

**CONTROLS ON METHYLATION OF GROUNDWATER HG(II) IN
HYPORHEIC ZONES OF WETLANDS**

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**Controls on Methylation of Groundwater
Hg(II) in Hyporheic Zones of Wetlands**
(WR07R008)

Project Completion Report

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Project Summary

Title: Controls on Methylation of Groundwater Hg(II) in Hyporheic Zones of Wetlands

Project I.D.: WR07R008

Investigators:

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Period of Contract: July 1, 2007 to June 30, 2009

Background/Need: This project addressed the groundwater-related problem of methylmercury (MeHg) formation in hyporheic zones, the subsurface regions of stream- and lake beds where the active exchange of surface and groundwater occurs. MeHg formation involves the methylation of Hg(II), in the hyporheic zone, by bacteria. Subsequent transport of the MeHg into surface waters leads to accumulation of this highly toxic substance in aquatic food webs.

Objectives: Our research was focused on determining the main factors controlling the bioavailability of inorganic Hg(II) for production of MeHg in wetland hyporheic zones. Our objectives included:

1. Experimentally determining rates of mercury methylation and demethylation using isotopic tracer techniques .
2. Determining whether observed MeHg concentrations were allochthonous or produced *in situ*.
3. Assessing the influence of strong Hg-binding ligands on methylation rate potential and determining whether sulfide or dissolved organic carbon (DOC) played the greatest role in regulating Hg(II) bioavailability (as probed by methylation rate potential measurements) to methylating bacteria.
4. Determining whether neutral complexes of mercury and sulfide, such as HgS^0 , are the most bioavailable form of Hg(II) to bacteria.
5. Comparing two geochemically different sites within the wetland, with different groundwater flow patterns, to determine if Hg(II) bioavailability and methylation were influenced by the contrasting redox conditions.

Methods: This study was conducted in the Allequash Creek Wetland, in the Trout Lake region of Vilas County, northern Wisconsin. We collected sediment cores from two sites, one in the groundwater-fed headwaters of Allequash Creek (“Upper Springs” site), and one in the riparian wetland roughly half-way between the headwaters and the creek’s discharge into Allequash Lake, 4 km downstream (“Middle Wetland” site). Cores were collected in July and October, 2008, and February and July, 2009.

Some cores were kept intact and were injected with isotopically labeled mercury for methylation and demethylation rate potential measurements. Others were sliced at 2 cm intervals, and porewater was extracted for measurements of geochemical parameters. Parameters measured included sulfide, sulfate, Fe(II), Fe(III), DOC, total Hg (HgT), MeHg, strong Hg-binding ligand concentrations, concentrations of negatively-charged Hg species, and major ion concentrations.

Results and Discussion: HgT and MeHg concentrations in porewater varied considerably, spatially and temporally, from 2.8 to 50.3 ng/L for HgT and from 0 to 1.8 ng/L for MeHg. HgT concentrations were generally higher at the upper springs site than at the middle wetland, but did not show any consistent trend with depth at either site. MeHg concentrations were generally higher at the middle wetland site than

at the upper springs, and tended to peak within the upper 4 cm of sediment before declining with increasing depth. This trend of declining MeHg concentration with depth was reversed in the February, 2009 sampling, with MeHg concentrations peaking either at the deepest (8-10 cm at the middle wetland site) or second-deepest (6-8 cm at the upper springs site) horizon sampled.

Methylation rate potentials, like HgT and MeHg concentrations, varied widely, both spatially and temporally, while demethylation rates were more uniform. The relative uniformity of demethylation rates suggests that demethylation is a process that either occurs under a wider range of redox conditions, than methylation, or is possibly carried out by a greater number of bacterial species. An alternate possibility is that demethylation has an abiotic component. The range of observed methylation rates was 0 to 9.5 fmol MeHg/g sediment/hr (0-34.5% of introduced spike/day), while the range of observed demethylation rates was 0.5 to 9.6 fmol MeHg/g sediment/hr (0-302.2% of introduced spike/day). Methylation rates were lowest in February, 2009, when porewater temperatures were lowest, however, demethylation rates in these samples were the highest measured. These findings indicate that methylation rates may be more sensitive to low temperatures than demethylation rates.

The poor correlation observed between porewater MeHg and HgT concentrations suggests that the abundance of HgT is not directly limiting to methylation. The relatively stronger correlations observed between MeHg and DOC concentrations, as well as between DOC concentrations and methylation rate potentials, suggest that DOC plays an important role in regulating the production of MeHg in this system, and perhaps also its retention in the porewater or export to the stream. Correlations between MeHg and sulfide concentrations, as well as between sulfide concentrations and methylation rates were also stronger than correlations between MeHg and HgT, suggesting that sulfide plays a role in regulating methylation. Strong correlations between MeHg concentrations and methylation/demethylation rates, combined with low MeHg concentrations in upwelling groundwater, suggest that the majority of the MeHg in this system is produced *in situ*. Multiple regression analysis showed DOC concentration to be the most important factor in explaining the variability in the observed methylation rates, in most cases, with sulfide being the second most important variable.

