Chapter 19

Geochemical and Biological Controls over Methylmercury Production and Degradation in Aquatic Ecosystems

J. M. Benoit[1], C. C. Gilmour[2], A. Heyes[3], R. P. Mason[3], and C. L. Miller[3]

[1]Chemistry Department, Wheaton College, Norton, MA 02766
[2]The Academy of Natural Sciences, Benedict Estuarine Research Center, St. Leonard, MD 20685
[3]University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, Solomons, MD 20688

It is the goal of this paper to discuss the more salient recent advances in the understanding of the controls of net CH_3Hg formation in natural systems. The discussion highlights the gaps in knowledge and the areas where progress in understanding has occurred. In particular, this chapter focuses on recent developments in Hg bioavailability and uptake by methylating bacteria, on the competing roles of sulfate and sulfide in the control of methylation, and in pathways for demethylation. The role of sulfide in influencing methylation is discussed in detail. In addition, the impact of other environmental variables such as pH, dissolved organic carbon and temperature on mercury methylation are discussed. Lastly, we provide a synthesis of the variability in the methylation response to Hg inputs across ecosystems. We suggest that although methylation is a function of Hg concentration, the range of methylation rates across ecosystems is larger than the range in Hg deposition rates. Overall, we conclude that factors in addition to the amount Hg deposition play a large role in controlling CH_3Hg production and bioaccumulation in aquatic ecosystems.

Introduction

Mercury (Hg) inputs to the environment have been increased dramatically since industrialization and anthropogenic sources of Hg to the atmosphere now dominate the input (*1-3*). While inorganic Hg is the major source of Hg to most aquatic systems, it is methylmercury (CH_3Hg) that bioconcentrates in aquatic food webs and is the source of health advisories worldwide that caution against the consumption of fish containing elevated CH_3Hg (*4-7*). Although a small fraction of the Hg in atmospheric deposition is CH_3Hg, the dominant source of CH_3Hg to most aquatic systems is *in situ* formation, or formation within the watershed (*8-12*). The current consensus, based mainly on temperature-dependency of Hg methylation and its response to biological substrates (*13-16*), is that biological methylation of inorganic Hg to CH_3Hg is more important than abiotic processes in natural systems. Biological methylation was first demonstrated in the late 1960's (*17*) and it is now generally accepted that sulfate reducing bacteria (SRB) are the key Hg methylators (*13,18-20*) although a number of organisms besides SRBs have been shown to produce CH_3Hg in pure culture from added Hg(II) (*21*).

The role of SRBs in methylation has been demonstrated through studies using specific metabolic inhibitors, addition of sulfate, and coincident measurement of sulfate reduction rate and CH_3Hg production. The addition of BES, a specific inhibitor of methanogens, was shown to increase Hg methylation while molybdate, a specific inhibitor of sulfate reduction, dramatically decreased CH_3Hg production in saltmarsh sediment (*13*). Since this early study, molybdate inhibition of mercury methylation, and coincident depth-profiles of sulfate-reduction rate and Hg methylation have demonstrated the importance of SRBs in estuarine, freshwater lake, saltmarsh, and Everglades sediments (*19,22-24*). Furthermore, addition of sulfate has been shown to stimulate mercury methylation in concert with stimulation of sulfate reduction, most notably during the whole lake sulfuric acid addition experiment in Little Rock Lake, WI (*18,25,26*) and in a series of short and long-term sulfate addition studies in freshwater ecosystems (*19,20,24,27*). However, while sulfate stimulates both sulfate reduction and mercury methylation at low sulfate concentrations, the build up of dissolved sulfide at high sulfate concentrations can inhibit Hg methylation (*28-30*). The mechanism of sulfide inhibition of Hg methylation is discussed in detail in this chapter. The sulfate addition experiments suggest that increased atmospheric sulfuric acid deposition in this century ("acid rain") may have lead to enhanced Hg methylation in remote freshwater ecosystems (*20,24*).

Overall, as many factors influence both methylation and the reverse reaction, demethylation, *in situ* CH_3Hg concentration is a complex function of its rate of formation and loss.

Community structure studies, using molecular probes and other techniques, have shown correspondence between the distribution of certain types of SRB and Hg methylation in sediments, and between sulfate reduction rate and Hg methylation rate (*31-34*). The primary site of methylation is just below the oxic/anoxic interface, which is often near the sediment surface in aquatic systems (*9,16,35-37*). It should be noted, however, that CH_3Hg can be produced in environments where sulfate reduction is low, such as upland soils, where other bacteria and fungi may be important methylators. However, little work has been done in these upland environments, as studies have rather focused on environments within aquatic ecosystems where the CH_3Hg produced has greatest likelihood of entering the aquatic food chain, and where sulfate reduction is a dominant degradation pathway for organic matter in sediments. Even though sulfate reduction and Hg methylation are linked, it should be noted that some SRB can methylate Hg while growing fermentatively (*38*).

One obvious mediator of Hg methylation rate is the concentration of inorganic Hg substrate, and its chemical form. Although there is a significant relationship between Hg and CH_3Hg across ecosystems, Hg does not appear to be largest source of variability in CH_3Hg production among ecosystems. The relationship between Hg and CH_3Hg concentrations in surface lake, river and estuary sediments and wetland soils across many ecosystems is weak but there is, on average, about 1% of the total Hg as CH_3Hg for the lower concentration (<500 ng/g) sites (Figure 1), which represent the range in Hg concentration of natural, unimpacted environments. The measured concentration at any time point is an integration of the impact of all the processes influencing CH_3Hg, such as differing loading rates (*39,40*) and methylation and demethylation rates (*16*), which vary spatially and temporarily (with season and temperature). Such variation is not accounted for in the data used in this plot, which include published and unpublished values from ongoing studies - see Figure caption for references. Only data collected and analyzed using trace-metal-free techniques were included here and the relationship is geographically biased, and favors contaminated systems. Additionally, as the data were not normally distributed, a log relationship is plotted ($r^2 = 0.41$; $p<0.01$; Fig. 1).

The data in Figure 1 appear to cluster into two sets, with Hg concentrations exceeding 500 ng g^{-1} having little increased impact on CH_3Hg production. For ecosystem types, the relationship has been found to be significant for estuaries ($r^2 = 0.78$, $p<0.01$), lakes ($r^2 = 0.64$, $p=0.01$) and rivers ($r^2 =0.68$, $p=0.01$) but not for wetlands ($r^2=0.29$, $p>0.05$) based on data in Figure

1. Overall, within single rivers or wetlands, or even clusters of similar ecosystems, significant relationships can exist, but the relationships currently have no predictive power, given the importance of other parameters, discussed below, in influencing methylation rate by controlling the availability of Hg to, and activity of, the methylating bacteria, and given our current level of understanding. Effective regulation of Hg pollution requires the ability to predict the relationship between Hg and CH_3Hg among ecosystems, a goal that researchers and modelers seem to be slowly approaching. Detailed investigations of the mechanisms of CH_3Hg formation, degradation, fate and transport are required, so that the factors controlling the levels of CH_3Hg in fish can be understood. Clearly, while many other factors influence Hg methylation, the supply and availability of Hg is a key parameter.

In addition to the effect of sulfide, other chemical factors influencing methylation include the supply of labile carbon (*57,58*) although the role of dissolved organic carbon (DOC) is complex. The distribution of methylation activity is tied to the distribution of biodegradable organic matter but complexation of Hg by DOC may influence Hg bioavailability. Maximal net methylation is often observed in surface sediments (*15,16*) where microbial activity is greatest due to the input of fresh organic matter. As a result, systems with high levels of organic matter production, such as wetlands, recently flooded reservoirs, or periodically flooded river plains, may exhibit extremely high rates of methylmercury production (*10,42,59,60*). New research on Hg complexation with DOC is highlighted below. Temperature is another important variable (*61*) as the temperature responses of methylation and demethylation have been reported to differ (*16,62*). However, seasonal changes in Hg complexation that affect methylation and demethylation differently could account for these observations.

Demethylation of CH_3Hg can occur via a number of mechanisms, including microbial demethylation and reduction by *mer* operon-mediated pathways, and by "oxidative demethylation processes" (*21,63-71*). In addition, photochemical CH_3Hg degradation in the water column has been demonstrated (*72*). The *mer*-based pathway is an inducible detoxification mechanism, while oxidative demethylation is thought to be a type of C1 metabolism. Recent research suggests that oxidative demethylation is the dominant process in uncontaminated surface sediments (*65,70,71*).

It is the goal of this paper to discuss the more salient recent advances in the understanding of the controls of net CH_3Hg formation in natural systems. Therefore, rather than being a complete review of the literature, this chapter will provide an in-depth examination of some of the pertinent recent papers and current developments, and will endeavor to highlight the gaps in knowledge and the areas where progress in understanding has occurred. In particular, this chapter focuses on recent developments in Hg bioavailability and uptake by

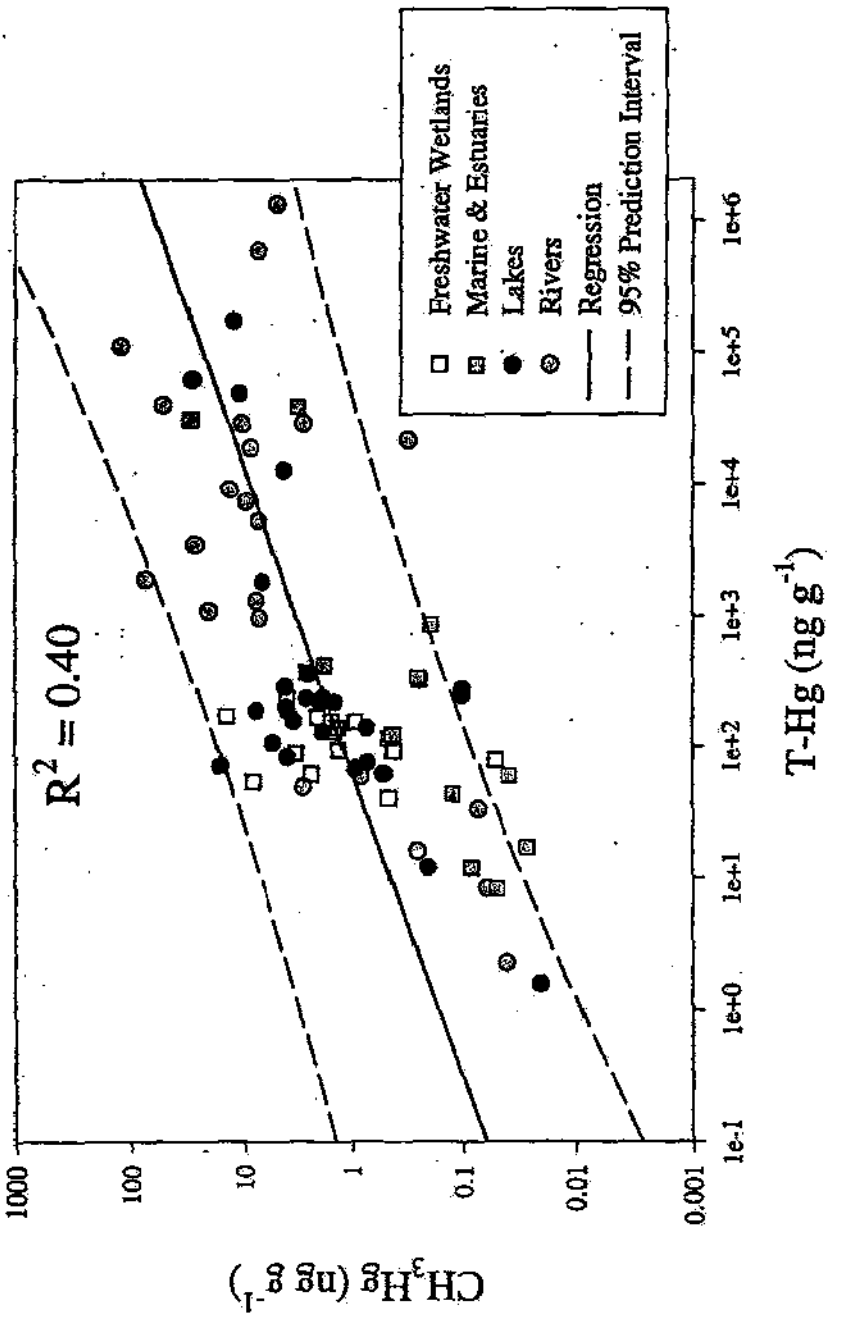

$R^2 = 0.40$
Freshwater Wetlands
Marine & Estuaries
Lakes
Rivers
Regression
95% Prediction Interval
CH_3Hg (ng g^{-1})
T-Hg (ng g^{-1})
1000
100
10
1
0.1
0.01
0.001
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6

