SEDIMENT FLUX AND PHOTODEGRADATION OF METHYLMERCURY IN TWO BOREAL DRAINAGE LAKES.

by

Patricia Sellers

A thesis submitted to the faculty of Graduate Studies in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Microbiology
The University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

SEDIMENT FLUX AND PHOTODEGRADATION OF METHYLMERCURY IN TWO BOREAL DRAINAGE LAKES

BY

PATRICIA SELLERS

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

DOCTOR OF PHILOSOPHY

ACKNOWLEDGMENTS

I thank the many people and institutions who have contributed to this effort over the years. Funding for my research was provided by the University of Manitoba (Ph.D. Fellowship), the Experimental Lakes Area (Graduate Fellowship), the Natural Sciences and Engineering Research Council of Canada, Ontario Hydro, Hydro Quebec, Manitoba Hydro, and the Atmospheric Environment Service. I am very grateful to the Department of Microbiology, the Freshwater Institute, the Experimental Lakes Area, and to Flett Research Ltd. for providing me with the resources that enabled me to complete this degree.

I thank the many people at the Freshwater Institute who always very helpful to me. I relied on the resourcefulness of Patricia Ramlal, Mike Capel, Mike Stainton and Morris Holoka when I was constructing and testing the "pCO_2 machine" in 1991 and 1992. Sharon Ryland and Donna Larocque were terrifically accomodating with ELA travel and housing requests. Susan Kasian was always a treat to visit with my statistical questions. Ken Beaty, Mark Lyng, and Eva Schindler provided me with hydrological and chemical data that was necessary for the completion of my thesis.

Several people helped me during my field seasons at the Experimental Lakes Area. Grant Edwards, Scott Higgins, Paul Humenchuk, Karen Scott, Sebastien Lamontagne and Vince St. Louis all provided me with helping hands in the field. Vince St. Louis, Allison MacHutchon, and Andrew Heyes helped me in the "clean room" laboratory at ELA.

I gratefully acknowledge the fact that I had a terrific advisory committee; Carol Kelly, John Rudd, Ray Hesslein, Bob Flett and Richard Sparling were always receptive, insightful, and helpful. I especially thank Carol and John for their boundless enthusiasm, for certainly there have been times when I needed it. I also thank them for their patience, for I know that my peculiarities were often not easy to factor into the graduate student equation. I also am very appreciative of Ray, whose resourcefulness and fun I gleefully soaked up during my pCO_2 days.

I thank my family for they, too, have been generously supportive, patient, and encouraging over the years. I especially thank my parents, Fred and Netty, for providing me with a one-room log shack at the edge of the Winnipeg River. Therein I enjoyed writing the first draft of my thesis.

I thank Marcia Kyle, Allison MacHutchon, Gordon Robinson and Karen Scott. Without their relentless and genuine friendship I most certainly would not have survived this thesis. During my embryonic stages as an ecologist I met David Schindler and Gordon Robinson, who have always encouraged me to explore and capture the spirit of the land. I thank them for that.

ABSTRACT

Current trends in the anthropogenic mobilization of mercury into the environment suggest that the widespread problem of mercury pollution will persist for decades. Methylmercury is of particular concern because this is the form of mercury that accumulates in fish and is toxic to fish consumers. Therefore, methylmercury is a potential health threat to humans who eat fish. Methylmercury accumulates in the fish of many lakes, but study of its production and loss has been hampered by the lack of quantitative measurement techniques. In this study, trace-level analytical techniques were used to estimate fluxes of methylmercury in lakes by two different methods: Direct measurement of sediment and water fluxes by *in situ* incubations of sediment cores and water column samples, and measurement of whole-lake rates of methylmercury production by whole-lake chemical mass balance. This study was conducted at the Experimental Lakes Area, northwestern Ontario.

Results of the sediment core incubations showed that the sediment-water methylmercury flux was naturally variable from site-to-site within a lake. This variability was likely due to small scale chemical and/or biological variability at the sediment-water interface, and means that extrapolation of core results to larger areas and time periods is uncertain.

During incubations of water column samples *in situ*, I discovered that methylmercury is abiotically photodegraded by sunlight. Methylmercury was primarily photodegraded by solar UV-A, and was directly proportional to methylmercury concentration and to solar irradiance. The photodegradation rate was not affected by

major differences in water chemistry, which means that *in situ* rates were easily predicted from methylmercury concentration and solar irradiance alone. Knowledge of this process has significantly changed our understanding of factors controlling methylmercury concentrations in different types of lakes, especially brown vs. clear lakes.

Measurements of methylmercury photodegradation, and inputs and outputs of methylmercury in an annual mass balance study showed that the methylmercury in an oligotrophic lake (Lake 240) was controlled by both in-lake processes (photodegradation and in-lake production) and external inputs. The relative importance of external inputs, photodegradation, and internal production changed from year to year, primarily due to differences in precipitation that affected external inputs. Previous mass balance studies have underestimated internal production because photodegradation was not included as a loss term.

TABLE OF CONTENTS

page

LIST OF FIGURES

LIST OF TABLES

Thesis format

With the exception of Chapter 2, this thesis is written in paper format. Therefore, each of Chapters 3, 4, and 5 will have an *abstract, introduction, materials and methods, results,* and *discussion* section. To avoid unnecessary repetition, a description of analytical methods common to all chapters is presented in Chapter 2. The preliminary results of my methylmercury photodegradation research have already been published in *Nature* (Sellers *et al.*, 1996). This topic is more completely discussed in Chapters 4 and 5, wherein I have referred to the *Nature* publication. The *Nature* publication is included in this thesis as an appendix (appendix A).

The appendices include another paper that has been published in *Limnology and Oceanography* (Sellers *et al.*, 1995). It is restricted to the appendix because it is about CO_2 measurement in and emission from lakes, and therefore not easily integrated into the main body of the thesis. It is included because it represents the research I conducted when I first began graduate studies as a Masters student and before I transferred into the PhD program in 1993.

Chapter 1

Introduction

Mercury as a global pollutant. The amount of mercury in the environment is a global concern because of its potential as a human health threat (Clarkson, 1990, 1992, 1994). In several regions of the world, humans whose main food source is fish, are potentially at risk due to consumption of fish with high mercury concentrations (e.g. Akagi *et al*, 1995; Fleming *et al*, 1995; Wheatley and Paradis, 1995). Environmental mercury pollution and fish contamination results from both natural and anthropogenic sources. Anthropogenic souces include industrial waste discharge, municipal incinerators, coal combustion, (Stein *et al*, 1996), gold and silver mining (Pfeiffer *et al*, 1993), and the creation of hydroelectric reservoirs (Hecky *et al*, 1991). Natural emission of Hg^0 from the earth's crust is estimated to be about 1/3 of the current anthropogenic emissions of Hg^0 to the atmosphere (Slemr *et al.*, 1985).

The detrimental impact on humans resulting from consumption of mercury-contaminated fish was first recognized in Japan in the 1950s when hundreds of people suffered from a neurological disorder which later became known as Minamata disease (Harada, 1995). Since that time, there have been hundreds of investigations into the impact of mercury pollution on the health of humans and aquatic ecosystems. Historically, much of the research conducted on lakes has focused directly on the occurrence, uptake and persistence of mercury in fish. There are two reasons for this. One is that humans eat fish. The other is that the measurement of the trace levels of mercury in media other than

fish is difficult, and only possible in lakes receiving large point-source inputs of mercury. Also, because nearly all the mercury that accumulates in fish is methylmercury (CH_3Hg^+; Grieb *et al.*, 1990; Bloom, 1992), many of the biogeochemical studies of mercury have focused on the production and destruction of that form.

More recently, the occurrence of high mercury levels in fish from remote lakes (Sorensen *et al*, 1990; Bodaly *et al*, 1993; Haines *et al*, 1994) suggests that atmospheric deposition alone can be sufficient to cause this problem (Sorensen *et al.*, 1990; Swain *et al.*, 1992). While atmospheric concentrations (Slemr *et al.*, 1995) and mercury emissions (Pirrone *et al.*, 1996) have declined on a global scale within this decade, levels are still generally 2-3 times higher than pre-industrial levels (Slemr and Langer, 1992; Slemr *et al.*, 1995). Atmospheric transport and deposition is a growing concern, especially on a regional scale, because mercury continues to be released to the atmosphere as a result of past gold and silver mining (Nriagu, 1994) and current industrial activities (Lindqvist, 1985; Chu and Porcella, 1995). Although the residence time of atmospheric mercury is relatively short (about 1 year; Lindqvist, 1985; Slemr *et al.*, 1985), the continuous cycling of mercury between the atmosphere and the biosphere (Lindqvist, 1985; Fitzgerald *et al.*, 1994) means that the problems of mercury pollution in lake environments may persist even if the human activities that mobilize mercury into the environment are reduced.

Mercury cycling in lakes. Methylmercury is one of several mercury species that exists in lakes. The other main species are inorganic: Elemental mercury (Hg^0) and Hg^{2+}. The relative distribution and concentration of the different mercury species is controlled by the complex interaction of methylation/demethylation, oxidation/reduction,

adsorption/desorption, precipitation/dissolution, uptake/excretion, and deposition/evasion reactions (Stein *et al.*, 1996). The extent to which these reactions occur in lakes is controlled by the physical, chemical and biological properties of the lake and its environment (Fig. 1). Further, all the transformations depicted in Fig. 1 may not be occurring in all lakes, or may not be detectable in all lakes because of the complexity of the interactions between species, and because of the limitation of the current analytical techniques.

Ionic and organic forms of methylmercury and Hg(II) can enter lakes directly from the atmosphere or watershed, or can be produced within. Ionic methylmercury (CH_3Hg^+) in the water column can covalently bind with dissolved organic carbon (DOC) and particulate organic carbon (POC), and the binding sites with organic molecules are most likely mercury-sulfide bonds (Zepp *et al.*, 1974; Hintelmann *et al.*, 1995). Methylmercury shows a strong affinity for the particulate phase. Measured partition coefficients (expressed as log K_d; K_d = concentration of mercury in particles/concentration in water) for CH_3Hg^+ are from ≈ 3 for sediment-water mixtures (Miskimmin, 1991), ≈ 4.2 for soil particles suspended in the water column (Mucci *et al.*, 1992), and ≈ 6.2 for suspended lake particles (Hurley *et al.*, 1994).

CH_3Hg-POC enters the food chain (Becker and Bigham, 1995; Mason *et al.*, 1996) or settles to lake sediments (Hurley *et al.*, 1994). CH_3Hg^+ can also be demethylated (Ramlal *et al..*, 1986; Korthals and Winfrey, 1987) or photodegraded (Sellers *et al.*, 1996). The latter two reactions are important because they reduce the amount of CH_3Hg^+ available for bioaccumulation.

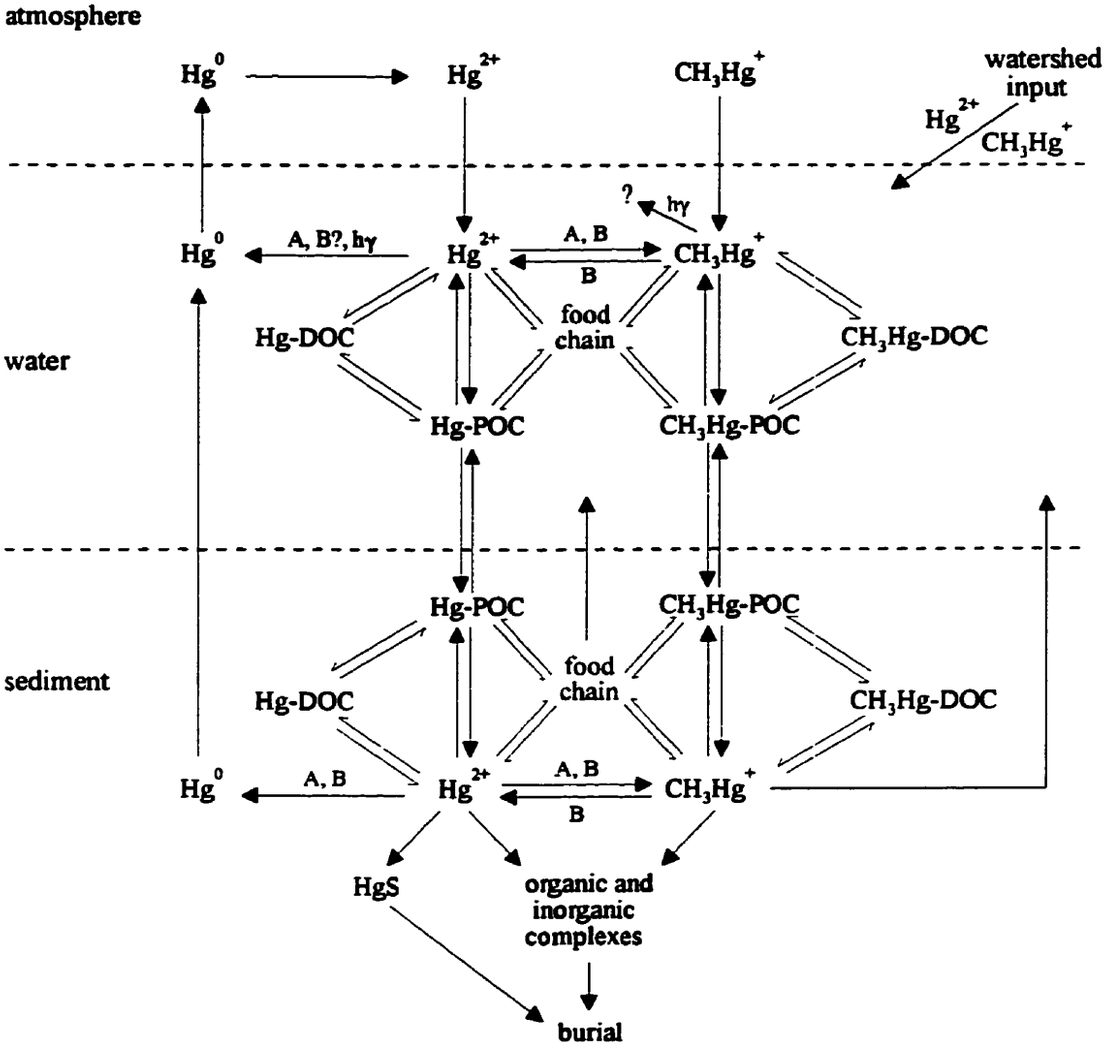


Fig. 1. Mercury cycling in drainage lakes. A = abiotic, B = biological for methylmercury production and destruction reactions.

In water and sediment, CH_3Hg^+ may be further biologically methylated to form dimethylmercury ($(CH_3)_2Hg$). Dimethylmercury is likely not a significant mercury species in soft-water (acid-sensitive) lakes because its formation is significant only at pH's above 7 (Fagerstrom and Jernelov, 1972). Although dimethylmercury has been measured in ocean waters (Mason and Fitzgerald, 1991), to date it has not been detected in lake waters (Fitzgerald *et al.*, 1991; Vandal *et al.*, 1991; Jocobs *et al.*, 1995). For this reason, dimethylmercury has not been included in Fig. 1.

Like CH_3Hg^+, ionic inorganic mercury (Hg^{2+}) in the water column can also bind with DOC and POC, and has a strong affinity for the particulate phase in lakes. The log K_d values for total mercury (mostly Hg^{2+}) are similar to those of CH_3Hg^+, ranging from 4.9 (Mucci *et al.*, 1995) to 5.8 (Hurley *et al.*, 1994) for suspended terrestrial and lake particles, respectively.

Hg^{2+} can be reduced to produce Hg^0. Hg^0 production from Hg^{2+} is mediated chemically (Alberts *et al.*, 1974; Allard and Arsenie, 1991) and biochemically (Fitzgerald *et al.*, 1994). Hg^0 production is also enhanced by sunlight in surface waters (Amyot *et al.*, 1994). Hg^0 is also a product of biological demethylation, but the contribution of this reaction to the total Hg^0 pool is thought to be small (Fitzgerald *et al.*, 1994). The end-products of methylmercury photodegradation are unknown, but one may be Hg^0. If this is so, then methylmercury photodegradation may also contribute significantly to Hg^0 production in surface waters. The solubility of Hg^0 in water is low; thus Hg^0 produced within the lake evades to the atmosphere. Production and emission of Hg^0 is an important process in lake mercury cycling (Vandal *et al.*, 1991; Fitzgerald *et al.*, 1994), especially

because it limits the amount of Hg^{2+} available for methylmercury production (Fitzgerald *et al.*, 1994). Atmospheric Hg^0 can be oxidized to Hg^{2+}, which can adsorb to particles and re-enter the lake through atmospheric fall-out.

Methylation, demethylation and volatilization reactions also occur in sediments. Hg^{2+} and CH_3Hg^+ can form inorganic and organic complexes, some of which may be taken up or ingested by benthic organisms and thereby enter the food chain (Becker and Bigham, 1995). The formation of other insoluble complexes, such as HgS, may limit the availability of mercury species for chemical and biological reactions. Inert complexes of mercury are part of the sediment pool.

Ultimately, the amount of methylmercury available for bioaccumulation in the food chain is controlled by the net balance of the formation and destruction processes of methylmercury, and these processes are discussed in the remainder of this chapter. Because destruction of methylmercury by (abiotic) photodegradation is the subject of this thesis, I will restrict my review of methylmercury destruction to biological demethylation.

Formation of methylmercury. Methylmercury is produced from the methylation of Hg^{2+} (Wood *et al.*, 1974; Ridley *et al.*, 1977). Both cellular (inside the cell) and acellular (outside the cell) mechanisms of methylation are known to occur. In acellular reactions, methyl groups are transferred from free methylcobalamin (Wood *et al.*, 1968; Imura *et al.*, 1971; Craig and Moreton, 1985; Choi and Bartha, 1993; Choi *et al.*, 1994), dissolved humic substances (Weber *et al*, 1985; Craig and Moreton, 1985), and possibly from methyltin compounds (Weber, 1993). Cellular methylation is the result of the interaction of intra-cellular methylcobalamin or other methylcorrinoids with Hg^{2+} (Berman

et al., 1990; Choi *et al.*, 1994). Choi *et al.* (1994) showed that methylation by methylcobalamin requires the participation of intracellular enzymes. However, the relative importance of cellular and ascellular methylation in lakes has not been measured. Acellular methylation has been demonstrated to be more important in some natural waters (Parkman *et al.*, 1994; Matilainen and Verta, 1995), but many more studies show that cellular methylation predominates in lake environments (e.g. Berman and Bartha, 1984; Callister and Winfrey, 1986; Xun *et al.*, 1987).

Methylators of mercury. Sulfate reducing bacteria are regarded as one of the main methylmercury producers in aquatic ecosystems (Compeau and Bartha, 1985; Gilmour and Henry, 1991). Some experimental additions of sulfate to sediment (Gilmour *et al.*, 1992; Gilmour and Riedel, 1995) and peat (Heyes *et al.*, 1996) samples have shown an increase in methylmercury production. Similarly, preliminary experiments show that sulfate additions to experimental plots in a wetland caused the methylmercury in surface peat porewater to increase 5 times above background levels after several days (B. Branfireun, pers. comm.). However, not all sulfate addition studies have shown an increase in methylation (e.g., Winfrey and Rudd, 1990), and this is probably because there exists an optimal concentration above which methylation of mercury is inhibited by solid HgS formation. For example, Gilmour *et al.* (1992) found that methylation was inhibited above 200 μM sulfate in creek sediments, Winfrey and Rudd (1990) found that methylation was inhibited with additions of 1 mM sulfate in lake sediments, and Heyes *et al.* (1996) found that methylmercury production in peat was inhibited by >2 mM sulfate.

It seems that the optimal concentration varies among environments, probably because of differences in chemical and/or physical conditions that affect microbial activity.

The strongest evidence to support sulfate reducers as principal methylators of mercury comes from the use of specific microbial inhibitors. Molybdate, an inhibitor of sulfate reducing bacteria, inhibits methylation in marine sediments (Compeau and Bartha, 1985, 1987), freshwater sediments (Gilmour *et al.* 1992; Gilmour and Riedel, 1995), and lake water (Matelainen, 1995). Experimental additions of bromoethanesulfonate (BES), a specific inhibitor of methanogenic bacteria, was shown to increase mercury methylation in marine sediments (Compeau and Bartha, 1985), presumably because the reduced competition between methanogens and sulfate reducers would shift the carbon and electron flow toward sulfate reducing bacteria. On the other hand Gilmour *et al* (1992) found that addition of BES to lake sediments decreased methylation slightly. Presumably this was because in low sulfate environments (freshwater), the syntrophic relationship between sulfate reducers and methanogens, which involves interspecies hydrogen transfer, accounts for a lot of the activity of the sulfate reducing population

In support of the hypothesis that sulfate reducers are principal methylators come from measurements of *in situ* methylmercury concentrations in lake sediment (Gilmour *et al.*, 1992) and water column profiles (Watras *et al.*, 1995a), which show that mercury methylation is located in the same strata as sulfate reduction. The maximum methylmercury concentration also occurs at the same depth as the minimum sulfate concentration (Watras *et al.*, 1995a).

Pure culture studies have also been conducted, which have shown that some (but not all) species of sulfate reducing bacteria (*Desulfovibrio*, *Desulfococcus*, and *Desulfobulbus* genera) methylate mercury (Gilmour *et al.*, 1992; Choi and Bartha, 1993; Choi *et al.*, 1994). However, four different methanogenic species and one acetobacterium that have been tested do not (G. Meichel, pers. comm.) even though they contain comparatively high cobalamin levels.

While there is much evidence to support the hypothesis that sulfate reducers are the major bacterial group responsible for mercury methylation, there are also reasons to believe that other bacteria may be important in certain lakes or under certain conditions within lakes. Gilmour *et al.* (1992) suggest than an optimum sulfate concentration for mercury methlation by sulfate reducers is 100 to 200 μM. Many lakes where methylation clearly occurs have sulfate concentrations below this optimum (e.g. most Canadian Shield Lakes; Kelly and Rudd, 1984). However, sulfate is not necessarily needed for sulfate reducers to live, nor for mercury methylation. Compeau and Bartha (1985) found that mercury methylation in sediment enrichment cultures were optimal in the presence of limiting sulfate and with fermentable substrates. Further, sulfate reducers are known to be active in sediment lacking sulfate, growing syntrophically with methanogens.

Because the cellular content of methylcorrinoids in many methanogenic and acetogenic bacteria is an order of magnitude greater than it is in sulfate reducing bacteria (Krautler *et al.*, 1988; Stupperich and Krautler, 1988; Stupperich *et al.*, 1988), it is premature to rule out other anaerobic bacteria as potentially important methylators of mercury. However, four methanogenic species and one acetogenic bacterium, all of which

contain high methylcorrinoids, do not methylate mercury (G. Meichel, pers. comm.). Still, this is a sampling of only a few of the many species living in lakes, and methanogenesis dominates microbial activity in many freshwater lakes (such as the Canadian Shield lakes (Kelly *et al.*, 1988) and release of methylcorrinoids with death of methane producing bacteria could lead to acellular methylation.

Finally, pure cultures of methanogens have also been shown to be inhibited by molybdate (Meichel *et al.*, 1996). This suggests that, in addition to inhibiting methylation by sulfate reducing bacteria, addition of molybdate to sediments may also have inhibited methylation by methanogenic bacteria (Gilmour *et al.*, 1992; Gilmour and Riedel, 1995).

Overall, the literature points to sulfate reducing bacteria as the principal methylators of mercury in the environment, and that they are most active as methylators when sulfate concentrations are low. The work from pure cultures studies also suggests that, in natural systems, sulfate reducing bacteria may be most active as methylators of mercury when they are growing fermentatively and not on sulfate. These conditions might maximize the availability for Hg^{2+} for methylation by limiting Hg precipitation with sulfides. If methylation is not dependent on the process of sulfate reduction, then this suggests that there is something else specific to methylating strains of sulfate reducers that favors mercury methylation. This cannot be methylcorrinoid content because, as stated above, it is an order of magnitude lower in sulfate reducers than it is in methanogenic or acetogenic bacteria. It may be that sulfate reducers are able to transport Hg^{2+} into their cells better than other anaerobic bacteria. It could also be that the fate of Hg^{2+} once inside the cell differs between bacteria. For example, it is possible that Hg^{2+} is preferentially

reduced (enzymatically) to Hg^0 rather than methylated in non-sulfate reducers but not in sulfate reducers. However, this would mean that the enzymes for Hg^{2+} reduction are synthensized in all bacteria and at *in situ* mercury concentrations. So far, this has not been demonstrated.

Destruction of methylmercury. Prior to the work presented here and by Sellers *et al.* (1996), the only known mechanism by which methylmercury was destroyed in lakes was biological demethylation. Demethylation is a biological, enzyme-mediated process whereas photodegradation is an abiotic, sunlight mediated process. Demethylation has the potential to occur in lake water and sediments (Winfrey and Rudd, 1990). The end-products of demethylation are typically Hg^0 and CH_4 (Robinson and Tuovenin, 1984) although CO_2 has also been observed (Oremland *et al.*, 1991; Oremland *et al.*, 1995).

Demethylators of methylmercury. The ability to demethylate mercury is widespread among microorganisms, although fewer genera have been isolated from sediments than from clinical settings such as hospitals (Robinson and Tuovenin, 1984). Clinical isolates include strains of *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces lividans*, and *Pseudomonas spp.* (Robinson and Tuovenin, 1984; Foster, 1987; Silver and Walderhaug, 1992). So far, sediment demethylating organisms have only been isolated from mercury polluted sediments, and include *Pseudomonas* species from river sediments (Tsai and Olson, 1990) and *Bacillus* species from marine sediments (Nakamura *et al.*, 1990). However, Oremland *et al.*, (1995) conducted substrate addition and inhibitor experiments which suggest that both sulfate reducing and methanogenic bacteria demethylate methylmercury oxidatively.

Assays of methylation and demethylation in lakes. Many of the investigations of methylation in lakes were made possible by the development of a radiochemical technique (using $^{203}Hg^{2+}$; Furutani and Rudd, 1980). This technique eliminated the necessity for long incubations periods (weeks) required with cold Hg^{2+} addition (e.g. Olson and Cooper, 1976). However, because of the low specific activity of added ^{203}Hg for the early studies, several times the natural Hg^{2+} concentration was added for detection of methylation and thus was not a true tracer technique. Also, the percent of the total Hg^{2+} that is available for methylation cannot be quantified, and may be very different after addition of $^{203}Hg^{2+}$. For these reasons, the *in situ* rates of methylation cannot be measured using the radiochemical technique. The same is true for the radioisotope technique using ^{14}C-methylmercury used for detection of demethylation (Ramlal *et al.*, 1986). The main advantage of the radioisotopic techniques, however, is that they can be used in a relative sense to quickly (and separately) to examine how different environmental parameters affect methylation and demethylation by natural microbial communities. Also, they can be used together to identify sites or conditions that have the greatest potential to favor net methylation (Ramlal *et al.*, 1986).

It is important to remember that the *MER* operon, which encodes the demethylating enzymes, is currently reported to be induced by methylmercury or Hg^{2+} levels of μg to mg per liter (Robinson and Tuovenin, 1984; Walsh *et al.*, 1988). These levels are achieved when the radiochemical assay of demethylation (described above) is used. However, these levels are much greater than the concentration found in unpolluted, natural lakes (ng L^{-1}), which are often the study sites for investigators using these

techniques. At present, however, induction of the *MER* operon at natural levels (1-2 ng L^{-1}) of Hg^{2+} is being observed (T. Barkay, pers. comm.), which suggests that induction by methylmercury at low concentrations is also possible however. Also, demethylation rates using ^{14}C-methylmercury show an immediate linear behavior in sediments (Ramlal *et al.*, 1986) but an initial lag in water (Xun *et al.*, 1987), which suggests that sediment must have the demethylating activity prior to addition of ^{14}C-methylmercury.

Very recently, a radio-tracer technique for the detection of methylation (using high specific-activity ^{203}Hg) has been developed (Stordal and Gill, 1995). The advantage of this techniques is that it measures rates of methylation without significantly increasing background concentrations, and therefore may provide a more realistic estimate of *in situ* rates (Stordal and Gill, 1995; Gilmour and Riedel, 1995). However, this tracer technique still does not measure *in situ* rates because the total amount of mercury available for methylation after $^{203}Hg^{2+}$ addition cannot be measured. Thus, the specific activity of the added isotope is unknown and therefore quantitative rates cannot be calculated.

The most promising technique currently being developed for measuring rates of *in situ* methylation (and demethylation) involves the use of stable mercury isotopes coupled with the high sensitivity detection method of gas chromatography and inductively-coupled plasma-mass spectrometry (GC-ICP/MS; Hintelmann and Evans, 1996). This method has many advantages over the previously discussed methods, including 1) isotope additions at or below *in situ* mercury concentrations, 2) changes in ambient methylmercury concentrations can be monitored while methylation (and demethylation) rates are being detected, and 3) the relative importance of individual mercury species available for

methylation can be assessed with simultaneous additions of labelled substrates (Hintelmann and Evans, 1996).

Whole ecosystem studies of net methylation (or net demethylation) have been made possible only in the last decade with the development of sensitive analytical techniques for the detection of mercury species in water at trace concentrations (e.g. Bloom and Fitzgerald, 1988; Bloom, 1989; Horvat *et al*, 1993). Although these techniques are burdened with many of the difficulties often associated with trace level analyses, the number of laboratories supporting them continues to grow because, for the first time, they allow for relatively easy measurement of mercury in natural environmental samples. The development of these techniques have shifted the focus of mercury research in lakes from laboratory incubation studies to field studies of whole ecosystems, which are necessary for understanding mercury cycling in lakes and prediction of the long-term impact of mercury pollution. Further, these techniques can be used to compliment radiochemical assays of methylation and demethylation (e.g. Ramlal *et al.*, 1993; Verta and Matilainen, 1995). More importantly however, they can be used to provide estimates of production of methylmercury in lakes, which include measurements of 1) methylmercury in incubated sediments (Gilmour and Riedel, 1995), 2) net flux from the sediment-water interface (Henry *et al.*, 1995), and 3) net whole-lake methylation (Sellers *et al.*, 1996).

Factors affecting methylation in lakes. To date, most of the studies of mercury methylation have been done with radiochemical additions of ^{203}Hg to samples. This approach may not only have the effect of increasing the Hg^{2+} to a final concentration that

greatly exceeds *in situ* concentrations (if it's not a tracer technique), but may also change the bioavailability of the total Hg^{2+} pool. These consequences should be kept in mind when examining and reviewing the effect of other environmental parameters on methylation (and demethylation; see next section) rates.

Many studies have shown that methylation of mercury is enhanced under anaerobic conditions when compared with aerobic conditions. This has been shown for methylation assays of sediments using cold chemical (e.g. Olson and Cooper, 1976) and radiochemical (Matelainen *et al.*, 1991, Regnell, 1994; Verta *et al.*, 1994) additions of Hg^{2+}. This has also been shown in lake water using $^{203}Hg^{2+}$ additions (Regnell and Tunlid, 1991). Simultaneous measurements of methylation in sediments and water have also shown that methylation is greater in the surface and subsurface sediment than in the overlying aerobic water (Korthals and Winfrey, 1987; Verta *et al.*, 1994). More recently, and in support of these earlier observations, profiles of *in situ* methylmercury concentrations in the water column of lakes show that the highest concentrations are found in anoxic hypolimnia (Watras and Bloom, 1994; Verta and Matelainen, 1995; Watras *et al.*, 1995a), which could be because of both *in situ* production and mineralization of settling particles. Also, measurements of sediment-water methylmercury flux is highest when water is anoxic (Henry *et al.*, 1995). However, it should be noted that increased concentrations and fluxes could also be due to lack of demethylation in anaerobic environments.

In addition to presence or absence of sulfate (discussed above), and the extent of lake anoxia, mercury methylation is affected by other environmental variables such as pH, temperature, availability of labile carbon, DOC, and Hg^{2+} concentration. Many studies

(reviewed by Winfrey and Rudd, 1990; Gilmour and Henry, 1991) have been done on the effect of pH on methylation because of the correlation between fish methylmercury concentrations and degree of lake acidification (e.g. Scheider *et al.*, 1979; Grieb *et al*, 1990). In lakes receiving acidic (sulfate) deposition, low pH is also correlated with elevated sulfate and the effect of sulfate on methylation may help to explain why the effect of pH is inconsistent among lakes. For example, a decrease in pH can depress methylation in subsurface sediments (Ramlal *et al.*, 1985; Steffan *et al.*, 1988) but can stimulate methylation at the sediment-water interface (Xun *et al.*, 1987). Xun *et al.* (1987) and Miskimmin *et al.* (1992) found that decreased pH stimulates methylation in surface waters, whereas Matilainen and Verta (1995) found no effect. Matilainen *et al.* (1991) found that a decrease in pH depresses methylation in water sampled above the sediment-water interface where as Regnell (1994) found that pH had no effect on methylation in water above intact sediment cores. Choi *et al.* (1994) showed that methylation in a strain of *Desulfovibrio desulfuricans* occurs above a pH of 6 and at an optimum pH of 6.5. This is higher than the pH of the water in acid lakes, and suggests that in these lakes especially, methylation is restricted to the sediments where the pH remains near 6.5 because of microbial consumption of H^+ associated with nitrate and sulfate reduction (Kelly *et al.*, 1984; Rudd *et al*, 1986).

The inconsistency of the results obtained within and among lakes makes it difficult to generalize about the effect of pH on mercury methylation. Although the cellular mechanism of methylation is directly affected by pH (Choi *et al.*, 1994), changes in pH in environmental samples may affect methylation indirectly by affecting the availability of

Hg^{2+} for methylation (see discussion on bioavailability below). Further, intra- and inter-lake differences of the effect of pH are confounded by differences in other chemical parameters, which also affect methylation. Clearly, these issues must be considered when one is examining or predicting the effect of pH on methylation or methylmercury accumulation in fish.

An increase in temperature and availability of labile organic carbon have the effect of increasing mercury methylation in sediments. This is not surprising because increased temperature and substrate concentration increase the activity of most bacteria. The effect of temperature has been shown by experimental incubations (Callister and Winfrey, 1986; Steffan *et al.*, 1988), and by spatial and temporal trends within lakes. Faster methylation rates occur in epilimnetic sediments than in hypolimnetic sediments (Matilainen *et al.*, 1991; Ramlal *et al*, 1993). Methylation rates are also highest during late summer when temperature and microbial activity at the sediment-water interface are highest (Korthals and Winfrey, 1987; Ramlal *et al.*, 1993). The effect of labile carbon has been demonstrated by experimental addition of organic substrates to incubating sediment (Furutani and Rudd, 1980; Callister and Winfrey, 1986; Regnell, 1994), and by examining the effect of increased organic input to sediments as a result of shoreline flooding (Ramlal *et al.*, 1986). It is also demonstrated by sediment depth profiles of methylating activity, which show that maximum activity occurs in the surficial floc at the sediment-water interface where labile organic carbon concentration and microbial activity is highest (Callister and Winfrey, 1986; Korthals and Winfrey, 1987).

The proportion of Hg^{2+} in unpolluted lake water and sediments that is available for methylation is unknown (Winfrey and Rudd, 1990; Gilmour and Henry, 1991), and continues to be an important limitation in the current understanding of mercury cycling in lakes. Xun *et al.* (1987) showed that microbial methylation of mercury in lake water was directly proportional to the amount of added Hg^{2+}. However, the high affinity of mercury for dissolved and particulate organic material (Zepp *et al.*, 1974; Miskimmin, 1991; Hurley *et al.*, 1994) suggest that much of the Hg^{2+} is unavailable for methylation, and that factors that affect this affinity may also affect methylation. For example, Miskimmin *et al.* (1992) showed that experimental addition of DOC to lake water samples inhibited methylation, and suggested that this is because DOC binds with Hg^{2+}, thereby making it unavailable for methylation. Matilainen and Verta (1995) also found that methylation is inhibited in DOC rich water. The binding of mercury to dissolved and particulate organic carbon in waters is affected by pH (Jackson *et al.*, 1980; Schindler *et al.*, 1980), and it has been suggested that a suppression of this binding by precipitation of DOC with other metals accounts for enhanced methylation with increased acidity in lake water (Winfrey and Rudd, 1980). In sediments, the binding of Hg^{2+} to sulfides and particulate carbon may limit sediment methylation (Ramlal *et al.*, 1985; Winfrey and Rudd, 1990; Matilainen *et al.*, 1991). The negative effect, in some cases, of lower pH on methylation in sediments may also be due to decreased availability of Hg^{2+} because of decreased solubility of Hg^{2+} at low pH (Ramlal *et al.*, 1985; Winfrey and Rudd, 1990). The decreased solubility is likely related to interaction with sulfides.

Recently, a luminescent biosensor has been developed by Selifonova *et al.* (1993) for estimation of the amount of Hg^{2+} available for intracellular reduction of Hg^{2+} to Hg^0 in polluted environmental samples. They found that the fraction of total Hg^{2+} available for reduction was 5 times smaller at the outlet than at the inlet of a mercury contaminated pond. In other words, the fraction of the total Hg^{2+} that entered the cells was not directly proportional to the amount of ambient (measured) Hg^{2+}, which was about 3 times smaller at the outlet than at the inlet of the pond. If the species of Hg^{2+} entering cells for reduction can also be methylated, then the study of Selifonova *et al.* (1993) suggests that the Hg^{2+} available for methylation in polluted and pristine lake environments cannot be predicted by the measured Hg^{2+} concentration.

There is only one study to date that reports on the species of Hg^{2+} that is available for methylation. Hintelmann *et al.* (1996) reported that dissolved mercury sulfides are easily methylated but solid HgS is not. More studies on the speciation of bioavailable Hg^{2+} are need to help predict the sites and conditions under which methylation occurs.

Factors affecting demethylation in lakes. Relative to methylation, far fewer studies on the effect of environmental variables on demethylation have been conducted, and so the knowledge of demethylation in lakes is more limited than it is for methylation. The effect of oxygen, for instance, is reported to be different among studies. Some studies show that demethylation in sediments is enhanced in the presence of oxygen (e.g. Compeau and Bartha, 1984; Ramlal *et al.*, 1986) while others show that sediment demethylation is depressed in the presence of oxygen (Regnell and Tunlid, 1991; Verta *et*

al., 1994) or that oxygen level has no effect on demethylation (Matilainen, *et al.*, 1991). The effect of oxygen on demethylation in lake water alone has not yet been examined.

An increase in acidity generally has the effect of decreasing demethylation in lake water and sediments (Ramlal *et al.*, 1986; Matilainen *et al.*, 1991; Miskimmin *et al.*, 1992). However, an increase in demethylation with decreasing pH in lake water (Xun *et al.*, 1987) or no change in demethylation with pH in lake sediments (Korthals and Winfrey, 1987) has also been reported. Presumably, only free (ionic, unbound) methylmercury is taken up by cells for demethylation in water and sediments. Therefore, the reported differences in the effect of pH may be related to the fraction of dissolved methylmercury that is free (ionic; bioavailable) and DOC-bound (unavailable), which increases with a decrease in pH (Hintelmann *et al.*, 1995).

Sediment demethylation seems to be favored by lower temperatures. Ramlal *et al.* (1993) found demethylation rates to be faster in hypolimnetic than in epilimnetic sediments, and also faster in the winter than in the summer. Korthals and Winfrey (1987) also found that maximum seasonal sediment demethylation did not coincide with maximum seasonal temperature, but Matilainen *et al.* (1991) did.

Organic rich sediments have higher demethylation rates than mineral sediments (Ramlal *et al.*, 1986; Korthals and Winfrey, 1987) or overyling water (Korthals and Winfrey, 1987), an effect that is likely related to overall microbial activity because it is affected by the availability of labile carbon.

Net methylation in lakes. Radiochemical assays of methylation and demethylation are of most value when they are used together to establish methylation/demethylation

ratios, which allow for the potential for net methylation to be assessed (Ramlal *et al.*, 1986; Gilmour and Henry, 1991). This is important because both methylation and demethylation determine the amount of methylmercury available for accumulation in lake biota. However, it has been (Furutani and Rudd, 1980; Ramlal *et al.*, 1986), and must continue to be, emphasized that these techniques provide a relative assessment only, and are not a quantitative measure of *in situ* rates. Still, their extensive use has allowed for several general statements about methylation and demethylation in lakes that are consistent with other observations, such as high methylmercury concentrations in fish in different lakes and reservoirs (Winfrey and Rudd, 1990; Gilmour and Henry, 1991). These include 1) biological processes dominate methylmercury transformations 2) demethylation is favored under aerobic conditions 3) methylation is favored under anaerobic conditions and 4) sediments are the dominant sites of net methylmercury production, and contribute methylmercury to the water column.

The more recent development of measurement techniques for *in situ* lake methylmercury (Bloom, 1989; Horvat *et al.*, 1993) initiated the testing of several of the above statements under *in situ* concentrations of mercury. Agreement between radiochemical assays of methylation and *in situ* methylmercury measurements means that the radiochemical methods can reflect the activities of natural populations (Ramlal *et al.*, 1993; Verta and Matilainen, 1995). Also, the view that anoxic conditions favors net methylation is supported by several measurements of relatively high methylmercury concentration in anoxic lake water (Watras and Bloom, 1994; Verta and Mailainen, 1995; Watras *et al.*, 1995). On the other hand, some of the other long-accepted views are not

supported by *in situ* measurements of methylmercury. For example, the dominant removal mechanism of methylmercury in clearwater lakes may be (abiotic) photodegradation rather than (biological) demethylation (Sellers *et al.*, 1996). Also, water column production of methylmercury in lakes with anoxic hypolimnia may be more important than sediment production (Henry *et al.*, 1995; Watras *et al.*, 1995a). Clearly, the development of measurement techniques for measurement of *in situ* concentration of methylmercury are helping greatly to expand the knowledge of mercury biogeochemistry, and, as stated above, are the only techniques available for quantifying *in situ* rates of mercury cycling.