Conclusions/Implications/Recommendations: The majority of the MeHg present at this site is produced *in situ*, in the hyporheic zone, however rates of production are highly variable by depth, sampling site, and season. In spite of predictions, generated by speciation modeling in previous studies, that sulfide will dominate Hg speciation (and therefore potential bioavailability) at this site, DOC appears to be the most important factor controlling Hg(II) methylation in this system. The findings of this study can aid in the creation of more targeted fish consumption advisories, can help improve mercury cycling computer models, and can lead to better wetland management practices to limit MeHg production. Future studies should focus on gaining a better understanding of the roles of DOC and sulfide in MeHg production and export.

Related Publications: Creswell, J.E., Kerr, S.C., Meyer, M.H., Babiarz, C.L., Shafer, M.M., Armstrong, D.E., and Roden, E.E. 2008. Factors controlling temporal and spatial distribution of total mercury and methylmercury in hyporheic sediments of the Allequash Creek wetland, northern Wisconsin. *Journal of Geophysical Research – Geosciences*. 113(G00C02):1-9.

Key Words: Methylmercury, mercury, wetland, hyporheic zone, dissolved organic carbon, methylation, sulfide

Funding: UWS Groundwater Research

Introduction

The accumulation of methylmercury (MeHg), the most toxic form of mercury (Hg), in aquatic food webs is a major problem in freshwaters across the U.S. (U.S. EPA, 2001). Production of MeHg occurs through the methylation of inorganic Hg(II) by bacteria in anoxic environments such as wetlands (Morel *et al.*, 1998, Benoit *et al.*, 1999). Groundwater and surface water have been demonstrated to transport some Hg(II) into these environments, but contribute little to no MeHg, implicating *in situ* production of MeHg as the primary source (Meyer, 2005; Stoor *et al.*, 2006; Armstrong *et al.*, 2006). Although information is emerging, the factors and mechanisms controlling MeHg production are not well understood. To design management strategies that would limit MeHg production, an understanding of factors regulating MeHg concentrations is essential.

Recent research provides some insight into the formation of MeHg in aquatic environments. Briefly, MeHg is produced in anoxic environments through bacterial activity, especially sulfate-reducing bacteria (Benoit *et al.*, 1999), but other species may also be involved, such as Fe(III)-reducing bacteria (FeRB) (Warner *et al.*, 2003; Fleming *et al.*, 2006; Kerin *et al.*, 2006). Factors believed to be important in controlling methylation rates include the activity of bacteria, concentration of Hg(II), and the speciation of Hg(II). Speciation is important because it influences the bioavailability of Hg(II) to bacteria (Hammerschmidt and Fitzgerald, 2004). Neutral species such as HgS^0 , $\text{Hg}(\text{HS})_2^0$, HgCl_2^0 , and $\text{Hg}(\text{OH})_2^0$ are believed to be bioavailable due to their lipophilic character and enhanced ability to pass through bacterial membranes (Morel *et al.*, 1998). Ionic species such as HgCl_3^- or HgHS_2^- are expected to be unavailable (Barkay *et al.*, 1997). In addition, binding to large organic molecules or natural dissolved organic matter is, under certain conditions, expected to prevent the uptake of Hg(II) by bacteria (Hammerschmidt *et al.*, 2004). In anoxic environments, Hg(II)-sulfide complexes are expected to dominate Hg(II) speciation due to the especially strong binding of Hg(II) to sulfide. Under these conditions, the bioavailability of Hg(II) should be mainly controlled by sulfide concentration and pH (Benoit *et al.*, 1999; Hammerschmidt and Fitzgerald, 2004). However, Hg(II) also associates with natural organic matter (NOM) through strong binding to reduced sulfur groups (Haitzer *et al.*, 2002), and our recent work indicates that Hg(II)-NOM complexes may play a role in regulating methylation, even in the presence of sulfide (Armstrong *et al.*, 2006; Chadwick, 2006; this study). The exact role of NOM on Hg(II) bioavailability is not clear. For example, the partitioning and transport of Hg(II) with NOM is well-established (cf. Hammerschmidt *et al.*, 2004; Babiarz *et al.*, 1998), and this binding might be expected to reduce the bioavailability of Hg(II). However, concentrations of MeHg also tend to correlate with NOM concentrations, which could suggest the opposite: that Hg(II) is more available in the presence of NOM. The MeHg-NOM correlation, however, could also be explained if NOM plays an important role in MeHg partitioning and transport. Possible explanations for the observed correlations between MeHg and NOM include: (a) non-equilibrium conditions at the time of sampling that allow Hg-NOM complexes in the presence of Hg(II) and sulfides (Hammerschmidt *et al.*, 2004); (b) covariation of Hg(II)-NOM complexes with a separate pool of bioavailable Hg(II); or (c) a NOM buffering effect on Hg(II) activities, whereby dissociation of Hg(II)-NOM complexes in association of microbial uptake provides a pool of Hg(II) for methylation.