Figure 1. Mercury (Hg) and methylmercury (CH_3Hg) in near surface (0-4 cm) sediment in freshwater wetlands from: North and South Carolina (41), Ontario, Canada (42), Florida Everglades (37); Marine and Estuarine sediments from: coastal N. and S. Carolina (34), The Chesapeake Bay and its Estuaries (43,44), coastal Florida (45), coastal Texas (46), Slovenia coast (47), coastal Poland (48) coastal Malaysia (48), Anadyr Estuary, Russia (48); Lakes: New Jersey (41), New York State (49),Wisconsin (41,50), California (51), Finland (52), Poland (48); Rivers: S. Carolina (41), Wisconsin (53), Nevada (54), Alaska (55), Germany (56), Poland (48).

methylating bacteria, on the competing roles of sulfate and sulfide in the control of methylation, and in pathways for demethylation. Lastly, we provide a synthesis of the variability in the methylation response to Hg inputs across ecosystems. We suggest that although methylation is a function of Hg concentration, the range of methylation rates across ecosystems is larger than the range in Hg deposition rates, and that factors in addition to Hg deposition play a large role in controlling CH_3Hg production and bioaccumulation in aquatic ecosystems.

Mercury Speciation and Methylation

Although mercury resistant bacteria possessing the *mer* operon have the ability to actively transport Hg(II), this operon is not present in SRB that methylate mercury (*38*). It is generally accepted that CH_3Hg is produced in an accidental side reaction of a metabolic pathway involving methylcobalamin (*73*), although this pathway has only been demonstrated in one SRB. Therefore, it is not likely that SRB have acquired an active transport for this toxin. A limited number of experiments with SRB support this idea (*38*). For this reason, diffusion across the cell membrane has been proposed as the important uptake mechanism (*30,38,74*). This hypothesis is consistent with studies that have demonstrated diffusion of neutral mercury complexes (chloride complexes) across artificial membranes and into diatom cells (*75-77*).

The diffusion hypothesis is also supported by the relationship between methylation and the distribution of neutral Hg sulfide complexes in sediments. It has been noted in many field studies that the rate of Hg methylation, and the CH_3Hg concentration in sediments, decrease as the sediment sulfide concentration increases (*28,29,36,37,74,78,79*). A number of mercury complexes exist in solution in the presence of dissolved sulfide, including HgS°, $Hg(SH)_2^\circ$, $Hg(SH)^+$, HgS_2^{2-} and $HgHS_2^-$ (*80-84*) and it is possible that inorganic Hg uptake by SRB occurs via diffusion of the dissolved neutral Hg complexes, such as HgS°. If so, then the bioavailability of Hg to the bacteria would be a function of sulfide levels, as this is the ligand controlling Hg speciation in solution in low oxygen zones where SRB are active. It has been shown through chemical complexation modeling that the speciation of Hg tends to shift toward charged complexes as sulfide levels increase (*74,84,85*), decreasing the fraction of Hg as uncharged complexes, and, as a result, the bioavailability of Hg to methylating bacteria.

The existence of neutrally charged Hg-S complexes, and the notion of decreasing bioavailability in the presence of sulfide, was demonstrated in the laboratory using a surrogate measure of membrane permeability, the octanol-water partition coefficient (K_{ow}; 85). These experiments showed that HgS° and

$Hg(SH)_2^\circ$, the neutral complexes present under the experimental conditions, had relatively high K_{ow}'s such that they would be taken up at rates more than sufficient to account for methylation in both pure cultures and in the field, based on the estimated permeability through the cell membrane, which is a function of K_{ow} (*30,74,86*). There is the potential for polysulfide formation in porewaters and the solubility of Hg in the presence of HgS(s) has been shown to be dramatically increased by the complexation of Hg with polysulfide species (*87*). However, these interactions do not appear to significantly enhance the concentration of neutral Hg species, as measured by changes in the K_{ow}, suggesting that the dominant polysulfide complexes are charged (e.g., HgS_xOH^- and $Hg(S_x)^{2-}$; *87*) and thus unavailable for uptake and methylation by SRB.

It should be noted here that theoretical chemical calculations suggest the neutral species in solution is $HOHgSH^\circ$ rather than HgS°, as it is likely that Hg would form a more stable linear complex (*88*). The results of Benoit et al. (*30*) are not in disagreement with this notion even though $HOHgSH^\circ$ would have a lower permeability, because of its larger molecular volume, than HgS°. The relationship between permeability and K_{ow} is extremely non linear (*89*) and $HOHgSH^\circ$ would have a permeability that is a factor of five less than that of HgS°. In the pure culture studies, estimated uptake was greatly in excess of methylation rate (Figure 2a) even if the neutral complex is considered to be $HOHgSH^\circ$.

Experiments with pure cultures and other studies indicate that not all the Hg taken up by the bacteria is methylated as there are other sinks within the cell for Hg (*30*). This is shown illustratively in Figure 2b, where Hg_C represents the pool of Hg available for methylation inside the cell. Given uptake of a neutral Hg complex ($[HgL_n^\circ]$), the intracellular steady state concentration of Hg_C is given by:

$$[Hg_C] = k_D.[HgL_n^\circ]/(k_B + k_M)$$

where k_M is the methylation rate constant, k_D is the diffusion rate, HgL_n° is the concentration of neutral complex in solution and k_B is the rate constant that incorporates all the other processes that are rendering Hg unavailable for methylation within the cell. The rate of formation of CH_3Hg, assuming no loss mechanisms, is then given by:

$$d[CH_3Hg]/dt = k_M.[Hg_C] = k_D.k_M.[HgL_n^\circ]/(k_B + k_M)$$

If k_M is much greater than k_B, then the rate of CH_3Hg formation is directly related to the rate of diffusion across the membrane. However, in the opposite case, the rate of methylation is dependent on both the rate of uptake and the rate at which Hg is being sequestered within the cell. However, there would still be a

Fig. 2a

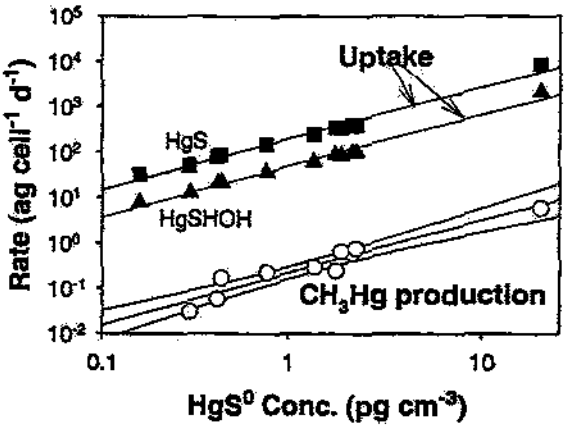


Fig. 2b

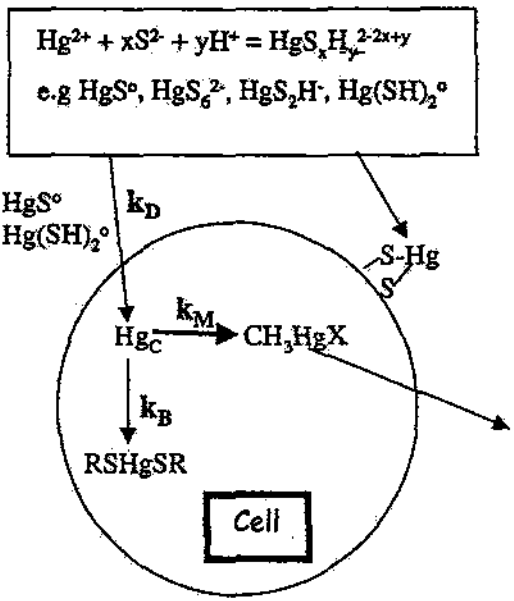


Figure 2. a) Estimated mercury (Hg) uptake rate, assuming passive diffusion of neutral Hg complexes - either modeled as HgS^o or $HOHgSH^o$, and the simultaneous Hg methylation rate in pure cultures of D. propionicus. b) Model representation of the assumed process of Hg uptake and methylation within this organism. The rate of methylation is designated as k_M; k_B is the combined rate of competing reactions that sequester Hg and make it unavailable for methylation and k_D is the uptake rate. See text for details.

linear relationship between the neutral complex uptake rate and methylation rate across a sulfide gradient, for example, if the rate constant, k_B, was relatively independent of the environmental conditions outside the cell, which is likely. At the limit, if $k_B >> k_M$, methylation will not occur to any measurable degree.

Studies with laboratory cultures of *Desulfobulbus proprionicus* across a sulfide gradient have shown that the uptake rate, as estimated from the dissolved Hg speciation and the estimated permeability of the complexes, and methylation rate changed in a similar fashion, and both increased with increasing HgS° concentration (Figure 2a), supporting the above hypothesis (*86*). These results also suggest that k_B is greater than k_M in this case and that a small fraction of the Hg taken up is methylated. The relative percent methylated would depend on a number of factors that would differ between organisms, due to differences in physiology, size and membrane composition. We propose that differences in Hg partitioning within cells may partially explain the large differences in methylation rates among various strains of SRB.

The hypothesis of neutral complex bioavailability controlling methylation begs the question why methylation appears to be confined mostly to SRBs in the environment. Clearly, these organisms dominate in the region where HgS° dominates the Hg speciation. Why then, in oxic or suboxic environments, where $HgCl_2^\circ$ and other neutral Hg complexes exist, is there is little evidence of methylation? In studies with diatoms, Mason et al. (*76*) demonstrated that little of the $HgCl_2$ taken up (<10%) reached the cytoplasm of the organisms, with most Hg being rapidly bound within the cellular membrane. On the other hand, CH_3HgCl was less strongly bound within the membrane and a greater fraction was found in the cytoplasm of the diatom (63%). The intracellular distribution is related to the rate and degree of reaction of the accumulated complex with cellular sites. The rate of sequestration (k_B), which would determine where the Hg would become bound within a cell, depends on the exchange reaction between the neutral complex and the cellular reaction site (RH); for example, $HgS^\circ + RH = HgR^+ + SH^-$. The kinetics of this process would be to some degree determined by the reaction mechanism (adjunctive or disjunctive), but are a strong function of the relative magnitude of the equilibrium constants for the accumulated complex and HgR^+. Given that most cellular binding sites for Hg are likely thiol groups, the rate of the exchange reaction should be faster for $HgCl_2$ than for HgS°, as the associated equilibrium constant ($HgCl_2^\circ + RH = HgR^+ + H^+ + 2Cl^-$) is much greater for $HgCl_2$. Furthermore, given the high stability of HgS°, its rate of dissociation will be slower than that of $HgCl_2$. Therefore, in the presence of HgS°, a higher fraction of the Hg diffusing across the outer membrane is transferred to the site within the cytoplasm where methylation can occur compared to other organisms, because of the kinetics of the intracellular reactions.

