ABSTRACT

Title of Thesis: TEMPORAL AND SPATIAL VARIABILITY OF METHYLMERCURY ACCUMULATION IN SMALL STREAM ECOSYSTEMS

Jacob Matthew Oster, Master of Science, 2018

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Maryland has state-wide fish consumption advisories for mercury, and there is a need to explain these trends. I explore two tools for characterizing MeHg conditions in Maryland. The first explores benthic macroinvertebrates as vectors of MeHg from sediments to fish. I examined macroinvertebrate communities over two years from two first-order streams differing in land-use and historical stream water MeHg concentrations. I assessed temporal and spatial variability in invertebrate populations in conjunction with an assessment of the distribution of MeHg in water and sediment. I tested a second tool, an autonomous continuous water sampler that would allow MeHg to be measured without laborious expeditions. I observed differences in concentrations of MeHg across trophic levels between watersheds and identified a candidate organism as a bioindicator of MeHg exposure risk and watershed MeHg condition, as well as a potential sampling mechanism for MeHg in aquatic ecosystems.

TEMPORAL AND SPATIAL VARIABILITY OF METHYLMERCURY ACCUMULATION IN SMALL STREAM ECOSYSTEMS

by

Jacob Matthew Oster

Thesis submitted to the Faculty of the Graduate School of the
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This thesis is dedicated to my grandfather Matthew O'Neill and in loving memory of my grandmother Ann O'Neill. They first sparked my interest in the outdoors during our fishing and birding outings, and their support throughout my education will always be treasured.

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Chapter 1

Introduction

Mercury is a metal that occurs in a variety of chemical forms or species that can cause physiological harm to organisms. Mercury sources to the environment are predominantly fossil fuel combustion, medical waste incineration, and gold mining in third world countries (Rood et al. 1995, Mergler et al. 2007). Elemental mercury (Hg) can disperse in the atmosphere, with residence time of 0.5-2 years before depositing on the earth's surface with precipitation (Carpi 1996). In the environment, Hg can be methylated into its more toxic subspecies, monomethyl mercury (MeHg), a known neurotoxin with the ability to bioaccumulate and biomagnify in aquatic food webs (Bloom 1992).The percentage of Hg present in organisms as MeHg has been shown to increase with trophic level due to the ease of passage across cell membranes and increased retention in tissues compared to other species of Hg but also because of slow rates of excretion (Van Wallegham et al. 2013). The process of Hg methylation is thought to be carried out by bacteria in anaerobic sediments of lakes, wetlands, and salt marshes (Mitchell and Gilmour 2008). Genes linked to the Hg methylation pathway in these microbes were characterized (Parks et al. 2013) but methods to identify and quantify the presence of methylating bacteria are ongoing. For example, Podar et al. (2015) looked for the genes in available genetic databases and found the genes for methylation in locations previously unknown to be sources of MeHg, including thawing permafrost soils, hot springs in Yellowstone, and bioreactors. Mercury methylation is likely occurring in places where MeHg cycling is either unknown or not well characterized. In fact, environments where Hg is methylated are often difficult to access and a need for seasonal sampling in these regions may require novel approaches.

While identifying and quantifying methylating bacteria in the environment has proven difficult, they are primarily found within anaerobic sediments and utilize a variety of compounds as sources for metabolism. Two documented types of methylators are iron-reducing and sulfur-

reducing bacteria (Mitchell and Gilmour 2008, Parks et al. 2013). These bacteria are hypothesized to reside at different levels within the sediment due to the availability of their preferred electron acceptors, namely oxygen, sulfide, sulfate, nitrates and iron (Braker et al. 2001, Benoit et al. 1999) and availability/quality of dissolved organic matter (DOM) substrates (Sunderland et al. 2006) along the aerobic/anaerobic gradient in benthic sediments. These aerobic/anaerobic gradients can occur within a small transition zone, usually a couple of centimeters (Figure 1.1), also known as the hyporheic zone. Oxygen is mostly available to the upper layers of sediment from the oxygenated water above. The most ideal electron acceptors (oxygen) are rapidly used up close to the surface, so alternative electron acceptors, (iron, which is preferred but in less quantity than sulfate in this environment), are used at the lower levels. Hg methylation has been mainly linked to location of active sulfate reduction but is inhibited by sulfide production (Langer et al. 2001, Sunderland et al. 2006), when concentrations of sulfate exceed 100μM (Gilmour et al. 1992).Organic carbon has also been found as an important factor in Hg and MeHg transformation and transfer, both in terms of complexation and influencing microbial activity (Sunderland et al. 2006). Water flowing from the bank or groundwater from beside or beneath the stream, respectively, can carry Hg from runoff through the different layers of sediment, exposing Hg to methylating bacteria for potential methylation. Once Hg is methylated, MeHg typically becomes bound to dissolved organic matter (DOM) ligands (Pettersson et al. 1995), and is transported across the layers as shown in the conceptual model below (Figure 1.1). MeHg is now available to higher trophic levels via the water column and in the sediment.

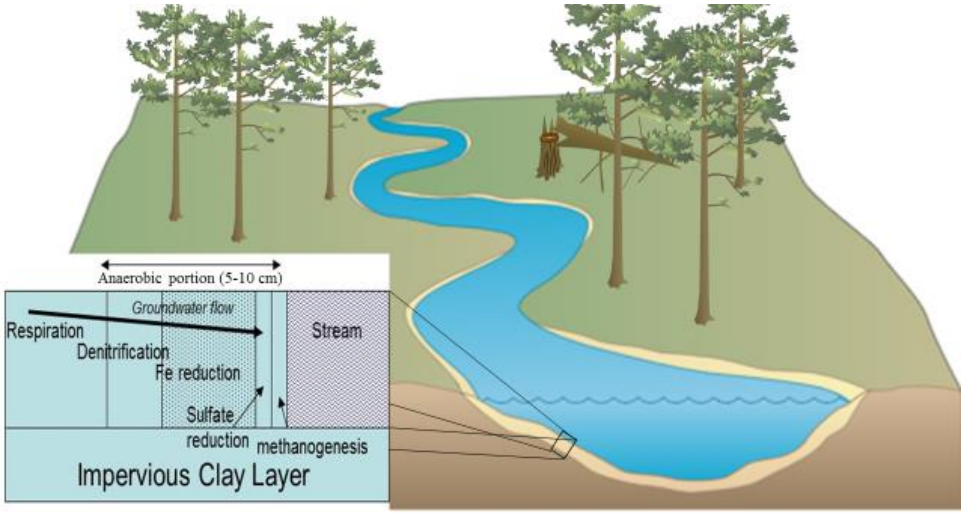


Figure 1.1 Proposed schematic of groundwater flow within anoxic sediments surrounding bodies of water (inset, courtesy of Andrew Heyes, forest stream graphics from Integration Application Network). This orientation can be from the impervious stream bed (clay in this instance) up through the stream bed or from the streambank to the stream. Water carrying Hg passes through the different levels of oxygen availability as shown by the large bold arrow. The horizontal double arrow above shows the extent of the anaerobic portion of the sediment before it reaches the stream (aerobic).

Several studies have reported small steams as being sources of MeHg to watersheds dating back to 1995 (Bishop et al. 1995, Branfireun et al. 1996) and concentrations can very among and within watersheds due to the presence of methylation and aerobic/anaerobic gradient "hotspots" (Shanley et al. 2008, Rolfhus et al. 2011). Bishop et al. (1995) found elevated MeHg levels (>1ng/L) in the top 5 cm of riparian sediments and in partially submerged sphagnum moss (>2ng/L) on the stream banks and concluded that biogeochemical processes within the sediments were important for controlling the export of MeHg from the watershed. Previous work examining Hg so far has used wells within the stream to measure composite or bulk Hg across the sediment layers (Bishop et al.1995, Heyes et al. 2010). However, other studies have found that stream biogeochemical processes change rapidly across the redox gradient, including processes that could affect MeHg concentrations (Roulet et al. 2000, Bishop et al. 1995). The hydromorphic control of MeHg production has been seen in wetland (Mitchell and Branfireun 2005) and stream chemistry (Seibert et al. 2009). Understanding the temporal and spatial processes within the

aerobic/anaerobic gradient is important to understanding how MeHg is exported from small streams and other water bodies.

At the time of the writing of Heyes and Gilmour (2010), few studies had looked at freshwater streams and their export to coastal zone ecosystems. To remedy this, they selected several watersheds within the Kilpatrick Marsh watershed, Edgewater, MD, to measure Hg deposition into the streams and Hg movement within the streams. They found elevated MeHg in small streams within the Kilpatrick Marsh watershed and measured differences in MeHg concentrations within streams of two first order watersheds with different land use. Concentrations and net export of MeHg were higher in a forested watershed than one dominated by agriculture and this difference is likely due to the differing watershed substrates, watershed history, and chemical processes happening within the watershed. Large amounts of organic matter from leaf litter tend to accumulate within the forested watershed, providing ideal conditions for the stream to go anoxic (10-20µg/L TOC) and provide ligands for Hg methylation and transport. In contrast, the agricultural site has a sandy substrate and residual nitrates from fertilizer are an order of magnitude higher in concentration in the agricultural watershed (10-150 µg/L NO_3- in the forested vs 1000-2000 µg/L in the agricultural) providing an alternate electron acceptor within the agricultural watershed which may reduce the presence of MeHg methylators (Heyes et al. 2010). The temporal pattern of MeHg concentrations in stream water of the watersheds are different, with the forested watershed having a strong seasonal pattern, with peak MeHg concentrations in the spring, whereas a slight increase in MeHg concentrations are seen spanning the summer in the agricultural watershed. Their work has also suggested that Hg methylation is driven by processes in the riparian zones, groundwater flow, and local precipitation. This pattern has been repeated fairly consistently over the past ten years in this watershed. Thus, seasonality and spatial variation could be important factors to consider when monitoring MeHg in stream water ecosystems.

Once MeHg is available to aquatic ecosystems, it can cause harm to organisms connected to these watersheds (Bloom 1992). Many studies have focused on the higher trophic levels and how organisms at these levels obtain, metabolize and excrete MeHg. MeHg can accumulate in the muscle tissue of fish, making piscivorous fish, birds, and humans at risk for exposure to elevated MeHg levels, which can lead to motor skill impairments, reduced fledging/survival rates of young, and mercury toxicosis in extreme cases (Evers et al. 2008, Roelke et al. 1991). Comparatively few studies have focused on lower trophic organisms, namely benthic macroinvertebrates (Henderson et al. 2011, George and Batzer 2008). These studies have found that MeHg percentages can range widely among species, spatial scales, and trophic levels. The majority of Hg in lower trophic levels, such as plankton, is inorganic Hg (>90%), but in intermediate trophic levels, such as mayflies and odonates, MeHg comprises a larger fraction (20-90%), of the total Hg (T-Hg) and in the highest trophic levels, such as fish and birds, over 90% of T-Hg is present as MeHg (George and Batzer 2008, Rolfhus et al. 2011). Predaceous invertebrates such as odonates typically have the highest MeHg levels among invertebrates, with most of the Hg present as MeHg (Rolfhus et al. 2011), but in some instances, omnivorous species such as amphipods will have higher MeHg concentrations than odonates or crayfish (George and Batzer 2008). Henderson et al. (2011) contrasted MeHg levels in insects from ponds with and without fish present. They found that invertebrates, especially odonates, can have higher MeHg levels when fish are absent from their ponds, indicating longer lifespans or higher relative trophic level reached within the ponds. This indicates that studying invertebrates with a species based approach is important to understand the processes driving MeHg accumulation in invertebrates and how other higher trophic levels (such as amphibians or birds) may be at risk if they feed on invertebrates from these ecosystems.

In addition, most Hg studies have focused on MeHg within lake and river food webs (Table 1.1), with fewer studies on small ephemeral bodies of water (Chumchal and Drenner

2015). In these ecosystems, the rate at which mercury accumulates within a food web can inform how much MeHg exposure risk is associated with different ecosystems at higher trophic levels. These rates have been described as the bioaccumulation factor (BAF) and biomagnification factor (BMF). The factors are calculated by taking the logarithm of the Hg concentration ratio between different trophic levels or first level consumers and the aqueous environmental Hg concentrations (Rolfhus et al. 2011), as shown in the equations below.

BMF = food web (n+1) trophic level Hg (ng/g dry weight) / food web (n) trophic level Hg (ng/g dry weight) (unit less)

BAF = first level consumer Hg (ng/kg dry weight) / aqueous Hg (ng/L), (unit l/kg)

Table 1.1 Bioaccumulation factors (BAF), and invertebrate MeHg levels for previous studies and their environments.

Study/Site	**BAF**	**Invertebrate MeHg levels**	**Sources**
Wisconsin Lakes	4.6-6.8	30-375 ng/g	Rolfhus et al. 2011
Marine systems	4.2-5.1	1.1 ng/g (zooplankton)	Hammerschmidt and Fitzgerald 2006
Maryland streams	2.69	4-56 ng/g	Mason et al. 2000

In Table 1.1, there is a wide range of BAF's and invertebrate MeHg levels. Marine systems have very low MeHg concentrations in the zooplankton, compared to the Wisconsin Lakes, even though both have similar BAF's. As highlighted in Table 1.1, the differences in magnification rates and concentrations of MeHg in the trophic levels between ecosystems could be attributed to concentrations at the lower trophic levels (indicating high background MeHg concentrations in the sediment or water), efficacy of transfer to higher trophic levels, and depuration rates in higher trophic levels. Rolfhus et al. (2011) noted that most magnification rate

studies have previously focused on higher trophic levels (fish and birds). Rolfhus et al. (2011) examined accumulation rates at lower trophic levels (zooplankton) and found the accumulation rates were highly variable (4.6-6.8) among the lakes due to variation in water Hg concentrations and invertebrate Hg concentrations (30-375ng/g).

Since vulnerable higher trophic level organisms are exposed to MeHg via consumption of MeHg contaminated food sources from aquatic ecosystems (Evers et al. 2008, Roelke et al. 1991, Mergler et al. 2007), characterizing the temporal and spatial variation of MeHg concentrations in these lower trophic levels is important (Chumchal and Drenner 2015). One challenge in doing this is having enough material to actually measure both concentrations of Hg and MeHg in a sample. To overcome this challenge, most studies on macroinvertebrates have focused on composite benthic macroinvertebrate samples grouped by feeding strategy or organisms, such as odonates and crayfish (George and Batzer, 2008). Some studies have examined methods to sample individual small organisms for simultaneous Hg and MeHg analysis, as most techniques for MeHg and Hg analysis require separating the sample into aliquots for Hg and MeHg analysis, a challenging feat for samples of small biomass (Taylor et al. 2008). Taylor et al. (2008) found that by digesting small sample biomasses (50-100 mg dry weight) in 4M HNO_3 acid, they were able to recapture most of the MeHg. Stronger HNO_3 is then added to the sample, followed by microwave digestion, which then allows for the measurement of T-Hg of the same sample. The results of Taylor et al. (2008) showed that MeHg reference standards were recovered at a rate consistent with established methods, such as distillation with Teflon vials (Horval et al. 1993). The approach of Taylor et al. (2008) is a promising technique for measuring variation among invertebrate species and individuals with small sample mass, which has been difficult to study previously as noted above.

The majority of total mercury (T-Hg) in higher trophic organisms (i.e. fish) is usually >90% MeHg, but in some lower trophic levels, such as crayfish, the ratio of MeHg to T-Hg is

lower, with 20-70% of total Hg being MeHg for some organisms (Mason et al. 2000). Most benthic organisms are not characterized for the MeHg:T-Hg ratios at the species level nor are the concentrations measured well documented over time in the macroinvertebrate communities, as sample biomass is usually a limiting factor. Most studies to date have only collected between one and three samples over the course of a year (Mason et al. 2000, George and Batzer 2008).

Invertebrates vary quite widely in their life histories, feeding strategies, lifespans, and tolerance to pollution. For example, amphipods (*Gammarus*) typically live 1-2 years and spend their entire lifespan within the stream. Amphipods are considered collectors, meaning they feed on fine particles depositing into the streams, and detritivores, feeding detritus in the stream. Other invertebrates, such as Hydropsychid caddisflies (also collectors), spend one winter as eggs within the stream, hatch in the spring, and emerge by summer. Predaceous dragonfly nymphs, such as *Cordulegaster*, may only reside in a water body up to a year before emerging while dobsonfly larvae (*Corydalus*) spend 2-3 years within the stream before emerging. Hydropsychid caddisflies, *Cordulegaster*, and *Corydalus* are examples of a subgroup of invertebrates (called emergents), which spend their larval stage within the stream and the adult phase out of the stream. Studying invertebrates with more precision at the individual level with the techniques shown in Taylor et al. (2008) can provide more clues to the fate of MeHg as it passes through the food web, as well as identify possible routes of exposure for higher trophic levels.

Assessing the MeHg condition of a watershed, that being MeHg production, export and organismal exposure, is expensive; requiring sampling of water, sediment, invertebrates, and fish throughout the year. A simpler approach would be to collect a subsample "indicator species" that could represent MeHg dynamics over a short period of time as an assay for MeHg export and exposure. Maryland's Department of Natural Resources (DNR) has been conducting annual surveys of benthic macroinvertebrate species richness and abundance since 1995 as part of the Maryland Biological Stream Survey (MBSS, Stranko et al. 2007). These surveys contribute to

Indices of Biotic Integrity which indicate overall stream health, but do not provide indicators for specific contaminants. Identifying species that could serve as bioindicators of MeHg contamination within small stream watersheds could provide DNR with a valuable risk assessment tool to observe MeHg cycling within the state of Maryland over the past two decades.

To pick a taxa to be used as a sentinel indicator, the MeHg transfer to the organisms that are present along with the organism's life history and ecology must be understood. For example Saouter et al. (1993) saw rapid transfer of MeHg to *Hexagina rigida*, and Chumchal et al. (2017) found odonate and chironomid MeHg levels respond quickly to changes water in level and drying within small semi-permanent ponds, as well as the presence and absence of predaceous fish. Invertebrates appear to respond quickly to seasonal fluctuations in MeHg availability; and because swings in MeHg have been observed in coastal plain watersheds (Heyes at al. 2010), timing of sampling of invertebrates for MeHg could be important for understanding temporal variation in MeHg export through the invertebrate community.

In this study I aim to quantify seasonal fluctuations of MeHg in the riparian sedimentary environment where Hg methylation is occurring in headwater streams while quantifying MeHg in resident macroinvertebrates. To do this I examined two watersheds with contrasting MeHg concentrations (as measured by Heyes et al. 2010): one surrounded by mostly forest and the other surrounded by agriculture. The approach was to sample whole sediment, sediment porewater, stream water, and invertebrates on a weekly or biweekly basis over 2 years to document the changes in the macroinvertebrate communities throughout the course of the spring and summer season and deduce whether the macroinvertebrates closely follow the seasonal fluctuations in stream water MeHg concentrations collected by traditional methods. In Chapter 2, I will discuss these linkages between MeHg in macroinvertebrates and the other matrices. In addition, I tested a continuous water sampler, referred to as an OsmoSampler (Jannasch et al. 2004), to continually collect stream sediment porewater to test whether such samplers could be used to reduce water

sampling effort. In Chapter 3, I will cover how I explored the OsmoSampler application in the laboratory and field settings.