Research objective and general approach

Using the "new" techniques for measurement of methylmercury in lakes (Bloom, 1989; Horvat *et al*, 1993), my general research objective was to estimate *in situ* rates of processes believed to significantly affect methylmercury concentrations in lake water. Processes known to affect lake methylmercury concentrations are either external (stream inflow, outflow, atmospheric deposition, and run-off) or internal, occurring in the lake sediment and water. However, the relative importance of external and internal processes are controlled by many site-specific variables (Rudd, 1995).

For reasons that will become apparent, my specific research objectives evolved concurrently with my research. Ultimately, however, I wanted to estimate the net mass flux of methylmercury from sediments to water in a lake. I wanted to do this because sediments are considered to be an important source of water column methylmercury for two reasons: 1) methylation is favored by anaerobic conditions (e.g. Matilainen *et al.*, 1991; Regnell, 1994), which exist in the subsurface sediments of lakes and 2) relative rates of net methylation are much higher in sediments than in the water column of lakes (e.g. Korthals and Winfrey, 1987; Verta *et al.*, 1994). Currently, however, there are very few estimates of whole-lake sediment release rates for methylmercury, and therefore the importance of sediments relative to other in-lake or external processes is poorly understood.

I attempted to estimate sediment methylmercury flux using two different approaches. The first was to incubate intact sediment cores and measure the change in the

mass of methylmercury in the water above the core over time. Such measurements can be used to estimate a whole-lake flux by extrapolation. The second approach was to conduct a whole-lake chemical mass balance. In this approach, sediment flux is estimated by the difference between losses of methylmercury from, and inputs of methylmercury to the water column of a lake.

Chapter 2

General Methods

In this chapter I describe the methods used for sample collection and analyses of methylmercury in lake water, which are common to chapters 3, 4, and 5. These common methods are described here only, and methods specific to each of these chapters are described within them. I also describe the field sites: Lake 979, which was used for the sediment core incubation work in chapter 3, and Lake 240, which was used for the photodegradation and mass balance work in chapters 4 and 5.

Study Area. My research was conducted at the Experimental Lake Area (ELA) which is located in northwestern Ontario (93°30'- 94° 00' W and 49°30'- 49°45' N). The central part of the ELA region is composed of 17 drainage basins, within which lie 57 lakes that are controlled by the Canadian Department of Fisheries and Oceans for experimental ecosystem research (Johnson and Vallentyne, 1971). The ELA is underlain by Precambrian granite that is overlain by glacial till (≈ 18 m) composed of quartz-feldspar sand and gravel (Brunskill and Schindler, 1971). Soils are 10-50 cm thick, and support forests composed mainly of jack pine, black spruce, trembling aspen, and white birch. Exposed hill tops and slopes have little or no soil. The ELA lakes vary considerably in size and chemistry (Cleugh and Hauser, 1971), but all are softwater drainage lakes and they represent the most chemically dilute and oligotrophic lakes of the world (Moss, 1980). Typical for the region, the ELA lakes are ice-covered for

about 5.5 months (November through April) during the winter. Mean annual (1969-1995) precipitation for the area is 678 mm (K. Beaty and M. Lyng, unpubl. data).

The two main study lakes were Lake 979 and Lake 240. Chemical and physical characteristics of these two lakes are given in Table 1. Lake 979 is a small pond in the centre of a riverine wetland (14.4 h). It does not stratify and has a water residence time of less than one month (see footnote of Table 1). Wetland vegetation is dominated by *Sphagnum* moss, with an over story of tamarck, black spruce, Laborador tea and leatherleaf (St. Louis *et al.*, 1996). Lake 979 is the experimental site of the Experimental Lakes Area Reservoir Project (ELARP; Kelly *et al.*, in press). The ELARP was designed to simulate the creation of a hydroelectric reservoir, and in June of 1993, the peatland surrounding Lake 979 was flooded by damming the lake outflow. The water level of Lake 979 was raised by 1.30 m as a result of flooding, increasing its surface area by 6.5 times. It was drawn down to 0.4 m above the pre-impoundment level in the fall of 1993. Spring flooding and fall drawdown occurred again in 1994 and 1995. Lake 979 receives most of its water from Lake 240, which is connected to Lake 979 via a short small stream (Fig. 1). Lake 979 was my study site in 1993 and 1994.

Lake 240 is much larger than Lake 979 (Table 1; Fig. 1) and has a longer residence time (Table 1). Unlike Lake 979, Lake 240 thermally stratifies in the summer months, resulting in an epilimnetic depth of 4-5 m. The shore of Lake 240 is composed almost exclusively of young (16 yr) jack pine forest as a result of a 1980 forest fire (Beaty, 1994). However, there are three distinct, small "pockets" of black spruce-Sphagnum wetland on the shore of the lake which survived the fire. Lake 240 receives about 60% of its water from two upstream lakes while the remainder comes from direct terrestrial run-off and precipitation. Twenty years of

Table 1. Chemical and physical characteristics of Lake 979 and Lake 240[1].

parameter	Lake 979	Lake 240
surface area (ha)[2]	2.38 (15.5)	44.1
maximum depth (m)[2]	1.45 (2.70)	13.1
mean depth (m)[2]	0.69 (0.79)	6.1
renewal time (yr)[3]	-	1.5*
PAR extinction coeff. (m^{-1})[4]	2.46 (0.88-3.65)	0.5 (0.36-0.68)
pH	6.24 (5.83-6.86)	7.12 (6.17-7.43)
DOC ($\mu mol\ L^{-1}$)[5]	1080 (610-1620)	570 (530-650)
chloride ($mg\ L^{-1}$)	0.37 (0.31-0.45)	0.35 (0.31-0.43)
sulfate ($\mu eq\ L^{-1}$)	2.61 (1.66-3.73)	3.76 (3.48-4.01)
alkalinity ($\mu eq\ L^{-1}$)	125 (102-149)	142 (138-149)

[1] averages and range (in parentheses) of physical and chemical data collected during 1994 ice-free season

[2] pre-flood (post-flood) values for Lake 979

[3] Lake 979 pre-impoundment renewal time calculated for the year of 1992 was 0.1 months. Post-impoundment renewal time calculated for a three-month period (July to September) was 0.5; 0.5 and 5.2 months for 1993, 1994, and 1995, respectively.

[4] PAR = photosynthetically active radiation.

[5] DOC = dissolved organic carbon.

* average from long-term (1969-1994) data set (K. Beaty and M. Lyng, unpubl. data).

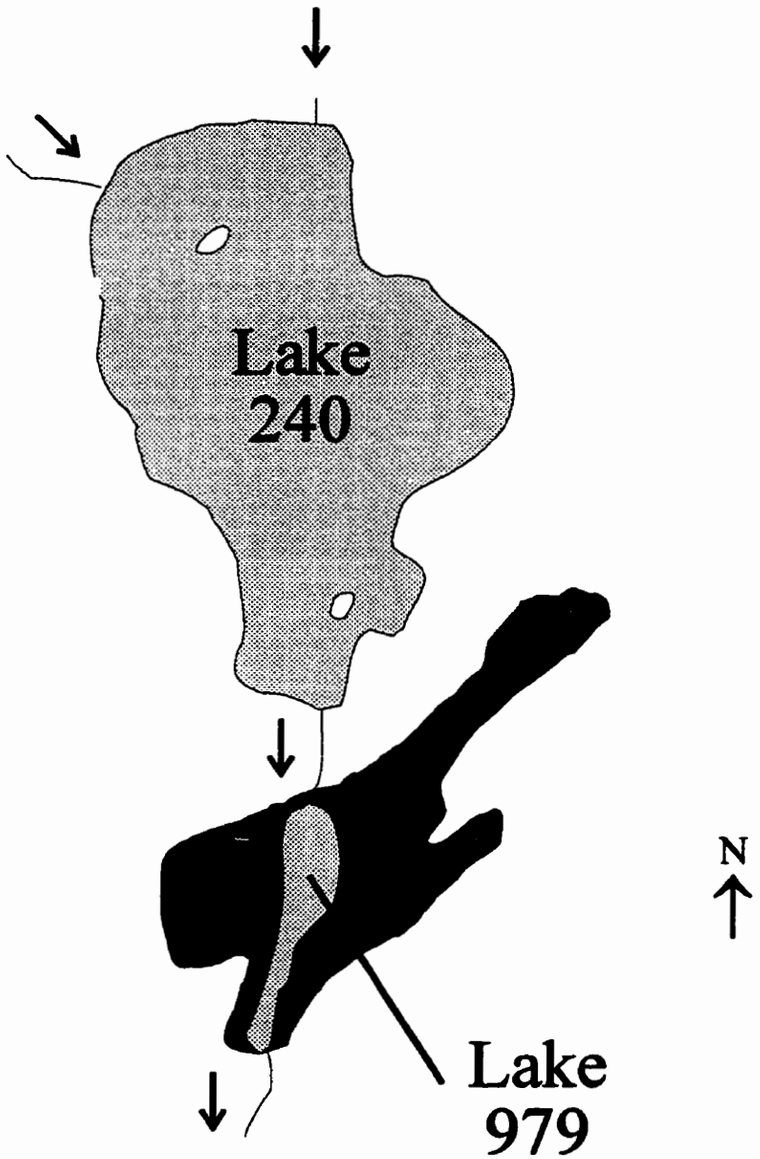


Fig. 1. The relative size and position of Lake 240 and Lake 979 with surrounding wetland. The arrows indicate the direction of water flow.

biological, chemical and physical data exist for this lake. Lake 240 was my study site in 1994 and 1995.

Sample collection and handling. Water for methylmercury analyses was collected in 125 or 250 mL Teflon® (PFA) bottles that were acid cleaned (six hours in hot nitric acid followed by six hours in hot 0.1% HCl solution). After cleaning, bottles were filled with in 0.1% HCl and kept sealed and double bagged (Ziploc®) until used. Sample bottles were cleaned between each use.

At all times during sampling and handling, care was taken to avoid contamination of the samples. This included the use of 1) talcum-free vinyl gloves designed for ultra-clean laboratory work (VWR Scientific), 2) two new Ziploc® bags for bottle storage (with and without sample), and 3) new plastic bags and clean plastic coolers for sample transport and storage. A "clean room" was designed and especially built in 1992 at the ELA for contamination-free trace metal analyses. Design features include filtered air, incandescent lighting and oil-based (low-mercury) paint.

Samples for methylmercury analyses were collected using the "clean hands, dirty hands" protocol (St. Louis *et al.*, 1994). This protocol requires two people, both of whom wear clean gloves. The gloves were kept in a Ziploc® bag which was transported to the field in a clean plastic container. The "dirty hands" person opened the plastic bags which enclosed the sampling equipment. She/he put on a pair of gloves before giving "clean hands" gloves to put on. "Dirty hands" touched only the outer Ziploc® bag which enclosed the sample bottle, and all other sampling equipment as was necessary. "Clean

hands" collected the sample, and was restricted to touching only the sample bottle and inner Ziploc® bag. After collection, all samples were frozen until they were analyzed.

Analytical technique. Samples were analyzed for methylmercury using the technique described in detail by Bloom (1989) and Horvat *et al.* (1993), which involves distillation extraction, aqueous phase ethylation, thermal desorption, GC separation and atomic fluorescence detection as elemental mercury (Hg^0).

For the distillation extraction step, up to 40 mL of sample was placed in a Teflon® (FEP) distillation vessel and 400 µL of 9 N H_2SO_4 and 200 µL of saturated KCl solution were added. The samples were capped and connected to receiving Teflon® vessels using 1/8 inch Teflon® tubing. The samples were then heated in an aluminum heating block at 135 - 140 C, and distilled into Teflon® (FEP) receivers through the Teflon® tubing with a stream of nitrogen. This extraction step took approximately 4.5 hours, at a distillation rate of about 7 to 8 mL per hour.

30 mL of the distillate were transferred to separate glass vessels (bubblers) and the methylmercury in the distillate was ethylated with 40 µL of 1% sodium tetraethylborate (in 2% KOH). For clear water samples such as Lake 240 water, which has lower mercury concentrations, distillate from 3 distillation vials was combined (total volume of samples = 105 mL) and ethylated in large bubblers. Before adding ethylating reagent, the distillate was buffered to a pH of 4.9 with sodium acetate buffer (400 µL). After the ethylating reagent was added, the bubblers were tightly closed, and ethylation was complete within 15 minutes. The efficiency of the ethylating reagent was checked several times and found

to be 100%. The ethylated methylmercury (now ethyl-methylmercury), a volatile species, was purged onto Tenax (35/60 mesh; Alltech) traps with a stream of nitrogen for 13 minutes. Gold traps were placed in line (upstream of the bubblers) to remove any mercury in the nitrogen. After collection, the Tenax traps were dried with a stream of nitrogen for 6 minutes. At this point, traps could be sealed and stored for a maximum of about 20 minutes.

For analyses of trapped ethyl-methylmercury, the Tenax traps were purged using UHP, oxygen-free helium, upstream of a gas chromatograph column (15% OV-3 chromasorb W, AW, DMCS; i.d. = 4 mm; length = 90 cm; temperature = 106 - 110 C). and an atomic fluorescence detector (Brooks Rand, Ltd; Bloom and Fitzgerald, 1988). All the ethylated mercury species were thermally desorbed from the traps using a heated wire coil, and ethyl-methylmercury was separated from other mercury species by the GC column. After the mercury species were eluted from the GC column, they were converted to Hg^0 with a combustion tube (glass wool $\approx$ 900 C) before entering the detector. The output from the detector was quantified as peak area using a standard integrator (Hewlett Packard 3396A).

Quantification of methylmercury in samples

1. *General.* Calculating the amount of methylmercury in the sample involved 1) comparing the peak area of the sample (already corrected for the presence of methylmercury in reagents in the sample) to that of a standard and 2) the application of an

extraction efficiency correction factor. Calculations were made according to the following equation:

$$\left[\left(\begin{array}{c}\text{sample}\\ \text{peak}\\ \text{area}\end{array} - \begin{array}{c}\text{distillation}\\ \text{blank}\\ \text{peak area}\end{array}\right) \times \frac{\begin{array}{c}\text{ng of}\\ \text{methylmercury}\\ \text{in standard}\end{array}}{\left(\begin{array}{c}\text{standard}\\ \text{peak}\\ \text{area}\end{array} - \begin{array}{c}\text{bubbler}\\ \text{blank}\\ \text{peak area}\end{array}\right)}\right] \div \begin{array}{c}\text{fraction of}\\ \text{methylmercury}\\ \text{recovered}\end{array} \qquad (1)$$

where the distillation blank peak area is corrected for the methylmercury in the distilled water used in blank determination.

2. *Determination of blanks*. Distillation blanks were determined by using distilled deionized water (DDW), instead of a sample, and H_2SO_4 and KCl at the distillation step. Bubbler blanks were determined by using DDW instead of distillate, plus ethylating reagent and buffer at the ethylation step. Blanks often contained no detectable methylmercury. When methylmercury was detected in both blanks, distillation blanks contained more methylmercury than bubbler blanks.

In 1993 and 1994, average sample values were much higher (14 to 45 times) than the blank values. In these years, blanks were performed more as a check for contamination of the DDW and reagents rather than for the purpose of calculating an amount of peak area for subtraction from the sample area (equation 1). Blanks were most frequently determined in 1995, when the methylmercury concentration in clear water samples was expected to be very low. However, because the number of distillations that could be performed was limited, often only one blank could be determined for each set of

analyses. In 1995-1996 period, a distillation blank value above instrument noise was detected 22 out of 43 times.

3. *Analyses of standards.* Methylmercury standard solution (1 ng per mL) in distilled water was made from stock solution (1000 ng per mL; Flett Research Ltd.) of methylmercuric chloride in isopropyl alcohol. For standard determination, standard solution was added to distilled water in the bubblers, ethylated, and further treated in the same manner as were the samples. The response of the detector is linear over the range of methylmercury in the samples (Bloom and Fitzgerald, 1988), so only one standard concentration was used per analytical run. Typically, 4 replicates of this standard concentration were analyzed per run.

4. *Sample extraction efficiency.* The efficiency of the distillation extraction of lake water samples was determined using a spike recovery method, or standard addition, method. In this method, one of the duplicate water samples is sacrificed by adding a known amount of methylmercury before the distillation begins. The amount of methylmercury recovered, less the amount originally in the sample is compared to the amount expected from the standard addition. Typically, two spike recoveries were determined for each analytical set of 3 or 6 samples. Overall, this averaged 99%. The range of yearly averages (95-103%; Table 2) was within the range reported by Horvat *et al.* (1993) for different types of water samples.

Although there was little difference in the annual averages of spike recoveries, 16-22% deviation from the average was observed on individual days within one year (Table 2). In 1993 and 1994, daily spike recoveries were used in the calculation of

Table 2. Per cent recovery of added methylmercury (as methylmercuric chloride) to water samples.

Year	n	average recovery (% ± 1 s.d.)
1993	66	95 ± 17
1994	164	103 ± 16
1995	142	100 ± 22
1996	38	96 ± 19

methylmercury concentration. However, in 1995 and 1996, the average spike recovery of 100% was used in the calculations. This was because comparison of samples known to have the same concentration but analyzed on different dates (and with different spike recoveries) often differed in concentration more than did samples analyzed on the same day. Preliminary data (not shown) showed that these concentration differences could be reduced by assuming 100% recovery. Therefore I concluded that the recovery of methylmercury standard added to samples was more variable than the recovery of the methylmercury originally in the sample. This is a puzzling phenomenon that has also been observed in other laboratories (e.g. N. Bloom, Frontier Geoschiences Inc., Seattle; pers. comm.).

5. *Sample detection limits*. A detection limit was determined from the long-term average of either the distillation or bubbler blank values. I defined the analytical detection limit as 2 times the standard deviation of the long-term average concentration of blanks. Two separate calculations were performed. One was for the data collected in 1993, the first year the analytical technique was operating, and, in comparison to the other years, was not as refined. The second was for data collected in 1995, the last year of analyses when the analytical technique had improved from previous years.

In 1993, the detection limit was calculated from bubbler blanks and was 0.08 ng L^{-1} at a blank level of 0.013 to 0.074 ng L^{-1}. In 1995, the detection limit was calculated from distillation blanks that were determined concurrently with the Lake 240 water analyses. In 1995, the sample detection limit was 0.02 ng L^{-1} at a blank level of 0.002 to 0.026 ng L^{-1}. 1995 was the only year during which samples that were at or near the

detection limit were collected (Table 3). These were samples from Lake 239 and Lake 240. The instrument detection limit was about 0.6 pg as Hg (Bloom, 1989) and was thus 0.0006 ng for a 100 mL distillate volume, or 0.006 ng L^{-1}.

6. *Analytical error*. Methylmercury analyses are time and labour intensive. In one analytical run, a maximum of 6 (e.g. 1993) or 12 (e.g. 1995) analyses could be performed. This means that, if duplicate analyses are performed (as was virtually always the case), a maximum of 3 (e.g. 1993) or 6 (e.g. 1995) samples could be analyzed per day. Thus, the nature of the technique is such that time does not permit the frequent analyses of water samples in replicates greater than two. I calculated the analytical error as the amount that a single analysis for a sample analysed in duplicate deviated from the average of the duplicate. This deviation was calculated from many sets of duplicates, and is expressed a percent of the average concentration (Table 3). This averaged 6 to 11% for all years, depending on sample type (Table 3). This number was arrived at whether the error was calculated from duplicate analyses of one water sample or single analyses of duplicate water samples (Table 3).

Table 3. Analytical error in duplicate samples based on year, water sample type, and type of analytical duplicate.

year	water sample type[1,2]	type of analytical duplicate[3]	average methylmercury in samples (ng L^{-1} ± 1 s.d.)	number of analytical duplicates	average analytical error[4] (% ± 1 s.d.)
1993	DW	D	0.56 ± 0.49	69	10 ± 9
1994	DW	D	1.79 ± 1.64	94	9 ± 9
1994	SW	D	1.63 ± 1.79	109	7 ± 11
1995	high SW	D	1.72 ± 1.04	247	6 ± 6
1995	low SW	S	0.35 ± 0.19	26	10 ± 7
1995	very low SW	S	0.041 ± 0.016	38	11 ± 10

SlowSW SW

[1] DW = deep water i.e. water above sediment and sediment pore water
SW = surface water i.e. water from surface of lake

[2] the terms 'high,' 'low,' and 'very low' refer to methylmercury concentrations that are relatively high, low, and very low.

[3] D = duplicate analyses for one water sample
S = single analyses for two replicate water samples

[4] Analytical error is calculated as the amount a duplicate sample deviates from the mean of the duplicates, expressed as percent of the mean.

Chapter 3

Measurement of methylmercury flux in intact sediment cores

ABSTRACT

Flux of methylmercury from sediment to water was measured using intact sediment cores taken from a small pond at the Experimental Lakes Area, northwestern Ontario. Fluxes were variable among cores within a set and between sets of cores. Cores incubated anaerobically tended to have higher methylmercury concentrations in the water overlying the sediment than did cores incubated aerobically, and also exhibited greater fluxes. At different times, positive and negative fluxes were observed in cores incubated both aerobically and anaerobically: Aerobic fluxes ranged from -29 to 95 ng m^{-2} d^{-1} and anaerobic fluxes ranged from -48 to 181 ng m^{-2} d^{-1}. Gradients (1 cm resolution) of methylmercury measured in sediment porewater did not consistently predict the direction and magnitude of the measured fluxes.

It was concluded that methylmercury sediment-water flux is naturally variable, probably because of the complex interaction of several chemical and biological processes at the sediment-water interface. The lack of agreement between gradients and fluxes suggests that a very fine layer at the surface is important in determining fluxes. The variability in flux means that the rates obtained from sediment-cores cannot be extrapolated to give an accurate whole-lake estimate. An alternative approach for the estimation of whole-lake sediment-water fluxes, such as a chemical mass balance, is recommended.

INTRODUCTION

In lakes, the amount of methylmercury available for bioaccumulation in invertebrates and fish is the result of its formation by methylation of Hg^{2+}, and its destruction by

demethylation and photodegradation. Methylation and demethylation are mediated primarily by bacteria. Insight into the occurrence of methylation and demethylation in lake ecosystems has been made possible by the use of the radioactive isotopes ^{203}Hg and ^{14}C-methylmercury for studying methylation (Furutani and Rudd, 1980) and demethylation (Ramlal *et al.*, 1986), respectively. These techniques are useful for comparing relative rates among different aquatic samples, for identifying potential sites of methylation or demethylation, and for testing hypotheses of how environmental factors control relative rates of methylation and demethylation. When both isotopes are used together, a methylation to demethylation ratio (M/D) can be calculated and thus potential sites of *net* methylation or demethylation can be identified (Ramlal *et al.*, 1986). Several studies using these techniques suggest that sediments are an important site of net methylmercury production in lakes (e.g. Korthals and Winfrey, 1987; Gilmour *et al.*, 1992; Ramlal *et al.*, 1993).

Isotopic methods cannot give quantitative estimates of methylation rates for two reasons. One is that the low specific activity of commercially available ^{203}Hg and ^{14}C-methylmercury requires that unnaturally high concentrations of these substrates must be used; addition of substrates at concentrations that significantly increase natural concentrations has the effect of artificially stimulating natural rates of methylation and demethylation. The other reason is that the specific activity of the isotope after it is added to the sample is unknown because an unknown proportion of the added substrate is bound to organic material or precipitated as HgS, which makes it unavailable for methylation.

Recently, the radioisotope technique for methylation has been improved with the use of high specific activity ^{203}Hg (Stordal and Gill, 1995). The amount of labelled mercury added to

samples can now be at trace levels (<10% of *in situ* mercury concentrations). However, the second impediment to measurement of natural rates (i.e. the bioavailability issue) has not been solved, and so it is still not possible to measure *in situ* rates of methylation. A radiotracer technique for measuring demethylation has yet to be developed and so only relative methylation rates can be determined with the radiotracer technique (Gilmour and Riedel, 1995; Stordal and Gill, 1995). Because the necessary isotopic techniques are not available, I attempted to estimate net methylation by directly determining the flux of methylmercury from sediments to overyling water as described below.

Analytical techniques for measurement of *in situ* concentrations of methylmercury in prisitne water samples have been developed in the last decade (Bloom, 1989; Horvat *et al.*, 1993b). These techniques have allowed for whole ecosystem mass balance studies, some of which have suggested that in-lake production of methylmercury is a significant process (Watras *et al.*, 1994; Henry *et al.*, 1995). Mercury methylation is favoured by anaerobic conditions (Winfrey and Rudd, 1990), and so the principal site of methylation in a lake is the sediments, the anoxic hypolimnion, or both, depending on the type of lake (Watras *et al.*, 1995a).

My objective in this study was to estimate the flux of methylmercury from the sediment to the water column by incubating undisturbed cores of sediment and measuring the change in methylmercury mass in the water above the core over time. As part of this effort, I examined core-to-core variability for each site and sampling date, and examined the relationship between methylmercury concentration (in the overlying water) and sediment flux. The main conclusion of this study is that measuring the change in mass of methylmercury in the water above incubated sediment cores provides highly variable results. The variability appears to be real,

rather than an artifact of the technique, but it makes extrapolation to larger lake areas and time periods quite uncertain.

METHODS

Study site. The study was conducted on Lake 979 during 1993 and 1994. Lake 979 is a small pond (2.4 ha) surrounded by a peatland (14.3 ha), the dominant vegetation of which is *Sphagnum* moss, Labrador tea (*Ledum groenlandicum* Oedes), black spruce (*Picea mariana* (Mill.)), and tamarack (*Larix laricina*;). For a more detailed description of the Lake 979 catchment see St. Louis *et al.* (1996). Lake 979 was chosen as the study site because the peatland surrounding Lake 979 is the experimental wetland of the Experimental Lakes Area Reservoir Project (ELARP), which began in 1991. ELARP was designed to simulate the creation of hydroelectric reservoir, and one of the objectives of this project is to examine the process by which fish in reservoirs become contaminated with methylmercury. The work described in this chapter was undertaken as a contribution to ELARP. On June 23, 1993, the Lake 979 wetland was experimentally flooded by damming the outflow of Lake 979. This increased the mean depth of Lake 979 from 0.69 to 0.79 m, the maximum depth from 1.45 to 2.70 m, and the surface area from 2.38 to 15.5 h. The wetland was again flooded in the spring of 1994, the second year of this study. The water column of Lake 979 does not thermally stratify.

Measurement of methylmercury flux. The sediment cores were take in Teflon® tubes made from cut off 2-L PFA-Teflon® jars (26.4 cm x 10.5 cm diameter; Savillex). Prior to each use, the Teflon® core tubes were soaked in 1% HCl for a minimum of 12 hours and then

soaked in 0.1 % KOH solution for a further 12 hour period. A final soaking in lake water (approximately 0.05 ng methylmercury L^{-1}) for a few hours was in an effort to equilibrate the binding sites on the core tubes with natural low concentrations of methylmercury that exist in ELA lakes. The sediment cores were taken within 10 m of the orginigal (pre-flood) shore at a lake water depth of about 0.7 m (pre-flood) or 1.7 m (post-flood). The sediment cored was very soft, dark colored gyttja. The sediment cores tubes were sealed at the bottom using butyl rubber stoppers with a Teflon® disk to prevent contact of the rubber with the sediment. During transport back to the laboratory, the cores were sealed tightly at the top with Tefon® lids and care was taken to minimize disturbance of the cores. In 1993, each incubation experiment consisted of two or three sediment cores. In 1994, there were four to six cores in each experiment. The sediment cores were taken within two or three meters of one another.

In the laboratory, the cores were incubated in the dark at *in situ* temperature and on a slowly rotating platform (1 rpm), which provides sufficient agitation to maintain natural levels of oxygen across the sediment-water interface (Rudd *et al.*, 1990). Cores incubate anaerobically were also rotated. Before incubations began, the cores were first equilibrated without any agitation for between 2 and 6 hours to allow the sediments to settle after transport to the field lab. After the equilibration period, the flux of methylmercury to/from the sediments was measured by following the change in mass of methylmercury in the water above the sediment over time. After an initial (time zero) water sample was taken, the height of the water above the core was adjusted to three cm by removing some of the water. The height of three cm was chosen because the water volume needed to be small enough to ensure a detectable change in methylmercury concentration over time.

For aerobic incubations, the uncapped cores were incubated with a 4-6 cm air-filled headspace. In 1994, two of the four sets of cores were incubated anaerobically. Anaerobic conditions were achieved by flushing the air space above the core with nitrogen for 10 to 15 minutes, and sealing the cores with screw-cap lids lined with Teflon® tape. Preliminary experiments showed that the oxygen in the water above the sediment was depleted within 5 to 6 hours of flushing of the headspace (this study; unpublished data). Anaerobic cores were sampled through one of the two ports in the lids, and anaerobic conditions were maintained during sampling by continuous flushing of the headspace with nitrogen gas. The water above the sediment was sampled at the beginning of the incubations, which lasted one to four days. The water was sampled at least one time after the initial sampling, but up to three more times for the longer incubations.

The water above the cores was sampled using ultra-clean sampling protocol required for analyses of methylmercury at trace levels (e.g. St. Louis *et al.*, 1994; chapter 2). In 1993, the water (at 1 cm above the core) was siphoned (using a Teflon® siphon tube), into an acid washed (hot concentrated HNO_3 followed by 1% HCl) Teflon® bottle. In 1994, plastic 100 mL syringes (ONCE®; Asik, Denmark; already tested for methylmercury adsorption/contamination) were used instead of a siphon. Care was taken to ensure that the sediments were not disturbed while sampling, and that sediment particles were not included in the sample. The sample was not filtered because I did not want to exclude any methylmercury that may have been released from the sediment and attached to suspended particles. The concentration of methylmercury in each sample was determined and the change in mass of

methylmercury above the core over time was determined in order to calculate flux in ng m^{-2} d^{-1}. If the change in concentration was less than 20%, the flux was considered to be zero.

Control tests were conducted to determine if a change in concentration of methylmercury in the water above the cores was due to flux of methylmercury to/from the sediment or to some other process. To test the possibility of attachment to or loss of methylmercury from the tube walls, I incubated four core tubes filled with sterilised Lake 979 water for two days, during which the water was sampled for methylmercury analyses four times. The second experiment was designed to ensure that the change in methylmercury in the water above the sediment was not due to processes which occurred in the water alone. For this experiment, the water above four sediment cores was subsampled for determination of methylmercury at the beginning of the incubation. The water was also subsampled into Teflon® bottles which were then incubated with the cores for 38 hours. The water in the bottles and above the sediment was analysed at the end of the incubation.

Measurement of sediment methylmercury. In 1993, some of the sediment cores were sliced (after incubation) into 1 cm sections using acid-washed Teflon® and glass labware. Sediment porewaters were extracted by centrifugation (6000 rpm for 10 minutes) in Teflon® bottles. The extracted porewater was filtered (0.45 μm; cellulose acetate) to ensure that sediment particulates would not be included in the porewater sample. For three of the cores, the solid sediment was also analysed for methylmercury.

Analytical. Methylmercury was quantified by the method of Bloom (1989) and Horvat *et al.* (1993a,b; see chapter 2 for details). Water samples for the first incubation (June, 1993) were analysed by Flett Research Ltd (Winnipeg, MB) and all other water samples were

analysed by the author at the Experimental Lakes Area. Whole sediment (0.1 to 1 g) was analysed by the author at Flett Research Ltd.

RESULTS

Control tests. The results of the core adsorption test showed that the walls of the core tubes do not absorb methylmercury from, nor release methylmercury to, sterilised lake water (Fig. 1).

The results of the experiment conducted to ensure that observed changes in water concentration above the sediment were because of water-sediment interactions, and not due to changes in the water alone, are given in Table 1. The results showed that when some of the water overlying sediment cores was incubated in the dark separately from the sediment cores, the methylmercury concentration did not change relative to the change that occurred in the water that remained on top the sediment (Table 1). At most, the water incubated separately changed from the initial concentration by 3 to 20%, whereas the water above the sediment changed by 30 to 321% (Table 1).

Sediment incubations. The time course of methylmercury concentration in the water above the cores is presented in Figs. 2 and 3. The initial concentration (Figs. 2 and 3) was sometimes higher than Lake 979 surface water, possibly because of disturbance during handling, or because of slight stratification of the Lake 979 water column. The initial concentration of methylmercury in the water above the cores varied greatly within a set of cores (Fig. 2 and 3). The direction of change in concentration was generally consistent within a set of sediment cores, and most incubations showed that the direction of change was the same

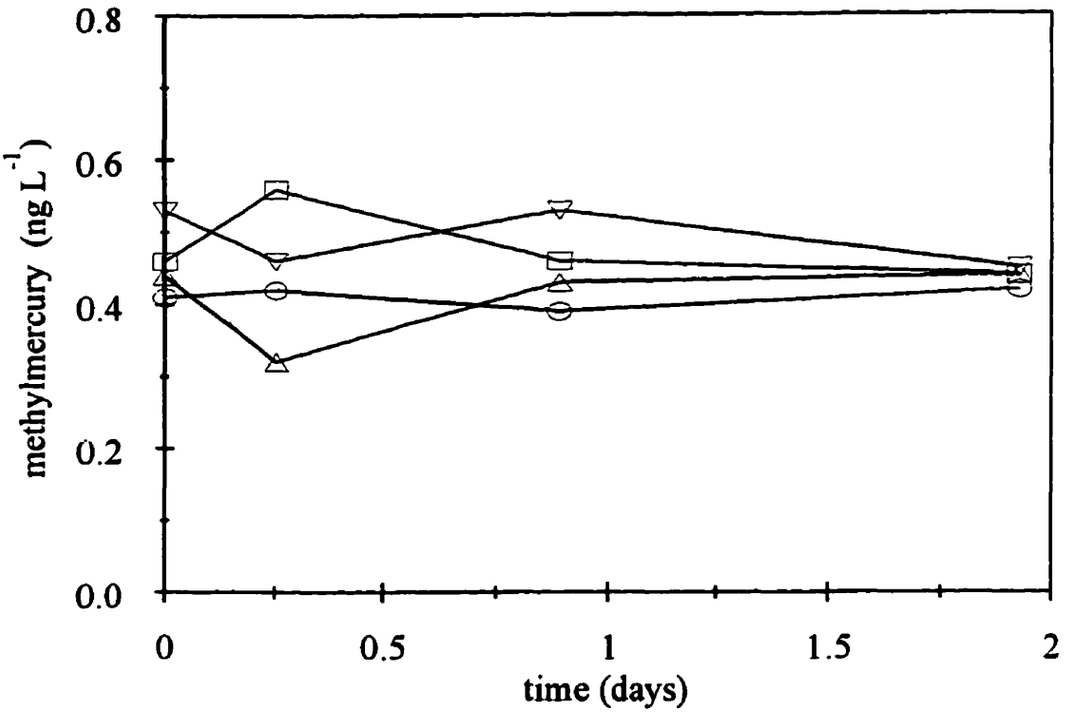


Fig. 1. Control test for methylmercury adsorption/contamination by Teflon® sediment core tubes. The concentration of methylmercury in sterilized Lake 979 water was analysed four times throughout the incubation in June, 1994. The data points represent the average concentration of duplicate analyses. Each duplicate analysis did not deviate from the average concentration of the duplicates by more than 20%.

Table 1. Methylmercury concentration in lake water from above sediment cores at the beginning and end of incubation, and the percent change in concentration from the initial concentration.

		water only [1]		sediment and water[2]	
	0 hours	38 hours		38 hours	
core number	(ng L^{-1})	(ng L^{-1})	% change	(ng L^{-1})	% change
1	1.50	1.25	17	4.38	192
2	0.98	0.95	3	2.29	134
3	2.15	2.03	6	1.48	31
4	0.66	0.79	20	2.78	321

[1] water incubated in Teflon® bottles

[2] water incubated on top of the sediment

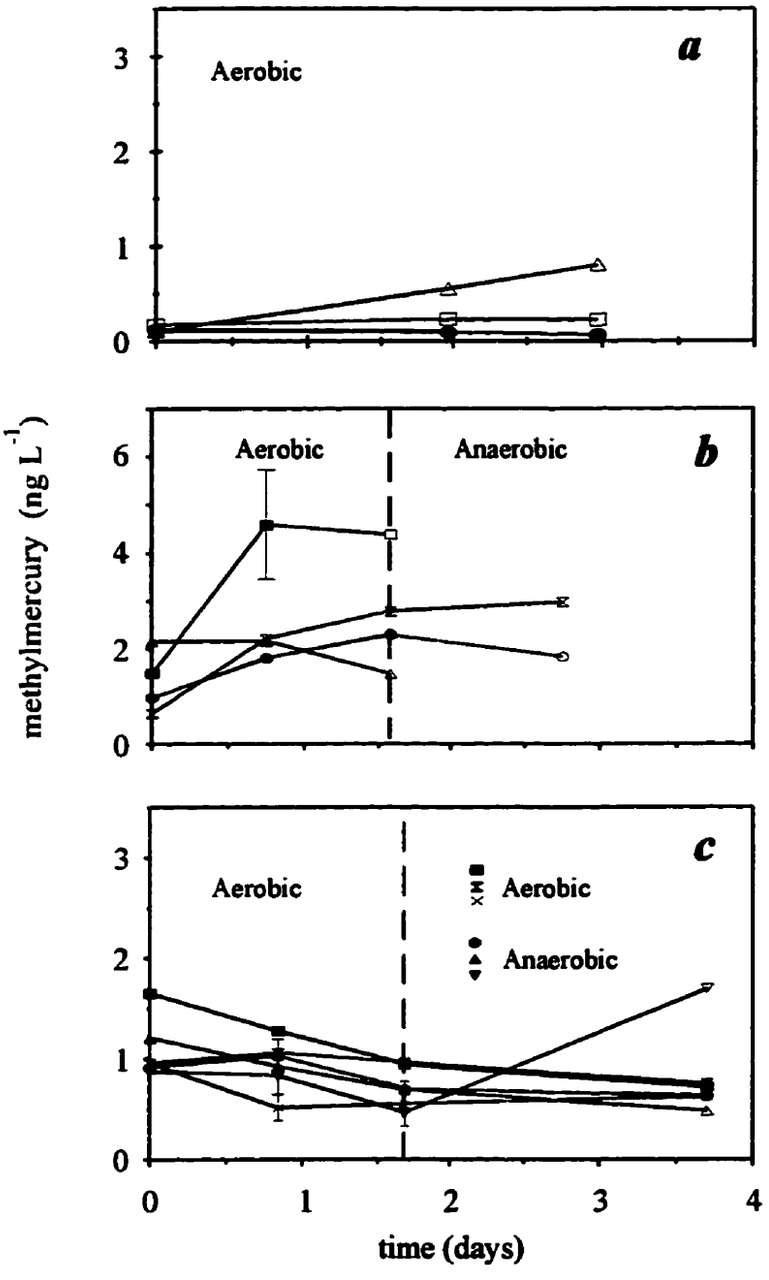


Fig. 2. The methylmercury concentration in lake water above sediment during sediment core incubations. Each panel represents a separate incubation, and each line within each panel represents one core. Closed symbols represent the average concentration of duplicate analyses; open symbols represent single analyses. Range bars between single anlyses are shown if the single concentrations deviate from the average concentration by more than 20%. The vertical line in *b* and *c* marks the time division between aerobic and anaerobic conditions. *a*, June 19, 1993; *b*, June 14, 1994; *c*, July 26, 1994.

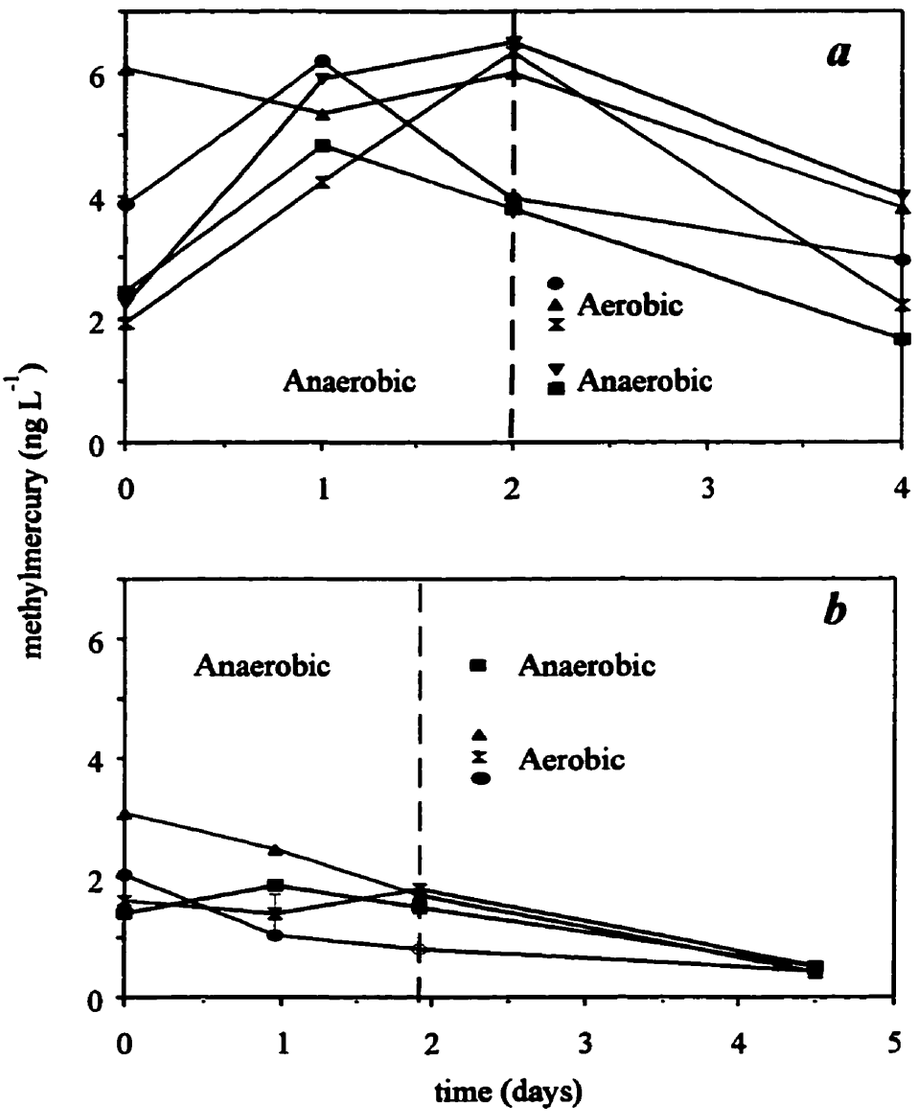


Fig. 3. The methylmercury concentration in lake water above sediment during sediment core incubations. Each panel represents a separate incubation, and each line within each panel represents one core. Closed symbols represent the average concentration of duplicate analyses; open symbols represent single anlyses. Range bars between single analyses are shown if the single concentrations deviate from the average concentration by more than 20%. The vertical lines mark the time division between anaerobic and aerobic conditions. *a*, June 21, 1994; *b*, Aug 25, 1994.

in at least one half of the cores (Figs. 2 and 3). The direction of change could not be distinguished between cores initially incubated aerobically (Fig. 2) and those initially incubated anaerobically (Fig. 3). Changing some of the cores within a set from aerobic to anaerobic or from anaerobic to aerobic conditions had no consistent effect on the direction of change in the methylmercury concentration (Figs 2c and 3). When the cores were incubated longer than three days, methylmercury concentrations in the cores tended to become more similar to each other, regardless of incubation conditions (Fig. 2 and 3). Concentrations also became more constant with time (Figs. 2 and 3).