Thus, it is not sufficient that the pathway for methylation exists within an organism but also that the Hg can be transported within the cell to the site of the reaction. As discussed below, methylation has been linked to the acetyl-CoA pathway in one bacterium (*73*) and a simple explanation of why some organisms which have this pathway do not methylate Hg is that the Hg is being bound to other intracellular sites before being transported to the site of methylation. Thus, there are kinetic and biochemical factors that influence the relative degree of methylation between organisms besides Hg bioavailability and Hg content in the medium. The kinetics and location of the intercellular reactions are an important modifier of the methylation rate and clearly more studies on the intracellular mechanisms of methylation are needed.

A simple model of Hg partitioning in sediments was developed to explore how Hg partitioning to solids impacts Hg concentration and bioavailability in sediment pore waters (*74*). The model developed used adsorption reactions as the mechanism controlling porewater concentration, since field measurements show that pure cinnabar equilibrium dissolution dramatically over predicts the concentration of Hg in porewaters. It should also be noted that there is little field evidence to support the formation of the pure HgS solid phase in environmental media (*90-92*). In the model, water column speciation was driven primarily by sulfide concentration. The adsorption of Hg was modeled with two types of binding sites, singly or double coordinated thiols, which could represent either an interaction with organic thiol and/or inorganic sulfide groups in the solid phase. This model was not only able to predict both the measured distribution of Hg in sediment porewaters in two ecosystems (the Patuxent River and the Florida Everglades) but the model-predicted concentration of neutral Hg-S complexes correlated well with the *in-situ* CH_3Hg concentration (*74*).

The model and the laboratory culture studies (*30,74,86*) cover the speciation of Hg in the presence of sulfide but it is known that Hg binds strongly to organic matter, and that dissolved organic carbon (DOC) impacts Hg methylation. The impact of organic content on Hg methylation appears to be complex (*16,57,58*). While Hg binds strongly to DOC, laboratory complexation studies using DOC isolates from the Florida Everglades suggest that this binding is not sufficient for Hg-DOC complexation to be important in systems where sulfide is present (*93,94*). Thus, while DOC has been shown to be the most important complexing ligand in surface waters in the absence of sulfide (*94,95*), it is likely to be unimportant in Hg complexation in sediment porewaters under typical DOC concentrations and >0.01 ΦM sulfide (*93,94*). However, binding of Hg to organic matter is important in the solid phase. Laboratory studies suggest that in oxic regions, organic complexation is much more important than binding of Hg to metal oxide phases in all except very low organic matter sediments (*96*). It has been suggested that Fe-S formation scavenges Hg in anoxic regions of the sediment (*97*) and that Hg binds strongly to pyrite such that, even when only

small amounts are present, it is the dominant solid phase binding Hg in sediments (*90*). These studies focused on regions of low organic content and, in contrast, our sediment sequential extraction studies (*96*) show that Hg is associated with the organic fraction even in the presence of significant solid sulfide phases (AVS and pyrite). Furthermore, it has been shown that the sediment particle-dissolved distribution coefficient (K_d) is a strong function of organic carbon (*46*). In the environment, concentrations of Fe, S and C typically co-vary in sediments, and all often correlate with Hg, and it is therefore difficult to ascertain from field data which is the ultimate controlling phase (*44,98*). Laboratory and field studies (*96,99,100*) suggest that the binding of Hg to organic matter involves interaction with the thiol groups of the organic molecules and thus, in a sense, the complexation of Hg to inorganic sulfide phases or to organic matter are comparable as both involve the interaction between Hg and a reduced S species.

The role of pH needs to be considered as the complexation with sulfide and thiols involves acid-base chemistry. An inverse correlation between lake water pH and mercury in fish tissues has been observed in a number of studies (*101 and references therein*) suggesting that pH influences methylation and demethylation in aquatic ecosystems. In some freshwater studies, methylation was reduced with decreasing pH (*27,35*) while the impact on demethylation was small. In other studies, increasing rates of mercury methylation in epilimnetic lake waters and at the sediment surface were found with lowered pH (*57,102,103*). Winfrey and Rudd (*35*) reviewed potential mechanisms for low pH effects on mercury methylation and suggested that changes in mercury binding could account for the seemingly conflicting results seen in all of these studies. They pointed out that lowering pH may lead to increased association of mercury with solid phases, decreased dissolved pore water mercury, and (presumably) to lower availability of Hg(II) to bacteria. The model discussed above (*74*) can be used as a simple predictor of the impact of pH on Hg methylation. Considering the reaction of Hg with the solid phase ($RSH + Hg^{2+} = RSHg^{+} + H^{+}$), and the dissolved speciation, the following overall reaction can be postulated:

$$RSHg^{+} + H_2O + HS^{-} = HgS^{\circ} + RSH + H^{+}$$

In the pH range of 7-10 (pK_{a1} ~7 for H_2S and assuming the pK for RSH is around 10), an increase in pH, at constant sulfide, will result in an increase in HgS° relative to $RSHg^{+}$ ($[HS^{-}]/[RSH]$ is essentially constant) and thus methylation should increase with pH. Below a pH of 7, decreasing pH (increasing $[H^{+}]$) leads to decreasing $[HS^{-}]$, and as a result, HgS° will decrease relative to $RSHg^{+}$ with pH i.e., methylation rate should decrease. This theoretical consideration supports the notions put forward by Winfrey and Rudd (*35*) and

suggests that a decrease in pH will lead to a decrease in methylation rate in sediments because of changes in the concentration of bioavailable Hg in porewaters. The magnitude of the effect will depend on the pH range as the impact of pH is more marked at low pH. Overall, these considerations suggest that sulfide concentration will have the most significant impact on Hg bioavailability in porewater but that other factors such as organic matter content, pH, temperature and the presence or absence of inorganic sulfide phases all play a role in controlling Hg bioavailability to methylating bacteria.

The conflicting influences of sulfate and sulfide on the extent of Hg methylation are well illustrated by the studies in the Florida Everglades (*37*). Studies over four years at eight sites that cover a large gradient in sulfate and sulfide showed that the highest methylation rates, and the highest $\%CH_3Hg$ in the sediment, were at sites of intermediate sulfate-reduction rates and sulfide concentration (Figure 3). In the Everglades, the north-south trend in sulfate concentration leads to a similar trend in sulfate reduction rate and porewater sulfide. As the sulfide concentration decreases, the relative concentration of predicted HgS° concentration increases. The peak in methylation rate results from the combination of the increasing availability of Hg to the SRB coupled with the decreasing sulfate reduction rate north to south. These results confirm the importance of both Hg speciation, and bacterial activity, in controlling Hg methylation rate. Overall, the sites with the highest Hg methylation are those that also have the highest fish CH_3Hg concentrations (*104*), confirming the direct link between the extent of Hg methylation and fish CH_3Hg levels.

Experiments in which Everglades sediments were incubated with additional sulfate or sulfide further demonstrated the interplay between bacterial activity and Hg speciation. In cores taken from a relatively low sulfate site, addition of sulfate stimulated methylation, and sulfate reduction, over that of unamended control treatments even though sulfide levels increased slightly (see example experiments in Figure 4). In these sulfate-limited sediments, the higher induced bacterial activity more than compensated for the slightly lower bioavailability of Hg at the higher sulfide levels. Addition of sulfide alone however resulted in inhibition of methylation. It is clear from this and other experiments that inhibition occurs at low ΦM sulfide concentrations in Everglades sediments. However, high rates of Hg methylation have been demonstrated in highly sulfidic saltmarsh sediments (*32*). Perhaps the high rates of sulfate-reduction in these sediments make up for the very low percentage of dissolved Hg that would exist as neutral species. For Everglades sites with higher ambient sulfate, addition of sulfate did not increase methylation but addition of more sulfide led to an inhibition of Hg methylation. The field data across sites show a decrease in methylation rate when concentrations of sulfide increase above 10 ΦM (Figure 3), consistent with the core incubation data. Overall, the results of the field and laboratory studies show that the balance between sulfate availability,

which controls SRB activity, and sulfide production and accumulation, which control Hg bioavailablity, are critical in modeling methylation rates. Ongoing mesocosm studies in the Everglades and in a boreal ecosystem should provide more quantitative equations for Hg cycling models.

Studies in a number of sites have now demonstrated a relatively strong relationship between the concentration of CH_3Hg in sediments and the instantaneous (short-term) rate of Hg methylation. Methylation rates can be estimated by Hg spike additions, either as a radioactive or a stable isotope, preferably to microbial communities held relatively intact (*18,41,103,105*) (Figure 5). The methylation rate constant, k_M, is calculated as the amount of new isotopic CH_3Hg formed per unit time, divided by the pool size of substrate. Methylation rate is derived by multiplying k_M by the total Hg pool size. Use of either custom-synthesized, high specific-activity ^{203}Hg, or the use of individual Hg stable isotopes combined with analysis by ICP-MS, allows methylation measurements to be done at relatively low, near ambient levels. In addition, when using stable isotopes it is possible to track both the *in situ* Hg and the added Hg spike and compare relative methylation rates (*106*). Furthermore, methylation and demethylation can be measured simultaneously in the cores if different isotopes are used for Hg and CH_3Hg (*41,105,106*).

Figure 5 shows relationships between methylation rates and ambient CH_3Hg concentration, in sets of 1m diameter enclosures at four sites across the Florida Everglades. In these studies, $^{202}HgCl_2$ was added to the surface water of the enclosures, and $CH_3{}^{202}Hg$ production was followed over time in surface sediments. Additionally, 5 cm sediment cores were removed from the enclosures for the estimation of instantaneous methylation rate, using $^{199}HgCl_2$ injected into the cores. The figure shows the concentration of $CH_3{}^{202}Hg$ in sediments in the spiked enclosures after 51 days, and production of $CH_3{}^{199}Hg$ in cores after 2 hours, both in comparison with *in situ* sediment CH_3Hg concentrations. Short term rates, net $CH_3{}^{202}Hg$ production after nearly 2 months, and the *in situ* concentration of CH_3Hg in the enclosures all provided the same information about the relative degree of methylation among sites. Measurement of short-term gross methylation, from an exogenous Hg spike, appears to be a good predictor of the relative steady state CH_3Hg concentration across sites within a specific ecosystem; i.e. a good relative measure of the propensity for methylation at each site.