Chapter 2

Temporal Variation of MeHg Concentration in the Riparian Zone of Two Watersheds

Introduction

The majority of human exposure to MeHg in the U.S. is through consumption of marine and estuarine fish (Carrington et al. 2004). In the state of Maryland, over 30 bodies of water have fish consumption advisories due to elevated mercury levels (MDE 2014). While the source of Hg to waterbodies is believed to be largely anthropogenic (Mason et al. 2000) processes within lakes and watersheds impact Hg methylation resulting in variations and seasonal fluctuations in MeHg productivity (Bishop et al. 1995). With thousands of headwater streams in Maryland, examining MeHg productivity through measurements of stream chemistry would be expensive. I propose to use macroinvertebrates as sentinels of MeHg productivity. Connections between fish MeHg concentrations across species and watersheds have shown no clear pattern in the few studies conducted and concentrations in the water are known to be episodic. Benthic macroinvertebrates, such as crayfish, bivalves, and other organisms have been used as sentinel indicators in other studies (Mason et al. 2000). However, MeHg and T-Hg levels in macroinvertebrates have not been well characterized, as the small biomass and concentration levels have made Hg characterization difficult in the past (George and Batzer 2007). The seasonal variation in MeHg concentrations in benthic macroinvertebrates is also not well understood. In order to better understand the MeHg concentrations in streams over time, I studied two small stream ecosystems with contrasting watershed characteristics in Maryland's coastal plain over the course of two summers to investigate the temporal dynamics of mercury accumulation and in the benthic macroinvertebrate communities.

To better explore the objective of examining the connections between MeHg concentrations in sediment, porewater, and invertebrates within the small streams, I strove to answer the following questions:

1. **Does MeHg accumulation in the hyporheic zone coincide with changes observed in the biota?**
2. **Are any organisms resident throughout the year, such as amphipods, that could be used to track seasonal shift in MeHg concentrations in the sediment and water of a single watershed? This is important as time of collection would affect the ability to compare watersheds.**
3. **Does MeHg concentrations in the organisms reflect differences in MeHg production, measured as MeHg concentrations, between the watersheds?**

Methods

Field Sites

Two sites within the Rhode River watershed in Edgewater, MD, of the mid-Atlantic Coastal Plain were selected for this study (Figure 2.1). The two first order streams have been studied for the past 40 years (Correll et al. 1992, Weller et al. 2003) and the watershed characteristics, nutrient discharge, and mercury concentrations within the streams have been well documented (Table 2.1.) by scientists associated with the Smithsonian Environmental Research Center (SERC) and the Chesapeake Biological Lab (CBL) (Heyes et al. 2010). For the past few years, the forested watershed (identified as "110" by SERC) typically exhibits higher MeHg levels in the water column, with the concentrations peaking between May and July historically (Figure 2.2). The agricultural watershed (identified as "109" by SERC) was predominately developed with corn and soy fields up until 15 years ago, when the property was acquired by

SERC. Following the acquisition, the property has been undergoing reforestation steps with various indigenous species of trees.

Watershed 109 has lower MeHg concentrations in water and does not show the same seasonal pattern as watershed 110 due to the history of nitrate fertilizers in the watershed providing alternate energy sources for microbes. These sites were selected due to their contrasting watershed histories, differences in MeHg concentrations, and other watershed characteristics to provide contrast and see the sensitivity of the invertebrate communities to watershed differences. I assumed the watersheds would have similar macroinvertebrate communities due to their relative proximity to one another (1.8 km away), being of a similar size, having similar hydrological regime and having sedimentary substrate.

Figure 2.1 The agricultural (left) and forested (right) stream beds (in blue) from above. Both watersheds have two transects (white bars) in which stream, well, and porewater samples collected. A v-shaped weir (yellow triangles) are downstream of the transects in both streams. Invertebrates were collected in between transects in both sites. Photos were taken using Google Earth Pro.

TABLE 2.1 Area and Land Cover for watersheds (Weller et al. 2003)

Watershed Name	**Area (km^2)**	**Developed land (%)**	**Cropland (%)**	**Grassland (%)**	**Forest (%)**
109	0.17	0	14.9	44.9	40.2
110	0.06	0	0	0	100

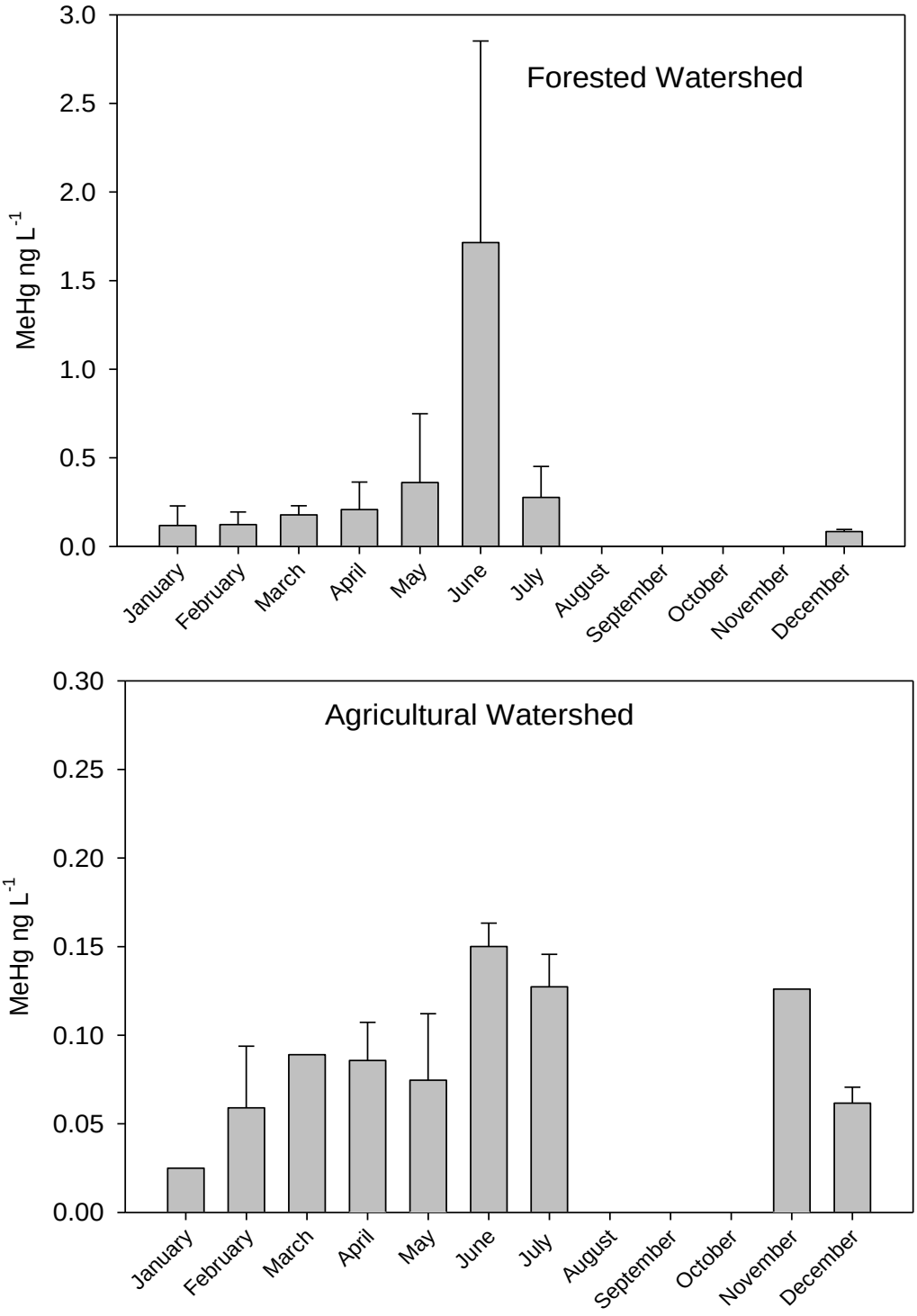


Figure 2.2. Average stream water MeHg concentrations in the agricultural (bottom) and forested (top) site from 2007 to 2008. Error bars denote standard deviation of within month samples. Peak MeHg concentrations occur in the May to July, with concentrations substantially higher in the forested site – note the factor of 10 difference in scale. Data from Heyes et al. 2010

Water Sampling

The watersheds are instrumented at the outflow with water flow recorders and water flow weighted samplers. 120° V-shaped weirs and stilling wells were installed at the bottom of the streams in the 1970's to measure flow rates from the streams. Using the water height in the stilling wells, depth monitors, and Campbell Scientific ® data loggers, flow and flow events were monitored and triggered water sampling once a certain flow threshold was reached. This data for my study period (2016 -2017) was still being processed at the time of this writing and was unavailable, so comments on flow will be based on field observations. Within each of these watershed, a series of well transects crossing the streambed and streambanks were established (Heyes et al. 2010). The research scientists at SERC and CBL have installed within stream and "out of stream" wells to monitor nutrient import and export from the stream and stream banks. The riparian zone wells collect water from the surface or stream bed to a depth of 50 cm. The streams sometimes stop flowing for periods in the summer, with the forested stream (110) stopping more frequently than the agricultural stream (109). Two transects in each watershed have been selected for use in this study; one close to the mouth of the stream and one closest to the head of the stream (Figures 2.1). The two transects are approximately 100 meters apart. For this study, only the central wells within the streambed were used.

Wells provide an evaluation of the "bulk" chemistry of water mixing between the stream and the stream bank and bed. To augment the well chemistry samples, porewater samples were collected using a polyetheretherketone 1/32" ID, 1/16" OD (PEEK) Tube© and 0.2 micron rhizon (Seeberg et al. 2005) array designed to sample across the aerobic and anaerobic boundary layers, likely the iron and sulfate reductions zones (Gilmour 2008) (Figure 2.3 A, B). The porewater arrays were installed in the stream bed at each transect, where wells were already placed, with ports at 0 (just above the sediment surface), and 2.5, 5.0 and 7.5 cm below the sediment-water interface (Figure 2.3 A.). PEEK lines and rhizones were cleaned with 12% HCl

prior to installation. Water samples were collected every two weeks (on average) (Table 2.2) drawn through PEEK lines using a Norm-Ject Single use syringe and stabilized with 0.5% HCl prior to analysis. I targeted different depths in porewater analyses to capture transitions in MeHg concentrations as water passes from the anoxic layers, which should contain Hg methylating bacteria (Podar et al. 2015, Parks et al. 2013).

Water samples from the wells and weirs were collected weekly with a Teflon tube connected to a peristaltic pump. 300-400mL were collected and stored in large acid washed Polyethylene Terephthalate (PETG) bottles. Samples were filtered through 0.45 μm combusted glass fiber filters and acidified to 0.5% with concentrated HCl. Water samples from the rhizones were held in the Norm-Ject syringes and transferred into the small screw top PETG vials, and acidified 0.5% with concentrated HCl. All water samples were refrigerated until analysis.

Sediment Sampling

In the Kilpatrick Marsh, located downstream of these watersheds, Mitchell and Gilmour (2008) found that the top 6 cm of sediment was most important layer for Hg Methylation. This is the likely location of the sulfur reducing bacteria, iron reducing bacteria, and possible methanogens that could be responsible for methylating mercury in this ecosystem. I focused on collecting the top 15 centimeters for our samples to capture this gradient in the anaerobic/aerobic gradient within the stream (Figure 4.A.).

Sediment cores were collected every two weeks (Table 2.2) from the stream bed sediment of each transect using BP 60 mL syringes. With the bottom nipple cut off so the syringe forms a cylinder that can later be extruded. The sediment cores were on average 10 cm deep in order to cover the anticipated aerobic and anaerobic layers within the sediment. Cores were aliquoted into four equal depths, usually in increments of 2.5 cm, weighed, and frozen until analysis. One replicate core was taken from each watershed.

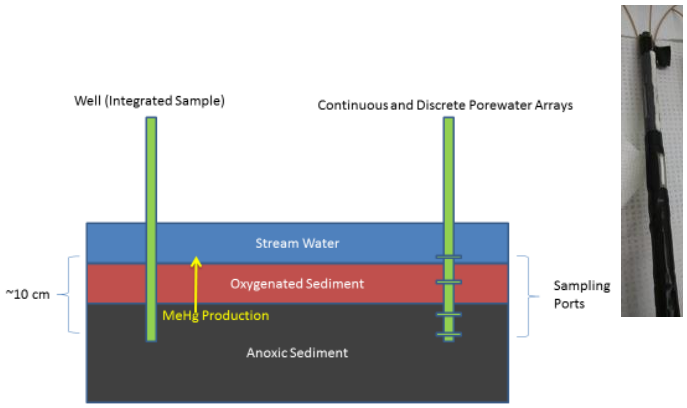


Figure 2.3. (Left) Traditional well sampling approach contrasted with discrete

Leaf Sampling

At the end of the 2016 sampling season (September) and before the beginning of the 2017 sampling season (Early April), leaves from within the stream channel and on the stream bank were collected around the transect lines. Leaves were stored in plastic bags and frozen until laboratory analysis.

Invertebrates

Benthic macroinvertebrates were collected every two weeks following protocols in Leslie and Lamp (2017). A plastic ring 20 cm in diameter and 12 cm deep was used to collect three sediment cores within the 100 m stretch. The core was placed in a five-gallon bucket and covered with stream water. The buckets were stored in a walk in refrigerator at 4° C until analysis. For analysis, the sediment and detritus were rinsed into a 750 micron sieve and live invertebrates were sorted and identified to the family or genus level using Merritt and Cummings et al. (2008). Samples were rinsed with nanopure water and freeze dried prior to analysis.

Table 2.2. 2016 sampling schedule. 2017 was similar, yet shorter in duration (April to June) and without OsmoSamplers.

Date	Discrete Porewater and Sediment Core Samples	OsmoSamplers	Benthic Macroinvertebrates
APRIL 14th	Deploy Sampling array for discrete porewater, collect sediment cores		Collect invertebrates
18th-21st	Deploy OsmoSamplers and collect invertebrates with dip net		
28th	Collect discrete samples and sediment cores		Collect invertebrates
MAY 12th	Collect sediment, porewater samples		Collect invertebrates
19th	Collect porewater samples		
26-28th	Collect sediment, porewater		Collect invertebrates
JUNE 9th	Collect discrete samples, sediment cores		Collect invertebrates
19th	Collect discrete samples		
24th	Collect sediment samples		Collect invertebrates
JUNE 7th	Collect discrete samples, sediment samples		Collect invertebrates
14th	Collect discrete samples, sediment samples		Collect invertebrates
29th	Collect discrete samples sediment samples		Collect invertebrates
AUGUST 12th	Collect discrete samples, sediment samples		
25th	Collect Final discrete samples and sediment samples	Retrieve OsmoSamplers	Collect Final invertebrates

MeHg Sample Preparation

Prior to MeHg analysis all samples (except macroinvertebrates) were distilled following Horval et al. (1993). For sediment and sediment, one to two grams of thawed wet sample was added to the Teflon distillation vials along with 1 ml of 9N sulfuric acid. For water samples, up to twenty mL of sample was added to the sample vial with 0.5 mL of 4M sulfuric acid. Samples were distilled at 190°C for up to 3 hours or until the receiver was filled to 22-25 mL. The distillation was refrigerated until lab analysis. For macroinvertebrates, freeze dried material was weighed into 3.5 mL microwave vials, to which was added 2mL of 4M nitric acid. Samples were placed in an oven at 60°C overnight. An aliquot of sample was then taken for MeHg analysis and measured. A separate sample for sediment was dried for later wet weight dry weight conversion.

T-Hg Sample Preparation

For sediment and leaf samples, 2-3 grams of material were added to a 75mL Erlenmeyer flask along with 5 mL of 50:50 concentration nitric/sulfuric acid. The samples were digested for up to 8 hours, after which the volume of the flask was brought up to 50 mL with 1 mL of bromochloride and the remainder with nanopure water. A subsample of the digest was poured into screw top PETG vials and stored at room temperature until analysis. For macroinvertebrates, once MeHg measurements were confirmed, usually the next day, an additional 1mL of acid was added to the vial and then microwave digested for 30 minutes in an Anton Paar Microwave at an increasing temperature ramp to 150°C for 20 minutes, with 10 minutes for cooling (Taylor et al. 2013).

MeHg and T-Hg Instrumental Analysis

All samples were analyzed via cold vapor atomic fluorescence spectrometry using a Tekran 2700 and Tekran 2600 for MeHg and T-Hg, respectively, following the EPA methods 1630 and 1631, respectively. For MeHg analysis, all of the distillate samples were poured into

brown amber vials, brought up to at least 20 mL of volume with nanopure water, buffered with acetate buffer, stabilized with ascorbic acid. Sodium tetraethylborate is added and the vial sealed. The vial is purged with argon and the head space gas trapped on Tenex within the Tekran 2700. Separation of mercury species is done using a capillary column, after which all species are converted to elemental Hg by pyrolysis before detection by fluorescence. For analysis of invertebrates 100-300 μL of the digest was added to 20 mL of nanopure water and run with the reagents listed above. Total mercury samples were prepared by addition of bromine monochloride (BrCl) to the aliquot 12 hours before analysis. For T-Hg detection, samples are placed in the same amber vials with hydroxylamine hydrochloride (to degrade any remaining BrCl) and stannous chloride to convert all mercury to elemental mercury, sealed and purged with argon. Mercury in the purge gas is trapped on gold coated beads to concentrate the Hg prior to release and detection by fluorescence inside the Tekran 2600. The detection limits for the surface water was 0.01ng/L, 0.05 ng/g in the sediments, and porewater was 0.05 ng/L. I used three standard reference materials to measure the recovery rate of Hg and MeHg in our analyses. TORT-3 and DERM 2 were used for invertebrate analyses with average recoveries around 90%. MESS-3 was used for sediment ant leaf analyses and average recoveries were approximately 90%.

Statistical Analyses

For most of the samples recorded at discrete time points (with the exception of amphipods), the sample size was small. Even over the breadth of the 2016 sampling season, there were less than 10 samples for most of the porewater samples at each location and depth with non-normal distribution. Thus, I chose to use Wilcoxon ranked-sum tests to compare all invertebrates, sediment and porewater samples across the sampling seasons between the watersheds to see if any differences were statistically significant. In addition, some preliminary correlations were drawn between amphipod MeHg concentrations to porewater and sediment MeHg concentrations

at different depths. Sample size was low for individual depths (4-10) in some instances, so correlations were more exploratory than conclusive.

Results

The concentrations of MeHg were higher in the sediment (0.249+/- 0.85ng/g vs 0.043+/- 0.075, wet weight) (Figures 2.4 and 2.5, Appendix A Table 1.) and macroinvertebrates (195+/- 132 ng/g vs 54 +/- 60.1 ng/g, dry weight) (Figure 2.6 and 2.7, Appendix A Table 4.) in the forested site than the agricultural site in 2016, respectively. At the forested site, the upper 5 cm of sediment generally had higher concentrations of MeHg compared to the agricultural site for most of the sampling season (Figure 2.5), with the agricultural site having a noted spike in concentrations in the deepest sediment samples (Figure 2.4) towards the end of the sampling season in 2016. A Wilcoxon rank sum test showed a p value of 8.962e-08, indicating the sediment samples for all values at all depths between the two sites are not equal. No such trends are as apparent in 2017 sediment sample data (Figures 2.8 and 2.9). There was little difference in MeHg concentration between catchments (Wilcoxon p-value = 0.081).