The hyporheic zone of streambeds is a subsurface, three-dimensional region in which the active exchange of typically oxic surface water and sub-oxic or anoxic groundwater produces sharp chemical gradients with depth (Alley *et al.*, 2002). These changing chemical conditions, along with a supply of labile organic matter, give rise to substantial biological activity, and can provide conditions conducive to production of MeHg. Our prior work at the Allequash Creek wetland in northern Wisconsin documents intense redox cycling in large areas of the hyporheic zone – highly reducing groundwater mixing with DOM and sulfate from surface water produces conditions that support active sulfate reduction, as well as zones of iron oxidation and reduction. Past studies in our group have also implicated the hyporheic zone as an important exporter of MeHg to surface water and downstream environments (Armstrong *et al.*,

2006; Meyer *et al.*, 2005). It is for these reasons that this study focused on Hg(II) methylation in the hyporheic zone of the Allequash Creek wetland.

The goal of our research was to determine the main factors controlling the bioavailability of inorganic Hg(II) for production of MeHg in wetland hyporheic zones. Our objectives included:

1. Experimentally determining rates of mercury methylation and demethylation using isotopic tracer techniques .
2. Determining whether observed MeHg concentrations were allochthonous or produced *in situ*.
3. Assessing the influence of strong Hg-binding ligands on methylation rate potential and determining whether sulfide or dissolved organic carbon (DOC) played the greatest role in regulating Hg(II) bioavailability (as probed by methylation rate potential measurements) to methylating bacteria.
4. Determining whether neutral complexes of mercury and sulfide, such as HgS^0 , are the most bioavailable form of Hg(II) to bacteria.
5. Comparing two geochemically different sites within the wetland, with different groundwater flow patterns, to determine if Hg(II) bioavailability and methylation were influenced by the contrasting redox conditions.

Procedures and Methods

Site Description: This study was conducted in the Allequash Creek wetland, in the Trout Lake basin, in Vilas County, northern Wisconsin (Figure 1). This area is in the Northern Highland Lake District of Wisconsin, and is dominated by glacial terrain. The creek is spring-fed, consisting of several groundwater point discharges at the base of a dominating hillslope (Armstrong *et al.*, 2006; Kerr, 2007; Creswell *et al.*, 2008). It receives groundwater discharge along most of its course, although the length and origin of groundwater flow paths discharging to the stream varies widely (Pint *et al.*, 2003). The wetland plant community is dominated by sphagnum moss, leatherleaf, tussocks sedge, and black spruce, while the surrounding hillslope is covered in a mix of coniferous and deciduous forest (Armstrong *et al.*, 2006; Creswell *et al.*, 2008).

Samples were collected at two locations within the wetland. The upper springs site is located in a beaver pond at the eastern end of the watershed, where the stream originates (Figure 1). The sediment here is relatively low in organic matter and iron, and oxidized sulfur compounds are more prevalent than sulfide species (Creswell *et al.*, 2008). Groundwater discharging to this area has traveled along relatively short flow paths of 25-50 years (Pint *et al.*, 2003), and is less reducing than groundwater at the other study site. The middle wetland site is defined by a three meter wide riparian zone on either side of the main stream channel, roughly half-way between the headwaters and the discharge point into Allequash Lake (Figure 1). This site is characterized by peat soils over six meters in depth, high concentrations of dissolved organic matter, higher iron concentrations, and a greater proportion of reduced sulfur compounds (Armstrong *et al.*, 2006; Creswell *et al.*, 2008). Groundwater discharging at this site has traveled along relatively long flow paths of 50-150 years (Pint *et al.*, 2003) and is characteristically anoxic and sulfidic. These sites were chosen in order to take advantage of data collected by prior studies in our research group (Meyer, 2005; Armstrong *et al.*, 2006; Meyer *et al.*, 2005; Creswell *et al.*, 2008; Kerr *et al.*, 2008), as well by the North Temperate Lakes Long Term Ecological Research (NTL-LTER) project and the U.S. Geological Survey's Water, Energy, and Biogeochemical Budgets (WEBB) project.

Field Methods: All sampling was carried out following trace metal clean techniques developed in our laboratory (Hurley *et al.*, 1996; Shafer *et al.*, 1999) to ensure sample integrity and to minimize contamination. Hyporheic zone sediment cores were collected from the upper springs and middle wetland sites during four different field campaigns in July and October, 2008, and February and July, 2009.

