262 ± 0.042	3.10 ± 0.10
	227	8.3	0.615 ± 0.000	12.51 ± 1.37
	239	7.1	2.712 ± 0.021	11.30 ± 0.50
Aug. 8	623	7.1	5.539 ± 0.051	38.41 ± 0.20
	373	7.1	4.385 ± 0.016	17.45 ± 1.67
Aug. 19	382	6.8	5.333 ± 0.028	30.17 ± 0.63
Aug. 16	239	7.0	4.655 ± 0.043	42.55 ± 1.33

* mean rate of two samples with range

Table 14 presents the results from 4 different lakes, L.239, 223, 302 N and 302 S. L.239 was chosen as the reference lake. The methylation rate in this lake was lower than that in acidified lakes. L.302 S displayed the highest methylation rates of the 4 lakes tested. Although L.223 had the lowest pH value, its methylation rate was only moderately higher than in the reference L.239. Demethylation rates were generally at the same level in all 4 lakes for these two examinations periods.

Table 15 shows that the rates of methylation in the other oligotrophic lakes were nearly the same as those in L.239 during our examinations in July and August, 1984. The eutrophic L.227 had the lowest methylation rates of all the lakes under study. Demethylation rates in other oligotrophic lakes were either similar to or lower than that in L.239.

3.5. Rates of methylation and demethylation in water profiles of several lakes.

"In situ" experiments on rates of methylation and demethylation in water profiles of reference L.239, eutrophic L.227 and acidified lakes 302 N and 302 S were done twice during the periods of Aug.25 to 29 and Sept. 14 to 16,1984. The results are presented in Tables 16 to 18. In both August and September, the acidified lakes had much higher rates of epilimnetic methylation than unacidified lakes. The reference L.239, however, displayed the highest demethylation rates of all four lakes examined(Tables 16, 17,18). L.302 S had a higher methylation rate and a lower demethylation rate than L.302 N(Tables 17 and 18). Methylation rates in eutrophic L.227 were the lowest of all the four lakes examined(Table 16).

Table 16 Profile of methylation(Rm) and demethylation(Rd) in L.227 on Aug. 25, 1984

Depth	T(°C)	Oxygen ug/L	*Rm ng Hg/L /12hs	*Rd ng Hg/L /12hs
1M(epi.)	23.0	9.66	2.189 ±0.019	22.58± 0.40
4M(therm.)	13.1	14.56	1.111 ±0.039	29.01± 4.10
6M(hyp.)	6.3	< .01	0.284 ±0.023	38.38± 2.50
10M(hyp.)	4.5	< .01	0.484 ±0.007	10.17± 1.33

Table 17 Profile of methylation(Rm) and demethylation(Rd) in Lakes 302 S, 302 N and 239 during Aug. 26 to 29, 1984

Lakes Date Depth	T(°C)	Oxygen ug/L	*Rm ng Hg/L /12hs	*Rd ng Hg/L /12hs
L.302 S, Aug. 26				
1M(epi.)	22.9	7.96	44.593± 0.082	18.34 ±0.27
6M(epi.)	22.9	8.46	33.858± 0.145	26.30 ±2.67
8M(therm.)	13.5	< .01	0.764± 0.016	14.23 ±0.32
10M(hyp.)	11.0	< .01	3.335± 0.012	6.05 ± 1.03
L.302 N, Aug. 27				
1M(epi.)	22.1	8.06	10.787± 0.314	23.06 ±0.97
6M(therm.)	14.3	11.96	9.808± 0.059	26.97 ±0.57
10M(hyp.)	6.9	< .01	0.886± 0.019	22.73 ±1.13
L.239, Aug. 29				
1M(epi.)	22.0	7.82	3.032± 0.059	112.98 ±13.27
7M(therm.)	15.6	7.94	37.529± 0.373	71.43 ±11.33
15M(hyp.)	6.0	8.29	2.181± 0.039	68.98 ±5.30

* mean rate of two samples with range

Table 18 Profile of methylation(Rm) and demethylation(Rd) in Lakes 302 S, 302 N and 239 during Sept.14 to 16, 1984

Lake Date Depth	T(^{o}C)	Oxygen ug/L	*Rm ng Hg/L /6hs	*Rd ng Hg/L /6hs
L.302 S, Sept. 15				
1M(epi.)	15.1	9.12	6.090 ± 0.069	8.11 ± 0.83
8M(epi.)	15.0	8.63	3.362 ± 0.044	10.83 ± 0.23
9.1M(the.)	13.5	8.51	1.163 ± 0.030	9.45 ± 1.00
L.302 N, Sept. 16				
1M(epi.)	14.3	9.53	4.363 ± 0.011	14.05 ± 0.10
7.2M(the.)	12.5	8.61	2.601 ± 0.039	28.03 ± 0.10
10M(hyp.)	7.1	< .01	1.640 ± 0.014	16.55 ± 0.37
L.239, Sept. 14				
1M(epi.)	14.9	9.33	3.907 ± 0.006	35.60 ± 0.17
8M(the.)	12.8	8.36	6.186 ± 0.117	20.48 ± 0.20
15M(hyp.)	5.1	7.16	0.456 ± 0.038	16.1 ± 0.037

*mean rate of two samples with range

Whether aerobic or anoxic, hypolimnion water of Lakes 227, 302 N and 302 S(anoxic) or L.239(aerobic) had the lowest activities of both methylation and demethylation. On the other hand, epilimnion water displayed the highest activities of both methylation and demethylation in L.227, 302 S and 302 N with the exception of the demethylation rate in L.302 N on Sept. 16. However, the highest methylation activity in L.239 were at the thermocline, while the highest demethylation activity was in the epilimnion(Table 17 & 18).