In the porewater of 2016, the forested site appears to have elevated levels of MeHg in the 0 cm and 7.5 cm from May to July, as expected (Figure 2.10, Appendix A Table 3). The agricultural site had some higher porewater MeHg concentrations in the lower 5.0 and 7.5 cm depths, but the surface water was much lower in the agricultural site (Figure 2.9 Appendix A Table 3) than the forested site throughout the year. Average porewater MeHg concentrations in the forested (0.746+/- 0.846 ng/L) and the agricultural site (0.377+/- 0.335ng/L) did not appear to be significantly different when compared using a Wilcoxon ranked sum test (p = 0.190.) Likewise, the patterns were not as apparent with the 2017 porewater samples (not shown). The upstream portion of the forested site consistently seems to have the highest concentrations of MeHg, both in the porewater and the sediment.

I found the diversity of the invertebrate communities was higher in the agricultural samples than the forested sites in both 2016 and 2017 (Figure 2.11, Appendix A Tables 6, 7, 8, and 9). The taxa most consistently found were amphipods (*Gammarus* sp.), oligochaete worms, isopods (*Caecidotea* sp.), caddisflies (Hydropsychidae), hellgrammites (*Cordyalus* sp.), Asiatic clams (*Corbicula* sp.), and water beetles (Hydrophilidae). To cover the ranges of trophic levels of interest and the taxa most commonly found, I focused the MeHg analyses on the following taxa: amphipods (omnivorous), caddisflies (collectors, shredders), hellgrammites (predators), and water beetles (predators).

Caddisflies were present in the beginning of the season in both years, but disappeared by the summer. Caddisfly MeHg appeared to have highest concentrations at the beginning of the field season but the MeHg concentrations decreased as did their abundance. Hellgrammites and water beetle MeHg concentrations seemed to remain consistently high, (depending on size) throughout the season but are present in low numbers. Amphipods were consistently found at sampling sites over the course of the 2016 sampling season. MeHg concentrations are higher for all taxa in the forested watershed than the agricultural site in 2016 (Figure 2.6 and 2.7, Appendix A, Table 4) with a Wilcoxon ranked sum test p value of 5.856e-12. The portion of MeHg as T-Hg varied among the species, with amphipods and megalopteran having the highest variability in percentage MeHg (Table 2.4). Correlation analyses between porewater, sediment and amphipod MeHg concentrations were performed (Table 2.5). Some relationships between these matrices and MeHg concentration in the amphipods are apparent, particularly the upstream sites but in most cases, no relationships were found. The small sample sizes for the sediment and porewater restrict the applicability of this approach.

In 2017, the agricultural site appears to have consistently higher species diversity than the forested site through the season, but the differences in MeHg concentrations across the trophic levels are less pronounced (Figures 2.12 and 2.13, Appendix A, Table 5). Using the Wilcoxon

ranked sum test, the between site invertebrate MeHg concentrations for all invertebrates in the entire season were found to be significantly different ($p = 3.389e-05$). Of the invertebrates collected in 2017, Hydrophilidae have the highest concentrations of MeHg, but only in a few instances (Figures 2.12 and 2.13, Appendix A, Table 5). Otherwise, concentrations seem to be lower in the forested site in comparison to 2016 for invertebrates, except for Hydrophilidae. The agricultural site has similar concentrations in 2017 and 2016, with the exception of the Hyrdophilidae.

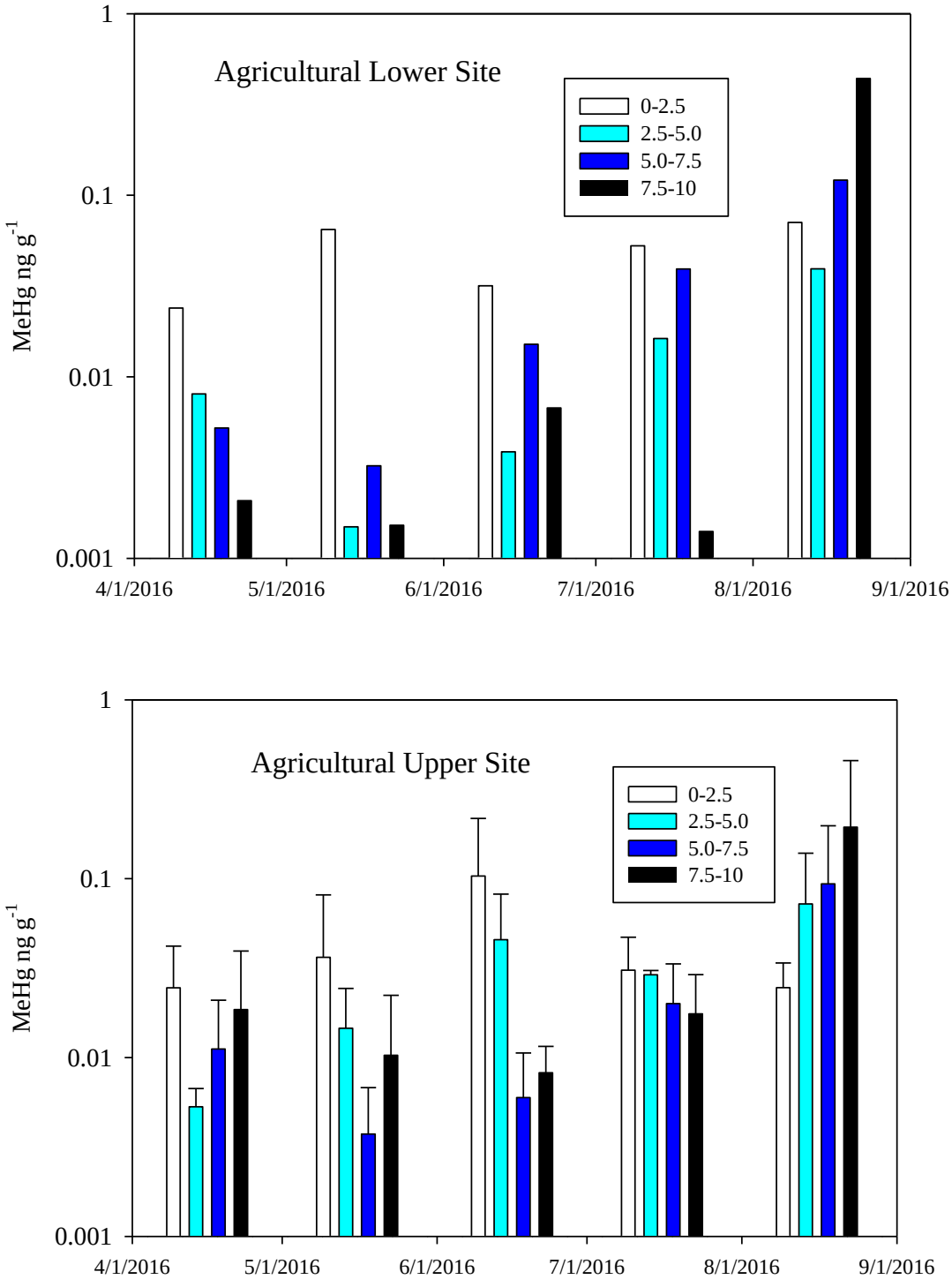


Figure 2.4. Sediment MeHg concentrations in ng/g (wet weight) across time for agricultural sites in 2016. The legend shows the depth in centimeters of the samples. The peaks in both occur in August.

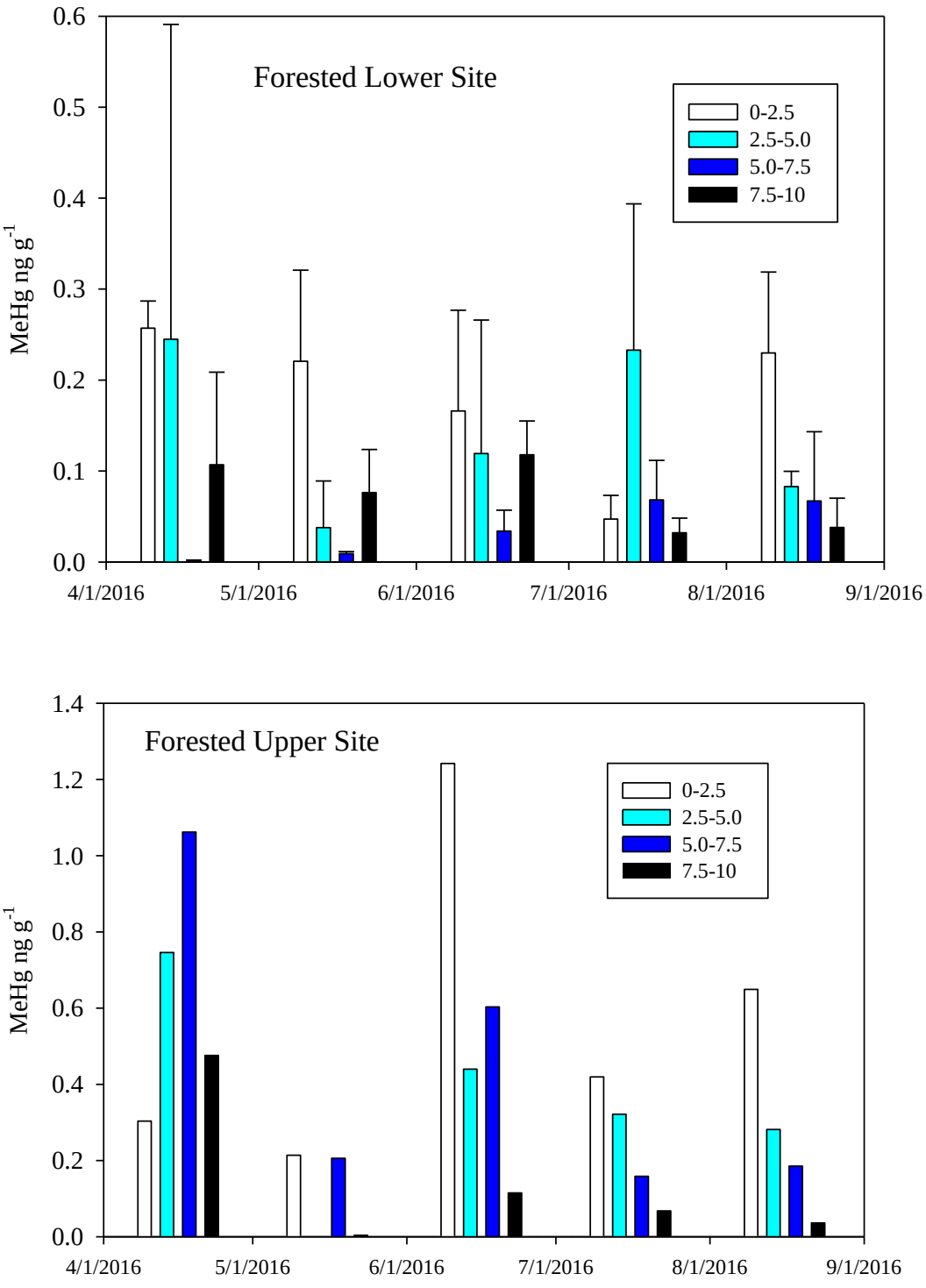


Figure 2.5 Sediment MeHg concentrations (wet weight) across time for forested sites in 2016. The legend shows the depth in centimeters of the samples error bars denote standard error of replicates.

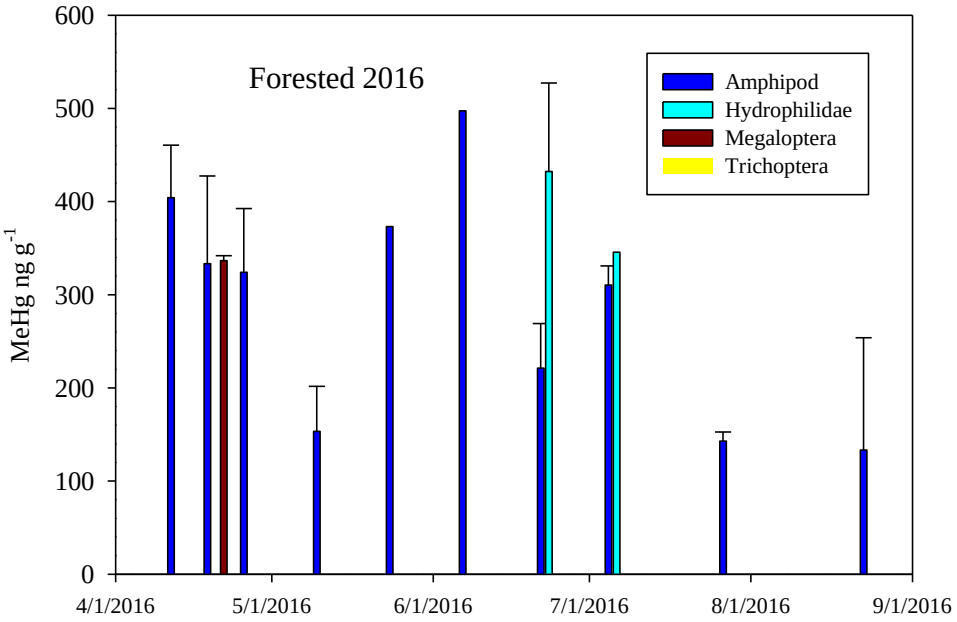


Figure 2.6 Macroinvertebrate MeHg concentrations for taxa across the 2016 season in the forested site. Concentrations are freeze dried mass.

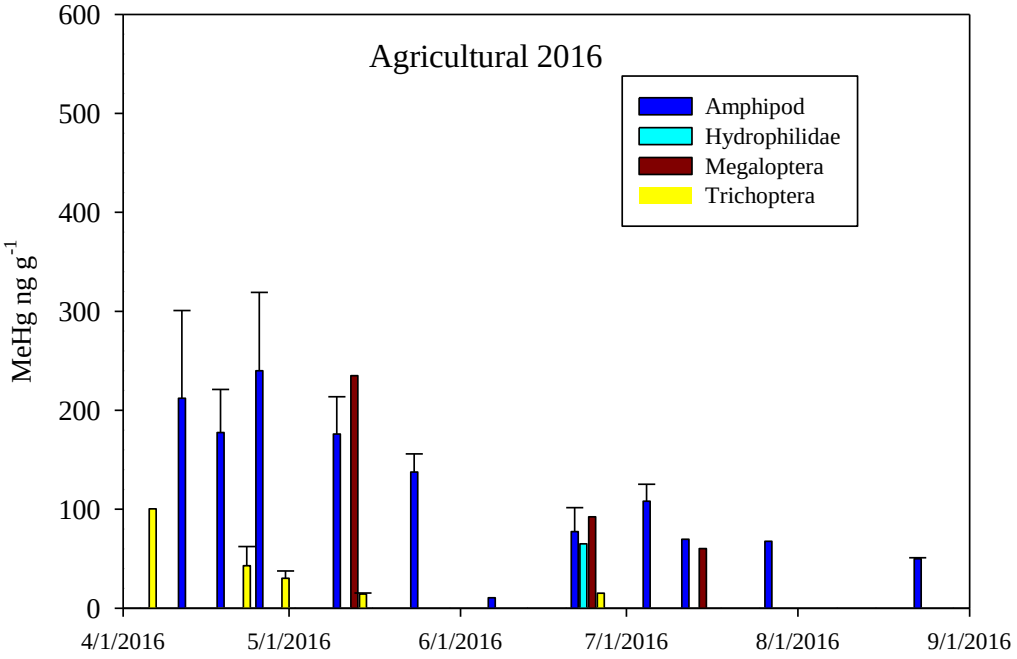
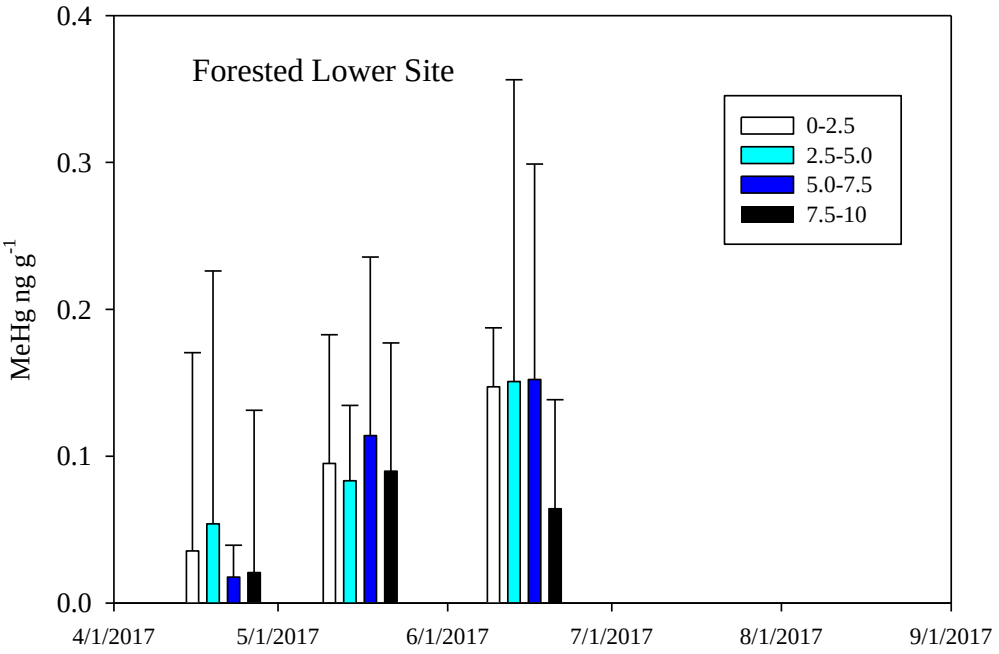


Figure 2.7 Macroinvertebrate MeHg concentrations for taxa across the 2016 season in the agricultural site. Concentrations are freeze dried mass.



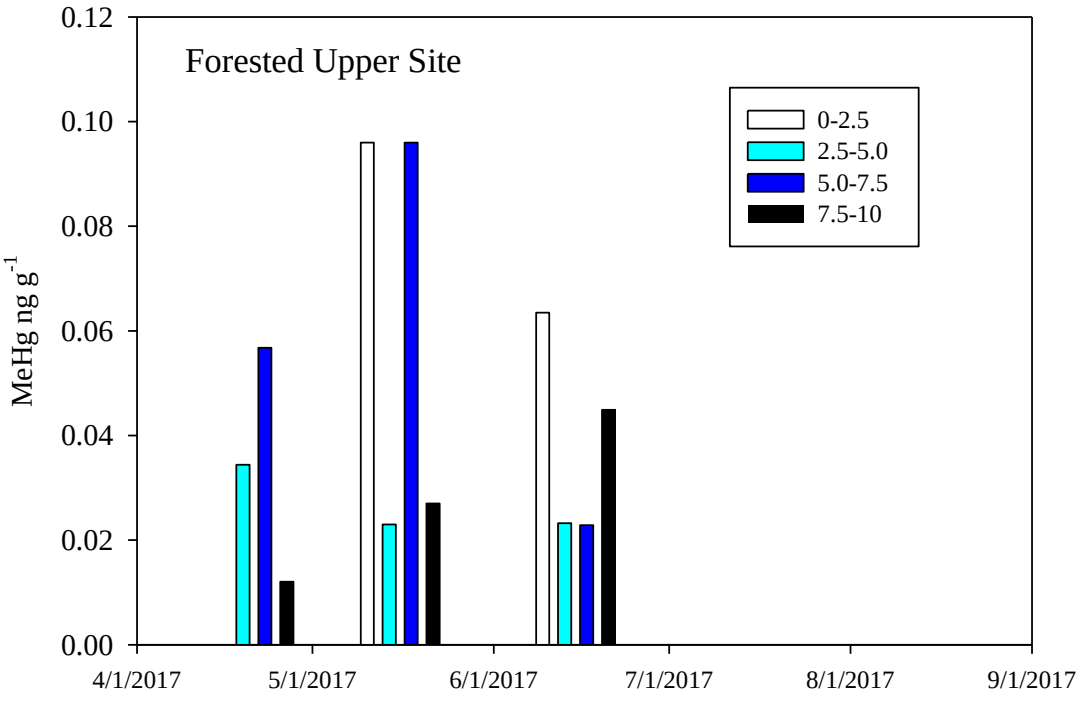


Figure 2.8 Sediment MeHg concentrations (wet weight) across time for forested sites in 2017. The legend shows the depth in centimeters of the samples and error bars denote standard error of replicates.