The relationship between new CH_3Hg production, as measured by short term incubation, and *in situ* CH_3Hg concentration remains strong within a single ecosystems over time. In the Experimental Lakes Area, Ontario, Canada (ELA) wetlands, we have observed a persistent relationship between new production and *in situ* concentration of CH_3Hg. However, the slope of the line changed seasonally. This was not a purely temperature dependent response, as CH_3Hg production and concentration peaked in the fall and not in the height of summer

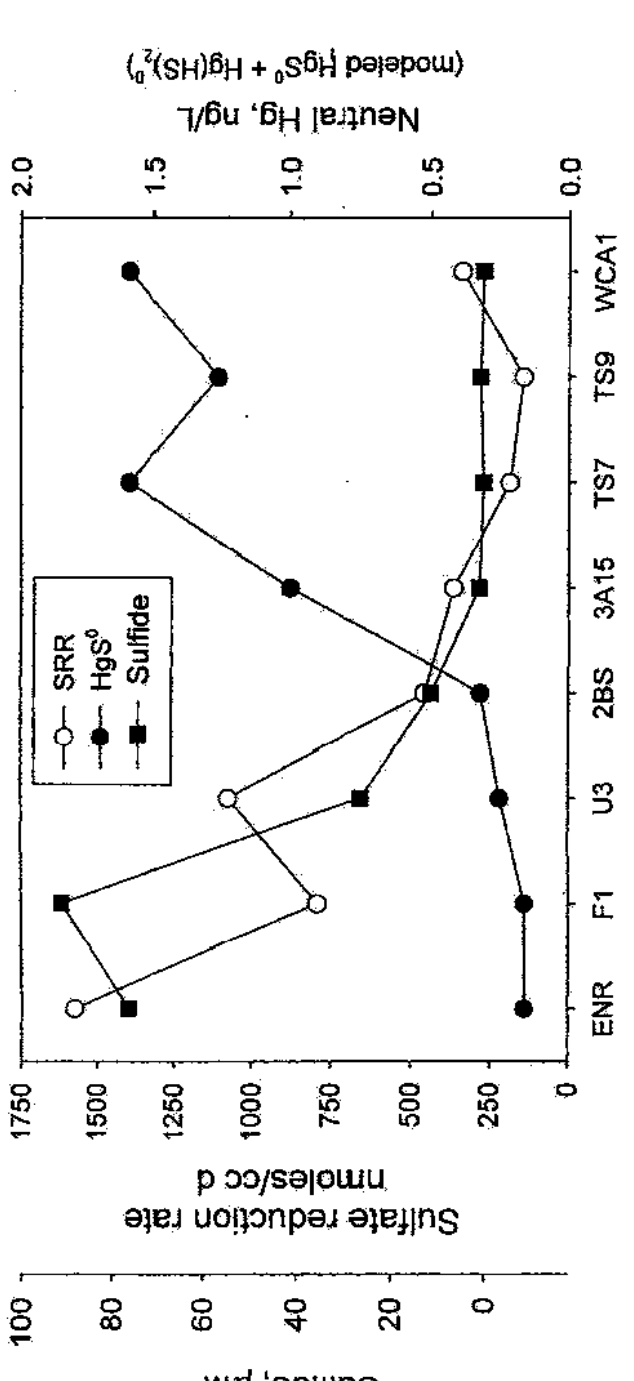
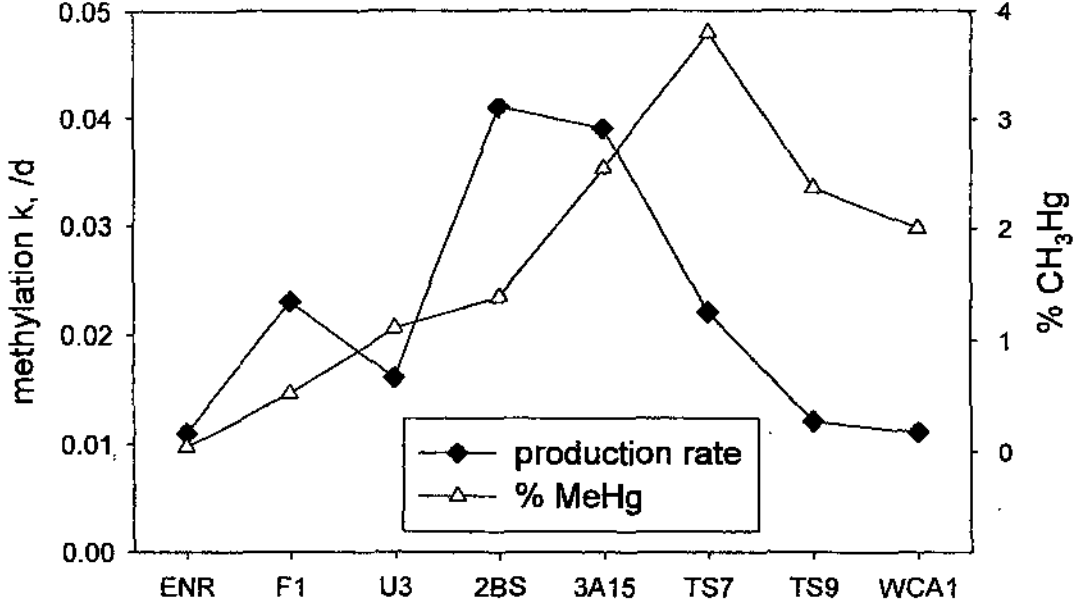

Sulfide, µM
100
80
60
40
20
0
Sulfate reduction rate
nmoles/cc d
1750
1500
1250
1000
750
500
250
0
SRR
HgS⁰
Sulfide
ENR
F1
U3
2BS
3A15
TS7
TS9
WCA1
Neutral Hg, ng/L
(modeled HgS⁰ + Hg(HS)₂⁰)
2.0
1.5
1.0
0.5
0.0

Figure 3. Measured sulfate reduction rate, porewater sulfide concentration, percent methylmercury (%CH_3Hg), mercury methylation rate and modeled porewater HgS^o in the upper 4 cm of Florida Everglades sediments at 8 ACME sites. Everglades sites are arranged from left to right by average surface water sulfate concentration (highest concentrations on the left). With the exception of the WCA 1 site, this represents a north to south transect, running from the Everglades Nutrient Removal Project (ENR) and Water Conservation Area 2A (F1, U3) in the north, through Water Conservation Areas 2B (2BS) and 3A (3A15), and to Taylor Slough in Everglades National Park (TS7, TS9) in the south. Data shown are averages from three years (1995-1998) of bi- to tri-annual sampling. Methods are described in ref. 37.

Continued on next page.


0.05
0.04
0.03
0.02
0.01
0.00
methylation k, /d
4
3
2
1
0
% CH_3Hg
production rate
% MeHg
ENR
F1
U3
2BS
3A15
TS7
TS9
WCA1

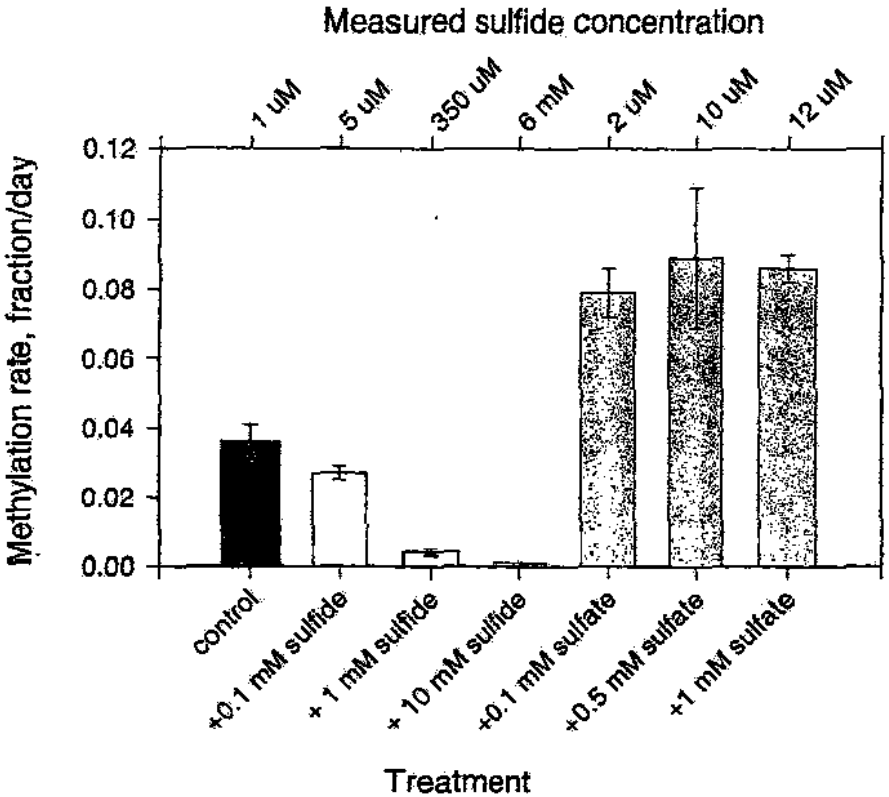
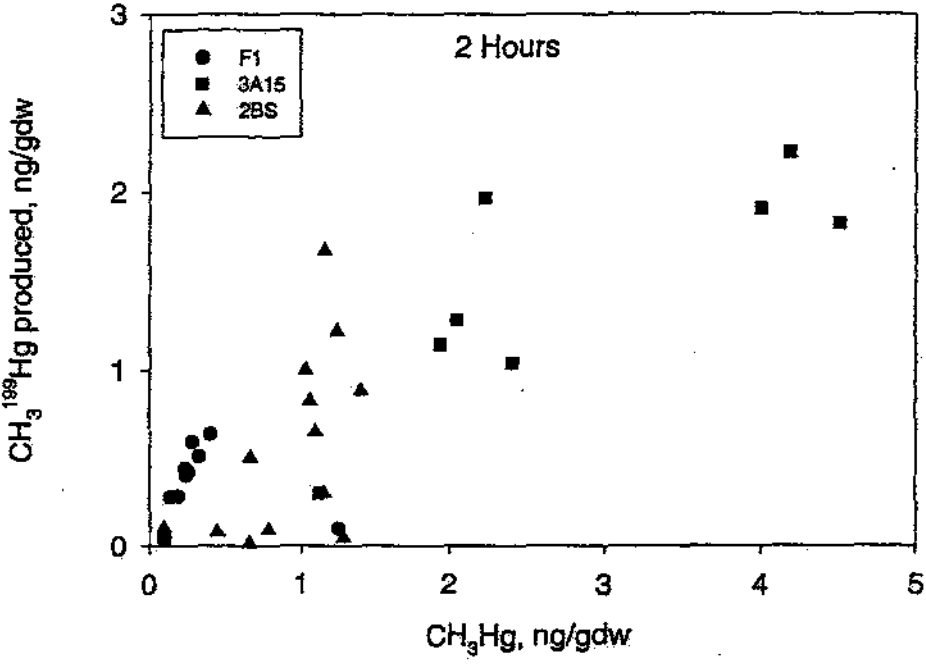


Figure 4. Methylmercury (CH_3Hg) production in Florida Everglades sediment cores after addition of either sulfate (light grey bars) or sulfide (white bars). Sediment cores, taken from the central area of the Loxahatchee National Wildlife Refuge (LNWR), were amended with either sodium sulfide or sodium sulfate (at neutral pH), by injection into the top 4 cm of sediment. The calculated concentration of the spikes in pore water after injection is shown on the bottom axis, based on measured porosity. After 1 hr of incubation with sulfate or sulfide, mercury methylation was estimated in the cores using high specific activity ^{203}Hg (18, 37). Methylation assays were conducted over 2 hr at ambient temperature. The final measured concentration of sulfide in sediment porewaters, three hours after the sulfate or sulfide spikes, is shown on the top axis. All measurements were made from triplicate cores for each treatment. Sediments spiked with sulfide sequestered much of it into the solid phase. Sediments spiked with sulfate produced measurable porewater sulfide, via sulfate reduction, within 3 hours. The LNWR is a very low-sulfate area within the Everglades. In these cores, addition of sulfate stimulated methylation even though sulfide levels increased slightly, while the addition of as little as 5 μM sulfide alone inhibited methylation.