To see if the activity that caused the change in concentration in the cores could be regained, the water on top the sediment cores was replenished with "new" pond water from Lake 979 at the end of one incubation, which was continued for another two days. No change in methylmercury concentration was observed for these cores (Fig. 4). Perhaps the source of bioavailable Hg^{2+} (e.g. overlying the water or oxidation reactions) was cut off.

Sediment water methylmercury fluxes. An estimate of *in situ* sediment methylmercury flux was made from the change in mass of methylmercury in the water above the sediment core for the period of time between the initial (time zero) and second sampling (Figs. 2 and 3). These estimates are presented in Table 2. The lowest concentration of methylmercury in the overlying water and the lowest flux was measured in the first incubation, which took place prior to flooding of Lake 979 (Table 2). Increasing the number of cores incubated did not decrease the magnitude of the flux range within a set of cores (Table 2). The fluxes were greatest in magnitude when the sediment cores were incubated anaerobically

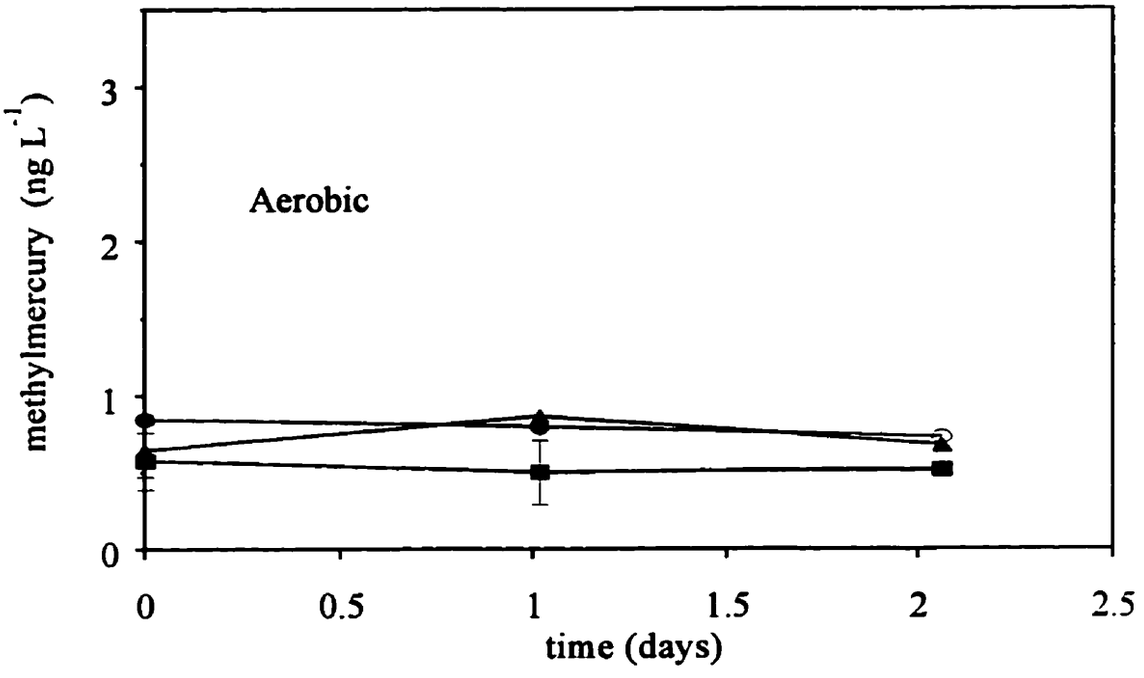


Fig. 4. The methylmercury concentration in lake water above sediment during sediment core incubations. The lines represent the continuation of aerobic incubations (Fig. 2c; July 31, 1994) after replenishing the lake water on top the sediment with "fresh" lake water. Closed symbols represent the average concentration of duplicate analyses; open symbols represent single analyses. Range bars between single analyses are shown if the single concentrations deviate from the average concentration by more than 20%.

Table 2. Calculated sediment-water methylmercury fluxes. A positive flux denotes methylmercury movement from the sediment to the water. For each set of cores, the aveage flux and the range of flux between cores is given.

Date	number of cores	Initial [CH_3Hg^+] (ng L^{-1})	average flux (ng m^{-2} d^{-1})	standard deviation (% of mean)	range of flux (ng m^{-2} d^{-1})	Incubation conditions
21-06-93	3	0.11 - 0.18	3	133	0 - 8	Aerobic/17 C
3-08-93	2	0.7 - 0.74	3	-	0 - 6	Aerobic/17 C
20-08-93	3	1.27 - 2.3	-16	69	-29 - -9	Aerobic/18 C
14-06-94	4	0.66 - 2.15	55	76	0 - 95	Aerobic/17 C
21-06-94	5	1.94 - 6.08	100	65	0 - 181	Anaerobic/17 C
26-07-94	6	0.88 - 1.65	-10	110	-24 - 0	Aerobic/18 C
25-08-94	4	1.41 - 3.08	-14	230	-48 - 24	Anaerobic/17 C

(Table 2, Fig. 5). There was no relationship between the concentration of methylmercury in the water and the methylmercury flux for aerobic or anaerobic incubations (Fig. 5).

Sediment methylmercury profiles. The concentration of methylmercury extracted from porewaters of the sediment at the end of three incubations is shown in Fig. 6. The methylmercury concentration in the pore water was highest in the top two centimeters of sediment, and decreased with increasing depth (Fig. 6). Methylmercury concentrations in porewaters of incubated sediment were highest before flooding of the Lake 979 wetland occurred (Fig. 6a), and were lower after flooding (Fig 6b, c). The apparent gradient of methylmercury across the sediment-water interface was determined from the difference between the porewater concentration in the first centimeter of sediment and the concentration in the water above the sediment. These gradients agree with the direction of the measured flux three out of five times (Table 3). Three sediment cores were analysed for methylmercury in the solid sediment. Solid sediment methylmercury was highest in the first two centimeters of sediment and decreased with sediment depth (Fig. 7).

DISCUSSION

The main objective of this study was to evaluate the use of intact sediment core incibation to quantify the flux of methylmercury from the sediment to the lake water. The most striking characteristic of the results was the high variability of flux among cores. There are three reasons to believe that the observed large variability in the fluxes among cores (Table 2) is natural, and not an artifact of the method used. One reason is that extreme care was taken during all stages of sampling and handling of the cores, and so error introduced by sediment

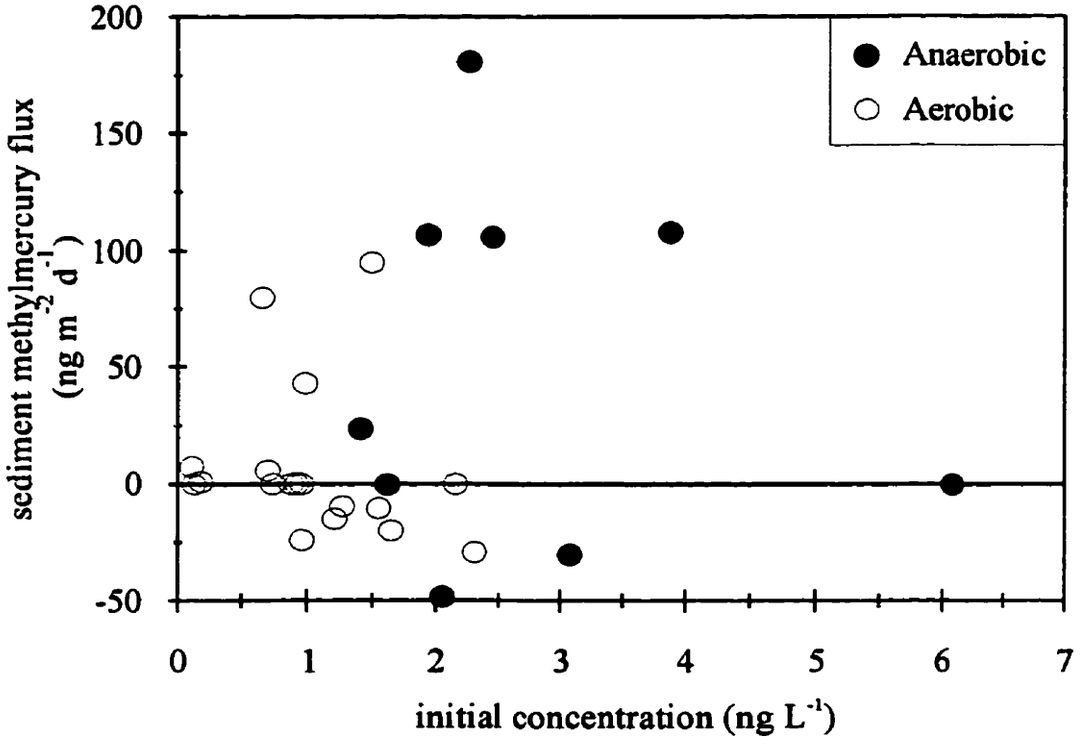


Fig. 5. The relationship between sediment-water methylmercury flux and the concentration of methylmercury in the water above the sediment at the beginning of the incubation.

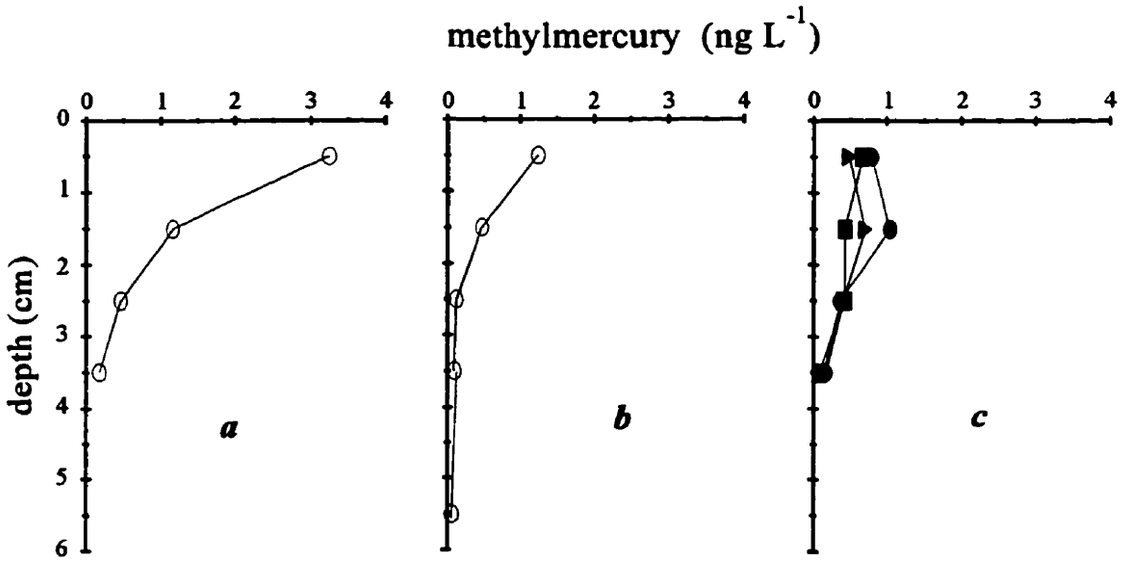


Fig. 6. Methylmercury in Lake 979 sediment pore water after 72 (*a*) or 24 hours (*b* and *c*) of sediment core incubation. Open symbols represent single anlyses; closed symbols represent the average concentration of duplicate analyses. For samples anlysed in duplicate, the single concentrations did not deviate from the average of the duplicates by more than 20%. *a*, June 22, 1993; *b*, Aug.3, 1993; *c*, Aug. 20,

Table 3. Apparent methylmercury gradients across the sediment-water interface derived from the concentration of methylmercury in the water above the sediment and the porewater concentration. These gradients are compare to the observed flux of methylmercury to/from the sediments.

Date (1993)	CH_3Hg^+ above sediment ($ng\ L^{-1}$)	porewater methylmercury ($ng\ L^{-1}$)	gradient ($ng\ L^{-1}\ cm^{-1}$)	measured flux ($ng\ m^{-2}\ d^{-1}$)
June 22	0.22	3.24	3.02	0.0
August 3	0.87	1.24	0.37	0.0
August 20	1.03	0.78	-0.25	-10.4
	0.85	0.48	-0.37	-29.1
	0.80	0.66	-0.14	-9.5

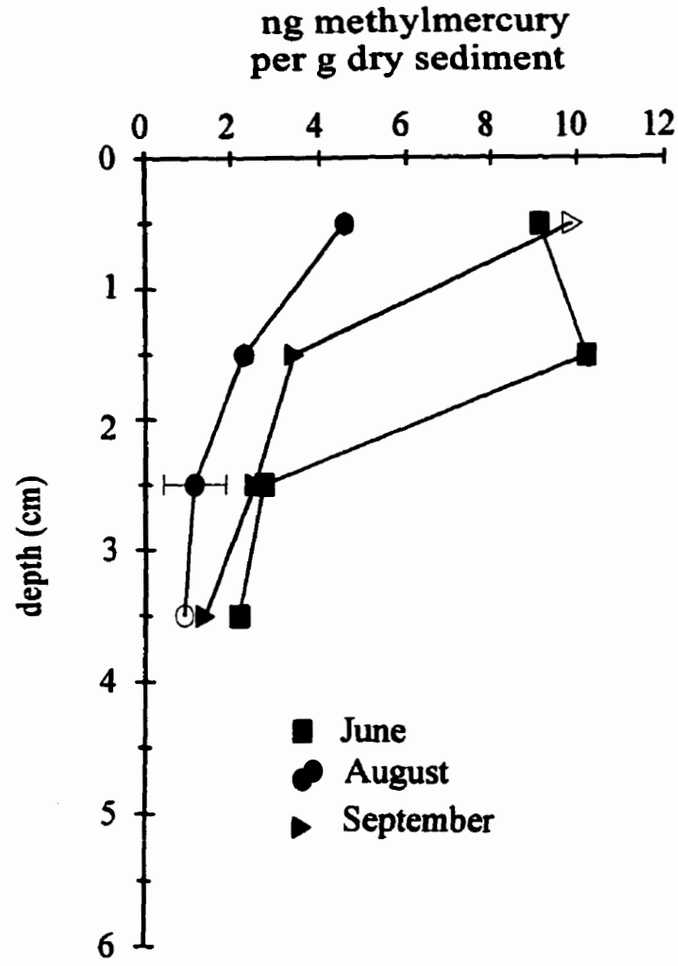


Fig. 7. Methylmercury concentration profile in Lake 979 solid sediment, 1993. Closed symbols represent the average concentration of duplicate analyses; open symbols represent single analyses. Range bars between single anlyses are shown if the single concentrations deviate from the average concentration by more than

particle re-suspension was likely minimal. If handling and sampling of the cores did cause particle re-suspension and variable methylmercury concentrations, then this should have been apparent at all sampling times. However, this was not the case in most of the prolonged incubations (Fig. 2c, 3b, and 4). Another reason is that methylmercury was not adsorbed to nor released from the inside walls of the core tubes (Fig. 1). Third, analytical error was generally less than 20% (chapter 2), and so can be eliminated as a significant source of the observed variability in the flux rates (Table 2). I therefore conclude that the measured fluxes were real but very variable.

The range of concentration of methylmercury in the water above the sediment and in methylmercury flux (Table 2) is similar to that observed by Sellers *et al.* (1994) who conducted the same incubations (before flooding) but with fewer cores that had lower methylmercury concentrations (10 to 60 times) in the water above the sediment. Therefore, increasing the number of sediment cores within an incubation while decreasing the duration of incubation did not reduce the variability among sediment cores within a set (Table 2). The only other study in which this method has been used for estimating methylmercury flux from sediments was conducted by Henry *et al.* (1995) who incubated two or three cores in a set, and also found large differences between measured fluxes.

There are several biological and chemical interactions at the sediment which may explain natural variability in methylmercury fluxes at the sediment-water interface. First, methylmercury is preferentially bound to organic molecules (Zepp *et al.*, 1974, Hintelmann *et al.*, 1995), and so site-to-site differences in organic content of sediment may account for the differences in the concentration of methylmercury between cores if methylmercury is released

from or adsorbed by the sediment during incubation. Second, differences in fluxes may also be attributed to differences or changes in chemical conditions and/or biological activities among the replicate cores. However, replenishing the cores with "fresh" lake water did not change the methylmercury concentration (Fig. 4). This suggests that either the methylmercury concentrations are not affected by the activity of microorganisms living within or on the sediment, or that the addition of lake water may not have changed the conditions enough for the stimulation of methylation or demethylation. Third, variability in sediment-water flux may be due to differences in natural rates of methylation in the sediment. Finally, there may be differences among cores in the redox cycling of inorganic metal complexes at the sediment-water interface. Redox reactions affect the solubility of metals and their complexes, and if methylmercury is bound to such complexes, then the solubility of methylmercury would simultaneously be affected. For example, redox sensitive iron and manganese complexes are present at the sediment-water interface, and changes in their dissolution/precipitation reactions affects the dissolution of trace elements which bind to them (Stumm, 1992).

Although the reasons for the observed variability in core fluxes (Table 3) are speculative at this time, it should be noted that the sediment-core incubation technique as it was used in this study is sensitive enough to detect a rate as low as 1.4 ng m^{-2} d^{-1}, which was the whole-lake rate obtained by mass balance in Lake 240, a nearby clear-water lake (chapter 5). This was the rate calculated for new production; flux of methylmercury from new production and recycling was estimated to be 2-5 times higher. Thus, positive rates obtained by core incubation in Lake 979 (Table 2) were often much higher than the average for Lake 240 as a whole. Also, negative fluxes were sometimes measured in Lake 979 cores (Table 2). I don't

think it is valid to simply average the core results to try to obtain a whole-lake average for Lake 979 because I don't know how representative this set was. It is possible that methylmercury fluxes in Lake 240 also vary from site to site and time to time; the mass balance approach provides only an average flux.

It has yet to be determined whether the measured fluxes are a result of diffusion of methylmercury along a concentration gradient which crosses the sediment-water interface, or is a result of production/consumption reactions on the sediment surface, or both. The direction (but not the magnitude) of the measured sediment-water flux matched the direction of the porewater gradient three out of five times (Table 3), which suggests that it may be possible to calculate sediment-water fluxes from these concentration gradients. However, this approach to estimating flux is hampered by many uncertainties. For example, the apparent gradient may not reflect the true gradient driving the flux, in which case the spatial resolution of the true gradient would need to determined. Other uncertainties and concerns are 1) the chemical and/or biological transformations of methylmercury as it crosses the sediment-water interface (Winfrey and Rudd, 1990; Gagnon, *et al.*, 1996), 2) the chemical form (and hence size) of the diffusing methylmercury species and 3) the influence of groundwater on *in situ* fluxes (Krabbenhoft and Babiarz, 1992; Hurley *et al.*, 1994; Branfireun *et al.*, 1996). While these uncertainties exist, it is unlikely that sediment-water concentration gradients can be used to calculate sediment contribution to the lake burden of methylmercury. Ultimately, however, it may be that both diffusive gradient flux and sediment surface flux contribute to changes in mass of methylmercury, and the gradient approach alone may underestimate the true flux.

Although porewater data (Fig. 6; Table 3) may not be may not be useful for estimating sediment-water flux in this study, both porewater and solid sediment data give insight into the production sites of methylmercury within the sediment, and suggest that the site of net methylmercury production is at or near the sediment-water interface (Fig. 6 and 7). The higher porewater concentrations observed before (Fig. 6a) compared with after (Fig. 6b) flooding suggest that the effect of flooding was to decrease sediment porewater methylmercury. If true, this may be the result of methylmercury release from the porewaters to the overlying water, and may have contributed to the increase in methylmercury concentration in the pond water that was observed as a result of flooding (J.W.M. Rudd, Freshwater Institute, Winnipeg, pers. comm.; Table 2). Alternatively, these differences may reflect temporal variation that is unrelated to flooding, which is suggested by the lower concentrations measured near the end of the summer months (Fig. 6c). Temporal variability is further suggested by the observation that, in the fall, porewater concentrations are comparatively low and uniform with depth in Lake 979 before flooding (V. St. Louis, State University of New York, Brockport, pers. comm). It is not known how flooding affected the movement of groundwater through these sediments, but it is possible that differences in pre- and post-flood porewater concentrations (Fig. 6) may also be due to changes in ground water movement.

The measured concentrations of methylmercury in the porewater (Fig. 6) are similar to measurements made in the surface sediments of a river (<1 to 5 ng L^{-1}; Gagnon *et al.*, 1996) and in the surface sediments of a softwater drainage lake (5 ng L^{-1}; Gilmour and Riedel, 1995). Measurements of methylmercury in the solid sediment (Fig. 7) are also similar to

measurements made for river sediments (Gagnon *et al.*, 1996) but an order of magnitude less than measurements made in other lake sediments (Gilmour and Riedel, 1995).

It is apparent that sediment-water methylmercury fluxes were much greater (in magnitude) after flooding of Lake 979 (Table 2). In this study, only one pre-flood incubation was conducted, and the mean concentrations and fluxes measured (Table 2) were within the ranges reported by Sellers *et al.* (1994; 0.10 to 0.40 ng L^{-1}, and -1 to 10 ng m^{-2} d^{-1}, respectively) prior to flooding. Negative fluxes (from the water to the sediment) were observed only after flooding, when the methylmercury concentrations in the water above the core were an order of magnitude higher (Table 2). However, both negative and positive fluxes were observed when the methylmercury concentration in the water exceeded 1 ng L^{-1} (Fig. 5). Therefore, this study does not confirm the results of Sellers *et al.* (1994) who observed negative fluxes only when the water concentrations exceeded 1-2 ng L^{-1}. The only pattern that is apparent from Fig. 5 is that sediment cores incubated anaerobically had a higher methylmercury flux, but the direction of flux, however, cannot be specified to anaerobic or aerobic conditions (Fig. 5). This is different from Henry *et al.* (1995) who measured a relatively large and positive flux only in one set of cores (out of three), which was incubated anaerobically. The high methylmercury concentration in the water above the core that is observed when the sediment is incubated anaerobically (Table 2) is consistent with the accepted view that methylmercury is produced when water and sediments are anoxic (Compeau and Bartha, 1984; Winfrey and Rudd, 1990; Regnell and Tunlid, 1991; Watras *et al.*, 1995a).

In conclusion, the high variability of core incubations results, while apparently real, makes it difficult to extrapolate results to larger areas and/or longer periods of time. An

alternative approach is recommended, such as the use of *in situ* sediment chambers. This approach would be likely be better for examination of spatial variability, and would include the effect of ground water influx in nearshore areas (Krabbenhoft and Babiarz, 1992; Hurley, *et al.*, 1994; Branfireun *et al.*, 1996). Another approach would be to conduct a whole-lake mass balance for methylmercury (Watras *et al.*, 1994; Hultberg *et al.*, 1994; Henry *et al.*, 1995; Sellers *et al.*, 1996), with sediment release rates set as the unknown. This is the approach that I decided to pursue (Chapter 5).

Chapter 4

Photodegradation of methylmercury in lake water

ABSTRACT

Methylmercury photodegradation in lakes was examined using Teflon® bottles filled with lake water and incubated *in situ*. Photodegradation was mediated primarily by solar UV (280-400 nm), and longer wavelengths were also effective. Photodegradation rates were the same for methylmercury in distilled deionized water and in lake water, suggesting that major differences in water chemistry do not affect rates. Experimental addition of H_2O_2 also had no effect methylmercury photodegradation. Photodegradation rates in a clear-water drainage lake were highest in the upper meter of the water column. In deeper waters, rates were much ower, but incubation results did not show a pattern completely consistent with that of sunlight attenuation. A linear relationship between methylmercury photodegradation rates and photosynthetically active radiation (PAR) was developed for PAR values ranging from 1 to 46 E m^{-2} d^{-1}. Together, these results suggest that whole-lake methylmercury photodegradation can be predicted if only three parameters are known: 1) depth integrated PAR, 2) depth of solar UV penetration, and 3) *in situ* methylmercury concentrations.

INTRODUCTION

Lake-water and sediment mercury exists as several inorganic and organic species, most of which are subject to both chemical and biological transformations (Stein *et al.*, 1996). Of particular interest is methylmercury because it is this form of mercury that accumulates in the biota, thereby having the potential to threaten the health of fish and fish consumers (Clarkson, 1994). Methylmercury is produced from the methylation of divalent inorganic mercury (Hg^{2+}),

which can occur biologically (Gadd, 1993), chemically (Nagase *et al.*, 1982; Weber, 1993), or photochemically (Hamasaki *et al.*, 1995). Until very recently, the only known mechanism for methylmercury destruction in lakes was bacterial demethylation (Winfrey and Rudd, 1990; Gadd, 1993; Stein *et al.*, 1996). However, Sellers *et al.* (1996; appendix A) have shown that an abiotic, sunlight-mediated process of methylmercury destruction also occurs in the surface waters of lakes. Unlike bacterial demethylation, photodegradation of methylmercury has been shown to occur at naturally low mercury concentrations (ng L^{-1}) in lakes (Sellers *et al.*, 1996; appendix A), and therefore may be the only mechanism by which methylmercury is destroyed in unpolluted lake environments. Further, Sellers *et al.* (1996; appendix A) showed that methylmercury photodegradation may be very important in regulating methylmercury concentration in some lakes. This is important because it is the combined balance of methylmercury production and destruction that controls methylmercury concentrations available for accumulation in food-chain organisms.

In this chapter I expand on the preliminary results of methylmercury photodegradation presented by Sellers *et al.* (1996; appendix A). Specifically, I re-examine the effect of methylmercury concentration and light quantity on photodegradation rates. I also examine the effect of light quality and some aspects of water chemistry on photodegradation rates. The main findings are that photodegradation rates are affected most by methylmercury concentration, and by the amount and type of solar irradiation. Differences in water chemistry have little effect on photodegradation rates, and overall the data suggest that methylmercury photodegradation could easily be included in mechanistic models of mercury cycling if a few of the appropriate environmental variables are measured.

METHODS

I. General approaches

A) *Analytical methods*: The methylmercury concentration in the water of each sample (100 mL) was usually analyzed in duplicate, but for a few of the replicate treatments only one analysis for each bottle was performed. The analytical technique used involves co-distillation extraction, aqueous phase ethylation, gas-chromatograph separation and atomic fluorescence detection of methylmercury as elemental mercury (Horvat *et al.*, 1993; for details see chapter 2).

B) *Incubation of samples*: For all experiments, lake water was incubated in PFA-Teflon® bottles and the concentration of methylmercury in the water was measured at discrete times throughout the incubation. For each experiment, 100 mL of lake water was distributed into (rigorously cleaned; chapter 2) 125 mL bottles and incubated *in situ*. With the exception of the photodegradation profiles in Lake 240, for which the bottles were incubated at discrete depths within the lake, the bottles were incubated on the surface of Lake 979. Because filtering has no affect on photodegradation rates (Sellers *et al.*, 1996; appendix A), lake water was filtered (0.45 μm cellulose-acetate) before incubation to minimize analytical variability. After filtering the methylmercury concentration was artificially elevated (using methylmercuric chloride stock solution) before it was distributed into the incubation bottles. The final concentrations did not exceed concentrations found in the ELA waters (1-3 ng L^{-1}; J.W.M. Rudd, Freshwater Institute,

Winnipeg, pers. comm.). The incubation bottles were sealed in two Ziploc® bags during the incubation and during storage before analyses.

Surface incubations typically lasted six to eight days while subsurface incubations lasted up to three weeks. For each treatment of each experiment, one bottle was retrieved from the lake at the beginning (time zero), middle, and end of the incubation. The trasmittance of mylar was measured several times, each time with a different piece, and showed no variation.

C) *Spectrophotometric measurements*: Transmittance (200 to 800 nm) measurements were made with a Milton Roy (Spectronic 3000) spectrophotometer. Trasmittance was measured through Ziploc® bag plastic film, mylar film (Herculene mylar, Azon), UF3-plexiglas (3mm) and PFA-Teflon® (1mm). The transmission spectrum of different pieces of mylar was the same.

D) *Measurement of solar irradiation*: Incident photosynthetically active irradiation (PAR) was measured continuously at a meteorological station near the study site (within 300 m) with a LiCOR terrestrial PAR sensor (190S). This instrument measures light in the visible range (400 to 700 nm) of the solar spectrum. Instantaneous measurements of light were taken several times at discrete depths in Lake 240 with a LiCOR underwater PAR quantum sensor (192S). These measurements were used to estimate the quantity of light at specific depths below the surface of Lake 240.

E) *Expression of photodegradation rates*: Photodegradation rates (in ng L^{-1} d^{-1}) were calculated as follows:

$$\text{photodegradation rate} = \Delta C / \Delta t_1 \qquad (1)$$

where ΔC is the change in mean methylmercury concentration (ng L^{-1}) between time zero and the first sampling of water samples analyzed in duplicate, and Δt_1 is the change in time between time zero and the initial sampling. For some experiments, the measured photodegradation rates are expressed as a percent of methylmercury photodegraded per day. This was calculated using the measured rate and initial concentration of methylmercury as follows:

$$\%\ \text{methylmercury photodegraded per day} = \text{ng } L^{-1}\ d^{-1} / \text{ng } L^{-1} \times 100 \qquad (2).$$

Expressed this way, differences in concentration, which affect rates of photodegradation (Sellers *et al.*, 1996; appendix A) are accounted for when rates are compared. If the measured concentration between time zero and the first sampling did not change by more than 15%, the rates (expressed using equation 1 or 2) were considered to be zero.

II. Experiments

A) *Wavelength exclusion experiments*: To determine the wavelengths of sunlight that mediate methylmercury photodegradation, incubated bottles were shielded using different screening materials (Fig. 1). The Ziploc® bags used for incubation do not impede transmission of UV or visible light (Fig. 1). Aluminum foil was to shield bottles from all wavelengths of sunlight (dark bottles), used as a shield. Mylar film was used to prevent

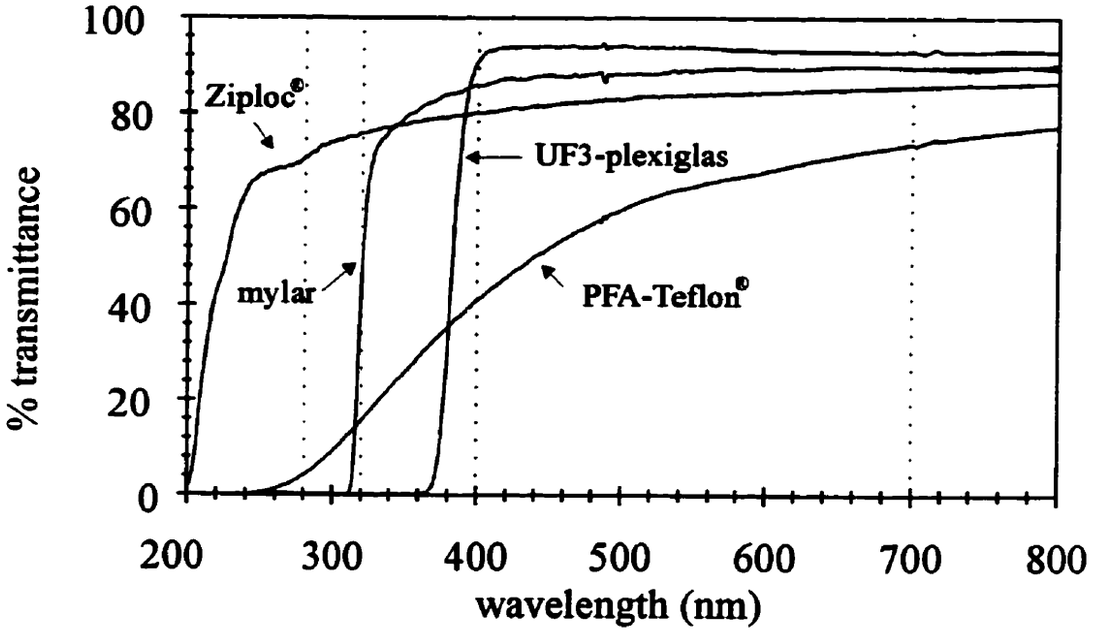


Fig. 1. Transmission spectra of Teflon® bottles (1mm), Ziploc® film, mylar film, and UF-3 plexiglas (3 mm). Vertical dotted lines mark the boundaries of UV-B (280-320 nm), UV-A (320-400 nm) and visible light (400-700 nm).

exposure of the bottles to UV-B (280 to 320 nm) and UF3-plexiglas to block nearly all UV (<400 nm). Bottles not exposed to UV-B were placed inside cylindrical cages (one for each bottle), around which mylar was wrapped. Bottles not exposed to any UV were incubated beneath a Plexiglas chamber (31 × 20.5 × 6.5 cm; wall thickness = 3 mm) which floated on top the surface of the lake. The transmission spectra of mylar and plexiglass (Fig. 1) did not change as a result of sunlight exposure during *in situ* incubations. It is important to note that the transmission spectrum of PFA-Teflon® suggests that only a small proportion of the UV-B passes through the Teflon® bottles. However, field measurements have shown that Teflon® bottles do not impede the transmission of sunlight within the visible light range (400 to 700 nm) of solar radiation, and that they transmit 66 and 78 % of solar UV-B and UV-A respectively (M. Amyot, pers. comm).

B) *Effect of water chemistry on photodegradation rates*: The effect of water chemistry on photodegradation rates was studied using three different water types that represented a wide range in the concentration of chemical constituents. The three waters used in this study were distilled deionized water, clear lake-water, and brown lake-water. The clear lake-water was collected from Lake 240, and the dark lake water was collected from Lake 979. The difference in some chemical parameters between these two lakes is given in Table 1.

C) *Hydrogen peroxide addition experiment*: To test if hydrogen peroxide (H_2O_2), (a photochemically produced oxidant in the surface waters of lakes (Cooper *et al.*, 1994)) participated in the chemical process of photodegradation, an H_2O_2 addition experiment was conducted. For this experiment, hydrogen peroxide was added to two sets of three

Table 1. Chemical characteristics of Lake 979 (dark) and Lake 240 (clear) water[1].

parameter	Lake 979	Lake 240
pH	5.95	6.93
DOC (μmol L^{-1})[2]	1480	601
chloride (mg L^{-1})	0.28	0.31
sulfate (mg L^{-1})	1.5	3.33
alkalinity (μeq L^{-1})	91	136

[1] averages of chemical data collected during 1995 ice-free season.

[2] DOC = dissolved organic carbon.

bottles to a final concentration of 1250 nmol L^{-1}. One set of bottles was incubated in the sunlight and the other was not. Parallel sets of bottles, which received no H_2O_2, were incubated simultaneously. Because the minimum night time $t_{1/2}$ of H_2O_2 in the lake from which the water was collected is about three hours (W. Donahue, pers. comm.), this concentration was chosen to ensure that the molar ratio of methylmercury to H_2O_2 was much greater than 1 at the beginning of the incubation. The initial concentration of methylmercury in these bottles was 0.012 nmol L^{-1}, and so the initial molar H_2O_2: methylmercury ratio was about 100 000: 1. Assuming that no new H_2O_2 is produced, this ratio would be 1:1 after two days of *in situ* incubation. However, new production of H_2O_2 within the bottles as a result of DOC-sunlight interaction (Cooper *et al.*, 1994; Scully *et al.*, 1996) would likely delay the net rate of H_2O_2 decay.

RESULTS

The results of field studies in which lake water was shielded from different wavelengths of solar radiation are shown in Fig. 2. Bottles that were shielded from only solar UV-B showed photodegradation rates that were either similar to or 35% lower than bottles that were exposed to all other wavelengths. Bottles shielded from both solar UV-A and UV-B (i.e. no UV) resulted in little or undetectable photodegradation when compared to bottles that were exposed to all sunlight or shielded only from UV-B. The dark bottles showed no detectable methylmercury photodegradation.

Ten attempts were made to characterize methylmercury photodegradation rates with depth in Lake 240. For most of these profiles, fixed depths of 0, 0.5, 1,3,5, and 7 m

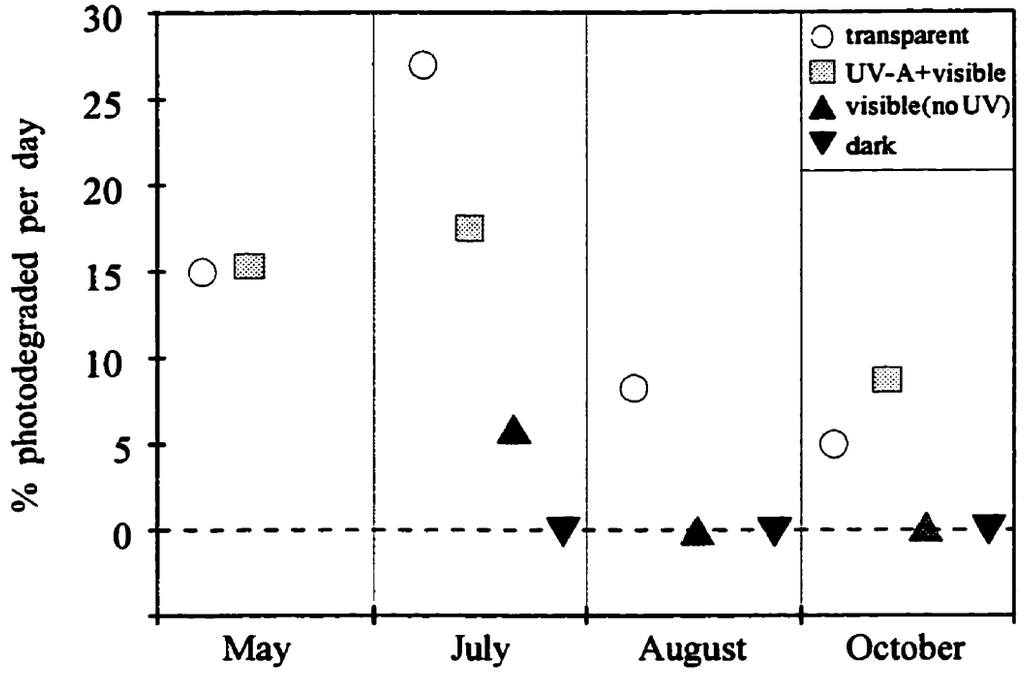


Fig. 2. Methylmercury photodegradation rates in lake water exposed to different wavebands of solar radiation. Bottles were incubated on the surface of a lake, and were either transparent to all sunlight, exposed to UV-A and visible sunlight (no UV-B), exposed to only visible sunlight (no UV), or shielded from all sunlight (dark).

were used. Three (Aug 5, Aug 18, Sept. 28) out of the 10 profiles had a photodegradation pattern consistent with the exponential attenuation of light (Fig. 3), only one of which (Sept. 28) was detailed in the first meter of water (Fig. 3b). Highest rates were measured at the at the top one meter of the water column (Fig. 3a,b). On May 25 and June 8, photodegradation was not detected at 0.5 and 1 m but photodegradation was detected at 3 m (Fig. 3a). On July 7, photodegradation was also not detected at 0.5 m but was at 1 and 3 m (Fig. 3a). On July 21, rates were higher at 3 m than at 1 and 0.5 m (Fig. 3a). Three out of seven times, the rates were higher at 5 than at 3 m, and 2 out of 3 times the rates were higher at 7 that at 5 m (Fig. 3a).

The relationship between methylmercury photodegradation rate and PAR was developed using four different data sets (Fig. 4). The relationship in Fig. 4a is from data already published (Sellers *et al.*, 1996, appendix A), but here the photodegradation rates are expressed as % photodegraded per day and cover a greater range in PAR. The relationship in Fig. 4b was developed from only the surface incubations of the photodegradation profiles conducted in Lake 240 (Fig. 3). The relationship in Fig. 4c was developed from surface incubations of all experiments, and consists of incubations on the surface of Lake 979 and those in Fig. 4b. The relationship in Fig. 4d was developed from all photodegradation rates measured, and consists of those detected below the surface of Lake 240 (Fig. 3) and the data of Fig. 4c.

The linear correlation between photodegradation rate and PAR is significant ($p<0.05$) in Fig. 4 ($r^2 = 0.95$, 0.43 and 0.69 for a, b, c, and d, respectively). The slope of the regression line is less in Fig. 4a (0.2) than it is in Figs. 4b (0.7), c (0.7), or d (0.6).