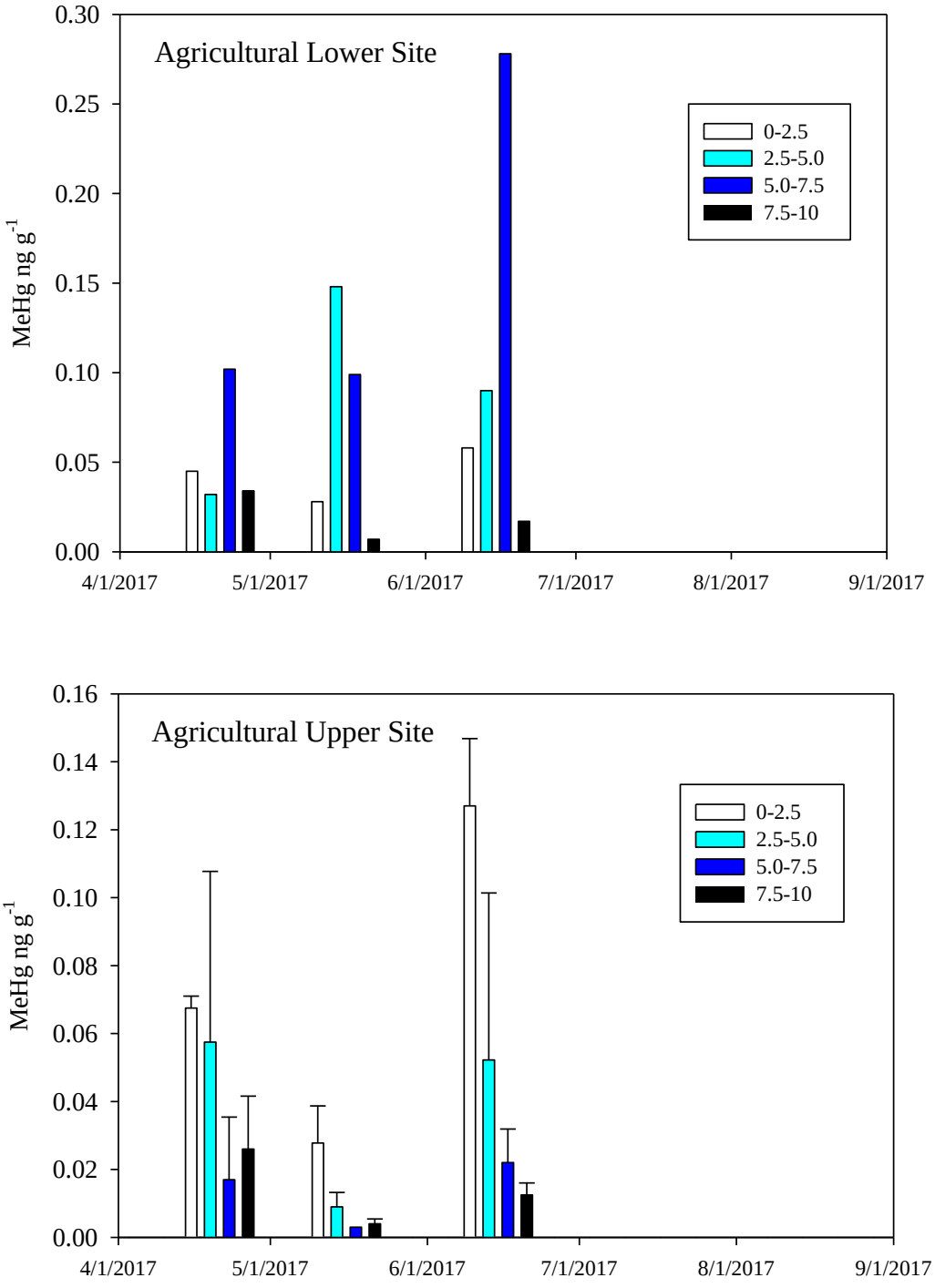


Figure 2.9 Sediment MeHg concentrations (wet weight) across time for agricultural sites in 2017. The legend shows the depth in centimeters of the samples and error bars denote standard error of replicates.

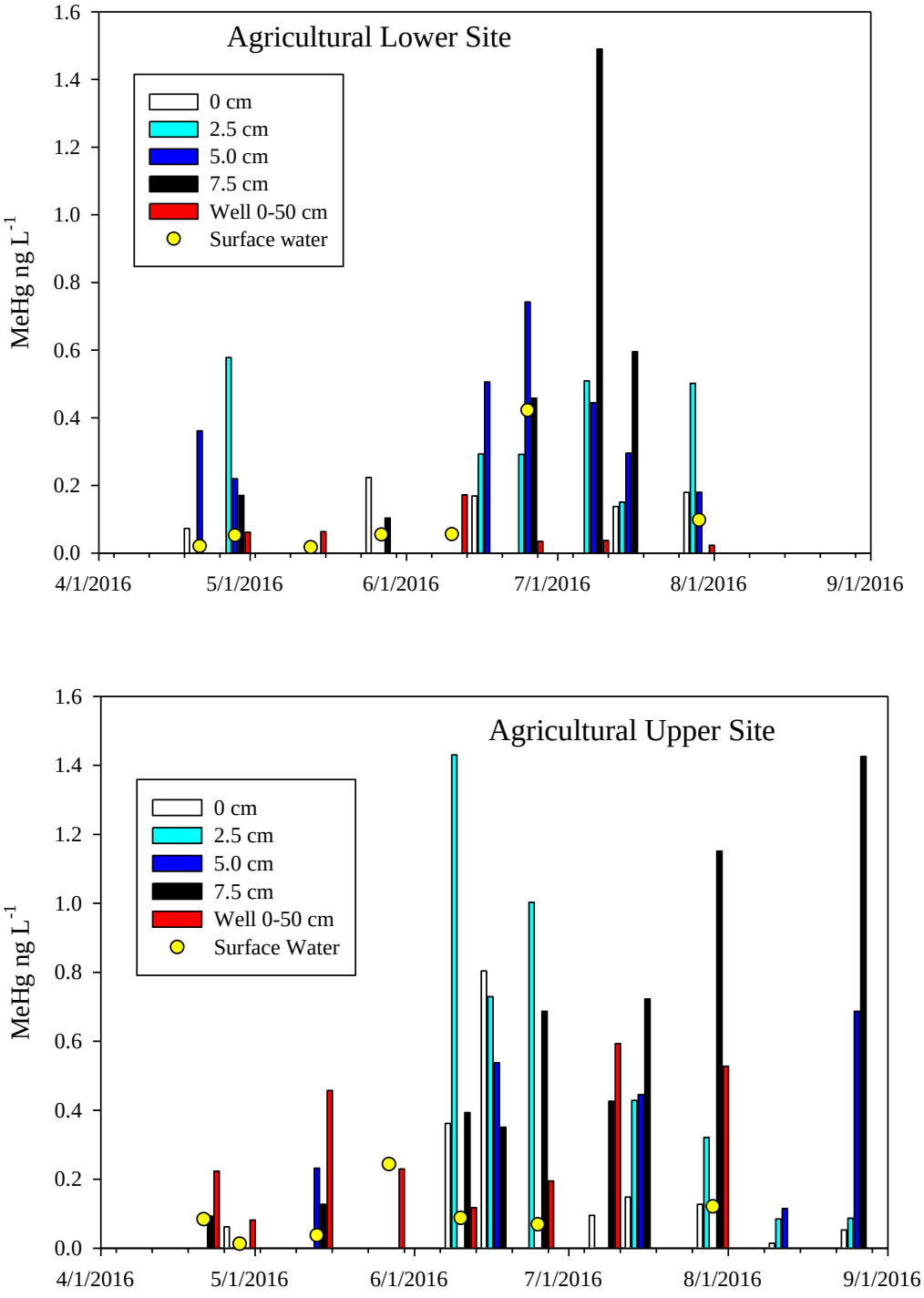


Figure 2.10 2016 Porewater MeHg profiles for the upstream and downstream transects within the agricultural sites. 0 cm denotes the samples taken in the porewater array at the sediment surface.

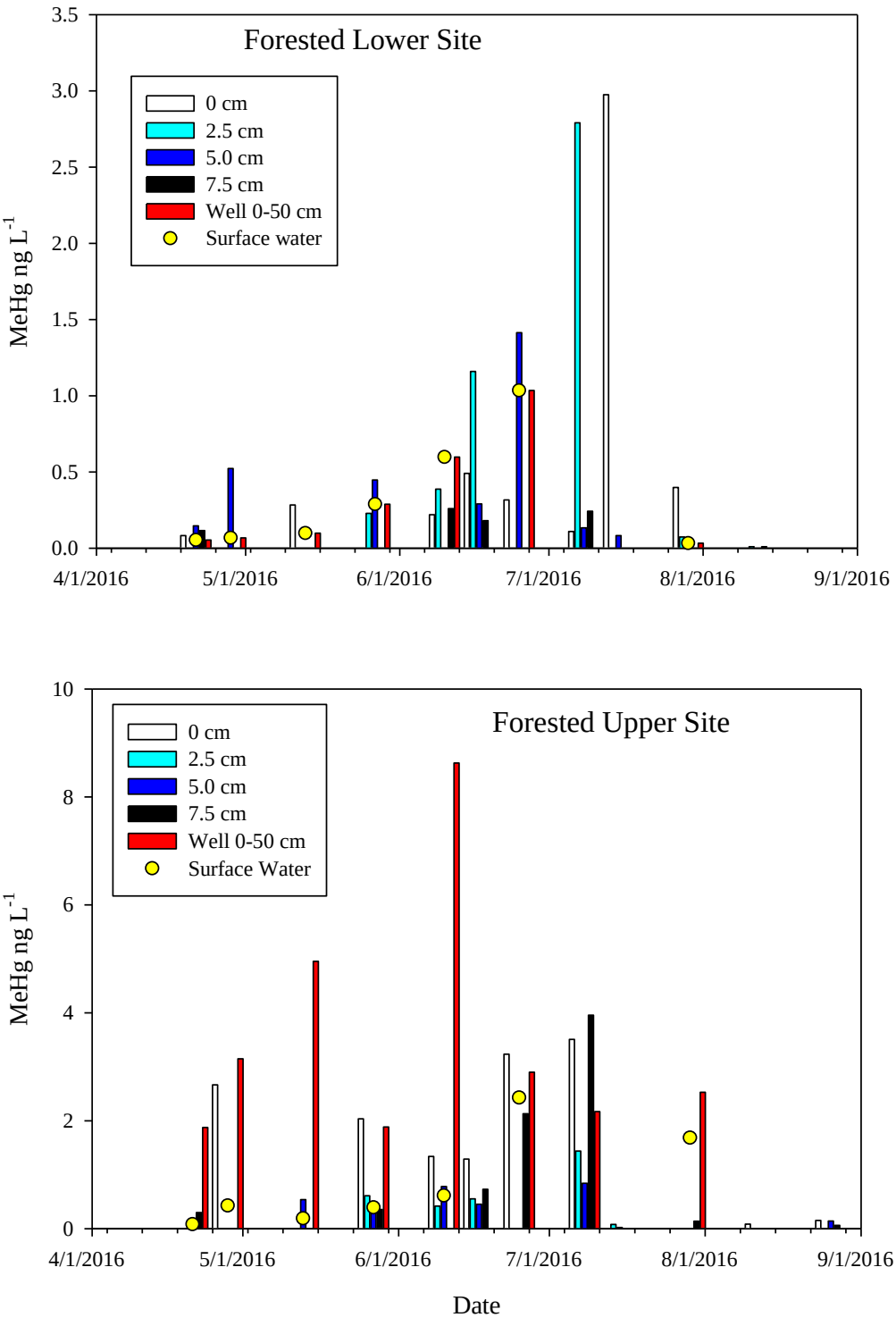


Figure 2.11 2016 Porewater MeHg profiles for the upstream and downstream transects within the forested sites. 0 cm denotes the samples taken in the porewater array at the sediment surface.

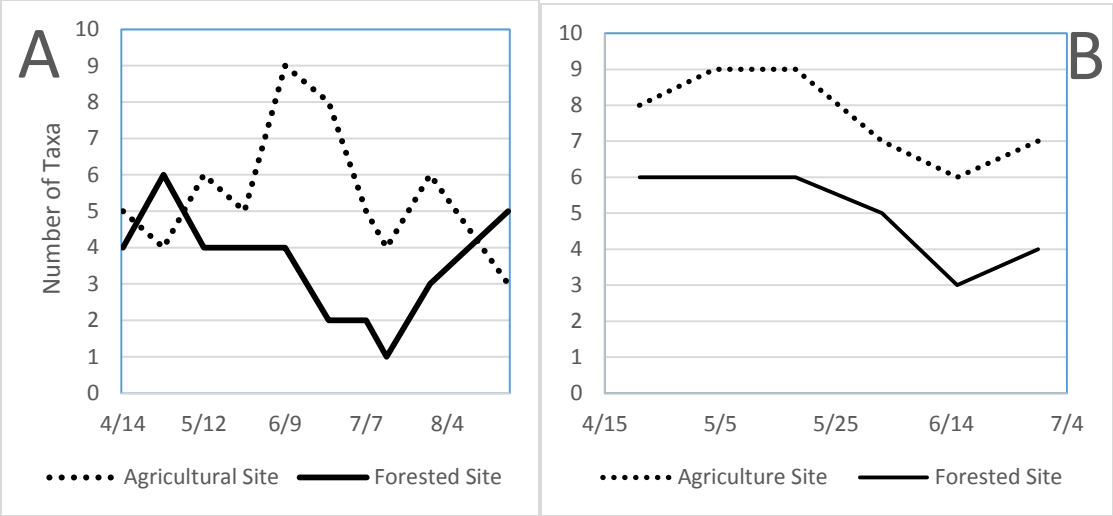


Figure 2.12 Number of taxa found in the field sites throughout the 2016 field season (A) and 2017 field season (B). Most taxa are genus or family level. The agricultural site contained communities with higher diversity for most of the samples collected.

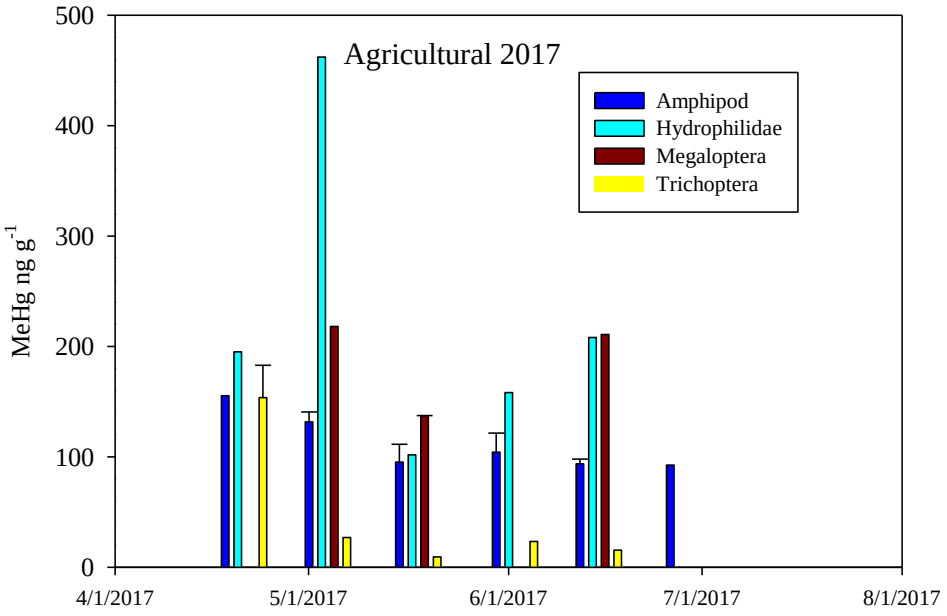


Figure 2.13 Average macroinvertebrate MeHg concentrations for four taxa collected in the 2017 season from the agricultural site. Concentrations are freeze dried weights.

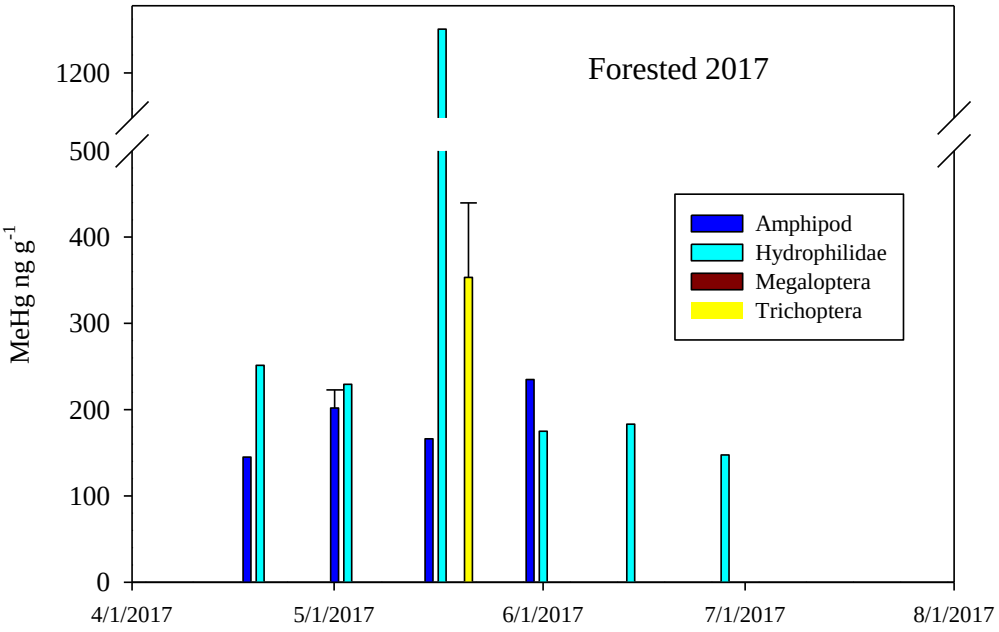


Figure 2.14 Seasonal MeHg concentrations for three taxa in the 2017 season in the forested site. Concentrations are freeze dried weights

Table 2.3. Leaf MeHg and T-Hg (ng/g, wet weight) for fall on 2016 and spring of 2017.