3.6.Effect of pH on rates of methylation in intact sediment cores

The sediment-water interface is considered likely as the most active site for mercury methylation in lake ecosystems(Robinson and Tuovinen 1984). Investigation of the effect of pH on methylation activity at the sediment-water interface was done by using intact sediment cores collected from L.302 S and L.239 at different depths. The pH of the overlying water in the core was adjusted where appropriate by addition of H_2SO_4 or HCl. $^{203}HgCl_2$ was added directly to the overlying water.

The distribution and diffusion of $^{203}Hg^{2+}$ in the sediment cores sampled from L.302 S are shown in Tables 19 and 20. Within 50 hours of incubatoin, almost all of the $^{203}Hg^{2+}$ in the sediment was found in the top 1cm, and its concentration increased as the incubation time extended from 24 to 50 hours. The core taken from the depth of 6M had a very flocculent surface layer and was highly organic while the core taken from the depth of 1.5M was very compact and sandy. The percentage of mercury diffusing into these two types of cores was different. Within 50 hours of incubation, a greater precentage of mercury was found in the

Table 19 $^{203}Hg^{2+}$ distribution and diffusion into intact sediment cores sampled at a depth of 6M in L.302 S on Aug. 17, 1984

Incubation time	Core Depth	Water pH	*DPM
24 hs.	1cm	5.7	1151.4
	2cm		101.7
	3cm		65.5
	4cm		36.6
	5cm		32.1
50 hs.	1cm	5.5	1240.3
	2cm		263.6
	3cm		69.7

* A sediment section of 1cm thick was suspended in 100ml water to which 10ml of 3M NaBr and 2ml of 2M $CuSO_4$ were added(Furutani and Rudd 1980). This suspension was shaken and after settling, 1ml of supernant was removed to measure DPM Which was corrected from background activity(50 DPM).

Table 20 $^{203}Hg^{2+}$ distribution and diffusion into intact sediment cores sampled at a depth of 1.5M in L. 302 S on Aug. 17, 1984

incubation time	Core Depth	water pH	*DPM
24 hs.	1cm	5.5	2184.1
	2cm		151.5
	3cm		65.4
	4cm		49.8
	5cm		34.3
50 hs.	1cm		4287.9
	2cm		492.4
	3cm		77.4

* A sediment section of 1cm thick was suspended in 100ml water to which 10ml of 3M NaBr and 2ml of 2M $CuSO_4$ were added(Furutani and Rudd 1980). This suspension was shaken and after settling, 1ml of supernant was removed to measure DPM which was corrected from background activity(50 DPM).

sediment in 1.5M cores than was detected in the sediment from 6M cores(Table 21).

Data on the production and distribution of (203-)methylmercury in intact sediment cores are presented in Tables 22 and 23. Nearly all methylmercury in sediment cores was found in top 1cm section, especially after 24 hours incubation. Tables 24 and 25 show the (203-)methylmercury production and distribution in intact sediment cores of overlying water and sediment. To our surprise, the control core samples which received 1ml of concentrated HCl still displayed high methylation activity, although the pH of overlying water was 1.0 as measured after 40 hours incubation. Most of methylmercury was found in the water in this case. Methylmercury concentration in the core samples was increased with the increase in incubation period from 24 hours to 50 hours.

Based on the above experiment, a further investigation on the effect of pH on methylation rate in L.239 sediment cores was done using an incubation period of 24 hours.The sediment cores were taken within a circular area of about 40cm diameter at a depth of 1M in L.239 on Aug.29, 1984. Table 26 shows that methylation rates were increased tremendously when the pH of the overlying water was decreased by the addition of H_2SO_4. Table 27 shows the results from a similar experiment. Here,core samples were taken at depths of 1.5M or 1M in L.239 on Aug.22,1984. The methylation rate increased when pH was lowered from 6.3 to 3.35 by addition of H_2SO_4 in the cores taken at 1M depth. The methylation rate decreased but did not cease when pH was lowered from 6.3 to 1.95 by addition of HCl.