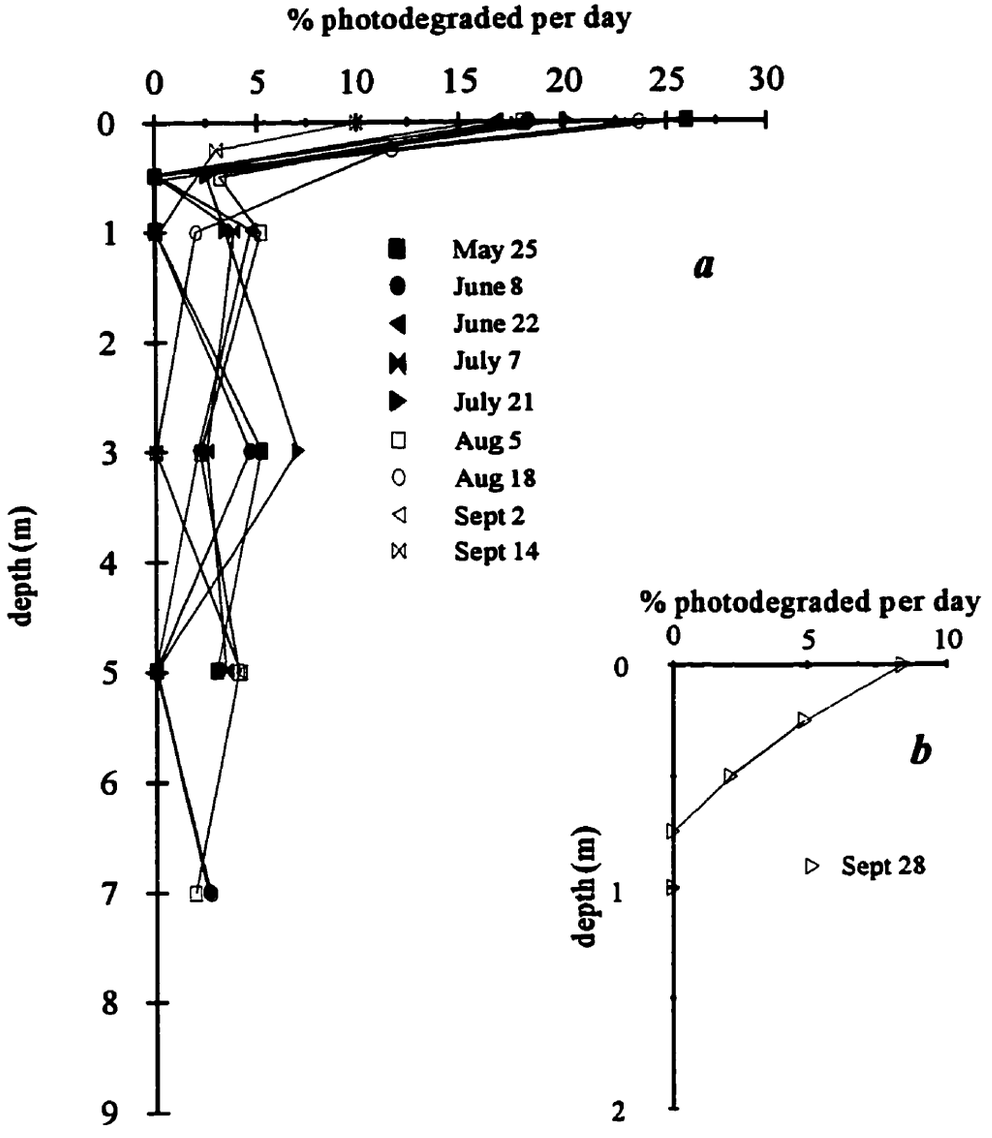


Fig. 3. Depth profiles of methylmercury photodegradation rates in bottles incubated in Lake 240 in 1995. The profile of September 28 (*b*) is shown separately because it is the only profile restricted to the first meter of water.

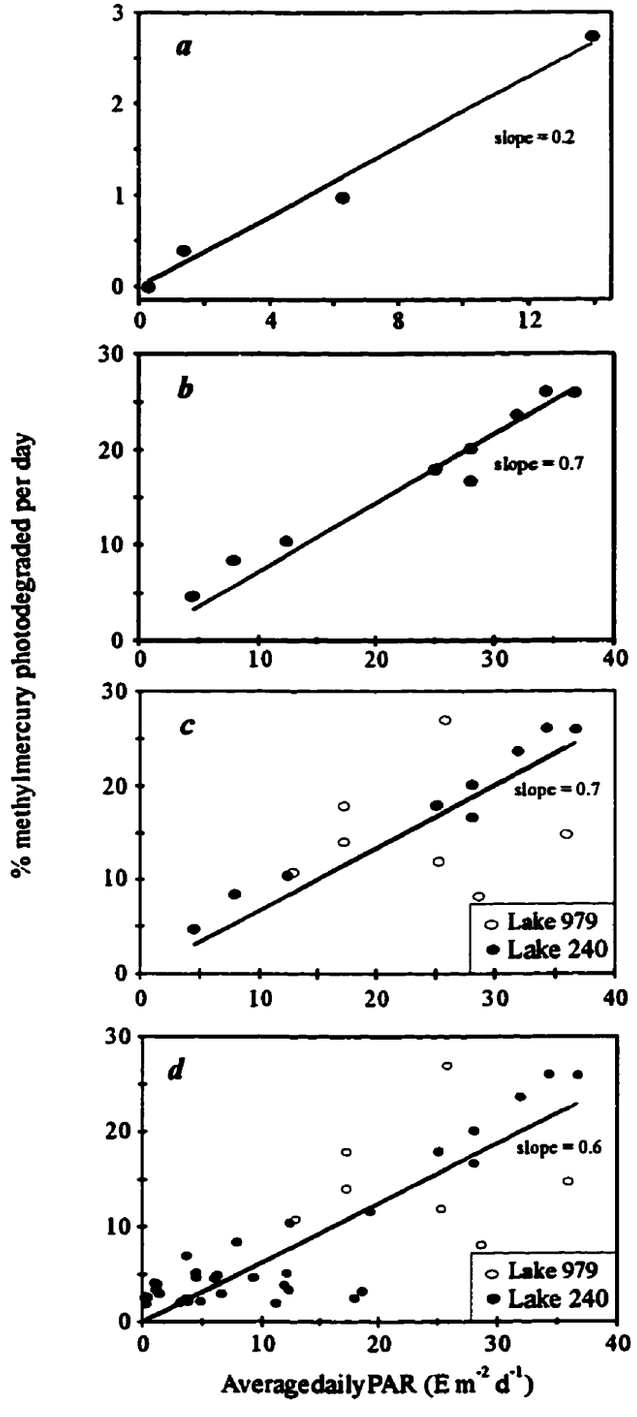


Fig. 4. The effect of sunlight intensity on methylmercury photodegradation rate. *a*, one photodegradation profile in Lake 240, 1994 (from Sellers *et al.*, 1996); *b*, surface incubations on Lake 240 in 1995; *c*, surface incubations on Lake 979 and Lake 240 in 1995; *d*, all surface and subsurface incubations in 1995. y-intercept is 0 for all regression lines. r^2 values are 0.98, 0.95, 0.43, and 0.69 for *a*, *b*, *c*, and *d* respectively. The linear correlations are significant at the 5% level.

Parallel incubations for a range of initial methylmercury concentrations in lake water (Lake 240) showed that methylmercury photodegradation was linearly dependent on concentration (Fig. 5).

No difference in methylmercury photodegradation rate was observed between distilled deionized water (DDW) and clear lake (Lake 240; Table 1) water (Fig. 6). Methylmercury was photodegraded at a slightly lower rate in the dark-colored lake (Lake 979; Table 1) water when compared to DDW and to clear lake water (Fig. 6).

The addition of H_2O_2 to lake water exposed to sunlight did not affect photodegradation rates (Fig. 7). In addition, the presence of H_2O_2 did not result in loss of methylmercury in bottles incubated in the dark (Fig. 7).

DISCUSSION

The photodegradation of methylmercury described here is one of the many sunlight mediated photochemical transformations that naturally occur in aquatic ecosystems. Others include the photodecomposition of DOC (Francko, 1990), metal-DOC complexes (Zafiriou *et al.*, 1984; Cotner and Heath, 1990), and organic pollutants (Zafiriou *et al.*, 1984; Ollis *et al.*, 1991). The photodegradation of other alkylated mercury and tin compounds is also known to occur in the presence of sunlight (Zepp *et al.*, 1973; Blunden and Chapman, 1982; Navio *et al.*, 1993; Suda *et al.*, 1993). Sunlight also stimulates production of Hg^0 (Amyot *et al.*, 1994) from Hg^{2+} (Munthe and McElroy, 1992).

This study is different from previous laboratory studies of methylmercury photodegradation (Inoko, 1981; Suda *et al.*, 1993) because here, natural sunlight and

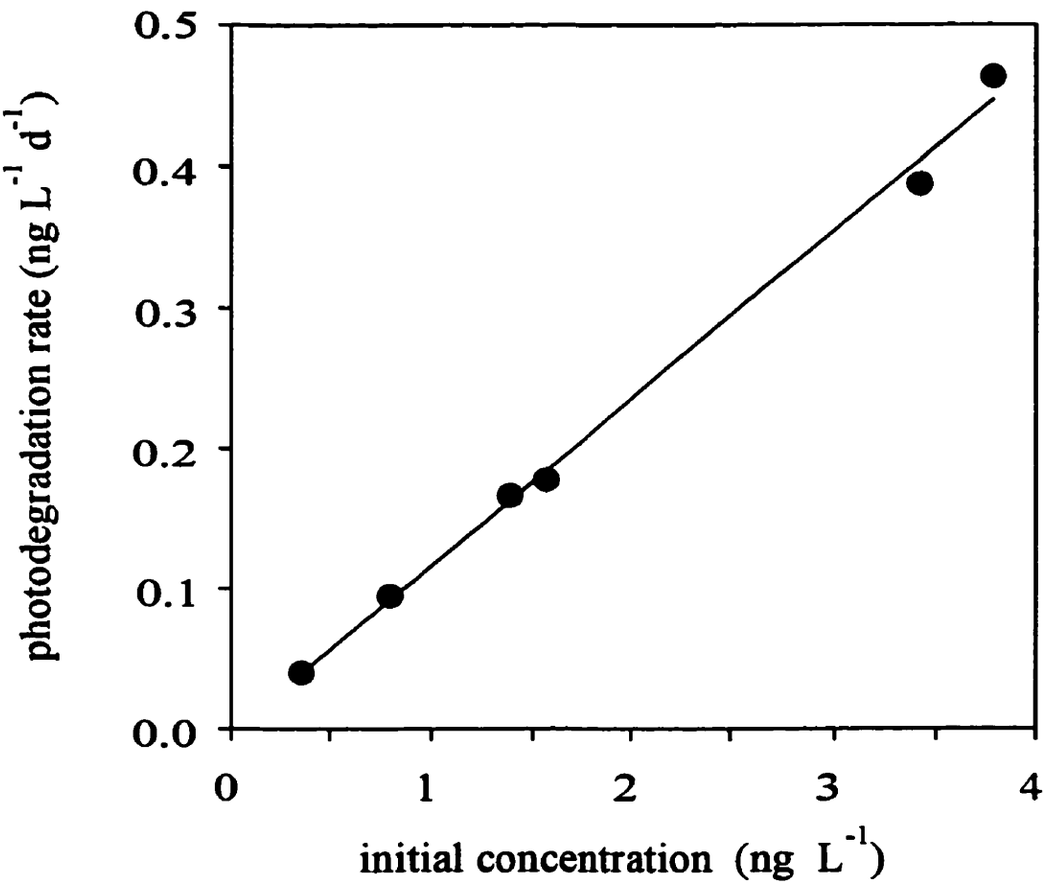


Fig. 5. The effect of methymercury concentration on the photodegradation rate in clear lake water (Lake 240). The light level is the same for all incubations. Regression line: y = 0; slope = 0.12. Average daily PAR was 25 E m^{-2} d^{-1}.

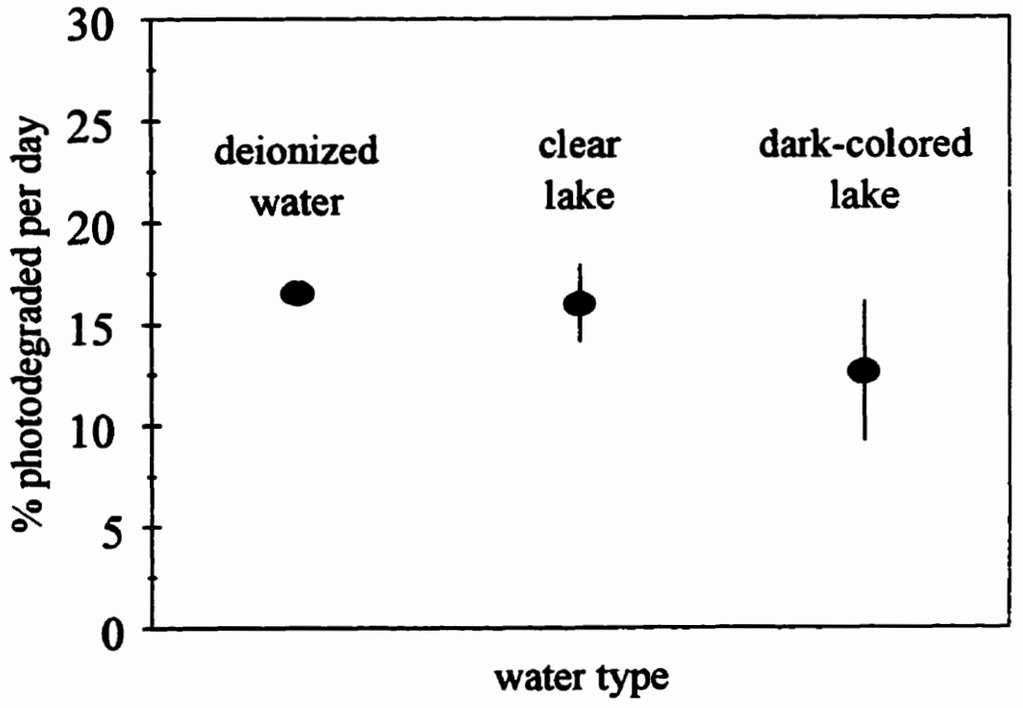


Fig. 6. Methylmercury photodegradation rates in water of different chemistry. The clear lake water was collected from Lake 240 and the dark lake water was collected from Lake 979. The range of rates for duplicate treatments is indicated by the lines. Duplicate rates were the same for deionized water and so no line is visible.

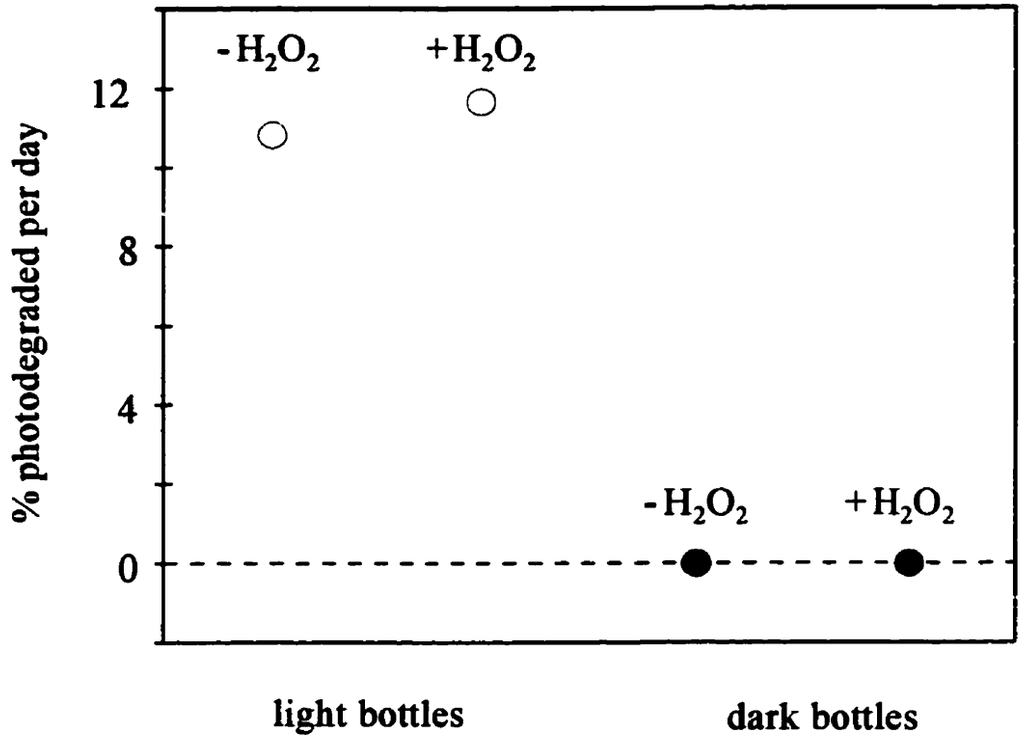


Fig. 7. Methylmercury photodegradation rates in lake water incubated with and without the addition of H_2O_2. Light bottles were exposed to sunlight and dark bottles were shielded from sunlight.

naturally occurring concentrations of methylmercury were used. In the previous studies, either artificial UV light (Inoko, 1981; Suda *et al.*, 1993), laboratory water (Inoko, 1981), or unnaturally (and very) high concentrations of methylmercury (Inoko, 1981; Suda *et al.*, 1993) were used. Also, methylmercury degradation was measured as the production of inorganic mercury (Inoko, 1981; Suda *et al.*, 1993) whereas in this study, the actual loss of methylmercury was measured. Despite these differences, there are similarities. For example, Suda *et al.* (1993) also showed that natural sunlight caused photodegradation in samples of concentrated seawater, and Inoko (1981) found that photodecomposition of methylmercuric chloride in solution followed first order kinetics.

It is likely that methylmercury is covalently bound to DOC in freshwaters (Zepp *et al.*, 1974), which also absorbs (Kouassi and Zika, 1992) and is photodegraded by (Francko, 1990) UV-sunlight. Therefore, it is not surprising that lake water shielded from UV sunlight showed less or no photodegradation of methylmercury (Fig. 2) than water exposed to full sunlight, and suggests that UV radiation is required for methylmercury photodegradation in lake water. The results of the UV-B exclusion experiments (Fig. 2) must be interpretted with caution because the transmission spectrum of PFA-Teflon made with a spectrophotometer (Fig. 1) show that only a small proportion of the UV-B is transmitted, whereas field measurements show that 66% of solar UV-B is transmitted (M. Amyot, pers. comm.). If the UV-B that is transmitted through the bottles does not contain enough energy to cause photodegradation, then there should be no difference in photodegradation rate in transparent bottles and bottles wrapped in mylar (UVA+ visible; Fig. 2). However, inconsistent results were obtained: Excluding solar UV-B (visible +

UV-A; Fig. 2) reduced photodegradation rates (by 35 %) in one experiment (July of Fig. 2), had no affect in another (May of Fig. 2), or increased rates slightly (October of Fig. 2) when compared to transparent bottles. These results, plus the fact that field measurements of solar UV-B transmission through these bottles were not made means that I cannot assess the role of UV-B in methylmercury photodegradation.

Using similar methods, Amyot *et al.*, (1994) reported that the exclusion of solar UV-B did not significantly reduce the rates of the photoproduction of dissolved gaseous mercury (DGM) in lake water incubated on the surface of a lake. However, these investigators also later found that UV-B may be important in DGM production in very clear lakes (Amyot *et al.*, 1996). If UV-B does mediate methylmercury photodegradation in lakes, it may be important in very clear lakes which have a greater UV-B penetration depth than do dark-colored lakes (Scully and Lean, 1994).

There is evidence to suggest that solar wavelengths longer than UV photodegrade methylmercury at low rates. First, in one of the three wavelength exclusion incubations, in which the samples were exposed to only visible light (July of Fig. 2), detectable photodegradation rates were measured (however, this could also suggest that the small amount of UV light transmitted through UF-3 Plexiglas (Fig. 1) was sufficient to cause photodegradation during this incubation). Second, methylmercury photodegradation was observed below the depth (0.8 to 1.5 m) to which UV-A is "extinguished" in Lake 240 (D. Lean, University of Ottawa, Ottawa, pers. comm; M. Stainton, Freshwater Institute, Winnipeg; Fig. 3). Third, the high correlation between methylmercury photodegradation

rates and visible light (Fig. 4) also suggests that wavelengths greater than 400 nm may also have sufficient energy to mediate methylmercury photodegradation.

I do not know what chemical form of methylmercury is photodegraded in lakes. The high affinity of methylmercury for DOC (e.g. Zepp *et al.*, 1974) and absorbance of UV-sunlight by DOC (e.g. Zepp, 1988) suggests that the photoreactive species is a methylmercury-DOC complex. Other possibilities are ionic forms of methylmercury, but neither methylmercuric chloride or methylmercuric hydroxide absorb wavelengths > 290 nm i.e. in the UV-B or UV-A range (D. Krabbenhoft, USGS, Madison, unpubl. data), which do mediate photodegradation (Fig. 2). However, methylmercury-sulfur (i.e. thiol) complexes do absorb light > 290 nm (D. Krabbenhoft, USGS, Madison, unpubl. data). It seems likely, therefore, that both methylmercury-DOC and methylmercur-thiol complexes are photoreactive in natural waters.

Although it is clear that most photodegradation of methylmercury occurs at the lake surface (Fig. 3), the maximum depth to which it occurs in Lake 240 is difficult to determine. Because UV sunlight appears primarily responsible for photodegradation, it seems likely that most of the photodegradation would occur to a depth equivalent to the depth to which UV radiation penetrates (approximately 1 m). In this study, most of the photodegradation was indeed observed within the first meter, but low rates were also detected at 3 m (Fig. 3a). I conducted only one detailed incubation profile within the first meter only (Fig. 3b), and additional experiments of this type are needed to determine the depth profile of methylmercury photodegradation in lake water.

The linear relationship between methylmercury photodegradation rate and PAR observed in this study (Figs. 4b-d) confirms the one described first by Sellers *et al.* (1996; appendix A; Fig. 4a). In this study, the relationship developed from bottle incubations on the surface of Lake 240 (Fig. 4b) is weakened by including bottles incubated on the surface of Lake 979 (Fig. 4c). The only difference between these incubations is that on Lake 240, no more than two bottlws were tethered to a float at any one time, whereas on Lake 979, bottles (as many as 10) were tethered to a raft. The presence of more bottles and/or the raft on Lake 979 may have caused some shading, which may explain why the correlation between PAR and photodegradation rate is weakened when Lake 979 surface incubations are included in this relationship (Fig. 4c).

The scatter that exists among the data points at the lower end of the relationship between photodegradation rate and PAR (Fig. 4d), which exists when subsurface Lake 240 incubations (Fig. 3) are included, is most likely because many of the photodegradation profiles do not follow the extinction pattern of light (Fig. 3). However, inclusion of subsurface photodegradation rates with surface photodegradation rates (Fig. 4d) improves the certainty of the relationship from that which is developed when only surface incubations are included (Fig. 4c).

The slope of the linear regression between PAR and photodegradation rate is much less in the first relationship developed (Fig. 4a; Sellers *et al.*, 1996, appendix A) than in the ones developed in this study (Fig. 4b-d). In this study, the slopes range from 0.6 to 0.7 (Figs. 4b-d) whereas in Sellers *et al.* (1996) the one derived from preliminary data (Fig. 4a) is 0.2. This difference may be because these relationships were arrived at using

slightly different methods. Sellers *et al.*, (1996; appendix A) developed this relationship from only one incubation experiment, in which the amount of PAR was varied by incubating bottles at different depths in Lake 240 while all the bottles had the same initial concentration. In the study described here, several photodegradation incubations, both on and below the surface of the lake, were conducted throughout the ice-free season. In this way, the amount of sunlight the bottles received was varied temporally (Fig. 4b,c) or temporally and spatially (Fig. 4d). The rates of photodegradation below the lake surface may have been influenced by the quality of light, which changes with depth. Regardless, the relationships between photodegradation rate and PAR developed in this study (Fig. 4b-d) are probably more reliable than the initial one (Fig. 4a), not only because they were developed from more data, but also because they include a greater range of PAR rates (up to 46 E m^{-2} d^{-1} vs. up to 13 E m^{-2} d^{-1} in Fig. 4a) and photodegradation rates (up to 28% photodegraded per day vs. up to 2.7% in Fig. 4a).

It is important to note that the slopes of the regression lines in the relationships between photodegradation rate and PAR developed in this study (Fig. 4b-d) are very similar (0.7 for Fig. 4b and c, 0.6 for Fig. 4d). The value of this slope is important because it can be used in the whole-lake estimation of photodegradation. I considered the relationship from Fig. 4d to be the most accurate in Fig. 4 because it includes the most data points. This relationship (slope = 0.6) was used for the estimation of *in situ* photodegradation for the methylmercury mass balance of Lake 240 (chapter 5). Further, because the rates are expressed as percent photodegraded per day means that the photodegradation rate is normalized for methylmercury concentration, and so only *in situ*

sunlight and methylmercury concentration are needed for a whole-lake estimate of methylmercury photodegradation (Chapter 5).

The linear relationship between methylmercury photodegradation rate and methylmercury concentration (Fig. 5), confirms the one described first by Sellers *et al.* (1996; appendix A). The relationship described here is for a lower range of concentrations (0.3 to 3.8 ng L^{-1} compared to 1.2 to 10.5 ng L^{-1}), but the slopes of the regression line are very similar (0.12 and 0.10 for this study and Sellers *et al,* (1996, appendix A), respectively).

There were several reasons to hypothesize that DOC would be involved in the chemical mechanism of methyl mercury photodegradation, and that photodegradation rates could be enhanced by the presence of DOC. One is that much of the methylmercury that exists in natural freshwaters is likely bound to DOC, especially where DOC concentrations are high (Zepp *et al.*, 1974; Driscoll *et al,* 1994; Watras *et al.*, 1995). Another reason is that DOC absorbs sunlight very strongly in the UV region (Zepp, 1988; Kouassi and Zika, 1992). In addition, the absorption of sunlight by DOC produces many chemically reactive oxidants, such as H_2O_2, which participate in the transformation of other inorganic and organic complexes (Zafiriou *et al.*, 1984; Cooper *et al.*, 1989; Hoigne *et al.*, 1989). Although the different waters used to examine the effect of water chemistry differed from one another in the concentration of other chemical parameters (Table 1), examining the effect of water chemistry on photodegradation rates (Fig. 6) was an indirect test of the effect of DOC. The fact that methylmercury photodegraded in distilled deionized water at rates similar to those of methylmercury in lake water (Fig. 6) suggests

that photodegradation does not require the participation of DOC or other chemical constituents, and that photodegradation occurs as a result of the direct interaction of methylmercury with sunlight (i.e. direct photolysis). If anything, the lower rates observed in dark-colored lake water (Fig. 6) suggest that a chemical characteristic peculiar to high DOC lake water can suppress methylmercury photodegradation.

Because H_2O_2 is photochemically produced from lake DOC (Cooper *et al.*, 1989; Hoigne *et al.*, 1989; Cooper *et al.*, 1994), the addition of H_2O_2 was another indirect test of the requirement of DOC in the photodegradation mechanism. However, H_2O_2 addition did not reduce or enhance photodegradation rates (Fig. 7), suggesting that DOC and H_2O_2 do not (indirectly and directly, respectively) participate in the chemical reaction of methylmercury photodegradation. Interestingly, Amyot *et al.* (1994) also found that addition of H_2O_2 did not stimulate the photoproduction of DGM. While it appears that DOC does not chemically participate in the photodegradation of methylmercury in lake water, it is important in regulating the amount of photodegradation that occurs in lakes by controlling the depth to which sunlight penetrates. In this way, DOC indirectly controls the effective depth of methylmercury photodegradation in lakes. This reduced light penetration probably is one reason highly colored lakes have high methylmercury concentration as compared to clear water lakes (Driscoll *et al.*, 1994; Watras *et al.*, 1995). Another reason for the high methylmercury concentration of dark-colored lakes is that methylmercury is transported into lakes from surrounding wetlands, which have a high concentration of methylmercury (St. Louis *et al.*, 1994, 1996).

The end products of methylmercury photodegradation in lake water have yet to be determined, and it is the fate of the Hg atom in the methylmercury molecule that is of most interest. Laboratory studies of methylmercury photodegradation show that mercury is converted to inorganic species (Inoko *et al.*, 1981; Suda *et al.*, 1993). If Hg^{2+} is a product of methylmercury photodegradation, it could subsequently be reduced chemically (Allard and Arsenie, 1991) or photochemically (Munthe and McElroy, 1992) to Hg^0. Alternatively, methylmercury photodegradation may produced Hg^0 directly (Inoko, 1981). In either scenario, methylmercury photodegradation may contribute to the photoproduction of Hg^0 (Amyot *et al.*, 1994) and if so, influences the emission of Hg^0 from lakes to the atmosphere (Vandal *et al.*, 1991; Schroeder *et al.*, 1992; Vandal *et al.*, 1995). However, measured rates of photoproduction of Hg^0 using similar incubation methods to those of this study are much faster (Amyot *et al.*, 1994), suggesting that photodegradation of methylmercury does not contribute directly to the production of Hg^0. On the other hand, estimated aerial rates of methylmercury photodegradation in Lake 240 (chapter 5) are similar to estimates of Hg^0 emission rates from a lake in one study (Vandal *et al.*, 1991) but not in others (Vandal *et al.*, 1995; Schroeder *et al.*, 1992; Lindberg *et al.*, 1996). It is clear that a determination of end-products must precede a realistic comparison of end-products and rates with other studies of Hg^0 evasion from lakes.

Typically, photochemical reactions are sensitive to changes in chemical and physical parameters (Zafiriou *et al.*, 1984). Although the specific effect of individual environmental variables were not tested in this study, I found that major differences in water chemistry did not affect methylmercury photodegradation rates. This suggests that

specific chemical parameters may need not be considered when estimating the amount of methylmercury photodegraded in a lake. So far, the only environmental variables found to significantly affect photodegradation rates are methylmercury concentration (Sellers *et al.*, 1996; appendix A; this study); light quantity (Sellers *et al.*, 1996; appendix A; this study) and light quality (this study). This means that it may be easy to include photodegradation in lake mercury cycling models (Hudson *et al.*, 1994) if only three parameters are known: depth-integrated PAR, the depth of UV penetration, and *in situ* methylmercury concentration.

Chapter 5

Cycling of methylmercury in a drainage lake: The relative importance of internal production, photodegradation, and external inputs

ABSTRACT

A detailed, chemical mass balance study of methylmercury was conducted for a clearwater drainage lake (Lake 240) at the Experimental Lakes Area, northwestern Ontario. External sources of methylmercury to Lake 240 were atmospheric deposition, direct terrestrial run-off, and two lakes: a larger, clearwater lake (Lake 239) and a smaller, brownwater lake (Lake 470). The outflow from Lake 240 and the inflows of the two upstream lakes were sampled weekly (spring), bi-weekly (summer), and monthly (winter). Losses of methylmercury were the lake outflow and photodegradation. Bi-weekly estimates of photodegradation for the ice-free period were made from a linear relationship between photosynthetically active radiation (PAR) and photodegradation rates, which was developed for a wide range of PAR measurements.

In 1995-1996, 7% of the externally supplied methylmercury and 38% of the water to Lake 240 came from Lake 239. In contrast, Lake 470 contributed 78% of the methylmercury but only 24% of the water. Lake 470 was the primary external source of methylmercury to Lake 240 because it is surrounded by wetlands, a significant source of DOC and methylmercury (St. Louis *et al.*, 1996; Hurley *et al.*, 1995). Losses of methylmercury from Lake 240 were dominated (>90%) by photodegradation. The mass balance estimate of in-lake production of methylmercury was 3 times greater than the total of all external inputs in this year. In wet years, however, external inputs should increase in importance. This study shows that the methylmercury concentration in Lake 240 was

largely controlled by two dominant internal processes: photodegradation and in-lake production.

During stratification, methylmercury accumulated in the hypolimnion of Lake 240, and the concentration profiles strongly suggest that the site of methylmercury release was the sediment-water interface rather than the water column. Using the mass balance estimate of in-lake production and the measured amount of methylmercury accumulated in the hypolimnion, a mathematical model for release of new and recycled methylmercury at the sediment water interface of Lake 240 was developed. This model suggests that the annual mass flux of new and recycled methylmercury was greater from epilimnetic than from non-epilimnetic sediments. It also predicted that the mass ratio of recycled to new methylmercury released from sediments is expected to be much smaller for epilimnetic than for non-epilimnetic sediments (0.7:1 and 5:1, respectively).

INTRODUCTION

Lakes receive methylmercury from the atmosphere, inflowing streams, run-off from their immediate terrestrial environment, and from production within. The relative importance of external and internal sources to lakes will depend on the amount of atmospheric loading (determined largely by their proximity to industries), catchment area, and the percentage of wetlands in the terrestrial catchment (Hurley, 1995; Rudd, 1995). In remote drainage lakes, wetlands can be a significant source of methylmercury (St. Louis *et al.*, 1994, 1996; Hurley *et al.*,

1995; Watras *et al.*, 1995d). Methods for quantifying external inputs of methylmercury to lakes are available. Bulk collectors for atmospheric deposition can be deployed, and inflow from streams and direct terrestrial run-off can be routinely sampled for methylmercury. However, methods for quantifying internal production have proved difficult to develop.

One approach to measurement of internal methylmercury production is to measure the rates at which methylmercury is produced in the water and sediment during experimental incubations. In doing so, either changes in ambient concentrations of methylmercury (e.g. Gilmour and Riedel, 1995; Henry *et al.*, 1995; Watras *et al.*, 1995a) or the transformation of isotopic tracers (Gilmour and Riedel, 1995; Stordal and Gill, 1995) are measured over time. The number of measurements of change in methylmercury concentration in intact sediment cores or chambers placed over sediments are few because the necessary analytical techniques have only recently become available. Even with current techniques, however, this type of measurement is still limited by the difficulty of measuring the change in methylmercury concentration against background concentrations, and by high variability (chapter 3) which makes extrapolation to the whole-lake uncertain. Isotope measurements have been hampered by the cost and difficulty of obtaining high-specific activity isotopes and quantification of the specific activity after addition to water and sediment (e.g. Gilmour and Riedel, 1995).

The other approach to estimating in-lake production of methylmercury is a mass balance approach. All methylmercury inputs to and outputs from the lake are

measured, and the difference between the mass of methylmercury entering the lake and the mass of methylmercury leaving the lake is an estimate of net production of methylmercury within the lake. Concurrent measurements of concentrations and/or methylation rates within the lake can be made to provide further information on seasonal and spatial patterns within the lake (e.g. Watras *et al.*, 1995a).

Only in the last decade have the analytical capabilities for measuring methylmercury at the trace levels found in natural waters become available (Bloom 1989; Horvat *et al.*, 1993). Since then, only a few mass balance studies of methylmercury in lakes have been reported (Hultberg *et al.*, 1994; Watras *et al.*, 1994; Henry *et al.*, 1995). However, all of these studies were conducted before the abiotic photodegradation of methylmercury by sunlight in lakes was known to occur (Sellers *et al.*, 1996, appendix A). Therefore, the exclusion of methylmercury loss by photodegradation in those studies means that the amount of methylmercury produced within those study lakes was likely (and perhaps seriously) underestimated (Sellers *et al.*, 1996, appendix A).

In a provisional budget, Sellers *et al* (1996, appendix A) used an average methylmercury concentration and long-term annual average water budget to estimate that in one year, the amount of methylmercury photodegraded in an oligotrophic lake was greater than the amount leaving the lake via the outflow, and greater than the amount received from external sources. They further estimated that, in order to maintain the methylmercury concentration observed in this lake,

internal production of methylmercury must have been about equal to external inputs of methylmercury to Lake 240.

The study presented in this chapter was undertaken to carry out a complete methylmercury budget for Lake 240. My objectives were to (i) to measure external inputs and losses of methylmercury, (ii) to measure losses of methylmercury, one of which was photodegradation, (iii) to use the data obtained to compare the relative importance of methylmercury photodegradation to other production and loss terms, and (iv) to estimate the amount of methylmercury produced within the lake using a mass balance approach. In this approach, internal production is estimated by the difference between the total loss of methylmercury from the lake and the total input of methylmercury to the lake. This mass balance study is more thorough than the provisional study (Sellers *et al.*, 1996, appendix A) because a larger number of measurements were done to determine the relationship between photodegradation rate and sunlight, and because real hydrologic inputs and losses of methylmercury were measured for the year rather than estimated from long-term data.

This study was conducted for a one year period, beginning in March 1995. The results showed that in this one-year period, the amount of methylmercury destroyed by photodegradation exceeded the amount that entered the lake from external inputs, that internal production of methylmercury contributed about 5 times more methylmercury to Lake 240 than did external inputs, and that methylmercury concentrations in Lake 240 were primarily affected by two in-lake processes: photodegradation and in-lake production of methylmercury.

METHODS

Study site. Lake 240 is an oligotrophic drainage lake situated in the boreal forest of northwestern Ontario at the Experimental Lakes Area (Johnson and Vallentyne, 1971). Lake 240 is part of a 723 h watershed characterised by wetlands, 6 lakes, and upland forests that are dominated by young jackpine (St. Louis *et al.*, 1994). It has one outflow, and receives water from two lakes, Lake 239 and Lake 470, which are connected to Lake 240 by short streams (Fig. 1). Lake 239 is an oligotrophic lake larger and deeper than Lake 240. Lake 470 is a smaller, brownwater lake that is surrounded by wetlands. Some hydrological and chemical characteristic of these three lakes are given in Table 1.

Hydrology of Lake 240. The water flow at all three outflows were measured continuously with a trapezoidal cut-throat flume, a twelve-inch Parshall flume, and a 120-degree v-notch weir for Lake 470, 239 and 240, respectively (K. Beaty and M. Lyng, unpubl. data.). For the period of this study, Lake 240 received 38% of its water from Lake 239 and 24 % from Lake 470. The amount of precipitation was also measured, and contributed 25% of the water to the lake while the remaining 13 % was received from overland run-off the adjacent forested uplands. Groundwater flux into Lake 240 is insignificant (K. Beaty, Freshwater Institute, Winnipeg, pers. comm.). Spring flow from all three lakes began in March of 1995. Water stopped flowing out of Lake 239 in late August and from Lake 240 in early September. The flow of water from Lake 470 was intermittent

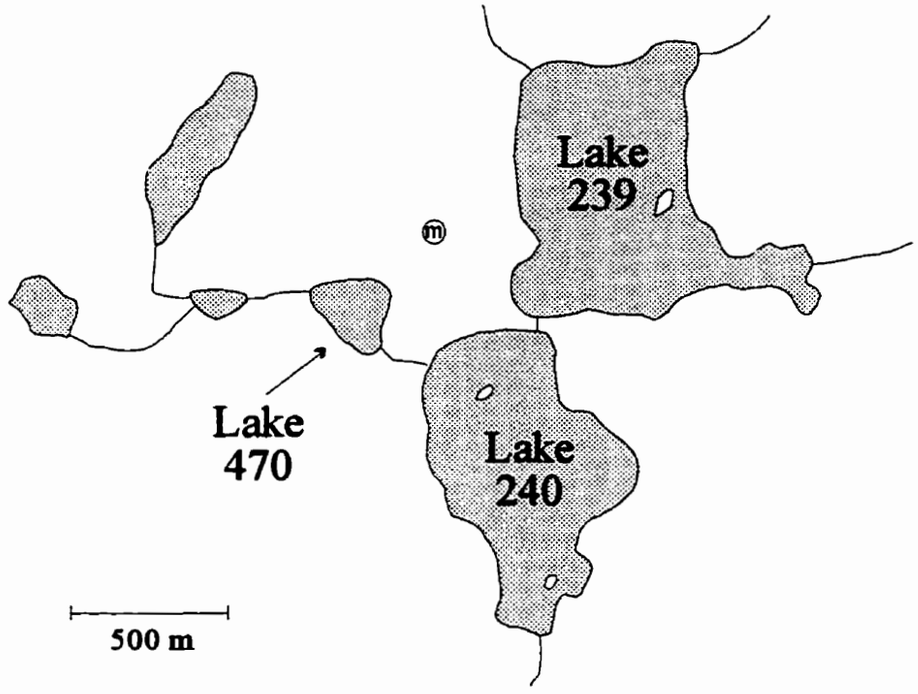


Fig. 1. The Lake 240 watershed, indicating relative position and size of the study lakes and meteorological station (ⓜ).

Table 1. Some characteristics of the study lakes.

Parameter	Lake 239	Lake 240	Lake 470
mean depth (m)	10.5*	6.1*	0.8$^{\Phi}$
maximum depth (m)	30.4*	13.1*	n.a.
surface area (h)	56.1*	44.1*	4.24$^{\Phi}$
residence time (yrs)	7.4$^{\delta}$	1.5$^{\delta}$	0.072$^{\theta}$
pH$^{\otimes}$	6.68 - 7.45	6.48 - 7.31	6.05 - 6.57
DOC (μmol L^{-1})$^{\otimes}$	440 - 620	550 - 640	840 - 1010

* Brunskill and Schindler, 1971

$^{\Phi}$ Beaty and Lyng, 1989

$^{\delta}$ 1970 to 1994 annual mean; K. Beaty and M. Lyng, unpubl. data.

$^{\theta}$ 1970 to 1990 annual mean; K. Beaty and M. Lyng, unpubl. data

$^{\otimes}$ from 18, 12, and 4 measurements made during 1995 for Lake 239, Lake 240 and Lake 470, respectively.

n.a. = not available

in late summer, and stopped flowing by December of 1995. Water flow from all three lakes began again with spring melt in March of 1996 at all three sites. Ice covered the lake from November 8, 1995 to May 17, 1996.