2 Hours
F1
3A15
2BS
$CH_3{}^{199}Hg$ produced, ng/gdw
CH_3Hg, ng/gdw
0
1
2
3
4
5

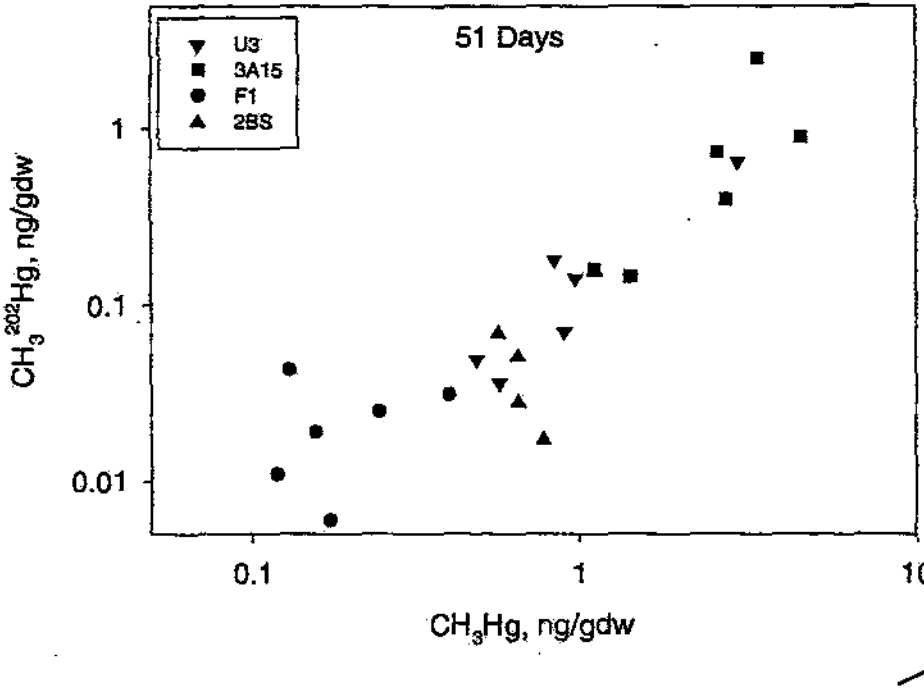

51 Days
U3
3A15
F1
2BS
$CH_3{}^{202}Hg$, ng/gdw
CH_3Hg, ng/gdw
0.01
0.1
1
10

Figure 5. Relationship between in situ methylmercury (CH_3Hg) concentration and methylmercury production from stable isotope spikes in Everglades surface sediments: a) $CH_3{}^{199}Hg$ production from ^{199}Hg injected into Everglades sediment cores after an incubation period of 2 hrs. vs. in situ CH_3Hg; b) $CH_3{}^{202}Hg$ in enclosure surface sediments 51 days after the enclosures were amended with ^{202}Hg vs. in situ native CH_3Hg. These measurements were made in experimental, 1m diameter enclosures at four sites across the Everglades, as part of the ACME project. Three enclosures at each site were spiked with ^{202}Hg in May 2000, with spikes ranging from half to two times the equivalent of 1 year of atmospheric deposition. (see text for details; from Krabbenhoft et al., unpublished data).

(Figure 6). While we do not know the reason for the seasonal trend, both the measured methylation and *in situ* concentration were responding in concert to the same controlling factors.

Overall, the rate of production of CH_3Hg from exogenous spikes is generally higher than the rate of methylation from *in situ* Hg pools. This probably reflects the higher bioavailability of the added Hg compared to that in the sediment (*106*). This is illustrated in Figure 7, which shows sediment CH_3Hg levels in a lake enclosure experiment conducted as part of the METAALICUS project in the ELA. The top panel shows *in situ* CH_3Hg as a percentage of total Hg in the sediments in each of the four enclosures throughout the summer. The enclosures were spiked with ^{200}Hg either in mid June (encl.1& 2) or biweekly throughout the summer (encl. 3 & 4). The middle panel shows $CH_3{}^{200}Hg$ as percentage of ^{200}Hg accumulated in sediments. The relative percentage of the added Hg that becomes methylated is initially much higher for the added Hg. Over time, the percentage decreases and this is likely a combination of both reduction in bacterial activity in the fall and a decrease in bioavailability of the Hg over time as it is cycled through the system (methylated, demethylated and complexed to strong binding ligands). With the caveat that the method of Hg addition in our spike experiments may not truly reflect reality, these data suggest that Hg newly deposited to ecosystems is more available for methylation than existing Hg pools. However, more work is needed to further ascertain the crucial question of the relative importance of newly added versus *in situ* Hg in contributing to the Hg that is methylated and bioaccumulated in aquatic systems.

Thus, we suggest that the *in situ* CH_3Hg concentration across a series of sites within an ecosystem can be used to predict which site is likely to be more active in terms of methylation, and likely in terms of bioaccumulation, all else being equal. However, there is too little information at present to determine the degree to which these relationships can be used in a quantitatively predictive fashion between ecosystems. Overall, in comparing across systems, the greatest difficulty is in assessing the pool of Hg available for methylation, which is crucial to estimating realistic accurate methylation rates. To this point, we have not been able to measure bioavailable pools of Hg to bacteria, nor have we been able to mimic the speciation of *in situ* Hg with added Hg. Therefore, short term production remains more qualitative than quantitative. Both of these questions are the focus of ongoing research.

Biological Controls over Methylation

Different organisms clearly have different rates of Hg methylation, even among the SRB, and not all SRB methylate Hg (*24,32*). A small number of Fe-reducing bacteria, that are phylogenetically similar to methylating SRB, have

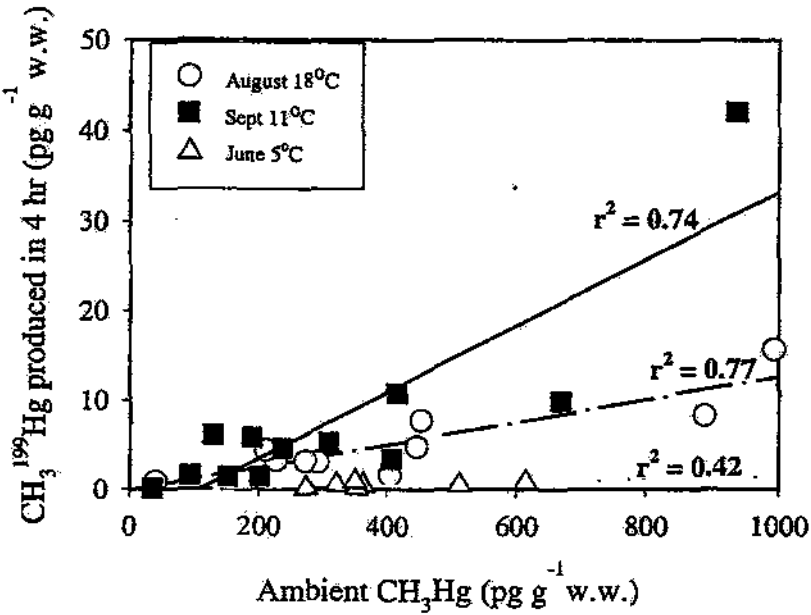


Figure 6. Native in situ methylmercury (CH_3Hg) concentration and excess $CH_3{}^{199}Hg$ produced from ^{199}Hg in 4 hrs, in peat collected in June, August and September, 2000, from a lakeside, sphagnum wetland (L115) at the Experimental Lakes Area (ELA) in northwest Ontario. Work was conducted as part of the METAALICUS project.

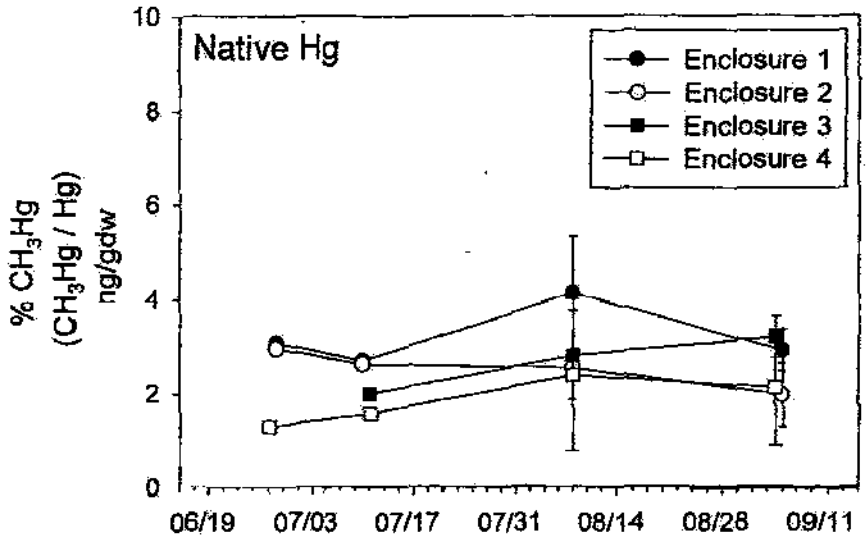


Figure 7. Sediment CH_3Hg levels in a lake enclosure experiment conducted in L329 in 2000 as part of the METAALICUS project in the Experimental Lakes Area (ELA), Ontario, Canada. The surface water of enclosures was spiked with ^{200}Hg either in mid-June (encl. 1 & 2) or biweekly throughout the summer (encl. 3 & 4). Spikes were equivalent to 1 year of atmospheric deposition. The top panel shows native in situ CH_3Hg as a percentage of total Hg in 0-4 cm depth sediments in each of four enclosures throughout the summer. The middle panel shows $CH_3{}^{200}Hg$ as percentage of ^{200}Hg accumulated in sediments. The bottom panel shows water temperature in the enclosure. A much higher percentage of the spike was found methylated in sediments than native Hg, especially within 2 months of the spike.

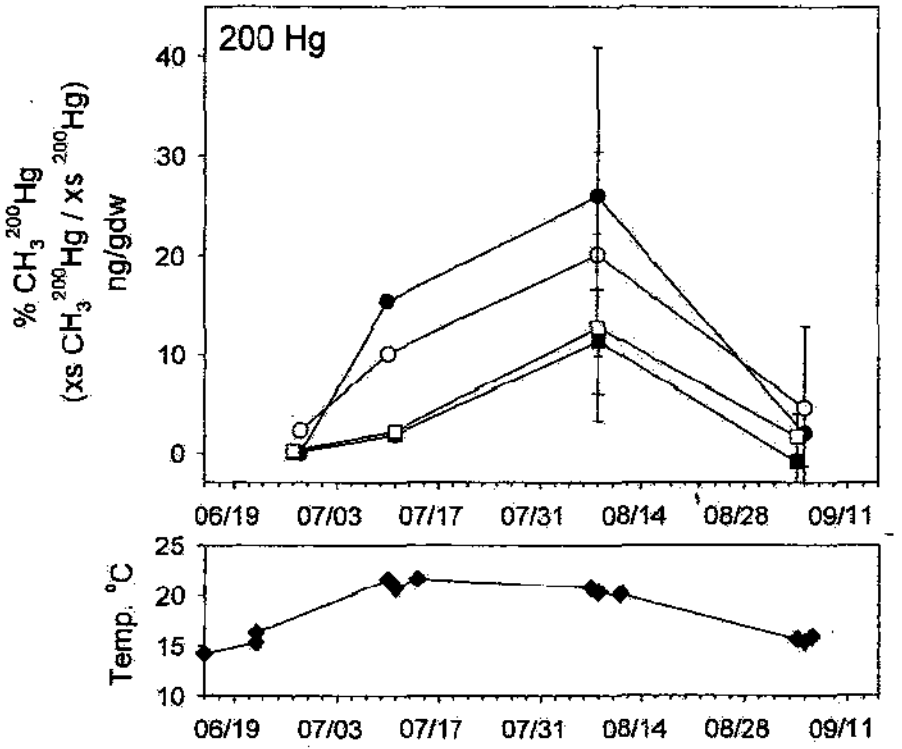


Figure 7. *Continued.*

been shown capable of methylating Hg in pure culture (*107*). While a number of organisms other than SRB have been shown to produce CH_3Hg in pure culture from added Hg(II) (see ref. *24* for a review) the relative rates of methylation by these organisms and their role in *in situ* methylation is unknown. Furthermore, while a number of SRB that are incomplete organic carbon oxidizers readily methylate Hg in culture (e.g. *Desulfobulbus propionicus*, (*30,86*) and *Desulfovibrio desulfuricans*, (*108*)), studies in the environment have suggested that they may not be the dominant Hg methylators. King et al. (*32*) showed in pure culture that, of the organisms they tested, *Desulfobacterium* methylated Hg at a substantially greater rate, under the conditions of their experiments, than the other species tested (*Desulfobacter*, *Desulfococcus*, *Desulfovibrio* and *Desulfobulbus*). *Desulfobacterium* is a complete acetate oxidizer and CH_3Hg was only produced in these cultures when sulfate-reduction was occurring. This contrasts results of others who have shown that *Desulfobulbus propionicus* can methylate Hg while growing fermentatively (*38,86*).