Table 21 The percentage of ^{203}Hg diffused into sediment of intact cores sampled at depths of 6M and 1.5M on Aug. 17, 1984

Lake depth	Incubation time(hs)	Total Hg in water ng Hg	Total Hg in sediment ng Hg	Hg % in sediment
6M	24	4633.3	1069.2	18.7 %
	50	3617.8	1163.2	24.3 %
1.5M	24	2592.9	1203.8	31.7 %
	50	1660.2	1596.4	49.0 %

Table 22 $CH_3{}^{203}Hg^+$ production and distribution in intact sediment cores sampled at a depth of 6M in L.302 S on Aug. 17, 1984

Incubation time	Core depth	water pH	*DPM
24 hs.	1cm	5.7	557.8
	2cm		43.4
	3cm		23.9
	4cm		7.8
	5cm		0
50 hs.	1cm	5.7	717.3
	2cm		141.5
	3cm		32.8

* The dilution factor(d = 8.148) from the extraction procedure was not used to correct the DPM in order to show clearly methylmercury production and distribution in the sediment cores. Background activity was corrected(50 dpm).

Table 23 $CH_3{}^{203}Hg^+$ production and distribution in intact sediment cores sampled at a depth of 1.5M in L.302 S on Aug. 17, 1984

Incubation time	Core depth	Water pH	*DPM
24 hs.	1cm	5.5	588.1
	2cm		46.5
	3cm		1.2
50 hs.	1cm		2834.2
	2cm		561.5
	3cm		41.9

* The dilution factor(d = 8.148) from the extraction procedure was not used to correct the DPM in order to show clearly methylmercury production and distribution in the sediment cores. Background activity was corrected(50dpm).

Table 24 CH_3Hg^+ production and distribution in intact sediment cores of overlying water and sediment sampled at a depth of 6M in L.302 S on Aug. 17, 1984

Incubation time	Water pH	Total CH_3Hg^+ in water ng	Total CH_3Hg^+ in sediment ng
24 hs.	5.7	3.313	8.345
40 hs.	1.0	9.341	1.902
50 hs.	5.7	30.743	11.213

Table 25 CH_3Hg^+ production and distribution in intact sediment cores of overlying water and sediment sampled at a depth of 1.5M in L.302 S on Aug. 17, 1984

Incubation time	Water pH	Total CH_3Hg^+ in water ng	Total CH_3Hg^+ in sediment ng
24 hs.	5.5	42.993	8.459
40 hs.	1.0	52.571	2.842
50 hs.	5.5	29.728	38.622

Table 26 Effect of pH on methylmercury production in intact sediment cores sampled at a depth of 1M in L.239 on Aug. 29, 1984

Incubation time	Initial water pH	Total CH_3Hg^+ in water ng	Total CH_3Hg^+ in sediment ng
24 hs.	6.3	1.165	2.528
	6.3	1.348	2.201
	6.3	1.535	2.941
	5.4	2.993	2.712
	4.7	3.287	2.013
	3.4	4.994	6.390

Table 27 *Effect of pH on methylmercury production in intact sediment cores sampled at depths of 1.5M and 1M in L.239 on Aug. 22, 1984

Lake depth (M)	Initial water pH	Total CH_3Hg^+ in water ng	Total CH_3Hg^+ in sediment ng
1.5	6.3	6.782	0.591
	2.0(HCl)	2.587	0.651
1.0	6.3	2.914	0.796
	3.4(H_2SO_4)	6.242	1.736

* 24 hours incubation

3.7. Effect of mercury concentration on rates of methylation and demethylation

Before July 1, 1984, the $^{203}Hg^{2+}$ addition used for methylation measurements was 1uCi/1ug Hg/100ul. However, the isotope $^{203}Hg^{2+}$ that arrived on July 1 from the supplier contained a lot of nonradioactive mercury. It was 1mCi/1.52mgHg on July 3. Accordingly, during the investigations of July and August, the isotope we used for methylation measurements contained more unlabled mercury. Because ^{203}Hg has a short half life of 46.7 days, it decays quickly. To compensate for a low activity, the isotope addition we used during investigation was as follows. From July 3 to July 18,it was 1uCi/1.52ug Hg/100ul. From July 19 to Aug. 6, it was 1uCi/1.91ug Hg/100ul. It was 1uCi/2.62ug Hg/100ul from Aug.7 to Aug.31. From Sept.10 to 20, it was 1uCi/1ug Hg/100ul. All caculations were decay-corrected.

The effect of mercury concentration on methylation was examined when a new isotope shipment ^{203}Hg arrived in September. For this evaluation, epilimnion water was collected from L.302 S and the sample was divided into 2 parts. Each part was further divided into a set of subsamples comprising 2 control and 4 tests portions. Each of the samples contained 150ml water. The first set received 100ul of 1uCi/1ug Hg/100ul, while the second set received 100ul of 1uCi/2.62ug Hg/100ul. After 12 hours incubation methylation rates were measured. The results are presented in Table 28. The methylation rate was calculated according to Equation 6. For the first set(receiving 1uCi/1ug Hg) Rm = 3.848 ng/L/d, and for the second set(receiving 1uCi/2.62ug Hg) Rm = 23.964 ng/L/d(Table 28). The rate was approximately 6 times greater for the second set than for the first set. The ratio of the methylated