Sample collection and analyses. Weekly samples of water for analyses of methylmercury concentration from the outflow of Lake 239, 240 and 470 were taken between March 16 and May 3, 1995 during high spring flow. Subsequently, bi-weekly samples were taken. Lake 240 profile samples were collected every 4 weeks beginning in May 8 to October 4. During ice cover, samples were collected monthly. Water was pumped from depth using a peristaltic pump equipped with platinum-cured silicone tubing (Cole-Parmer) that had been pre-washed with 0.1% HCl. Samples were collected in perfluoralkoxy (PFA) Teflon® bottles (125 or 250 mL) that had been washed in hot concentrated nitric and stored in 0.1% hydrochloric acid. To prevent contamination, ultra-clean sampling protocol that included the use of gloved hands and Ziploc® bags was used (St. Louis *et al.*, 1994).

Lake-water samples were collected for dissolved sulfate and oxygen analyses were pumped from depth using using a peristaltic pump equipped with platinum-cured silicone tubing (Cole-Parmer). Sulfate was analysed by the Experimental Lakes Area Chemistry Lab (E. Schindler) using the method of Stainton *et al.* (1977). Oxygen was analysed by the author using a scaled-down version of the Winkler method as described by Stainton *et al.* (1977). Sample for oxygen analyses were drawn from the silicone tubing using 20 mL

glass syringes fitted with an 18 g needle. The syringes were sealed from the atmosphere using small serum stoppers.

To collect samples of water flowing directly into Lake 240 from the adjacent uplands (direct run-off; DRO), water flowing down the hillside was gathered by deflector walls that were permanently attached to the bedrock on the shore of the lake. Two of these DRO collectors were constructed on the east shore within 20 m of the lake edge (Fig. 1). The deflector walls of each collector site were made of two plywood boards (approx. 2.5 cm x 20 cm x 200 cm) that were secured to the bed rock with fiber-glass cloth and resin in the shape of a V, angled approximately 150 degrees apart from each other. The fiber-glass covered walls were then painted with epoxy-paint. Water that collected within the confinement of the walls ultimately flowed down a section of plastic eaves-trough (approx. 60 cm), out the end of which DRO was sampled during or shortly after rain events. As a precaution against contamination from catchment construction material, sample collection did not begin until the walls had been first washed by an intensive rain event. Catchment number 1 was sampled three times during the study and catchment number 2 was sampled twice during the study period.

Stream inflow, outflow, epilimnetic and catchment samples were frozen until they were analysed for methylmercury concentration. Samples were analysed using the co-distillation extraction method of extraction followed by atomic fluorescence detection (Horvat *et al.*, 1993), as outlined in Chapter 2. The

detection limit, determined as 2 times the standard deviation of the mean of long-term average of blank concentrations, was 0.02 ng L^{-1} at a blank concentration range of 0.002 to 0.026 ng L^{-1}. When the concentration of methylmercury was below the detection limit, it is reported as one half of this value i.e. 0.01 ng L^{-1}, and this value was used in all mass balance calculations when methylmercury was below detection.

Solar Radiation measurements. Total incident photosynthetically active radiation (PAR) was continuously monitored at a nearby (300 m) meteorological station with a LiCOR terrestrial PAR sensor (190S; E. Schindler, Freshwater Institute, Winnipeg, pers. comm.). Depth profiles of instantaneous PAR were measured several times during the ice-free season using a LiCOR underwater PAR quantum sensor (192S).

Photodegradation incubations. The concentration of methylmercury in Lake 240 is very low (Sellers *et al.*, 1996, appendix A) and so direct measurement of photodegradation rate at *in situ* concentrations is impossible with current analytical techniques. Because photodegradation rate is linearly proportional to methylmercury concentration and solar radiation (Sellers *et al.*, 1996, appendix A; chapter 4), my approach was conduct a series of *in situ* photodegradation bottle incubations using Lake 240 water, in which the methylmercury concentration had been artificially increased by low-level addition of methylmercury. The water in the incubation spiked bottles had an initial concentration that ranged from 1 to 3 ng L^{-1} methylmercury, which is within the

range typical for ELA lakes and streams (J. Rudd, Freshwater Institute, Winnipeg, pers. comm.). Photodegradation rates determined from these incubations were then corrected to *in situ* rates using the straight line relationship between photodegradation rate and PAR and the *in situ* methylmercury concentrations. To do this, several photodegradation incubations were conducted throughout the 1995 ice-free season. Each incubation, which lasted from six to eight days, began on a different date. For each of these incubations, water collected from the surface of Lake 240 was filtered through a 0.45 μm cellulose acetate filter. Filtering of lake water does not affect photodegradation rates (Sellers *et al.*, 1996, appendix A), and so I used filtered water for these incubations in order to reduce analytical variability that may be enhanced by the presence of particles. Methylmercuric chloride stock solution (in distilled water) was added to the filtered lake water to elevate the methylmercury concentration. The water was then distributed in 125 mL PFA-Teflon® bottles that were sealed in two Ziploc® bags and incubated on the surface of Lake 240 (most incubations) or Lake 979, or below the surface of Lake 240. Both the Teflon® bottles and Ziploc® bags were transparent to UV and visible sunlight (chapter 4). For each incubation, three bottles were used: one bottle was analysed for methylmercury at the beginning, a second bottle was analysed part way through the incubation, and a third bottle was analysed at the end of the incubation. The bottled incubation samples were frozen until analyses. Duplicate analyses of methylmercury concentration were performed on each bottle.

Expression of photodegradation rate. For each photodegradation incubation, a time course of methylmercury concentration was plotted. Photodegradation rates (ng L^{-1} d^{-1}) were calculated from the change in methylmercury concentration (ΔC) between time zero and the first sampling (Δt) as follows:

$$\text{photodegradation rate} = \Delta C/\Delta t \qquad (1).$$

The measured photodegradation rates are expressed as a percent of methylmercury photodegraded per day using the initial methylmercury concentration as follows:

$$\%\ \text{photodegraded per day} = \text{ng } L^{-1}\ d^{-1} \div \text{ng } L^{-1} \times 100 \qquad (2).$$

Calculation of mass of methylmercury photodegraded in the Lake 240 water column during the open-water season. Depth-averaged (1m) photodegradation rates were estimated for 12 two-week and 2 one-week periods throughout the ice-free season. To do this, the depth averaged (1m) PAR was estimated using both instantaneous PAR and total incident PAR measurements. The instantaneous PAR measurements were used to estimate the percent of incident PAR within the first meter, which would account for any reflection of PAR at the lake surface. Next, the average percent 1-m light penetration was applied to the total incident PAR that was measured for each period. Finally, the

depth-averaged PAR and *in situ* methylmercury concentration were used to estimate the depth-averaged *in situ* photodegradation rate for each period from the relationship between the percent methylmercury photodegraded per day and PAR (see Fig. 3 below) using the following equation:

$$PR = \begin{matrix}\text{fraction} \\ \text{photodegraded} \\ \text{per day}\end{matrix} \times \begin{matrix}\textit{in situ} \\ \text{methylmercury} \\ \text{concentration}\end{matrix} \quad (3).$$

The mass of methylmercury (M, in ng) photodegraded during each period was calculated using the following equation:

$$M = PR \times V \times t \quad (4)$$

where PR (ng L^{-1} d^{-1}) is the depth integrated photodegradation rate for the period, V is the volume of water in the first meter of Lake 240 (in litres) and t is the duration of the period (in days).

Methylmercury photodegradation is mediated primarily by the UV-A range (320 to 400 nm) of the solar spectrum (chapter 4). In Lake 240, the 1% light penetration depth of incident UV-A is 0.8 to 1.5 m (D. Lean, University of Ottawa, Ottawa; M. Stainton, Freshwater Institute, Winnipeg, pers. comm.). For this reason, I chose 1 m for the effective depth of methylmercury photodegradation in this estimate. However, if photodegradation rates are better correlated with UV irradiance than PAR, then using the relationship between photodegradation and

PAR may overestimate photodegradation rates because UV light is "extinguished" at a shallower depth than PAR. On the other hand, the high correlation between PAR and photodegradation rate (see Fig. 3 below; Sellers *et al.*, 1996, appendix A; chapter 4) suggests that photodegradation may have occurred below 1 m because even at 3 m, there is 10 to 15% of incident PAR. Indeed, photodegradation was measured below 1 m in this lake (Sellers *et al.*, 1996, appendix A; chapter 4). Therefore, limiting the effective depth of photodegradation to 1 m would offset any overestimate imposed by using the relationship shown in Fig. 3.

Calculating the mass of methylmercury in the inputs to and outflow from Lake 240. The mass (M) of methylmercury entering Lake 240 from Lake 239, and Lake 470, and the mass leaving Lake 240 via the outflow was calculated using the following equation:

$$M = \Sigma(C_i \times V_i) \qquad (5)$$

where methylmercury concentration (C_i) is in ng L^{-1} and represents the mean concentration of methylmercury between in unfiltered samples of two consecutive sampling times. The volume (V_i) is in litres, and represents the discharge of water during the period between the two consecutive samplings. The mass (M) of methylmercury for the budget year was calculated by summing the masses

determined for each period. The periods were 1 week long in the spring, 2 weeks long during the summer, and 1 month long during the winter. There were a total of 14, 16, and 19 periods for Lake 239, Lake 240, and Lake 470, respectively.

The amount of methylmercury that Lake 240 received from DRO was also estimated using equation (5). The volume of DRO water flowing into Lake 240 was estimated by multiplying the discharge to area ratio of nearby, hydrologically monitored catchments with the area of land represented by DRO samples. The mean methylmercury concentration of all DRO samples was applied to the discharge of DRO for the study period.

The methylmercury concentration in precipitation was not measured in this study. Rather, the concentration applied to measured precipitation volumes were taken from data presented by St. Louis *et al.*, (1995), and was 0.043 and 0.047 ng L^{-1} for rain and snow respectively.

Calculation of internal production of methylmercury. The mass balance equation used to estimate internal production of methylmercury is as follows:

$$P_I = O + D - \Sigma I + \Delta M \qquad (6)$$

where P_I is internal methylmercury production, O is loss via outflow, D is photodegradation, I is external input, and ΔM is change in storage, which is assumed to be zero. External inputs are Lake 470 outflow, Lake 239 outflow, DRO, and wet deposition

(Fig. 1). Each of the terms in the mass balance equation above (equ. 6) has an error associated with it. The error associated with the lake outflow measurements of ions and water is estimated to be 20% and the error associated with the DRO term is estimated to be 10-15% (Rudd *et al.*, 1990). The error associated with methylmercury analyses is 10-15% (chapter 2). The error on the slope of the line in the regression between photodegradation rate and sunlight (see Fig. 3, below), from which whole-lake photodegradation was estimated, is 6%. I had no way of estimating the error assocated with the ΔM term of the mass balance.

Calculation of hypolimnetic methylmercury accumulation during stratification. The amount of methylmercury that accumulated in the hypolimnion of Lake 240 was calculated from the concentration profiles (determined from unfiltered water samples) during the period of stable stratification and from the volume of lake water for intervals of hypolimnion. For each 1 m interval (or fraction thereof) below the top of the hypolimnion, the mass (M) was calculated using equation (5) where C_i is the methylmercury concentration and V_i is the volume of water for each depth interval. Total hypolimnetic mass is the sum of the masses in each depth interval. A sediment methylmercury production rate (P_S, in ng m^{-2} d^{-1}) was calculated using the following equation:

$$P_S = \Delta M \div A \div \Delta t \qquad (7)$$

where ΔM is the change in hypolimnetic mass between sampling dates, A is the hypolimnetic sediment area estimated from lake bathymetry, and Δt is the number of days between sampling dates.

RESULTS

Concentration of methylmercury in study lakes. The methylmercury concentration of surface water leaving Lake 240 ranged from <0.02 to 0.076 ng L^{-1} (Table 2). In general, concentrations were lower during the summer months than during the spring and winter months (Fig. 2a), possibly because of increased methylmercury photodegradation and decreased external loading to the epilimnion during the summer. The concentration of methylmercury leaving Lake 239 was lower than for Lake 240, and was typically below the detection limit (Fig. 2b), ranging from <0.02 to 0.046 ng L^{-1} (Table 2). Lake 239 is a much larger lake and so concentrations may be lower in this lake than in Lake 240 because the longer residence time of Lake 239 (Table 1) means that methylmercury is subjected to more photodegradation. The concentration of methylmercury leaving Lake 470 was the highest measured, ranging from 0.17 to 0.85 ng L^{-1} (Fig. 2c). The concentration was about 10 times higher in Lake 470 than in Lake 240 and Lake 239 (Table 2) because Lake 470 is a small lake (Fig. 1) surrounded by wetlands, and wetlands are known to be sites of methylmercury production (St. Louis *et al.*, 1994, 1996; Hurley *et al.*, 1995; Watras *et al.*, 1995d). No seasonal pattern in

Table 2. Methylmercury concentration in outflow and epilimnetic samples of Lake 240, and in outflow samples of Lake 239 and Lake 470.

Sample site	number of samples	average ($ng\ L^{-1}$)	range ($ng\ L^{-1}$)
Lake 240 epilimnion	6	0.041	<0.020 - 0.061
Lake 240 outflow	14	0.037	<0.020 - 0.076
Lake 239 outflow	13	0.021	<0.020 - 0.046
Lake 470 outflow	20	0.350	0.160 - 0.850

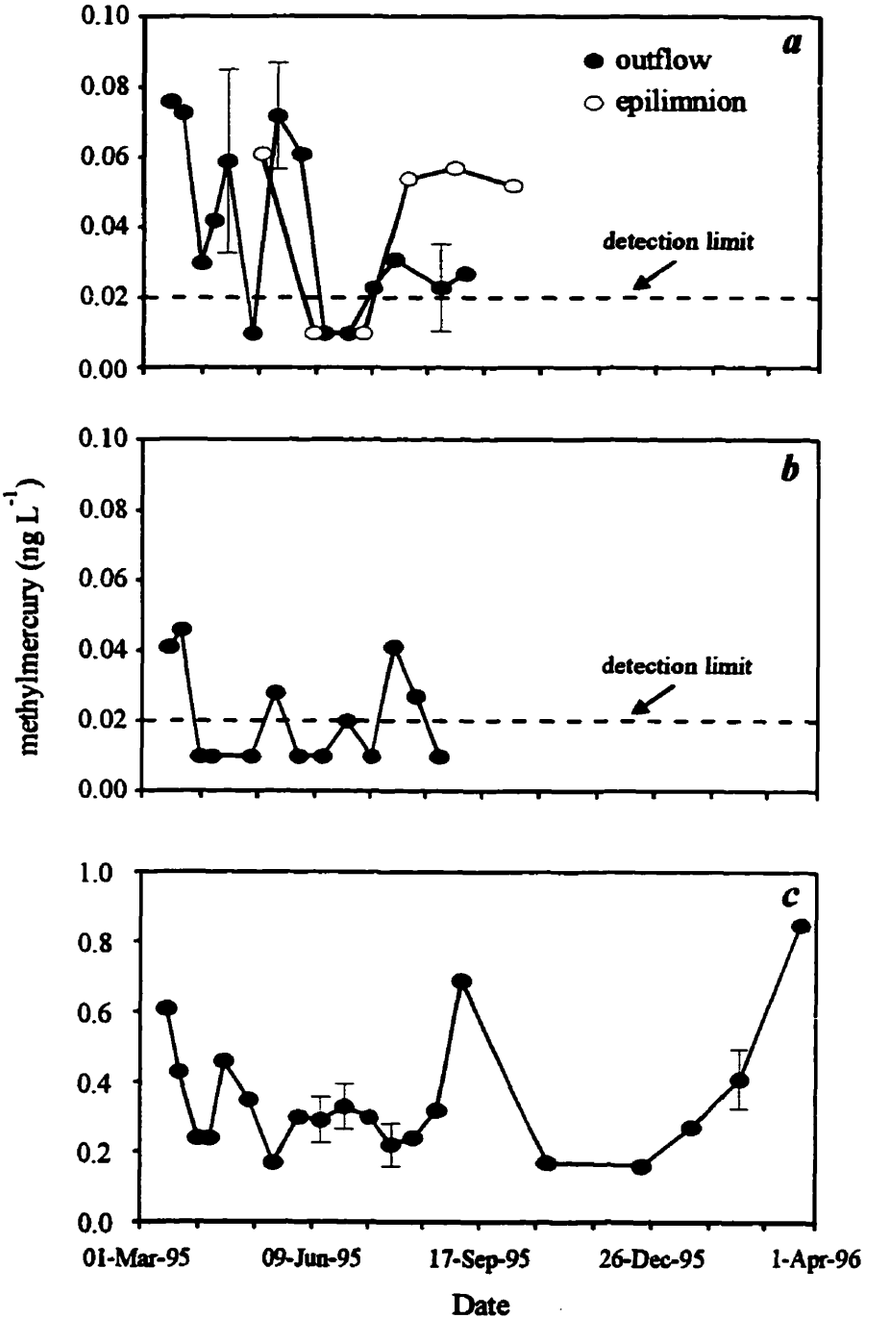


Fig. 2. Methylmercury concentration in whole water samples of a) Lake 240 outflow and epilimnion, b) Lake 239 outflow and c) Lake 470 outflow. Symbols represent the average concentration of duplicate analyses. Range bars between single analyses are shown if single concentrations deviate from the average concentration by more than 15%. Water stopped flowing out of Lake 239 and Lake 240 in late summer 1995, and did not begin again until April 1996 (after the study period ended).

methylmercury concentration was discernible for Lake 239 or Lake 470 (Fig. 2 b and c). The surface water concentrations of Lake 240 taken from the centre of the lake ranged from <0.02 to 0.061 ng L^{-1} and were lowest during the month of June (Fig. 2a).

Mass of methylmercury photodegraded during ice-free season. Forty-one photodegradation bottle incubation experiments were conducted between May 25 and October 16, 1995, and the rates measured ranged from 2 to 27% methylmercury photodegraded per day (Fig. 3). These rates were linearly related to the amount of PAR (<1 to 37 E m^{-2} d^{-1}) to which the bottles were exposed (Fig. 3). This relationship between photodegradation rate and rate of PAR (Fig. 3) was used to determine average photodegradation rates in the top 1 m of Lake 240 at *in situ* concentrations (equation 3) for time periods of 1 to 2 weeks. These *in situ* rates, estimated for each of the 14 periods during the ice-free season, ranged from 1.2 to 9.6 pg L^{-1} d^{-1} (Table 3) and averaged 3.6 pg L^{-1} d^{-1}. The total amount estimated to be photodegraded from May 1 to November 8, 1995, was 290 mg (Table 3).

Mass of methylmercury entering and leaving Lake 240. The mass balance calculations showed that Lake 240 received 78% of its externally supplied methylmercury from Lake 470, another 11 % from the atmosphere, 7% from Lake 239, and 4% from direct DRO (Table 4). The largest loss of methylmercury from Lake 240 resulted from photodegradation (92%), while only 8% left the lake via

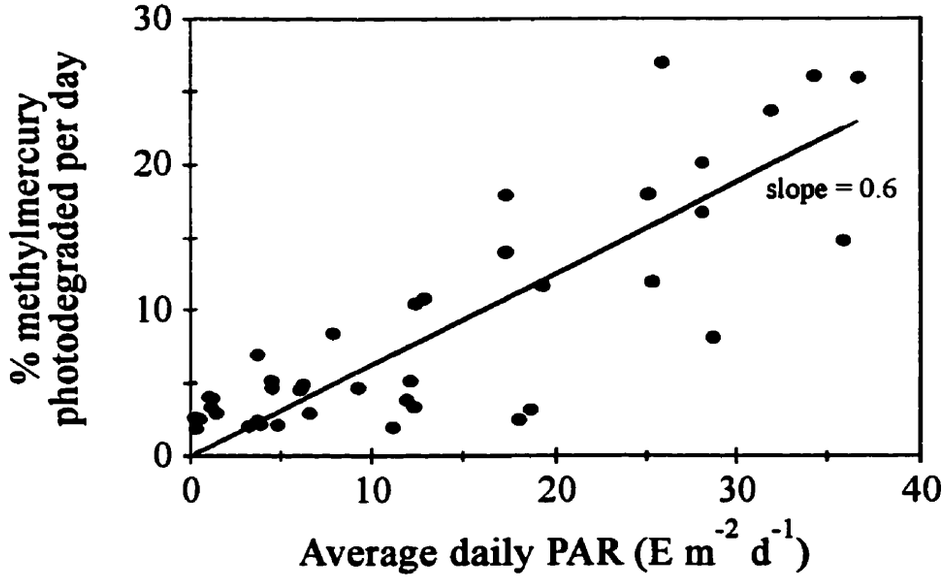


Fig. 3. The relationship between photosynthetically active radiation (PAR) and methylmercury photodegradation rate. The linear correlation ($r^2 = 0.6$) is significant at the 5% level. The y-intercept of the regression line was forced through zero. .

Table 3. Inputs for the calculation of methylmercury photodegraded in the first meter of Lake 240 during the ice-free period (May 1 to November 8) of 1995.

Period (month/day)	Incident PAR ($E\ m^{-2}\ d^{-1}$)	Depth (1m) integrated PAR ($E\ m^{-2}\ d^{-1}$)	Methylmercury concentration ($ng\ L^{-1}$)	Depth (1 m) integrated photodegradation rate ($pg\ L^{-1}\ d^{-1}$)	Mass of methylmercury photodegraded (mg)
5/1 to 5/8	27.1	17.6	0.061	6.4	22
5/9 to 5/23	27.4	17.8	0.049	5.2	34
5/24 to 6/8	38.3	26.1	0.023	3.6	25
6/9 to 6/23	47.3	30.6	0.010	1.8	12
6/24 to 7/7	21.7	14.7	0.010	0.9	5
7/8 to 7/21	27.1	18.8	0.021	2.4	14
7/22 to 8/3	33.0	22.6	0.043	5.8	32
8/4 to 8/17	31.6	21.8	0.055	7.2	43
8/18 to 8/31	25.4	17.1	0.057	5.9	35
9/1 to 9/17	22.6	10.4	0.056	3.5	25
9/18 to 10/4	13.5	7.0	0.054	2.3	16
10/5 to 10/18	11.3	6.9	0.054	2.2	13
10/19 to 11/1	7.0	4.2	0.054	1.4	8
11/2 to 11/8	6.8	3.7	0.054	1.2	3
					Σ 290

Table 4. Water and methylmercury (MeHg) inputs to/losses from Lake 240 for a one year period (March 1995 to March 1996).

	drainage area[1,2] (ha)	water discharge[2] (m^3)	% of total water input	average MeHg[3] (ng L^{-1})	import or export of MeHg (mg)	% of measured MeHg inputs or losses
Lake 239	393	326 454	38	0.021	+ 6	7
Lake 470	168	205 831	24	0.350	+ 63	78
direct run-off	118	108 770	13	0.030	+ 3	4
wet deposition	--	211 592	25	0.045	+ 9	11
outflow	--	553 141	--	0.037	- 24	- 8
photodegradation	--	--	--	--	- 290	- 92
Net internal production estimate of MeHg = Σ outputs - Σ inputs = 310 - 81 = 230 mg						

[1] total drainage area of the Lake 240 watershed is 723 ha.

[2] K. Beaty and M. Lyng, unpublished data

[3] ranges given in Table 2.

the outflow (Table 4). The only source of methylmercury not measured was net internal methylmercury production. This was estimated by mass balance (equation 6) to be 230 mg (Table 4). Applying this value to the surface area of the whole lake sediment gave a net sediment flux of 1.4 ng m^{-2} d^{-1} (Table 5).

Hypolimnetic methylmercury accumulation. Lake 240 was thermally stratified by June 8, at which time the depth of the top of the hypolimnion was 7.5 m. This depth increased as the season progressed, and on Aug 31, was 9 m. During of stratification, concentrations of methylmercury ranged from <0.02 to 0.057 ng L^{-1} in the epilimnion and from 0.024 to 0.96 ng L^{-1} in the hypolimnion (Fig. 4a). Hypolimnetic concentrations increased over time (Fig. 4a). With the exception of one profile (Oct 4), the methylmercury concentration increased with increasing depth in the hypolimnion (Fig. 4a). Although particulate concentration was not measured, I noted that the hypolimnetic samples that had the highest concentration of methylmercury (Fig. 4a) also had the highest concentration of green-pigmented particles.

Typically, dissolved sulfate and oxygen decreased in the hypolimnion with time (Fig. 4b,c), showing the opposite trend to that of methylmercury. Dissolved oxygen was still detectable in the deep water on the last sampling date (Fig. 4b).

Sediment methylmercury flux rates were calculated from the hypolimnetic concentration profiles for the period from June 8 to August 31. The total mass of methylmercury in the hypolimnion (> 8 m) was 9.8 mg on June 8 and 70.1 on

Table 5. Estimates of sediment areal fluxes of methylmercury. Whole lake fluxes were estimated from the output-input mass balance estimate (Table 4) of net internal production (230 mg). The flux of methylmercury from the sediment-water interface of the hypolimnion was estimated from the methylmercury concentration profiles (Fig. 4a) and lake bathymetry.

Sediment surface	Method of estimation	Period of estimation	Estimated methylmercury flux ($ng\ m^{-2}\ d^{-1}$)
whole-lake sediments	mass balance	1 yr	1.4[Φ]
hypolimnetic sediments	hypolimnetic profiles during stratification[*]	84 days[θ]	5.7[⊗]

[*] Total methylmercury accumulated in hypolimnion during stratification = 61 mg

[θ] Calculated from June 8 to Aug 31.

[⊗] Monthly values are 0.6, 7.8, and 11.1 for June 8 to July 7, July 7 to Aug 3, and Aug 3 to Aug 31, respectivly. This estimate includes "new" and "recycled" methylmercury.

[Φ] whole-lake flux is the net flux of "new" methylmercury expressed per square meter of sediment.

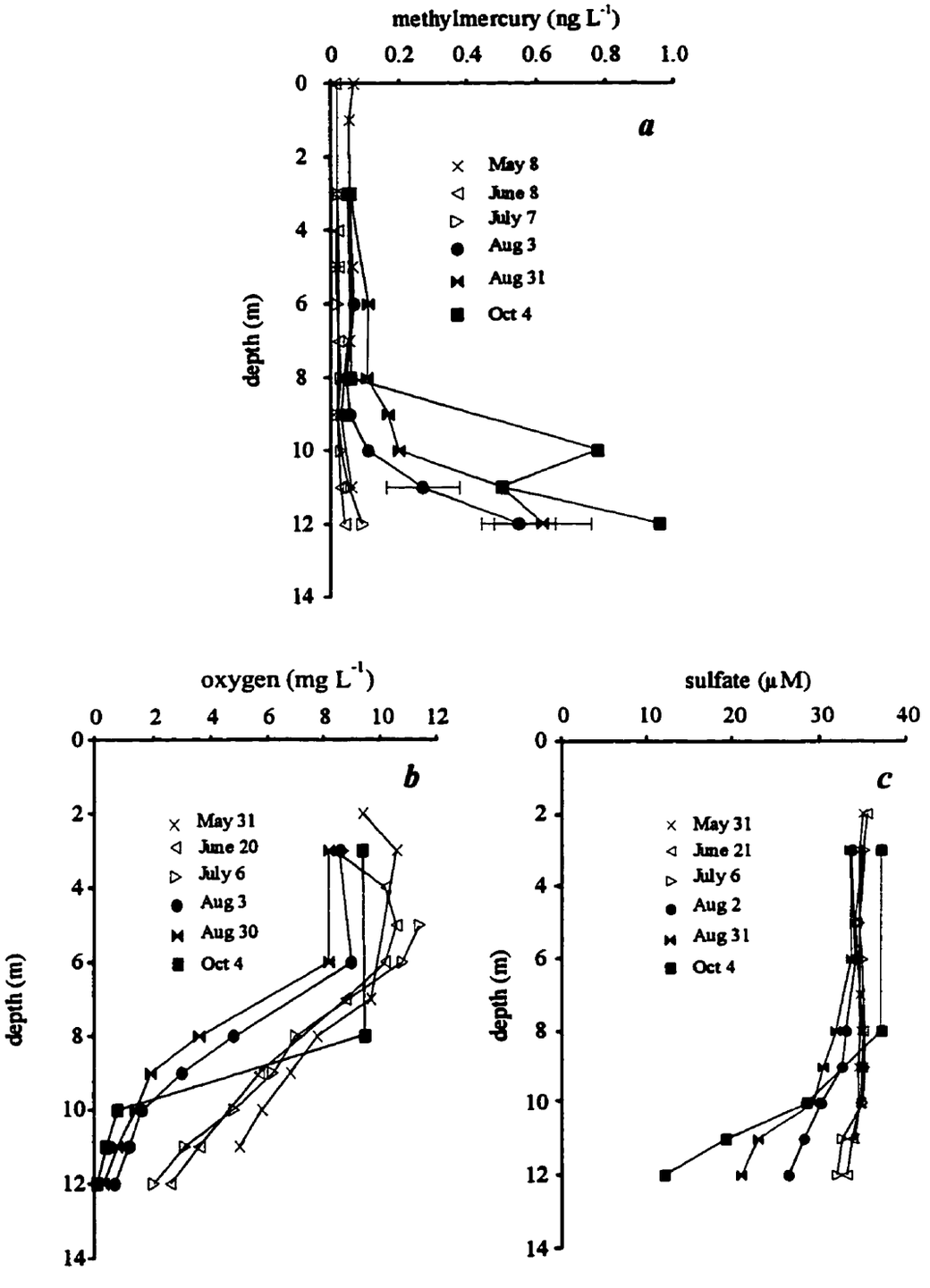


Fig. 4. Methylmercury (*a*), oxygen (*b*), and sulfate (*c*) concentrations in whole water samples in Lake 240, 1995. Symbols represent the average concentration of duplicate analyses in *a* and single anlyses in *b* and *c*. Range bars between single anlalyses in *a* are shown if the single concentrations deviate from the average concentration by more than 15%.

August 31. The total amount that accumulated in the hypolimnion between these two dates was therefore the difference of these masses (60.3 mg), from which a hypolimnetic sediment areal flux rate was calculated to be 5.7 ng m^{-2} d^{-1} (Table 5).

DISCUSSION

The inputs to Lake 240 from the outflows of Lakes 470 and Lake 239 were quite different from each other in their methylmercury contribution to Lake 240 (Table 3). The relatively greater importance of the Lake 470 input compared to Lake 239 was expected (Sellers *et al.*, 1996, appendix A). The association of methylmercury with DOC (e.g. Driscoll *et al.*, 1994; Watras *et al.*, 1995d) and the higher concentration of DOC in Lake 470 than in Lake 239 (Table 1) suggested that Lake 470 would contribute more methylmercury to Lake 240. The relationship between methylmercury export and wetlands (St. Louis *et al.*, 1994; 1996; Hurley *et al.*, 1995; Watras *et al.*, 1995d) also suggested that Lake 470 would be more important because unlike Lake 239, Lake 470 is surrounded by wetlands and its water chemistry is greatly influenced by these wetlands. For a study such as this, frequent measurements of methylmercury export from small lakes such as Lake 470 would be more important than from larger clear lakes because smaller lakes respond quickly to rain/drought events within a season, which would increase the variability of their water chemistry, including methylmercury concentrations (Fig. 2).

The mass balance showed that in-lake production was the dominant contributor of methylmercury to Lake 240, exceeding external inputs by a factor of 3 (Table 3). The site of release of this new methylmercury was most likely in the sediments for the following reasons: First, many studies (e.g. Korthals and Winfrey, 1987; Winfrey and Rudd, 1990; Matelainen *et al.*, 1991; Regnell, 1994; Watras and Bloom, 1994; Henry *et al.*, 1995; Verta and Matelainen, 1995) have shown that net methylation is favored under anaerobic conditions. This would restrict the site of methylation in Lake 240 to the anoxic subsurface of epilimnetic and hypolimnetic sediments, because the hypolimnetic water does not become anoxic during stratification. Second, whole-water incubations carried out in the dark (Fig. 1a in appendix A) rarely showed any increase in methylmercury over time, which means that net methylation (if both methylation and demethylation are occuring) is zero in the water column. However, the fact that radiochemical assays of demethylation in the water column show that an enzyme induction period is required (Xun *et al.*, 1987), means that demethylation is most likely not occurring in the water column at *in situ* concentrations. In the absence of demethylation, I would have expected to see an increase in methylmercury in water samples incubated in the dark if methylation was occurring. However, this was not seen (Fig. 1a in appendix A), and together this information suggests that methylation is not occurring in the water column. Third, methylmercury concentration profiles suggest that hypolimnetic sediments are a strong souce of

water colum methylmercury as the ice-free season progresses (Fig. 4a). For these reasons, the diagramatic representation of the methylmercury mass balance in Lake 240 (Fig. 5) places the site of internal methylmercury release in the sediments.

The dominant site of mercury methylation may be the epilimnetic sediments because of the combined effect of subsurface anoxic conditions (which exists in all sediments) and higher temperatures (Ramlal *et al.*, 1993). However, other environmental variables also affect methylation rates, and it is the combined interaction of all variables which will affect where methylation occurs in a lake. These other variables include the supply of mercury available for methylation (Xun *et al.*, 1987), DOC (Miskimmin *et al.*, 1992), pH (e.g. Xun *et al.*, 1987; Gilmour and Henry, 1991), dissolved sulfate (Gilmour *et al*, 1992; Watras *et al.*, 1995a), microbial activity (Regnell, 1994) and carbon availability (Callister and Winfrey, 1986).

The annual estimate of photodegradation of methylmercury for Lake 240 in this study (290 mg) was about half of that estimated in the provisional methylmercury budget (680 mg) conducted by Sellers *et al.* (1996, appendix A). The greater estimate of photodegradation in the provisional budget was because a higher average methylmercury concentration was used (0.07 ng L^{-1} in Sellers *et al*, 1996 (appendix A) compared with 0.01 to 0.06 ng L^{-1} (Fig. 2a) in this budget) and because the relationship between photodegradation rate and PAR was different. In the provisional budget, Sellers *et al.* (1996) used the average methylmercury concentration in 1994, a wetter year in which

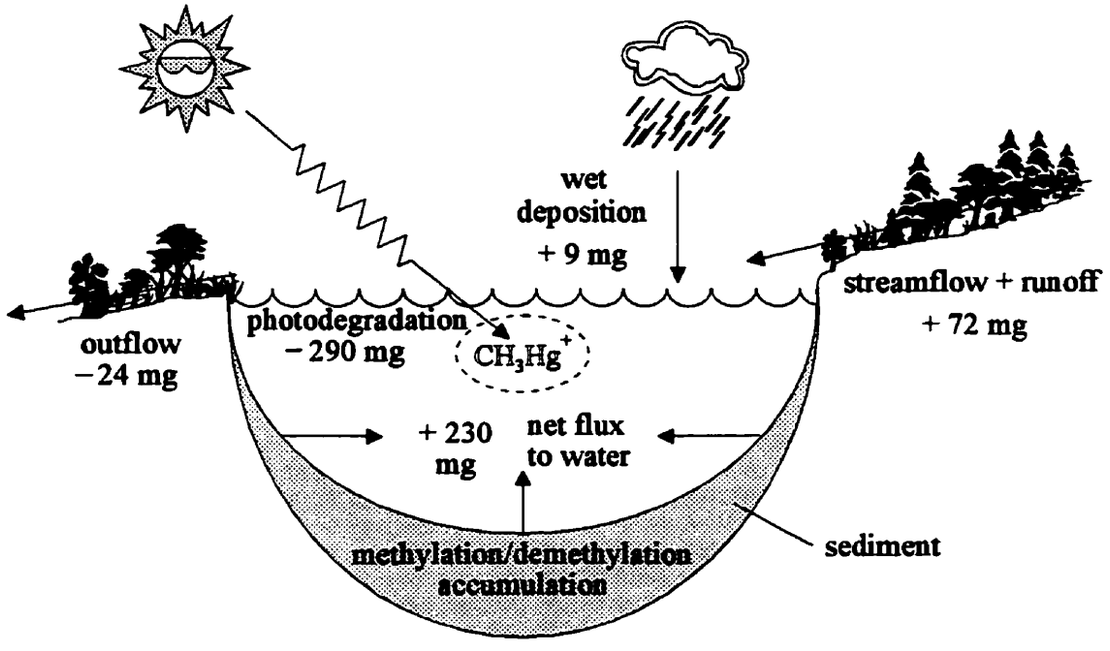


Fig. 5. Diagram of methylmercury mass balance budget for 1 year (March 1995 to March 1996) in Lake 240.

Lake 240 probably received more methylmercury from its wetland watershed (Lake 470). Also, the initial relationship between photodegradation rate and PAR in Sellers *et al.* (1996; appendix A) was developed from four photodegradation rates measured in one depth-profile photodegradation incubation. In this study, this relationship was developed with 41 rate measurements made from several surface and subsurface incubation experiments. In the provisional budget, Sellers *et al.* (1996) used a slope of 0.2 in the calculation of whole lake photodegradation rates. If Sellers *et al.* (1996, appendix A) had used the information provided in this study (i.e. a slope of 0.6 (Fig. 3) and an *in situ* concentration of 0.04 ng L^{-1} (Table 2), they would have calculated that 1170 mg of methylmercury was photodegraded in one year instead of 680 mg. Accordingly, their mass balance estimate of net internal production (equation 7) would have been 940 mg instead of 450 mg. The factor of two difference between these two estimates of internal production emphasizes the importance of a reliable estimate of photodegradation in the mass balance budget. It also suggests that methylation rates in the lakes may be variable from year to year.

Estimates of external inputs of methylmercury and loss via the outflow were much higher (about 5 times for both) in the provisional budget reported for this lake (Sellers *et al.*, 1996; appendix A) than they are in this study. This was primarily because the long-term averages of water flows that were used in the provisional budget were much higher (3 times) than those measured for the specific year of this study, which was a very dry year. It is not uncommon for water flows to differ greatly from one year to another at the

ELA. Thus, the relative importance of both photodegradation and internal production of methylmercury in Lake 240 compared to external inputs from one year to the next will largely be controlled by interannual variability in precipitation as it affects the amount of water, and therefore methylmercury flowing into the lake.

In this budget, Lake 240 was a net sink for methylmercury because less methylmercury left the lake via the outflow than entered the lake via the external inputs (Fig. 5). However, this is a simplified view of in-lake processes. If I had not included photodegradation of methylmercury in the mass balance study described here, I would have calculated net in-lake production to be negative and concluded that the lake itself was not an important contributor of methylmercury. In fact, both the amount of methylmercury photodegraded (290 mg) and the amount of net internal production (230 mg) in Lake 240 were 3 to 26 times greater than any other input or loss term (Fig. 5). This is important to remember in view of the results of previous mass balance studies. Hultberg *et al.*, (1994) concluded that external inputs into a drainage lake in Sweden, where atmospheric deposition of mercury is high, were sufficient to account for all of the methylmercury in fish, suggesting that in-lake production was unimportant. Watras *et al.* (1994) concluded that in-lake methylation and recycling were very important in a seepage lake in northern Wisconsin. Henry *et al.* (1995) found that both external inputs and in-lake production were important contributors to mercury in a polluted lake, and also concluded that recycling was an important process. In all of these studies,

The shape of the concentration profiles of methylmercury during summer stratificaiton (Fig. 4a) strongly suggests that the sediments are the source of hypolimnetic methylmercury in Lake 240. Most of the flux occurred during late stratification (see footnote of Table 4) when particulate flux to the hypolimnion and microbial decomposition at the sediment-water interface was highest (Fig. 4 b,c), and so the 61 mg of methylmercury that accumulated during the 84 days of stratification was probably a large part of the total for the year. While some of this methylmercury was probably a result of recycling from particles rather than new production alone, this 61 mg was an important source of methylmercury to the water column of Lake 240, on the same order as external inputs (Table 3).

It is very likely that the methylmercury that accumulated in the hypolimnion of Lake 240 during stratification (Fig. 4) was distributed over the entire lake depth during fall turnover. If this occurred, the methylmercury that was released from the hypolimnetic sediments would also have been available for photodegradation, particle adsorption, phytoplankton uptake/trophic transfer and loss via the outflow.

Current model for methylmercury production and recycling in Lake 240. The mass balance budget of methylmercury in Lake 240 (Fig. 5) is expanded in Fig. 6 to include hypothetical estimates of the separate contribution of epilimnetic and non-epilimnetic sediments to the release of "new" as well as "recycled" methylmercury. To construct this model, the measured amount of methylmercury released by the hypolimnetic

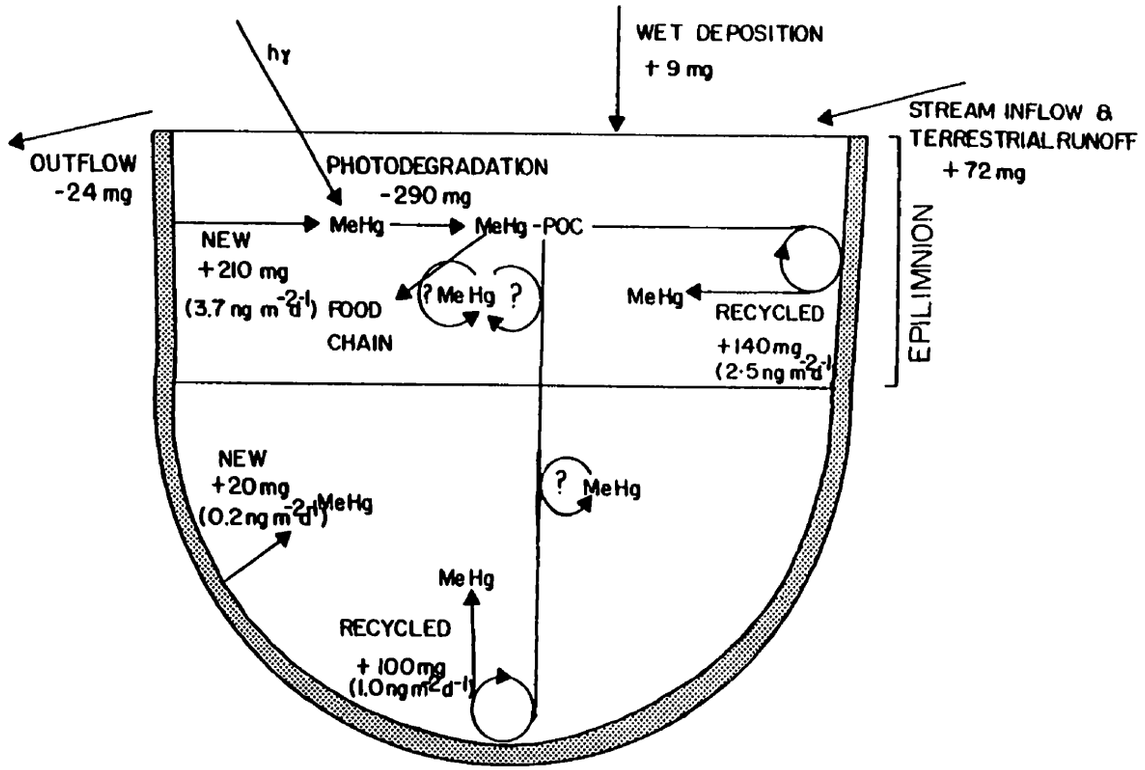


Fig. 6. Hypothetical model for the flux of new and recycled methylmercury at the sediment-water interface in Lake 240. The rates are given on an annual basis. Note that the release of new methylmercury for the whole-lake is 230 mg (Fig. 5).

sediments during stratification (61 mg; Table 4) and the amount of whole-lake net internal production (230 mg) estimated from the mass balance budget (Fig. 5), which must be new methylmercury, was used.