King et al. (*32*) also found that marine sediments amended with acetate produced more CH_3Hg than sediments amended with lactate, or unamended controls. Acetate-amended slurries were dominated by *Desulfobacterium* and *Desulfobacter*. Macalady et al. (*33*), using polar lipid fatty acid analysis, also found that *Desulfobacter*-like organisms were important Hg methylators in sediments of a Hg-contaminated freshwater system, Clear Lake, California. It appears from these results that the organisms capable of complete oxidation of acetate are potentially more efficient methylators in the environment.

However, it is clear that there is some aspect of the mechanism of Hg methylation that allows some bacteria to methylate Hg while others do not. The ability to methylate Hg is not confined to one phylogenetic group of sulfate-reducing bacteria but is scattered throughout the phylogenic tree of sulfate-reducing eubacteria (*41*). Furthermore, phylogenetically similar organisms have differing abilities to methylate Hg -e.g., *Delsulfovibrio gigas*, *D. vulgaris*, *D. salexigens* and *D. desulfuricans aestuarii* do not methylate Hg but *D. desulfuricans* LS (*109*) and ND132 (*24*) do. A pathway for methylation has been demonstrated for only one organism (*Desulfovibrio desulfuricans* LS). Berman et al. (*109*) showed that mercury methylation is an enzymatically catalyzed process *in vivo*, and suggested, based on the selective inhibition of mercury methylation in *D. desulfuricans* LS, that methylation is mediated by a cobalt porphyrin in this organism. Further work (*73,79*) led the group to propose that Hg methylation in this organism occurs via transfer of a methyl group from methyl-tetrahydrofolate to cobalamin to Hg. The methyl group may originate from serine or via the acetyl-CoA synthase pathway.

Mercury methylation by cell extracts of *D. desulfuricans* LS was 600-fold higher compared to free methylcobalamin (*73*), and thus it is not merely the presence of cobalamin that instills the ability to methylate Hg at a significant

rate. Indeed, cobalamin is not unique to SRB. Cobalamin and high levels of acetyl-CoA enzymes are present in methanogens and acetogens, and indeed, cell extracts of a methanogen, have been shown to methylate Hg (*110*). However, these organisms are not thought to play a large role in environmental methylation, based on selective inhibitor studies (*13,19,22,37*). Furthermore, it is not known whether the corrinoid protein found in strain LS is always present in the SRB that do methylate Hg. It has been suggested that SRBs that methylate Hg possess a distinct or highly specific enzyme to catalyze this step. However, the identity of the enzyme responsible for methyl transfer to Hg in most methylators is not known.

Recent studies have shown that of the complete acetate oxidizers, *Desulfococcus multivorans* (1be1), *Desulfosarcina variabilis* (3be13) and *Desulfobacterium autotrophicans* all contain the acetyl-CoA pathway and methylate Hg while *Desulfobacter hydrogenophilus* does not methylate Hg but does have the pathway. Similarly, for SRBs that are not complete oxidizers, there is a correspondence between the presence of the acetyl-CoA pathway and the ability to methylate Hg for *Desulfovibrio africanus*, *D. sulfuricans* LS and *D. vulgaris* (Marburg). However, there are also organisms that methylate Hg that do not have this pathway (*Desulfobulbus propionicus* (1pr3) and *D. propionicus* (MUD)). Thus the acetyl-CoA pathway cannot be the only mechanism for Hg methylation *in vivo*.

Given the reactivity of Hg, it is obvious that the Hg will not be present inside cells as the free metal ion, Hg^{2+}. Thus, the transfer of the methyl group to Hg likely involves the interaction with Hg bound to a ligand, or to an enzyme. There are a number of mechanisms for methyl transfer within cells, but as the Hg is likely in the +2 state within the bacteria, the methyl group needs to be transferred as a radical or as a carbanion, and this restricts the methyl transfer process to that involving electrophilic attack by Hg(II) on cobalamin (*110*). If Hg was in the +1 state, then it could directly substitute for Ni(I) in the normal operation of the acetyl-CoA pathway i.e., be involved in a nucleophilic attack on the corrinoid methyl group. This is an intriguing but untested notion. Alternatively, it may be that the Hg is bound to a particular enzyme or thiol group in some organisms that places it in the correct location for transfer, or that steric hindrance prevents the transfer of Hg in some organisms but not in others. In the acetyl-CoA pathway, the methyl group is transferred to carbon monoxide dehydrogenase (CODH) and if Hg were bound to the active site of the CODH, the transfer of the methyl group directly to the Hg could occur. These ideas are speculative, and further studies should focus on identifying the location of Hg within the cell during methylation.

Bacterial Demethylation

Microbial degradation via the *mer* operon is the best-studied pathway of CH_3Hg degradation. The operon is widely distributed in nature, often co-existing on transposons that also contain antibiotic resistance genes (*111*). Variants on the *mer* operon that include the *mer*B gene confer "broad spectrum" resistance to a variety of organomercury compounds including methyl- and ethylmercury chloride via organomercurial lyase (*21,112*). Microbial degradation of methylmercury occurs through the cleavage of the carbon-mercury bond by the enzyme organomercurial lyase followed by reduction of Hg(II) by mercuric reductase to yield methane and Hg^0 (*21*). The physiology and genetics of *mer*-mediated CH_3Hg degradation and mercury resistance have been extensively reviewed elsewhere (*69,111-115*). While the biochemistry of the commonly studied *mer* operon is fairly well understood, newer studies of the distribution of the *mer* operon in the environment are revealing unexpected polymorphism and genetic diversity (*116-121*).

As many as half of the bacteria from Hg-contaminated sites may contain the *mer* operon (*111*). However, another mechanism also appears to mediate CH_3Hg degradation. While methane and Hg^0 are the primary products of *mer*-mediated Hg demethylation, CO_2 has also been observed as a major methylmercury demethylation product by Oremland and co-workers (*64,70,71*). These authors suggested that methylmercury degradation can occur through biochemical pathways used to derive energy from single carbon substrates, and they termed this process "oxidative demethylation", i.e.:

$$4CH_3Hg^+ + 2H_2O + 4H^+ = 3CH_4 + CO_2 + 4Hg^{2+} + 4H_2$$

As a presumptive C1 metabolic pathway, oxidative demethylation is not an active detoxification pathway for CH_3Hg, unlike *mer*-mediated demethylation and Hg reduction. A variety of aerobes and anaerobes (including sulfate reducers and methanogens) have been implicated in carrying out oxidative demethylation, and oxdative demethylation has been observed in freshwater, estuarine and alkaline-hypersaline sediments (*64,65,70*). However, the identity of the organisms responsible for oxidative demethylation in the environment remains poorly understood. Further, no organism has been isolated that carries out this pathway.

Pak & Bartha (*66*) confirmed the ability of two sulfate reducing bacterial strains and one methanogen strain to demethylate mercury in pure culture. They argued that the CO_2 seen in these studies resulted from oxidation of methane released from CH_3Hg after cleavage via organomercurial lyase by anaerobic methanotrophs in the sediments and that CO_2 was a secondary product and not the primary product of demethylation. However, Marvin-DiPasquale et al. (*71*)

found that the rate of CO_2 production from CH_3Hg far exceeded the rate of CO_2 production from CH_4 in sediments from two of their study ecosystems, under both aerobic and anaerobic conditions.

The relative importance of *mer*-mediated versus oxidative demethylation is poorly understood (*71*). In highly contaminated environments, the *mer* operon is more prevalent among the microbial community, and Hg(II) reduction activity is enhanced (*111*). However, the rate of microbial Hg^0 production in the environment may not always be proportional to *mer* transcription (*123*). Overall rates of microbial activity, the presence of Hg-reducing genes divergent from commonly used probes, and the bioavailability of CH_3Hg to cells also play a role. In systems that are not highly contaminated, oxidative demethylation appears to dominate, under both aerobic and anaerobic conditions. The Hg concentrations that would cause a switch from one pathway to the other are only loosely defined. Most studies of Hg demethylation via oxidative demethylation have employed ^{14}C-labeled CH_3Hg and thus only the carbon products are traceable. The end-product of oxidative demethylation has been presumed to be Hg(II), but that has not been confirmed. Demethylation studies using CH_3Hg containing a specific stable Hg isotope should help resolve that issue.

Bioluminescent Sensors for Mercury

"Bioreporters" are genetically engineered microorganisms designed to rapidly assess the bioavailable concentration of contaminants, or the rate of contaminant degradation. In these bioreporters, the bioluminescence operon (*lux*) is inserted as the sensor component into the biodegradation or resistance pathways of interest. When the pathway is expressed, the *lux* genes are expressed concurrently. A relatively simple measurement of light production can then be used to assess, for example, expression of a metal resistance gene or a hydrocarbon degradation pathway. Clearly, the potential advantage of bioreporters over chemical measurement lies in the possibility of determining bioavailable or bioactive concentrations of the contaminant of interest. Selifonova et al. (*124*) constructed the first Hg bioreporters, fusing the promotorless *lux* operon from *Vibrio fischeri* into the Tn21 mercury resistance operon (*mer*), and using *E. coli* as the host strain. The organisms showed semi-quantitative response to Hg in contaminated natural waters, at concentrations as low as a few nM. In constructing these first Hg bioreporters, the importance of understanding Hg transport pathways was recognized as being crucial if bioreporters were to be used to assess Hg bioavailability. The *mer* operon consists of a sequence of genes that encode active Hg transport and Hg reduction (*merA*), plus regulatory genes (*merR* and *merD*). Selifonova et al. constructed a set of three *mer-lux* fusions with and without the transport and reductase genes

(*124*). Interestingly, light production in response to Hg by strains with and without the transport genes was similar, suggesting Hg uptake was occurring by pathways other than *mer*-based active Hg transport. However, complicating factors, such as the potential energy and counter-ion requirements of Hg transport may cloud interpretation of these data.

Continued development of this bioreporter demonstrated that Hg-dependant light production in this strain was cell density-dependant (*125*) and dependant on the chemistry of the assay medium (*126*). The strain used in these studies contained a *mer-lux* fusion without Hg transport genes (pRB28). Reduction of cell density to about 10^5 cells per ml (at the high end of the range of cell densities found in natural waters) in Hg assays reduced the number of competitive binding sites for Hg, and therefore improved the sensitivity of the assay, into the pM range. Dissolved organic carbon also decreased the bioavailability of Hg to this strain. The assay buffer was manipulated to show that neutral Hg-Cl complexes induced more light production than negatively charged complexes, suggesting that uptake by this strain, under these conditions, was via diffusion.

Because light production is energy-dependent in these biosensors, it is necessary to separate factors that influence cellular activity in general from factors that influence Hg bioavailability specifically (*127,128*). Barkay et al. have used constitutive controls to achieve this goal, constructing an isogenic strain (pRB27) in which *lux* expression is constitutive, and therefore Hg-independent (*126*). Another approach is to construct biosensors with only a partial *lux* operon, so that the aldehyde precursors to *lux*-mediated light production are not produced by cells, but are supplied externally (*129*). This reduces the energy requirements of light production, but requires additional alteration of test media, and potentially affects Hg speciation. In order to examine Hg bioavailability under a wider range of conditions, Kelly et al. and Scott et al. transferred the pRB27 and pRB28 *mer-lux* fusions of Barkay et al. into *Vibrio anguillarum* (*130,131*). This host strain has wide salinity tolerance, and is a facultative anaerobe. Refinement of assay conditions also improved sensitivity to <0.5 pmol bioavailable Hg L^{-1}. This strain has been used to examine the bioavailability of trace level additions of Hg(II) to natural lake waters, and to examine the bioavailability of Hg in unamended natural waters. The percentage of ambient total Hg in lake and rain water available was found to be very low, as was the bioavailability of tracer additions of Hg to natural waters. Finally, as a first step in understanding Hg bioavailability in the conditions in which methylation occurs, Golding et al. (*132*) have worked with *E. coli* and *Vibrio anguillarum* bioreporters under anaerobic conditions. In some circumstances, Hg(II) uptake by both strains appears to occur via

facilitated transport. This suggests that Hg uptake by these strains occurs by a different mechanism than Hg uptake by methylating SRB, which occurs via diffusion of neutral species (*30,86*). Differences in medium content could potentially account for these differences as, for example, facilitated transport of Hg bound to amino acids has been shown to occur across membranes of higher organisms (*133*).