Table 28 The effect of mercury concentration on rate of methylation for L.302 S epilimnion water sampled on Sept. 11, 1984

$^{203}Hg^{2+}$ addition per sample	DPM from test samples	DPM from control samples	Ratio methylated
1uCi/1ug Hg	308.5	62.2	
	279.4	68.1	
	306.0		
	281.9		
mean	294.0	65.2	
			0.000132

Rm = (s-b)(d)(a)(g) ---------- Eq. 6
= (294.0-65.2)x5.6x(1/2220)x(1000/150)
=3.848 ng/L/d

1uCi/2.62ug Hg	608.8	71.5	
	628.2	77.7	
	591.9		
	645.1		
mean	618.5	74.6	
			0.000278

Rm = (s-b)(d)(a)(g) ---------- Eq. 6
= (618.5-74.6)x5.6x(2.62/2220)x(1000/150)
= 23.964 ng/Ld

(203-)mercury to the total (203-)mercury was also increased from 0.000132 to 0.000278(Table 28). It clearly indicats that high Hg^{2+} concentration will result in very high methylation rate.

A further investigation on the effect of $^{203}Hg^{++}$ concentration on methylation rate and the effect of $^{14}CH_3Hg^+$ concentration on demethylation rate were conducted.

Again, the examination of the effect of $^{203}Hg^{++}$ concentration on methylation was done by using L.302 S epilimnion water samples. The isotope solution used in this study had a specific radioactivity of 1uCi/1ug Hg. The range of mercury concentration added to the sample was from 0 to 66.667ug/L. The results of Sept.13 and Sept.18,1984 were shown in Fig.20. The methylation rate increased exponentially when the $^{203}Hg^{++}$ concentration increased. Even when the $^{203}Hg^{2+}$ concentration increased until 66.667ug Hg/L water, the methylation rate still increased exponentially in the epilimnion water samples collected on Sept. 18, 1984(Fig. 21). The result of Sept. 18 in Fig. 20 is only an enlargement of part of the result shown in Fig. 21.

The examination on the effect of $^{14}CH_3Hg^+$ concentration on demethylation rate was done using L.302 S epilimnion water samples. The isotope solution used in this study had a specific radioactivity of 10000dpm/o.1ug Hg as CH_3Hg^+. The range of methylmercury concentration used was from 0 to 7.467ug Hg/L. The results of experiments on Sept.13 and Sept.18,1984 are presented in Fig.22. The demethylation rate increased linearly when methylmercury concentration increased from 0 to 3.733ug Hg/L water and the increase slowed down at a high methylmercury concentration of 7.467ug Hg/L water(Fig.22).

Fig. 20 Effect of Hg^{2+} concentration on rate of methylation for L.302 S epilimnion water sampled on Sept. 13 and Sept. 18. Incubation time, 12hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