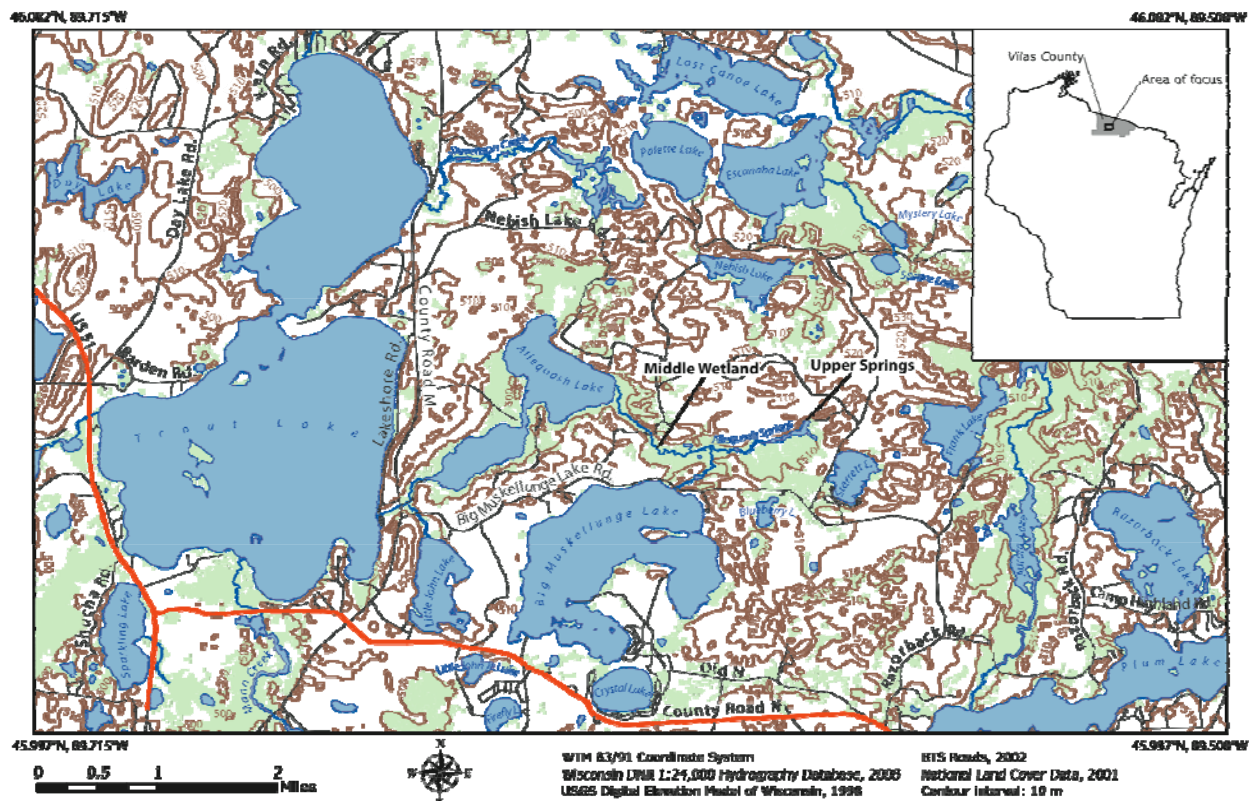


Figure 1: Map of the study area, showing the locations of the sampling sites. Wetlands, as delineated in the National Land Cover Database, are shown in green. Contour interval: 10 m.

Laboratory and Analytical Methods: Cores were transferred to the nearby UW Trout Lake Station for processing. Four replicate cores from each site were collected for geochemical analysis. These were sectioned in five 2 cm increments in an anoxic glove box, and composited by depth horizon. Porewater was removed from the composited core sections by centrifugation, and was then passed through acid-cleaned 0.45 μm filters using all-plastic syringes. Porewater and sediments were then subsampled for geochemical analyses, described below. In most cases, porewater was kept in the glove box until it was preserved for storage and transport, in order to minimize the oxidation of redox-sensitive analytes.

Methylation/Demethylation Rate Potential Measurements: Two cores from each site were collected for this analysis. The cores for methylation rate potential measurements were injected with pH-neutral amendments of isotopically enriched Hg(II), (^{200}Hg) which were diluted to the appropriate concentration using Allequash Creek water. Injections were made through silicone septa in the wall of the core tube. Cores for demethylation rate measurements were injected with isotopically enriched amendments of MeHg (Me ^{199}Hg), prepared as described above. Amendments typically increased the HgT and MeHg burden of the sediment by 1-2%. Cores were incubated at porewater temperature for 7-13 hours. Incubations were terminated by slicing and freezing.

Sediments were analyzed following the direct distillation method (Horvat *et al.*, 1993). After distillation, samples analyzed by GC-ICP-MS, following standard methods for isotopic Hg measurement (Hintelmann *et al.*, 1995). In samples amended with ^{200}Hg (II), any $^{200}\text{MeHg}$ measured was methylated during the incubation. In samples amended with Me ^{199}Hg , the difference between the introduced Me ^{199}Hg and what remained at the end of the incubation was what was demethylated (Hintelmann *et al.*, 2000).

HgT/MeHg: Samples for mercury and methylmercury analysis were analyzed using Cold Vapor Atomic Fluorescence Spectrometry (CVAFS), following established methods (Babiarz *et al.*, 1998; Bloom and Telliard, 2001a; Bloom and Telliard, 2001b).

Reducible Mercury Titrations: This method provided an estimate of the concentration of strong mercury-binding ligands in porewater. It involves amending replicate porewater samples with a range of Hg(II) concentrations, and allowing the amended mercury to equilibrate with the natural ligands overnight. Samples are then reduced with SnCl₂, a weak reductant, purged with nitrogen, and the evaded Hg is measured. The ligand concentrations are calculated from the difference between introduced and measured Hg (Lamborg *et al.*, 2003; Lamborg *et al.*, 2004). Because the reductant used in this analysis is weak, the ligands measured are operationally defined to be strong. Although sulfide species would generally be considered strong Hg-binding ligands in hyporheic porewater, these samples were not stored under anoxic conditions prior to analysis, thus it is assumed that all sulfide present was oxidized.

Ion Exchange Resin: Porewater samples were passed through columns containing diethylaminoethyl resin in order to isolate negatively charged aqueous complexes (e.g. DOM, free anionic Hg species) from porewater (Chadwick, 2006). After passing through the columns, the porewater, containing only positive and neutral aqueous complexes, was analyzed for HgT. The negatively charged complexes were eluted from the resin using weak acid and were also analyzed for HgT.