The first step in constructing this model was to divide the lake into two strata. This was done so that eplimnetic sediments can be distinguished from non-epilimnetic sediments (Fig. 6), based on the assumption that methylmercury release rates from metalimnetic and hypolimnetic sediments were similar. This assumption was considered valid because during late stratification (Aug 3 to Aug 31) areal release rates of methylmercury (calculated from the concentration profiles (Fig. 4a)) were similar for metalimnetic and hypolimnetic sediments (7.2 and 8.6. ng m^{-2} d^{-1}, respectively).

The first execution of the model estimated the total annual amount of methylmercury released from non-epilimnetic sediments (Fig. 6). The mass of methylmercury that was released from the hypolimnetic sediments (61 mg; Table 4) during stratification was probably the result of both new production and recycling of methylmercury from sedimented particles. The amount released during stratification was assumed to be equivalent to the annual amount, and the resulting calculated flux rate (1.2 ng m^{-2} d^{-1} over 14.3×10^4 m^2) was applied to the area of the metalimnetic sediments (14.2×10^4 m^2) to arrive at total annual mass flux for sediments below 4 m of 120 mg. This is considered to be the total amount (i.e. new plus recycled) released in this lower stratum of the Lake 240 (Fig. 6).

Ramlal *et al.* (1993), working in boreal lakes very close and similar to the ELA lakes, showed that epilimnetic methylation rates in a lake similar to Lake 240 were about 20 times faster per unit area in epilimnetic than in hypolimnetic sediments. This ratio was applied to the whole lake estimate of new methylmercury production in Lake 240 (230 mg; Fig. 5) to calculate the proportion of this new methylmercury that was released from the (epilimnetic) sediments above 4 m (area = 15.6×10^4 m^2) and the sediments below 4 m (28.5×10^4 m^2). The annual mass flux of new methylmercury from the epilimnetic sediments was thus estimated to be 210 mg at a rate of 3.7 ng m^{-2} d^{-1} (Fig. 6), and annual mass flux of new methylmercury from the sediments below 4 m was calculated to be 20 mg at a rate of 0.2 ng m^{-2} d^{-1} (Fig. 6). This estimate of new production from sediments below 4m was much smaller than the estimate of total sediment release of methylmercury (120 mg), and the difference is expected to come from recycling (100 mg at a rate of 1.0 ng m^{-2} d^{-1}; Fig. 6).

In order to estimate the amount of methylmercury release from epilimnetic sediment that is due to recycling, it was assumed that release from particles during decomposition is proportional to total decomposition of organic carbon. In a lake similar to Lake 240, Ramlal *et al.* (1993) found that decomposition rates in epilimnetic sediments were about 2.5 times higher than in hypolimnetic sediments. To calculate the amount of recycled methylmercury at the sediment-water interface of the epilimnion in this model (Fig. 6), it was assumed that is also true for Lake 240, and that the rate of methylmercury recycling at the sediment-water interface is directly proportional to sediment respiration

rates. By this approach, and again using the non-eplimnetic sediment recycling rate of 1.0 ng m^{-2} d^{-1} (Fig. 6), the annual mass of methylmercury recycled at the eplimnetic sediment surface was estimated to be 140 mg at a rate of 2.5 ng m^{-2} d^{-1} (Fig. 6). All the estimated sediment flux rates (Fig. 6) are within the range measured in previous studies that used intact sediment cores to measure the methylmercury flux from the sediment surface to the overyling water (Henry *et al.*, 1995; chapter 3).

This model predicts that the ratio of recycled methylmercury release to new methylmercury production were very different for epilimnetic and non-eplimnetic sediments. For epilimnetic sediments, this was 0.7 : 1, whereas for non-epilimnetic sediments, this was 5 : 1. (Fig. 6). If the ratio for non-epilimnetic sediments (5 : 1) is applied to the measured hypolimnetic flux rate (presumably from both new and recycled methylmercury) in Lake 240 (5.7 ng m^{-2} d^{-1}; Table 5), then the rate at which new methylmercury was released from hypolimnion during stratification was 1.1 ng m^{-2} d^{-1}. This agrees very well with the areal rate estimated for new methylmercury for the whole-lake by mass balance (1.4 ng m^{-2} d^{-1}). This agreement supports the mass blance approach for estimation of new methylmercury production, and suggests that the whole-lake estimate for Lake 240 (230 mg; Fig. 5) is reliable.

For the whole lake, the model shows that the ratio of recycled to new production was about 1 : 1. This is different from Henry *et al.* (1995), who estimated sediment recycling to be about 40% of new production in a eutrophic,

urban lake. However, it should be noted that the ratio of recycled to new methylmercury in this model (Fig. 6) are influenced by the choice of relative rates of mercury methylation and respiration for epi and non-epilimnetic sediments. I chose relative rates (20:1 for methylation and 2.5:1 for respiration for epi and non-epilimnetic sediments) from measurements made by Ramlal *et al.* (1993) in a lake similar to Lake 240. Over the range of their study lakes, Ramal *et al.* (1993) measured methylation rates 20-40 times higher and respiration rates 2-4 times higher in epilimnetic than in hypolimnetic sediments. If I had used these ratios in any combination, the most that the modelled ratio of recycled to new production (1:1; Fig. 6) would have changed is 35%.

Obviously, the release of recycled methylmercury is the most uncertain term in the whole-lake model (Fig. 6). To do a sound estimate of recycling below 4 m that was independent of the first, I used available measurements for sediment decomposition and the percent of methylmercury in particulates. The methylmercury content in POC (0.025 μg per g C; J. Rudd, unpubl. data) was used. If methylmercury is released from sedimenting POC in the same proportion as is carbon (as CO_2), and the rate below 4 meters was 5 280 μmol m^{-2} d^{-1} on an annual basis (based on summer and winter rates measured by Kelly *et al.*, 1984), these particles could contribute methylmercury at a potential rate of 1.6 ng m^{-2} d^{-1} (from recycling) at the non-epilimnetic sediment surface for the whole year. This is an estimate of the maximum potential recycling rate from POC at the surface of non-eplimetic sediments. This is 60% higher than the model estimate (Fig.

6), and means that this recycling estimate is not an impossible one. I would expect the potential rate to be higher than the actual rate because probably not all the methylmercury in POC is released back into the water column—some is likely demethylated and we know that some accumulates in the sediments.

If the same reasoning is applied to the hypolimnion alone (> 8 m; sediment C release rate of 12 500 μmol m^{-2} d^{-1}; Kelly *et al.*, 1984) during the period of stratification (84 days), when new methylation should be at its highest, the estimate of methylmercury recycled is 45 mg. Again, this is a potential maximum from recycling of methylmercury in POC. This amount is smaller than the measured total during stratification (61 mg), which likely includes both new and recycled methylmercury.

The value of the model presented here (Fig. 6) is that it provides the first estimates of the relative contribution of epilimnetic and non-epilimnetic sediment to new and recycled water column methylmercury in a lake where losses due to photodegradation were included in the budget. It also provides estimates to which past and future empirical and model estimates of methylmercury fluxes in lakes can be compared. It should be noted that the model (Fig. 6) does not include an estimate for the amount of methylmercury that is recycled in the water column itself, which is likely to have occurred (Hurley *et al.*, Watras *et al.*, 1994; Henry *et al.*, 1995). Therefore, the model estimate of the amount of methylmercury recycled at the sediment-water interface of Lake 240 (Fig. 6) is likely an underestimate of the total amount of methylmercury recycled in the lake in

one year. Water column recycling is not easily measured because of rapid turnover rates from particle sorption-desorption reactions and consumption.

While a model such as this (Fig. 6) may assist in developing the current understanding of methylmercury cycling in lakes, the major importance of the study presented in this paper lies in the net mass balance budget (Fig. 5), which shows the importance of internal processes in affecting the methylmercury concentration in Lake 240. The estimates made in this study were from many more measurements of photodegradation than in the provisional budget for this lake (Sellers *et al.*, 1996, appendix A), and therefore are more reliable. This study has also shown the effect of year to year variability in external run-off. Both studies identified photodegradation and in-lake production as important components of the methylmercury cycle in this lake.

Chapter 6

Summary and Conclusions

Anoxic subsurface sediments are expected to be an important source of methylmercury in many lakes because methylmercury production is mainly an anoxic process (Korthals and Winfrey, 1987; Winfrey and Rudd, 1990). However, the contribution of sediments to the methylmercury pool in the water column of lakes remains poorly understood because the appropriate techniques for *in situ* measurements of flux of methylmercury from the sediments have not been developed. Quantifying the methylmercury flux from sediments would enable one to assess the relative importance of in-lake (sediment) and external sources of lake methylmercury.

For the research component of my doctoral thesis, my objective was to determine whole-lake, net sediment flux of methylmercury using two different methods. My first approach was to incubate intact sediment cores and to measure the change in mass of methylmercury in the water above the cores over time. In this approach, the intent was to use the results of these core incubations to estimate a whole lake flux by extrapolation. My second approach was to conduct a whole-lake methylmercury mass balance. In this latter approach, the contribution of the sediments is estimated by the difference between other losses and inputs of methylmercury to the lake. During the initial work on this whole-lake mass balance, while investigating net methylmercury changes with time in sediments and water, I discovered a previously unknown process in the methylmercury cycle. The following is a summary of the main findings and conclusions of my research.

Sediment-water methylmercury fluxes are very variable and whole lake fluxes cannot be accurately predicted from sediment core incubations. Sediment-core incubations showed that sediment-water fluxes of methylmercury were very variable in space and time, and therefore unsuitable for extrapolation to whole lake or to long term scales. Measured fluxes varied over short time periods both among cores within a set and between sets of cores incubated under the same conditions. The measured fluxes in cores taken from Lake 979 ranged from -48 to 181 ng m^{-2} d^{-1}. The direction of flux did not necessarily depend on incubation conditions (i.e. aerobic vs. anaerobic), but the highest fluxes were observed when cores were incubated anaerobically. This variability was real, and is likely because of small-scale variability in microbial activities and mercury availability at the sediment-water interface. In a nearby lake (Lake 240), the average flux of methylmercury from sediments was estimated to be 2-5 ng m^{-2} d^{-1} based on a mass balance approach. The wide range of core results suggests that such an average value does not give a full picture of fluxes that may occur in certain times and sites, and vice versa. Because of the variability, it is not recommended that the sediment core incubation method be used for estimates of whole-lake methylmercury fluxes from sediments.

Methylmercury is photodegraded in lakes. I discovered that methylmercury was photodegraded abiotically by sunlight in the surface waters of lakes. Prior to this discovery, it was believed that methylmercury is destroyed by biological demethylation alone, which has not been demonstrated to occur at the low mercury concentrations found in most lake waters. The fact that photodegradation occurs at low, *in situ* concentrations,

means that it likely occurs in all lakes, and may be the only mechanism by which methylmercury is destroyed in the water column of unpolluted lakes.

Methylmercury photodegradation rates can be easily predicted. Methylmercury photodegradation rates were positively affected by solar irradiance and methylmercury concentration, and were the same in sterile as in non-sterile water samples. Rates were not affected by major differences in water chemistry. The wavelengths of sunlight that seemed to be the most effective in causing methylmercury photodegradation were those in the UV (280-320 nm) region. However, these wavelengths were not solely responsible, and rates were linearly related to PAR, which is a common limnological measurement. Because photodegradation appears to be affected by only a few environmental parameters, it is likely that this process can be included easily in mathematical models that predict methylmercury concentrations in water and fish.

Because methylmercury photodegradation is affected by water clarity but not by water chemistry, the amount of color in lakes is an important consideration when predicting whether or not significant photodegradation is occurring within a lake. This is because sunlight penetrates to shallower depths in colored lakes compared to clear lakes, and so the water column of colored lakes would have less of the solar energy required for photodgradation to occur. Because color is a surrogate for light extinction, and because color is often correlated with DOC, DOC concentration (which is a common limnological measurement) would be also be a good indicator of the extent of photodegradation. Photodegradation would be more important in clear lakes than in brown, high DOC lakes, and in part, explains why clear lakes have lower methylmercury concentration than dark

lakes. It should also be remembered that lakes with higher DOC often receive drainage from wetlands which are high external contributors of methylmercury (St. Louis *et al.*, 1994, 1996).

A chemical mass balance is a better way to estimate methylmercury flux than is extrapolation from sediment core incubations. The mass balance approach to estimate net sediment flux of methylmercury was more reliable than the sediment-core incubation method, and showed that both internal and external sources of methylmercury to the lake were important. In the mass balance approach, however, one has to be sure to quantify all important input and loss terms. An estimate of methylmercury loss by photodegradation must be included in mass balance studies of lakes, especially in those of clear-water lakes. If not included, the relative importance of other input and loss terms cannot be assessed accurately.

The relative importance of external inputs, internal production, and photodegradation is likely to vary from year to year. I showed that in Lake 240, a remote clear-water drainage lake, photodegradation and internal production were more important than external inputs in regulating methylmercury concentration. However, the relative importance of external inputs to Lake 240 varied in different years because of differences in precipitation. Thus, in dry years, external inputs are likely unimportant but in very wet years, even in clear water lakes, external inputs can be important sources of methylmercury to the lake ecosystem. During wetter years, increased photodegradation because of higher methylmercury concentration in lakes (due to increased external input) would be offset by decreased solar irradiance. The variability of internal production from

year to year has not yet been assessed, and will require multiple years of mass balance measurements. It is expected that this rate might change in concert with organic input to the sediments, both autochthonous and allochthonous.

Photodegradation reduces the amount of methylmercury available for bioaccumulation. If photodegradation did not occur, then the methylmercury concentration in the water column of lakes would be much higher. Because zooplankton concentrations are in equilibrium with water concentrations (M. Paterson, Freshwater Institute, Winnipeg, pers. comm.), the methylmercury in concentration in fish would be higher if photodegradation did not occur.

The knowledge that methylmercury is photodegraded by sunlight has practical applications. Methylmercury photodegradation should be a consideration when sites for hydroelectric reservoir construction are being determined. For example, reservoirs that flood peat soils have high concentrations of colored DOC, which impedes light penetration and photodegradation. This inhibition of photodegradation would augment the problem of high methylmercury levels in fish from reservoirs. Further, methylmercury pollution by direct discharge of industrial effluent could be alleviated by passing waste water through holding ponds before final discharge. These holding ponds should be shallow to maximize the potential for methylmercury photodegradation by solar irradiance.

Concluding remarks

Our perception of the mercury cycle in lakes has been fundamentally changed as a result of the research presented here. I have identified photodegradation as a loss

mechanism of methylmercury in lakes, and as a key link in the methylmercury cycle of clear water lakes. It was previously believed that biological demethylation dominated methylmercury destruction in lakes, but now it seems that abiotic photodegradation may be equally or more important in lake water because biological demethylation has still not been detected at the low mercury concentration found in most lakes. Further, inclusion of photodegradation in a methylmercury mass balance study allowed me to quantify in-lake net production of methylmercury and to demonstrate that this process can be as important in supplying methylmercury to lakes as external inputs. This means that external inputs of methylmercury cannot be the sole consideration when attempts are made to understand and to regulate methylmercury levels in lakes; internal processes (photodegradation and internal production) must also be considered.

REFERENCES

Akagi, H., O. Malm, F.J.P. Branches, Y. Kinjo, Y. Kashima, J.R.D. Guimaraes, R.B. Oliveira, K. Haraguchi, W.C. Pfeiffer, Y. Takizawa and H. Kato. 1995. Human exposure to mercury due to goldmining in the Tapojos River basin, Amazon, Brazil: Speciation of mercury in human hair, blood and urine. **Water Air Soil Pollut.** 80: 85-94.

Alberts, J.J., J.E. Schindler, R.W. Miller, and D.E. Nutter. 1974. Elemental mercury evolution mediated by humic acid. **Science** 184: 895-897.

Allard, B. and I. Arsenie. 1991. Abiotic reduction of mercury by humic substances in aquatic systems - an important process for the mercury cycle. **Water Air Soil Pollut.** 56: 457-464.

Amyot, M., G. Mierle, D.R.S. Lean, G.A. Gill, and D.J. McQueen. 1996. Modelling the effect of light on volatile mercury production in lakes. IN R. Ebinghaus, G. Petersen, and U. von Tumpling. Fourth International Conference on Mercury as a Global Pollutant; 1996 Aug 4-8; Hamburg. Hamburg: Bundesminister fur Bildung, Wissenschaft. p. 130.

Amyot, M., G. Mierle, D.R.S. Lean, and D.J. McQueen. 1994. Sunlight-induced formation of dissolved gaseous mercury in lake waters. **Environ. Sci. Technol.** 28: 2366-2371.

Beaty, K.G., and M.E. Lyng. 1989. Hydrometeorological data for the Experimental Lakes Area, northwestern Ontario, 1982 to 1987. **Can. Data Rep. Fish. Aquat. Sci.** 759: v + 280 p.

Becker, D.S. and G.N. Bigham. 1995. Distribution of mercury in the aquatic food web of Onandaga Lake, New York. **Water Air Soil Pollut.** 80: 563-571.

Berman, M. and R. Bartha. 1984. Levels of chemical versus biological methylation of mercury in sediments. Bull. **Environ. Contam. Toxicol.** 36: 401-404.

Berman, M., T. Chase Jr., and R. Bartha. 1990. Carbon flow in mercury biomethylation by *Desulfovibrio desulfuricans*. **Appl. Environ. Microbiol.** 56: 298-300.

Bloom, N. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. **Can. J. Fish. Aquat. Sci.** 46: 1131-1140.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. **Can. J. Fish. Aquat. Sci.** 49: 1010-1017.

Bloom, N. and W.F. Fitzgerald. 1988. Determination of volatile mercury species at the picogram level by low-temperature gas chromatography with cold-vapour atomic fluorescence detection. **Anal. Chim. Acta.** 208: 151-161.

Blunden, S.J. and A.H. Chapman. 1982. The environmental degradation of organotin compounds - a review. **Environ. Technol. Lett.** 3: 267-272.

Bodaly, R.A., J.W.M. Rudd, R.J.P. Fudge and C.A. Kelly. 1993. Mercury concentrations in fish related to size of remote Canadian Shield lakes. **Can J. Fish. Aquat. Sci.** 50: 980-987.

Branfireun, B.A., A. Heyes, and N.T. Roulet. 1996. The hydrology and methylmercury dynamics of a Precambrian Shield headwater peatland. **Water Resour. Res.** 32: 1785-1794.

Brunskill, G.J., and D.W. Schindler. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. **J. Fish. Res. Bd. Canada** 28: 139-155.

Callister, S.M. and M.R. Winfrey. 1986. Microbial methylation of mercury in Upper Wisconsin sediments. **Water Air Soil Pollut.** 29: 453-465.

Choi, S.-C., and R. Bartha. 1993. Cobalamin-mediated mercury methylation by Desulfovibrio desulfuricans LS. **Appl. Environ. Microbiol.** 59: 290-295.

Choi, S-C., T. Chase Jr., and R. Bartha. 1994. Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS. **Appl. Environ. Microbiol.** 60: 1342-1346.

Chu, P., and D.B. Porcella. 1995. Mercury stack emissions from U.S. electric utility power plants. **Water Air Soil Pollut.** 80: 135-144.

Clarkson, T.W. 1990. Human health risks from methylmercury in fish. **Environ. Toxicol. Chem.** 9: 957-961.

Clarkson, T.W. 1992. Mercury: Major issues in environmental health. **Environ. Health Perspect.** 100: 31-38.

Clarkson, T.W. 1994. The toxicology of mercury and its compounds. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution Integration and Synthesis.* Ann Arbour: Lewis. p. 631-641.

Compeau, G. and R. Bartha. 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. **Appl. Environ. Microbiol.** 48: 1203-1207.

Compeau, G.C. and R. Bartha. 1985. Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediments. **Appl. Environ. Microbiol.** 50: 498-502.

Compeau, G.C. and R. Bartha. 1987. Effect of salinity on mercury-methylating activity of sulfate-reducing bacteria in estuarine sediments. **Appl. Environ. Microbiol.** 53: 261-265.

Cooper, W.J., C. Shao, D.R.S. Lean, A. S. Gordon, and F.E. Scully. 1994. Factors affection the distribution of H_2O_2 in surface waters. IN: L.A. Baker (ed). *Environmental Chemistry of Lakes and Reservoirs*. ACS Symp. Ser. 237: 391-422.

Cooper, W.J., R.G. Zika, R.G. Petasne, and A.M. Fischer. 1989. Sunlight-induced photochemistry of humic substances in natural waters: Major reactive species. IN: P. MacCarthy and I.H. Suffet (eds). *Aquatic humic substances: Influence on fate and treatment of pollutants*. ACS Symp. Ser. 219: 333-362.

Cotner, J.B. and R.T. Heath. 1990. Iron redox effects on photosensitive phosphorus release from dissolved humic matters. **Limnol. Oceanogr.** 35: 1175-1181.

Craig, P.J., and P.A. Moreton. 1985. The role of speciation in mercury methylation in sediments and water. **Environ. Pollut. Ser. B** 10: 141-158.

Driscoll, C.T., C. Yan, C.L. Schofield, R. Munson, and J. Holsapple. 1994. The mercury cycle and fish in the Adirondack lakes. **Environ. Sci. Technol.** 28: 136A-143A.

Fagerstrom, T. and A. Jernelov. 1972. Some aspects of the quantitative ecology of mercury. **Water Res.** 6: 1193-1202.

Fitzgerald, W.F., R.P. Mason, and G.M. Vandal. 1991. Atmospheric cycling and air-water exchange of mercury over mid-continental lacustrine regions. **Water Air Soil Pollut.** 56: 745-767.

Fitzgerald, W.F., R.P. Mason, G.M. Vandal, and F. Dulac. 1994. Air-water cycling of mercury in lakes. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution Integration and Synthesis*. Ann Arbour: Lewis p. 203-220.

Fleming, L.E., S. Watkins, R. Kaderman, B. Levin, D.R. Ayyar, M. Bizzio, D. Stephens, and J.A. Bean. 1995. Mercury exposure in humans through food consumption from the Everglades in Florida. **Water Air Soil Pollut.** 80: 41-48.

Foster, T.J. 1987. The genetics and biochemistry of mercury resistance. **Crit. Rev. Microbiol.** 15: 117-140.

Francko, D.A. 1990. Alteration of bioavailability and toxicity by phototransformation of organic acids. IN: E.M. Perdue and E.T. Gjessing (eds). *Organic Acids in Aquatic Ecosystems*. Dahlem: John Wiley and Sons. p. 167-177.

Furutani, A. and J.W.M. Rudd. 1980. Measurement of mercury methylation in lake water and sediment samples. **Appl. Environ. Microbiol.** 40: 770-776.

Gadd, G.M. 1993. Microbial formation and transformation of organometallic and organometalloid compounds. **FEMS Microbiol. Rev.** 11: 297-316.

Gagnon, C., E. Pelletier, A. Mucci, and W.F. Fitzgerald. 1996. Diagenetic behavior of methylmercury in organic-rich coastal sediments. **Limnol. Oceanogr.** 41: 428-434.

Gilmour, C.C. and E.A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. **Environ. Pollut.** 71: 131-169.

Gilmour, C.C., E.A. Henry, and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. **Environ. Sci. Technol.** 26: 2281-2287.

Gilmour, C.C. and G.S. Riedel. 1995. Measurement of Hg methylation of sediments using high specific-activity ^{203}Hg and ambient incubation. **Water Air Soil Pollut.** 80: 747- 756.

Grieb, T.M., C.T. Driscoll, S.P. Gloss, C.L. Schofield, G.L. Bowie, and D.B. Porcella. 1990. Factors affecting mercury accumulation in fish in the Upper Michigan Peninsula. **Environ. Toxicol. Chem.** 9: 919-930.

Haines, T.A., V.T. Komov, and C.H. Jagoe. 1994. Mercury concentration in perch (*Perca fluviatilis*) as influenced by lacustrine physical and chemical factors in two regions of Russia. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution Integration and Synthesis*. Ann Arbour: Lewis. p.397-407

Hamasaki, T., H. Nagase, Y. Yoshioka, and T. Sato. 1995. Formation, distribution, and ecotoxicology of methylmetals of tin, mercury, and arsenic in the environment. **Crit. Rev. Environ. Sci. Technol.** 25: 45-91.

Harada, M. 1995. Minamata Disease: Methylmercury poisoning in Japan caused by environmental pollution. **Critic. Rev. Toxicol.** 25: 1-24.

Hecky, R.E., D.J. Ramsay, R.A. Bodaly, and N.E. Strange. 1991. Increased methylmercury contamination in fish in newly formed freshwater reservoirs. IN

T.Suzuki *et al* (eds). *Advances in Mercury Toxicology*. New York: Plenum Press p. 33-52.

Henry, E.A., L.J. Dodge-Murphy, G.N. Bigham, S.M. Klein, and C.C. Gilmour. 1995. Total mercury and methylmercury mass balance in an alkaline, hypereutrophic urban lake (Onandaga Lake, NY). **Water Air Soil Pollut**. 80: 509-518.

Heyes, A., T.R. Moore, J.W.M. Rudd, and C.A. Kelly. 1996. Sulfate stimulation of methylmercury production in incubated peat? IN R. Ebinghaus, G. Petersen, and U. von Tumpling. Fourth International Conference on Mercury as a Global Pollutant; 1996 Aug 4-8; Hamburg. Hamburg: Bundesminister fur Bildung, Wissenschaft. p. 173

Hintelmann, H. and R.D. Evans. 1996. Simultaneous determination of mercury methylation and methylmercury demethylation rates in sediments by GC-ICP/MS. IN R. Ebinghaus, G. Petersen, and U. von Tumpling. Fourth International Conference on Mercury as a Global Pollutant; 1996 Aug 4-8; Hamburg. Hamburg: Bundesminister fur Bildung, Wissenschaft. p. 42.

Hintelmann, H., P.M. Welbourn, and R.D. Evans. 1995. Binding of methylmercury compounds by humic and fulvic acids. **Water Air Soil Pollut**. 80: 1031-1034.

Hoigne, J., B.C. Faust, W.R. Haag, F.E. Scully, and R.G. Zepp. 1989. Aquatic humic substances as sources and sinks of photochemically produced transient reactants. IN: P. MacCarthy and I.H. Suffet (eds). *Aquatic humic substances: Influence on fate and treatment of pollutants*. ACS Symp. Ser. 219: 363-380.

Horvat, M., L. Liang, and N.S. Bloom. 1993a. Comparison of distillation with other current isolation methods for the determination of methylmercury compounds in low level environmental samples. Part I. Sediments. **Anal. Chim. Acta** 282: 135-152.

Horvat, M., L. Liang, and N.S. Bloom. 1993b. Comparison of distillation with other current isolation methods for the determination of methylmercury compounds in low environmental samples. Part II, Water. **Anal. Chim. Acta**. 282: 153-168.

Hudson, R.J.M., S.A. Gherini, C.J. Watras, and D.B. Porcella. 1994. Modeling the biogeochemical cycle of mercury in lakes. The mercury cycle model (MCM) and its application to the MTL study lakes. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution: Integration and Synthesis*. Ann Arbour: Lewis. p. 473-523.

Hultberg, H, A. Iverfeldt, and Y-H Lee. 1994. Methylmercury input/output and accumulation in forested catchments and critical loads for lakes in southwestern Sweden. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution: Integration and Synthesis*. Ann Arbour: Lewis. p. 137-152.

Hurley, J.P., J.M. Benoit, C.L. Babiarz, M.M. Shafer, A.W. Andren, J.R. Sullivan, R. Hammond, and D.A. Webb. 1995. Influences of watershed characteristics on mercury levels in Wisconsin Rivers. **Environ. Sci. Technol.** 29: 1867-1875.

Hurley, J.P., D.P. Krabbenhoft, C.L. Babiarz, and A.W. Andren. 1994. Cycling of mercury across the sediment-water interface in seepage lakes. IN: L.A. Baker (ed). *Environmental Chemistry of Lakes and Reservoirs*. ACS Symp. Ser. 237: 425-449.

Hurley, J.P., C.J. Watras, and N.S. Bloom. 1994. Distribution and flux of particulate mercury in four stratified seepage lakes. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution: Integration and Synthesis*. Ann Arbour: Lewis. p. 69-82.

Imura, N., E. Sukegawa, S-K. Pan, K. Nagao, J-Y. Kim, T. Kwan, and T. Ukita. 1971. Chemical methylation of inorganic mercury with methylcobalamin, a vitamin B_{12} analog. **Science** 172: 1248-1249.

Inoko, M. 1981. Studies on the photochemical decomposition of organomercurials-methylmercury(II) chloride. **Environ. Pollut.** B. 2: 3-10.

Jackson, T.A., G. Kipphut, R.H. Hesslein, and D.W. Schindler. 1980. Experimental study of trace metal chemistry in soft-water lakes at different pH levels. **Can. J. Fish. Aquat. Sci.** 37: 387-402.

Jacobs, L.A, S.M. Klein, and E.A. Henry. 1995. Mercury cycling in the water column of a seasonally anoxic urban lake (Onandaga Lake, NY). **Water Air Soil Pollut.** 80: 553-562.

Johnson, W.E. and J.R. Vallentyne. 1971. Rationale, background, and development of experimental lake studies in northwestern Ontario. **J. Fish. Res. Bd. Canada** 28: 123-128.

Kelly, C.A. and J.W.M. Rudd. 1984. Epilimnetic sulfate reduction and its relationship to lake acidification. **Biogeochemistry** 1: 63-77.

Kelly, C.A., J.W.M. Rudd, and D.W. Schindler. 1988. Carbon and electric flow via methanogenesis, SO_4^{-2}, NO_3^-, Fe^{3+}, and Mn^{4+} reduction in the anoxic hypolimnia of three lakes. **Arch. Hydrobiol. Beih.** 31: 333-344.

Kelly, C.A., J.W.M. Rudd, A. Furutani, and D. W. Schindler. 1984. Effects of lake acidification on rates of organic matter decomposition in sediments. **Limnol. Oceanogr.** 29: 687-694.

Korthals, E.T. and M.R. Winfrey. 1987. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. **Appl. Environ. Microbiol.** 53: 2397-2404.

Kouassi, A.M. and R.D. Zika. 1992. Light-induced destruction of the absorbance property of dissolved organic matter in seawater. **Toxicol. Environ. Chem.** 35: 195-211.

Krabbenhoft, D.P. and C.L. Babiarz. 1992. The role of groundwater transport in aquatic mercury cycling. **Water Resour. Res.** 20: 3119-3128.

Krautler, B., H.-P. E. Kohler, and E. Stupperich. 1988. 5'-Methylbenzimidazolyl-cobamides are the corrinoids from some sulfate-reducing and sulfur-metabolizing bacteria. **Eur. J. Biochem.** 176: 461-469.

Lee, Y-H., and H. Hultberg. 1990. Methylmercury in some Swedish surface waters. **Environ. Toxicol. Chem.** 9: 833-841.

Lee, Y-H., and A. Iverfeldt. 1991. Measurement of methylmercury and mercury in run-off, lake, and rain waters. **Water Air Soil Pollut.** 56: 309-321.

Lindberg, S.E., T.P. Meyers, and J. Munthe. 1996. Micrometeorological studies of air/surface exchange of mercury vapor over Lake Gardsjon, Sweden and in the Florida Everglades. IN: R. Ebinghaus, G. Petersen, and U. von Tumpling. Fourth International Conference on Mercury as a Global Pollutant; 1996 Aug 4-8; Hamburg. Hamburg: Bundesminister fur Bildung, Wissenschaft. p. 132

Lindqvist, O. 1985. Atmospheric mercury - a review. **Tellus** 37B: 136-159.

Mason, R.P. and W.F. Fitzgerald. 1991. Mercury speciation in open ocean waters. **Water Air Soil Pollut.** 56: 779-789.

Mason, R.P., J.R. Reinfelder, and F.M.M. Morel. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. **Environ. Sci. Technol.** 30: 1835-1845.

Matilainen, T., M. Verta, M. Niemi, and A. Uusi-Rauva. 1991. Specific rates of net methylmercury production in lake sediments. **Water Air Soil Pollut.** 56: 595-605.

Matilainen, T. 1995. Involvement of bacteria in methylmercury formation in anaerobic lake waters. **Water Air Soil Pollut.** 80: 757-764.

Matilainen, T. and M. Verta. 1995. Mercury methylation and demethylation in aerobic surface waters. **Can. J. Fish. Aquat. Sci.** 52: 1597-1608.

Meichel, G., R. Maurice, C. Kelly and R. Sparling. 1996. Molybdate inhibition of sulphate reducing bacteria grown without sulphate and its implications for understanding mercury methylation results in low sulphate environments. IN: R. Ebinghaus, G. Petersen, and U. von Tumpling. Fourth International Conference on Mercury as a Global Pollutant; 1996 Aug 4-8;Hamburg. Hamburg: Bundesminister fur Bildung, Wissenschaft. p. 413.

Miskimmin, B.M. 1991. Effect of natural levels of dissolved organic carbon (DOC) on methyl mercury formation and sediment-water partitioning. **Bull. Environ. Contam. Toxicol.** 47: 743-750.

Miskimmin, B.M., J.W.M. Rudd, and C.A. Kelly. 1992. Influence of dissolved organic carbon, pH, and microbial respiration rates on mercury methylation and demethylation in lake water. **Can. J. Fish. Aquat. Sci.** 49: 17-22.

Moss, B. 1980. *Ecology of Fresh Waters*. New York: Halsted. 332 p.

Mucci, A., M. Lucotte, S. Montgomery, Y. Plourde, P. Pichet, and H. Van Tra. 1995. Mercury remobilization from flooded soils in a hydroelectric reservoir of northern Quebec, La Grande-2: results of a soil resuspension experiment. **Can. J. Fish. Aquat. Sci.** 52: 2507-2517.

Munthe, J. and W.J. McElroy. 1992. Some aqueous reactions of potential importance in the atmospheric chemistry of mercury. **Atmos. Environ.** 26A: 553-557.

Nagase, H., Y. Ose, T. Sato, and T. Ishikawa. 1982. **Sci. Total Environ.** 24: 133-142.

Nakamura, K., M. Sakamoto, and H. Uchiyama and O. Yagi. 1990. Oganomercurial-volatilizing bacteria in the mercury-polluted sediment of Minamata Bay, Japan. **Appl. Environ. Microbiol.** 56: 304-305.

Navio, J.A., F.J. Marchena, C. Cerrillos, and F. Pablos. 1993. UV photolytic degradation of butyltin chlorides in water. **J. Photochem. Photobiol. A: Chem.** 71: 97-102

Nriagu, J.O. 1994. Mercury pollution from the past mining of gold and silver in the Americas. **Sci. Total Environ.** 149: 167-181.

Ollis, D.F., E. Pelizzetti, and N. Serpone. 1991. Photocatalyzed destruction of water contaminants. **Environ. Sci. Technol.** 25: 1523-1529.

Olson, B.H. and R.C. Cooper. 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. **Water Res.** 10: 113-116.

Oremland R.S., C.W. Culbertson, and M.R. Winfrey. 1991. Methylmercury decomposition in sediments and bacterial cultures: Involvement of methanogens and sulfate reducers in oxidative demethylation. **Appl. Environ. Microbiol.** 57: 130-137.

Oremland, R.S., L.G. Miller, P. Dowdle, T. Connell, and T. Barkay. 1995. Methylmercury oxidative degradation potentials in contaminated and pristine sediments of the Carson River, Nevada. **Appl. Environ. Microbiol.** 61: 2745-2753.

Parkman, H., P. Ostlund, M-O Samuelsson, and A. Iverfeldt. 1994. Methylmercury in a permanently stratified Fiord. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution: Integration and Synthesis.* Ann Arbour: Lewis. p. 107-118.

Pfeiffer, W.C., L.D. Lacerda, W. Salomons, and O. Malmi. 1993. Environmental fate of mercury from gold mining in the Brazilian Amazon. **Environ. Rev.** 1: 26-37.

Pirrone, N., G.J. Keeler, and J.O. Nriagu. 1996. Regional differences in worldwide emissions of mercury to the atmosphere. **Atmos. Environ.** 30: 2981-2987.

Ramlal, P.S., C.A. Kelly, J.W.M. Rudd, and A. Furutani. 1993. Sites of methyl mercury production in remote Canadian Shield lakes. **Can. J. Fish. Aquat. Sci.** 50: 972-979.

Ramlal, P.S., J.W.M. Rudd, A. Furutani, and L. Xun. 1985. The effect of pH on methyl mercury production and decomposition in lake sediments. **Can. J. Fish. Aquat. Sci.** 42: 685-692.

Ramlal, P.S., J.W.M. Rudd, and R.E. Hecky. 1986. Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. **Appl Environ. Microbiol**. 51: 110-114.

Rasmussen, J.B., L. Godbout, and M. Schallenberg. 1989. The humic content of lake water and relationship to water shed and lake morphometry. **Limnol. Oceanogr**. 34: 1336-1343.

Regnell, O. 1994. The effect of pH and dissolved oxygen levels on methylation and partitioning of mercury in freshwater model systems. **Environ. Poll**. 94: 7-13.

Regnell, O., and A. Tunlid. 1991. Laboratory study of chemical speciation of mercury in lake sediment and water under aerobic and anaerobic conditions. **Appl. Environ. Microbiol**. 57: 789-795.

Ridley, W.P., L.J. Dizikes, and J.M. Wood. 1977. Biomethylation of toxic elements in the Environment. **Science** 197: 329-332.

Robinson, J.B., and O.H. Tuovinen. 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: Physiological, biochemical, and genetic analyses. **Microbiol. Rev**. 48: 95-124.

Rudd, J.W.M. 1995. Sources of methylmercury to freshwater ecosystems: A review. **Water Air Soil Pollut**. 80: 697-713.

Rudd, J.W.M., C.A. Kelly, D.W. Schindler, and M.A. Turner. 1990. A comparison of the acidification efficiencies of nitric and sulfuric acis by two whole-lake addition experiments. **Limnol. Oceanogr**. 35: 663-679.

Rudd, J.W.M., C.A. Kelly, V.L. St. Louis, R.H. Hesslein, A. Furutani, and M.H. Holoka. 1986. Microbial consumption of nitric and sulfuric acids in acidified north temperate lakes. **Limnol. Oceanogr.** 31: 1267-1280.

Scheider, W.A., D.S. Jeffries, and P.J. Dillon. 1979. Effects of acidic precipitation on Precambrian freshwaters in southern Ontario. **J. Great Lakes Res.** 5: 45-51.

Schindler, D.W., R.H. Hesslein, R. Wagemann, and W.S. Broecker. 1980. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. **Can. J. Fish. Aquat. Sci.** 37: 373-377.

Schroeder, W., O. Lindqvist, J. Munthe, and Z. Xiao. 1992. Volatilization of mercury from lake surfaces. **Sci. Total Environ.** 125: 47-66.

Scully, N.M. and D.R.S. Lean. 1994. The attenuation of ultraviolet radiation in temperate lakes. **Arch. Hydrobiol. Beih.** 43: 135-144.

Scully, N.M., D.M. McQueen, and D.R.S. Lean. 1996. Hydrogen peroxide formation: The interaction of ultraviolet radiation and dissolved organic carbon in lake waters along a 43-75^{o}N gradient. **Limnol. Oceanogr.** 41: 540-548.

Selifonova, O., R. Burlage, and T. Barkay. 1993. Bioluminescent sensors for detection of bioavailable Hg(II) in the environment. **Appl. Environ. Microbiol.** 59: 3083-3090.

Sellers, P., R.H. Hesslein, and C.A. Kelly. 1995. Continuous measurement of CO_2 for estimation of air-water fluxes in lakes: An in situ technique. **Limnol. Oceanogr.** 40: 575-581.

Sellers, P., C.A. Kelly, J.W.M. Rudd, and A.R. MacHutchon. 1996. Photodegradation of methylmercury in lakes. **Nature** 380: 694-697.

Sellers, P., C.A. Kelly, J.W.M. Rudd, and V. St. Louis. 1994. Are aquatic sediments sites of net methylation or demethylaion? Abstract in IN: The Third International Conference on Mercury as a Global Pollutant. Whistler. British Columbia.

Silver, S. and M. Walderhaug. 1992. Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. **Microbiol. Rev.** 56: 195-228.

Slemr, F. and E. Langer. 1992. Increase in global atmospheric concentrations of mercury inferred from measurements over the Atlantic Ocean. **Nature** 355: 434-436.