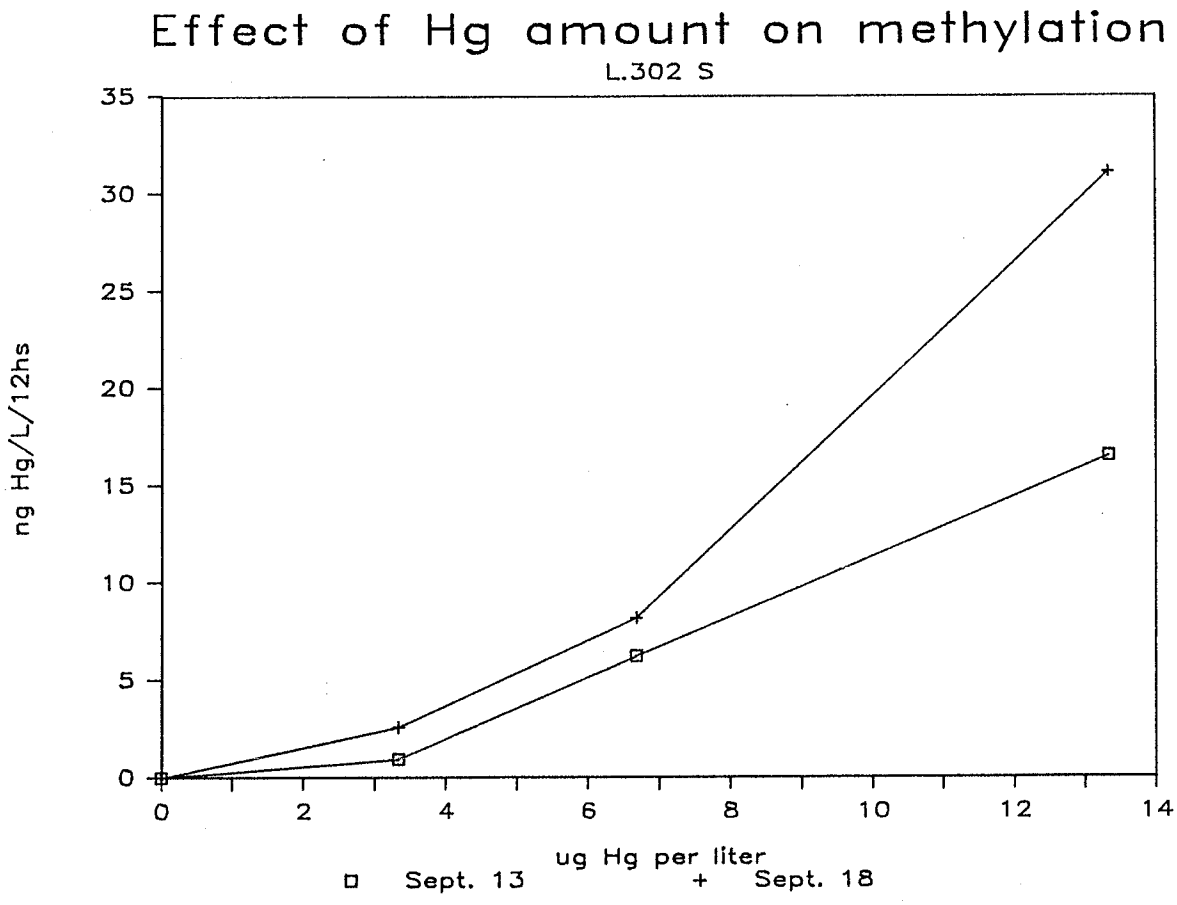

Effect of Hg amount on methylation
L.302 S
ng Hg/L/12hs
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
ug Hg per liter
Sept. 13
Sept. 18

Fig. 21 Effect of Hg^{2+} concentration on rate of methylation for L.302 S epilimnion water sampled on Sept. 18. Incubation time, 12hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

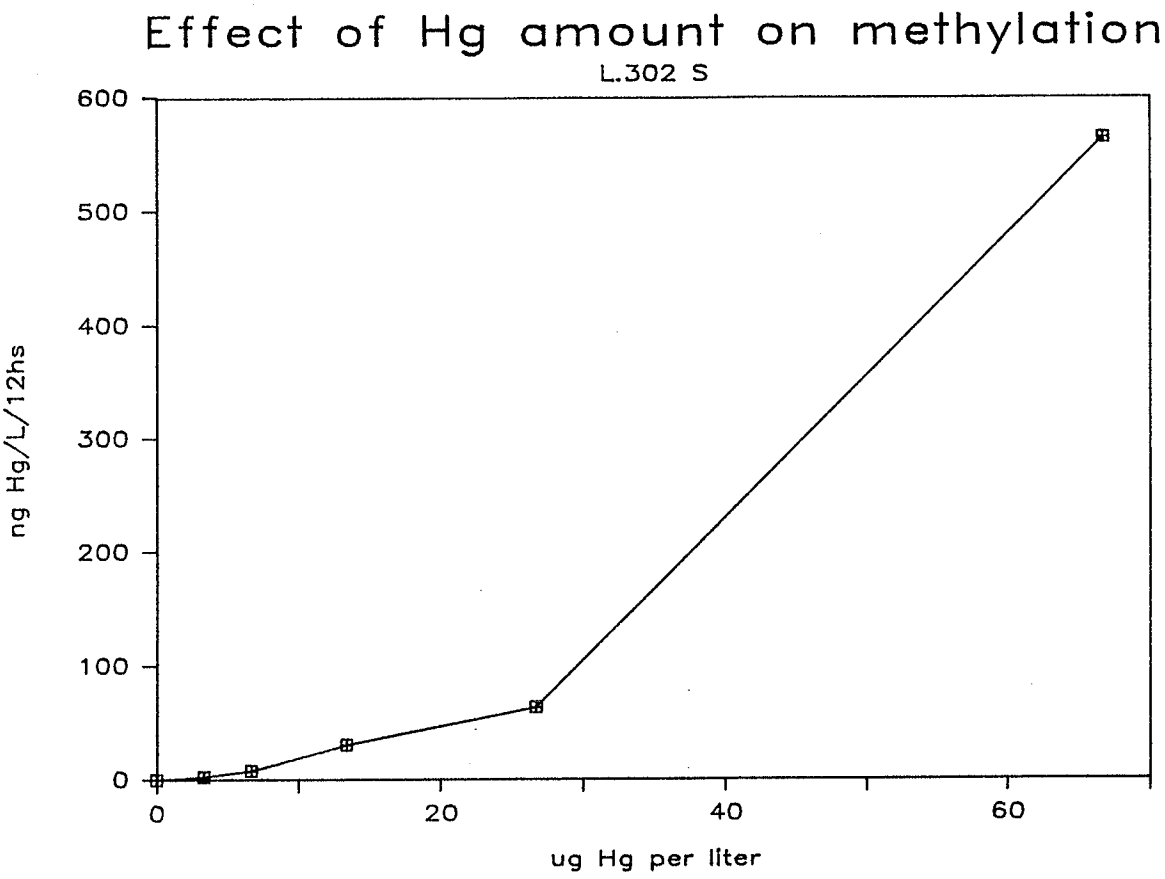
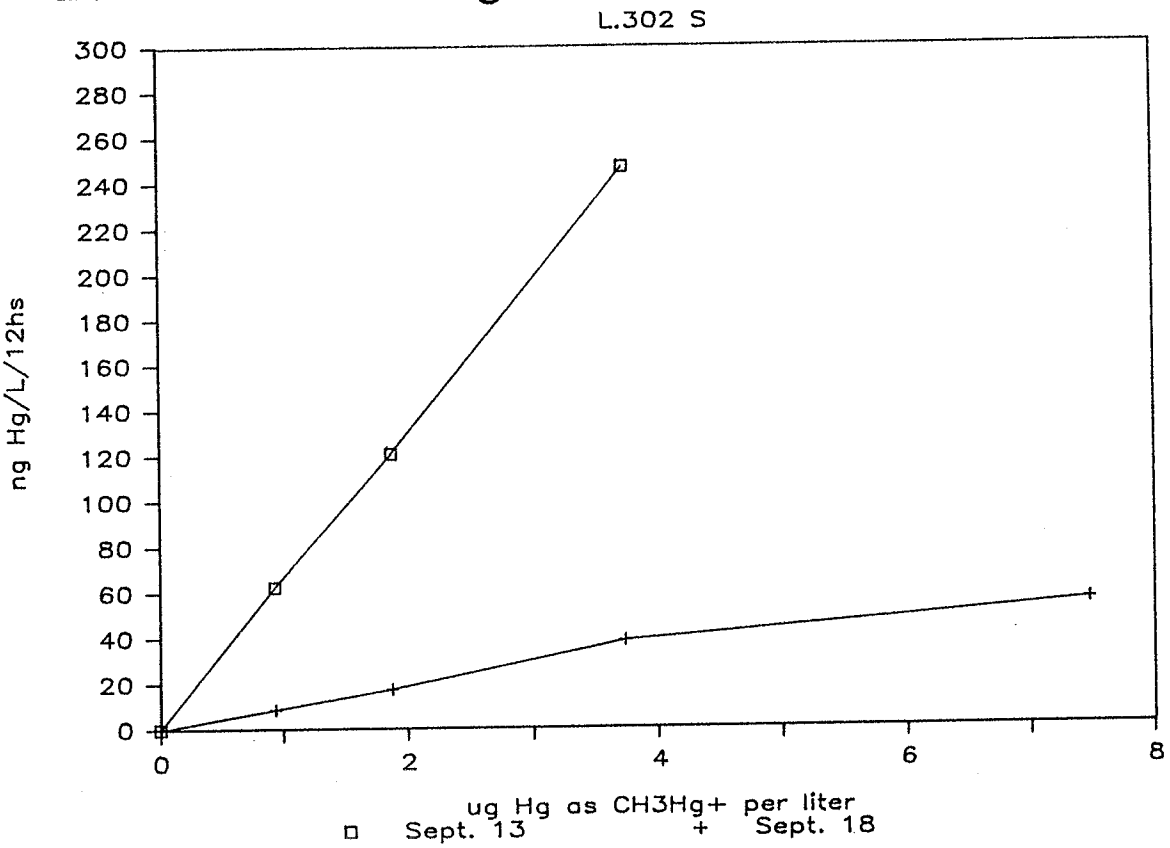