Ancillary Geochemistry: Iron and sulfide in porewater were measured colorimetrically, following the methods of Stookey (1970) and Cline (1969), respectively. Dissolved organic carbon was measured using a Shimadzu TOC-V/CSH instrument. Sulfate concentrations were measured by ion chromatography. Major ions and metals were measured by high resolution ICP-MS.

Results and Discussion

HgT and MeHg concentrations in porewater varied considerably, spatially and temporally, from 2.8 to 50.3 ng/L for HgT and from 0 to 1.8 ng/L for MeHg (Figure 2). HgT concentrations were generally higher at the upper springs site than at the middle wetland, but did show any consistent trend with depth at either site. MeHg concentrations were generally higher at the middle wetland site than at the upper springs, and tended to peak within the upper 4 cm of sediment before declining with increasing depth. This trend of declining MeHg concentration with depth was reversed in the February, 2009 sampling, with MeHg concentrations peaking either at the deepest (8-10 cm at the middle wetland site) or second-deepest (6-8 cm at the upper springs site) horizon sampled. MeHg concentrations showed little apparent correlation with HgT concentrations within each core, but did generally correlate well with DOC, sometimes positively, sometimes negatively. This finding suggests that DOC either plays an important role in MeHg formation in this system, or that it is responsible for MeHg retention in the hyporheic porewaters sampled. The lack of correlation between MeHg and HgT suggests that Hg(II) concentration is not a directly limiting factor in MeHg production at this site.

Methylation rate potentials, like HgT and MeHg concentrations, varied widely, both spatially and temporally, while demethylation rates were more uniform. The relative uniformity of demethylation rates suggests that demethylation is a process that either occurs under a wider range of redox conditions, than methylation, or is possibly carried out by a greater number of bacterial species. An alternate possibility is that demethylation has an abiotic component. The range of observed methylation rates was 0 to 9.5 fmol MeHg/g sediment/hr (0 to 34.5% of introduced spike/day), while the range of observed demethylation rates was 0.5 to 9.6 fmol MeHg/g sediment/hr (0 to 302.2% of introduced spike/day). These methylation rate potentials span a wider range than those found in nearshore marine sediments (1.4 to 8.2% d⁻¹; Hammerschmidt and Fitzgerald, 2004) and Ontario lakes (1.2 to 1.6 % d⁻¹; Hintelmann *et al.*, 2000). The mean of the methylation rates in this dataset (3.9% d⁻¹) is comparable to that in the marine sediment

dataset (3.8% d⁻¹), but is higher than that of the Ontario dataset (1.4% d⁻¹). Demethylation rates measured in this study (208.3% d⁻¹ mean) are higher than in the Ontario lakes study (~40% d⁻¹). Methylation rates in this study were lowest in February, 2009, when porewater temperatures were lowest, however, demethylation rates in these samples were the highest measured. These findings indicate that methylation rates may be more sensitive to low temperatures than demethylation rates.

Methylation rate potentials only twice showed a strong positive correlation ($r^2 > 0.7$) with porewater (upper springs site, October, 2008) or sediment (upper springs site, July, 2008) MeHg concentrations. There were no strong negative correlations between sediment or porewater MeHg concentrations and demethylation rate potentials. If the MeHg present within a given core section were produced within that section, one would expect it to correlate positively with the methylation rate potential, and negatively with the demethylation rate potential. Given the observed lack of correlation, however, we conclude that the highly dynamic flow patterns in this system rapidly transport MeHg from where it is produced to other locations in the hyporheic zone or stream. In spite of the lack of correlation between methylation rate potentials and hyporheic zone MeHg concentrations, the very low MeHg concentrations found in the upwelling groundwater at both sites lead us to conclude that the majority of the MeHg observed in the hyporheic zone was methylated *in situ*. The mean MeHg concentration in upwelling groundwater measured in 2003 and 2004 was 0.09 ng/L (Meyer *et al.*, 2005), while the mean porewater concentration measured in this study was 0.46 ng/L.

Site/Date	DEAE+/0	DEAE-	DOC	Sulfide	r ²
July, 2008	-0.010	-0.021	<i>5.508</i>	1.131	0.452
October, 2008	0.007	0.029	-2.605	<i>3.927</i>	0.911*
February, 2009	0.004	0.006	-0.555	0.031	0.922*
Upper Springs (All Dates)	-0.004	-0.001	<i>-1.234</i>	-0.111	0.455
Middle Wetland (All Dates)	0.017	-0.044	<i>7.674</i>	2.761	0.553

Table 1: Multivariate regression results. The geochemical parameters listed were regressed against methylation rates. Numbers in the DEAE+/0, DEAE-, DOC, and Sulfide columns are regression coefficients. The largest coefficients from each regression are shown in italics. The r² value in each row corresponds to fit of the multivariate regression to the data. Regressions were run by date, in which case data from both sites were included, and by site, in which case data from all dates were included. * indicates a significant result at the p=0.05 level.