Slemr, F., W. Junkermann, R.W.H. Schmidt, and R.Sladkovic. 1995. Indication of change in global and regional trand of atmospheric mercury concentrations. **Geophys. Res. Lett.** 22: 2143-2146.

Slemr, F., W. Seiler, and G. Schuster. 1985. Distribution, speciation, and budget of atmospheric mercury. **J. Atmos. Chem.** 3: 407-434.

Sorenson, J.A., G.E.Glass, K.W.Schmidt, J.K. Huber, and G.R. Rapp, Jr. 1990. Airborne mercury depostion and watershed characteristics in relation to mercury concentrations in water, sediments, plankton, and fish of eighty Northern Minnesota Lakes. **Environ. Sci. Technol.** 24: 1716-1727.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, N.S. Bloom, and R.J. Flett. 1994. Importance of wetlands as sources of methylmercury to boreal forest ecosystems. **Can. J. Fish. Aquat. Sci.** 51: 1065-1076.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, R.J. Flett, and N.T. Roulet. 1996. **Production and loss of methylmercury and loss of total mercury from boreal forest catchments containing different types of wetlands. Environ. Sci. Technol.** 30: 2719-2729.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. The chemical analyses of fresh water, 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25.

Steffan, R.J., E.T. Korthals, and M.R. Winfrey. 1988. Effects of acidification on mercury methylation, demethylation, and volatilization in sediments from an acid-susceptible lake. Appl. Environ. Microbiol. 54: 2003-2009.

Stein, E.D., Y. Cohen and A.M. Winer. 1996. Environmental distribution and transformation of mercury compounds. Crit. Rev. Environ. Sci. Technol. 26: 1-43.

Stordal, M.C. and G.A. Gill. 1995. Determination of mercury methylation rates using a ^{203}-Hg radiotracer technique. Water Air Soil Pollut. 80: 725-734.

Stumm, W. 1992. *Chemistry of the solid-water interface: Processes at the mineral-water and particle-water interface in natural systems.* New Yor: John Wiley and Sons, Inc. 428 p.

Stupperich, E., H.J. Eisinger and B. Krautler. 1988. Diversity of corrinoids in acetogenic bacteria. Eur. J. Biochem. 172: 459-464.

Stupperich, E. and B. Krautler. 1988. Pseudo vitamin B_{12} or 5-hydroxybenzimidazolyl-cobamide are the corrinoids found in methanogenic bacteria. Arch. Microbiol. 149: 268-271.

Suda, I., D. Suda, and K. Hirayama. 1993. Degradation of methyl and ethyl mercury by singlet oxygen generated from sea water exposed to sunlight or ultraviolet light. **Arch. Toxicol.** 67: 365-368.

Swain, E.B., D.R. Engstrom, M.E. Brigham, T.A. Henning, and P.L. Brezonik. 1992. Increasing rates of atmospheric mercury deposition in midcontinental North America. **Science** 237: 257: 784-787.

Tsai, Y-L. and B.H. Olson. 1990. Effects of Hg^{2+}, CH_3Hg^+, and temperature on the expression of mercury resistance genes in environmental bacteria. **Appl. Environ. Microbiol.** 56: 3266-3272.

Vandal, G.M., W.F. Fitzgerald, K.R. Rolfhus and C.H. Lamborg. 1995. Modelling the elemental mercury cycle in Pallette Lake, Wisconsin, USA. **Water Air Soil Pollut.** 80: 529-528.

Vandal, G.M., R.P. Mason and W.F. Fitzgerald. 1991. Cycling of volatile mercury in temperate lakes. **Water Air Soil Pollut.** 56: 791-783.

Verta, M. and T. Matilainen. 1995. Methylmercury distribution and partitioning in stratified Finnish forest lakes. **Water Air Soil Pollut.** 80: 585-588.

Verta, M., T. Matinainen, P. Porvari, M. Niemi, A. Uusi-Rauva, and N.S. Bloom. 1994. Methylmercury sources in boreal lake ecosystems. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution: Integration and Synthesis*. Ann Arbour: Lewis. p. 119-136.

Walsh, C.T., M.D. Distefano, M.J. Moore, L.M. Shewchuk, and G.L. Verdine. 1988. Molecular basis of bacterial resistance to organomercurial and inorganic mercuric salts. **FASEB J** 2: 124-130.

Watras, C.J. and N.S. Bloom. 1994. The vertical distribution of mercury species in Wisconsin lakes: Accumulation in plankton layers. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution: Integration and Synthesis*. Ann Arbour: Lewis. p. 137-152.

Watras, C.J., N.S. Bloom, S.A. Claas, and K.A. Morrison, C.C. Gilmour, and S.R. Craig. 1995a. Methylmercury production in the anoxic hypolimnion of a dimictic seepage lake. **Water Air Soil Pollut**. 80: 735-745.

Watras, C.J., N.S. Bloom, R.J.M. Hudson, S. Gherini, R. Munson, S.A. Claas, K.A. Morrison, J. Hurley, J.G. Wiener, W.F. Fitzgerald, R. Mason, G. Vandal, D. Powell, R. Rada, L. Rislov, M. Winfrey, J. Elder, D. Krabbenhoft, A.W. Andren, C. Babiarz, D.B. Porcella, and J.W. Huckabee. 1994. Sources and fates of mercury and methylmercury in Wisconsin lakes. IN: C.J. Watras and J.W. Huckabee (eds). *Mercury Pollution: Integration and Synthesis*. Ann Arbour: Lewis 153-177.

Watras, C.J., K.A. Morrison, and N.S. Bloom. 1995b. Mercury in remote Rocky Mountain lakes of Glacier National Part, Montana, in comparison with other temperate North American regions. **Can. J. Fish. Aquat. Sci.** 52: 1220-1228.

Watras, C.J., K.A. Morrison, and N.S. Bloom. 1995c. Chemical correlates of Hg and methyl-Hg in northern Wisconsin lakes under ice-cover. **Water Air Soil. Pollut.** 84: 253-267.

Watras, C.J., K.A. Morrison, J.S. Host, and N.S. Bloom. 1995d. Concentration of mercury species in relationship to other site-specific factors in the surface waters of northern Wisconsin Lakes. **Limnol. Oceanogr.** 40: 556-565.

Weber, J.H. 1993. Review of possible paths for abiotic methylation of mercury(II) in the aquatic environment. **Chemosphere** 26: 2063-2077.

Wheatley, B. and S. Paradis. 1995. Exposure of Candadian aboriginal peoples to methylmercury. **Water Air Soil Pollut.** 80: 3-11.

Winfrey, M.R. and J.W.M. Rudd. 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. **Environ. Toxicol. Chem.** 9: 853-869.

Wood, J.M. 1974. Biological cycles for toxic elements in the environment. **Science** 183: 1049-1052.

Wood, J.M., F. Scott Kennedy, and C.G. Rosen. 1968. Synthesis of methylmercury compounds by extracts of a methanogenic bacterium. **Nature** 220: 173-174.

Xun, L., N.E.R. Campbell, and J.W.M. Rudd. 1987. Measurements of specific rates of net methyl mercury production in the water column and surface sediments of acidified and circumneutral lakes. **Can. J. Fish. Aquat. Sci.** 44: 750-757.

Zafiriou, O.C., J. Joussot-Dubien, R.G. Zepp, and R.G. Zika. 1984. Photochemistry of natural waters. **Environ. Sci. Technol.** 18: 358A-371A.

Zepp, R.G., 1988. Environmental photoprocesses involving natural organic matter. IN: F.H. Frimmel and R.F. Christman (eds).*Humic substances and their role in the environment.* New York: John Wiley & Sons. p. 193-214.

Zepp, R.G., G.L. Baughman, N. Wolfe and D.M. Cline. 1974. Methylmercuric complexes in aquatic systems. **Environ. Lett.** 6: 117-127.

Zepp, R.G., N.L. Wolfe, and J.A. Gordon. 1973. Photodecomposition of phenylmercury compounds in sunlight. **Chemosphere** 2: 93-99.

Appendix A

Photodegradation of methylmercury in lakes

Published in *Nature*, volume 380, pp 694-697, 1996

co-authors: C.A. Kelly, J.W.M. Rudd, and A.R. MacHutchon

Methylmercury can accumulate in fish to concentrations that threaten human health[1]. Fish mercury concentrations are high in many reservoirs[2] and acidified lakes[3], and also in many remote lakes[4,5] —a fact that may be related to increased atmospheric deposition of anthropogenically mobilized mercury during the past few decades[6]. Although sources of methylmercury to lakes and reservoirs are known[7], in-lake destruction has not been demonstrated to occur at the low concentrations found in most water bodies. Here we report *in situ* incubations of lake water that show that methylmercury is decomposed by photodegradation in surface waters. This process is abiotic and the rate is first-order with respect to methylmercury concentration and the intensity of solar radiation. In our study lake, the calculated annual rates of methylmercury photodegradation are almost double the estimated external inputs of methylmercury from rain, snow, streamflow and land-runoff, implying the existence of a large source of methylmercury from bottom sediments. Photodegradation could also be an important process in the mercury cycle of other aquatic systems. This discovery fundamentally changes our understanding of aquatic mercury cycling, and challenges the long-accepted view that microbial demethylation dominates methylmercury degradation in natural fresh waters.

Recent development of highly sensitive analytical capabilities[8] have enabled us to examine the stability of methylmercury in lake water at naturally occurring, low concentrations. At the Experimental Lakes Area (ELA) in northwestern Ontario, we

incubated lake water in Teflon bottles (which transmit sunlight[9]) and found that methylmercury concentration decreased in the sunlight but not in the dark (Fig. 1*a*). Filtering lake water with 0.45 μm filters, which removed all photosynthetic organisms and most bacteria, had no effect on the photodegradation rate (Fig. 1*b*). The fact that the reaction occurred in filtered water suggested that the photodegradation was abiotic. To confirm the abiotic nature of the reaction, we conducted an experiment using unfiltered, sterilized lake water and found that sterilization did not inhibit photodegradation (Fig. 1*c*).

The light dependence of the reaction was demonstrated further in an experiment in which one set of bottles was exposed to sunlight, a second set was dark, and a third set was moved from dark to light during the experiment. Loss of methylmercury occurred only during the periods when bottles were exposed to sunlight (Fig. 1*d*).

We also determined that photodegradation rates were first-order with respect to methylmercury concentrations (Fig. 2*a*) and to levels of photosynthetically active radiation (PAR, Fig. 2*b*). The lake water used in all of these experiments (Figs. 1 and 2) had a pH of 6.1-6.3 and a dissolved organic carbon (DOC) concentration of 1,190-1,470 μmol L^{-1}. Several comparisons of photodegradation rates in different ELA lakes spanning a range of water chemistry (e.g., DOC 250-1,210 μmol L^{-1}; pH 6.0-7.5; alkalinity 127-143 μeq L^{-1}) showed no differences in rates due to water chemistry (P.S., C.A.K., J.W.M.R. and A.R.M., unpublished data).

In situ incubations showed that methylmercury photodegradation rates decreased with depth below the lake surface, and corresponded closely to the exponential decrease in

Fig. 1. Methylmercury concentrations in lake water incubated in light and dark (aluminum foil covered) Teflon bottles on the surface of the lake. Each panel represents a separate experiment in which all bottles were incubated under the same light and temperature conditions. The experiments were conducted on different dates. Water treatments: *a*, unfiltered water; *b*, unfiltered water incubated in the light and the dark, and filtered water incubated in the light; *c*, unfiltered, sterilized water; *d*, filtered water in the light and the dark, or transferred from dark to light. In *a* and *c*, there were two sets of bottles incubated for each treatment. The lines represent separate sets, with each data point representing a single analysis from a single bottle incubated. The low initial value for one of the light treatments in *c* is due to analytical error. The data points in *d* represent duplicate analyses for a single bottle at each point in time, and range bars indicate where these duplicate anlyses are larger than the data points.

METHODS: All sampling, manipulation and analyses of water were performed using ultra-clean techniques[8]. Water was incubated with a 50% headspace under the following temperature (mean daily) and light conditions: *a* and *c*, 17.5 C and 23 E m^{-2} d^{-1}; *b*, 16.5 C and 21 E m^{-2} d^{-1}; *d*, 20.0 C and 25 E m^{-2} d^{-1}. For *b* and *d*, the concentration of methylmercury was elevated by addition of methylmercuric chloride stock solution (made

using distilled water). For *c*, water and bottles were sterilized by autoclaving, and were not opened until analyses. Lake water used for these experiments had a pH of 6.14, a dissolved organic carbon concentration of 1,410 μmol L^{-1} and a chloride concentration of 0.4 mg L^{-1}. Methylmercury concentration was determined by distillation extraction, aqueous phase ethylation and atomic fluorescence detection[8]. A control experiment ensured that methylmercury did not adsorb to the teflon bottles: after incubation, acid (9 N H_2SO_4) was added to sample bottles to a final concentration of 0.09 N. No methylmercury was released back into solution (data not shown).

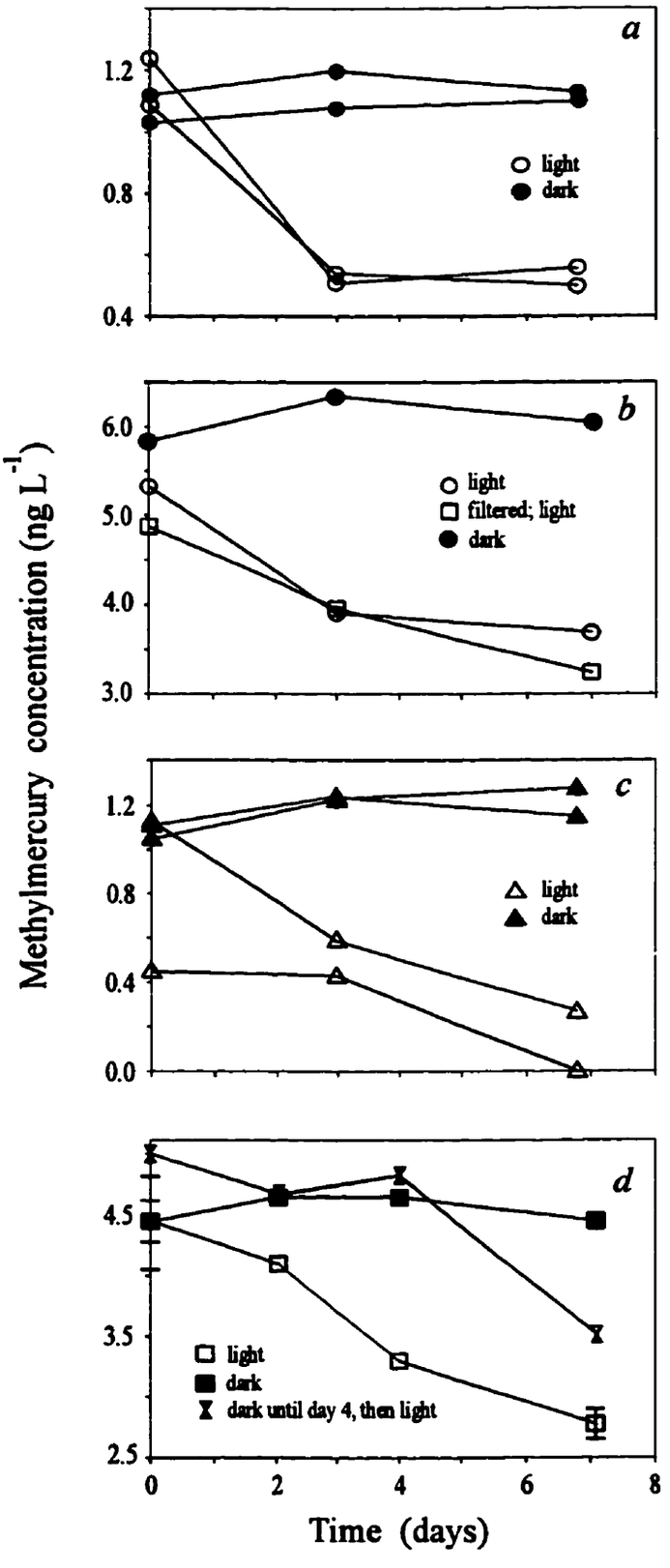
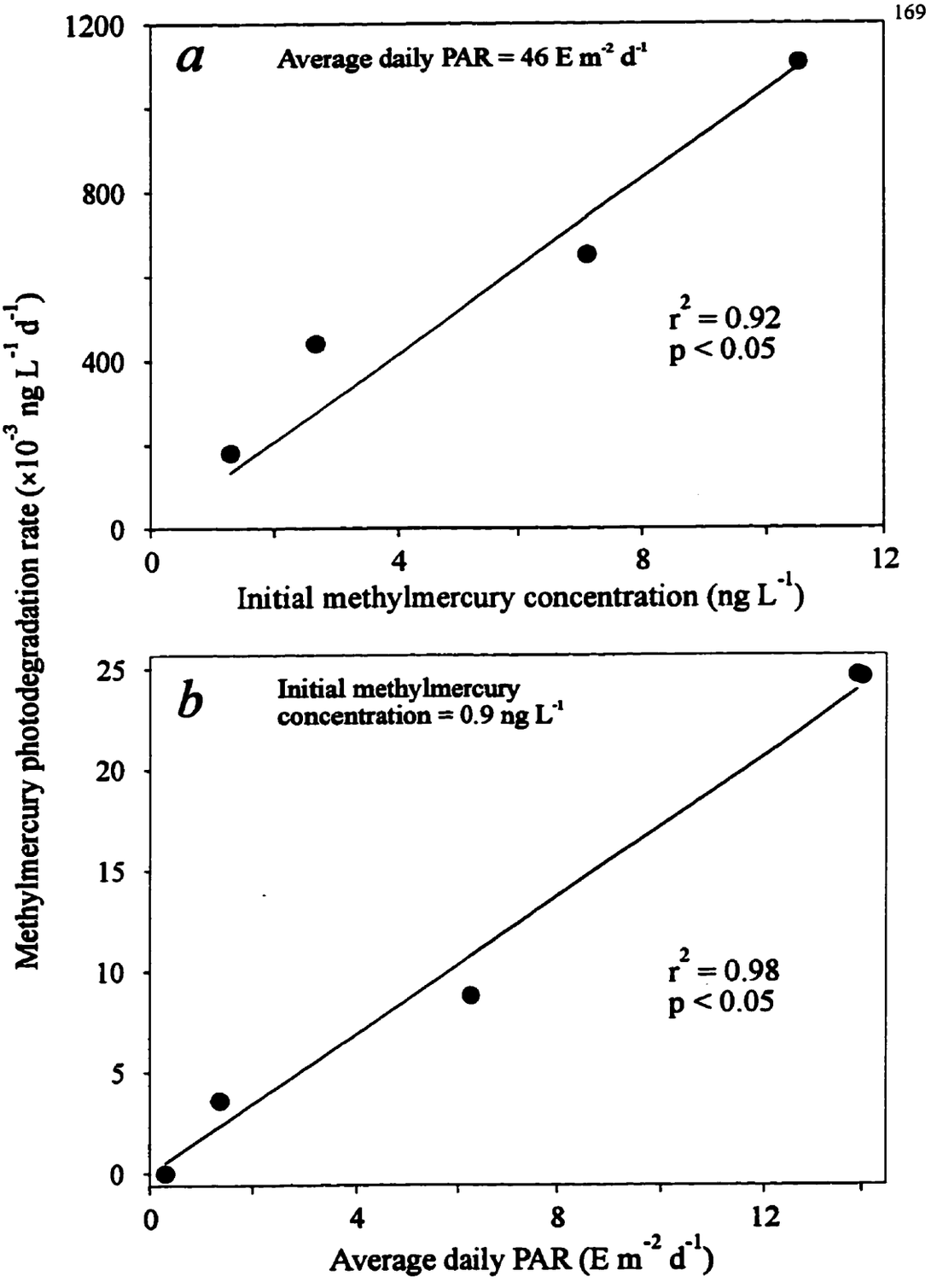

a
1.2
0.8
0.4
light
dark
b
6.0
5.0
4.0
3.0
light
filtered; light
dark
c
1.2
0.8
0.4
0.0
light
dark
d
4.5
3.5
2.5
light
dark
dark until day 4, then light
0
2
4
6
8
Time (days)
Methylmercury concentration (ng L-1)

Fig. 2 *a*. The effect of initial methylmercury concentration on the photodegradation rate (the light level is the same for all incubations). r^2, square of the correlation coefficient; p, level of significance. *b*, The effect of light intensity on methylmercury photodegradation rate. The initial methylmercury concentration is the same in all bottles.

METHODS: *a*, Unfiltered, methylmercury-amended, replicate lake water samples were incubated on the lake surface for 4 days. For each of four methylmercury concentrations, there were three bottles and one bottle was analyzed (in duplicate) on days 0, 2, and 4. Rates given were calculated by linear regression on the three time points. *b*, Bottles containing filtered lake water were incubated at discrete depths to obtain different light levels. The PAR at each depth was calculated using surface radiation, and the average light extinction coefficient. This coefficient was determined with a LiCOR quantum sensor from nine light profiles taken during the incubation period. The surface bottles were incubated for 16 days and all others 21 days because of lower light levels. The lake temperature (°C) range at each incubation depth is as follows: 0 m, 9.2-15.2; 1 m, 9.3-15.3; 3 m, 9.3-15.4; 5 m 9.4-15.4; 9 m 9.5-14.1. Replicate bottles were incubated at each depth, and were collected and analyzed (in duplicate) on three dates. Rates given were calculated from the average concentration on each date by linear regression of the three points in time.


a
Average daily PAR = 46 E m-2 d-1
r2 = 0.92
p < 0.05
1200
800
400
0
0
4
8
12
Initial methylmercury concentration (ng L-1)
Methylmercury photodegradation rate (×10-3 ng L-1 d-1)
b
Initial methylmercury concentration = 0.9 ng L-1
r2 = 0.98
p < 0.05
25
20
15
10
5
0
0
4
8
12
Average daily PAR (E m-2 d-1)

light intensity with depth (Fig. 3). Because we have shown that methylmercury photodegradation is consistent with standard rate laws for photochemical reactions, it should be possible to add this process to mechanistic models of mercury cycling in lakes and reservoirs[10].

The end-products of methylmercury photodegradation in natural waters have not been identified. In laboratory experiments done at high methylmercury concentrations (micrograms per litre), a variety of mercury and carbon species are photo-produced[11]. In natural waters, however, the low concentrations of methylmercury (0.05 to 3.0 ng L^{-1} in ELA lakes and streams) are bound with complexing agents which are also present only in trace quantities[12]. Therefore, the chemistry of the reaction in natural waters may be different than in the laboratory experiments cited.

It is important to understand the difference between the process of photodegradation of methylmercury described here and the process of photoproduction of elemental mercury (Hg^0; ref. 9). Photoproduction of Hg^0 is thought to occur primarily by reduction of inorganic mercury (Hg^{2+}; refs 13, 14), and flux of Hg^0 to the atmosphere is an important mechanism by which mercury is lost from lakes[14]. Photodegradation of methylmercury may or may not produce Hg^0. If it does, the estimated production rate of Hg^0 in our study would be 1.5 $\mu g\ m^{-2}\ yr^{-1}$. This is similar to the flux of Hg^0 to the atmosphere measured in two previous studies of lakes[14,23], but smaller than Hg^0 production rates in another study[9]. Of course, identification of methylmercury photodegrdation end-products is necessary for a realistic interpretation of these rate

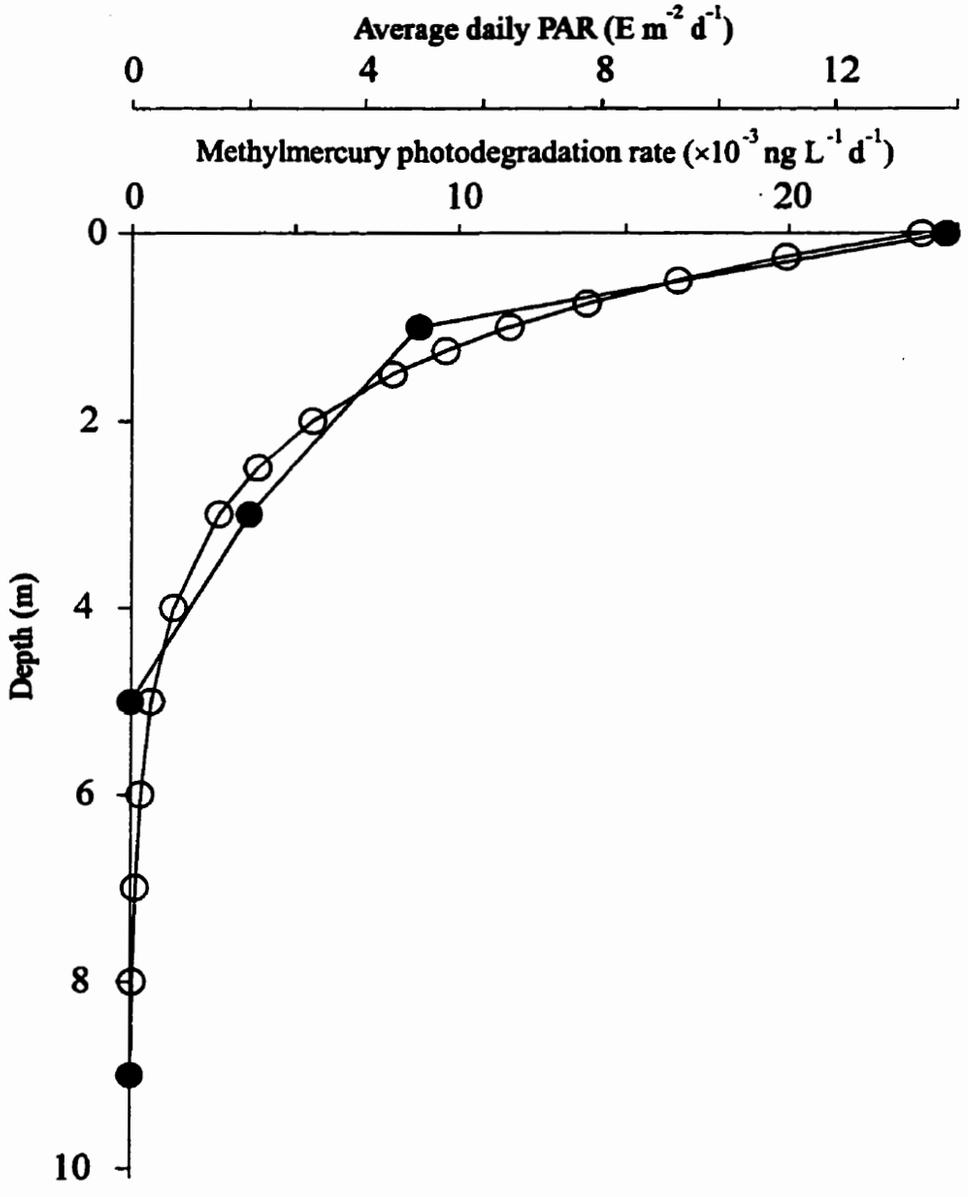


Fig. 3. Depth profiles of photodegradation rate (●) and PAR (○) in Lake 240 (ELA). Same data as in Fig. 2*b*.

comparisons. Even so, the immediate significance of methylmercury photodegradation methylmercury photodegradation is that it decreases the concentration of methylmercury, which is the form of mercury that is most easily accumulated in fish and that is the most toxic to fish consumers.

Two lines of evidence show that abiotic photodegradation is a much more important process in epilimnetic lake water than is biological demethylation, which can occur in the dark. First, our experiments repeatedly showed that methylmercury concentrations in unfiltered water were stable in the dark (for example, Fig. 1*a*), indicating that biological demethylation was either absent or was occurring at rates undetectable with our methods. Second, biological demethylation can be detected in lake water using ^{14}C-methylmercury at very high (1,900 ng L^{-1}) concentrations (incubated in the dark), but the turnover rate measured is about 350 times slower than it is for photodegradation[15]. The data we present here therefore call into question the long-accepted view that biological demethylation is the dominant removal mechanism of methylmercury in lake water. However, biological demethylation may be important in sediments where methylmercury degradation rates measured using ^{14}C-methylmercury in the dark are much higher than in the water column[15,16].

Given that numerous studies of demethylation using ^{14}C-methylmercury have been done in the past[17], one may question why photodegradation has not been observed before. The reason is that in all previous studies the samples were incubated in the dark[17].

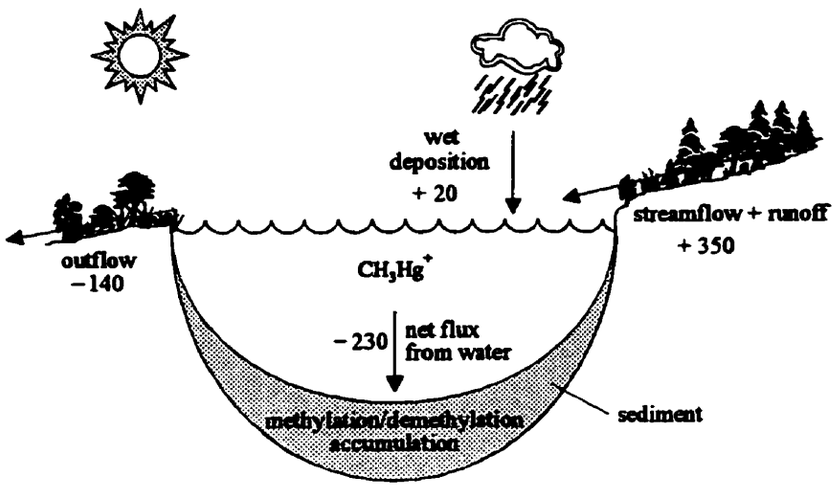
To estimate whether the observed photodegradation rates could have a significant effect on the methylmercury concentration in lakes and reservoirs, we calculated a methylmercury turnover rate for the epilimnion of a typical ELA lake (Lake 240) during summer stratification of 1994. We used the following Lake 240 data: (1) the average epilimnetic methylmercury concentration (0.07 ng L^{-1}), (2) the average incident PAR (35 E m^{-2} d^{-1} [18,19]) and (3) the average extinction coefficient for PAR (0.5 m^{-1}) in the epilimnion (0-4 m depth). We also used the relationships between photodegradation rate and methylmercury concentration (Fig. 2a), and between photodegradation rate and light intensity (Fig. 3). From these data we estimated that the average photodegradation constant in Lake 240 was 0.043 d^{-1} for the ice-free stratified season. This means that the methylmercury in the epilimnion of Lake 240 would be replaced about 7 times during the period of summer stratification.

We made a preliminary comparison of the importance of photodegradation relative to the annual masses of methylmercury flowing into and out of Lake 240 at ELA (Fig. 4). Using previously published[20] and unpublished data, we estimated that the total methylmercury input (direct deposition, streamflow, and runoff) was ≈370 mg yr^{-1} and outflow was ≈140 mg yr^{-1}. Thus, if photodegradation is not included in the methylmercury cycle (Fig. 4*a*), one would conclude that there is a net flux of methylmercury from the water column (net destruction and/or storage). But when photodegradation is included (Fig. 4*b*), there must be an in-lake source of methylmercury of about 450 mg yr^{-1}. Because the entire water column of Lake 240 is oxic, and because

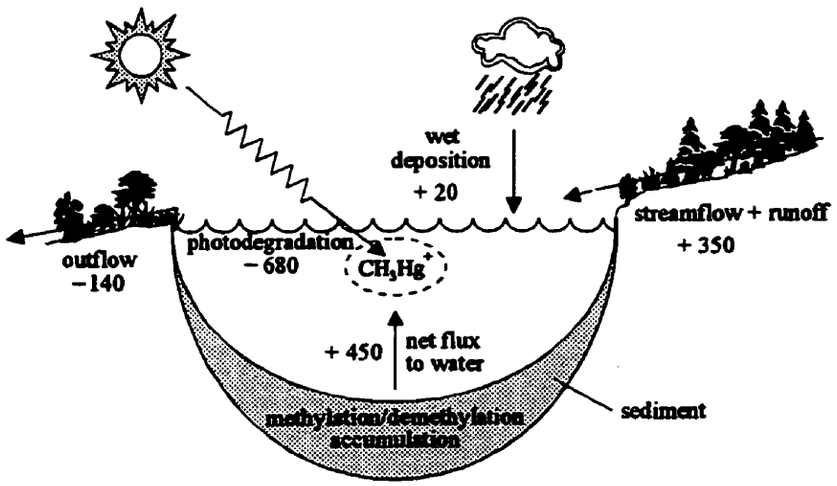
Fig. 4. Preliminary annual mass balance budget for methylmercury (mg per year) in Lake 240 without (a) and with (b) the inclusion of photodegradation in the methylmercury cycle. The net flux of methylmercury to/from the sediments is the net result of methylation, demethylation, and accumulation in the sediments.

METHODS: Direct wet deposition and terrestrial runoff were estimated from previously published data for the Experimental Lakes Area[20]. Stream inflows were calculated from measured volumes and concentrations (unpublished data). Lake 240 has a surface area of 44.1 h, and for 1994 had an average epilimnetic methylmercury concentration of 0.07 ng L^{-1}, an average DOC concentration of 575 μmol L^{-1}, and an average pH of 7.06. An annual photodegradation rate was calculated as described in text.

a Lake budget without photodegradation



b Lake budget with photodegradation



mercury methylation is primarily an anoxic process[21], this in-lake source is expected to be the sediments at a rate of ≈1 $\mu g\ m^{-2}\ yr^{-1}$. This mass balance shows that during the year of our study, the flux from sediments must be similar in importance to external inputs as a source of methylmercury to this lake.

Knowledge of photodegradation may be useful in the design of methods to mitigate methylmercury problems. For example, where effluent water contains high concentrations of methylmercury, it could be retained in shallow ponds prior to discharge. Coincidentally, this should also decrease the concentration of inorganic mercury because of photoreduction of Hg^{2+} to volatile Hg^0 (ref. 9). Knowledge of methylmercury photodegradation should also be used as a criterion for site selection of reservoirs, which commonly contain mercury contaminated fish. For example, peatland sites are not recommended because water in reservoirs that flood peatlands is stained brown by dissolved organic matter. This staining reduces light penetration and the beneficial impact of photodegradation.

Our finding that photodegradation is an important sink for methylmercury in lakes has important implications with respect to the understanding of mercury cycling in aquatic ecosystems. For example, a recent study[22] (completed before this work) found that external methylmercury inputs to a Swedish lake are equal to the annual accumulation in fish, and consequently it was concluded that in-lake production of methylmercury was unimportant. We should also have concluded that in-lake methylation was unimportant for Lake 240, if we had not known that photodegradation was an important sink for

methylmercury. Other recent mass balance studies have likely also underestimated in-lake methylation rates because methylmercury photodegradation was unknown[23,24]. The fact that in-lake methylation is more important than previously thought means that attempts to control methylmercury levels in lakes must take into account internal as well as external sources of methylmercury.

References

1. Clarkson, T.W. in *Mercury Pollution: Integration and Synthesis,* Lewis Publishers, Chelsea, MI. pp. 631-641 (1994).
2. Hecky, R.E., D.J. Ramsay, R.A. Bodaly, & N.E. Strange. in *Advances in Mercury Toxicology* Plenum Press, New York. pp. 33-52 (1991).
3. Wiener, J.G. *Trans. 52nd. N.A. Wild. & Nat. Res. Conf.* 645-657 (1989).
4. Bodaly, R.A., J.W.M. Rudd, R.J.P. Fudge, & C.A.Kelly. *Can. J. Fish. Aquat. Sci.* **50,** 980-987 (1993).
5. Haines, T.A., V.T. Komov, & C.H. Jagoe. in *Mercury Pollution: Integration and Synthesis,* Lewis Publishers, Chelsea, MI. pp. 397-407 (1994).
6. Swain, E.B., D.R. Engstrom, M.E. Brigham, T.A. Henning, & P.L. Brezonik. *Science.* **257,** 784-787 (1992).
7. Rudd, J.W.M. *Water Air Soil Pollut.* **80,** 697-713 (1995).
8. Horvat, M., L. Liang, & N.S. Bloom. *Anal. Chim. Acta* **282,** 153-168 (1993).

9. Amyot, M., G. Mierle, D.R.S. Lean, & D.J. McQueen. *Environ. Sci. Technol.* **28,** 2366-2371 (1994).

10. Hudson, R. J. M., S.A. Gherini, C.J. Watras, & D.B. Porcella. in *Mercury Pollution: Integration and Synthesis,* Lewis Publishers, Chelsea, MI. pp. 473-523 (1994).

11. Inoko, M. *Environ. Pollut. B, Chem. Phys.* **2,** 3-10 (1981).

12. Zepp, R.G., G.L. Baughman, N.L. Wolfe, & D.M. Cline. *Environ. Lett.* **6,** 117-127 (1974).

13. Xiao, Z.F., J. Munthe, D. Stromberg, & O. Lindqvist. in *Mercury Pollution: Integration and Synthesis,* Lewis Publishers, Chelsea, MI. pp. 581-592 (1994).

14. Vandal, G.M., R.P. Mason, & W.F. Fitzgerald. *Water Air Soil Pollut.* **56,** 791-803 (1991).

15. Xun, L., N.E.R. Campbell & J.W.M. Rudd. *Can. J. Fish. Aquat. Sci.* **44,** 750-757 (1987).

16. Ramlal, P.S., J.W.M. Rudd, & R.E. Hecky. *Appl. Env. Microbiol.* **51,** 110-114 (1986).

17. Winfrey, M.R. & J.W.M. Rudd. *Environ. Toxicol. Chem.* **9,** 853-869 (1990).

18. Fee, E.J. *Can. Tech. Rep. Fish. Aquat. Sci.* **1740,** 27 p (1990).

19. Shearer, J.A., E.R. DeBruyn, D.R. DeClercq, D.W. Schindler, & E.J. Fee. *Can. Tech. Rep. Fish. Aquat. Sci.* **1341,** 58 p (1985).

20. St.Louis *et al. Can. J. Fish. Aquat. Sci.* **51,** 1065-1076 (1994).

21. Gilmour, C.G, E.A. Henry, & R. Mitchell. *Environ. Sci. Technol.* **26,** 2281-2287

(1992).

22. Hultberg, H., A. Iverfeldt, & L.H. Lee. in *Mercury Pollution: Integration and synthesis,* Lewis Publishers, Chelsea, MI. pp. 313-322 (1994).

23. Watras, C.J. *et al.* in *Mercury Pollution: Integration and Synthesis,* Lewis Publishers, Chelsea, MI. pp. 153-177 (1994).

24. Henry, E.A., L.J. Dodge-Murphy, G.N. Bigham, S.M. Klein, & C.C. Gilmour. *Water Air Soil Pollut.* **80,** 509-518 (1995).

ACKNOWLEDGEMENTS. We thank R. Harris, R.E. Hecky, R.H. Hesslein, D.M. Rosenberg, D.W. Schindler, R. Sparling and V.L. St. Louis for their comments on an earlier version of this manuscript. We especially than K.J. Scott XVII for her input on experimental design. This work was supported by a Natural Sciences and Engineering Research operating (C.K. and J.R.), a University of Manitoba Fellowship (P.S.), and a Department of Fisheries and Oceans subvention grant.

Appendix B

Continuous measurement of CO_2 for estimation of air-water fluxes in lakes: An in situ technique

Published in *Limnology and Oceanography*, volume 40, pp.575-581, 1995

co-authors: R.H. Hesslein and C.A. Kelly

Abstract

Three parameters must be known to use the thin boundary-layer model (or other bulk transfer models) for CO_2 flux between water and air: the concentration of dissolved CO_2 ($CO_{2(aq)}$); the concentration of CO_2 in the air immediately above the water ($CO_{2(atm)}$); and the wind velocity, which is used to determine the appropriate transfer coefficient. These parameters change hourly, and from day to day in a nonlinear fashion, and so frequency of measurements is an important factor in determining the accuracy of flux estimates for any period. To achieve a high frequency measurement, we developed a self-contained, solar powered, in situ sampling system that continuously measures and records $CO_{2(aq)}$, $CO_{2(atm)}$ and windspeed. Unique to this technique is an underwater, in situ equilibration chamber (ISEC). The ISEC was tested in a shallow wetland pond, where changes in both $CO_{2(aq)}$ and $CO_{2(atm)}$ were large. The data obtained showed that large errors may result from extrapolating flux calculations made from short term data (e.g. daily) to longer time periods.

Introduction

The most commonly used method for estimating gas flux across the air-water interface is the thin boundary layer (TBL) model, which requires windspeed measurements, and of the concentrations of the gas in air and water. Gas concentration data are usually acquired by periodic sampling, with the frequency of sampling usually limited by the accessibility to the site and time available for analyses.

When measurements are made for the purpose of calculating CO_2 flux in lakes where diel changes in water and air are small, limitations on sampling frequency may not be serious. However, there are two common situations in which very frequent measurements in both air and water may be necessary: eutrophic lakes and shallow ponds - both of which have strong diel changes due to high rates of photosynthesis and respiration. Even where diel changes are not so strong (as in oligotrophic lakes), other considerations, such as large changes in windspeed, may make sampling frequency important.

Because the CO_2 flux is proportional to the *difference* between the air and water concentrations, monitoring CO_2 concentrations in air may also be important. Often this concentration is not measured, but is assumed to be the current global mixing ratio (356 ppm; C.D. Keeling, pers. comm.). For small lakes in protected areas, the CO_2 in the air above the water may be influenced by surrounding forest or wetland, especially during periods of low windspeed. In oligotrophic lakes, the CO_2 in air and water are often close to equilibrium (Hesslein *et al.*, 1991); thus, small changes in either may change the direction of the flux during the day-night cycle.

Dissolved CO_2 ,$CO_{2(aq)}$, can be calculated from measurements of other components of the CO_2 system (i.e. pH, dissolved inorganic carbon, and carbonate alkalinity) or directly quantified. Because of potential errors with the indirect method, the direct quantification of $CO_{2(aq)}$ is preferable for both marine and freshwater systems (Dickson and Riley 1978; Herczeg and Hesslein 1984; Stauffer 1990). The method most commonly

used for direct quantification is equilibration of a water sample with a small head space and the measurement of the pCO_2 in this head space (Hesslein et al. 1991). pCO_2 is then used to calculate $CO_{2(aq)}$ using Henry's Law.