Effect of Hg amount on methylation
L.302 S
600
500
400
300
200
100
0
ng Hg/L/12hs
0
20
40
60
ug Hg per liter

Fig. 22 Effect of CH_3Hg^+ concentration on rate of demethylation for L.302 S epilimnion water sampled on Sept. 13 and Sept. 18. Incubation time, 12 hs. Means of duplicate samples are plotted, vertical lines show ranges of the differences. Very small ranges are not shown.

Effect of CH3Hg amount on demethylation



Discussion

Methylation and demethylation activities were consistently found in water columns of ELA lakes during the summer of 1984. Furutani and Rudd (1980) found methylation activity only occasionally in the water column of a mercury polluted lake they examined. This finding might have been the result of the long incubation period(4 days) employed or due to the relatively low mercury levels(0.41ug Hg/270ml) in their experiment, a concentration of nearly 4.5 times lower than that used in our experiments.

That the observed methylation was likely of biological origin is demonstrated by our control experiments where the methylation reaction was inhibited completely either by autoclaving or by the addition of concentrated HCl(Table 28). Chemical methylation is not inhibited or influenced by autoclaving the methylation agents such as fulvic acid or humic acid(Nagase et al. 1982).

Methylation activity increased as the water pH was lowered from about 7.0 to 5.0 in lakes not receiving acid addition(Figs. 5-9 and Tables 8-10) and decreased as the water pH was raised from about 5.5 to 8.5 in acidified lakes(Fig. 10 and 11 and Tables 11 and 12). DeSimone et al.(1973) demonstrated that the methylcobalamin-dependent methylation has a optimum pH around 4.7. It is not surprising, then, that methylation activity is faster at pH values around 5.0 in water samples, as the methylcobalamin excreted by bacteria will react with Hg^{2+} more quickly at this pH. This may also explain our finding that when the pH of L.239 water was decreased to pH 3.47, the methylation rate decreased(Fig. 7) because the pH was now below the stated optimum for methylcobalamin-dependent activity. Other possibilities, of course,

should be considered. For example, at pH 5.0, Hg^{2+} may be more readily taken up by the methylating bacteria.