Reducible mercury titrations showed considerable variability in the strong Hg-binding ligand concentrations (1.73-113.09 nN, mean: 40.62 nN). These data fall mostly within the range found in previous studies (0.3 to 60 nM; Lamborg *et al.*, 2003; Lamborg *et al.*, 2004), and the mean value from this study is similar to the value measured for a Minnesota bog, the most similar site for which published data exist (60 nN). Although there were generally no discernable trends with depth, by season, or between sites in these data, it is important to note that all of the measured ligand concentrations are at least an order of magnitude higher than the highest measured HgT concentration. We assume that the majority of the ligands measured using this method are organic (Lamborg *et al.*, 2003) and correspond to a fraction of the measured DOC concentrations. This finding of strong ligand concentrations well in excess of HgT concentrations, in sediments in which methylation is nonetheless taking place, supports the hypothesis that there is a complex relationship between Hg(II), DOC, and methylation. Based on the assumption that strong ligand binding of Hg(II) makes it unavailable for methylation, we would not expect to see methylation in waters with strong ligand concentrations in excess of HgT concentrations. The fact that methylation is occurring in these porewaters, however, indicates either that some ligand-bound Hg(II) is being made available for methylation, or that non-equilibrium conditions exist between Hg(II) and strong organic ligands in this system, leaving a fraction of Hg(II) free for methylation.

The ion exchange resin data do not show discernable trends with depth, by season, or between sites. At the upper springs site in February, 2009, the positive correlation between the concentration of positive and neutral Hg species with the methylation rate potential ($r^2=0.78$) was stronger than the correlation of the methylation rate with any of the other parameters considered (negatively charged Hg species, DOC, sulfide). This correlation implies that, in this instance, positive and neutral Hg species do a better job of explaining the observed variability in methylation rates than the other factors. While this result may suggest an important role for neutrally charged Hg-S complexes in controlling methylation, the fact that such a strong correlation occurred only once prevents us from drawing broad conclusions.

Multivariate regression analysis of methylation rates against selected geochemical parameters (Table 1) shows that DOC is most often the most significant factor in explaining the observed variability in methylation rates. Sulfide is generally the second most significant factor. It is interesting to note, however, that the sign of the relationship between DOC and methylation rate varies. This relationship suggests that DOC sometimes stimulates and sometimes inhibits methylation.

Conclusions and Recommendations

Selected conclusions, as they relate to our research objectives, are outlined below:

1. Experimentally determining rates of mercury methylation and demethylation using isotopic tracer techniques .

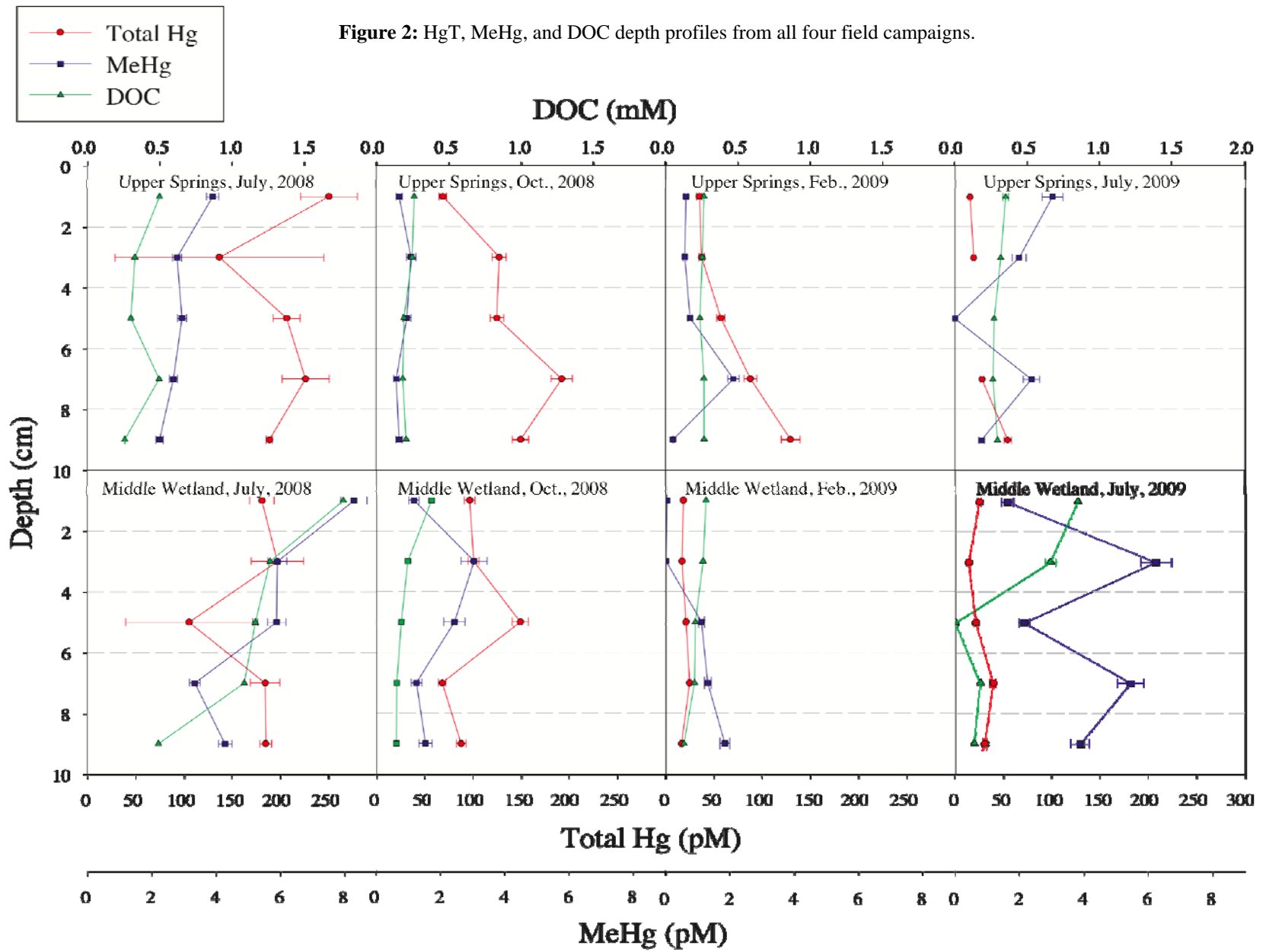
The range of observed methylation rates was 0 to 9.5 fmol MeHg/g sediment/hr (0 to 34.5% of introduced spike/day), while the range of observed demethylation rates was 0.5 to 9.6 fmol MeHg/g sediment/hr (0 to 302.2% of introduced spike/day).