Sample ID	MeHg	THG	MeHG:THg
Forested Upstream 2016	0.601	3.313	0.181
Forested Downstream 2016	0.497	3.108	0.160
Agriculture Upstream 2016	0.640	2.600	0.246
Agricultural Downstream 2016	0.491	2.683	0.183
Agricultural Middle 2016	0.494	2.203	0.224
Forested Middle 2016	0.422	4.347	0.097
Forested up stream out of stream 2017	0.230	2.574	0.090
Forested upstream in stream 20n17	3.194	3.587	0.890
Forested downstream out of stream 2017	0.324	2.984	0.109
Forested downstream in stream 2017	2.195	2.618	0.839

TABLE 2.4. MeHg:T-Hg ratios for samples collected.

Sample	**MeHg:T-Hg**
Leaves	11-80%
Trichoptera	33-66%
Amphipod	29%-90%
Hydrophilidae	21%-53%
Megaloptera	15-90%

Discussion

1. **Does MeHg accumulation in the hyporheic zone coincide with changes observed in the biota?**

In 2016 MeHg concentrations in sediment and water phases of the forested watershed followed the same pattern as Heyes et al. (2010) had reported. In the forested watershed, a peak in MeHg concentrations was observed in the upper layers between June and July, with concentrations decreasing towards the end of the season. Concentrations in the different layers of sediment and porewater appear to vary widely within each watershed, with few clear patterns arising. Precipitation has a strong influence on the headwater stream, with water levels high in the early spring and subsided almost completely by the mid-summer, with the forested site drying out in the lower half of the streambed and weir by July and the upper stretch by August. In 2017, both sites were completely dry by the end of June. As the water level drops MeHg concentrations remain the same in the surface layers of the sediment of the forested watershed but MeHg concentrations increase in the lower layers of the agricultural site. This is also reflected in the porewater of both watersheds, although water levels drop so far the upper sediments dry out in

the forested catchment. Despite these late season increases in MeHg concentration at the agricultural site, MeHg concentrations at the forested site remain higher than the agricultural site. Within each watershed, while the patterns in MeHg remain similar between the two sites, the timing and magnitudes are slightly different. This indicates that local biogeochemistry varies markedly within these watersheds. The most marked differences in concentrations are within the forested site, where porewater concentrations at the upper site are more than twice the lower site. The concentrations in the porewater and sediment of the upstream portion of the forested site may contribute to elevated MeHg levels downstream, although I did not investigate fate and transport processes and how this source impacts MeHg export downstream. It is more likely a "hotspot" in upstream portion may have some unique chemistry absent at the downstream portion of the watershed and the agricultural site in general (Mitchel et al. 2008).

Of the invertebrates, amphipods appeared to mirror the trends in the porewater and sediment MeHg in each of the watershed most consistently (Figure 2.10). Amphipods MeHg levels increase prior to the rise in MeHg in the porewater (Figures 2.14 and 2.15) and are higher in concentration in some instances than even the predaceous hellgrammites and water beetles. Decomposing leaves are a potential substrate for invertebrates (Tiegs et al. 2008). Deposition of leaves is an important pathway of Hg to the forest (Graydon et al. 2012), as Hg depositing from the atmosphere binds strongly to organic material, especially leaves, and may be scavenged from the atmosphere and released into the streams upon leaf decomposition (Heyes et al. 2010).

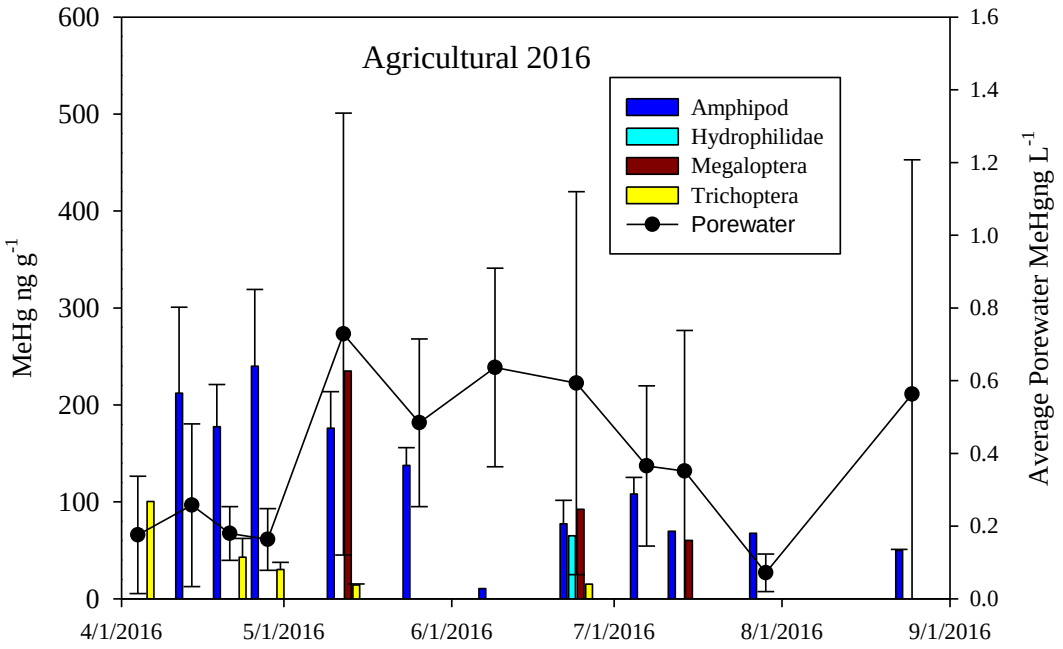


Figure 2.15 Macroinvertebrate MeHg concentrations for taxa across the 2016 season in the agricultural site, along with the average MeHg concentrations of the porewater from upstream and downstream.

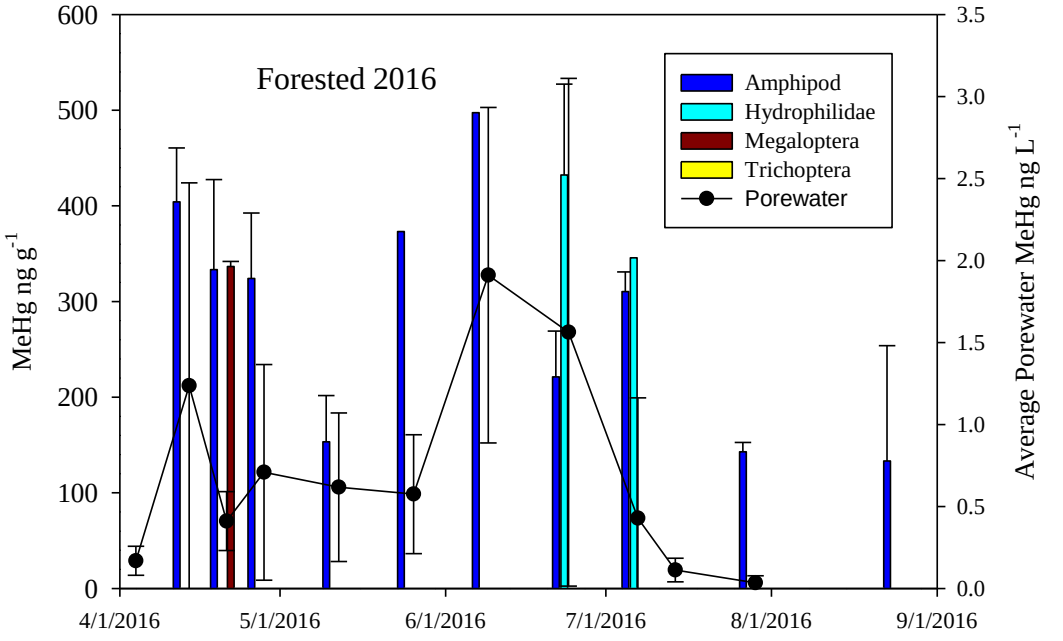


Figure 2.16 Macroinvertebrate MeHg concentrations for taxa across the 2016 season in the forested site, along with average MeHg concentrations of the porewater from upstream and downstream.

2. **Are any organisms resident throughout the year, such as amphipods, that could be used to track any seasonal shift in MeHg concentrations in the sediment and water of a single watershed? This is important as time of collection would affect the ability to compare watersheds.**

Amphipods did appear to have a difference in MeHg concentrations between the two watersheds corresponding to the general differences in the MeHg, indicating they may be of use to assess the MeHg condition of the watersheds. However, the timing of the elevated concentrations in the invertebrates was not always in sync with the elevated concentrations of the sediment and porewater. One explanation for this is the sources of MeHg to amphipods shifts over time. Leaf decomposition could provide a source of MeHg not captured in the sediment and porewater. Leaf samples collected at the beginning of the 2017 field season had elevated MeHg levels, which could explain why MeHg levels were high at the beginning of the field seasons in amphipods and caddisflies, as these invertebrates are likely consuming the leaf litter. Factors controlling MeHg in invertebrates likely also depend on life history and feeding strategy, shifting location as the food source changes. Leaf mats appear to decrease in number over the spring and summer and water levels drop forcing amphipods into the wetter sediment. Amphipods may in fact be an excellent indicator capturing the shifting sources that would be difficult to capture using water and/or sediment.

Seasonal shifts in water levels also impact species diversity in 2016 as seasonal patterns appear to be driven by the water level and precipitation (Figures 2.11). It is likely that amphipods would be a useful tool for indicating the level of MeHg exposure in small streams if they are sampled in the April to May period, as they showed more variation in 2016 than the higher trophic levels (Megaloptera and Hydrophilidae in Figure 2.12 and 2.13), their life history makes them more ideal to collect year round (in comparison to the caddisflies), and they seem to show some association with MeHg levels in the sediment and porewater, possibly due to feeding

strategies as detritivores (Table 2.5). In addition, since the other taxa may be susceptible to drying periods in the stream, amphipods may be able to cope with short drying periods and return quickly to the water column, making them an ideal candidate for MeHg sampling. Amphipods in the forested site have very high concentrations, on the same order of magnitude of small fish or crayfish in other studies, which could have serious implications for taxa that feed on the amphipods. Fish are a common predator of amphipods, but in these streams, it is likely that amphibians, birds, or other invertebrates could prey on the amphipods.

3. Does MeHg concentrations in the organisms reflect differences in MeHg production, measured as MeHg concentrations, between the watersheds?

MeHg concentrations of sediment and invertebrates were significantly different between the two watersheds in 2016.The forested watershed saw MeHg concentrations in these matrices nearly an order of magnitude higher than concentrations in the agricultural watershed. Porewater concentrations were not statistically different ($p = 0.081$), but this could be due to a small sample size of porewater collected ($n = 5$ for most porewater samples) and the high variability. Furthermore, I averaged porewater concentrations over the sampling depths, which likely include zones with no methylation activity, diluting the signal. I did not try and select peak concentrations as bulk sediment is collected in most studies. Sediment and invertebrate concentration were markedly different between the watersheds, and mostly clearly depicted in the upper stretch of the forested watershed, (Figure 2.6). The trends in the concentrations in the sediment did not always coincide or correlate with the concentration trends in the porewater, indicating there may be some interstitial processes that are not captured in this study or is a collection artifact because the samples were not directly "related" (porewater not separated from the sediment that was measured but rather originating from the *in situ* stream collection).

The MeHg levels measured in amphipods in this study where similar to that measured by George and Batzer (2007) who concluded amphipod MeHg levels were higher due to amphipods being detritivores, so that amphipods would likely have a closer association with the sediment than the other invertebrates. The biomagnification factor, if I use the MeHg concentration of the amphipods and the average concentration of the porewater samples, is 2.305 l/kg for the forested watershed and 1.918 l/kg for the agricultural watershed in 2016, which is close to the value reported for Mason et al. (2000).

Conclusions and Future Work

I was able to observe increases in the concentration of MeHg in the sediment and porewater, most often in the mid to late summer at the sites. MeHg concentrations in the sediment and invertebrates varied significantly between the two watersheds in 2016. Amphipods (*Gammarus*) appeared to respond most strongly to the variations in the MeHg concentrations, with other taxa having a more muted response. This difference is likely due to feeding strategy and life history, as most of the other taxa were either predaceous, long lived (1+years), or emerge from the stream. Amphipods match the MeHg trends shown in the discrete porewater sampling and sediment to a degree. With high variability and noise in the sediment and amphipods, assessing exact connections is difficult. MeHg concentrations were significantly different in both the amphipods and sediment between the two sites and both varied over time. These amphipods could be useful tools for MeHg risk assessment in waterbodies with little previous study. Timing of sampling is important for determining MeHg load to amphipods, so timing of sampling for determining bioaccumulation and biomagnification rates; food web risk assessment, and MeHg condition of the watershed is important to consider for future studies. Organisms that feed on amphipods from these watersheds, such as fish, amphibians, or wading birds, could be at risk for MeHg exposure.

Future work should use stable isotopes to delineate connections in the food web to see what organisms are at risk for MeHg exposure, as well as determine the base food source for the organisms in the watersheds. In addition, other studies could measure the DOC, dissolved oxygen concentration, oxygen isotopes, and trace metals to see what cofactors could be present with MeHg in the sediments and could facilitate or impede MeHg transfer. Finally, this study of invertebrates should be expanded to other watersheds to see if there are similar trends between MeHg in the sediment, porewater, and other biota to see if the applicability of amphipods as a risk indicator species on a larger scale, including watersheds where fish are present. If this study could be connected with the MBSS invertebrate studies, these connections could be examined on a larger scale.

Table 2.5. Correlations between amphipod MeHg levels and the MeHg levels of porewater and sediment for 2016. Values with an absolute value greater than 0.7 are highlighted. The sample size for the sediments and porewater is limited to four due to the sampling design.

			Upstream Sediment			Downstream Sediment			
		Surface	2.5cm	5.0 cm	7.5 cm	Surface	2.5cm	5.0 cm	7.5 cm
Agricultural Site	amphipod MeHg	-0.40293	-0.73014	-0.33221	-0.30352	-0.57882	-0.32399	-0.46686	-0.33957
	log amphipod MeHg	-0.50562	-0.83298	-0.40094	-0.37026	-0.41247	-0.39549	-0.53077	-0.40686
Forested Site	amphipod MeHg	-0.23802	0.052143	0.510921	0.373619	0.408575	-0.09569	-0.95434	0.750419
	log amphipod MeHg	-0.15318	0.126899	0.536761	0.395574	0.23069	0.042965	-0.89575	0.773443
		Upstream porewater				Downstream porewater			
		7.5 cm	5.0 cm	2.5 cm	surface	7.5 cm	5.0 cm	2.5 cm	surface
Agricultural Site	amphipod MeHg	-0.62353	-0.91683	-0.61317	-0.39334	-0.54585	-0.59101	0.964774	-0.75965
	log amphipod MeHg	-0.69303	-0.91143	-0.66165	-0.47292	-0.57835	-0.70468	0.993215	-0.5722
Forested Site	amphipod MeHg	-0.20652	0.760512	-0.59868	0.352393	0.325031	0.42129	-0.11658	-0.91672
	log amphipod MeHg	0.002032	0.889574	-0.70481	0.511893	0.517967	0.518848	0.112337	-0.95529

Chapter 3

OsmoSampler Viability as a Long-Term Remote Water Sampling Technique for Methylmercury

Introduction

Routine water sampling for MeHg is important to understand seasonal mercury cycling (Heyes et al. 2010). However, traditional MeHg sampling at the low level trace metal concentrations requires stabilization with HCl within 24 hours of collection (Wilde et al. 2004), which can make routine sampling laborious in remote locations. Podar et al. (2015) identified unique and new locations for Hg methylation, such as bio reactors, hot springs, and caves. Carrying out MeHg studies in these remote locations could be difficult with traditional methods, and ideally, a continuous water samplers would be used that could preserve the sample in situ. Osmotically powered water samplers (OsmoSamplers) may be one such tool for continuous water sampling when routine discrete sampling may not be feasible or be cost prohibitive. OsmoSamplers were designed to continuously sample water in remote locations for extended periods of time (up to a year) with little maintenance, no power source, and greater adaptability than most remote and continuous water samplers, such as peristaltic pumps (Johnson and Colleti 2002), or optic sensors (Massoth et al. 1995). OsmoSamplers are traditionally deployed on the seafloor (Jannasch et al. 2004) or the bed of an estuary (Gelesh et al. 2016)and can measure a variety of chemical species in water, including trace metals and ions (Jannasch et al. 2004) or dissolved gases (Gelesh et al. 2016). As of this study, the use of OsmoSamplers for measuring Hg in the environment has not been examined. In this chapter, I explore the feasibility of OsmoSamplers as a method for continuously sampling MeHg in the water and sediment porewater of a headwater stream with inline addition of acid for MeHg stabilization. I completed laboratory trials to assess the practicality of the OsmoSampler acid addition and a field trial paired with discrete water sampling to assess the reliability of OsmoSamplers in comparison to traditional methods.

Methods

OsmoSamplers typically are constructed of chambers separated by semipermeable membranes (Figure 3.1A).

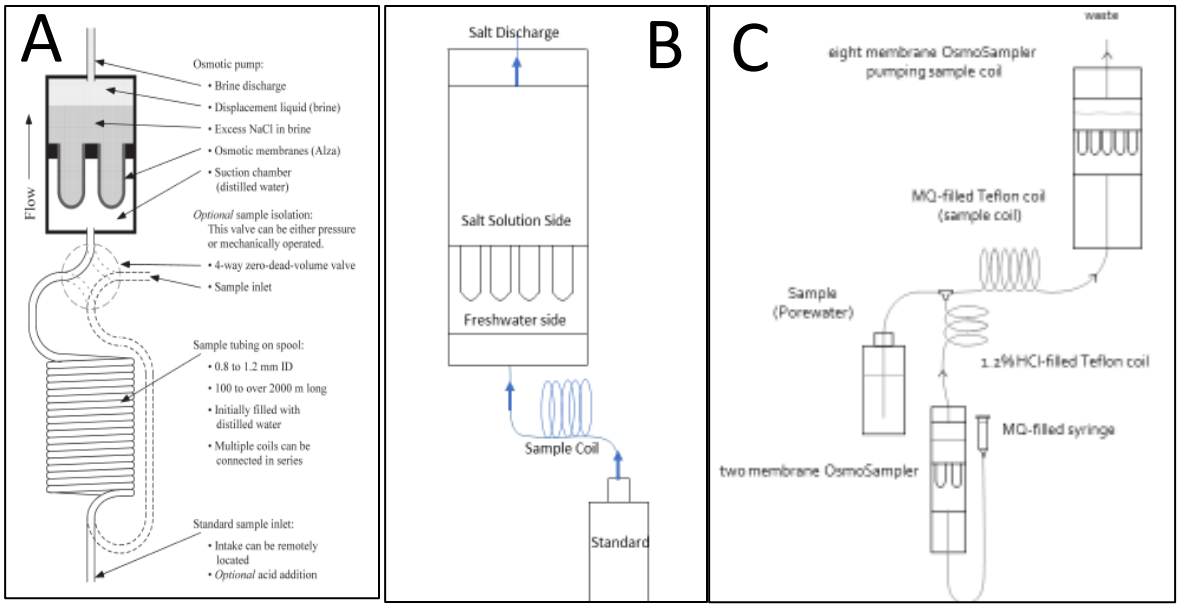


Figure 3.1. A) OsmoSampler. A salt chamber is located above semipermeable membranes (cylinders in figure) forming the "udder". A freshwater chamber is located below the udder where the tubing connects to the main body. Water is pulled from the tubing upwards across the membrane and excess water is discharged from an outlet above the salt chamber (Jannasch et al. 2004). B). Typical OsmoSampler Deployment. One large pump is connected to the standard or sample source by a sampling coil. Sample is pulled into the sample coil by the osmotic pressure in the OsmoSampler. C).OsmoSampler Deployment Design selected for field work, utilizes a small osmotic pump operating in reverse, discharging HCl solution in line into main sampling coil to preserve the sample.