On the other hand, demethylation activity decreased whether pH was raised or lowered from the ambient pH(Fig. 8-11 and Tables 8-12). This is probably due to the adaptation of bacteria to the environmental pH. Any shift in pH experimently induced may delay the induction of enzymes responsible for demethylation.

Methylation activities have been demonstrated to be cyclic in pattern in sediment samples(Spangler et al. 1973), human faeces(Edwards and McBride 1975) and even in pure culture experiments(Hamdy et al. 1975), due to the concomitant degradation of methylmercury by bacterial demethylation activity(Spangler et al. 1973). Data in this study show that methylation activity is also cyclic in pattern in the water samples of inland lakes. The combination of methylation and demethylation studies in L.302 S water samples revealed that methylmercury concentration reached its highest value by the 3rd day of incubation and decreased quickly due to the very marked increase in demethylation activity at the same time(Fig.14-17). This result also supports the finding that Hg^{2+} is an alternative inducer of demethylation enzymes(Clark et al. 1977 and Weiss et al. 1977). Because of the cyclic methylmercury production, longer incubation for comparing the effect of pH on the activities of methylation and demethylation gave questionable results(Fig. 12 and 13). However, longer incubation disclosed that although demethylation activity was lowered by lowering the pH for 1 day's incubation, the activity would increase very quickly once the bacteria adapted the new pH environment(Fig. 13).

Methylation activity is also directly related to the Hg^{2+} concentration available. Methylcobalamin-dependent chemical methylation has shown that methylation rate increased linearly with the increase in Hg^{2+} concentration(DeSimone et al. 1973). Nagase and colleagues(1982) found that both humic acid and fulvic acid extracted from sediment methylated mercury and that the rates increased linearly with the increase in inorganic mercury concentration. Rudd et al.(1983) demonstrated that methylation rates in surficial sediments was directly related to Hg concentration over several orders of magnitude. However, data in our study show that the methylation rate increased exponentially with an increase in Hg^{2+} concentration in L.302 S water samples(Fig. 20 and 21). Demethylation activity is also related to CH_3Hg^+ concentration, and the rate increased linearly as CH_3Hg^+ increased(Fig.22).The reason behind these phenomena warrants further study.

In lake water profiles, methylation activity was much higher in the epilimnion water than in the hypolimnion possibly due to low temperature in hypolimnion(Tables 16-18). In most cases, the most active site for methylation is in the epilimnion, but L.239, deep and very oligotrophic, had the highest methylation activity at the thermocline. Demethylation activity did not change as much as did methylation activity in water profiles and its highest activity is usually found in the epilimnion(Table 16-18).

The water samples taken from acidified lakes always had higher methylation activity than did the samples taken from circumneutral lakes(Tables 13-15). This is to be expected since, in our experience, slight acidity seems to favour lake water methylation. Other

posibilities, however, cannot be ignored. For exsample, acidified and circumneutral lake may harbour quite different microbial poppulations. Furhter examination of this possibility is also warranted.

Eutrophic L.227 had the lowest methylation activity of all the lakes under study. This may be due to the high pH value in its epilimnion. This finding concurs with that of Rudd and Turner(1983) who found that fish accumulated less mercury in enclosures in which water pH was raised by stimulating primary production. However, fish did accumulate more mercury in enclosures in which fertilizer stimulated primary production did not change pH although the growth rate of fish was enhanced as compared with unfertilized control enclosures. In summery, it seems that high level of nutrients in water will increase methylation activity, but methylation rate will decrease when much pH is increased.

Data in this study show that methylation and demethylation rates were all decreased in sediment samples taken from three ELA lakes when the ambient pH was lowered. The demethylation rate was decreased to a lesser extent than the methylation rate(Table 2-7). Ramlal(1983) found a similar result for her methylation assay. Her study also suggested that lower methylation at lowered pH is probably due to the binding of mercuric ion to sulfide. Furutani et al.(1984) demonstrated that reduction in pH did not decrease the microbial activity in sediment samples as measured by CO_2 and CH_4 production, but rather decreased the methylation rate. On the other hand, methylation rates increased when sediment pH was raised to 7.0. These experimental results do not explain why fish in "acid rain" stressed lakes contain increased amount of methylmercury.

A likely explanation for the above experiments is that Hg^{2+} was thoroughly mixed with sediment floc and low pH reduced the amount of mercury available for methylation in two possible ways: a) an acid condition favors the dissociation of FeS and, in consequence, Hg^{2+} is sequestered by sulfide binding(Ramlal et al. in press); or (b) a low pH increases the probablity for organic particles to coagulate and Hg^{2+} associated with or bound to these organic materials is buried in the coagulant, and thus becomes less available for methylation by bacteria(Hakanson 1974 and Miller 1975). However, the above conditions will not occur in the natural environment. Firstly, inorganic mercury will settle to the top of sediment and some will diffuse into deeper layers(Miller 1975). Secondly, sediment in acidified lakes is usually not acidified due to the presence of sulfate and nitrate reducing bacteria, which consume protons(Kelly et al. 1984).