2. Determining whether observed MeHg concentrations were allochthonous or produced *in situ*. Based on our results, we conclude that the majority of the MeHg present in the Allequash Creek wetland hyporheic zone is produced *in situ*. Hyporheic porewaters are a mixture of surface and groundwater, thus porewater MeHg could theoretically be transported into the hyporheic zone from either source. Because the stream flow is composed almost entirely of groundwater, however, MeHg transport in upwelling groundwater is the primary possible source for allochthonous inputs. The very low measured concentrations of MeHg in upwelling groundwater thus led us to the conclusion that MeHg is being produced in the hyporheic zone.

3. Assessing the influence of strong Hg-binding ligands on methylation rate potential and determining whether sulfide or dissolved organic carbon (DOC) played the greatest role in regulating Hg(II) bioavailability (as probed by methylation rate potential measurements) to methylating bacteria.

While we measured organic ligands in much greater concentrations than HgT in our porewater samples, methylation was still taking place in this system, suggesting either that Hg(II) complexation with organic matter does not prevent methylation, or that non-equilibrium conditions exist, allowing some Hg(II) not to be complexed with organic matter. DOC often showed a strong correlation with methylation rates (Table 1), both in univariate and multivariate models, suggesting that it plays an important role in controlling rates of methylation, however the relationship between DOC and methylation was sometimes positive and sometimes negative. Sulfide concentrations were also correlated with methylation rate potentials, but generally not as strongly as DOC. However, correlations between sulfide and methylation rate potentials were positive in every instance, suggesting strongly that increased sulfide concentrations lead to increased methylation. It is thus not entirely clear which geochemical parameter plays the most significant role in regulating Hg(II) bioavailability, however, sulfide appears to be the primary promoter of Hg(II) methylation.

Figure 2: HgT, MeHg, and DOC depth profiles from all four field campaigns.