In order to pump, OsmoSamplers rely on an osmotic gradient between two chambers of water, one with nanopure or distilled water and the other with oversaturated saline solution, separated by the semi-permeable membrane. Water is drawn across the permeable membrane from the distilled water chamber into the saline chamber, creating a pressure difference in the distilled water chamber. To sample environmental water continuously, the distilled chamber is usually connected on to thin tubing (1/8" Outer Diameter (OD)) filled with water (Figure 3.1A). The surface area of the semipermeable membranes and the temperature of the OsmoSamplers regulate how much volume of sample is pumped daily. OsmoSamplers are sensitive to changes in

temperature (Jannasch et al. 1994). OsmoSampler experiments and field trials are run with temperature loggers to monitor changes in temperature as a method for correcting pumping rates of the samples. Based on the expected temperatures, OsmoSampler size, and length of deployment, a sampling coil, usually copper or Teflon depending on the desired sample, is prefilled with nanopure water and attached to the freshwater chamber. Sample is then drawn into the sampling coil and displaces the nanopure water into the OsmoSampler freshwater chamber and excess saline solution is expelled out from the saline chamber (Figure 3.1A).

Sample resolution depends on the diffusion coefficient within the sample coil (Taylor 1953). Taylor (1953) found that as long as flow rates are less than 1mL per day and tube inner diameter (ID) is less than 0.5 mm, molecular diffusion along the tubing is minimal due to the fluid flow. The pumping rates for the OsmoSamplers allow for resolution of about a week, depending on the variability and concentration of the chemical species of interest.

For these experiments, I used Teflon 1/8" OD 300m long sampling coils. Teflon coils were pre-cleaned with 6% trace metal grade HCL for up to 8 hours. Sample coils were rinsed with at least 3 times their volume of nanopure water. The effluent pH was monitored with pH strips to assure the effluent was the correct pH. The eight-membrane form of the OsmoSampler pumps about 1 mL of sample a day in to the sample coil. If paired with a four-membrane pump adding acid at a rate of 0.5mL per day, (Figure 3.1B), a sample could be stabilized with HCl, allowing for long deployment times at remote locations where routine discrete sampling may not be feasible. The acid addition coils were filled with 1.2% trace metal grade HCl so that upon addition into the sample coil, the final HCl concentration would be between 0.4 and 0.6%. The eight membrane OsmoSamplers and two-membrane OsmoSamplers pumped an average of 1.1mL/day and 0.4 mL/day, respectively.

Lab Trials

I assembled several OsmoSampler arrays in a laboratory setting to measure the reliability of the OsmoSamplers. The first of the methods used in the lab were a single OsmoSampler drawing a non-acidified MeHg standard (Figure 3.1B). The goal of this experiment is to see if MeHg is recoverable without acid or if it is lost or degraded. The second was similar to the first, a single pump sampling a MeHg standard (Figure 3.1 B) pre-acidified with 0.5% HCl. This experiment's goal is to see if MeHg is recoverable from the Teflon coils if it is already acidified. Studies have shown that MeHg is stable unacidified for about a week, but the preferred method is to acidify (Heyes, personal communication). The unique feature of the OsmoSamplers is that a second pump can be added to the sample collection stream as a way to introduce preservative in situ (Robidart et al. 2013). Therefore, I carried out a third experiment that added a smaller pump with an acid addition Teflon line adding 1.2% HCl to the non-acidified standard (Figure 3.1C, Figure 3.2). The goal of this experiment is to see if the MeHg can be preserved when HCl is added in-line via the smaller pump. Two replicates of each setup were used, for a total of 6 OsmoSampler Arrays. The lab experiments were run for 6 weeks. In the first 2 weeks, the standard used was a MeHg concentration of 6.21 ng/L MeHg. The second 2 weeks, a standard of 0.235 ng/L MeHg solution was used. And in the final 2 weeks, nanopure water was used as the standard. For the acidified standards, the target HCl % was 0.5% HCl. In the acid addition, the smaller pump discharge was connected to a coil filled with 1.2 % trace metal grade HCl solution. As the pumping rate of the smaller pumps was usually 25-30% the pumping rate of the large pumps, the target sample HCl concentration would be between 0.3-0.4% HCl.

Figure 3.2. Pictures of the lab experimental acid addition setup, the Erlenmeyer flask contains the standard, the coils are in the middle, and the pumps are on the left.

In addition, I ran two more laboratory experiments. In the first, an acid addition array (Figure 3.1C) was run with the MeHg standard mixed with a humic acid ligand to represent how the MeHg would likely occur in the field sites. The ligand and standards were stored in Mylar bags to prevent UV degradation. In the second, the HCl was added in line via a smaller than normal diameter tube (1/16" OD, 1/32"ID PEEK© line) in line with the main sampling coil to test if the OsmoSampler would draw in sample based on the ratio of the size difference between the two sampling coils (Figure 3.3), thus eliminating the need for the smaller pump. At the completion of the experiments, the Teflon sample coils were sectioned into 1 m sections, yielding about 1 mL of sample per meter. Samples were run similar to the porewater methods detailed in Chapter 2, by adding up to 20 mL of nanopure water, distilling following procedures in Horval et al. (1993), and running on the Tekran 2700 following EPA protocol 1630. At these small volumes, MeHg was difficult to recover in measurable concentrations, so I switched to sectioning the coils in 5m sections, taking a subsample of 0.5m for ion chromatography analysis. I ran the small 0.5m subsample on a Dionex-1000 Ion Chromatograph to determine chloride concentrations as a method for checking the mixing rates of the acid with the sample in experiments where acid was added in line.

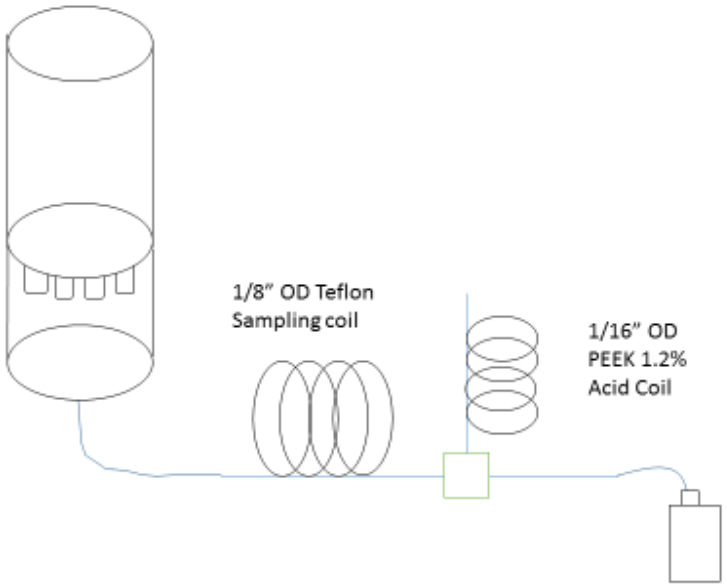


Figure 3.3. Acid Addition OsmoSampler setup using smaller diameter PEEK tubing filled with acid to determine success rate without acid addition pump. The setup is similar to the acid addition set up (figure 12 c), but without the small pump facilitating acid addition, instead relying on the large pump to draw from the two lines based on the proportion of the line diameters.

Field Trial

I installed OsmoSampler acid addition arrays (Figure 3.1C) at the forested site at the Smithsonian Environmental Research Center (see Chapter 2), assuming I would have more success detecting mercury in the elevated concentrations at this site based on historic data (Heyes et al. 2010). I deployed 8 OsmoSamplers acid addition arrays in the summer of 2016 in tandem with the discrete porewater sippers at the same transects (Figures 2.1.) and porewater depths (Figure 2.3A.). This means an OsmoSampler was sampling at the same depth as the discrete porewater samplers mentioned in Chapter 2. Four sets of two OsmoSampler arrays were each stored in a 5 gallon bucket with an iButton temperature logger (Part Number: DS1921G-F5, sensitivity +/- 0.5°C) Two sets of buckets were installed upstream and two downstream by partially burying the buckets and filling the buckets with water to stabilize the OsmoSampler temperature and protect from heavy flow events (Figure 3.4).

Figure 3.4 Field deployment of OsmoSamplers at one of the forested stream sites. Two sets of OsmoSamplers are in each of the buckets, to cover all the depths sampled by the discrete porewater array (shown as clump of syringes).

The OsmoSamplers were deployed on April 21st, 2016 at the beginning of the discrete porewater sipper sampling. OsmoSampler sampling coils were connected to a tether with rhizones for sampling at the same levels as the porewater sippers. They were retrieved on August 26th, the same day the sipper sampling ceased for the 2016 season. If discrete porewater samples had any additional sampler remaining after MeHg analysis, a subsample was taken for IC analysis for comparison with OsmoSampler Cl and SO_4 values. Pumping rate corrections were applied based on the average weekly temperatures, aligning with MeHg Peak concentrations, assuming eight membrane and two membrane OsmoSamplers have base pumping rates of 1.2mL and 0.5 mL per day, respectively, at 21°C.

Results

The acidified standard trial (with no acid addition in line) appeared to be consistent with the measured standard concentrations (Figure 3.5, Appendix B, Table 1) this indicates that once stabilized with HCl, MeHg does not appear to stick to the Teflon in the coils or degrade.

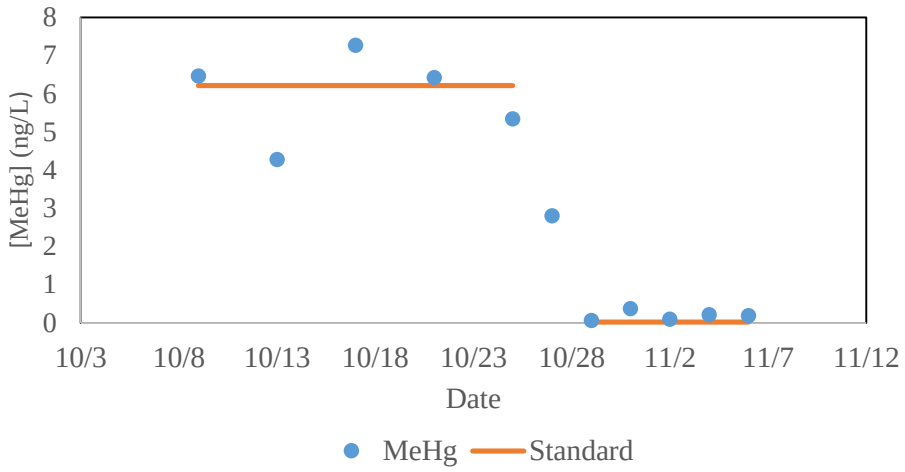


Figure 3.5. MeHg concentration measurements for the Acidified Standard OsmoSampler Test and the standards used. The concentrations of the standard are shown in the red line and the concentrations measured in the OsmoSampler are shown as the points.

The acid addition process (Figure 3.6) showed mixed results between the non-acidified standard and acidified standard tests. Concentrations recovered varied for the high concentration (6.23 ng/L) standard, with concentrations deviating above and below the standard concentration. The concentrations do appear to be close to the correct order of magnitude. These results indicate that the sample can be acidified and preserved, but the mixing rates may not always be equal.

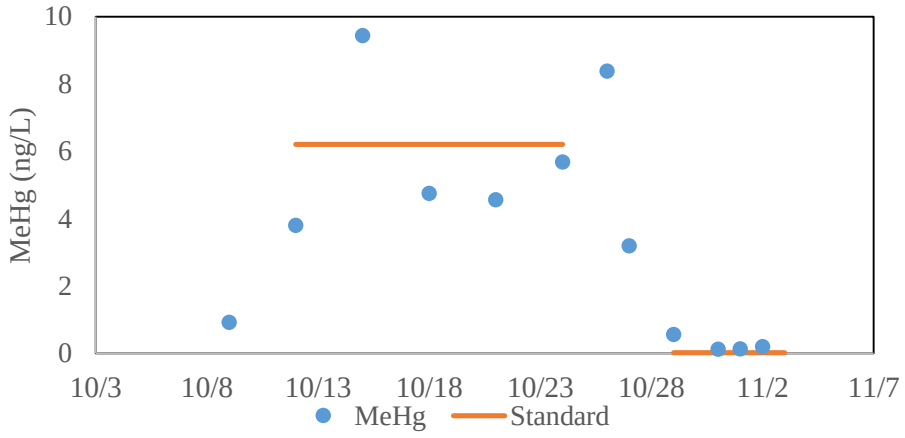
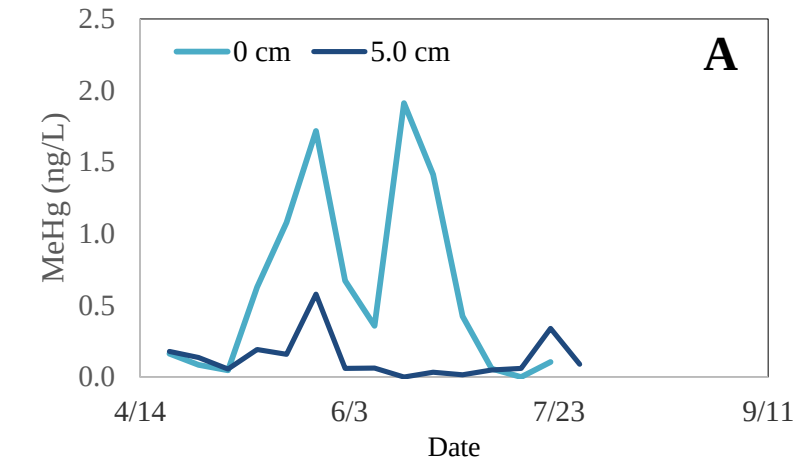


Figure 3.6. MeHg concentrations for the OsmoSampler in-line Acid Addition Experiment. As in Figure 3.5, the standard concentrations are shown in the red line, and the measured concentrations are shown as blue dots

The non-acidified standard with no acid addition, the acid addition with a different ligand (humic acid), and the acid addition experiment with two lines of differing thickness did not yield any MeHg from their sections. The likely explanation for the non-acidified standard is that MeHg is degraded or binds to the Teflon without acidification (Appendix B. Table 1). The acid addition with the different ligand likely had complications due to the ligand binding to the inside of the Mylar bag (data not shown). This procedure should be rerun with an alternative standard container. In the PEEK tubing experiment, sample was drawn from the main tube, but the acid was not drawn in from the PEEK tubing, (measured by volume changes in syringes on both tubes, Appendix B. Table 6). This indicates, mixing did not occur when a small pump is not attached to the line of acid due to some fluid dynamics within the union, justifying the need for the small pump acid addition.

For the field experiments, three of the sampling coils had no acid when measured with pH paper (pH was 7 for all samples). Bubbles were found in the lines of those three coils, indicating a leak in the fittings or tubing somewhere. Based on the pH results, the pumps did not work as needed. Based on the analysis from the non-acidified lab experiment, it is unlikely that MeHg was preserved, so I did not run the samples from those three coils for MeHg. It is possible that other chemical species, such as sulfate (Appendix B, Tables 2,3,4), were collected, but those samples have not been run at the time of this writing. Five of the eight coils deployed were successfully acidified in the field. The two OsmoSampler coils with the most promising results (based on comparison with the porewater values) are shown in Figure 3.7A, along with the corresponding discrete porewater measurements (Figure3.7B). The additional OsmoSampler results from the coils with acid found in the coils are shown in Figure 3.8 (Appendix B, Table 5).



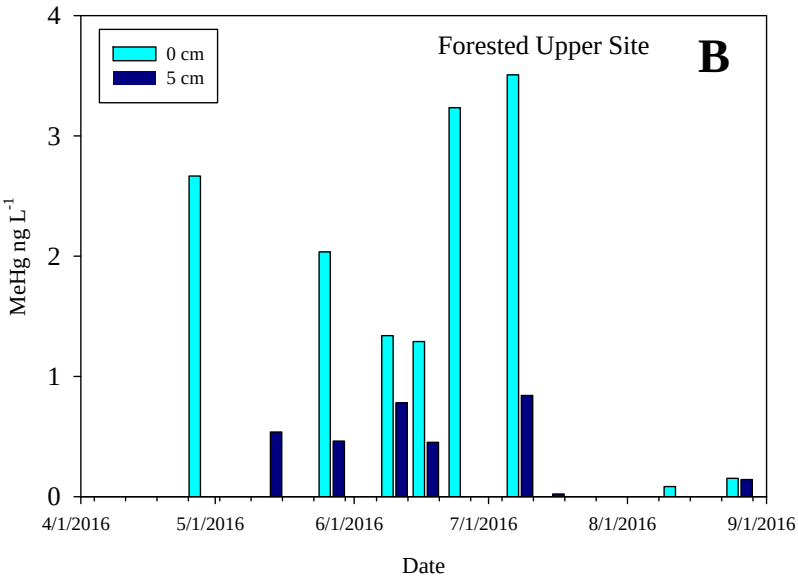


Figure 3.7. A) Field OsmoSampler porewater MeHg concentrations (ng/L) for upstream depths in the forested watershed. **B)** The corresponding porewater MeHg concentrations results.