Genetic engineering using the *mer* operon has also been applied to Hg bioremediation. The advantage of strains constructed with the *mer* operon lies in the specificity of the *mer*-based uptake and detoxification systems, which are often unaffected by the presence of other metals. For example, a mercury bioaccumulator has been engineered (*134*), as a potential aid in mercury bioremediation. To produce the bioaccumulator, an *E. coli* strain was constructed to express a *mer*-based Hg^{2+} transport system and to overexpress pea metallothionine (MT), which protects the cells from Hg toxicity and allows for continued accumulation of Hg-MT within cells. Accumulation of Hg by the strain was not affected by metal chelators such as EDTA and citrate. Organisms that overexpress organomercurial lyase have also been constructed as potential aids in clean-up of organomercury contaminated sites (e.g. *135*).

Mercury biosensors are a potentially valuable tool for assessing Hg bioavailability. To date they have been used to demonstrate that Hg complexation has a large influence on Hg bioavailability to these cells, and that only a small fraction of Hg dissolved in natural waters is generally available for uptake. Also, previously unidentified pathways for Hg transport may need to be considered. More studies should lead to a fuller understanding of Hg transport pathways in cells without the *mer*-based transport system, and allow comparison of those systems with the transport systems of methylating and demethylating microorganisms, and the broader spectrum of microorganisms at the bottom of the food web. An important issue in understanding the results of Hg biosensor studies is the role of Hg transport pathways coded by the *mer* operon and those of the host organism. The bioavailability of Hg to methylating organisms is perhaps the key to modeling Hg methylation rates. Bioreporters can potentially be used to define that fraction of the ambient Hg pool if it can be shown that Hg uptake by methylators and bioreporters are similar. This should be the focus of continued research. However, CH_3Hg production itself may be the best "bioreporter" of Hg bioavailability to methylating bacteria. Since CH_3Hg production by these microorganisms occurs intracellularly, CH_3Hg production depends on Hg transport and serves as a sensor for Hg bioavailability. We have used *D. propionicus* in this way to examine uptake of neutral HgS complexes (*30,86*).

References

1. Pacyna, J.M. In *Global and Regional Mercury Cycles: Sources, Fluxes and Mass Balances*; W. Baeyens; R. Ebinghaus; O. Vasiliev, Eds.; Kluwer Academic Publishers, Dordrecht, 1996, pp. 161-178.
2. Mason, R.P.; Fitzgerald, W.F.; Morel, F.M.M. *Geochim. Cosmochim. Acta* **1994**, *58*, 3191-3198.
3. Fitzgerald, W.F.; Engstrom, D.R.; Mason, R.P.; Nater, E.A. *Environ. Sci. Technol.* **1998**, *32*, 1-7.
4. Lindqvist, O.; Johannson, K.; Aastrup, M.; Andersson, A.; Bringmark, L; Hovsenius, G.; Hakanson, L.; Meili, M.; Timm, B. *Water Air Soil Pollut.*, **1991**, Special Issue, Vol. 55.
5. U.S. EPA. Mercury Study Report to Congress. EPA-452/R-97-004, US EPA Office of Air, 1997, Washington, DC.
6. Clarkson, T.W. *J. Trace Element Exp. Med.* **1998**, *11*, 303-317.
7. Mahaffey, K.R. *JAMA* **1998**, *280*, 737-738.
8. St. Louis, V.L.; Rudd, J.M.W.; Kelly, C.A.; Beaty, K.G.; Flett, R.J.; Roulet, N.T. *Environ. Sci. Technol.* **1996**, *30*, 2719-2729.
9. Krabbenhoft, D.P., Benoit, J.M., Babiarz, C.L., Hurley, J.P., Andren, A.W. *Wat. Air Soil Poll.* **1995**, *80*, 425-433.
10. St. Louis, V.L.; Rudd, J.M.,W.; Kelly, C.A.; Beaty, K.G.; Bloom, N.S.; Flett, R.J. *Can. J. Fish. Aquat. Sci.* **1994**, *51*,1065-1076.
11. Driscoll, C.T., Holsapple, J., Schofield, C.L., Munson, R. *Biogeochem.*, **1998** *40* , 137-146.
12. Waldron, M.C., Coleman, J.A., Breault., R.F. *Can. J. Fish Aquat. Sci.*, **2000**, *57*, 1080-1091.
13. Compeau, G.; Bartha, R. *Appl. Environ. Microbiol.* **1985**, *50*, 498-502.
14. Berman, M.; Bartha, R. *Bull. Environ. Contam. Toxicol.* **1986**, *36*, 401-404.
15. Ramlal, P.S.; Kelly, C.A.; Rudd, J.W.M.; Furutani, A. *Can. J. Fish. Aquat. Sci.* **1992**, *50*, 972-979.
16. Korthals, E.T.; Winfrey, M.R. *Appl. Environ. Microbiol.* **1987**, *53*, 2397-2404.
17. Jensen, S.; Jernelov, A. *Nature*. **1969**, *223*, 753-754.
18. Gilmour, C.C.; Riedel, G.S. *Water Air Soil Pollut.***1995**, *80*, 747-756.
19. Gilmour, C.C.; Henry, E.A.; Mitchell, R. *Environ. Sci. Technol.* **1992**, *26*, 2281-2287.
20. Branfireun, B.A.; Roulet, N.T.; Kelly, C.A.; Rudd, J.W.M. *Global Biogeochem. Cycles.* **1999**, *13*, 743-750.
21. Robinson, J.B.; Tuovinen, O.H. *Microbiol. Rev.* **1984**, *48*, 95-124.
22. Chen, Y.; Bonzongo, J.-C.J.; Lyons, W. B.; Miller, G.C. *Environ. Toxicol. Chem.* **1997**, *16*, 1568-1574.

23. King, J.K.; Saunders, F.M.; Lee, R.F.; Jahnke, R.A. *Environ. Toxicol. Chem.* **1999**, *18*, 1362-1369.
24. Gilmour, C.C.; Henry, E.A. *Environ. Poll.* **1991**, *71*, 131-169.
25. Watras, C.J. and 21 others. In *Mercury Pollution: Intergration and Synthesis.*, C.J. Watras and J.W. Huckabee, Eds., Lewis Publishers, Boca Raton, 1994, pp. 153-177.
26. Urban, N.R., Brezonik, P.L., Baker, L.A., Sherman, L.A. *Limnol. Oceanogr.*, **1994**, *39*, 797-815.
27. Steffan, R.J.; Korthals, E.T.; Winfrey, M.R. *Appl. Environ. Microbiol.* **1988**, *54*, 2003-2009.
28. Compeau, G.; Bartha, R. *Appl. Environ. Microbiol.* **1987**, *53*, 261-265.
29. Compeau, G.; Bartha, R. *Bull. Environ. Contam. Toxicol.*, **1983**, *31*, 486-493.
30. Benoit, J.M.; Mason, R.P.; Gilmour, C.C. *Appl. Environ. Microbiol.* **2001**, *67*, 51-58.
31. Devereaux, R.; Winfrey, M.R.; Winfrey, J.; Stahl, D.A. *FEMS Microbial. Ecol.* **1996**, *20*, 23-31.
32. King, J.K.; Kostka, J.E.; Frischer, M.E. *Appl. Environ. Microbiol.* **2000**, *66(6)*, 2430-2437.
33. Macalady, J.L. Mack, E.E., Nelson, D.C., Scow, K.M. *App. Environ. Microbiol.*, **2000**, *66*, 1479-1488.
34. Kotska, unpubl
35. Winfrey, M.R; Rudd, J.W.M. *Environ. Toxicol. Chem.* **1990**, *9*, 853-869.
36. Benoit, J.M.; Gilmour, C.C.; Mason, R.P.; Reidel, G.S.; Reidel, G.F. *Biogeochem.* **1998**, *40*, 249-265.
37. Gilmour, C.C.; Riedel, G.S.; Ederington, M.C.; Bell, J.T.; Benoit, J.M.; Gill, G.A.; Stordal, M.C. *Biogeochem.* **1998**, *40*, 327-345.
38. Henry, E.A. Ph.D. Thesis, Harvard University, **1992**.
39. Allan, C.J.; Heyes, A. *Water Air and Soil Pollut.* **1998**, *105*, 573-592.
40. Allan, C.J.; Heyes, A.; Roulet, N.T.; St. Louis, V.L.; Rudd, J.W.M. *Biogeochem.* **2000**, *52*, 13-40.
41. Heyes, A.; Gilmour, C.C.; Mason, R.P. Unpublished data.
42. Heyes, A.; Moore, T.R.; Rudd, J.W.M.; Dugoua, J.J. *Can J. Fish. Aquat. Sci.* **2000**, 57, 2211-2222.
43. Mason, R.P.; Lawson, N.M; Lawrence, A.L.; Leaner, J.J.; Lee, J.G.; Sheu, G-R. *Mar. Chem.* **1999**, 65, 77-96.
44. Mason, R.P.; Lawrence A.L. *Environ. Toxicol. Chem.* **1999**, *18*, 2438-2447.

45. Kannan, K.; Smith, R.J. Jr.; Lee, R.F.; Windom, H.L.; Heitmuller, P.T.; Macauley, J.M.; Summers, J.K. 1998. *Environ. Contam. Toxicol.*, **1998**, *34*, 109-118.
46. Bloom, N.S.; Gill, G.A.; Driscoll, C.; Rudd, J.; Mason, R.P. *Environ. Sci. Technol.* **1999**, *33*, 7-13.
47. Hines, M.E.; Horvat, M.; Faganeli, J.; Bonzongo, J.C.J.; Barkay,T.; Major, E.B.; Scott, K.J.; Baily, E.A.; Waewick, J.J.; Lyons, W.B. *Environ. Research*, **2000**, *83*,129-139.
48. Kannan, K; Falandysz. *Water Air Soil Pollut.* **1998**, *103*, 129-136.
49. Henry, E.A.; Dodge-Murphy, L.J.; Bighma, G.M.; Klein, S.M.; Gilmour, C.C. *Water Air Soil Poll.* **1995**, *80*, 489-498.
50. Watras, C.J.; Back, R.C.; Halvorsen, S.; Hudson, R.J.M.; Morrison, K.A.; Wente, S.P. *Sci. Tot. Environ.* **1998**, *219*, 183-208.
51. Suchanek, T.H.; Mullen, L.H.; Lamphere, B.A.; Richerson, P.J.; Woodmansee, C.E.; Slotten, D.G.; Harner E.J.; Woodward, L.A. *Water Air Soil Pollut.* **1998**, *104*, 77-102.
52. Verta, M.; Matilainen, T. *Water Air Soil Poll.* **1995**, *80*, 585-588.
53. Hurley, J.P.; Cowell, S.E.; Shafer, M.M.; Hughes, P.E. *Environ. Sci. Technol.* **1998**, *32*, 1424-1432.
54. Bonzongo, J.-C.; Heim, K.J.; Chen, Y.; Lyons, W.B.; Warwick, J.J.; Miller, G.C.; Lechler, P.J. *Environ. Toxicol. Chem.* **1996**, *15*, 677-683.
55. Hines, unpublished
56. Hintelman H.; Wilken, R.D. *Vom Wasser* **1994**, *82*, 163-173.
57. Miskimmin, B.M.; Rudd, J.W.M.; Kelly, C.A. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 17-22.
58. Miskimmin, B.M. *Bull. Environ. Contam. Toxicol.* **1991**, *47*, 743-750.
59. Guimaras, J..R.D.; Meili, M.; Hylander, L.D.; Silva, E.D.E.; Roulet, M.; Mauro, J.B.N.; de Lemos, R.A. *Sci. Total Environ.* **2000**, 261, 99-107.
60. Roulet, M.; Guimaraes, J.R.D.; Lucotte, M. *Water Air and Soil Pollution*, **2001**, *128*, 41-60.
61. Bodaly, R.A., Rudd, J.M.W., Fudge, R.J.P., Kelly, C.A. *Can. J. Fish. Aquat. Sci.* **1993**, *50*, 980-987.
62. Ramlal, P.S.; Kelly, C.A.; Rudd, J.W.M.; Furutani, A. *Can. J. Fish. Aquat. Sci.* **1993**, *50*, 972-979.
63. Barkay, T., Liebert, C., Gillman, M. *Appl. Environ. Microbiol.* **1989**, 196-1202.
64. Oremland, R. S.; Culbertson, C.W.; Winfrey, M.R. *Appl. Environ. Microbiol.* **1991**, *57*, 130-137.
65. Oremland, R.S.; Miller, L.G.; Dowdle, P.; Connel, T.; Barkay, T. *Appl. Environ. Microbiol.* **1995**, *61*, 2745-2753.