When they sampled wetland ponds on the Hudson Bay Lowlands, Hamilton *et al.* (1994) found that large changes in $CO_{2(aq)}$ in each 24-h period were caused by complex interactions between light and dark biological cycles and daily wind patterns. At least 4-5 samples per day were needed to define properly the diel pattern of $CO_{2(aq)}$, and frequent sampling was difficult due to the remote location. At the Experimental Lakes Area (ELA) of northwestern Ontario, wetland lakes and ponds also exhibit large changes in $CO_{2(aq)}$ throughout the day (unpubl. data). To facilitate frequent sampling, we developed a technique for the continuous and direct measurement of $[CO_2]$ in water and air. For $CO_{2(aq)}$, we constructed a gas-filled, water-tight, gas- permeable, in situ equilibration chamber (ISEC). The $[CO_2]$ in this underwater chamber was measured alternately with the $[CO_2]$ in the air just above the water. The sampler is portable, automated, and powered by solar-recharged batteries. The data acquired during field testing were used to address the following questions: How frequently does sampling need to be done in order to estimate flux accurately? How important is it to measure the air concentration of CO_2, as well as the water concentration? Field tests of the ISEC technique were conducted in an experimental wetland pond (Lake 979) at the ELA in northwestern Ontario (Johnson and Vallentyne 1971).

Methods

The ISEC was designed so that CO_2 inside the gas-filled ISEC would equilibrate with CO_2 dissolved in the lake water fast enough to allow $CO_{2(aq)}$ changes in the water to be reflected accurately by changes inside the ISEC. For the ISEC to reach >95% of equilibrium after 1 h, which we considered to be adequate temporal resolution, a $t_{1/2}$ of ≤ 12 min, and hence an equilibration rate constant (k_{eq}) ≥ 0.0578 min^{-1}, was targeted in its design. Factors that affect the $t_{1/2}$ are the permeability and thickness of the membrane and the ratio of equilibrating surface area to total volume. The total volume is the ISEC volume plus the non-equilibrating volume, which includes the sample cell of an infrared gas analyzer (IRGA) and the connecting tubing. The ISEC volume thus needs to be a large proportion of the total volume. The mathematical relationship of these parameters is:

$$k_{eq} = \frac{D \times S \times A_s}{V_{tot} \times Z} \qquad (1).$$

D is the membrane diffusivity of CO_2 in cm^2 sec^{-1}, S is the membrane solubility of CO_2 in cm^3 cm^{-3}, A_s is the surface area of the ISEC in cm^2, V_{tot} is the total volume in cm^3, and Z is the membrane thickness in cm. The theoretical $t_{1/2}$ of the ISEC we designed was 2.3 min.

The ISEC was tested in the laboratory with an 91-liter aquarium, two thirds of which was filled with water and sealed from ambient air using a Plexiglas cover. The ISEC was placed in the water, and the gas inside the ISEC was continuous with the

sample cell of an IRGA by using copper tubing (0.32 cm). This tubing extended from the ends of the ISEC to the IRGA through ports in the Plexiglas. The gas inside the ISEC was circulated through the IRGA using a peristaltic pump. The water in the aquarium was circulated, until turbulence was visible (to simulate lake conditions). When tested in the laboratory, the ISEC responded with at $t_{1/2}$ of 12 min. The discrepancy between the theoretical and the observed $t_{1/2}$ is likely the result of an aqueous laminar boundary layer on the surface of the membrane, which existed despite the turbulence induced in the test conditions. This boundary layer would increase the effective thickness of the membrane. The boundary layer thickness would need to be approximately 230 μm in order to account for the difference between the theoretical and observed $t_{1/2}$,. This thickness is typical of aqueous boundary layers at the air-water interface (Broecker *et al.*, 1980; this study) and of epiphytic bottoms (Turner *et al.*, 1991) of ELA lakes.

The cylindrical ISEC (length, 30 cm; diam, 2 cm; mass ≈ 110 g; Fig. 1) consists of an internal frame constructed with galvanized wire cloth (No. 8 mesh; wire diam, ≈. 0.5 mm; Greening Donald) and protected with rust-proof paint. The frame supports a covering of Nitex nylon cloth (mesh size, 16 μm^2) overlain with a silicone polycarbonate membrane (1 mil thick; MEM 213, Membrane Products Co.). The Nitex serves to provide additional support for the membrane and to protect the membrane from the wire. All components and seams of the ISEC, including copper end caps and tubing, are secured with silicone sealant.

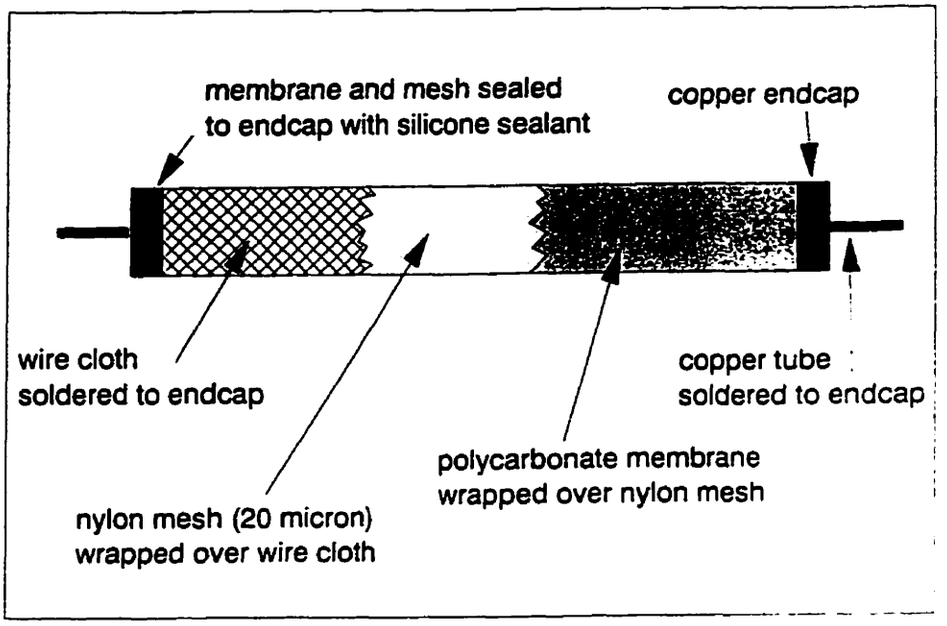
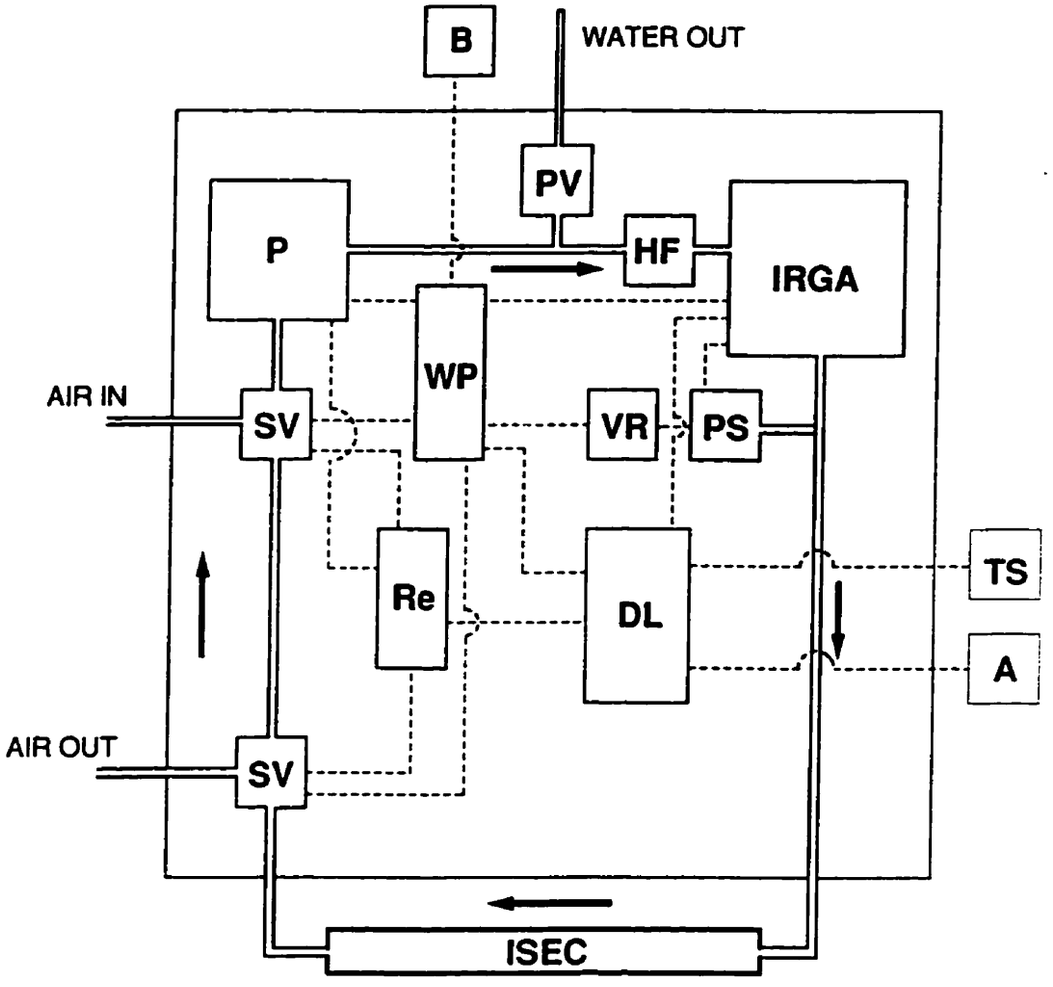


Fig. 1. Diagram of the ISEC.

In addition to the ISEC, major components of the sampling system (Fig. 2) included a portable IRGA (model 6262, LiCor Inc.; current draw, 1 A), a data logger/system controller (model CR10, Campbell Sci.; current draw, 35 mA), a temperature sensor (model 107B, Campbell Sci.), and a peristaltic pump (model 7533-30, Masterflex, Cole-Parmer; current draw = 2.5 A). After initial testing, this pump was replaced with an air-sampling piston pump (model AS-300SS, Spectrex USA) equipped with a voltage regulator, which draws much less current (≈ 150 mA). The internal volume of the ISEC (≈ 95 ml) is continuous with the pump and with the sample cell of the IRGA through copper tubing (0.32 cm) and represents ≈ 80% of the total volume. Wherever possible, copper tubing and Swagelok fittings were used to connect sampler components, to ensure minimal diffusive loss or gain of CO_2.

The pump (flow rate, ≈ 120 ml min^{-1}) mixed the ISEC contents with the non-equilibrating volume (5-6 times per hour for 2 min) and circulated the chamber contents through the IRGA at the time of analyses. The pump head was fitted with Norprene tubing (size 14, Masterflex, Cole-Parmer), which connected directly with the copper tubing. A hydrophobic filter (0.45 μm; water-breakthrough at 110.32 kPa; Acro 50, Gelman Sci.) was situated in line between the pump and the IRGA; it filtered the sample stream and prevented water from entering the IRGA in the event of water seepage into a faulty ISEC. In the event of a leak, a relief valve (set at 27.58 kPa) placed in-line immediately upstream of the filter, would alleviate the water pressure. The water would be diverted through an outlet port and allowed to discharge into the pond. Solenoid

Fig. 2. Block diagram of sampler. Dotted lines represent electrical connections, arrows indicate direction of circulation, and the largest box represents the fiberglass case. Key: P—pump; B—battery; RV—relief valve; HF— hydrophobic filter; IRGA—infrared gas analyzer; PS—pressure sensor; VR—voltage regulator; TS—temperature sensor; A—anemometer; ISEC—in situ equilibration chamber; SV—solenoid valve; Re— relay; DL—data logger; WP—wiring panel.


B
WATER OUT
PV
P
HF
IRGA
WP
AIR IN
SV
VR
PS
Re
DL
TS
A
AIR OUT
SV
ISEC

valves (model V5R66620, Honeywell) were place in line and served to direct the air flow so that $CO_{2(atm)}$ just above the water surface (≈ 20 cm) could be measured every 4 h. Sampling lines (copper tubing) for air intake and vent were situated between the ISEC and the pump. The internal pressure of the IRGA was measured using a pressure sensor (model 142SC30A, SenSym; current draw = 10 mA) equipped with a voltage regulator. The IRGA was calibrated in the laboratory using standardized mixtures of CO_2 in reconstituted air (80% nitrogen, 20% oxygen; Linde). Drift of the calibration was negligible.

The data logger recorded signals from the IRGA, temperature sensor, and an anemometer (model 12102, RM Young). The data logger provided the time base and also controlled the operation of the pump and solenoid valves through solid state relays (D1D12, Crydom).

For field operation, the components were secured on a wooden platform (26 × 26 cm) enclosed in a fiber-glass and waterproof case (model 92900, Skydyne). Holes were drilled through the case to accommodate Swagelok fittings (for ports) and electrical leads. A floating raft held the case, a 12-V deep-cycle battery (105 A-h), the anemometer, and a solar panel (model MMVBAEOOO, Solarex; 51 × 107 cm; 3.5 A at 15.4 V). The average current draw of the system was ≈ 1.6 amps, with a peak draw of 3.5 amps. In addition, we found it necessary to power the data logger and the pressure sensor separately with a smaller (e.g. motorcycle) battery. This battery was not recharged by the solar panel.

The ISEC and the temperature probe were suspended in the water through slots in the raft ≈20 cm below the water surface. Dense foam (externally) and fiberglass (internally) were used to insulate the case. Insulation was necessary to prevent condensation of water vapour in the sample cell of the IRGA, which could occur at night if the air temperature fell below that of the water. The raft was anchored in the middle of Lake 979 (mean depth = 0.8 m; area = 2.39 h). The ISEC was cleaned (with water and soft sponge) or replaced every 3-4 d, after which time only very small amounts of microbial growth on the surface of the membrane could be seen.

The pCO_2 within the ISEC was measured every hour. A simultaneous recording

$$CO_{2(aq)} = pCO_2 \times K_H. \qquad (2)$$

of water temperature was made for the correct choice of Henry's Law constants (K_H; Harned and Davis 1943) in the calculation of $CO_{2(aq)}$ as:

where $CO_{2(aq)}$ = mol liter^{-1}, pCO_2 = atm and K_H = mol liter^{-1} atm^{-1}.

The accuracy of the ISEC $CO_{2(aq)}$ measurements was tested against a previously established method for discrete water samples (Hesslein *et al.*, 1991). Briefly, at times of IRGA analyses, surface water was collected near the raft by puncturing the stopper of an evacuated 160-ml serum bottles containing 8.9 g of KCl for preservation and 10 ml of nitrogen to ensure the proper headspace volume for later gas analyses. Between two and four replicate samples were collected at nine discrete times during 5 d in August 1992.

In the laboratory, the samples were shaken to equilibrate the gas and liquid phases. The headspace was subsampled (0.2 ml) using a Pressure-Lok syringe (Mandel Scientific), and this analyzed for pCO_2 with a flame ionizing gas chromatograph (GC), equipped with a methanizer (Schimadzu Mini 2). The GC was standardized using certified standards of 82 to 2,897 ppm CO_2 reconstituted in air (Linde). Henry's Law constants used to calculate $CO_{2(aq)}$ were corrected for the presence of the KCl (Harned and Davis 1943).

Fluxes of CO_2 across the air-water interface were estimated using the TBL model (eg. Liss and Slater 1974). In this model, the flux (F) is assumed to be limited by the rate of diffusion of a gas across a stagnant film of water at the air-water interface, and it is proportional to the concentration gradient of the gas across the film. Use of the TBL model requires that the $[CO_2]$ at the base of the water film (C_w), the concentration at the

$$F = k_{CO_2} \times (C_w - C_{sw}) \qquad (3)$$

water surface (C_{sw}; which is assumed to be in equilibrium with the air above it) and a gas exchange coefficient be known:

where k_{CO2} is the gas exchange coefficient for CO_2 (cm h^{-1}). k is empirically derived using various tracer techniques in surface waters, as summarized by Wanninkhof (1992).

We used the relationship between windspeed and k_{CO2} derived by measuring losses of sulfur hexafloride (SF_6) from a small lake at different windspeeds (Wanninkhof *et al.*, 1991). Values of k_{CO2} were calculated from k_{600}, which is the k measured with SF_6 and

normalized to a Schmidt number (Sc) of 600. Sc equals the kinematic viscosity of water

$$k_{600} = 0.76 \times u \qquad (4)$$

at a temperature divided by the diffusivity of the gas at that temperature. For CO_2 at 20 °C, Sc = 600. If windspeed is < 3 m s^{-1} :

$$k_{600} = (5.6 \times u) - 14.4 \qquad (5)$$

If windspeed ³ 3 m s^{-1}:

where u is the windspeed measured over a 15-min interval just prior to the time of CO_2

$$k_{CO_2} = k_{600} \times \left[\frac{600^{0.67}}{(Sc_{CO_2})^{0.67}}\right] \qquad (6)$$

measurement. Values of k_{CO2} were calculated from k_{600} values according to the following

$$k_{CO_2} = k_{600} \times \left[\frac{600^{0.5}}{(Sc_{CO_2})^{0.5}}\right]. \qquad (7)$$

equations. For windspeeds < 3.0 m s^{-1}:

where Sc_{CO2} is the in situ Sc for CO_2. For windspeeds ≥ 3.0 m s^{-1}:

Instantaneous fluxes were calculated from windspeeds measured every 15 min and were integrated over a 1-h time period.

Results

The ISEC technique proved to be a reliable and accurate method of measuring $CO_{2(aq)}$ in a system where the $CO_{2(aq)}$ was changing at rates of several mmol CO_2 liter^{-1} h^{-1}. For the nine occasions on which $CO_{2(aq)}$ was measured by both the ISEC and by taking discrete samples, there was no statistically significant difference between the mean measurement of the two methods, as tested by a standard *t* test (Fig. 3; $t = 2.306$; $p < 0.05$). This agreement showed that, as expected from measurements of the ISEC equilibration half time, gas exchange across the membrane was rapid enough that the $[CO_2]$ in the ISEC did not lag behind changes in in situ $CO_{2(aq)}$. Also, microbial growth, which might have occurred on the ISEC, did not affect $CO_{2(aq)}$ measurements over periods of 3-4 d.

Measurements of $CO_{2(atm)}$ were made every 4 h and ranged from 369 to 557 ppm (Fig. 4A). In general, values were fairly close to the global average during the day (Fig. 4A), when windspeeds were high (Fig. 5A). It then increased at night as conditions became calmer. This pattern, together with small diel temperature changes, caused the values of C_{sw} (Eq. 3) to follow the same pattern, ranging from a low of 13 μmol liter^{-1} in the day to a high of 20.3 at night (Fig. 4B). The range of values for both these measurements differed from one day to the next - again linked to windspeed. For example, on 18 August, the $CO_{2(atm)}$ ranged from 375 to 560 ppm, while on the windier day 230, the range was only 375-400 ppm (Fig. 4A,B).

Fig. 3. Comparison of $CO_{2(aq)}$ determined by the GC-bottle and ISEC methods in August of 1992 on Lake 979. The number of bottle samples is indicated by *n*; bars represent 95% C.I.

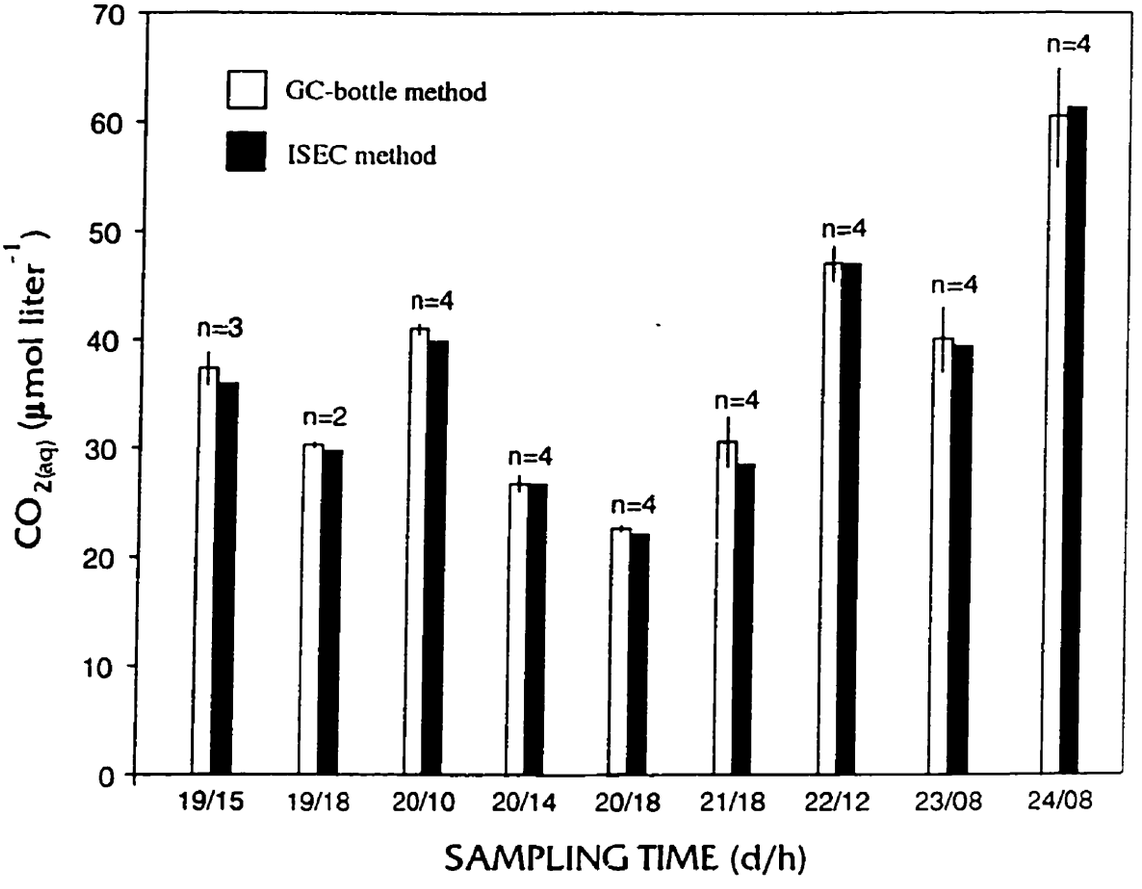
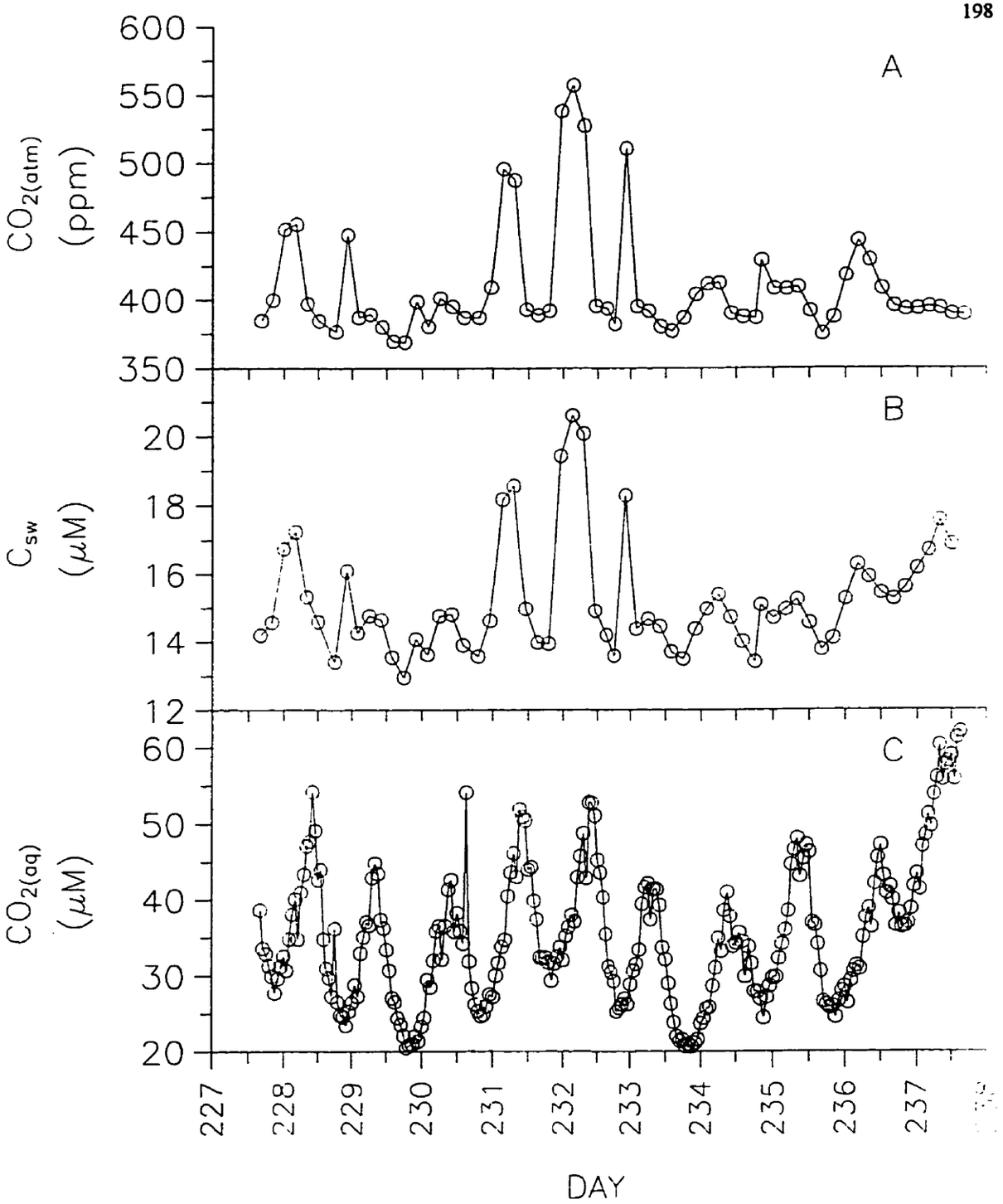

70
60
50
40
30
20
10
0
GC-bottle method
ISEC method
$CO_{2(aq)}$ (μmol liter^{-1})
n=3
n=2
n=4
n=4
n=4
n=4
n=4
n=4
n=4
19/15
19/18
20/10
20/14
20/18
21/18
22/12
23/08
24/08
SAMPLING TIME (d/h)

Fig. 4. A continuous record of (A) $CO_{2(atm)}$, (B) C_{sw} and (C) $CO_{2(aq)}$ for Lake 979, 14-25 August.


600
550
500
450
400
350
$CO_{2(atm)}$ (ppm)
A
20
18
16
14
12
C_{sw} (μM)
B
60
50
40
30
20
$CO_{2(aq)}$ (μM)
C
227
228
229
230
231
232
233
234
235
236
237
DAY

The pattern of $CO_{2(aq)}$ concentrations was quite different from $CO_{2(atm)}$. On most days, $CO_{2(aq)}$ peaked during morning (≈0900 hours), and then began to decline (Fig. 4C). Lowest concentrations were recorded in the evening near dusk (2000 hours). For the measurement period, $CO_{2(aq)}$ concentrations ranged from ≈20 to 60 μmol liter^{-1} (at 20 °C and 1 atm, 20 μmol liter^{-1} is equivalent to 510 ppm).

The values of C_{sw} (Fig. 4B) were less than $CO_{2(aq)}$ (Fig. 4C) at all times; thus, there was a net flux of CO_2 from the pond to the atmosphere throughout the measurement period. Because of large changes in both $CO_{2(aq)}$ (Fig. 4C) and windspeed (Fig 5A), calculated CO_2 fluxes also changed greatly throughout each day, ranging from ≈30 to 4,000 μmol m^{-2} h^{-1} (Fig. 5B).

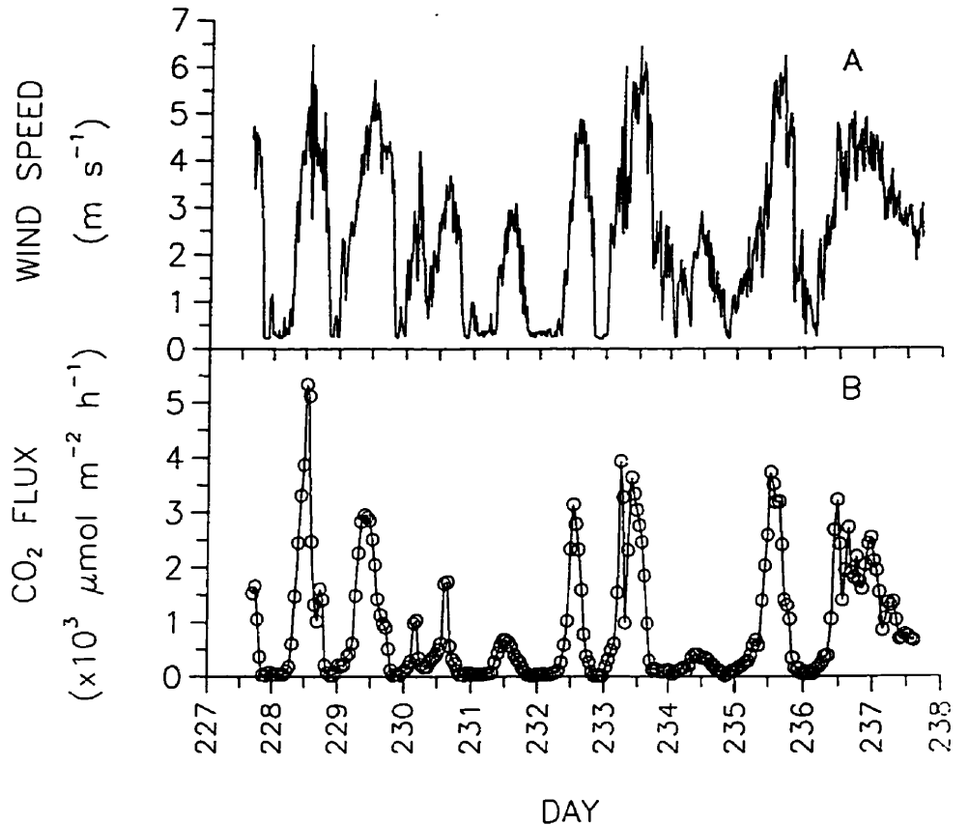
Discussion

This work had two major objectives: to develop and to test in the field a sampler for automated, continuous measurement of CO_2 in water and air, and to use the data obtained to evaluate the relative accuracy of flux estimates made using continuous versus less frequent measurements.

The ISEC sampler performed well in the field. The power requirements could be satisfied with standard solar panels, and the temperature inside the case could be kept warm enough at night to prevent condensation within the sample cell of the IRGA.

A major question concerning the effectiveness of this sampler was whether the equilibration of CO_2 inside the ISEC would be rapid enough to measure accurately the

Fig. 5. A continuous record of windspeed and CO_2 flux for Lake 979, 14-25 August 1992.


WIND SPEED (m s^{-1})
CO_2 FLUX (x10^3 μmol m^{-2} h^{-1})
A
B
0
1
2
3
4
5
6
7
227
228
229
230
231
232
233
234
235
236
237
238
DAY

changes in $CO_{2(aq)}$. Clearly, the rate of equilibration was adequate (Fig. 3), even in Lake 979 where the $CO_{2(aq)}$ was changing at about 1-2 μmol liter^{-1} h^{-1}. Even more rapid changes have been observed in shallow wetland ponds on the Hudson Bay Lowlands (Hamilton *et al.*, 1994), but most lakes would not change this rapidly. It should be noted that the particular membrane material and thickness used here was chosen for use in the type of system where the ISEC was deployed; it would be possible, with different material or ISEC dimensions, to build an ISEC with a faster response time.

The ISEC was not tested to see how long it could be left unattended without microbial growth on the membrane that would affect the pCO_2 measurements. Presumably, the length of time would depend on the activity of the microbial population of the water in which it is suspended. For remote operation, a small cylinder of gaseous sterilizing agent could be inserted in line between the IRGA and the ISEC (Fig. 1) and programmed to periodically clean the membrane; methyl bromide is suggested because it is least harmful to the optics of the IRGA (LiCor Inc. pers. comm.).

The continuous data set that we obtained with the ISEC technique allowed us to address the question of how frequently the parameters needed for flux calculations [$CO_{2(atm)}$, $CO_{2(aq)}$, and windspeed] needed to be measured. First, the data obtained were used to test the necessity of a continuous measurement of $CO_{2(atm)}$ above the water surface. One might expect that a complete record of this measurement would be important because it increased at night to values that were 6-46% higher than daytime values during each 24-h period (Fig. 4A). However, when $CO_{2(atm)}$ was assumed to be

constant [using the average $CO_{2(atm)}$ observed at high windspeeds, 383 ppm], the calculated daily fluxes were essentially the same (Table 1) for two reasons. First, in Lake 979, in which $CO_{2(aq)}$ was much higher than the air equilibrium $CO_{2(aq)}$, the use of an average value for $CO_{2(atm)}$ did not affect the gradient across the air-water interface enough to influence the magnitude of the CO_2 flux. Second, the greatest difference between the measured $CO_{2(atm)}$ and the assumed value occurred at night, when fluxes (Eq. 3) were always low due to low windspeed. Infrequent measurements of $CO_{2(atm)}$ at high windspeeds, or an assumed global mean concentration (356 ppm), thus, would be adequate for Lake 979, and probably for most systems in which the values of $CO_{2(aq)}$ concentrations are high compared to the equilibrium values.

In contrast to the case of $CO_{2(atm)}$, measurement of $CO_{2(aq)}$ needed to be made frequently. $CO_{2(aq)}$ concentrations changed by factors of 2.0-2.5 in each 24-h period (Fig. 4C) in Lake 979. Thus, one or two measurements per day would not be adequate to calculate an accurate daily flux. In a previous study on shallow wetland ponds (Hamilton *et al.*, 1994), samples were taken six times in a 24-h period: at dusk, dawn, midmorning, noon, late afternoon, and dusk of the following day. Simulation of this sampling scheme with our data (using 15-min time-averaged winspeeds at the selected sampling times) for several 24-h periods, showed that this approach would have been within 15% of the estimate from the ISEC on 5 out 7 d (Table 2). More frequent samples would improve the estimate on the other 2 d. Although the diel change of $CO_{2(aq)}$ in oligotrophic lakes is comparatively small, the importance of frequent measurements in each 24-h period should

Table 1. A comparison of daily fluxes using measured and constant values of $CO2_{(atm)}$. Fluxes are in μmil m^{-2} d^{-1}.

1992	Measured $CO_{2(atm)}$	Constant $CO_{2(atm)}$ [1]
16 Aug	27,300	26,900
17 Aug	10,700	10,800
18 Aug	5,630	5,850
19 Aug	16,100	16,600
20 Aug	32,900	32,900
21 Aug	4,580	4,730
22 Aug	29,900	29,900

[1] Constant of 383 ppm; $pCO2 = 0.0003727$ atm.

Table 2. A comparison of daily fluxes calculated using continuous and discontinuous sampling schedules. Fluxes are in mmol m^{-2} d^{-1}.

1992[1]	Continuous sampling[2]	Discontinuous sampling[3]
16-17 Aug	467	314
17-18 Aug	250	266
18-19 Aug	713	695
19-20 Aug	1,430	2,740
20-21 Aug	209	237
21-22 Aug	1,300	1,500
22-23 Aug	1,120	1,080

[1] Sampling beginning at dusk and continuing for 24 h

[2] Samples taken every hour

[3] Samples taken at 2000, 0600, 1000, 1300, 1700, 1900 hours

not be overlooked. In these lakes, the CO_2 gradient across the air-water interface is not large (Hesslein *et al.*, 1991) and the direction of this gradient (which indicates whether the flux is into or out of the lake) is thus sensitive to small changes in $CO_{2(aq)}$ and $CO_{2(atm)}$. Therefore, frequent measurements of both $CO_{2(aq)}$ and $CO_{2(atm)}$ may be required to accurately determine the magnitude and direction of CO_2 flux. With the ISEC technique, measurements of $CO_{2(atm)}$ can be obtained simultaneously with $CO_{2(aq)}$ measurements. The accuracy of CO_2 measurement by the IRGA (1-2%) would be adequate for detecting these small CO_2 gradients.

Although accurate estimates of net daily fluxes would be possible with frequent discrete sampling within each 24-h period, the most serious error in extrapolation of flux calculations from shorter to longer periods occurred when we assumed that the flux measured on a single day was typical of daily fluxes for a whole week (Table 3). This was because daily fluxes, calculated by integrating hourly fluxes over a 24-h, period showed large changes from one day to the next (Table 3). To demonstrate the importance of a continuous record for calculation of gas fluxes from such rapidly changing systems, we extrapolated a weekly flux from each daily measurement. We then compared each of these weekly estimates with the actual integrated flux for the week (Table 3). These estimates ranged from 25 to 181% of the actual flux, and showed that relying on measurements for a single day to extrapolate to a longer time period could cause serious errors.

Table 3. A comparison of actual and estimated weekly CO2 fluxes. The actual weekly flux is calculated from hourly fluxes integrated over the 7-d period. The estimated weekly flux is found by extrapolating from a single day.

1992	Daily flux[1]	Weekly flux estimates[2,3]	Actual weekly flux[2]
16 Aug	27,300	191,100	127,110
17 Aug	10,700	74,900	
18 Aug	5,630	39,410	
19 Aug	16,100	112,700	
20 Aug	32,900	230,300	
21 Aug	4,580	32,060	
22 Aug	29,900	209,300	

[1] $\mu mol\ m^{-2}\ d^{-1}$

[2] $\mu mol\ m^{-2}\ week^{-1}$

[3] Daily flux × 7.

The ISEC technique has the potential to be modified to measure dissolved methane (CH_4), which also changes over each 24-h period (Hamilton *et al.*, 1994). Dissolved CH_4 could be measured by oxidizing CH_4 to CO_2 with a heated metal catalyst (e.g. copper oxide) inserted in line of the continuous loop (Fig. 2). Alternatively, a CH_4-detecting IRGA could be added in series with or in place of the CO_2-detecting IRGA.

Dissolved CO_2 measurements in lakes have only recently been made on a widespread basis, and the frequency of measurement has ranged from only one or a few times a year (most often) up to weekly (Cole *et al.*, 1994). CO_2 flux has been estimated less frequently. The ISEC technique is a simple and reliable means by which our knowledge of dissolved CO_2 and CO_2 fluxes can be expanded. Its in situ operation eliminates field and analytical time normally required for discrete $CO_{2(aq)}$ determinations. The ISEC is especially useful for measurement of $CO_{2(aq)}$ in remote lakes and ponds, for which access is limited. The 24-h fluxes can be used to estimate net daily community production, while longer term records can be used to address the question of which lakes are net sources or sinks of atmospheric CO_2.

References

Broecker, W. S., T-H. Peng, G. Mathieu, R. Hesslein, and T. Torgersen. 1980. Gas exchange rate measurements in natural systems. Radiocarbon **22**: 676-683.

Cole, J. J., N. F. Caraco, G. W. Kling, and T. K. Kratz. 1994. Carbon dioxide supersaturation in the surface waters of lakes. Science **265**: 1568-1570.

Dickson, A. G., and J. P. Riley. 1978. The effect of analytical error on the evaluation of the components of the aquatic carbon-dioxide system. Mar. Chem. **6**: 77-85.

Hamilton, J. D., J. W. M. Rudd, C. A. Kelly, R. H. Hesslein, and N. T. Roulet. 1994. Flux to the atmosphere of CH_4 and CO_2 from wetland ponds on the Hudson Bay Lowlands (HBL). J.Geophys. Res. **99**: 1495-1510.

Harned, H. S., and R. Davis Jr. 1943. The ionization constant of carbonic acid in water and the solubility of carbon dioxide in water and aqueous salt solutions from 0° to 50°. Am. Chem. Soc. J. **65**: 2030-2037.

Herczeg, A. L., and R. H. Hesslein. 1984. Determination of hydrogen ion concentration in softwater lakes using carbon dioxide equilibria. Geochim. Cosmochim. Acta **48**: 837-845.

Hesslein, R. H., J. W. M. Rudd, C. Kelly, P. Ramlal, and K. A. Hallard. 1991. Carbon dioxide pressure in surface waters of Canadian lakes, p. 413-431. *In* S.C. Wilhelms and J.S.Gulliver [eds.], Air-water mass transfer. Am. Soc. Civ. Eng.

Johnson, W. E., and J. R. Vallentyne. 1971. Rationale, background and development of experimental lake studies in Northwestern Ontario. J. Fish. Res. Bd. Can. **28**: 123-

128.Liss, P. S., and P. G. Slater. 1974. Flux of gases across the air-sea interface. Nature 247: 181-184.

Stauffer, R. E. 1990. Electrode pH error, seasonal epilimnetic pCO_2, and the recent acidification of the Maine lakes. Water Air Soil Pollut. 50: 123-148.

Turner, M. A., E. T. Howell, M. Summerby, R. H. Hesslein, D. L. Findlay, and M. B. Jackson. 1991. Changes in epilithon and epiphyton associated with experimental acidification of a lake to pH 5. Limnol. Oceanogr. 36: 1390-1405.

Wanninkhof, R. 1992. Relationship between windspeed and gas exchange over the ocean. J. Geophys. Res. 97: 7373-7382.

Wanninkhof, R., J. Ledwell, and J. Crusius. 1991. Gas transfer velocities on lakes measured with sulfur hexaflouride, p. 441-455. *In* S.C. Wilhelms and J.S. Gulliver [eds.], Air-water mass transfer. Am. Soc. Civ. Eng.