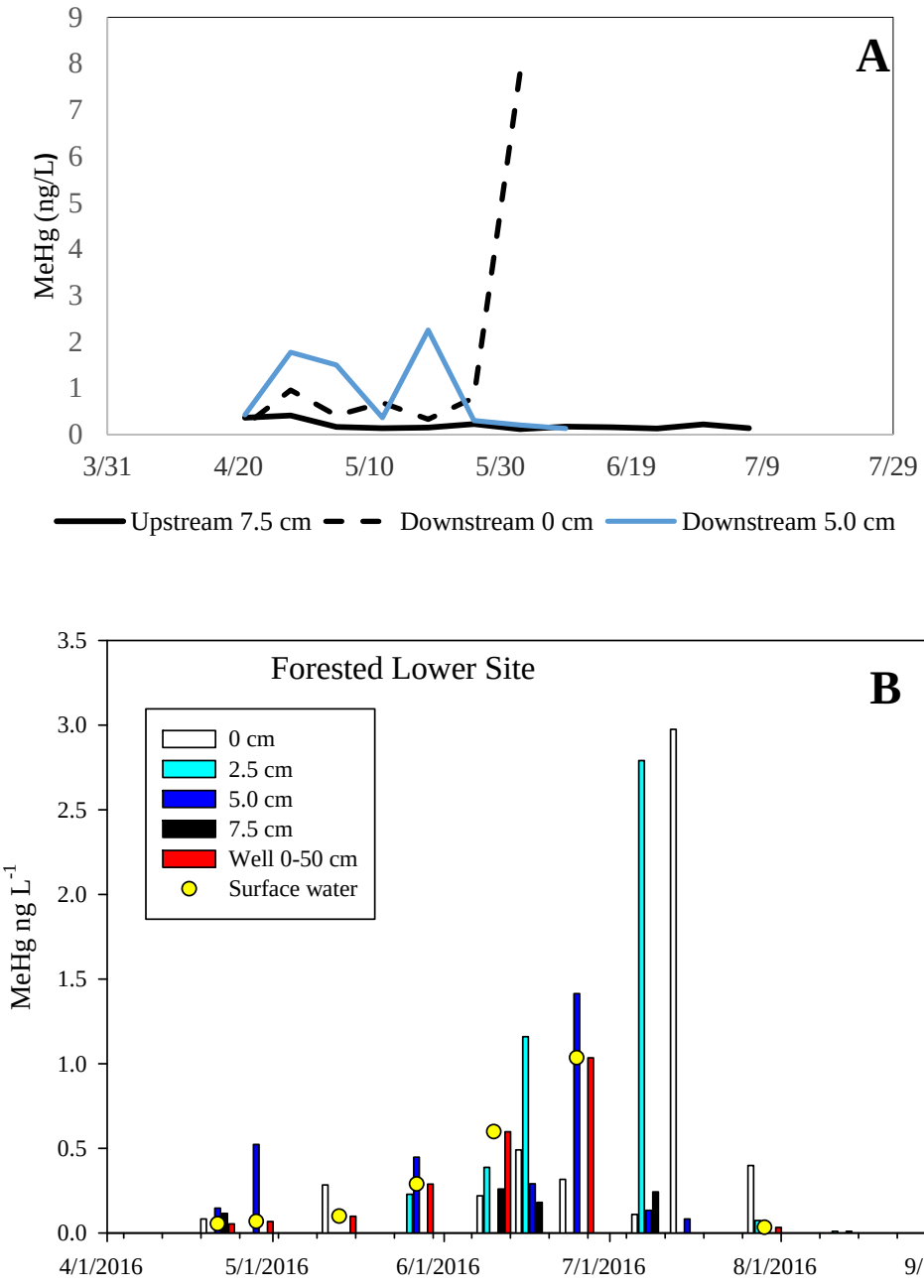


Figure 3.8 A). OsmoSampler MeHg concentrations (ng/L) for additional arrays. **B)**. Porewater MeHg concentrations (ng/L) for the downstream forested site.

It is likely that the upstream 7.5 cm array in Figure 3.8. A did not collect any sample. The 5.0cm depth may have obtained some sample, but the concentrations are higher than those found

for most of the samples at depth in the lower forested site. . The OsmoSamplers collecting water from the 0 cm and 5 cm depths may have been functioning correctly but the sudden change in MeHg in samples collected beyond May suggests the ports were compromised in some way. Based on these initial results, three out of the eight OsmoSampler may have worked with varying degrees of success. The temperatures recorded by the temperature loggers showed a wide range of temperatures over the field season (Figure 3.9).

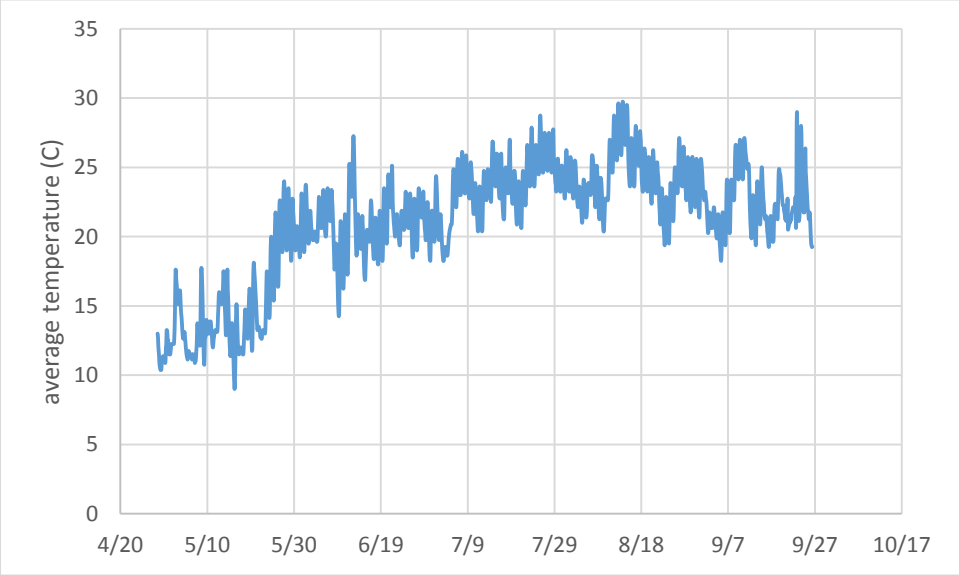


Figure 3.9 Average temperature logger measurements for the duration of the 2016 field experiment. Temperature appears to be fairly episodic, despite storing the OsmoSamplers in five-gallon buckets filled with water.

Discussion

This is the first known use of OsmoSamplers as a technique for sampling MeHg from sediment porewater in stream ecosystems. OsmoSamplers were shown in some of the lab and field experiments to successfully capture MeHg from the sampling environment, indicating these could be a useful sampling approach in appropriate conditions. However, there was a range of success rates for the lab and field experiments. Lab experiments initially showed promising results (Figure 3.5 and 3.6) but some variability that could be due to the temperature fluctuations

(Figure 3.9), or variability of flow rates between the large and small pump as temperature swings. It is possible that the smaller two-membrane pumps are more susceptible to swings in temperature than the eight-membrane pumps. The variability in pumping rates could cause acid to not mix well with the sample as it's collected, causing bubbles in the line or poor preservation of sample.

With these considerations in mind, I do have some indication that the OsmoSamplers worked in the field (Figure 3.7 and 3.8.) and if perhaps deployed in an environment where the water level and temperature are more stable, the OsmoSamplers could be a useful tool for monitoring seasonality in MeHg concentrations in a more stable temperature environment. Even though I stored the OsmoSamplers in 5 gallon buckets filled with water, I still observed fluctuations in temperature (Figure. 3.9). Combined with variability in stream water levels, these could explain the low success rates in this field site. Once bubbles enter the sampling lines, the time series created by the samplers can be interrupted or slowed. This could explain the lack of sample in some of the OsmoSampler as flow dropped in the sites. Only three of the 8 OsmoSampler arrays were successful in acquiring measurable MeHg levels (Figure 3.7 and 3.8, Appendix B, Table 5.), with two appearing to match the order of magnitude and timing of the MeHg peaks in the discrete porewater arrays, and a third with the same order of magnitude, but different timing. The concentration and pattern is consistent with the other measures of surface water and porewater, and given the spatial variability described in Chapter 2, indicative of the environment. These samplers would probably perform at higher success rates when submerged in the stream bed and with constant flow of water. However, I was able to collect MeHg samples using these OsmoSamplers, indicating that in the correct conditions, this could be a useful sampling strategy for MeHg in the water column.

Conclusions and Future Work

I was able to observe increases in the concentration of MeHg in the porewater using OsmoSamplers. OsmoSamplers may be limited by water depth and temperature stability in their application. Based on the application of OsmoSamplers in other studies, these could be powerful tools for determining watershed MeHg condition and seasonal trends in MeHg concentrations. Before widespread application, additional tests should be run to see if the substrate and ligands that MeHg would bind to would affect the recovery of MeHg within the coils. In addition, testing the OsmoSampler in environments where the concentration of MeHg is lower, the temperature is more stable, and the water level is deeper would be useful for demonstrating the application of the OsmoSampler in other environments.

APPENDIX A: MeHg and Hg Concentrations

Table 1. MeHg concentrations (ng/g) for sediment collected in 2016

	Date				
Sample Location	April	May	June	July	August
109T1-4	0.024	0.065	0.032	0.053	0.071
109T1-3	0.008	0.001	0.004	0.016	0.039
109T1-2	0.005	0.003	0.015	0.039	0.121
109t1-1	0.002	0.002	0.007	0.001	0.439
109T2A-4	0.037	0.068	0.184	0.019	0.031
109T2A-3	0.006	0.021	0.071	0.028	0.119
109T2A-2	0.004	0.002	0.009	0.029	0.167
109T2A-1	0.004	0.002	0.011	0.009	0.380
109T2B-4	0.012	0.005	0.023	0.042	0.018
109T2B-3	0.004	0.008	0.020	0.030	0.025
109T2B-2	0.018	0.006	0.003	0.011	0.020
109T2B-1	0.033	0.019	0.006	0.026	0.008
110T1A-4	0.278	0.292	0.088	0.066	0.167
110T1A-3	0.000	0.002	0.015	0.347	0.095
110T1A-2	0.000	0.007	0.018	0.099	0.013
110T1A-1	0.035	0.110	0.091	0.044	0.015
110T1B-4	0.236	0.150	0.244	0.029	0.293
110T1B-3	0.490	0.074	0.223	0.119	0.071
110T1b-2	0.002	0.011	0.050	0.038	0.121
110T1B-1	0.179	0.043	0.144	0.021	0.061
110T3-4	0.303	0.214	1.242	0.420	0.649

110T3-3	0.746	0.001	0.440	0.321	0.281
110T3-2	1.062	0.206	0.603	0.159	0.185
110T3-1	0.476	0.004	0.115	0.068	0.036

Table 2. Sediment MeHg (ng/g) concentrations for 2017

	Date	MeHg	
Sample Location	4/21/2017	5/16/2017	6/15/2017
109T1-4	0.045	0.028	0.058
109T1-3	0.032	0.148	0.090
109T1-2	0.102	0.099	0.278
109t1-1	0.034	0.007	0.017
109T2A-4	0.065	0.036	0.141
109T2A-3	0.022	0.012	0.087
109T2A-2	0.004	0.003	0.029
109T2A-1	0.015	0.003	0.015
109T2B-4	0.070	0.020	0.113
109T2B-3	0.093	0.006	0.018
109T2B-2	0.030	0.003	0.015
109T2B-1	0.037	0.005	0.010
110T1A-4	0.107	0.157	0.119
110T1A-3	0.095	0.047	0.296
110T1A-2	0.056	0.028	0.256
110T1A-1	0.171	0.028	0.117
110T1B-4	0.298	0.033	0.176
110T1B-3	0.338	0.120	0.005
110T1b-2	0.025	0.200	0.048
110T1B-1	0.015	0.152	0.012
110T3-4		0.096	0.063
110T3-3	0.034	0.023	0.023
110T3-2	0.057	0.096	0.023
110T3-1	0.012	0.027	0.045

Table 3. MeHg Porewater values (ng/L) for 2016

Sample Location	4/21	4/28	5/13	5/27	6/10	6/17	6/25	7/8	7/15	7/29	8/12	8/26
109T1-4		0.17		0.10			0.46	1.49	0.60	0.00		
109T1-3	0.36	0.22				0.51	0.74	0.44	0.30	0.18		
109T1-2		0.58				0.29	0.29	0.51	0.15	0.50		
109t1-1	0.07			0.22		0.17			0.14	0.18		
109T2-4	0.09		0.13		0.39	0.35	0.69	0.43	0.72	1.15		1.43
109T2-3			0.23			0.54			0.45		0.12	0.69
109T2-2					1.43	0.73	1.00		0.43	0.32	0.08	0.09
109T2-1		0.06			0.36	0.80		0.10	0.15	0.13	0.01	0.05
110T1-4	0.12				0.26	0.18		0.24			0.01	
110T1-3	0.15	0.52		0.45		0.29	1.41	0.13	0.08	0.03		
110T1-2				0.23	0.39	1.16		2.79		0.07	0.01	
110T1-1	0.08		0.28		0.22	0.49	0.32	0.11	2.97	0.40		
110T3-4	0.30			0.35		0.73	2.13	3.96		0.14		0.06
110T3-3			0.54	0.46	0.78	0.45		0.84	0.02			0.14
110T3-2				0.61	0.42	0.55		1.44	0.08			

110T3-1		2.67		2.03	1.34	1.29	3.23	3.51			0.08	0.15

Table 4. Macroinvertebrate MeHg and T-Hg concentrations (ng/g) for 2016

Sample ID	Date	Site	Taxa	MeHg (ng/g)	T-Hg ng/g	MeHg/T-Hg	# of bugs	Sample Weight (g)
SO_6916_A1	6/9/2016	110	A	497.39	795.57	0.63	2	0.0007
S9_62416_A1	6/24/2016	109	A	91.36	160.13	0.57	5	0.0052
S9_62416_A2	6/24/2016	109	A	99.80	167.16	0.60	10	0.0070
S9_62416_A3	6/24/2016	109	A	77.06	144.33	0.53	5	0.0051
S0_62416_A1G	6/24/2016	110	A	254.98	336.02	0.76	5	0.0045
SO_62416_A2	6/24/2016	110	A	187.37	21.39	8.76	10	0.0061
S9-62416_A1	6/24/2016	109	A	36.99	88.06	0.42	5	0.0059
S9_62416_A2	6/24/2016	109	A	81.38	136.77	0.60	10	0.0074
S0_7716_A1	7/7/2016	110	A	324.91	395.62	0.82	3	0.0033
S0_7716_A2	7/7/2016	110	A	295.98	292.98	1.01	10	0.0060
S9_7716_A1	7/7/2016	109	A	120.20	137.32	0.88	5	0.0042
S9_7716_A2	7/7/2016	109	A	95.94	94.91	1.01	10	0.0056
S9_71416_A1	7/14/2016	109	A	26.82	32.55	0.82	5	0.0053

S9_71416_A2	7/14/2016	109	A	69.67	67.64	1.03	10	0.0064
S0_72916_A1	7/29/2016	110	A	153.94	124.69	1.23	5	0.0070
S0_72916_A2	7/29/2016	110	A	136.02	129.15	1.05	1	0.0048
S0_72916_A3	7/29/2016	110	A	138.91	127.25	1.09	5	0.0131
S9_72916_A1	7/29/2016	109	A	67.62	78.55	0.86	2	0.0031
S0_82516_A1	8/25/2016	110	A	48.03	54.25	0.89	2	0.0037
S0_82516_A2	8/25/2016	110	A	218.54	193.58	1.13	6	0.0048
S9_82516_A1	8/25/2016	109	A	50.76	59.89	0.85	5	0.0071
S9_82516_A2	8/25/2016	109	A	49.26	6753.92	0.01	3	0.0041
S0_42816_M1	4/28/2016	110	M	259.43	4.50	57.69	1	0.0760
S9_71416_M1	7/14/2016	109	M	60.30	64.20	0.94	2	0.0061
S9_51216_m1	5/12/2016	109	M	235.03	209.90	1.12	1	0.0070
S9_62416_m1	6/24/2016	109	M	92.33	84.06	1.10	2	0.0019
S0_82516_M1	8/25/2016	110	M	251.82	210.24	1.20	1	0.0031
S9_42816_T1	4/28/2016	109	T	24.88	14.59	1.71	2	0.0513
S9_42816_T2	4/28/2016	109	T	28.38	25.66	1.11	2	0.0233
S0_42116_T1	4/21/2016	110	T	333.00	283.18	1.18	1	0.0334

S0_42116_T2	4/21/2016	110	T	340.37	318.44	1.07	2	0.0098
S942116_T1	4/21/2016	109	T	28.63	31.81	0.90	1	0.0117
S942116_T2	4/21/2016	109	T	64.88	72.86	0.89	1	0.0029
S942116_T3	4/21/2016	109	T	35.29	42.49	0.83	1	0.0101
S942116_T4	4/21/2016	109	T	35.47	44.04	0.81	1	0.0083
S9_62416_T1	6/24/2016	109	T	15.11	20.15	0.75	1	0.0118
S9_41416_T1	4/4/2016	109	T	100.45	95.64	1.05	1	0.0035
S9_51216_T1	5/12/2016	109	T	14.01	16.93	0.83	1	0.0113
S9_51216_T2	5/12/2016	109	T	13.74	15.29	0.90	1	0.0121
S9_51216_T3	5/12/2016	109	T	15.41	29.10	0.53	1	0.0088
S0_42116_D1	4/21/2016	110	D	307.27	229.64	1.34	1	0.0107
S9_62416_H1	6/24/2016	109	H	65.02	70.66	0.92	1	0.0151
S062416_H1	6/24/2016	110	H	365.12	257.13	1.42	1	0.0271
S062416_H2	6/24/2016	110	H	499.40	342.22	1.46	1	0.0150
S07716_h1	7/7/2016	110	H	345.77	247.28	1.40	1	0.0162
S9-41416-A1	4/14/2016	109	A	274.80	285.45	0.96	2	0.0047
S9-41416-A2	4/14/2016	109	A	149.48	291.73	0.51	10	0.0015

S0-41416-A1	4/14/2016	110	A	372.71	79.32	4.70	1	0.0029
S0-41416-A2	4/14/2016	110	A	461.03	234.07	1.97	2	0.0030
S0-41416-A3	4/14/2016	110	A	441.29	157.81	2.80	2	0.0064
S0-41416-A4	4/14/2016	110	A	341.53	1324.19	0.26	4	0.0015
S0-42116-A1	4/21/2016	110	A	296.70	587.17	0.51	1	0.0024
S0-42116-A2	4/21/2016	110	A	305.29	291.35	1.05	4	0.0067
S0-42116-A3	4/21/2016	110	A	283.91	70.46	4.03	8	0.0100
S0-42116-A4	4/21/2016	110	A	500.81	440.33	1.14	3	0.0036
S0-42116-A5	4/21/2016	110	A	279.66	1247.77	0.22	1	0.0019
S9-42116-A1	4/21/2016	109	A	178.50	175.28	1.02	1	0.0052
S9-42116-A2	4/21/2016	109	A	184.77	46.69	3.96	3	0.0141
S9-42116-A3	4/21/2016	109	A	202.55	170.13	1.19	2	0.0057
S9-42116-A4	4/21/2016	109	A	217.35	448.24	0.48	2	0.0052
S9-42116-A5	4/21/2016	109	A	104.79	504.62	0.21	14	0.0026
S0-42816-A1	4/28/2016	110	A	307.28	346.41	0.89	2	0.0033
S0-42816-A2	4/28/2016	110	A	361.39	183.89	1.97	2	0.0034
S0-42816-A3	4/28/2016	110	A	309.40	265.53	1.17	3	0.0031

S0-42816-A4	4/28/2016	110	A	229.57	338.69	0.68	9	0.0032
S0-42816-A5	4/28/2016	110	A	412.95	320.60	1.29	2	0.0029
S9-42816-A1	4/28/2016	109	A	150.60	289.80	0.52	6	0.0025
S9-42816-A2	4/28/2016	109	A	336.07	310.47	1.08	1	0.0028
S9-42816-A3	4/28/2016	109	A	264.36	83.02	3.18	1	0.0051
S9-42816-A4	4/28/2016	109	A	208.45	305.57	0.68	2	0.0023
S0-51216-A1	5/12/2016	110	A	151.03	389.29	0.39	6	0.0027
S0-51216-A2	5/12/2016	110	A	111.58	126.50	0.88	11	0.0041
S0-51216-A3	5/12/2016	110	A	235.47	125.71	1.87	3	0.0030
S0-51216-A4	5/12/2016	110	A	142.87	85.98	1.66	10	0.0052
S0-51216-A5	5/12/2016	110	A	125.37	179.02	0.70	9	0.0032
S9-51216A1	5/12/2016	109	A	219.34	143.04	1.53	5	0.0046
S9-51216-A2	5/12/2016	109	A	172.59	237.93	0.73	5	0.0035
S9-51216-A3	5/12/2016	109	A	135.84	226.20	0.60	6	0.0034
S9-52616-A1	5/26/2016	109	A	139.06	130.61	1.06	8	0.0043
S9-52616-A2	5/26/2016	109	A	142.61	85.82	1.66	7	0.0052
S9-52616-A3	5/26/2016	109	A	160.81	104.53	1.54	4	0.0059

S9-52616-A4	5/26/2016	109	A	109.75	108.23	1.01	9	0.0055
S9-52616-A5	5/26/2016	109	A	135.85	145.47	0.93	1	0.0061
S0-52616-A1	5/26/2016	110	A	373.13	3866.04	0.10	1	0.0002
S9-6916-A1	6/9/2016	109	A	10.52	145.37	0.07	1	0.0056

Table 5. Macroinvertebrate MeHg and T-Hg (ng/g) for 2017.