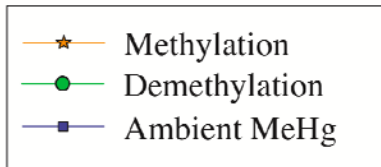
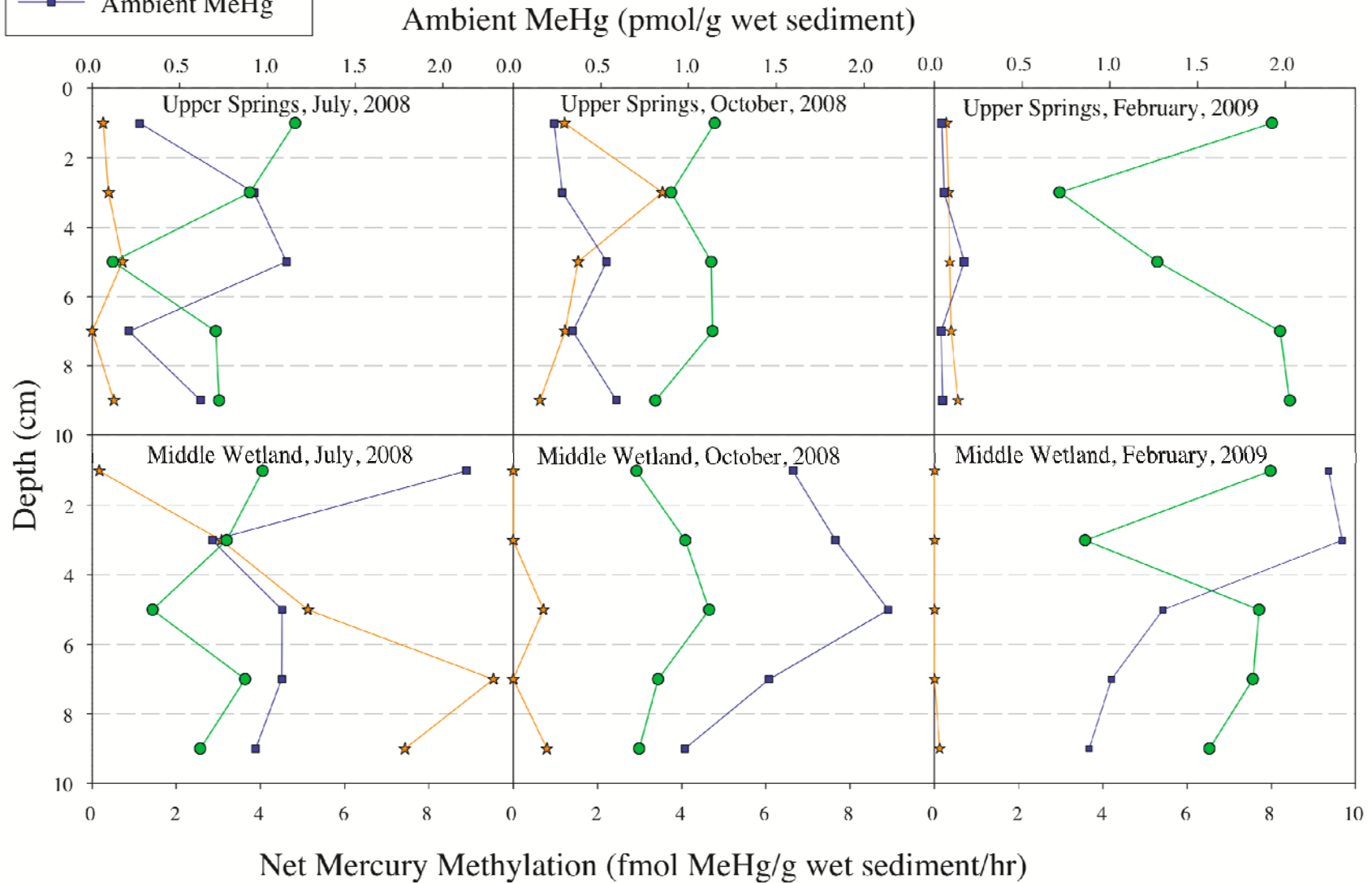


Figure 3: Methylation and demethylation rate potential and ambient (non-isotopically amended) MeHg depth profiles.



4. Determining whether neutral complexes of mercury and sulfide, such as HgS^0 , are the most bioavailable form of Hg(II) to bacteria.

There was no clear correlation between the concentration of neutral and positive Hg complexes and methylation rates. It is thus unclear, based on the data presented here, whether these complexes are more bioavailable, and therefore more readily methylated.

5. Comparing two geochemically different sites within the wetland, with different groundwater flow patterns, to determine if Hg(II) bioavailability and methylation were influenced by the contrasting redox conditions.

While there are clear and consistent geochemical differences between the two sites studied, methylation rates at one site do not consistently differ from the other. This finding suggests that methylation and bioavailability are highly variable, and likely the result of several different factors that can change rapidly at each site.

Further investigation of controls on methylation in hyporheic zones should involve experimental manipulation of hyporheic sediments from the Allequash Creek wetland, in order to determine the specific influence of each on methylation and bioavailability. The key finding of this study, from a management perspective, is that the majority of the MeHg in this system is produced internally, rather than imported from external sources. The findings of this study can aid in the creation of more targeted fish consumption advisories, can help improve mercury cycling computer models, and can lead to better wetland management practices to limit MeHg production.

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Appendix A: Awards, Publications, Reports, Patents, and Presentations

Publications:

Creswell, J.E., Kerr, S.C., Meyer, M.H., Babiarz, C.L., Shafer, M.M., Armstrong, D.E., and Roden, E.E. 2008. Factors controlling temporal and spatial distribution of total mercury and methylmercury in hyporheic sediments of the Allequash Creek wetland, northern Wisconsin. *J. Geophys. Res. - Biogeosciences*. **113**:1-9.

Presentations:

Creswell, J.E., Babiarz, C.L., Shafer, M.M., Roden, E.E., and Armstrong, D.E. 2007. Mercury Methylation, Demethylation, and Bioavailability in the Hyporheic Sediments of a Northern Wisconsin Wetland. Poster presentation. American Geophysical Union Fall Meeting, San Francisco, CA, 10-14 December, 2007.

Creswell, J.E., Babiarz, C.L., Shafer, M.M., and Armstrong, D.E. Measurements of mercury methylation rates and bioavailability in the Allequash Creek Wetland, Northern Wisconsin. Poster presentation. American Geophysical Union Fall Meeting, San Francisco, CA, 15-19 December, 2008.

Creswell, J.E., Babiarz, C.L., Shafer, M.M., Armstrong, D.E., and Roden, E.E. Mercury methylation, demethylation, and bioavailability in the hyporheic sediments of a northern Wisconsin wetland, USA. Oral presentation. Ninth International Conference on Mercury as a Global Pollutant, Guiyang, China, 7-12 June, 2009.

Creswell, J.E., Babiarz, C.L., Shafer, M.M., Armstrong, D.E., and Roden, E.E. Bioavailability of mercury to methylating bacteria in a northern Wisconsin wetland: Results from a year of field sampling. Oral presentation. American Chemical Society National Meeting, Washington, D.C., 16-20 August, 2009.