66. Pak, K.-R.; Bartha, R. *Bull. Environ. Contam. Toxicol.* **1998**, *61*, 690-694.
67. Pak, K.-R.; Bartha, R. *Appl. Environ. Microbiol.* **1998**, *64*, 1013-1017.
68. Spangler, W.J.; Spegarelli, J.L.; Rose, J.M.; Miller, H.M. *Science.* **1973**, *180*, 192-193.
69. Moore, M.J.; Distefano, M.D.; Zydowsky, L.D.; Cummings, R.T.; Walsh, C.T. *Acc. Chem. Res.* **1990**, *23*, 301-308.
70. Marvin-Dipasquale, M.C.; Oremland, R.S. *Environ. Sci. Technol.* **1998**, *32*, 2556-2563.
71. Marvin-DiPasquale, M.; Agee, J.; McGowan, C.; Oremland, R.S.; Thomas, M.; Krabbenhoft, D.; Gilmour, C.C. *Environ. Sci. Technol.*, **2000**, *34*, 4908-4916.
72. Sellers, P.; Kelly, C.A.; Rudd, J.W.M.; MacHutchon, A.R. *Nature.* **1996**, *380*, 694-697.
73. Choi, S.-C.; Chase, Jr., T; Bartha, R. *Appl. Environ. Microbiol.* **1994**, *60*, 4072-4077.
74. Benoit, J.M.; Gilmour, C.C.; Mason, R.P. *Environ. Sci. Technol.* **1999**, *33*, 951-957.
75. Gutknecht, J.J. *J. Membr. Biol.* **1981**, *61*, 61-66.
76. Mason, R.P.; Reinfelder, J.R.; Morel, F.M.M. *Water Air Soil Pollut.* **1995**, *80*, 915-921.
77. Mason, R.P.; Reinfelder, J.R.; Morel, F.M.M. *Environ. Sci. Technol.* **1996**, *30*, 1835-1845.
78. Craig, P.J. Moreton, P.A. *Mar. Poll. Bull.* **1983**, *14*, 408-411.
79. Choi, S-C.; Bartha, R. *Bull. Environ. Contam. Toxicol.* **1994**, *53*, 805-812.
80. Schwarzenbach, G.; Widmer, M. *Chim. Acta.* **1963**, *46*, 2613-2628.
81. Paquette, K.; Helz, G. *Water Air Soil Pollut.* **1995**, *80*,1053-1056.
82. Paquette, K. 1994. Ph.D. Thesis. The University of Maryland, 1994.
83. Dyrssen, D. *Mar. Chem.* **1988**, *24*,143-153.
84. Dyrssen, D; Wedborg, M. *Water Air Soil Pollut.* **1991**, *56*, 507-520.
85. Benoit, J.M.; Mason, R.P.; Gilmour, C.C. *Environ. Toxicol. Chem.* **1999**, *18*, 2138-2141.
86. Benoit, J.M.; Gilmour, C.C.; Mason, R.P. *Environ. Sci. Technol.* **2001**, *35*, 127-132.
87. Jay, J.A.; Morel, F.M.M.; Hemond, H.F. *Environ. Sci. Technol.* **2000**, *34*, 2196-2200.
88. Tossell, J.A. *J. Phys. Chem. A.* **2001**, *105*, 935-941.
89. Stein, W.D.; Lieb, W.R. *Transport and Diffusion Across Cell Membranes*, Harcourt Brace Jovanovich, London, **1986**.

90. Huerta-Diaz, M.A.; Morse, J.M. *Geochim. Cosmochim. Acta.* **1992**, *56*, 2681-2702.
91. Bono, A.B. M.S. Dissertation, McGill University, 1997. 177 pp.
92. Dmytriw, R., Mucci, A., Lucotte, M., Pichet, P. 1995. *Wat. Air Soil Pollut.* **1995**, *80*, 1099-1103.
93. Benoit, J.M.; Mason, R.P.; Gilmour, c.C.; Aiken, G.R. *Geochim. Cosmochim. Acta* **2001**, *65*, 4445-4451.
94. Reddy, M.M., Aiken, G.R *Wat. Air Soil Poll.* **2000**, *132*, 89-104.
95. Hudson, R.J.M.; Gherini, S.; Watras, C.; Porcella, D. In *Mercury as a Global Pollutant: Towards Integration and Synthesis*. C.J. Watras and J.W. Huckabee, Eds.; Lewis Publishers: Boca Raton, **1994**, pp. 473-526.
96. Miller, C.L.; Mason, R.P. ACS 222nd Meeting, Chicago, August, **2001**; ACS abstract, *41*(2), 514-518.
97. Mikac, N.; Kwokal, Z.; May, K..; Branica, M. *Mar. Chem.* **1989**, *28*, 109-126.
98. Muhaya, B.B.M.; Leemakers, M.; Baeyens, W. *Water Air Soil Poll.* **1997**, *94* , 109-123.
99. Xia, K.; Skyllberg, U.L.; Bleam, W.F.; Bloom, P.R.; Nater, E.A.; Helmke, P.A. **1999**, *33*, 257-261.
100. Kim, C.S.; Shaw, S.; Rytuba, J.J.; Gordon, E.B. Jr. ACS 222nd Meeting., Chicago, Il, August 26-30, **2001**, *4*, 497-503.
101. Spry, D.J.; Wiener, J.G. *Environ. Pollut.* **1991**, *71*, 243-304.
102. Ramlal, P.S.; Rudd, J.W.M.; Furutani, A.; Xun, L. *Can. J. Fish. Aquat. Sci.* **1985**, *42*, 685-692.
103. Xun, L.; Campbell, N.E.R.; Rudd, J.W.M. *Can. J. Fish. Aquat. Sci.* **1987**, *44*, 750-757.
104. Royals, H.E.; Lange, T.R. ACS Abstract, 1995 Meeting, Washington, DC, **1995.**
105. Hintelmann, H.; Evans, R.D.; Villeneuve, J.Y. *J. Anal. Atomic Spec.* **1995**, *10*, 619-624
106. Hintelmann, H.; Keppel-Jones, K.; Evans, R.D. *Environ. Toxicol. Chem.*, **2000**, *19*, 2204-2211.
107. Gilmour, C.C.; Riedel, G.S., Coates, J.S.; Lovley, D. Abstract, Amer. Soc. Microbiol., New Orleans, **1996**.
108. Choi, S.C.; Bartha, R. *App. Environ. Microbiol.* **1993**, *59*, 290-295.
109. Berman, M.; Chase, T.; Bartha, R. *Appl. Environ. Microbiol.* **1990**, *56*, 298-300.
110. Wood, J.M.; Kennedy, F.S.; Rosen, C.G. *Nature*. **1968**, *220*, 173-174.
111. Liebert. C.A. Hall, R.M., Summers, A.O. *Microbio. Mol. Biol. Rev.*, **1999**, *63*, 507-522.

112. Barkay, T. 2000. In *Encyclopedia of Microbiology*. 2nd edition. Academic Press, San Diego, pp. 171-181.
113. Summers, A.O. *Ann. Rev. Microbiol.* **1986,** *40*, 607-634.
114. Foster, T.J. *CRC Crit. Rev. Microbiol.* **1987**, *15*, 117-140.
115. Silver, S., Phung. L.T. *Ann. Rev. Microbiol.* **1996**, *50*, 753-789.
116. Osborn, A.M., Bruce, K.D., Strike, P., Ritchie, D.A.. *Appl. Environ. Microbiol.*, **1993**, *59*, 4024-4030.
117. Bruce, K.D. *Appl. Environ. Microbiol.* **1997**, *63* , 4914-4919.
118. Liebert, C.A. Wireman, J., Smith, T., Summers, A.O. *Appl. Environ. Microbiol.* **1997**, *63*, 1066-1076.
119. Ravel, J., DiRuggiero, J., Robb, F.T., Hill, R.T. *J. Bacteriol.* **2000**, *182*, 2345-2349.
120. Ravel, J., Schrempf, H., Hill, R.T. *Appl. Environ. Microbiol.* **1998**, *64*, 3383-3388.
121. Reyes, N.S., Frisher, M.E., Sobecky, P.A. *FEMS Microbial Ecol.* **1999**, *30*, 273-284.
122. Hines, M.E., Horvat, M., Faganeli, J., Bonzongo, J-C.J, Barkay, T., Major, E.B., Scott, K.J., Bailey, E.A., Warwick, J.J., Lyons, W.B. *Environ. Res.* **2000**, *83*, 129-139.
123. Jeffrey, W.H., Nazaret, S., Barkay, T. *Microbial Ecol.* **1996**, *32*, 293-303.
124. Selifonova, O.; Burlage, R.; Barkay, T. *Appl. Environ. Microbiol.* **1993**, *59*, 3083-3090.
125. Rasmussen, L.D.; Turner, R.R.; Barkay, T. *Appl. Environ. Microbiol.* **1997**, *63*, 3291-3293.
126. Barkay, T.; Gilman, M.; Turner, R.R. *Appl. Environ. Microbiol.* **1997**, *63*, 4267-4271.
127. de Weger, L. A.; Dunbar, P.; Mahafee, W.F.; Lugtenberg, B.J.J.; Sayler, G.S. *Appl. Environ. Microbiol.* **1991**, *57*, 3641-3644.
128. Hill, P. J.; Rees, C.E.D.; Winson, M.K.; Stewart, G.S.A.B. *Biotechnol. Appl. Biochem.* **1993**, *17*, 3-14.
129. Virta, M.; Lampinen, J.; Karp, M. *Anal. Chem.* **1995**, *67*, 667-669.
130. Kelly, C.A.; Scott, K.J.; Holoka, M.; Rudd, J.W.M. In preparation.
131. Scott, K.J.; Rudd, J.W.M.; Kelly, C.A. In preparation.
132. Golding, G.R.; Kelly, C.A.; Sparling, R.; Loewen, P.C.; Rudd, J.W.M.; Barkay, T. *Limnol. Oceanogr.* In press.
133. Leaner, J.J. PhD Dissertation, University of Maryland, College Park, 2001, 182 pp.
134. Chen, S.; Wilson, D.B. *Appl. Environ. Microbiol.* **1997**, *63*, 2442-2445.
135. Horn, J.M.; Brunke, M.; Deckwer, W.; Timmis, K.N. *Appl. Envir. Microbiol.* **1994**, *60*, 357-362.