Sample ID	Date	Site	Taxa	# of bugs	Sample Weight (g)	average weight	MeHg ng/g	T-Hg ng/g	MeHg/T-Hg
S0_42117_A1	4/21/2017	110	A	1	0.001	0.0012	145.06	273.09	0.53
S0_42117_H1	4/21/2017	110	H	2	0.009	0.0047	251.38	1168.88	0.22
S9_42117_H1	4/21/2017	109	H	1	0.019	0.0192	195.09	768.80	0.25
S9_42117_T1	4/21/2017	109	T	1	0.063	0.0630	111.25	334.60	0.33
S9-42117-T2	4/21/2017	109	T	1	0.061	0.0612	195.98	293.12	0.67
S9-42117-A1	4/21/2017	109	A	4	0.002	0.0005	155.37	173.05	0.90
SO_50417_H1	5/4/2017	110	H	1	0.005	0.0052	229.34	1076.23	0.21
SO_50417_A1	5/4/2017	110	A	1	0.003	0.0031	216.69	511.85	0.42
SO_50417_A2	5/4/2017	110	A	7	0.001	0.0002	187.04	159.73	1.17

S9_50417_A1	5/4/2017	109	A	10	0.002	0.0002	130.63	98.69	1.32
S9_50417_A2	5/4/2017	109	A	7	0.003	0.0005	141.10	266.71	0.53
S9_50417_A3	5/4/2017	109	A	10	0.002	0.0002	123.40	106.01	1.16
S9_50417_M1	5/4/2017	109	M	1	0.010	0.0098	218.02	721.70	0.30
S9_50417_T1	5/4/2017	109	T	2	0.026	0.0129	26.91	75.05	0.36
S9_50417_H1	5/4/2017	109	H	1	0.002	0.0017	462.10	919.93	0.50
S9_51817_A1	5/18/2017	109	A	5	0.005	0.0010	104.45	226.61	0.46
S9_51817_A2	5/18/2017	109	A	9	0.006	0.0006	93.93	129.56	0.72
S9_51817_A3	5/18/2017	109	A	10	0.005	0.0005	83.07	138.14	0.60
S9_51817_A4	5/18/2017	109	A	12	0.004	0.0003	77.31	91.48	0.85
S9_51817_M1	5/18/2017	109	M	1	0.021	0.0213	93.76	601.94	0.16
S9_51817_M2	5/18/2017	109	M	1	0.031	0.0308	180.67	602.17	0.30
S9_51817_T1	5/18/2017	109	T	1	0.018	0.0185	9.31	22.40	0.42
S9_51817_H1	5/18/2017	109	H	1	0.004	0.0035	101.83	394.24	0.26
S0_51817_A1	5/18/2017	110	A	3	0.001	0.0003	166.14	-86.29	-1.93
S0_51817_H1	5/18/2017	110	H	1	0.001	0.0011	1395.13	2623.19	0.53
S0_51817_D1	5/18/2017	110	D	1	0.171	0.1712	414.28	0.00	#DIV/0!

S0_51817_DX1	5/18/2017	110	D	1	0.005	0.0047	292.38	653.64	0.45
S9_60217_A1	6/2/2017	109	A	6	0.007	0.0011	117.43	303.51	0.39
S9_60217_A2	6/2/2017	109	A	8	0.006	0.0007	116.72	154.59	0.76
S9_60217_A3	6/2/2017	109	A	10	0.005	0.0005	101.96	166.06	0.61
S9_60217_A4	6/2/2017	109	A	12	0.005	0.0004	80.57	110.21	0.73
S9_60217_H1	6/2/2017	109	H	1	0.017	0.0174	158.16	428.99	0.37
S9_60217_T1	6/2/2017	109	T	1	0.017	0.0173	23.42	58.38	0.40
S0_60217_A1	6/2/2017	110	A	1	0.001	0.0007	234.98	9.39	25.03
S0_60217_M1	6/2/2017	110	M	1	0.142	0.1418	173.14	1381.64	0.13
S0_60217_H1	6/2/2017	110	H	1	0.021	0.0213	175.00	626.24	0.28
S9_61517_A1	6/15/2017	109	A	10	0.006	0.0006	96.21	241.94	0.40
S9_61517_A2	6/15/2017	109	A	11	0.006	0.0006	96.07	232.59	0.41
S9_61517_A3	6/15/2017	109	A	14	0.009	0.0006	88.84	302.58	0.29
S9_61517_M1	6/15/2017	109	M	1	0.045	0.0447	210.80	654.76	0.32
S9_61517_H1	6/15/2017	109	H	2	0.032	0.0159	208.00	542.85	0.38
S9_61517_T1	6/15/2017	109	T	1	0.016	0.0164	15.50	39.23	0.40
S0_61517_H1	6/15/2017	110	H	5	0.017	0.0034	183.20	733.34	0.25

S9_62917_A1	6/29/2017	109	A	10	0.007	0.0007	92.46	298.69	0.31
S0_62917_H1	6/29/2017	110	H	1	0.004	0.0043	147.42	402.75	0.37

Table 6. Invertebrates found in the forested site for 2016

Date	Amphipod	Isopod	Corbicula	Oligochaete	Diptera	Megaloptera	Coleoptera	Cambarus	Odonata	Chironomid	Trichoptera	Hydrophilidae
4/14	8	21	20	0	1	0	0	0	0	0	0	0
4/21	40	100	20	20	0	0	0	0	1	0	3	0
4/28	9	40	2	0	0	1	0	0	0	1	1	0
5/12	31	30	6	0	1	0	0	0	0	0	0	0
5/26	1	4	0	1	0	0	0	1	0	0	0	0
6/9	2	4	4	8	0	0	0	0	0	0	0	0
6/24	51	22	12	44	3	0	3	1	0	0	0	0
6/24	0	3	0	4	0	0	0	0	0	0	0	1
7/7	25			5								
7/14				4								
7/29	34	4										1
8/25	13			7		1				1		1

Table 7. Invertebrates found in the agricultural site for 2016

Date	Amphipod	Isopod	Corbicula	Oligochaete	Diptera	Megaloptera	Coleoptera	Cambarus	Tipulidae	Planaria	Chironomid	Hydrophilidae	Trichoptera
4/14	10	31	8						2				1
4/21	19	80	25				1		7		5		7
4/28	8	70		2									4
5/12	21	10		5		3				1			6
5/26	42	55		10		1			1	20			
6/9	44	25	4	9		2	1		3	4			1
6/24	200	5			2	1	1			6			
6/24	65	6	6	12		2	1			6			1
7/7	43	3		1						23		1	
7/14	27	3				1						1	
7/29	2	1	1	1						1		1	
8/25	16		10									1	

Table 8. Macroinvertebrates found in the forested site for 2017

Date	Amphipod	Isopod	Corbicula	Oligochaete	Diptera	Megaloptera	Coleoptera	Chironomid	Hydrophilidae	Odonata
4/21	1	17	19	94	2				2	
5/4	9	24	1	3				3	1	
5/18	3	40		14	2				1	1
6/2	1		11	4		1			1	
6/15		3		21					5	
6/29				6	11		6	1	1	

Table 9. Macroinvertebrates found in the agricultural site for 2017

Date	Amphipod	Isopod	Corbicula	Oligochaete	Diptera	Megaloptera	Cambarus	Tipulidae	Planaria	Chironomid	Hydrophilidae	Trichoptera
4/21	5	43	10	102				1		12	2	2
5/4	30	128	2	81		1			1	6	1	2
5/18	51	53	6	76	1	2			23	1		1
6/2	40	32	15	76					22		1	1
6/15	95	11		2		1					5	1
6/29	24	23	3	81		6	1				2	

Appendix B. Chloride and Sulfate Values for OsmoSamplers and Discrete Porewater.

Table 1. OsmoSampler 2015 Lab Experiment MeHg (ng/L) results (without HCl corrections)

Sample ID	Date	MeHg
A1 2+3	11/3	0.00
A1 4+5	11/2	0.20
A1 6	11/1	0.14
A1 7	10/31	0.12
A1 8	10/29	0.56
A1 9	10/27	3.20
A1 10	10/26	8.38
A1 12	10/24	5.68
A1 14	10/21	4.56
A1 17	10/18	4.75
A1 18	10/15	9.43
A1 20	10/12	3.80
A1 21	10/9	0.92
A2 4+5	11/6	0.19
A2 6+7	11/4	0.21
A2 8	11/2	0.10
A2 9	10/31	0.37
A2 10	10/29	0.07
A2 11	10/27	2.80
A2 12	10/25	5.34
A2 14	10/21	6.42
A2 16	10/17	7.27
A2 17	10/13	4.28
A2 20	10/9	6.46
S1C1 2+3	11/14	0.14
S1C1 4+5	11/11	0.11
S1C1 6+7	11/8	0.51
S1C1 8	11/5	0.17
S1C1 9	11/2	0.54
S1C1 10	10/30	0.09
SC1 11	10/27	1.37
S1C1 12	10/24	1.29
SC1 13	10/21	0.12
S1C1 14	10/18	0.03
S1C1 15	10/15	0.00
S1C1 16	10/12	0.19
SC1 17	10/9	0.56

S2C2 3	11/11	0.12
S2C2 4	11/10	0.43
S2C2 5	11/9	0.33
S2C2 6	11/8	0.49
S2C2 7	11/6	0.39
S2C2 8	11/4	0.56
S2C2 10	11/2	0.78
S2C2 11	10/31	0.71
S2C2 12	10/29	0.81
NA1 8+9		0.04
NA1 10+11		0.07
NA1 12+13		0.02
NA1 14 + 15		0.06
NA1 16+17		0.02
NA1 18+19		0.01
NA1 20 +21		-0.04
NA1 22+23		0.09
NA1 24		0.04
NA1 25+27		0.00
NA1 26		-0.10
NA1 28		0.08
NA1 30		0.10
NA1 33		-0.07
NA1 35		-0.01
NA2 1 + 2		0.41
NA2 3+4		0.38
NA2 5+6		0.69
NA2 9+10		0.56
NA2 11+12		0.16
NA2 13+14		0.11
NA2 15 + 16		0.64
NA2 17+18		0.18
NA2 19+21		0.04
NA2 20		0.03
NA2 22		0.09
NA2 23+24		-0.05
NA2 25		-0.07
NA2 27+28		-0.05

Table 2. OsmoSampler SO_4 concentrations (mM). Samples are not dated due to wide spread values.

Sample Number	Upstream 0cm	Downstream 0cm	Downstream 5.o cm	Upstream 5.0 cm	Upstream 7.5 cm
1	3.919	0.697	0.266	0.064	0.284
1	4.252	0.677	0.247	0.015	0.278
2	1.177	0.815	0.391	-	0.293
2	1.166	0.791	0.354	0.020	0.311
3	0.875	0.643	0.366	0.028	0.340
3	1.038	0.673	0.368	0.028	0.292
4	0.907	0.363	0.307	0.020	0.080
4	0.903	0.373	0.260	0.018	0.067
5	0.885	0.270	0.261	0.119	0.073
5	0.878	0.255	0.260	0.027	-
6	0.894	0.246	0.299	0.027	0.077
6	0.881	0.277	0.264	0.020	0.082
7	0.985	0.332	0.273	0.088	0.085
7	0.910	-	0.259	-	0.088
8	0.888	0.273	-	0.064	0.090
8	0.887	0.384	0.331	0.059	0.076
9	0.905	0.415	0.785	0.021	0.100
9	0.906	0.466	0.383	0.056	0.090
10	-	-	0.432	-	0.082
10	-	0.437	-	0.026	0.104
11	-	0.248		-	0.087
11	0.898	0.303		-	-
12	-	0.254		0.026	0.129
12	-	0.244		-	-
13	-			0.028	0.253
13	0.879			0.038	-
14				0.054	0.333
14				0.028	0.282
15				0.042	0.365
15				0.015	-
16				-	0.277
16				0.175	1.737
17				0.232	1.710
17				0.128	0.093
18				-	0.099
18				0.254	
19				0.157	

19				-	
20				-	
20				-	
21				0.013	
21				-	
22				0.144	
22				0.168	
23				0.021	
23				0.206	
24				0.153	
24				0.176	
25				-	
25				0.133	
26				0.224	
26				0.187	

Table 3. OsmoSamplers Cl (mM) concentrations for 2016.

Sample #	Upstream 0cm	Downstream 0cm	Downstream 5.o cm	Upstream 5.0 cm	Upstream 7.5 cm
1	53.966	8.707	93.474	54.076	86.281
1	58.713	8.686	93.459	52.935	87.683
2	41.526	19.124	22.347	27.009	59.272
2	41.522	19.110	21.956	31.150	58.171
3	34.959	37.174	36.341	28.294	38.707
3	37.668	36.980	35.276	28.317	9.282
4	15.924	104.300	108.874	33.875	9.406
4	15.914	102.189	107.251	32.387	37.773
5	20.973	83.370	110.043	26.611	47.657
5	20.985	83.367	107.096	25.955	50.125
6	53.863	8.306	137.269	32.445	26.360
6	52.316	8.253	138.647	31.584	26.216
7	46.876	7.798	138.451	33.466	50.458
7	46.929	7.651	136.332	32.994	50.593
8	76.237	7.690	7.715	24.569	56.550
8	73.874	7.690	7.773	24.527	55.447
9	35.467	7.667	15.223	32.271	97.430
9	33.503	7.662	7.759	32.327	98.495
10	70.964	7.781	7.684	33.145	95.785
10	69.205	7.654	7.674	33.564	94.442
11	18.818	7.768		33.589	100.134
11	18.624	7.757		33.153	98.609

12	12.463	7.674		24.707	49.696
12	12.471	7.754		24.971	48.607
13	12.495			32.931	6.860
13	12.520			32.812	6.858
14	12.492			33.716	6.767
14	12.489			33.580	6.760
15	12.518			46.956	6.881
15	12.599			47.144	6.818
16				0.064	6.750
16				0.054	6.760
17				0.043	5782.543
17				0.051	5763.146
18				0.020	151.130
18				0.084	147.313
19				0.079	
19				0.095	
20				0.038	
20				0.043	
21				0.057	
21				0.052	
22				0.087	
22				0.108	
23				0.144	
23				0.079	
24				0.041	
24				0.034	
25				0.038	
25				0.080	
26				0.054	
26				0.036	

Table 4. Discrete porewater Cl and SO_4 concentrations (mM) for the downstream (T1) and Upstream (T3) sites of the forested watershed for 2016.

Sample ID	Date	Cl (mM)	SO4 (mM)
110 T1_A1_04_21B	4/21/2016	0.274	0.232
110 T1_A4_04_21B	4/21/2016	0.299	-
110 T1_B1_04_21B	4/21/2016	0.209	0.366
110 T1_1_06_10B	6/10/2016	0.274	0.190
110 T1_1_07_08b	7/8/2016	0.164	0.163

110 T1_2_07_08B	7/8/2016	0.154	0.046
110 T1_1_07_14B	7/14/2016	0.145	0.166
110 T1_3_07_15B	7/15/2016	0.119	0.112
110 T1_1_07_29B	7/29/2016	0.098	0.173
110 T1_4_08_12B	8/12/2016	0.170	0.157
110 T3_3_05_13B	5/13/2016	0.156	0.092
110 T3_1_05_27B	5/27/2016	1.667	0.088
110 T3_1_06_17B	6/17/2016	0.215	0.056
110 T3_4_06_17B	6/17/2016	0.214	-
110 T3_2_06_17B	6/17/2016	0.262	-
110 T3_3_06_17B	6/17/2016	0.475	0.074
110 T3_1_07_14B	7/14/2016	0.158	-
110 T3_2_07_29B	7/29/2016	0.192	-
110 T3_1_08_12B	8/12/2016	0.201	0.029
110 T3_4_08_26B	8/26/2016	0.188	-
110 T3_1_08_26B	8/26/2016	0.178	-
110 T3_3_08_26B	8/26/2016	0.196	-

Table 5. MeHg (ng/L) concentrations for the OsmoSamplers in the forested watershed for 2016

sample #	ng/g
T3D4-01	0.136
T3D4-02	0.218
T3D4-03	0.128
T3D4-04	0.153
T3D4-05	0.172
T3D4-06	0.112
T3D4-07	0.222
T3D4-08	0.147
T3D4-09	0.134
T3D4-10	0.160
T3D4-11	0.407
T3D4-12	0.363
T1D1-01	7.781
T1D1-02	0.790
T1D1-03	0.324
T1D1-04	0.684
T1D1-05	0.406
T1D1-06	0.954
T1D1-07	0.190
T1D3-01	0.125
T1D3-02	0.202
T1D3-03	0.298
T1D3-04	2.250
T1D3-05	0.357

T1D3-06	1.495
T1D3-07	1.772
T1D3-08	0.422
T3D1 1	0.076
T3D1 2	-0.003
T3 D1 3	-0.041
T3D1 4	0.539
T3D1 5	0.992
T3D1 6	1.630
T3D1 7	0.584
T3D1 8	0.268
T3D1 9	1.823
T3D1 10	1.325
T3D1 11	0.337
T3D1 12	-0.029
T3D1 13	-0.088
T3D1 14	0.017
T3D3 1	0.096
T3D3 2	0.053
T3D3 3	-0.028
T3D3 4	0.108
T3D3 5	0.075
T3D3 6	0.495
T3D3 7	-0.024
T3D3 8	-0.022
T3D3 9	-0.084
T3D3 10	-0.050
T3D3 11	-0.068
T3D3 12	-0.034
T3D3 13	-0.024
T3D3 14	0.256
T3D3 15	0.005

Table 6. Daily syringe volume measurements for PEEK tubing experiment. Sample intake and PEEK Tubing should be decreasing by 0.6 and 0.4 mL daily, respectively, and the discharge should be increasing by 1.0 mL daily. Peek tubing was checked for obstruction, and none were

Syringe Location	**Sample coil intake**	**Peek Tubing**	**OsmoSampler Discharge**
Date	volume (mL)		
6/20/2016	3	3	1
6/21/2016	1.9	3	1.5
6/22/2016	1.3	3	2
6/25/2016	0->3	3	
6/27/2016	0.9->3.3	2.9	5.2
6/30/2016	1.2	2.9	7.1
7/1/2016	0.6	2.8	8
7/5/2016	0	2.9	10.8

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