To examine the question of the effect of pH on methylmercury production at the sediment-water interface, a nearly natural experimental condition might be approximated by using intact sediment cores with overlying water receiving the experimental acid additions. Miller(1975) found that over a period of 110 days, approximately 25-50% of mercury added to the water was taken up by the sediment in intact sediment cores, most of which was found to be concentrated at the sediment-water interface. In contrast to this slow uptake by undisturbed sediments, almost all mercury was taken up by shaken sediments. The author ascribed this phenomenon to the greater surface area exposed in the latter case. Rudd et al.(1983) demonstrated that the methylation rate was highest near the sediment-water interface in sections of sediment cores. This could be attributed to the high microbial activity

at that site. Data produced by this study support these previous findings. Both mercuric ion and methylmercury found in the sediment of intact cores were mainly at sediment-water interface. The difference between the absorption of mercury into the sediment in intact cores taken from a depth of 1.5M and 6M in the epilimnion of L.302 S is probably due to the properties of the cores. The cores from the 6M depth were flocculent and more organic while those from 1.5M were sandy and more compact. Because the sediment in the cores from 6M contained more organic material it likely had more microbial activity than did the cores from 1.5M .A higher microbial activity, in turn, might suggest that a stronger and sharper Eh gradient existed at the sediment-water interface for the 6M cores and this Eh gradient might have prevented more Hg^{2+} from diffusing into the sediments(Hutchinson 1957).

Miller and Akagi(1979) found that methylmercury released into the overlying water in a sediment-water system was nearly doubled when the water pH was lowered from 6 to 5, although methylmercury production in the system was not influenced over the same pH range. Our data show a different result to that found by Miller and Akagi. Methylmercury production in the overlying water was nearly doubled for a decrease in pH of approxiately 1.0 unit, although methylmercury production in the sediment did not increase,even when the pH of overlying water was redused from 6.3 to 4.7. Sediment production of methylmercury did increase when the pH was further lowered to 3.4(Tables 26 and 27). The difference between these two studies is that we only used a 1day incubation period and they used 7, 14 and 21days incubation periods. Because they used longer incubation times, they concluded that changing values of the pH in natural sediment-water systems did not affect the

total amount of methylmercury produced, and that the increased amount of methylmercury in the overlying water was due to changing the partition of methylmercury between water and sediment. Our data suggest that low pH not only influences partition of methylmercury but also increases methylmercury production in the sediment-water system.

Akagi et al.(1979) also reported that the removal of methylmercury by fish increased the net methylmercury production in sediment-water system. In the natural environment, methylmercury will be removed from the place where it is produced by water circulation and by absorption by organisms(Miller et al. 1977 and Parks et al. 1984). Therefore, short incubations are most likely to reveal the true rate of methylmercury production at sediment-water surfaces.

The result from this sediment-water system indicates the effect of pH changing in lake on methylation at sediment-water interface, not in sediment. In sediment, methylation rate is likely to decrease when pH is lowered because mercury concentration is reduced by increased sulphide binding(Ramlal et al. in press). Rudd(Pers. comm.) found that pH of sandy and compact sediment did not changed, although that of flocculent and more organic sediment decreased when lake was acidified.

The methylation activity in the water column of lakes was confirmed in this study, although the importance of methylation in water has been noticed before. Rudd and Turner(1983) demonstrated that ^{203}Hg was bioaccumulated 8- to 16-fold faster when the sediment was absent than when it was either suspended in the water or present at the bottom of large "in situ" enclosures. Their results imply the removal of Hg2+ by the sediment and also the importance of methylation in the water column. However before answering whether methylation in water or at the

sediment-water interface plays a more important role in methylmercury accumulation in fish bodies, further investigation is needed.

The results from this study indicate that acidic precipitation is most likely to cause increased concentrations of methylmercury in fish harvested from lakes of low pH in two ways: a) lowering the ambient pH in lake waters will increase microbial methylation activities in the water column and at the sediment-water interface. Demethylation activity in the water column on the other hand was decreased by lowering the pH as shown for 1day incubations. b) the increased loading of mercury which usually accompanies by acidic precipitation will increase methylation activity exponentially in the water column. Because demethylation rates only increased linearly with an increase in CH_3Hg^{2+} concentration in the water column, the increased amount of Hg^{2+} might increase the net methylmercury production. Acidification of lakes would also increase mercuric ion concentration in the water column by inhibiting the transfer of Hg^{2+} from water to bottom sediment(Jackson et al. 1980 and Schindler et al. 1980). Our data indicate that the combination of increased input of mercury and lowered pH by "acid rain" in lakes is most likely the reason why the fish in "acid rain"-stressed lakes contain more methylmercury.

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